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Abstract No.1

Determination of thermodynamic parameters of extraction of mercury compounds from fish species in the Anzali Wetland

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Fish is an important source of nutrients for humans. It is an excellent source of proteins, vitamin D, selenium and, especially, long-chain n-3 fatty acids eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid. However, some fish species can also contain harmful substances such as methylmercury. These contaminants are present at low levels in the aquatic ecosystem, but bioconcentrate in the aquatic food chain. Methylmercury, as an organic mercury, mainly originates from biotic methylation of inorganic mercury promoted by algae, bacteria or fungi in the ocean and in sediments of freshwater system. Methylmercury the availability and toxicity to living organisms are increased owing to its lipophilic and protein-binding properties and it is readily accumulated by biota and biomagnified along food-chains. The main route of $MeHg^+$ poisoning in humans is fish consumption.
Mercuric compounds distributed throughout via the bloodstream to all tissues in the body. In muscles, mercuric ions have also strong tendency to bond to sulfhydryl groups of amino acids of protein molecules. So, their bonds to this group in Cysteine Amino acid of different protein muscles tissue in fishes and stored. Based on Pearson principle, mercuric and sulphide ions of Cysteine amino acid of proteins can form covalent bond with each other. Bonding energy between mercuric and sulphide is enthalpy type. Hence, by digestion muscle tissue fish in range of temperatures (331- 365 K) can be obtained bonding energy. In this work, mercury compounds were determined in muscle tissues of common carp, northern pike, goldfish and white fish in the Anzali wetland using a cold vapor atomic absorption spectrometry. Logarithmic plot of mercury concentrations versus 1/T can be given ΔH°, ΔS° and ΔG° of extraction that related to the mercury compounds bond with SH group of protein.

Key words: Methylmercury, Muscle, Common carp, Northern pike, Goldfish, Anzali Wetland, cold vapor atomic absorption spectrometry, Thermodynamic.

Abstract No.2

Thermodynamics of binding silver ions with jack bean urease

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A thermodynamic study of silver ions upon interaction of jack bean urease (JBU) was carried out at two temperatures of 27°C and 37°C in Tris buffer (30 mM; pH7.0) using an isothermal titration calorimetry. There is a set of twelve identical and non-interacting binding sites for silver ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 185 μM and -16.7 kJ/mol at 27°C and 229 μM and -16.3 kJ/mol at 37°C, respectively. The molar entropy of binding is +15.7 J K^{-1} mol^{-1}at 27°C and +17.1 J K^{-1} mol^{-1} at 37°C. Hence, the binding process of silver ion to JBU is not only enthalpy driven but also it is entropy driven, which the role of entropy driven should be more effective by increasing the temperature.

Keywords: Urease, Silver ion, Isothermal titration calorimetry, Binding constant, Enthalpy of binding, Entropy of Binding.

Abstract No.3

Competition binding study of aspirin and amlodipine to human serum albumin in multi-drug therapy: a molecular dynamic approach

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The competition between drugs can be analyzed by molecular modeling and bioinformatics tools. The effect of the displacement of one drug from the complex of the other with human serum albumin (HSA) has been described on the comparison of inhibition constants for the binary and ternary systems. The aim of this work was to determine the competition between amlodipine (AML) and aspirin (ASA) in muli-drug therapy. Binding of AML and ASA can result in decrease or increase of the affinity of the second drug to HSA this can be caused by the location in the same binding site on HSA or by the conformational changes the protein structure. Crystal structure analysis has revealed that HSA consists of a single polypeptide chain of 585 amino acid residues and comprises three structurally homologous domains (I–III): I (residues 1–195), II (196–383), and III (384–585) that assemble to form a heart-shaped molecule. Sub-domain IIA is a high affinity of binding sites for drugs. ASA also known by trade name ASA and AML are used in our work. Autodock4 was carried out to calculate possible interactions between the protein and AML and ASA. The results show interaction of AML and HSA in site II, around the ASP259, ASP255 that the best inhibition constant of protein (K_i) and docking energy are 5.25 μM and -7.2 kcal/mol, respectively. The ASA located in sub-domain IIA around the Lys199 with the best K_i and docking energy are18.51μM and-6.46 kcal/mol.
The results indicate that this drugs bind in different sites in sub-domain II with different affinity and obviously we have a ternary system. Our results have been confirmed by spectroscopic analysis.

Keywords: Albumin, Aspirin, Amlodipine, Molecular modeling

Abstract No.4

Effects of low frequency electromagnetic field on tubulins assembly and structure

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However, the low frequency electromagnetic fields (ELF) effects on animals and humans, have been studied widely, their molecular effects on structural and functional biomolecules are still unknown. Microtubules are one of the most important cytoskeletal proteins that are conductive and posses certain electric double layer and might be involved in molecular traffics in the cytoplasm. According to our results maximum polymerization identified at 217 Hz when MTs incubated for 30 min, followed by those of 100 and 50 Hz. However, the trend reversed in 5 min and to a lower level in 10 min incubated groups. The results of TEM micrographs showed normal MTs but their length had increased as a function of frequency rise. Here ELF effects on the structure and function of microtubule studied by CD, TEM and Fluorescence spectroscopy are presented to address the possible involvement of MTs in neural cell function and memory loss occurrence in Alzheimer.

Key words: Tubolins, ELF, Alzheimer.

Abstract No.5

Investigation of amylogenic properties of tTG

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Tissue transglutaminase (tTG) as a multifunctional enzyme is a member of transglutaminase family. All members of this family, except one, catalyze the calcium dependent formation of a ε-(γ-glutamyl) lysine isopeptide bond which can be inhibited by GTP. Several reports have previously shown that this enzyme is involved in apoptosis. Moreover, it has been shown that the expression of tTG is elevated in some neurodegenerative diseases such as Parkinson, Huntigtun, and

Alzheimer. Several proteins including α-synuclein, Huntigtin, β-amyloid (Aβ), actin, and tau are among the substrates of tTG. In people affiliated with Parkinson, cross linked bonds catalyzed by tTG are also present in lewy bodies. In the brain of Alzheimer patients, tTG was seen to co-localize with senile plaques and showed isopeptide activity. There has not been any extensive studies on investigation of amyloidgenic properties of tTG itself, or searching for inhibitors affecting the isopeptide bond formation. We have investigated the amylogenic property of tTG at various conditions. To this end, the cDNA of tTG was amplified by PCR and subcloned into the expression vector pET-28a. The recombinant protein was purified using affinity chromatography. Various techniques such as dye binding assay, circular dichroism (CD) and electron microscopy were employed to examine the amyloidgenic properties and fibril formation of the recombinant protein.

Key words: Tissue transglutaminase, neurodegenerative diseases, amyloid, isopeptide bond, fibril formation.

Abstract No.6

A new application for the fluorimetric liquid chromatographic method using benzoin for the analysis of N2-(2-carboxyethyl)-L-arginine in fermentation broth

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N2-(2-carboxyethyl)-L-arginine synthase (CEAS), an unusual thiamin diphosphate (ThDP)-dependent enzyme, catalyses the first step in the biosynthesis of the β-lactamase inhibitor clavulanic acid in Streptomyces clavuligerus. The first step of the biosynthesis of the clavulanic acid is the condensation of two primary metabolites, D-glyceraldehyde 3-phosphate and L-arginine, to give N2-(2-carboxyethyl)-L- arginine (CEA). The analysis of the CEA as a guanidino compound is quite difficult due to its poor delectability owing to the absence of a strong chromophore and fluorophore. In this research, a sensitive fluorimetric method for determination of monosubstituted guanidine compounds based on their reaction with benzoin is introduced. It has been shown good precision and sensitivity and required only minimal sample handling. Liquid chromatographic analysis of N2-(2-carboxyethyl)-L-arginine (CEA) in fermentation broth of Streptomyces clavuligerus RFL35 using benzoin as a fluorogenic reagent. The method is based on reversed-phase HPLC, after pre-column derivatization of N2-(2-carboxyethyl)-L-arginine (CEA) in fermentation broth of Streptomyces clavuligerus RFL35 with benzoin and fluorescence detection, it has been shown good precision and sensitivity and required only minimum sample handling. The retention time of CEA was 4.7 min. This finding may help to find the optimum fermentation medium ingredients for increasing of clavulanic acid production by Streptomyces clavuligerus. Also, it may facilitate studying mechanism of N2-(2-carboxyethyl)-L-arginine synthase, the thiamin diphosphate (ThP2)-dependent enzyme that catalyzes unusual N-C bond forming reaction.

Keywords: Streptomyces clavuligerus RFL35, N2-(2-carboxyethyl)-L-arginine, Benzoin.

Abstract No.7

Binding Propertise of a New Anti-tumor Component (2,2′_bipyridine octyldithiocarbamato pd(II) nitrate) with Calf Thymus DNA

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Metal-based drugs have been used in therapeutic medicine for several hundreds of years and are used in contemporary society for the treatment of a large variety of human ailments, e.g., cancer, diabetes, and rheumatoid arthritis, as well as in diagnostic medicine. Dithiocarbamates have also been investigated for anti-cancer potential, most notably platinum and palladium dithiocarbamates. Based on cytotoxicity assays, often the metal-containing dithiocarbamate compounds show greater potency than cisplatin but, are not as cytotoxic as other standard drugs.

Thus the interaction of a new palladium (II) antitumor complex of formula [pd(bpy)(oct-dtc)]No3 (where bpy is 2,2′-bipyridine and oct-dtc is octyldithiocarbamate) with calf thymus DNA was studied by isothermal titration UV-visible spectroscopy in Tris-HCl buffer, pH7 containing 25 mmol/L Sodium chloride. There is a set of 6 binding sites (g) for the complex on DNA (per 1000 nucleotides) with positive binding cooperativity. n, the Hill coefficient finds out to be 5.92 at 300 K and 6.18 at 310 K respectively. Kapp, the apparent equilibrium constants are 0.052 and 0.058 (mol/L)-1 at 300 K and 310 K respectively. The above complex can denature the DNA and the concentration of this complex in the midpoint of transition, [L] 1/2, is decreased by increasing temperature, from 0.093mmol/L at 300 K to

0.086mmol/L at 310 K. The conformational stability ($\Delta G^\circ H_2O$) of DNA upon interaction with cited complex determined to be 11.84 and 12.11 kJ/mol at 300 K and 310 K respectively. Thus DNA is more stable at 310 K i.e. presence of complex led to decrease the stability of the DNA. M-values (a measure of complex strength for DNA denaturation) are 0.172 and 0.205 (kJ/mol).(mol/L)-1 at 300 K and 310 K respectively. The molar enthalpy ($\Delta H^\circ_{denaturation}$) of DNA denaturation by cited complex in the range of 300 K and 310 K is determined to be 3.65kJ/mol. In addition, the calculated molar entropy ($\Delta S^\circ H2O$) of DNA denaturation by the complex is -0.03 kJ/mol.K at 300 K.

Key words: dithiocarbamate compounds, Calf Thymus DNA, therapeutic medicine.

Abstract No.8

Interaction of a mixed-ligand palladium complex of 2,2/_bipyridine and dithiocarbamate with Calf Thymus DNA

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Cisplatin is one of the most potent anticancer agents available today and is widely used in the treatment of many malignancies, including testicular, ovarian, bladder, head and neck, small cell and non-small cell lung cancers. However, its use is associated with severe side effects, such as acute nephrotoxicity and chronic neurotoxicity. Numerous strategies have been made to reduce the toxicity of platinum based drugs. One of them is using a variety of sulfur containing ligands such as dithiocarbamates.

Thus the interaction of a new palladium(II) antitumor complex of formula [pd(bpy)(hex-dtc)]No_3 (where bpy is 2,2'-bipyridine and hex-dtc is hexyldithiocarbamate) with calf thymus DNA was studied by isothermal titration UV-visible spectroscopy in Tris-HCl buffer, pH 7 containing 25 mmol/L Sodium chloride. There is a set of 7 binding sites (g) for the complex on DNA (per 1000 nucleotides) with positive binding cooperativity. n, the Hill coefficient find out to be 3.15 at 300 K and 2.77 at 310 K respectively. K_{app}, the apparent equilibrium constants are 0.26 and 0.17 $(mol/L)^{-1}$ at 300 K and 310 K respectively. The above complex can denature the DNA and the concentration of this complex in the midpoint of transition ,$[L]_{1/2}$, is decreased by increasing temperature, from 0.025 mmol/L at 300 K to 0.024 mmol/L at 310 K. The conformational stability (ΔG°_{H2O}) of DNA upon interaction with complex determined to be 9.95 and 8.9 kJ/mol at 300 K and 310 K respectively. Thus DNA is more stable at 300 K i.e. presence of complex led to decrease the stability of the DNA. m-values (a measure of complex strength for DNA denaturation) are 0.308 and 0.397 (kJ/mol).$(mol/L)^{-1}$ at 300 K and 310 K respectively. The molar enthalpy of DNA denaturation by cited complex at 300 K and 310 K is determined to be 40.46 kJ/mol. In addition, the calculated molar entropy (ΔS°_{H2O}) of DNA denaturation by the complex is 0.1 kJ/mol.K at 300 K.

Key words: anticancer agents, dithiocarbamates, thermodynamic parameters.

Abstract No.9

A novel method for measurement of protein-protein interaction by resonance light scattering

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Developing new techniques for measurement of protein-protein interaction (PPI) is one the most important aspect in biological sciences. Because, in one hand, it constitutes the central role in many biological processes and therefore to the integrity of living cells and in other hand understanding the molecular principles and different factors governing these interactions are of great important in industrial application. Some methods for studies of PPI are fluorescence resonance energy transfer (FRET), circular dichroism (CD), fluorescence anisotropy and calorimetery. Since the introduction of resonance light scattering (RLS) technique in 1993 it has been used for several purposes. However, the method using RLS for measurement of PPI has not been reported so far. Here we present a new simple and sensitive method based on RLS technique for measurement of PPI. The rationale for this method is based on the RLS relation with macromolecule volume according to RLS formula: $I_{RLS} = \left(32\pi^3 V^2 n^2 N / 3\lambda_0^4\right)\left[(\delta_n)^2 + (\delta_k)^2\right]$.
When other factor assumed fixed the RLS intensity is directly proportional to the square of macromolecule volume. So, if the PPI occur, the volume of the formed complex will be larger than each monomer alone and therefore the RLS intensity will increase. In this study we used human serum albumin (HSA) and serum transferrin (ST) as a model for evaluating this method. As we expected, the RLS intensity had increasing trend by titrating HSA by ST. Plotting the intensity versus ST concentrations produced a linear line with slope

proportional to binding constant of the complex. Furthermore, we demonstrate lomefloxacin that strengthen this complex formation. These results are in good accordance with our previous works based on second derivative fluorescence and spherical polar Fourier methods. Currently, we are going to study the above experiments based on FRET and CD techniques in order to further ascertain of this method.

Key words: Protein-protein interaction, resonance light scattering, Human serum albumin.

Abstract No.10

Second derivative fluorescence spectroscopy and Spherical Polar Fourier correlations studies of protein-protein interaction between two drug carrier proteins induced by a fluoroquinolone

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From a biopharmaceutical point of view studying the interactions of drugs with blood carrier proteins, such as albumin, are very important step in drug industry since these proteins play important role in the transport and disposition of drugs. However, to our knowledge the effect of a drug on protein-protein interaction (PPI) in serum has not been taken so far and most of the researches are focused on the interaction of a drug with a carrier protein. Here we tested the effect of lomefloxacin, a fluoroquinolone antibiotic, on the interaction between human serum albumin (HSA) and serum transferrin (ST). We analyzed the H factor from second derivative fluorescence spectra for the mixture and algebraic average of HSA and ST in various concentrations of LMF. It was found that some kind of PPI has been occurred and the fluor residues of proteins are either located in the interface of the formed complex or are transferred to the core of the protein. For further considerations we measured the stoke shifts of the fluor residues in the same groups as above to probe the changes in their microenvironment upon complex formation. Interestingly it was more blue-shifted for the mixture of HSA-ST which further emphasis the previous results. Also, we measured the changes in surface hydrophobicity by the aid of ANS and it was found that protein-protein complex formation is from hydrophobic regions. Moreover, we performed a molecular dynamic study of the interaction between these two proteins based on Spherical Polar Fourier correlations (SPF) algorithm to predict the possible protein-protein interaction sites in order to have a better understanding of this interaction. Here, we demonstrated that drug may induced protein-protein complex formation which may interfere with the natural function of carrier proteins. These results are useful pieces of information for pharmaceutical companies.

Key words: Protein-protein interaction, fluoroquinolone, fluorescence spectroscopy, Spherical Polar Fourier correlations.

Abstract No.11

Human transferrin as a carrier protein for lomefloxacin: fluorescence spectroscopy and molecular modeling studies

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Serum Transferrin (ST) and human serum albumin (HSA) are two carrier proteins in the blood which affects the absorption, distribution, metabolism and excretion properties of intrinsic and extrinsic ligands. So, it is important to characterize the interaction of drugs with these proteins. Due to the importance of lomefloxacin (LMF), a fluoroquinolone antibiotic, the interaction of this to HSA has been studied before however; a deep literature survey reveals that, till now there is not any report on the interaction of LMF with ST. In fact, most of the reports are on the interaction of metal ions with this protein and there are just a few reports that show it as a drug carrier. Here we studied the interaction of LMF with HSA and ST. We determined the number of binding site and binding affinity based on fluorescence quenching method. From second derivative fluorescence spectra the hydrophobicity changes in the microenvironment of Trp and Tyr was estimated. Also, the conformational changes induced by LMF in these proteins were studied by synchronous fluorescence. Since the structure of holo-transferrin has not been resolved, we have modeled its structure and we used it in next step for molecular modeling studies. For HSA the structure was available at Protein Data Bank (PDB). From molecular modeling we have determined the binding energy and the binding cavity of LMF on these proteins. In conclusion, the binding capacity and binding affinity of LMF for HSA are lower and higher, respectively compared to those for ST. LMF interaction with these proteins is accompanied by some conformational changes. Finally we show that, although, the affinity of LMF to ST is much lesser than of

that to HSA, but it is still enough to be carried by ST and this protein can also act as a carrier for this drug.

Key words: Human transferrin, fluorescence spectroscopy, molecular modeling.

Abstract No.12

A novel view for calculation of ligand-protein binding parameters by resonance light scattering

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Since many biological phenomena depend on the interactions of ligands to proteins, it is of importance to develop new sensitive and simple method for determination of binding parameters. Some techniques commonly used for this purpose are equilibrium dialysis, UV spectrophotometery, fluorescence spectroscopy and calorimetery. Resonance light scattering (RLS) is a sensitive instrumental technique. RLS used for several purposes such as analytical applications and also there is one report on the application of this technique for determination of the inclusion constant of drug to oligosaccharides; however to our knowledge the method using RLS for calculation of ligand- protein binding parameters has not yet been reported. This method is based on the fact that the intensity of RLS is proportional to the volume of the macromolecule. So, the increase in the concentrations of the ligand-protein complex can intensify RLS spectra. Based on this physical property we determine the number of binding site and the binding affinity of the lomefloxacin as a drug to human serum transferrin as a carrier proteins which is equal to 1.63 and $7.493 \times 10^3\ \mathrm{M}^{-1}$ respectively. Also we determined these parameters by fluorescence spectroscopy method and interestingly these were in good accordance to that of RLS. The calculated value from fluorescence spectroscopy was as 1.55 and $7.613 \times 10^3\ \mathrm{M}^{-1}$ for number of binding site and the binding affinity respectively. As a result the RLS method can be used for calculation of the ligand-protein binding parameters, however further research by adopting other ligands and proteins are needed to establish such method.

Key words: ligand-protein binding, resonance light scattering, fluorescence spectroscopy, calorimetery.

Abstract No.13

An electrochemical acetylcholine nanobiosensor based on nanoshells hollow nickel microspheres-Nafion nanocomposite

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Electrocatalytic oxidation of acetylcholine (ACh) on different nickel-based composites was investigated. The morphology and the structure of the catalyst (Ni nanoshells) was examined by scanning and transmission electron microscopies (SEM and TEM). Cyclic voltammetry and chronoamperometry were employed to study the oxidation process. The results showed that ACh was irreversibly oxidized on nickel nanoshells-carbon microparticles-Nafion composite with the highest catalytic activity. The catalytic rate constant and the transfer coefficient for the electrocatalytic oxidation of ACh and the diffusion coefficient for ACh were obtained using cyclic voltammetry, steady-state polarization measurements and chronoamperometry. A sensitive and time-saving sensing procedure was developed for the analysis of ACh. The nanocomposite showed high sensing performance with a sensitivity of 48.58±0.43 mA M^{-1} and a limit of detection of 49.33 nM.

Key words: acetylcholine nanobiosensor, Ni nanoshells, SEM, TEM.

Abstract No.14

Cytotoxicity and rich DNA-binding studies of 1,10-phenanthrolinebutyldithiocarbamato palladium(II) complex

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cis-Diamminedichloroplatinum(II), or cisplatin, has become one of the most important chemotherapeutic agents for the treatment of a wide spectrum of solid tumors. The toxic side effects associated with this compound let to the development of second generation drugs [1]. In response to this need, palladium complex of the type [Pd(phen)(But-dtc)]NO_3 (where But-dtc is buthyldithiocarbamate and phen is 1,10-

phenanthroline) has been synthesized[2] and interacted with highly polymerized calf thymus DNA.

The interaction of DNA by this antitumor complex has been investigated in aqueous solution using fluorescence, electronic absorption titration and gel filtration methods. The results of fluorescence titration suggest that the above metal complex presumably intercalate into DNA through the planar 1,10-phenanthroline ligand present in its structure. Studies of gel filtration method show that the binding of this complex with DNA is strong enough and do not break readily. This compound can denature DNA and the concentration in the midpoint of transition $[L]_{1/2}$ decreased by increasing the temperature, from 0.0134 mM at 300K to 0.0127 mM at 310K. The conformational stability of DNA in the interaction with this complex ($\Delta G^{\circ}_{(H_2O)}$) are in the range of 9.846 kJ/mol and 19.61 kJ/mol at 300 and 310K respectively. The values of m (a measure of ligand strength for DNA denaturation) are in the range of 589.6 and 710.1 (kJ/mol).$(mol/L)^{-1}$ at 300 and 310K respectively. Also the enthalpy of DNA denaturation by the complex ($\Delta H^{\circ}_{conformation}$ or $\Delta H^{\circ}_{denaturation}$) in the range of 300K and 310K is find out to be 37.58 kJ/mol. In addition, the calculated entropy ($\Delta S^{\circ}_{(H_2O)}$) of DNA denaturation by complex is 0.055 at 300K. The negative value of entropy change is related to the less disorder of denatured DNA with respect to the native DNA. There is a set of 5 binding site (g) for the complex on the DNA with positive cooperativity in binding. n, the Hill coefficient (as a criterion of cooperativity) find out to be 1.97 and 1.60 at 300K and 310K respectively. K_{app}, the apparent equilibrium constant are 6.69 mM^{-1} at 300K and 4.36 at 310K respectively. The complex show 50% cytotoxic concentration (Cc_{50}) value against chronic myelogenous leukemia cell line, K562, much lower than that of cisplatin.

Key words: *cis*-Diamminedichloroplatinum(II), calf thymus DNA, electronic absorption titration.

Abstract No.15

Binding studies of dihydropyrimidinone drivationes to serum albumin by fluorescence, molecular docking and QSAR

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Binding of dihydropyrimidinone drivationes was investigated by fluorescence spectrophotometry, molecular docking as well as QSAR at pH4 and pH 7 and 27°C and 37°C. Binding constant was obtained from: $F_0/(F_0 - F) = (1/f)K[Q] + 1/f$ Structure of ligands were optimized by AM1 implemented in Hyperchem 7.0. Molecular docking was performed by Autodock 3.0. Docking energy sorted based on their values. Two binding sites characterized by binding probability or number of binding clusters which compatible with literature. Correlation between experimental binding energy (ΔG_{exp}) and docking free energy (ΔG_{doc}) evaluated by SPSS and correlation coefficient was 0.81. Over 1400 molecular descriptors were calculated by Dragon 3.0. Correlation between ΔG_{exp} and ΔG_{doc} with cited descriptors was studied by multiple linear regression (MLR). An equation between experimental and calculated descriptor was derived. The correlation coefficient between predicted and experimental data was obtained as 0.94 and 0.95 for ΔG_{exp} and ΔG_{doc} respectively. Classification of descriptors was done by principal component analysis (PCA). Results showed that smaller, more aromatic, less compact and more HOMO energy molecules have higher free energy of binding as following.

$$\Delta G_{\text{binding}} = 2.76 - 0.001(Volume) + 1.56(Aromaticity) - 10.32(Folding) + 0.03(E_{HOMO})$$

Key words: Dihydropyrimidinone derivatives, Principal component analysis, QSAR, Human serum albumin, Molecular docking.

Abstract No.16

The Inhibition of Advanced Glycation End Products (AGEs) of Human Serum Albumin by Morphine: Spectroscopic Study

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Hyperglycemia in diabetes causes non-enzymatic glycation of free amino groups (lysine residues) of proteins and leads to their structural and functional changes, resulting in complications of the diabetes. Human serum albumin (HSA) incubates with glucose forming glycated HSA (GHSA). We examined the structural modifications of GHSA in the presence and the absence of morphine. We incubated HSA with glucose (40 mM) in presence and absence of morphine at 37°C for 42 days. To study the structural changes, Far-UV CD was used to measure changes in the secondary structure of HSA and GHSA, fluorescence spectrophotometer for detection of fluorescent advanced glycation end

products (AGEs) and UV spectrophotometer was used for quantification of the reacted lysine side-chains. The GHSA shows an increase in fluorescence intensity because of formation of AGEs and changes in secondary structure compared to non-glycated HSA. The results indicate that morphine inhibits the formation of fluorescent AGEs and it can also reduce the changes of HSA structure in hyperglycemic condition. Furthermore, morphine reduces the amount of modified lysine side chains.

Key words: Diabetes, Glycation, Human serum albumin, Far-UV CD, Fluorescence and UV- Visible spectroscopy

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Abstract No.17

A fluorescence spectroscopic investigation of Human holo-transferrin upon interaction with Ropinirole hydrochloride in the absence and presence of different ions

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The binding of Ropinirole hydrochloride (REQUIP) to Human holo-transferrin (hTf) in the absence and presence of various kind of ions (Fe^{3+}- Al^{3+}- Cr^{3+}- Co^{2+}- Mg^{2+}) under physiological conditions (pH=7.4) has been investigated by using fluorescence spectroscopy. hTf is a single chain glycoprotein containing N-lobe and C-lobe with 679 amino acids and molecular mass of ~ 80 kDa present in plasma at a concentration of about 35 μM. hTf is only 30% iron-saturated and the vacant sites can bind other metal ions. REQUIP is an orally administered non-ergolin dopamine agonist and the molecular weight is 296.84 Da. REQUIP as a treatment for Parkinson's disease and Rest legs syndrome (RLS).

The intrinsic fluorescence of hTf was quenched by REQUIP in the absence and presence of different ions, which were rationalized in terms of the static-dynamic quenching with two set of binding sites for hTf-REQUIP and Fe^{3+}, Al^{3+}, Cr^{3+}, Co^{2+} ions and the dynamic quenching fluorescence with one set of binding site for hTf-Mg^{2+}-REQUIP complex. The number of binding sites and the Stern-Volmer quenching constant of the hTf-REQUIP complex were determined 0.84, 0.59, 1.159×10^{7} M^{-1} and 5.257×10^{6} M^{-1}. The n value and K_{SV} in the presence of Fe^{3+}, Al^{3+}, Co^{2+}, Cr^{3+}, Mg^{2+} ions were calculated 0.71, 0.53, $1.282\times10^{7}M^{-1}$, 5.876×10^{6} M^{-1}; 0.98, 0.66, 1.041×10^{7} M^{-1}, 5.491×10^{6} M^{-1}; 1.03, 0.67, 1.286×10^{7} M^{-1}, 6.092×10^{6} M^{-1}; 1.31, 0.50, 1.121×10^{7} M^{-1}, 4.248×10^{6} M^{-1} and 0.74, 3.459×10^{6} M^{-1}, respectively.

The red shift in maximum wavelength and decreased in fluorescence intensity in the absence and presence all the ions, revealed increased polarity of the fluorophores, changed and disturbed the microenvironment of tryptophan and tyrosin residues and less hydrophobic of fluorophores microenvironment. The hTf-Co^{2+}-REQUIP complex indicated the most quenching fluorescence, therefore in presence of Co^{2+}, the most interaction between hTf and REQUIP was shown and in the presence of Mg^{2+}, the less than other ions quenching and interaction between hTf-REQUIP was shown. This study is expected to provide important insight into the interaction of the physiologically important hTf with REQUIP and different ions to use in various therapeutic projects.

Key words: Human holo-transferrin, Ropinirole hydrochloride, static-dynamic quenching.

Abstract No.18

A synchronize study of interaction between HSA with to anti-breast cancer drugs (estradiol and paclitaxel): Fluorescence and molecular modeling approaches

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HSA can bind and carry through the bloodstream poorly water-soluble drugs, including anticoagulants, tranquilizers and anesthetics. HSA often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro. It has been shown that the distribution, free concentration and the metabolism of various drugs can be significantly altered as a result of their binding to HSA. Drug interaction with proteins will in most cases significantly affect the elimination rate of the drug. Up to now, extensive investigations on interactions between proteins and components of living systems or pharmaceutical molecules have been carried out, because such studies can provide information on the features that affect the therapeutic effect of drugs. Drugs which are administered in multidrug therapy may alter each others binding to the transporting protein. Therefore, it is necessary to determine both the location of the binding sites and the possible interactions for each individual drug. The competition between two drugs for the binding sites on HSA may result in decrease in binding and hence increase in the concentration of free biologically active fraction of one or both the drugs. Here we studied the synchronize interaction of estradiol and paclitaxel with HSA. Paclitaxel is an anticancer and estradiol employ in treatment of breast

cancer, hypoestrogenism and preventation of osteoporosis. The quenching of HSA fluorescence is accompanied by the blue shift of maximum protein fluorescence in the presence of paclitaxel. The blue shift of tryptophanyl maximum of emission wavelength points to a decrease of polarity within the binding site of paclitaxel. The binding constant values and the number of binding sites were determined from the fluorescence intensity changes. Estradiol influences on the binding site of HSA by paclitaxel. In the presence of estradiol, the HSA binding site for paclitaxel is significantly decreased than when estradiol is absent. On the other hand in the presence of estradiol, binding affinity paclitaxel to HSA has increased. Molecular dynamic studies confirmed our results obtained by fluorescence technique.

Key words: HSA, estradiol, paclitaxel, Fluorescence spectroscopy, molecular modeling.

Abstract No.19

Different Modes of Interaction of *Saffron* Carotenoides with I-DNA motif

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Saffron is the dried stigmas of a flower scientifically identified as *Crocus sativus* L. It has various biological and medicinal properties including antitumor activity. One of the saffron targets is ctDNA. Our previous studies indicated that saffron carotenoids and monoterpenaldehydes bind to the DNA minor groove. Recently we attempted to investigate the interaction of two major saffron carotenoides (crocin and crocetin) with specific oligonucleotides. Here the results of the interaction of CCC-repeat sequences with the named components is presented. Circular dichroism (CD) studies strongly suggested that the CCC-repeat sequences, with different lengthes, adapt a four stranded I-motif structure. This structure has a critical function in the c-ki-ras protooncogene promoter, besides the human telomers. Our results showed that the mentioned saffron components interact with this structure. Crocin stabilized the selected sequences with i-motif structure, but interaction of crocetin resulted in the destabilization of this structure and precipitate formation. In conclusion, saffron secondary metabolites (crocins and crocetin) interact with particular oligonucleotide structures and induce some conformational changes in them. This is possibly the reason fro various properties of these and other saffron components.

Keywords: Saffron, Carotenoides, I-motif, Circular Dichroism.

Abstract No.20

The effect cationic and anionic porphyrins on the structure and activity of adenosine deaminase

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The effect of meso-tetrakis(3-N-methyl-pyridyl) porphyrin and meso-tetrakis(4-sulfonatophenyl)porphyrin on the structure and activity of adenosine deaminase(ADA) was investigated by UV-Vis, circular dichroism (CD), fluorescence specectrophotometry and molecular dynamics as well as molecular docking at 37 ^{o}C and different pH. Kinetic results showed that both organic ligands act as non-competitive inhibitors. Increasing the pH make enhancing the inhibition constant (K_I) for both ligands. The inhibition constant for cationic are 13.08, 29.86, 45.61, 83.17, 132.7 $\mu M^{-1}$and for anionic are 4.31, 5.05, 17.08, 39.75, 102 μM^{-1} at pH 4, 5, 6, 7, 8, respectively. On the other hand, ligand binding was studied by fluorescence specectrophotometry. The results of binding were compatible with kinetic results. Increasing the ligand concentration, causes decreasing the fluorescence intensity. Circular dichroism specectrophotometry showed that reducing the pH decreases the secondary structure of ADA. Anionic porphyrin reduces the secondary structure more than the cationic porphyrin. Molecular docking was used as a complementary to fluorescence specectrophotometry in order to estimation of docking energy and binding site. The result showed that free energy of docking for cationic is more negative than anionic porphyrin.

Keywords: Porphyrin, Adenosine deaminase, Fluorescence, Circular dichroism, Electrostatic and hydrophobic interaction, Inhibition constant.

Abstract No.21

Analysis of non-Michaelis behavior of yeast alcohol dehydrogenase activity

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Alcohol dehydrogenases (ADH) catalyze reversible oxidation of alcohols to their corresponding aldehydes. ADHs are found in many organisms. In yeast, alcohol dehydrogenase (YADH, EC 1.1.1.1) is a NAD^+-dependent oxidoreductase (150KDa). YADH is one of the first enzymes which has been purified and crystallized. Regarding to the importance of YADH in the biotechnological processes and its applications in food industry, many researchers survey characterization of YADH activity but much information is not available about YADH activity. The purpose of this research is to investigate and characterize kinetic parameters of YADH activity. The activity measurements were performed in 0.01M sodium pyrophosphate buffer (pH 8.5, 25°C), 1.5 mM NAD^+ and various ethanol concentrations at 25ºC using Shimadzu UV-1800 spectrophotometer equipped with cell holder temperature controller. Analysis of results indicated that the enzyme had non-Michaelis behavior. The enzyme activity contained two phases that the first phase takes place in wide range of low ethanol concentrations and the second phase was in higher ethanol concentrations. The first and second phase showed negative and positive cooperativity, respectively. Hill coefficient, CL_{max}, S_{max}, S_{50} were determined using saturation curve, Hill and Clearance plot. We deduced the activity of YADH has been changed in different substrate concentrations via allosteric effect of the substrate.

Keywords: alcohol dehydrogenase, non-Michaelis, Hill coefficient, Clearance.

Abstract No.22

The competitive interaction studies between aspirin and tamoxifen with human serum albumin: A fluorescence spectroscopic approach

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Combination of several drugs is often necessary especially during long-them therapy. The competition between drugs can cause a decrease of the amount of a drug bound to albumin. This results in an increase of the free biological active fraction of the drug. The aim of the presented study was to describe the competition between aspirin and Tamoxifen in binding to human serum albumin. The binding sites for aspirin and tamoxifen in tertiary structure of human serum albumin with the use of spectrofluorescence analysis. Therefore the analysis of the Ksv and n are necessary for the formulation of drugs. It was found that aspirin has two set of binding sites in HSA, while tamoxifen has one set of binding site in HSA .In order to find out the changes in the fluorescence of HSA bound with a drug in the presence of another drug the quenching curves in the ternary system. The quenching of HSA fluorescence in the ternary systems (HSA-ASA) [TMX] and (HSA-TMX) [ASA], differs from that in the binary systems ASA-HSA and TMX-HAS respectively. It was found that the presence of ASA changes the number of classes of TMX binding sites in the structure of HSA.Tamoxifen has one set of binding site in HSA while in the presence of ASA it has two sets. There are two set of binding sites for ASA in HSA structure and the presence of Tamoxifen dose not change this value. In other words, ASA can cause the increase of the values of quenching constant Tamoxifen-HSA complex. The competition of aspirin and tamoxifen in binding to human serum albumin should be taken in to account in the multi-drug therapy.

Key words: aspirin, tamoxifen, human serum albumin, fluorescence spectroscopy.

Abstract No.23

Second derivative fluorescence spectroscopy investigation of conformational changes of hTf upon interaction with an anti-breast cancer drug

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The second derivative fluorescence spectroscopy is a sensitive and reliable technique for monitoring and characterizing the transitions that take place in the environments of tyrosines and tryptophans (mainly tryptophans) in proteins was investigated. The second derivatives of the emission scans of human serum transferrin (hTf) was studied under different pH conditions at two wavelength 280, 295 nm. The fluorescence intensity of hTf gradually decrease upon increasing the

concentration of Tamoxifen and the emission λ_{max} is shifed to high wavelengths at all pH because of the unfolding of protein. An analysis of fluorescence second derivative spectra suggests that features two negative bands (I, II) at 280, 295 nm. The origion of this bands is presumably due to the transition of the electrons back to the different vibrational levels of the ground state. As the concentration of tamoxifen is increased, the position and the intensity of the shortest wavelength band are altered. Specifically, an increase in the intensity of the shortest wavelength band is observed along with a red shift, indicating the binding of tamoxifen to hTf and conformational changes induced by the interaction leading to further expoture of tryptophan residue to the polar solvent and the binding site of tamoxifen on hTf was very close to tryptophan residue. From the second derivative, it is observed the negative band (I) is most sensitive to the changes in the tertiary structure of hTf on addition of tamoxifen and the loss in the intensity at band (I) with no change at band (II) when the pH is lowered, relates to the partial unfolding of hTf. The presence of tyrosine on the spectra is noticeable at wavelength below 350 nm because tyrosine second derivative spectrum shows a minimum at around 300 nm and a maximum about 325 nm and therefore contribute to tryptophan second derivative spectra distortion in the 290-350 nm region. Parameter H is used to monitor apparent changes in second derivative fluorescence spectra when structural transitions are induced in these proteins and it is associated with changes in the degree of polarity in the environments of all the tryptophans in a protein. Thus, second derivative fluorescence spectroscopy should help in revealing characteristics of the structure and/or function of these proteins and tool to identify partially unfold states of proteins during formulation utilizing order of magnitiude lower concentrations compared to such other technique as near UV CD.

Key words: fluorescence spectroscopy, hTf, anti-breast cancer drug.

Abstract No.24

Preparation of curcumin loaded human serum albumin nanoparticles

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Curcumin is a pigment compound in curcuma *longa* L. that act as cancer chemopreventive agent. It has been widely used as a spice and inflammation, skin wounds and drugs. Also, curcumin has antimicrobial and antiviral properties and considered as cancer chemopreventive agent. In spite of wide biological application of curcumin, its poor solubility in water at acidic and physiological pH, and its rapid hydrolysis under alkaline conditions limits its usage. Albumin has long been the center of attention of pharmaceutical industry due to its ability to carrier various drug molecules and alters their pharmacokinetic properties. So, vehicle generated from albumin is appropriate delivery system in human body.

The present study is aimed at developing and exploring the use of HSA to synthesize curcumin nanoparticles. Addition of organic solvent to aqueous albumin solution near the isoelectric point reduces the dielectric constant of the media and causes aggregation or agglomeration. Then nanoparticles of HSA are formed after 5 times sonication for 2 minutes. The effect of some experimental conditions such as HSA concentration, pH, and the molar ratio of acetone to HSA were investigated. Size distribution of particles was measured with TEM.

Flocculation was observed when 4% HSA was employed and it was difficult to redisperse this flocculated even by sonication. The majority of the nanoparticles had a size distribution from 50-200 nm. The results showed that pH and the different volume of acetone has no significant effect on the size of nanoparticles. Encapsulation efficiency of curcumin-albumin nanoparticles and their size show that nanoparticles obtained from 4% albumin solution are the best carrier system for curcumin.

Key words: Curcumin, human serum albumin, cancer chemopreventive.

Abstract No.25

Intermediates monitoring for poly-alanine in the presence of guanidine chloride

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All-atom molecular dynamics simulation of poly-Alanine (PA) was investigated in absence and presence of 0.224, 0.448, 0.673, 0.897 and 1.122 M of guanidine chloride (GC) at different temperatures by Gromacs 3.3 molecular dynamics at constant pressure and temperature (NPT). The initial helix structure converted to random coil in the studied time interval. Increasing the temperature increases the

rate of helix to coil phase transition, while increasing the concentration of denaturant decreases the cited conversion. Results of calculated heat capacity (Cp) showed that transition temperature increases by increasing the denaturant concentration. Which are 300, 315 and 330 K at 0.224, 0.673, 1.122 M respectively. In some conditions (i.e. higher temperature) helix partially denatured to the coil and then another regular structure such as beta and/or helix structure created following converted to random coil. Thus two peaks will be observed in the plot of *C*p versus temperature. One peak related to transition of helix to partial denatured and another related to conversion of beta to random coil.

Key words: Phase transition, polyalanine, thermal stability, heat capacity, molecular dynamics.

Abstract No.26

The second derivative fluorescence spectroscopic investigation of human holo-transferrin upon interaction with cyclophosphamide at different pH

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Human serum transferrin is a single-chain glycoprotein with molecular mass of ~80kDa and has a major role as iron transport. Cyclophosphamide is an anti-breast cancer. Second derivative fluorescence spectroscopy is a sensitive and reliable technique for monitoring and characterizing the transitions that take place in the environments of aromatics amino acids (mainly tryptophan) in proteins. One advantage of using this technique lie is the possibility of monitoring process in proteins, which involve relatively small changes in the tryptophan environments but which may not be clearly visible in the fluorescence spectra. The value of H is $H=h/h'$. H is the different in intensities between the minimum around 320-350 nm and the shoulder at 370 nm. h' is the difference in intensities between the maximum at 400 nm and the minimum around at 320-350 nm. In our study in three different pH at 6.4, 7.4 and 8.4 the H value increase by increasing of drug concentration indicate that the polarity of fluorophore decrease. Therefore the second derivative fluorescence spectroscopic can demonstrate the microenvironments of fluorophore of proteins at different condition and we can investigate the conformational changes of protein upon interaction with ligands.

Key words: human holo-transferrin, cyclophosphamide, fluorescence spectroscopy.

Abstract No.27

A comparison between two carrier blood proteins (HSA and hTf) upon interaction with cyclophosphamide at three different pH: A fluorescence spectroscopy approach

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Human serum albumin (HSA) is a carrier protein and most important, abundant constituent of blood plasma and serves as a protein storage component. Human serum transferrin is a carrier protein and has a major role as an iron transport. Fluorescence spectrum of HSA and hTf was quenched with increasing of cyclophosphamide. In the study the interaction between HSA and cyclophosphamide in pH 7.4 two series of binding site and apparent binding constant are obtain. They are K_{sv1}= 8.6×10^8 M^{-1}, K_{sv2} =6.3×10^8 M^{-1} and n_1=0.99, n_2=0.97. But in pH 8.4 and 6.4 there is just one set of binding site and apparent binding constant obtain. In pH 8.4, n value is 0.99 and K_{sv} value is 4.1×10^8 M^{-1}, and in pH6.4, n value is 0.99 and K_{sv} value is 10^5 M^{-1}. At two other pH (6.4 and 8.4) there are two set of binding sites and apparent binding constant obtain. In pH 8.4 K_{sv} value are 1.07×10^8 $M^{-1}$and 4.04×10^7 M^{-1}, and n value are 0.97 and 0.99 respectively. At pH 7.4 the values of K_{sv} are 6.08×10^7 M^{-1} and 46.5 M^{-1} and n values are 0.98 and 0.98 respectively. In pH 6.4, K_{sv}=1.3×10^8 M^{-1},and n=0.99.This data indicate that the affinity of HSA and hTf to cyclophosphamide in pH 6.4 is more than the other pHs and in the otherwise cyclophosphamide binds to HSA more than hTf because HSA have important role in carrier the ligands than the hTf.

Key words: HSA, hTf, cyclophosphamide, fluorescence spectroscopy.

Abstract No.28

Study on interaction of DNA from calf thymus with 1,10-phenanthroline diimine palladium(II) complex of short hydrocarbon chain ethyldithiocarbamate ligand as potential antitumor agent

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The platinum complexes such as cisplatin (CDDP) and carboplatin are the subject of much attention because of their beneficial effects in the treatment of cancer. Although CDDP exhibits strong activities against ovarian, gastric, and prostate cancers, it has serious problems of nephrotoxicity and emesis. To reduce these side effects, agreat deal of effort has been focused on the preparation of new complexes as well as on methods of administration. In the present study, we evaluated the effectiveness of the interaction of calf thymus DNA with a new palladium (II) antitumor complex of formula [Pd(Phen)(Et-dtc)]NO_3 (where Phen =1,10-phenanthroline and Et-dtc=ethyldithiocarbamate) in 10 mmol/L of Tris-HCl buffer of pH=7.0.

The complex show 50% cytotoxic concentration (Cc_{50}) value against chronic myelogenous leukemia cell line, K562, much lower than that of cisplatin. This water soluble complex has been characterized by spectroscopic and non spectroscopic methods and interacted with calf thymus DNA using UV-Vis isothermal titration method in Tris-HCl buffer solution (pH=7.0) at 300 and 310 K. In these interaction studies, binding parameters, thermodynamic parameters, and the types of bindings between this agent and DNA are described as follow:
The above compound can denature DNA and the concentration of this ligand in the midpoint of transition ($[L]_{1/2}$) is decreased by improving temperature, from 0.0101 mM at 300K to 0.0099 mM at 310K. The conformational stability of DNA in the interaction with ligand ($\Delta G^{\circ}_{(H_2O)}$) determined to be 8.85 kJ/mol and 12.39 kJ/mol at 300 K and 310K respectively. Thus DNA is more stable at 310K i.e. presence of ligand led to less stability of DNA. Values for m, (a measure of ligand strength for DNA denaturation) are 883.5 and 1216 (kJ/mol).$(mol/L)^{-1}$ at 300K and 310K respectively. The enthalpy of DNA denaturation by this complex ($\Delta H^{\circ}_{conformation}$ or $\Delta H^{\circ}_{denaturation}$) in the range of 300 and 310K find out to be 96.89 kJ/mol. In addition, the calculated entropy ($\Delta S^{\circ}_{(H_2O)}$) of DNA denaturation by this complex is 0.29 kJ/mol.K at 300 K. The positive value of entropy change is related to the more disorder of denatured DNA with respect to the native DNA. There is a set of 6 binding sits (g) for the complex on the DNA with positive cooperativity in binding. n, the Hill coefficient (as a criterion of cooperativity) find out to be 1.43 at 300K and 1.24 at 310 K respectively. $K_{app,}$ the apparent equilibrium constant are 1.90 mM^{-1} and 1.77 mM^{-1} at 300K and 310K respectively. Fluorescence studies showed that this complex does intercalate in DNA. Gel filtration suggests them to bind with DNA and the binding is strong and irreversible.

Key words: calf thymus DNA, 1,10-phenanthroline diimine palladium(II), Thermodynamic paramaters.

Abstract No.29

Thermal unfolding molecular dynamics simulation of spinach plastocyanin

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Thermal denaturation of spinach plastocyanin was investigated by molecular dynamics simulation in order to study of unfolding mechanism of this protein. Molecular dynamics simulation was carried out in high temperature within 18 ns by using Gromacs software. The radius of gyration (Rg) and Root Mean Deviation (RMSD) have been used to follow the unfolding process. Moreover, distributions of Rg were used to identify states during the simulation. By using Gaussian curve fitted to distribution of Rg curves, three state and two transition ensemble was detected during the simulation.

Key words: spinach plastocyanin, sodium phosphate, MD simulation.

Abstract No.30

Study on chaperoning effect of the N-terminal propeptide precursor of a novel thermolysin-like metalloprotease

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The proper folding of some proteins requires the assistance of other proteins called molecular chaperones, while the folding of others needs an intramolecular chaperone (IMC). Extracellular proteases are generally synthesized as inactive precursors consisting of a signal peptide, an N-terminal propeptide, and/or a C-terminal propeptide, along with a mature region displaying catalytic activity. Typically, the N-terminal propeptides of proteases act as intramolecular chaperones and/or inhibitors of the cognate mature enzymes, and this is the case for subtilisin, α-lytic protease, carboxypeptidase Y, cathepsins L, and all thermolysin-like, neutral zinc-metalloproteases (TNPs) identified so far. In previous studies, the gene of a novel haloalkaline zinc-metalloprotease (SVP2) from the moderately halophilic bacterium, *Salinivibrio proteolyticus* has been cloned, sequenced, and classified as a member of family M4, which includes a large group of TNPs that are produced by both Gram-positive and Gram-negative bacteria. Newly synthesized SVP2 precursor undergoes several proteolytic processing events to yield mature SVP2, from which the N-terminal propeptide usually comprising 199 amino acids are removed. To define the role of the propeptide of SVP2 in its processing and folding, we constructed a new vector with truncated form of nucleotide sequence of *SVP2* precursor gene (ΔN-SVP2), and the effects of this propeptide deletion on casienolytic activity, processing, stability, and accumulation inside and outside of the cell were examined. Our results indicated that, although the N-terminal propeptide of SVP2 precursor shares 48% identity with that of other TNPs, which assists the refolding of protease, inhibits the folded protein to process its C-terminal propeptide, and shows a stronger inhibitory activity toward mature proteases, the SVP2 propeptide does not reveal similar functions, suggesting it makes different structure upon folding.

Key words: chaperoning, thermolysin-like metalloprotease, protein folding.

Abstract No.31

Analysis of pH Dependence Activity of Alcohol Dehydrogenase

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The activity of an enzyme is profoundly affected by pH. The effect of pH can be irreversible inactivation, occurring at extreme pH extents or reversible inactivation due to pH effects on kinetic parameters of enzymatic reaction. The essence of studying pH effects on enzyme is undoubtedly due to the vast application of them in industry undeniable. The biotechnological potentials of alcohol dehydrogenases encouraged us to investigate the effects of pH on its activity and stability. In this study, the effects of pH on PQQ dependent alcohol dehydrogenase purified from Acetobacter sp. Strain SSM15 was investigated. Enzyme assay was colorimeterically performed in Mcilvaine buffer with ethanol as substrate at 25 °C. According to the log v_{max} versus pH graph, the optimaum pH of ADH activity was achieved. The pK_{es} values, related to the protonation constant of the enzyme-substrate complex, were determined from the slope of the graph which is in concert with the data reported from its structural studies. The pK_{es1} and pK_{es2} were calculated 4.1 and 7.2, respectively. The pH profile of the enzyme stability was obtained by enzyme incubation in different pH for 24 hr. Concerning the pH dependence of enzyme activity, the enzyme can be considered as a diprotic type, which is reactive in monoionized form.

Key words: Alcohol dehydrogenase, PQQ, pH effects, pK_{es}, Activity, Stability.

Abstract No.32

Prediction of residues on bovine carbonic anhydrase as binding sites in reacting with sodium dodecyl sulfate based on molecular dynamics and docking simulations

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The main objective of this study is to specify binding site and define the binding affinity of sodium dodecyl sulfate (SDS) into bovine carbonic anhydrase (BCA) using the P_i values of residues and the free binding energy. P_i values obtain by the simulation procedure. The

residue i with $P_i>1$ has been considered to have affinity toward the SDS ligand, while with $P_i<1$ it have no affinity. So, the calculated P_i values for BCA residues and docking simulation to be sed to prove that. In the docking scheme, the SDS ligand docked into each of the residues with $P_i>1$, which are located on different regions of the surface of BCA. Moreover, the Blind Docking method was employed, the results of which were in good agreement with the above docking.

Key words: bovine carbonic anhydrase, sodium dodecyl sulfate, molecular dynamics, docking simulations.

Abstract No.33

Investigation of the association behaviors between lomefloxacin and human serum albumin: A fluorescence spectroscopic study

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Protein plays an important role in life processes and investigation of the interaction between small molecules and protein has been the focus of extensive research in recent years. The binding between probes and proteins is of ever increasing importance in the pharmaceutical industry, sensor and scientific communities. Albumins, the most abundant protein in the plasma, are characterised by a low content of tryptophan and methionine, a high content of cysteine and preponderance of charged acidic and basic amino acids. The interaction between lomefloxacin and human serum albumin (HSA) has been studied by steady fluorescence spectroscopy. The binding of lomefloxacin to HSA quenches the tryptophan residue fluorescence and the results show that both static and dynamic quenching occur together with complex formation. The binding constant and binding sites of lomefloxacin to HSA at pH 7.4 are calculated and showed two set of binding sites, according to the double logarithm regression curve. In addition, the distance between the lomefloxacin and HSA is estimated to be 1.32 nm using Foster equation on the basis of the fluorescence energy transfer. On the other hand the fluorescence spectra show that the microenvironment of the tryptophan and tyrosine residues has obvious changes, which obeys the phase distribution model. Finally, the thermodynamic data show that lomefloxacin molecules enter the hydrophobic cavity of HSA via hydrophobic and electrostatic interactions. The interaction between lomefloxacin and HSA induced an obvious reduction of the protein alpha helix and beta sheet structures.

Key words: lomefloxacin, human serum albumin, fluorescence spectroscopy.

Abstract No.34

Binding of curcumin to beta casein, a route to make a functional food

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International food information council (IFIC) defines the functional food as "foods that provide health benefits beyond basic nutrition". Recently a world wide attempts is performed to devise novel encapsulation materials for multi–target drugs with low solubility and hence their incorporation into food. In this regard physico-chemical properties of biopolymers such as carbohydrates and proteins render them a good candidate to achieve this purpose. In this study we speculated the virtue of the interaction between curcumin, a potent anticancer and anti inflammatory natural polyphenol, and beta-casein using U.V-Vis and fluorescence spectroscopies at different temperatures. Obtained binding isotherm plots revealed that hydrophobic interactions are the main factors contributed in beta-casein upon interaction with curcumin. Augmenting the temperatures up to 37^0C increased the number of bound curcumin up to 4 times. ANS fluorescence explained this phenomenon very well since surface hydrophobicity is enhanced at upper temperatures. These findings imply that casein as a natural biopolymer from milk can be a good matrix for increasing the solubility of curcumin in pharmacology.

Key words: curcumin, beta-casein, solubility, hydrophobic surface, functional food.

Abstract No.35

Hemoglobin from the *Acanthopagrus latus*

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Hemoglobins (Hbs) are members of the globin superfamily devoted to the transport of oxygen to cells. Except for the Antarctic fish belonging to the icefish family, these proteins are present in all vertebrates. Unlike the majority of mammals, which produce a single major hemoglobin (Hb) component (90% of the Hb content of the erythrocyte), many fish species have multiple Hb components. Multiple hemoglobin system in many fishes may be important in physiological adaptation to variable environments. The purpose of this research is to investigate pattern of hemoglobins from *Acanthopagrus latus* and purified them. Blood samples were collected by heparinized syringes and then hemoglobin was extracted. Polyacrylamide gel electrophoresis showed that hemolysates of erythrocytes from *Acanthopagrus latus* were mixtures containing 3 hemoglobin components. SDS-PAGE showed that different polypeptide chains are involved in the hemoglobin molecules. Three different hemoglobins were purified by ion-exchange chromatography of the hemolysates.

Keywords: fish hemoglobin, electrophoresis, chromatography, Multiple hemoglobin.

Abstract No.36

Isolation, Purification, and Enzyme entrapment of Different Peroxidase Isozymes from *Raphanus sativus l.niger*

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Peroxidase exists in different amount in herbal sources. Peroxidase is a glycoprotein which contains heme and includes different cation and anion isoenzymes that categorized in 3 groups: acid (pI<4), neuter (4<pI<10) and alkali (pI> 11). This enzyme is resistant against variable pH and has high thermal stability and has many applications in laboratory diagnosis for measurement hormones and bacterial toxins with immunoassay method in ELISA technique. In this study, we isolate and purify different peroxidase isoenzymes from *Raphanus sativus l.niger*. The crude enzyme was extracted from raw root plant by homogenization and concentrated crude juice using ammonium sulfate precipitation, the active fractions were collected by assaying peroxidase special method, three isoenzymes observed in primary gel electrophoresis then with purification and apply ion exchange chromatography include Q and SP- Sepharose, we observed two peroxidase isoenzymes. Isolation process of these two isoenzymes was done using SP-Sepharose chromatography procedure. In addition, we examine the detection of hydroxy aromatic derivations (phenol and benzidin) using entrapment of the peroxidase in polyacrylamide gel. Using a solution contains sodium acetate buffer and hydrogen peroxide, a peace slide of entrapment enzyme in gel in, can clearly detect phenol and benzidin in very short time. Therefore, we suggest using this model; ones could easily eliminate phenol and benzidin as primary pollution of much industrial sewage. We hope that by using abundant and cheap sources, enzyme production in our country will progress and be cost effective and we will be able to eliminate our industrial pollutions.

Key words: peroxidase, extraction, isolation, isoenzymes, acrylamide gel, phenol and benzidin.

Abstract No.37

An Extremely Thermostable Protease with an alkaline pH Profile from a hyperthermophilic *Bacillus sp.* MLA64 isolated from "Dig Rostam" Hot Spring in Iran

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This work reported a new extremely thermostable protease from a newly isolated hyper thermophilic *Bacillus sp.MLA64* from "Dig Rostam" hot spring in Iran. The protease was purified with an 11.4-fold increase in specific activity and 28% recovery. The molecular weight of the purified enzyme was estimated to be 24 kDa. The enzyme was extremely stable and highly active in the temperature range from 40 to 100° C with an optimum temperature at 95°C. The enzyme was highly active over a wide range of pH from 8.0 to 12.5, with an optimum at pH 9.5. The thermostability of the enzyme was not enhanced in the

presence of $CaCl_2$, indicating that the enzyme is calcium independent. CD spectroscopy data revealed no conformational change occurred in the secondary structure of enzyme after 30 min incubation at 95°C. The enzyme showed high stability towards non-ionic surfactants and relative stability against anionic surfactant SDS. In addition, the enzyme was relatively stable towards oxidizing agents. The N-terminal amino acid sequence of the first 20 amino acids of the purified protease showed low homology with other bacterial peptidases, suggesting that the enzyme can be a new protease.

Key words: *Bacillus sp.MLA64,* "Dig Rostam" hot spring, Purification, Characterization, extremely thermostable protease, Alkaline pH profile.

Abstract No.38

Inhibition of ceruloplasmin by lead: A role for lead toxicity

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Inhibition of human ceruloplasmin (CP) activity by lead (pb) was studied. Pb inhibited CP activity progressively up to 0.9 mM concentration where about 80% of the enzyme activity was lost. The inhibition was non- competitive with respect to p-phenylendiammonium dichloride (PPD) as substrate. Preincubation of the enzyme with millimolar concentrations of sulfhydryl groups containing compounds such as glutathione (1.2 mM, 12mM), or β-mercaptoethanol (1.2mM) protected enzyme inactivation against pb. In addition, fluoroscopy study was carried out on the native and pb-inhibited enzyme. Maximum emission spectrum of the inhibited enzyme showed an increased level of about 76% with regard to the native enzyme. The change observed in the enzyme fluorescence intensity following inhibition of the enzyme by pb was prevented in the presence of the sulfhydryl compounds. The data suggest that a conformational change in the native enzyme due to pb binding caused enzyme inactivation and sulfhydryl groups on the enzyme probably are involved in inhibition by pb.

Regarding the fact that CP is a multifunctional protein with an enzymatic role, and its involvement in several metabolic pathways, lead toxicity, at least in part, may by due to inhibition of this enzyme.

Key words: Enzyme, Ceruloplasmin, Lead, Inhibition, Toxicity.

Abstract No.39

A novel disposable functionalized-carbon nanotube/ionic liquid modified screen-printed biosensor for glucose detection

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Screen-printed glucose biosensors have been fabricated using carboxyl functionalized-multi-walled carbon nanotubes (MWCNT) and a room temperature ionic liquid of 1-allyl-3-methylimidazolium bromide ([almim] Br). The nanocomposite ([almim] Br-MWCNT) was formed by simply grinding the MWCNT with the [almim] Br. The enzyme glucose oxidase (GOx) and [almim] Br-MWCNT were mixed and then the mixture was cast on the surface of graphite screen printed electrode; therefore, biosensor is prepared in a one-step fabrication procedure. The prepared nanocomposite was used for the study of GOx direct electron transfer. The result suggested that GOx could be tightly adsorbed on the modified screen printed electrode. The modified electrode exhibited a quasi-reversible cyclic voltammogram corresponding to GOx with a formal potential of -427 mV in 0.1 M phosphate buffer solution at pH 7.0. Under the optimized experimental conditions, the proposed biosensor exhibited a relatively high sensitivity (40.33 μA μM^{-1}) toward glucose and a long-term stability. The biosensor dynamic range was from 0.033- 0.14 μM with a very low detection limit of 1 nM.

Key words: screen-printed biosensor, glucose detection, carboxyl functionalized-multi-walled carbon nanotubes.

Abstract No.40

Surfactants effect on the absorption spectra of tetra- sulfonated copper phthalocyanine

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Phthalocyanines are classical pigments that owing to the ease of preparation and modification, high stability and extraordinary physical properties, have been widely studied. Non-aggregated phthalocyanines have received considerable attention. These compounds, normally with bulky substituents, possess good solubility, which can facilitate the purification and characterization processes. The non-aggregate nature can also prevent undesirable effect arising from staking of molecules. In this work the effect of ionic surfactants (0-20 □m) on the adsorption spectra of copper-tetrasulfonatophthalocyanine was investigated at different pH, ionic strength and temperature using UV-Vis spectrophotometry (GBC-916). It is observed that intensity of peaks decreases due to addition of SDS, while in the presence of DTAB, it increases at low concentration and decreases at higher concentration of DTAB. It seems that it is due to interaction of the negatively charged sulfate group which exist in pigment molecule and positively charged surfactant. Such electrostatic interactions were not observed in SDS. By addition of urea and acetamide, the intensities of soret and Q bands decrease, and with addition of phthalocyanine, intensity of peaks increases. Plotting the absorbance versus concentration in the range of 9.9×10^{-6} M to 3.3×10^{-4} M resulted the straight line which representing of low aggregation. The similar results was observed at pH=2 while at pH=1.2 a distinct difference was observed in the absorption spectra. Also, in this case by addition of DTAB, it did not observe the increasing the absorption spectra.

Key words: Phthalocyanine, Aggregation, Surfactant, Electronic spectrophotometery, Mesomeric effect.

Abstract No.41

Limited proteolysis as a probe of conformational changes in pyruvate kinase from an Iranian *Geobacillus*

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Pyruvate kinase (ATP: pyruvate 2-O-phosphotransferase, EC 2.7.1.40) catalyzes the irreversible and a control point in the regulation of glycolytic pathway. The analysis of conformational transitions in different condition using limited proteolysis was carried out on a pyruvate kinase from the Iranian thermophilic *Geobacillus* that was cloned and over expressed in *Escherichia coli* with either trypsin or thermolysin. These proteases cleaved the enzyme into few major fragments in the same chain regions, which is suggested to be flexible loops. The optimum temperature of the enzyme was 50-70 °C. In 60 °C, it is completely digested with thermolysin. Sorbitol and glycine have a limited effect on protection of complete digestion of enzyme in 60-65 °C, without any effect on production of major fragments produced in absence of these additives. On the other hand, MgCl2 protects this enzyme from thermal inactivation at 60 °C.

Key words: Pyruvate kinase, limited proteolysis, thermolysin, additives.

Abstract No.42

Cloning and expression of the allergen *Che a 1* from *Chenopodium album* in *Escherichia coli*

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Chenopodium album (*Salmeh*) is a fast-growing weedy annual plant in the genus Chenopodium. *Chenopodium album* pollen represents a predominant allergen source in Iran. The main *Chenopodium album* allergens have been described as *Che a 1*, *Che a 2* and *Che a 3*. The aim of this work was to clone the *Che a 1* in *Escherichia coli* to be a launch for over producing the recombinant allergen. The cloning, production and purification of recombinant allergen in *E .coli* is an economical method which may provide sufficient amount of highly purified proteins for diagnostic and therapeutic purposes. In order to clone this allergen, the pollens were subjected to RNA extraction. A full-length fragment encoding *Che a 1* was prepared by polymerase chain reaction of the first strand cDNA synthesized from *Chenopodium*

album extracted pollen total RNA. Cloning was carried out by inserting the cDNA into the pET21b (+) vector, and transformed into *E. coli* /Top10. For analysis, the constructed plasmid containing *Che a 1* was subjected to sequencing. The result of PCR confirmed the existence of *Che a 1* in *E. coli* /Top10 included pET21b (+) vector. The BL21 (DE3)/pET21b (+) system was selected to express the *Che a 1* protein. In conclusion, the cDNA of the major allergen of the *Chenopodium album* pollen, *Che a 1*, was successfully cloned. This study is the first report of using *E. coli* as a prokaryotic system for *Chea 1* cloning.

Key words: allergen Che a 1, recombinant allergen, gene expersion.

Abstract No.43

Synthesis and characterization of three novel nickel (II) complexes as models for tyrosinase

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Tyrosinases are copper containing metalloproteins, having a diamagnetic spin-coupled copper paired in the active centre and are able to oxidize various phenolic compounds and thus are great interest for different biotechnological applications. Several binuclear copper chelating complexes have shown to act as model compounds of the enzyme active site. In the present work we substituted the tow copper with tow nickel atoms and interested to study the effect of changing the metal centers. Thus we prepared three binuclear nickel complexes of the type [(dien) Cu (μ-diam) Cu (dien)] $(NO_3)_4$, (where dien is diethylentriamine and μ-diam is 1,4-diaminobutane, 1,6-diaminohexane and 1,8-diaminooctane). These complexes have been synthesized by reaction of nickel nitrate with dien and subsequent addition of bridging diamine. These complexes have been characterized by the chemical analysis, conductivity measurements, ultraviolet-visible, infrared and ^{1}H NMR spectroscopy. The infrared and ^{1}H NMR spectral studies of these complexes have ascertained the modes of binding of the dien and diamine ligands to nickel centers. The molar conductance values of these nickel complexes in conductivity water suggest them to be 1:3 electrolytes. Studies of catalytic properties of these complexes in comparison with tyrosinase are in progress.

Key words: Tyrosinase, diethylentriamine, nickel (II).

Abstract No.44

Direct electrochemistry of chemically modified Laccase immobilized on carbon nanotube-ionic liquid composite

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Laccase (Lac) was modified using Woodward's reagent K (WRK) as a specific modifier of carboxyl residues. The modified Lac was immobilized on glassy carbon electrode using carbon nanotube-ionic liquid composite. Then direct electrochemistry of the immobilaized Lac was investigated. Using cyclic voltammetry a pair of quasi-reversible redox peaks were obtained at approximately -0.09 and +0.01 V vs. Ag/AgCl. The peak current ratio (I_{pa}/I_{pc}) was 0.97 and ΔE_p was 0.1 V in acetate buffer solution (pH 5) at the scan rate of 0.1 V. s^{-1}. The peak currents were linear versus scan rate in the range of 10 ~ 250 mV s^{-1}. This exhibits that the Lac was adsorbed on electrode surface. The electrochemical parameters of immobilized Lac including charge transfer coefficient (0.36), apparent heterogeneous electron transfer rate constant (1.6 ± 0.08 s^{-1}) and formal potential (-0.029 V) were determined.

The enzyme layer on electrode was quite stable and the peak currents of continuous cyclic voltammetric experiments were reproducible. The weak cathodic peak represented an irreversible electrochemical behavior while by increasing the scan rate it tended to more symmetrical behavior. This result suggested that electron transferring in oxidized species is faster than that in reduced state.

Key words: Laccase, Woodward's reagent K, Carbon nanotubes, Ionic liquid, Direct electrochemistry.

Abstract No.45

Study of the effect of hyperthermia in the presence gold nano particles and cisplatin on MM200 melanoma cell line

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Novel approaches to treat cancer that are effective with minimal toxicity profiles are needed. In the present study we evaluated gold nano-particles (GNP_s) in human melanoma cell lines (MM200) to determine: intrinsic cytotoxity of the GNP_s (50 nm diameters) and microwave-induced heating of intracellular GNP_s to produce thermal destruction of melanoma cells. We examined the anti-tumor effects of combining cisplatin and GNP_s with microwave-mediated hyperthermia in cell cultured MM200 melanoma cells. Cell culture divided in to eight groups: group 1; no treatment (control); group 2: cisplatin alone; group 3: one hyperthermia treatment; group 4: GNP_s alone; group 5: cisplatin with microwave; group 6: cisplatin with GNP_s; group7: cisplatin with GNP_s and hyperthermia; group 8: GNP_s with hyperthermia. Then with MTT assay, assessed cell proliferation per group. In addition, to clarify the rules of the every agents in MM200 in the presence of nano-particle and hyperthermia we used from SDS page electrophoresis of the samples before and after agents effects for understanding the protein mechanism of hyperthermia in the presence of nano-particles.

Key words: hyperthermia, gold nano particles, cisplatin, melanoma cell line.

Abstract No.46

A novel view of the simultaneous interaction of two anti-breast cancer drugs with human serum albumin: Spectroscopic approaches

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Human serum albumin (HSA) is the most important and abundant constituent of blood plasma. It is a globular protein composed of 585 amino acid residues in three homologous α-helical domains (I,II,III). Information on the interaction of HSA with drug can help us better understand the absorption and distribution of drug. Therefore, it has become an important research field in chemistry, life science, and clinical medicine. Acetaminophen, commonly known as Tylenol, is a medication used to treat fewer and mild to moderate pain. Acetaminophen is available alone and in combination with other medications to treat symptoms of colds, flu, headache and osteoarthritis. Fluoxymestrone is a male hormone used to treat delayed male puberty or to treat a testosterone/androgen deficiency. In woman, this medication is used to treat breast cancer. The interaction between acetaminophen with HSA at physiological conditions (pH=7.4) investigated by fluorescence spectroscopy. Quantitative analysis of binding parameters (e.g. quenching constants) indicates the affinity to the binding site. The binding of acetaminophen to HSA quenches the tryptophan residue fluorescence at 280 nm, and the results show the static quenching occurs with complex formation. The binding constant and binding sites of acetaminophen to HSA are calculated. In addition, the binding constants and binding sites for acetaminophen with being of fluoxymestrone in interaction to HSA at 280 nm were determined. Analysis of quenching of fluorescence of HSA in the binary system showed that fluoxymestrone affect the complex formed between acetaminophen and HSA. On the basis of K_a and K_q values in was concluded that fluoxymestrone may probably cause decrease affinity of acetaminophen to serum albumin. Static quenching for the binary system calculated. The binding constants of acetaminophen-HSA and fluoxymestrone complexes with it calculated for the second class of binding sites.

Key words: Human serum albumin, Spectroscopic techniques, Fluoxymestrone.

Abstract No.47

β-turn types prediction in proteins using statistical model of LDA and Artificial Neural Network

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Protein secondary structure prediction is an intermediate step in prediction of tertiary structure from amino acid sequence. B- turn is a very important element of protein structure, then the prediction of β-turns and their types are crucial process of the secondary structure prediction. The aim of the present study is predicting β-turn types using a two- stage hybrid model that comprise the linear discriminant analysis (LDA) and the artificial neural network (ANN). The databank was used in this study, consisted of 565 non homologous Protein chains which prepared using the PAPIA system. The percentage of the occurrence for 20 types amino acids in different positions of β-turn sequence (that is i, i+1, i+2, i+3) utilized as the structural parameters. Initially, LDA(a statisrical technique) analyzed 100 structural parameters and selected 40 significant parameters. The selected parameters were then used as input into a three layered feed-forward neural network. After optimization, the ANN architecture was consisted of 40,15 neurons for input and hidden layer respectively. The output layer comprise 5 neuron each of which related to 5 types of β-turn types including NS, I, II, VIII and IV of β-turn. Performance measures (MCC, prediction accuracy, sensitivity, specificity and probability of correct prediction) used for the evaluation of established model. MCC values for types I, IV, VIII and prediction accuracy values in types I and NS were the better than obtained results in previous established model (multi nomial logistic regression and ANN). In conclusion, a two–stage hybrid model was introduced that was able to predict β-turn types with high accuracy. This model is simpler and takes less time compared with the previous established model. Therefore it will highlights its potential usefulness to be applied in protein secondary structure prediction.

Key words: β-turn types Prediction, Linear discriminant analysis, Artificial neural network.

Abstract No.48

Studies on interaction between propranolol and HSA in presence and absence of magnetic field

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Human serum albumin (HSA) is the most abundant carrier protein of the blood with a high affinity for a wide range of metabolites and drugs and have many physiological functions. Propranolol, 1-[isopropylamino-3-[1-naphthyloxy]-2-propanol], is a adrenoceptor antagonist (-blocker), which is widely used in the treatment of several diseases such as cardiac arrhythmia, angina pectoris, sinus tachycardia, thyrotoxicosis, hypertrophic subaortic stenosis and hypertension. The effects of static magnetic fields (SMFs) on biological systems have been a topic of considerable interest for last two decades. The increasing production of electric (EMFs) and magnetic fields (MFs) due to the increasing use of electronic devices in homes and work places, is encouraging studies on the influences of magnetic fields on living organisms with a goal to protect better human health against their probable unfavorable effects.
In this paper, the interaction between propranolol HSA and in physiological buffer (pH 7.4) was investigated by UV and fluorescence absorption spectroscopy. In order to understand the effect of magnetic field, all experiments were also done in the presence of magnetic field (3 mT); and the binding parameters were determined for both cases.
The Hill equation was employed to analyze the data obtained, for determining of the binding capacity, g, binding constant, K, and Hill constant, n_H,.

The results showed that the values of g and n_H increased slightly in the presence of magnetic field, and the binding constant in both method, uv and fluorescence spectroscopy, was decreased in the magnetic field exposed situation.

Key words: propranolol, HSA, magnetic field.

Abstract No.49

Effects of magnetic field on the interaction between Amlodipine and hemoglobin

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Hemoglobin is the major protein component of red blood cell and as an allosteric tetrameric protein; it has an important role in carrying oxygen from lungs to different tissues, and it reacts with other gasses and also with several organic molecules. Amlodipine, which is calcium channel blocker, used for the treatment of hypertension, angina pectoris and cerebrovascular disease. In recent times, many studies concerning magnetic fields effects on biological objects were carried out, because in modern society, due to its impossibility of avoiding exposure to magnetic field produced by transmission and distribution of electric power and devices used inside houses and work places.

In this work, we investigated the interaction between amlodipine and hemoglobin in the absence and presence of magnetic field (52 mT) using UV and fluorescence absorption spectroscopy. To analyze the UV data, obtaining the binding capacity, g, binding constant, K, and Hill constant, n_H, Scatchard and Hill equations were employed. The results revealed that the value of g was the same in both case, in the presence and absence of magnetic field, but n_H and K decreased slightly in the presence of magnetic field. In the case of fluorescence spectroscopy, the fluorescence intensity was found to be decreased in the presence of magnetic field.

Key words: magnetic field, Amlodipine, hemoglobin.

Abstract No.50

Kinetic studies of lactoperoxidase interaction lead ion

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Lactoperoxidase (LPO) which is an enzyme of the mammalian peroxidase family is known as an antibacterial enzyme, and it can be used as a biopreservative agent in food, feed specialties, cosmetics and related products. Lead (Pb), a heavy metal with no known physiological function in human body, is considered as one of the most hazards that affect all biological systems through exposure from air, water, and food source. The aim of this investigation was to study the effect of Pb on the LPO activity isolated from bovine milk in vitro. LPO purified using bath wise chromatography on phosphor cellulose with specific activity of 1.1 U/mg protein. LPO activity was determined in the absence and presence of different concentrations of Lead acetate, and Lineweaver-Burk double reciprocal plot was drawn according to the data obtained.

Pb^{2+} inhibited LPO activity progressively up to 0.8 mM concentrations where about 85% of the enzyme activity was lost. The inhibition was found to be non-competitive with respect to 2, 2′-azion- bis (3-ethylbenez- thiazoline-6- sulfonic acid (ABTS). Above data suggest a conformational change in the enzyme due to Pb^{2+} binding caused enzyme inactivation and sulfhydryl groups on the enzyme molecule probably are involved in the inhibition of the enzyme by Pb^{2+}.

Key words: Lactoperoxidase, Lead, inhibition, non- competitive.

Abstract No.51

Using utrophin (dystrophin homologue) immunohistochemistry in diagnostic field

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Genetic defaults on Xp21 which result to absence of dystrophin leads to Duchenne Muscular Dystrophy (DMD).these defects may result to decrease in dystreophin product or structural defects in it and there for a mild form, becker muscular dystrophy (BMD) occurs. Many years ago Utrophin in muscle tissue identified.this protein is homologue to dystrophin and named dystrophin related protein(DRP).utrophin expression is limited to neuromuscular junction and myotendinus in normal muscle tissue but it up regulates and labeled adjacent to the majority of muscle fibers in absence or decreased amount of dystrophin. Because of the high degree of sequence similarity between these homologues utrophin could compensate for the lack of

dystrophin.amino acid sequence of utrophin results several structural motifs similar to those in dystrophin.for example the N-terminals binds to actin, rod domains have multiple triple helical repeats and cysteine-rich and C-terminal domains interact with DAPs. 65% and 73% homology of utrophin to dystrophin by comparing nucleotide and amino acid sequence, respectively was useful results for our study. Absence of dystrophin detected by immunohistochemistry is sufficient for the diagnosis of DMD patients which their genetic defects weren't detectable but for BMD, Immunohistochemistry test ,using dystrophin antibody isn't diagnostic.Due to the upregulation of utrophin in BMD patients compared with normal muscle fibers and homology between dystrophin and utrophin, parallel immunostain using utrophin antibody could be helpful for the diagnosis. In this study 15 clinically suspected BMD patients were undergone muscle biopsy.The muscle samples were examined for absence or presence of dystrophin and utrophin using IHC method. The results were compared with western blot analysis.It has been shown that utrophin immunostain is important in diagnosic panel for BMD and could be a concomitant method for western blot analysis in diagnostic fields.

Key words: utrophin, immunohistochemistry, diagnostics.

Abstract No.52

A Protein Fold Combined Classifier Based on Information Content of Sequence Extracted Features and PSSM

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Protein function is related to its chemical reaction with surrounding environment including other proteins. On the other hand, this depends on the spatial shape and tertiary structure of protein and folding of its constituent components in space. Correct Identification of protein domain fold solely using extracted information from protein sequence is a complicated and controversial task in current computational biology. A combined classifier based on Information content of extracted features from protein primary structure has been introduced in facing this challenging problem. In the first stage of our proposed two tier architecture, there are several classifiers each of them is trained with a different sequence based feature vector. In comparison with previous works, besides the predicted secondary structure, hydrophobicity, van der Waals volume, polarity, polarizability, and different dimensions of pseudo-amino acid composition vectors, the position specific scoring matrix (PSSM) has been used to improve correct classification rate. Using K-fold cross validation on training dataset related to 27 famous folds of SCOP, the 28 dimensional probability output vector from each evidence theoretic K-NN classifier is used to determine the information content or expertness of corresponding feature for discrimination in each fold class. In the second stage, the output of classifiers for test dataset will be fused using Choquet fuzzy integral operator to make better decision for target fold class. The expertness factor of each classifier in each fold class has been used to calculate the fuzzy integral operator weights. Results make it possible to provide deeper interpretation about the effectiveness of each feature for discrimination in target classes for query proteins.

Key words: Protein folding, position specific scoring matrix, Protein Fold Combined Classifier.

Abstract No.53

An efficient purification method for high recovery of h-GCSF from recombinant *E. coli*

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Human G-CSF, a single chain polypeptide containing 174 amino acid residues (MW=18,800, pI=6.1), is one of the hemopoietic growth factors. Development of inexpensive and simple culture media is always favorable for commercial production of recombinant proteins in *E. coli.* The high-level expression of eukaryotic proteins in *E. coli* often leads to formation of insoluble inclusion bodies (IBs) in the cytoplasm or periplasm. Recovery of active material from (IBs) is often difficult and involves two general steps: 1) protein solubilization in a denaturant and 2) protein refolding.

On a commercial scale, reducing the number of protein purification steps is practical and economical because each purification step not only increases the final product, but also causes successive

yield losses of the recombinant protein. In this research, we developed an efficient and scalable procedure for production and purification of recombinant human GCSF (rh-GCSF) of *E. coli.* This process includes: an optimized batch culture with LB and glucose 10 g/l with expression level 40%, cell harvesting, cell lyse with high pressure homogenizer, two-step washing, IB solubilization, refolding, and finally protein purification by FPLC with cation exchanger column. The new developed method leads to purification of 720 mg pure protein from 1.8 g l^{-1} rh-GCSF in each batch. Hence, the recovery yield was about 40% and purity over than 99%. To our knowledge, this is one of the highest yields and production levels of the purified recombinant proteins, which has been reported for human recombinant protein via expression in *E. coli*. Also by this method, we can produce a protein that its characteristics would structurally be preserved.

Key words: Human G-CSF, protein purification, protein refolding.

Abstract No.54

Purification and characterization of hemoglobin components of the Caspian Sea sturgeon *(Acipenser persicus* and *Acipenser stellatus)* blood: Spectroscopic Study

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Hemoglobin (Hb) multiplicity is a commonly used index of phylogenetic differentiation and molecular adaptation in fish and enables it to adapt itself with different ecological conditions. In the current study the Hbs of two Iranian Sturgeon species have been investigated. After extraction of Hb from blood, the polyacrylamide gel electrophoresis(SDS-PAGE) and cellulose acetate electrophoresis methods as well as isoelectric focusing have been used to confirm Hb multiplicity in these fishes. It is shown that although both species have multiple Hbs with different isoelectric points, the dominant Hb can be identified from others. Comparison of these dominant Hbs with human Hbs using electrophoresis on cellulose acetate and visual analysis of densitometer patterns showed that dominant Hb in *Acipenser stellatus* has similar band with human HbA whereas it is similar to human HbF for *Acipenser persicus*. Ion-exchange chromatography method has been used to Hb purification for these species and the accuracy of method confirmed with isoelectric focusing (IEF) and SDS-PAGE electrophoresis. Structural studies using fluorescence spectrophotometery showed that the Hbs of these fishes have similar properties with each other and clear differences with human Hbs. Also it is showed that human HbA is more hydrophobic than dominant purified Hbs of fishes. Based on circular dichroism analysis (CD) investigation, the percentage of alpha-helix and beta-sheet substructures of Hbs has been measured.

Key words: Hemoglobin, Spectroscopic Study, *Acipenser persicus, Acipenser stellatus.*

Abstract No.55

Purification of bovine lactoperoxidase and its circular dichroism spectroscopic studies

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Lactoperoxidase is one of the most prominent enzymes in bovine milk. It is a glycoprotein with a single chain and heme prosthetic group. It has 612 amino acid residues that giving the molecular mass of 78 kDa. It catalyses oxidizing halide and pseudohalide ions such as thiocyanate by hydrogen peroxide to produce potent antimicrobial which can inactivate a wide range of microorganisms in a lactoperoxidase system (LP-s). LP-s also is identified as a natural antimicrobial system. The field of actual and potential application of these natural antimicrobial systems is very broad. It can be used as natural antimicrobial tool in different industries such as food products, dairy products, cosmetics, drugs and medicine.

In this study we used Amberlite CG-50 ($NH4^+$ form) resin, CM-Sephadex C-50 ion-exchange chromatography and Sephadex G-100 gel-filtration chromatography to purify lactoperoxidase from skimmed bovine milk. Purification degree for the purified lactoperoxidase was shown by SDS-PAGE and $R_z(A_{412}/A_{280})$ value. The R_z (A_{412}/A_{280}) value was 0.8. Then the secondary structure of the enzyme was studied using circular dichroism (CD). The contents of secondary structure are as followed: 67.7% beta-structure, 19% alpha-helix, 13.3% unordered

structure, which is indicative of no changes in the secondary structure during purification processes.

Key words: bovine lactoperoxidase, circular dichroism spectroscopic studies.

Abstract No.56

The Structural Characterization of Recombinant Human Granulocyte Colony Stimulating Factor

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Human granulocyte colony-stimulating factor (h-GCSF) stimulates proliferation and differentiation of neutrophil precursor cells as well as activation of mature granulocytes for more efficient immune responses. The biochemical and biophysical characterization of recombinant protein is required, when they are developed for human clinical use. A number of techniques can be used to determine the biophysical properties of protein and to examine their biochemical and biological integrity. The results of these experiments are compared with those obtained using naturally occurring proteins to make sure that the recombinant protein has the desired characteristics of the naturally occurring one. In this study, the purified protein was characterized by using Neupogen® and PDgrastim as reference standards. This research investigates the characterization of final product of rh-GCSF as characterization analysis: Bacterial endotoxin test, CD measurement, Disulfide bond analysis, Analysis of monomer and aggregates form of rh-GCSF. Also purity was measured by SDS-PAGE, Western blotting and quantified by Bradford.

An efficient, scalable and cost-effective procedure for production and purification of rh-GCSF in *E. coli* were utilized. The quantitative analysis shows that the purified protein yield was 400 mg from 1 g of cell dry mass (40%) by Bradford, SDS-PAGE (gel densitometry) and Western blotting and the purity was more than 99%. According to the inspection chromatogram, obtained peak conforms to the molecular weight of rh-GCSF. Disulfide bonds are in correct position, rh-GCSF and reference standard chromatograms overlap with each other. The overlaid CD spectra show that the rh-GCSF was on par to the reference standards.

The obtained results approved that the rh-GCSF protein isolated in this study was highly pure and comparable with the innovator products, Neupogen® and PDgrastim. Based on the above results, the product has been found to be adequate for preclinical studies.

Key words: Human granulocyte colony-stimulating factor (h-GCSF), structural study, Spectroscopy.

Abstract No.57

Angiotensin II Type 1 Receptor Polymorphism: The Response to Diabetes in Coronary Artery Disease Patient and Prevalence of Metabolic Syndrome by the Adult Treatment Panel III and International Diabetes Federation Definitions in Iranian population

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In Iranian population, diabetes, hypertension and metabolic syndrome (MS) are common and are major risk factors for coronary artery disease (CAD); the contribution of gene polymorphisms of the rennin angiotensin system is controversial and may differ among populations. We investigated the role of angiotensin II type 1 receptor in aggravated risk of CAD in diabetic patients and prevalence of MS by the Adult Treatment Panel III (ATP III) and International Diabetes Federation (IDF) definitions in 428 Iranian population. The A1166C gene polymorphisms of this cross-sectional study were detected using polymerase chain reaction-based protocols. The genotype and allele frequencies, important clinical characteristics and laboratory values were compared with three groups: 184 CAD patients, 109 Diabetes with CAD and 135 normal peoples. According to ATP III and IDF, MS characterizing considered in all group. Demographic data and risk factors were determined by history, physical examination and laboratory tests. There were significant differences between groups in all risk factors except BMI and gender. The A/C allele of the AT1R, A1166C polymorphism was associated with diabetes to increased risk of CAD (A allele p=0.018, Regression: 0.005, odd ratio (OR):6.4; 95% (CI):1.7-24.1) and (C allele p=0.05, Regression: 0.04, odd ratio (OR):0.2; 95% (CI):0.13-0.4). A and C allele show very notable differences in male and female (p<0.0001). MS in ATP III defined with p=0.022, and IDF defined with p=0.006, indicated significant differences between three groups, with MS showing different

prevalence in man and women ($p<0.001$). We conclude that Angiotensin II type 1 receptor increase the occurrence of diabetes with CAD in homozygous 1166C. In Iranian population MS is highly prevalent. IDF definition seems to be more pertinent than ATP III for screening CAD and diabetes risks. Clinical and Para-clinical data indicated in this population indicate a high level of CAD risk factors.

Key words: Polymorphism, Angiotensin II Type 1 Receptor, Diabetes.

Abstract No.58

Nanotoxicology and Spectroscopy Studies of interaction between Silver Nanoparticles and DNA

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The interaction between silver nanoparticle with calf thymus DNA (CT DNA) was studied by UV-visible, fluorescence, far UV circular dichroism (CD) spectroscopies at physiologic temperature of 37 ºC. By the analysis of UV-visible titration and thermal denaturation studies of DNA, it was found that silver nanoparticle can form a new complex with double-helical CT DNA and increase the T_m value of DNA. This kind of binding may cause a slight change of the conformation of DNA. The fluorescence emission spectra of intercalated ethidium bromibe (EB) with increasing concentration of silver nanoparticle at 37 ºC represented a significantly reduction of the ethidium intensity and quenching of EB fluorescence. Also, CD results suggested that silver nanoparticle can significantly change the helicity conformation of CT DNA and then induce the alteration of nonplanar and tilted orientations of DNA bases, resulting in the changes of DNA base stacking and act as an intercalator. Spectroscopic results represented that binding of silver nanoparticle to CT DNA resulted significantly changes on the structure and conformation of DNA and act as an intercalator via increasing of stability of DNA by increasing of T_m, quenching of EB fluorescence intensity and alteration of CD spectra. Also, the anti-tumor property of silver nanoparticle was studied by testing it on human tumor cell line K562. The 50% cytotoxic concentration (Cc_{50}) of silver nanoparticle was determined using MTT assay after 24 h incubation time. Results of the present study may provide useful information to design better anticancer compounds using metal nanoparticles with lower side effects in the future.

Key words: Silver nanoparticle; CT DNA; Ethidium bromibe.

Abstract No.59

A calorimetric study of the binding of Cyanide ions to Jack Bean Urease

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Urease has the historical distinction of being the first enzyme crystallized. Jack bean urease (JBU) is a urea amidohydrolase, isolated as a crystalline enzyme by James Sumner. This enzyme is a metalloenzyme with two nickel ions per subunit, where nickel ion plays an essential role in catalysis. Urease hydrolyzes urea to form carbamate and ammonia, carbamate spontaneously degrade to CO_2 and a second molecule of ammonia. Cyanide ion was studied as an effector of Jack bean urease at 27°C in 30 mM Tris buffer, pH=7 by Isothermal Titration Calorimetry (ITC). For a set of identical and independent binding sites, we have before shown three different methods of ITC data analysis. The simple novel model was used for CN^-+JBU interaction over the whole range of CN^- concentrations. It was found that cyanide ion acted as a noncooperative inhibitor of urease, and there is a set of 12 identical and independent binding sites for CN^- ions. The dissociation equilibrium constant is 750 µM. The molar enthalpy of binding is ΔH = -13.6 kJ mol^{-1}. In the second ITC data analysis method for a set of identical and independent binding sites, the dissociation equilibrium constant for CN^-+JBU interaction obtained 750 µM. The molar enthalpy of binding was ΔH = -13.6 kJ mol^{-1}. The dissociation equilibrium constant and the molar enthalpy of binding values obtained from this two methods are equal.

Key words: calorimetry, Cyanide ions, Jack Bean Urease.

Abstract No.60

Production, Extraction and Purification of Prodigiosin Pigment from *Serratia marcescens PTCC1111*

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Prodigiosins are a family of naturally occurring tripyrrole ring-containing red pigments produced by microorganisms, especially *Serratia marcescens*. They have a common pyrrolyldipyrrolylmethene skeleton. Its chemical formula is $C_{20}H_{25}N_3O$ and it is insoluble in water. Thus this pigment can be extracted by organic solvents such as ether, chloroform, acetone, ethanol, methanol, petroleum. These pigments are emerging as a novel group of compounds having distinct biological activities (antibacterial, antimycotic,immunomodulating, anti-tumor, antimalarial and nuclease). Hence they are very important in pharmacy. For production of prodigiosin, *Serratia marcescens* was grown in a nutrient broth. Bacterial cells were extracted by centrifugation. Supernatant was evacuated and cells were retained for extraction of the pigment. Acidic methanol was added and the solution was then filtrated with vacuum pump. It was centrifuged again and evaporated. Finally the pigment was purified by column chromatography and characterized with thin-layer chromatography and NMR methods.

Extraction demonstrated that from 1500 ml nutrient broth culture, 6/75 mgr pure pigment can be achieved. The results suggest that such method is more feasible and faster, and produces pigments of higher purity, compared to that of conventional methods.

Key words: *Serratia marcescens*, Prodigiosin, pigment, extractraction, purification.

Abstract No.61

The combining of fluorescence spectroscopic, synchronize fluorescence, resonance light scattering, circular dichroism and molecular dynamic techniques are the best methods for anti-cancer drugs binding protein investigation

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Folding and binding are two of the most fundamental aspects of protein behavior. Biological function is generally possible only when a protein is folded into a specific three-dimensional conformation. It is important to realize that the specific function of a protein molecule can be well-governed by rare fluctuation into a particular subset of conformational substances. Biological function involves the interaction with other molecules: enzymes bind their substrates and products and carrier proteins bind their ligands. In the circulatory system drugs are transported as complexes with carrier blood proteins. Drugs, which are administered in multi-drug therapy, may alter each others binding to the transporting proteins. Therefore, it is necessary to determine both the location of the binding sites and the possible interactions for each individual drug. One of the branch research of our group in Biophysical research lab is the binding of various kind of anti-cancer drugs (neutral and synthetics) to the carrier blood proteins (human serum albumin, human serum transferrin, human lactoferrin) as alone and simultaneously have been examined by fluorescence spectroscopic, synchronize fluorescence, resonance light scattering, circular dichroism and molecular dynamic techniques at physiological pH with the aim of analyzing the role of the different interactions in the drug complexation process with these proteins. Combination of several drugs is often necessary especially during long-term therapy. The competition between two drugs for the binding sites on human serum albumin and transferrin may result in decrease in binding and hence increase in the concentration of free biologically active fraction of one or both the drugs. On the other hand binding can also have a significant impact on the pharmacokinetics of drugs, since these proteins have a limited number of regions for high-affinity binding. Moreover the protein is a flexible molecule and binding of a drug often affects the simultaneous binding of other drugs. Information about such an influence is important since an alteration in protein binding may change the volume of distribution, clearance and elimination of a drug and may modulate its therapeutic effect. However it is important to classify and identify drug-binding sites in order to evaluate the risk of drug interactions. The aim of our group study is to analyze the interactions of anti-breast cancer drugs with blood carrier proteins and to evaluate the mechanism of competitive binding of those to proteins. The effects of drugs on the proteins were estimated and the binding and quenching properties of drug-serum albumin complexes are determined.

Key words: spectroscopic techniques, molecular dynamic techniques, drugs binding protein investigation.

Abstract No.62

Upregulation of NF-kB1/RelA in human bronchial wall of mustard gas induced patients

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Sulfur mustard (SM) is a chemical warfare agent which has been used during Iran-Iraq war against Iranian troops. Nowadays there are more than 40000 people suffering from SM lesions, especially pulmonary disorder in Iran. SM disturbs scavenge of ROS and eventually causes chronic obstructive pulmonary disease (COPD) which is one of the most abundant inflammatory disease. Nuclear factor kB (NF-kB)/ Rel family is one of the most important proteins involved in inflammatory responses. They are members of DNA-binding protein factors that are required for transcription of many proinflammatory molecules. Existence of NF-kB is a well known marker in inflammatory status in animal model systems to expose the pathobiology of lung diseases. In this study we sought to address the expression of NF-kappaB1/RelA and presence of inflammation in bronchial wall biopsies of SM exposed patients. We considered NF-kappaB1/RelA as the primary heterodimer in lung inflammation. Ten normal individuals and twenty SM induced patients were comprised. Expression of NF-κB1/RelA in healthy and SM induced samples were measured by semi quantitative RT-PCR, Real-time PCR. Expression levels of NF-κB1 and RelA in SM exposed patients were upregulated about 2.53±0.32 and 3.83±0.87 folds respectively in compare to normal samples (P□0.05).

This is the first study about the induction of inflammatory molecules in patients exposed to SM. Here we suggest that over expression of NF-κB1/RelA molecules in COPD induced by SM, may follow an inflammatory procedure in bronchial wall of these patients at mRNA levels.

Key words: bronchial wall, mustard gas, NF-κB1, RelA, COPD.

Abstract No.63

Fluorescence spectroscopy study of human hemoglobin upon interaction with an anti-breast cancer drug

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Hemoglobin is the major component of red blood cell and as an allosteric tetrameric protein**;** it has an important role in carrying oxygen from lunge to different tissues. Hemoglobin was initially thought to reversibly bind only with oxygen, but they were later shown to react with other gasses such as CO and NO and with several organic molecules such as phospholipids and other membrane lipid.Tamoxifen is a non-steroidal anti-estrogen drug that is widely used in the treatment and prevention of breast cancer. It is currently used for the treatment of the both early and advanced ER^+ (estrogen receptor positive) breast cancer. Here, interaction between hemoglobin and tamoxifen was investigated at two pH (pH 7.4 and pH 8.4) using fluorescence spectroscopy. Fluorescence measurements were carried out in a Jasco 2500, Hitachi fluorescence spectrophotometer. The excitation wavelength was 280 nm, and the emission spectra were read at 300-600 nm. For describing the fluorescence quenching by tamoxifen using the Stern-Volmer and Scatchard equation. The results of Sten-Volmer and Scatchard plots reveal that K_{SV} and binding constant decreased with increasing pH. The intrinsic Fluorescence of Hb primarily originates from β-37 Trp that plays a key role in the quaternary State change upon ligand binding. Changes in emission spectra tryptophan are common in response to protein conformational transitions, substrate binding. Linear Stern-Volmer plots may either reveal the occurrence of just a binding site for quencher in the proximity of the fluorophore, or indicate the existence of a single type of quenching. Scatchard plots lead us to analyze the binding data by fitting the data to Hill equation for multi-set of binding site. Therefore, the difference between binding affinities two interaction is showed change structure of HB.

Key words: Fluorescence spectroscopy, human hemoglobin, Tamoxifen.

Abstract No.64

Calculation of the pK_a values of carbonic anhydrase residues in presence of various concentrations of sodium dodecyl sulfate: molecular dynamics simulations

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The effect of sodium dodecyl sulfate (SDS) on the pKa values of carbonic anhydrase residues is the subject of this work. We have studied the ionization behavior of carbonic anhydrase residues at different concentrations of sodium dodecyl sulfate by means of molecular dynamics simulation. The average structure of carbonic anhydrase in absence and presence of various concentrations of SDS obtained from Molecular dynamic simulation. To calculate residue pKa values we were used the empirical method developed by Hui Li *et al*. In this method, the desolvation effects and intra-protein interactions, which cause variations in pK_a values of protein ionizable groups, are empirically related to the positions and chemical nature of the groups proximate to the pK_a sites. From the obtained results it was established that the manner of effect of SDS on BCA is different in various concentrations.

Key words: pKa, sodium dodecyl sulfate, carbonic anhydrase.

Abstract No.65

Tetra sulfunated nickel phthalocyanine assemblies and its binding to human serum albumin

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The physicochemical properties of Ni (II) sodium tetra sulfunated phthalocyanine ,NiPcTS, was investigated at various experimental conditions such as various concentration of NiPc and ionic strength in10mM phosphate buffer, pH 7.4 at 25°C. The results represents the low tendency of this phthalocyanin for formation of assembles due to increasing of concentration and the formation of H-type aggregate due to increasing of ionic strength. The interaction of NiPcTS with human serum albumin (HSA) was also studied by Uv-Vis spectroscopic technique in various molar ratios at different temperature and various ionic strength. The results represent the formation of a 1:1 complex from HSA -NiPcTS and the predominant role of electrostatic forces in the interaction. The binding constants (K) were obtained by analysis of optical absorption spectra of mentioned complex at various HSA concentrations using SQUAD software. The thermodynamic parameters were calculated by van't Hoff equation.

Key words: HSA, electrostatic interactions, thermodynamic parameters.

Abstract No.66

Structural study of firefly luciferase in the presence of additives by Circular dichroism spectropolarimetery

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Firefly luciferase is one of the most interesting enzyme in which has important roles in many fields, such as medicine, biotechnology, diagnostics, research, food and other industries. The main problem that always limits its application is its instability at room temperature or during handling with enzyme. In this study firefly luciferase from *photinus pyralis* has been used. Structural studies have been conducted by means of circular dichroism (CD) spectropolarimetery both for far UV-CD and near UV-CD. Also thermal denaturation study of luciferase has been investigated by CD spectropolarimetery at 222nm. All studies were performed in the presence of glycine in comparison to trehalose and $MgSO_4$ as stabilizers at different concentrations.Structural study of firefly luciferase at both far and near UV-CD revealed no prominent effect on the secondary and tertiary structure. Some small changes in compactness and rigidity which were observable at some specific concentrations of additives, but it was negligible in average. As it is predictable for compatible additives, they have no significant effect on protein structure that is shown in this experiment.

Thermal unfolding study of firefly luciferase revealed that there are two melting points which indicate a three-state unfolding in luciferase structure (as it is proved recently via calorimetric studies of

firefly luciferase by Nano DSC III). Glycine also enhanced first and second T_m and ΔG^o of unfolding as well as the trehalose and $MgSO_4$. The result showed that these additives stabilize firefly luciferase as both T_m and ΔG^o increased upon addition of these compounds.

Key words: Firefly luciferase, circular dichroism, thermal unfolding, T_m, ΔG, stabilization, glycine.

Abstract No.67

Conventional and stopped-flow fluorescence study of firefly luciferase in the presence of additives

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Firefly luciferase is an interesting enzyme in which has important roles in many fields. In this study firefly luciferase from *photinus pyralis* has been used. In this study, Structural studies of luciferase have been conducted by two important techniques as stopped-flow florescence and conventional fluorescence spectroscopy. The effect of glycine as a stabilizer has been studies in comparison to the trehalose and $MgSO_4$ (reported earlier) at different concentrations. Rate constant of luciferase refolding obtained by diluting 1 volume of unfolded enzyme solution to 30 volumes by refolding buffer. It showed that refolding constant of luciferase is changed upon addition of additives. Trehalose increased the rate constant of refolding relative to intact protein meanwhile, $MgSO_4$ decreased the rate constant. Glycine also increased the rate constant of refolding, but its effect was not as high as trehalose. This phenomenon is explained as an increment in viscosity of solvent for trehalose and glycine, but preferential hydration was more prominent in the case of $MgSO_4$.

Fluorescence spectroscopy of luciferase in the presence of trehalose at different concentration showed interesting phenomenon, meanwhile there was no changes in luciferase spectra in the presence of $MgSO_4$ and glycine. As it showed earlier, fluorescence spectra of luciferase increased concomitantly by increment of trehalose concentration with a slightly red-shift. This phenomenon (increase in the intensity accompanied by red-shift) is observed in Hometric protein family with few numbers of Trp in their protein sequence. This was explained by the effect of self-quenching, which could be eliminated after local structural alteration (or denaturation and unfolding).To testify this phenomenon in luciferase, list of potentially self-quenching interactions between Trp and other groups has been prepared in which some interaction may result in a self quenching phenomenon. We conclude this phenomenon as a structural changing effect locally on luciferase structure upon addition of trehalose which may lead in an increase of intensity with red-shift, but the way it may alter the structure is different with that of glycine.

Key words: Firefly luciferase, stopped-flow fluorescence, self-quenching, refolding rate constant.

Abstract No.68

High efficiency yield of artificial peroxidase-like enzyme induced by mixed gemini 12-2-12/SDS micelle and imidazol: Biomimetic approach

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The heme group has fundamental role in activation of hemoproteins and hemoenzymes such as horseradish peroxidase (HRP). Heme in the absence of protein environment has also catalytic role but alone heme exposing in the solvent and substrate and makes heme to inactive-oxo complexes. An alternative approach to the preparation of robust biocatalysts consists in the encapsulation of metalloporphyrins into water-soluble micelles that mimic the polypeptide envelope, which protects the catalytic center of natural enzymes. In these studies, engineered mixed gemini 12-2-12/SDS micelle in the absence and the presence of imidazol at various guaiacol concentrations as a substrate were performed and the relevant Michaelis–Menten constant (K_m) were obtained. The turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_m) of aforementioned artificial enzyme were measured by Lineweaver-burk plot.

The biocatalyst with the components of hemin-gemini 12-2-12/SDS micelles indicates that cationic gemini 12-2-12 has improved the catalytic efficiency nearly 8 times respect to hemin-SDS. This indicates that the presence of both negative and positive charges around the active site, as mimicking the native peroxidase will increase the catalytic efficiency toward the native HRP efficiency. But the optimum productivity in a peroxidatic reaction is due to biocatalyst with the components of hemin-gemini 12-2-12/SDS micelles-imidazol with 19.8 $\mu M^{-1}s^{-1}$ catalytic efficiency that is about 27% relative to native HRP. The imidazol moiety as histidin representative in the native HRP

will increase the reactivity and catalytic efficiency of this artificial enzyme. This high efficiency is due to both mimicking of positive and negative charge distribution and imidazol agent. In this paper we report the kinetic parameters for this novel artificial enzyme.

Key words: Gemini micelle surfactant, SDS, Heme, Imidazol, HRP peroxidase, Artificial enzyme.

Abstract No.69

The changes of hemoglobin species ratio related to its conformational changes upon superparamagnetic iron oxide nanopraticle binding

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Magnetic nanoparticles offer many attractive possibilities for biomedical applications. The effect of magnetic nanoparticles on the human hemoglobin species was studied in the presence of a 100 mM phosphate buffer (pH 7.0) by different spectroscopic techniques. Spectroscopic studies showed that different concentrations of magnetic nanoparticles cause to increase the amount of deoxy - and decrease the amount of oxy forms of hemoglobin. These nanoparticles have no significant effects on the met- conformation of hemoglobin. The changes in oxy/deoxy ratio upon magnetic nanoparticle binding are related to the conformational changes of the pyrrole ring system. The improvement in the deoxy/oxy ratio, after adding magnetic nanoparticles, may be attributed to the incorporation of COO^- terminal from the oleic acid coat of nanoparticle.

Key words: Hemoglobin, Superparamagnetic iron oxide, Nanopraticle, Binding.

Abstract No.70

Separation of the proteolytic enzymes of ficin from fig

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Fig latex contains a group of cystein proteases named ficin (E.C.3.4.4.12). This enzyme can be used in drug and food industries for production of digestive drugs,meat tenderization and cheese production,So separation of this enzyme was studied.Latex and fig fruit extraction were collected. Protein Content of them was determined by UV spectrophotometry method. Then precipitation was done by ammonium sulfate. The precipitate was colleced by centrifugation and suspended in phosphate buffer containing EDTA and Nacl and followed by ion-exchange chromatography. The results were analyzed by electrophoresis on polyacrylamide gel (SDS-PAGE) and gel staining with R-250 coomasiee.It showed that purified enzymes had molecular weight about 25 kD. This study led to the gain of a procedure for separation and purification of proteolytic enzymes of fig fruits.This enzyme can be used for casein hydrolysis and preparation of meat digestive drugs.

Key words: cystein protease, Ficin, Fig, Ion-exchange chromatography.

Abstract No.71

Antioxidant peptides derived from camel casein

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It is well known that the oxidative stress due to free-radicals is considered to be responsible for many chronic diseases such as cardiovascular diseases, diabetes, cataracts, neurodegenerative disorders, and certain types of cancer and aging. Caseins are a major source of bioactive peptides. These peptides can be produced *in vivo* during gastrointestinal digestion or *in vitro* through food processing using specific enzymes. The peptides obtained from enzymatic hydrolysis of milk proteins can act as an antioxidant. Peptides with antioxidant activity possess great potential for use as natural antioxidants in food products without any side effects.

In this paper we report the antioxidant activities and kinetic parameters for hydrolysis of camel and bovine caseins using chymotrypsin and obtained peptide fractions. The total antioxidant capacity (TAC) of caseins and their low-molecular-weight fractions

were investigated using spectrophotometry ABTS-based method (reduction of the cation radical of 2,20-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid)).The results indicate the overall antioxidant activity of camel caseins and their hydrolysis were higher than bovine caseins and peptide fraction between 5-10 kDa showed the highest antioxidant activity. It can be concluded that camel caseins or their hydrolysates can be used as a novel ingredient for producing nutraceuticals and natural drugs with high antioxidant activity.

Key words: antioxidant peptides, free-radicals, Camel Casin, kinetic parameters.

Abstract No.72

The biophysical chemistry interaction of silver nanoparticles and doxorubicin

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Breast cancer, which affects an important percentage of human beings, occurs when abnormal cells grow out of control in one or both breasts. Anthracyclines, particularly doxorubicin (DOX) are widely used antibiotics for medical treatments of breast cancer. In this research, we have studied the interaction between silver nanoparticles and anticancer drug of doxorubicin using UV-visible spectroscopy, fluorescence spectroscopy and circular dichruism (CD) at 37 °C. We have determined the binding constant (K_a=215.34 mM^{-1}) and enthalpies of this interaction. The interaction of doxorubicin with varying silver nanoparticle concentration represented one binding sites. Altogether, our data indicated that there is a strong interaction between silver nanoparticles and DOX.

Key words: Doxorubicin, Silver nanoparticles, Thermodynamic parameters.

Abstract No.73

Impact of the Major Phosphorylation Site on Chaperoning Function and Allergenicity of Beta-Casein

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Beta-CN (β-CN) molecule is a single chain protein of known sequence containing a cluster of five phosphoseryl residues in the N-terminal hydrophilic domain. This protein is one of the highly allergenic components of cow's milk which possesses multiple sequential antigenic determinants (epitopes) in its primary structure. Moreover β-CN is member of intrinsically unstructured protein (IUP) family exhibiting chaperone-like activity *in vitro.* In this study as expressed and purified from E. coli, β-CN lacks the phosphoryl residues, because the prokaryotic host does not realize post-translational phosphorylation of the eukaryotic protein (β-CN). Subsequently, the impact of phosphoryl residues on IgE mediated immune reactivity (allergenicity) and chaperoning function were investigated and compared using the recombinant and native β-CNs. Spectroscopic measurement and enzyme-linked immunosorbant assay (ELISA) were performed in order to compare chaperoning abilities and allergenicity of the beta-caseins respectively. The results exhibit major roles played by the cluster of phosphoseryl residues in both chaperoning activity and in shaping of the allergenicity profile of β-CN. Consequently this study suggests the major phosphorylation site as one of the important antigenic determinant elements along the primary structure of beta-casein. Moreover phosphoseryl cluster plays significant role in amphipathic character and subsequently chaperoning function of this molecule.

Key words: Beta-casein, Chaperone-like activity, Allergenicity, Phosphoseryl cluster.

Abstract No.74

Molecular dynamics study of transition conformation in Human serum albumin denaturation

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Human serum albumin (HSA) is most abundant protein in human blood plasma, is produced in the liver and comprises about half of the blood serum protein. HSA is soluble in serum and is important in regulating

blood osmotic pressure. HSA serves as carriers for molecules with low water solubility, including hydrophobic hormones, unconjugated bilirubin, free fatty acids, calcium ions, and some exogenous chemicals such as drugs. In the present work, we used molecular dynamics simulation methods to study the structural alterations and nature of forces involved in the transition from native to denatured states of HSA. Gromacs *version 3.3.3. package* installed over UBUNTU Linux version 8.10 (Intrepid) on a Intel ® Pentium ® M based PC at 1.6 GHz with 469.5 MiB of Ram package, and ffgmx force field was used in the present work. The coordinates used for HSA was obtained from RCSB Protein Data Bank, with PDB ID: 3CX9. The protein was equilibrated in a cubic box with 9.581nm x 5.959nm x 9.717nm dimensions. Energy minimization was carried out using steep integrator and F_{max} were chosen 1000 for 1000 step. Molecular dynamics with all-bond constrain for 200ps and finally no constrain were used to simulate done for up to 4ns. Our results show heating up the albumin solution exerts vast alterations in the system leading to denaturation of albumin. Stepwise refinement of simulation trajectories revel cooperative events during denaturation. Increase in kinetic energy at 52°C leads to decrease in solvent-protein H. Bond cause a simultaneous increase in protein-protein H. Bond. However obvious decrease in gyration radius and in solvent accessible surface area (SAS) proves the formation of a more compacted conformation in albumin before denaturation takes place. More increasing in temperature causing H. Bond breakdown, converting regular structures to random ones, and finally leading to completely denatured structures, in about 70°C (T_m). We also studied the position changing of Tryptophan-212 during the simulation. Trp(212) is known to lie in the hydrophobic pocket of HSA and is located on the surface of sub domain 4 in domain II. Outgoing of Trp(212) at the melting temperature is in accordance with denatured conformation.

Key words: Molecular dynamics, HSA, gyration radius, hydrophobic pocket, denaturation.

Abstract No.75

A Novel Approach to Quantify the Chaperone-Like activity

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Molecular chaperones form a family of proteins believed to evolve towards prevention of protein unfolding and aggregation in denaturing conditions. Consequently, chaperones play important role in preventing of the serious problems so called aggregation diseases such as Alzheimer's, Parkinson's, and Huntington's, Creutzfeldt - Jakob disease, cataract and type II diabetes. In this study the ability of bovine beta-casein to prevent aggregation of pancreatic insulin was considered as a sign of its chaperone-like activity. The chemical-induced aggregation of insulin was detected by measuring of the increase in optical density at 360 nm as a function of time. For quantitative estimation of chaperone-like activity of beta-casein, k_1 and A_{lim} were derived from the aggregation curves, with the assumption that, as proposed already, aggregation follows completely first order kinetics. A_{lim} is the limiting value of absorbance (A) at $t\rightarrow \infty$ and k_1 is the rate constant of the first order reaction. The $k_1.A_{lim}$ product is the initial rate of aggregation and it is expressed in unit of absorbency per time unit. To quantify chaperone-like activity of beta-casein at different molar ratios of chaperone/target protein, $k_1.A_{lim}$ of each experiment was divided individually per $(k_1.A_{lim})_0$ of the control experiment (absence of beta-casein) and subtracted from unit. The resulting values varied from zero (in the absence of casein chaperone) to one (where $k_1.A_{lim}= 0$). These values, increasing from 0 to 1 with the increase of the chaperone/substrate ratio, are correlated directly with the chaperone-like activities of beta-casein chaperone. The percentage of chaperone-like activities can be obtained by multiplying the obtained values by 100. Advantage of the current approach is to apply combination of key parameters (k_1 and $A_{lim)}$ in measuring of chaperone-like activity.

Key words: Chaperone-like activity, Quantification, First order rate constant (k_1), Limiting value of absorbance (A_{lim}).

Abstract No.76

The Importance of a Flexible Loop in Kinetic Pathway of Refolding of Iranian Firefly Luciferase and Its Thermodynamic Stability

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In order to elucidate the effect of a flexible β-strands connecting loop on the stability of folded state and kinetic pathway of refolding of Iranian firefly luciferase (*Lampyris turkestanicus*), kinetics and

thermodynamic studies were carried out, using stopped-flow and conventional fluorescence spectroscopy respectively. All kinetics and thermodynamic data were analyzed by kaliedagraph analysis software. According to thermodynamic study, the free energy of protein unfolding in water ($\Delta G_{U-N}^{H_2O}$) as a measure of its stability, was determined. Results of kinetic study indicate that refolding pathway of luciferase consists of two steps in which early events of protein folding contains of conversion of unfolded into intermediate state; followed by conversion of intermediate to final folded state via rate limiting transition state. Finally, the free energy of intermediate, transition and folded state as well as their relative accessible surface area were calculated using appropriate kinetics and thermodynamics equations. These findings suggest that during refolding process of luciferase, closure of this loop is essential for facilitating the condensation of two β-strands.

Key words: Loop, Stability, refolding, kinetics, luciferase, thermodynamic, kaliedagraph.

Abstract No.77

Effect of sucrose and trehalose on stability, kinetics properties, thermal aggregation and structure of *Photinus pyralis* firefly luciferase

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In this study to improve the thermostability of firefly luciferase, sugars as stabilizing additives were used. The combination of sucrose and trehalose were found to have a strong stabilizing effect on firefly luciferase activity and prevention of thermoinactivation. These additives also increase optimum temperature. The presence of both additives was found suitable to inhibit thermal aggregation of firefly luciferase and decreasing of bioluminescence decay rate. On the other hand, in order to understand the molecular mechanism of thermostabilization, the effect of combination of sucrose and trehalose on secondary structure of luciferase was investigated by FTIR which shows some changes in secondary structure.

Key words: Firefly luciferase, Bioluminescence, Stabilization, FTIR.

Abstract No.78

Synthesis of novel series of cytotoxic imidazole-based compounds, importance of the electron withdrawing elements in their anti-proliferation activities

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With the aim to establish a relation between anti-proliferation activity and nature of the compounds, this study was carried out in order to synthesis a novel series of cytotoxic imidazole-based compound. The anti-proliferation activities of the synthetic compounds were examined and compared using Escherichia coli (DH5α) and human erythromyeloblastoid leukemia cell line (K562) as the target cells. The antibacterial activity of the ligands was assessed spectrocopically on microtiter plate-based assay. To evaluate the cytotoxic effect of the ligands on the leukemic cell line, 3-(4,5-dimethtl-2-thiazolyl)-2,5-di-phenyl-2H-tetrazoliun bromide (MTT) based assay was used. The results of this study showed that the existence of hydrogen accepting electron withdrawing elements in the structure of synthetic compounds could enhance significantly their anti-proliferation properties. This study may provide novel information for future development of more efficient cytotoxic compounds.

Key words: Imidazole-based compounds, electron withdrawing element, Anti-proliferation.

Abstract No.79

Chaperone-Like Activity of Beta-Casein; a Mechanistic Perspective

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Recently, a novel function for caseins has been proposed as "molecular chaperones" protecting several proteins against heat, chemical and UV light induced aggregations.
The primary structure of beta-casein has a highly amphiphilic character, playing a crucial function in aggregation and micellisation processes of this casein. Using different techniques including chemometric studies, fluorescence and Uv-Visible spectroscopy, the importance of dissociation ↔ association process, as well as the existence of an intermediate species as beta-casein/target protein complex were confirmed in the chaperoning mechanism of this casein. The formation of this complex was also extremely temperature sensitive. In overall this study confirmed presence of chaperone-active monomeric- and chaperone-inactive multimeric species for beta-casein.

Key words: Chaperone, Beta-casein, Mechanism.

Abstract No.80

Improvement of carbon nanotubes performance for enzyme based electrochemichal biosensors using ionic liquid

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Carbon nanotubes (CNTs) as a type of highly conductive nanomaterials and excellent electrocatalytic abilities are used in electrochemical biosensors. They are able to promote electron transfer rate when used as an electrode material. The abilities of CNTs could be even more promoted when they are composed with the materials so called ionic liquid. Recently, researchers developed biosensors with high sensitivity and good biocompatibility using a mixture of CNTs and ionic liquids. In the present report by composing amine functionalized-multiwalled carbon nanotubes with a room temperature ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate), a biocompatible nano-composite was obtained. This nano-composite was examined as matrix for immobilization of different enzymes such as catalase, glucose oxidase and choline oxidase on glassy carbon electrode. The prepared nano-composite could efficiently mediate the electron transfer between the enzyme sand electrodes. Establishment of fast electron transferring between redox enzymes and electrode surface can be obtained when the electrode is modified with such a nano-composite. Using this method not only a pair of well-defined, quasi-reversible redox peak was observed for the immobilized enzymes but also the electrochemical parameters such as charge transfer coefficient (α) and apparent heterogeneous electron transfer rate constant (k_s) could also be estimated. The formal potential for catalase, glucose oxidase and choline oxidase were obtained as -467.5 and -0.395 V versus Ag/AgCl in the phosphate buffer solution, respectively. Under the optimized experimental conditions, the immobilized enzymes exhibited relatively high affinity towards their substrates. The proposed biosensors showed sensitivities of 156.05, 1449 and 70.51 μA mM^{-1} cm^{-2} toward hydrogen peroxide, glucose and choline, respectively.

Key words: CNTs, electrochemical biosensors, nanocomposite, enzyme immobilization.

Abstract No.81

Effect of ethylenediamine on mushroom tyrosinase activity

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Tyrosinase (EC 1.14.18.1), also known as phenoloxidase (PO), is a copper-containing, mixed-function oxidase widely distributed in microorganisms, animals, and plants that is a key enzyme involved in the browning that occurs upon bruising or long-term storage of plants. Tyrosinase catalyzes both the hydroxylation of monophenols and the oxidation of o-diphenols into o-quinones, and is involved in the

formation of pigments such as melanins Cutaneous hyperpigmentation is a common disorder due to excess melanin production by the enzyme tyrosinase. Tyrosinase inhibitors have become increasingly important in medicinal, agricultural, and cosmetic products, primarily in relation to its roles in hyperpigmentation. Accordingly, there is an urgent need to design and screen tyrosinase inhibitors with higher bioactivities, and to elucidate the mechanisms of inhibition. Here we examined the effect of ethylenediamine on both activities of mushroom tyrosinase (MT) as a model enzyme for human tyrosinase at 20°C in 10 mM phosphate buffer solution, pH 6.8 for gaining more insights about mechanism and inhibition of the enzyme. L-Dopa and L-Tyrosine were used as substrates of catecholase and cresolase activities, respectively. The results show that ethylenediamine inhibits competitively both activities of the enzyme with inhibition constant (K_i) of 0.125 and 0.089 μM for catecholase and cresolase activities, respectively. It is consumed that ethylenediamine increases the electron density around the copper ion at the active site, which changes the properties of metal ion so that decreases the enzyme activity.

Key words: Mushroom Tyrosinase, Inhibition, cresolase, catecholase, Ethylenediamine.

Abstract No.82

Investigation of amorphous aggregation of target proteins in the presence and absence of the molecular chaperone and the crowding agent

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Protein aggregation is a phenomenon wherein the protein loses its native structure and adopts a non-native conformation leading to aggregate formation. β-Casein acts as a molecular chaperone stabilizing target proteins under stress conditions, through the protein-protein interaction and formation of a soluble complex. In this study, the effects of β-casein during its interaction with a variety of stressed target proteins (ovotransferrin, insulin, α-lactalbumin and catalase) in the presence and absence of the macromolecular crowding agent, dextran, are examined. The aggregation of proteins induced by 20 mM DTT and heating. Interaction between the mentioned proteins is investigated by visible absorption spectroscopy, intrinsic fluorescence spectroscopy, extrinsic fluorescence emission (ANS binding) and HPLC. The results show, the rate and extent of aggregation of target proteins due to the DDT and heat treatment are enhanced in the presence of dextran. Thus, dextran accelerates destabilization of target proteins. Furthermore, in this situation, β-casein is less effective in preventing the aggregation and precipitation of target proteins. Thus, dextran may cause the structural changes to β-casein, which reduces its ability to interact with the target proteins. These data indicate the poorer chaperone performance of β-casein in the presence of a crowding agent and support the hypothesis that β-casein interacts more effectively with slowly aggregating rather than rapidly aggregating target proteins. It is proposed that dextran-induced changes of protein conformation and the rate of intermolecular association are in a kinetic competition with the chaperoning activity of β-casein.

Key words: molecular chaperone, protein aggregation, protein stabilization.

Abstract No.83

Detection and dosimetry of gamma ray through reduction of cytochrome C based on superoxide radical anion production

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Reactive oxygen species (ROS) including the superoxide radical anion ($O^{\cdot-}_2$) can result in inflammation and inflict cell injury that includes DNA damage mediated by Fenton chemistry. As a result, gamma ray monitoring is of great importance in various fields such as: radiology, environmental monitoring and medical treatments. Gamma rays as ionizing radiation causes formation of reactive oxygen species; $O^{\cdot-}_2$ through radiolysis process of water in absorbing medium such as watery medium or living cells. Therefore, measurement of $O^{\cdot-}_2$ can be used for detection and dosimetry of gamma ray. Despite that, detection of $O^{\cdot-}_2$ is still a challenging problem. The direct detection of $O^{\cdot-}_2$ is commonly difficult due to their high reactivity and thus a short life time period.

In this study, the reaction of $O^{\cdot-}_2$ with cytochrome C (cyt C) was exploited for determination of $O^{\cdot-}_2$. For determination of $O^{\cdot-}_2$, the cyt C was dissolved in phosphate buffer and injected into the cuvette of spectrophotometer. Thereafter, in order to produce $O^{\cdot-}_2$, potassium superoxide solution in dimethylsulfoxide (DMSO) was added in cyt C solution in cuvette. The amount of $O^{\cdot-}_2$ was determined

spectrophotometrically by measuring the cyt c absorbance at the wavelength of 550 nm. Beer-Lambert equation with molar extinction coefficient of 2.1×10^4 M^{-1} cm^{-1} was used for calculation of $O^{\cdot-}_2$ concentration. Finally, the calibration curve was prepared by plotting the absorbance versus $O^{\cdot-}_2$ concentration. Due to production of $O^{\cdot-}_2$ by gamma ray, it seems that this method is feasible for detection and dosimetry of gamma ray, too. Further study in this field is in current.

Key Words: Detection, dosimetry, gamma ray, cytochrome C, superoxide radical.

Abstract No.84

Detection of Gamma Rays Emitted by Cobalt-60 Using a Modified Horseradish Peroxidase Based Biosensor

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Gamma ray monitoring is of great importance in various fields such as radiology, environmental monitoring and medical treatments. Moreover, its detection in determination of the absorbed dose in the body exposed and also in radiography of pipeline weld bounding in industrial instruments is essential.

In this investigation, for monitoring of gamma ray a hydrogen peroxide biosensor based on determination of H_2O_2 was developed. The biosensor consisted of modified horseradish peroxidase (HRP) immobilized at the surface of glassy carbon electrode. Anthraquinone 2-carboxylic acid (AQ) was used as a novel electron shuttling mediator for modification of HRP. In the presence of Cobalt-60 as a gamma emitter radioisotope, H_2O_2 was generated by radiolysis process in watery medium. For this reason application of proposed biosensor was feasible for gamma ray detection. As a result, in the presence of Cobalt-60, the biosensor responses were studied by chronoamperometry. In the presence of Cobalt-60 radioisotope and at the applied potential of -550 mV vs Ag/AgCl, a cathodic current was obtained due to amperometric detection of H_2O_2. This was regarded as biosensor response and it was linear over the range of 0.25 to 5 Gy.

Key words: Cobalt-60, Horseradish Peroxidase, hydrogen peroxide, Biosensor.

Abstract No.85

The Role of GADD45A Expression in Repairing of Gamma Ray–Induced DNA Damage of Human Lymphocyte

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Exposure to ionizing radiation (IR) produces several forms of cellular DNA damage, including formation of uracil, apurinic/apyrimidinic sites, 8-oxoguanine, single-strand and double-strand breaks. Changes of genes expression are one of the biological responses of cells after ionizing radiation (IR) exposure. The up-regulation of some repair genes may be implicated in repair of Gamma ray-induced DNA damage. GADD45 genes are implicated in response to environmental stresses that can be result in cell cycle arrest, repair or apoptosis. The over expression of GADD45A was previously shown to be associated with the IR damage response in human cells.

In the present study, radiation-induced early transcription of GADD45A was studied in human lymphocyte cells. The peripheral blood was extracted from volunteered donors using heparinated syringe. Then, it mixed with hanks buffer and the lymphocyte cells were extracted and washed using Ficoll protocol. The lymphocyte cells cultured in the 25 cm^2 cell culture flasks, each flask containing more than 1 million lymphocyte cells and RPMI1640 medium. Thereafter, the cells irradiated at different doses of gamma ray emitted from ^{60}Co. After irradiation, both the irradiated and the non-irradiated cell samples were incubated for 4 hrs at 37 °C prior to RNA extraction. Total RNA were isolated and cDNA were synthesized. In order to evaluation DNA repair and determination of the expression of GADDA45A at various doses of gamma ray, the synthesized cDNA was used for Real-time PCR experiments using syber green dye.

Key words: GADD45A, Gamma Ray, DNA Damage, Human Lymphocyte.

Abstract No.86

Cottrell Equation Validity for Modified Horseradish Peroxidase in the Presence of Gamma Ray Radioisotopes

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In the presence of low energy gamma emitter radioisotopes of thallium-201 (^{201}Tl) and technetium-99m (^{99m}Tc) hydrogen peroxide (H_2O_2) was generated by radiolysis process in potassium phosphate buffer. For measurement of H_2O_2, modified horseradish peroxidase (HRP) was immobilized at the surface of glassy carbon electrode (GCE). For modification of HRP, anthraquinone 2-carboxylic acid (AQ) was used as a novel electron shuttling mediator. The cathodic current was measured chronoamperometrically. The chronoamperometry experiments were carried out in the presence of each one of the radioisotopes independently. The results showed that, by increasing the dose rate of radioisotopes, the response of GCE were increased, too. This behavior of biosensor was completely corresponded with the Cottrell Equation as given by:

$$i(t) = \frac{nFACD^{1/2}}{\pi^{1/2} t^{1/2}}$$

Where, i(t) is chronoamperometric current as a function of time, n, F, A, C, D and t are mole number, Faraday constant (96500 C/mole), biosensor surface area (m^2), concentration of analyte (here H_2O_2), diffusion constant (m^2/s) and time (s), respectively. Except C (the concentration of H_2O_2 generated in buffered solution by the radioisotopes), all other factors were constant in all of the experiments. As a result, the concentration of generated H_2O_2 was directly affected the GCE responses (*i(t)*). Hence, our results were completely matched with Cottrell Equation.

Key Words: Cottrell Equation, Gamma Ray, Horseradish Peroxidase, hydrogen peroxide.

Abstract No.87

Non-reducing carbohydrates suppress nanofibrilar AGE formation in human serum albumin glycation

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AGE accumulation results from a combination of hyperglycemia in diabetic complications and glycation of proteins. Glycation involves the formation of chemically reversible early glycation products with proteins, Schiff bases and Amadori adducts. These early adducts underwent slow and complex rearrangements to form advanced glycation end-products (AGEs).
β-cyclodextrin (β-CyD) and trehalose are used both in food and drug industry. Here, the effects of these natural non-reducing carbohydrates on glycation reactions of human serum albumin (HSA) in the presence of glucose studied. This study performs using circular dichroism, absorbance and fluorescence techniques. The characterization study of AGEs was performed by determination of auto-fluoresence, febrile formation, and the number of modified lysine and arginine side chains. Our results indicated that β-CyD and trehalose reduced the formation of AGEs (AGE fluorescent) and nanofibril formation of HSA in the presence of glucose. Thus, β-CyD and trehalose improved helical structure of HSA through interactions with HSA and altered solute-protein interactions. Together our data suggest the presence of trehalose or CyDs in foods may provide beneficial effects in diabetes by diminishing AGEs formation.

Key words: Diabetes, Trehalose, β-cyclodextrin, Glycation, HSA, Glucose.

Abstract No.88

Aggregation behavior of tetrakis (N,N´,N´´,N´´´-tetramethyl tetra-2,3-pyridino) porphyrazine copper(II) and its interaction with ct-DNA: A thermodaynamic approach

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The association behavior of tetrakis (N, N´,N´´,N´´´-tetramethyl tetra-2,3-pyridino) porphyrazine copper (II) ([Cu (II) 2,3-tmtppa]$^{4+}$) with calf thymus DNA (ct-DNA) was investigated in aqueous solutions at 25°C and various ionic strengths. The results show that [Cu(II) 2,3-tmtppa]$^{4+}$does not have any affinity for aggregation due to increasing of salt concentration and exists as monomers even in homogeneous

aqueous solutions of high ionic strengths (more than 1M NaCl). Interaction of [Cu (II) 2,3-tmtppa]$^{4+}$ with ct-DNA has been also studied in 1mM aqueous phosphate buffer of pH 7.0, using optical absorption and resonance light scattering (RLS) spectroscopies and thermal denaturation experiments. The appearance of hypochromicity of less than 10% and bathochromicity shift of $\Delta\lambda \leq 2$ nm in [Cu (II) 2,3-tmtppa]$^{4+}$ UV-vis spectra, increasing of thermal melting point of DNA, and no change in RLS spectra of porphyrazine due to interaction with DNA, represent the minor outside groove binding mode without any stack aggregate formation. The binding constant (K) was obtained by analysis of the optical absorption spectra of the complex at various DNA concentrations using SQUAD software. K value was estimated to be $2.34 \times 10^5 \pm 0.06$ M^{-1} at 25°C. The thermodynamic parameters were calculated by van's t Hoff equation. The enthalpy and entropy changes were 41.83 ± 3.28 kJ/mol and 242.08 ± 9.88 J/mol.K at 25°C, respectively. The results indicate that the process is entropy driven and suggest that hydrophobic interactions are the main driving forces for the complex formation. Increasing of the ionic strength due to addition of NaCl destabilized porphyrazine-DNA complexes indicating the competition of Na^+ ions with porphyrazine complexes for occupation of minor groove binding sites.

Key words: calf thymus DNA, entropy driven process, thermodynamic parameters.

Abstract No.89

Association Study of rs6994992 from NRG1 gene with multiple sclerosis in a population of Iran.

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Human multiple sclerosis (MS) is a complex disease with a multifaceted etiology and heterogeneous pathology. Demyelinated central nervous system (CNS) lesions are the pathologic hallmark of MS and are accompanied by varying degrees of inflammation, reactive gliosis, oligodendrocyte death, axonal loss, complement activation, and antibody deposition. Remyelination follows the pathological loss of myelin in diseases like multiple sclerosis (MS). This phenomenon occurs in many MS lesions but becomes increasingly incomplete/inadequate and eventually fails in the majority of lesions in damaged areas. Understanding and stimulating the remyelination process are therefore important goals in MS research. It seems that NRG1-ERBB signaling may have a critical role in differentiation of oligodendrocyte progenitor cells (OPCs) and thus in remyelination process. Since association study is effective approach to unravel the genetic contribution to the etiology of complex diseases, we decide to study association rs6994992 (SNP8NRG243177) with multiple sclerosis. The SNP of rs6994992 is a functional promoter variant in NRG1 gene and it has been suggested that it is associated with schizophrenia predisposition. It interferes with a *cis*-regulatory serum response element and resides in the 5- flanking putative promoter region of and it has been suggested that it may involved in remyelination process.
Peripheral blood was collected from 135 subjects with MS and 190 healthy controls. All subjects were diagnosed with definite MS by Poser and McDonald criteria by a specialist. A PCR-RLFP technique was used to genotype rs6994992 in extracted DNA.
We found a difference in allele frequency for this SNP between MS patients and control group.

Key words: MS, Demyelinated central nervous system, regulatory serum response element.

Abstract No.90

Evidence around RNA-binding feature of artemin: A bioinformatic study

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Artemin is one of abundant stress protein found in *Artemia* and partly responsible for the amazing tolerance of this brine shrimp in harsh environmental conditions. Artemin's functions during *Artemia* embryo development are not clearly understood. Although a common ancestor has been suggested for artemin and ferritins based on their low sequence similarity, their functions are substantially different. There are some evidences showing the binding of artemin to RNA at high temperatures *in vitro*, suggesting a role in RNA protection. As *Artemia* enters diapause stage, artemin appears in the cysts and soon after termination of sever conditions and turning of cysts into larva, it disappears. It has been reported that metabolic dormancy occurs in *Artemia* cysts under the diapause condition, and processes like DNA replication, transcription and translation are substantially suppressed during this period. PUF proteins belong to PUF super-family (CDD id: cl02427) consisting of two families. One of these families includes proteins which contain Pumilio-family RNA binding repeats (CDD id:

pfam00806) while the other family includes proteins which contain Pumilio-like repeats (CDD id: smart00025). These repeats are necessary and sufficient for sequence specific RNA binding. PUF proteins are conserved among different organisms and regulate various aspects of development by controlling mRNA stability and repressing translation through sequence-specific interactions with mRNAs. In the present study, we investigated the possibility of presence of similar features between artemin and RNA-binding proteins. Analyses revealed some similarities, including presence of PUF repeats, helix distribution pattern in these repeats and presence of RNA-proteins interacting critical residues, between artemin and PUF proteins. Our findings highlight functional differences between artemin and ferritins, putting into doubt possibility of common ancestry for them; and can justify the tendency of artemin for binding RNAs and its expression *in-vivo* during diapauses.

Key words: Artemin, RNA-binding proteins, PUF proteins, Bioinformatic study.

Abstract No.91

Determination of amino acids extracted from the single cell protein produced by treated wheat straw

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With the increasing world population, the necessity of using ligno-cellulosic wastes for production of single cell protein (SCP) as animal feed seems to be important. Essential amino acids play a very important role in animal nutrition program. Amino acids are effective over growth and repair of the tissues and performance of the immune system. In the present work wheat straw was treated with NaOH 2% at 100 ºC and used as substrate for solid state fermentation. Then, SCP was produced by treatment of wheat straw with *Pleurotus florida.* Total protein analysis showed an amount of 62.8 gr protein per 100 gr of dried SCP. Then the protein content of the SCP was extracted and hydrolyzed with HCl 6 normal for 48 hours under 110 ºC. Thereafter, the amino acids of protein were analyzed by using A-200 Amino Nova analyzer. The results indicated that the ratio of essential amino acids to total amino acids was 65.6%. The percentage of essential amino acid ingredient of extracted proteins was as follows: Lysine: 9.5, Histidine: 19.8, Threonine: 0.6, Valine: 6.6, Methionine: 2.1, Isoleucine: 7.3, Leucine: 6.8, Phenylalanine: 4.3 and Arginine: 8.3. This indicates that the produced SCP could be a suitable substitute in the food program of animal feed.

Key words: Single cell protein, Essential amino acids, Wheat straw, Lignocellulosic wastes, Animal feed.

Abstract No.92

Effects of new designed anti-cancer Pd(II) complexes with different aliphatic tails on Human serum albumin

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The pharmacokinetics and pharmacodynamics of any drug will depend, largely, on the interaction that has with human serum albumin (HSA), the most abundant plasma protein. The interaction between new synthesized Pd(II)-complexes, 2,2'-bipyridin octyl dithiocarbamato Pd(II) nitrate (Octpd), 2,2'-bipyridin Butyl dithiocarbamato Pd(II) nitrate (ButPd), 2,2'-bipyridin Ethyl dithiocarbamato Pd(II) nitrate (EtPd), anti-tumor components, with human serum albumin, a carrier protein, were studied at different temperatures of 27 and 37 ºC by fluorescence spectroscopy, far UV circular dichroism (CD) spectrophotometric and differential scanning calorimetry (DSC) techniques. By the analysis of fluorescence intensity, it was observed that Pd(II)-complexes have strong abilities to quench the intrinsic fluorescence of HSA through a dynamic quenching procedure. The binding parameters were evaluated by fluorescence quenching method. The thermodynamic parameters, including □H°, □S° and □G° were calculated by fluorescence quenching method, indicated that hydrophobic forces play a major role in the interaction of Pd(II) complexes with HSA. Far-UV-CD results represented that Pd(II)-complexes induced decreasing in content of α helical structure of protein. The binding of new designed drugs (Pd(II) complexes) on blood carrier protein of HSA resulted significantly alterations on the structure and conformation of protein via decreasing of stability of HSA by decreasing of T_m, red shift in maximum fluorescence intensity, decreasing in content of □-helical structure and increasing of nonpolar or accessible hydrophobic surface of HSA to solvent.

Key words: Pd(II) complex, Human serum albumin, fluorescence intensity, calorimetry.

Abstract No.93

Automorphism Group and Topological Indices of the Chemical Graph of Fullerenes

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In an earlier paper, the authors of this paper designed a MATLAB program for computing symmetry of molecules. They applied this program to calculate the symmetry of the fullerene C80.
In this paper, using a well-known result on graphs, we write another MATLAB program for computing the automorphism group of some fullerene graphs, which has better running time. The PI, Wiener and Schultz indices of these chemical graphs are also computed.

Key words: Fullerenes, topological indices, automorphism.

Abstract No.94

Inhibition of mushroom tyrosinase with nitroanilines and their vanillin derivatives

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Tyrosinase also known as polyphenol oxidase (PPO) is a copper-containing mono-oxygenase, which is responsible for melanization in animals and the enzymatic browning of fruit. It displays two distinct enzymatic activities: the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of the latter to *o*-quinones (diphenolase activity). Tyrosinase inhibitors have been used as depigmenting agents for pigmentation disorders, skin whitening agents, anti-browning substances for foods and beverages of plant origin and alternative insect control compounds. There are several reports on inhibitory effect of phenolic, benzoic acid and salicylic acid compounds, but there are not any investigations which show the inhibitory effect of nitroanilines on the enzyme, although amino derivatives of benzoic acid were reported as tyrosinase inhibitors. We, therefore, for the first time investigated the inhibitory effect of 2-nitroaniline (**a**), 3-nitroaniline (**b**), and 4-nitroaniline (**c**), as well as their newly synthesized vanillin derivatives (2-nitrobenzenaminium 4-formil-2-metoxyphenolate (**d**), 3-nitrobenzenaminium 4-formil-2-metoxyphenolate (**e**) and 4-nitrobenzen aminium 4-formil-2-metoxyphenolate (**f**)) on the oxidation of dopamine hydrochloride by mushroom tyrosinase. Among them, compound **c** exhibited the most inhibitory effect while **a** acted as an activator. For these compounds, the IC_{50} follows the order of **c** < **e** < **a** = **f** < **b**. Compound **c** and **e** were competitive while **a**, **f** and **b** were un-competitive inhibitors. The results indicate that the relative positioning of amino and nitro groups is important in the inhibition of the enzyme.

Key words: Tyrosinase, Inhibition, Nitroaniline.

Abstract No.95

Characterization of a new thermophilic maltogenic amylase from a hot spring *Geobacillus* isolate

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Maltogenic amylases (EC 3.2.1.133) are exo-acting enzymes, exhibiting the novel enzymatic properties that are clearly discernible from other α-amylase family members; they show both transglycosylation and hydrolysis activities on various substrates specially cyclodextrins (CD), which makes them useful for the preparation of branched oligosaccharide mixtures and novel carbohydrates. In contrast with other amylases, only a few maltogenic amylases and related enzymes have been characterized mainly from *Bacillus* and *Thermus* sp. In our study, a thermophilic maltogenic amylase producing bacterium was isolated from Gheynarge hot spring and identified as a *Geobacillus sp.*, based on microbiological tests and 16S rDNA sequence. Relative activity of the enzyme was far more with ß-cyclodextrin in comparison with starch, amylopectin, amylose, and glycogen substrates. The temperature profile of the enzyme is very broad (from 35 to 70 °C) with 65 °C being the optimum temperature, which is different from those of *Bacillus subtilis* (45 °C), *Bacillus sp*. WPD616 (50 °C), *B. licheniformis* (50 °C), *B. stearothermophilus* (55 °C) and *Thermus sp*. IM6501 (60 °C) maltogenic amylases. The enzyme is optimally active at a wide range of pHs (from 4.5 to pH 9). It was more thermostable than other maltogenic amylases that have ever been characterized. The maltogenic amylase encoding gene was cloned and sequenced. The protein consists of 589 amino acids. The deduced amino acid

sequence of the enzyme showed a very high sequence homology to other maltogenic amylases, especially those from *Thermus sp*. IM6501. Considering the low level of amino acid substitutions, the sequence analysis provided useful information regarding its higher thermostability.

Key words: Thermophilic, Thermostability*, Geobacillus,* Maltogenic amylase.

Abstract No.96

Interactions of Proteins with Membranes: Insight from All-Atom and Coarse-Grained Molecular Dynamics Simulations

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Interactions of protein with biomembranes play a vital role in various biological processes such as the transport of peptide across membrane, fusion, and signal transductions. Studying interactions of small peptide with lipids is a requisite first step toward the understanding of more complex processes seen in larger membrane proteins such as folding, pore formation and self-assembly of membrane proteins. Experimental techniques such as NMR, X-ray scattering have been used to study the relationship between the structure and function of membrane proteins. Computational methods such as all-atom and coarse-grained molecular dynamics simulations have complemented experimental techniques. On the other hand, MD can provide insight about protein-membrane interactions at length scale that is not realizable through most experimental techniques. Here, we present the results of CG and atomistic molecular dynamics simulations of antimicrobial peptides interacting with dipalmitoylphosphatidylcholine (DPPC) and palmitoyloleoylphosphatidylglycerol (POPG) phospholipid bilayers. The peptides we have chosen for our study are Piscidins, antimicrobial amphipathic cationic peptides which were first isolated from fish. Circular dichroism and NMR experiments have shown that Piscidins adopt an amphipathic alpha helical conformation with hydrophobic and hydrophilic residues on opposing sides in TFE and DPC micelles. The aims of the present work are to explore its spontaneous insertion into the biomembranes during an equilibrium MD study. From the MD simulations of Piscidins with DPPC and POPG bilayers, we have identified key interactions that dominate the lipid-peptide interactions. Lysines, histidines and arginines are primarily responsible for antibacterial activity in these peptides. The simulations have recognized strong interactions among Arg 7, His11, Lys 14 and Thr 15 in Piscidin with the oxygen atoms of the lipid head group regions, which can be characterized by hydrogen bonding. Coupling these simulation results with experimental data will allow us to further elucidate the details of membrane protein insertion, positioning, and stabilization.

Key words: biomembranes, antimicrobial peptides, MD simulations.

Abstract No.97

Effect of Positive charge on the Thermodynamic Stability and Folding Kinetic of Firefly Luciferase

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Design and synthesis of novel proteins by genetic engineering techniques, allows complex problems in biology to be answered by structure-activity relationships in an analogous manner. The fact is one of applications of physical-organic chemistry. This approach has been applied to study the folding pathway and stability of the wild type firefly luciferase (*Photinus pyralis)* and four mutants, which residues have different charges (Arg356, Lys356, Glu356, and Gln356). The changes in stability of mutants are measured via the changes in free energy of unfolding of the protein. Kinetic measurements are made based on the folding and unfolding of wild-type and mutants. Combination of kinetic and thermodynamic data enable the role of side chains in the stabilization of the final, transition state of the proteins to be measured.Comparative study of chemical denaturation of native and mutant luciferases by intrinsic and extrinsic fluorescence, circular dichroism, DSC, stopped-flow kinetics, and φ-value revealed that insertion of positively charged residues(Arg,Lys) in the flexible loop(352-358), plays a significant role on the stability of (*P. pyralis*) luciferase and pathway of folding with a redshift.

Key words: thermodynamic, Luciferase, kinetic, stability.

Abstract No.98

Molecular dynamics simulation of Human protein Z complex with Protein Z-dependent protease inhibitor

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Human protein Z (PZ) is a single chain protein with 360 amino acid residue with four functional domains: N-terminal Gla domain, two epidermal growth factor (EGF)-like domains (EGF1 and EGF2 domains), and a serine protease (SP)-like domain. PZ is structurally homologous with coagulation factors VII, IX, X and anticoagulant protein C. PZ has no proteolytic activity, because the lacks of critical histidine and serine residues in catalytic site, and hence it could not be a zymogene for serine protease activity in coagulation. However PZ acts as cofactor in inhibition of coagulation factor X (f Xa) by the Protein Z- dependent protease inhibitor (ZPI). Abnormal changes in ZP amount (increase or decrease) had been reported for ischemic stroke and Non-Arteriotic Anterior Ischemic Optic Neuropathy (N-AION). Molecular dynamic simulation is a powerful method used to study the structural changes of protein complexes in different conditions. NAMD (NAnoscale Molecular Dynamics) version 2.6, under CHARMM22 force filed is installed on an Intel ® Pentium ® M based PC at 1.80 GHz with 768 MiB of Ram package. PZ-ZPI complex coordinates was obtained from RCSB Protein Data Bank with PDB ID: 3F1S. The protein was minimized and equilibrated in a cubic box with 92.78 × 105.86 × 116.30 Å dimensions. Energy minimization was carried out using steep integrator and fmax were choosed 1000 for 20000 step. Molecular dynamics with all-bond constrain for 200ps and then with no constrain were done for up to 2ns. Our results show that PZ-ZPI complex is stabilized primarily by electrostatic or salt bridges interactions i.e. about 14 salt bridges in complex interface. Calcium ions as cofactors change the complex conformation to possibly active one. However, calcium ions bind to negative residues specifically at the complex interface via electrostatic bonds, inducing structural alterations predominantly concomitant with about 4% decrease in regular structures in PZ and 2% increase in ZPI proteins which means the formation of a new conformation for PZ-ZPI complex. There is about 7% decrease in complex hydration in the presence of calcium ions, confirming new conformation with somehow different properties. RMSD curve confirm the stability and reliability of simulation and the complex conformation**.**

Key words: molecular dynamics, Human protein Z, protein conformation.

Abstract No.99

Chemometric study of the mobile phone radiofrequency effect on normal and β-thalassemia hemoglobins

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Widespread use of mobile phones has increased the human exposure to electromagnetic fields (EMFs). In this study, the effect of mobile phone radiofrequency (910 MHz and 940 MHz) on structure of normal and β-thalassemia hemoglobins was investigated by circular dichroism(CD). The characterization of the unfolding pathway under thermal stress in two exposed and unexposed states has been shown using chemometric methods. The CD spectra obtained under denaturing conditions corresponding normally to a mixture of the denatured polypeptide, the intermediate structures and native form which make the resolution and characterization of intermediates of folding pathway. Several chemometric techniques such as singular value decomposition, evolving factor analysis and multivariate curve resolution successfully applied to study the conformational changes of normal and β-thalassemia hemoglobins exposed to EMFs. Multivariate curve resolution chemometric technique was performed on CD spectra under thermal stress, to obtain the required information about the number and change in concentrations of the species involved. Chemometric analysis of CD spectra of hemoglobins under thermal stress, led to the existence of three different molecular species including native (N), intermediate (I) and denature (D) species. Appearance and buildup of two molecular species I and D were connected to the disappearance of N-species. The concentration of intermediate species of both normal and β-thalassemia hemoglobines exposed to radiofrequency of 940 MHz is higher than the corresponding concentrations exposed to radiofrequency of 910 MHz. Also the concentration of intermediate species of both normal and β-thalassemia hemoglobins exposed to EMFs is much higher than their concentrations in the unexposed state. These findings are in agreement with our previous work which revealed that exposing to mobile phone EMFs can change the structure of hemoglobin.

Key words: Mobile phone, Hemoglobin A, β-thalassemia Hemoglobin, Circular dichroism, chemometrics, Multivariate curve resolution.

Abstract No.100

The role of fetal rat cerebrospinal fluid on differentiation and proliferation of rat pheochoromocytoma cells

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In the early stages of brain development, cells within the ependymal lining of the neural tube are thought to secrete cerebrospinal fluid (CSF). It is well documented that fetal CSF contains many neurotrophic and growth factors which are known as modulators of neurogenesis, differentiation and brain extracellular microenvironment. Rat pheochromocytoma PC12 cells have been widely used as an in vitro model of neuronal differentiation since the cells undergo differentiation to sympathetic neuron-like cells in response to NGF, bFGF, EGF, TGF-α and GDNF. We hypothesized that prenatal CSF could have differentiational effect on PC12 cells, therefore CSF was removed by tapping the cisterna magna of Wistar rat fetuses (E17-E20) then centrifuged. PC12 cells were cultured in RPMI-1640 with 10% FBS, 100 unit/ml of penicillin, 100 mg/ml of streptomycin and 5% CO_2 at 37 ºC. CSF at different ages was added to the medium at 7, 10, 25% (v/v). The cell viability and cell proliferation were measured by MTT assay. The neuronal differentiation of PC12 cells were showed by changes of neurite outgrowth. Viability and cell proliferation were significantly elevated in PC12 cells cultured in CSF supplemented medium in E18 compared with control ones. A significant neuronal-like outgrowth appeared as early as day 3 after the application of the CSF supplemented medium E17. It was shown that CSF neurotrophic factors can support normal neurogenesis and promotes proper brain development, neuronal differentiation and brain cyto-architecture owing to its close contact with germinal epithelium. It has been reported that CSF can be a survival material on its own with any medium for cerebral cortex primary cultures. Our data are in the same line with pervious studies that clarify crucial role of CSF neurotrophic factors in neuronal differentiation and cell proliferation. Taken together we address PC12 neuronal differentiation and cell proliferation to CSF induction by its components especially growth factors.

Key words: cerebrospinal fluid, PC12 cells, neuronal differentiation, cell proliferation.

Abstract No.101

Design of Disulfide Bridge in Luciferase for Screening of a Novel Enzyme using Site Directed Mutagenesis

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Firefly luciferase (EC 1.13.12.7) catalyses the oxidation of a benzothiazole substrate (beetle luciferin) in the presence of magnesium ions, ATP and molecular oxygen .The product, oxyluciferin, is generated in an excited state which then decays to the ground state with the emission of a photon. The North American firefly luciferase is used extensively for measuring microbial contamination, and over the last decade or so there has been intense commercial interest in developing bioluminescence-based technologies as a replacement for more conventional screening techniques. However, several factors limit further application and development of this technology, including the low stability of the enzyme both *in vitro* and *in vivo*, a low turnover number and a high *K*m for the substrate ATP.

One of the major goals of protein engineering is to design proteins with enhanced stability and activity. It has been shown for many naturally occurring proteins that disulfide bonds can enhance protein stability considerably. In this work we have been attempted to increase thermal stability of firefly luciferase by designing disulfide bonds and then its effect on the BL spectra, thermostability, kinetic and structural properties were analyzed.

Key words: Luciferase, thermostability, site directed mutagenesis.

Abstract No.102

Circular permuted luciferase

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Circular permutation (CP) in the protein structure is a rearrangement of the amino acid sequence, such that the original amino- and carboxyl-termini of the polypeptide seem to be linked and new ones created elsewhere Circular permutation(CP) can occur because the N- and C-termini of proteins often end near each other.

Firefly luciferase is a monomeric enzyme that catalyzes the oxidation of luciferin with molecular oxygen in the presence of ATP and Mg2+ to emit visible light. Luciferase is a useful reporter in numerous species and in wide variety of cells.
As caspase3 has an important role in apoptosis we have developed a circulary-permuted firefly luciferase artificially joined at the original termini by cloning caspase3 recognition site. The function and structure of this luciferase has been studied now.

Key words: Circular permutation, Firefly luciferase, apoptosis.

Abstract No.103

The study of Fe^{2+}, Ca^{2+} and Mg^{2+} ions effects on secretion of bacterial lipase

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Lipases are lipolytic enzymes that hydrolysis the long chain acylgelycerols to glycerol and fatty acids. There is a location in structure of Pseudomonas aeroginosa lipase for binding to Ca^{2+} and so it is predictable that metal ion can affected the enzyme activity or secretion. To study of these effects, influence of Ca^{2+}, Mg^{2+} and Fe^{2+} ions in chloride salt form were investigated on both secretion and activity of enzyme. In this study, secretion of this enzyme in clinical samples (91 strains of *Pseudimonas aeruginosa* were isolated from burn infections) was investigated by using of colorimetric screening. To predict the amount of secreted lipase, a standard graph should be drawn. So few exact concentrations of a commercial lipase (in this study Pseudomonas cepacia lipase) were prepared. Logarithm of lipase concetration is linearly related to the halo diameter on agar plates. Resultant equation was used as standard lipase activity graph. In this study in comparison with standard graph, 1 sample had most lipase secretion and selected for next studies. Results showed that addition of both Ca^{2+} and Mg^{2+} ions increase the enzyme activity in low concentrations (up to 3mM) but existence of Mg^{2+} ion in the medium had not an important role on enzyme secretion. Ca^{2+} ion had effective role in both cases while Fe^{2+} ion prevented bacterium from growth and reproduction. So it can say that divalent metal ions such as what mentioned had not similar effect on lipase. Some of them like Ca^{2+} ion acts as an activator in enzyme secretion or activity but Fe^{2+} ion inhibited the enzyme behavior.

Key words: Lipase, Divalent ions, Nutrient agar plate.

Abstract No.104

Seed Mediated Sequential Synthesis and Aging Investigations of Monodispersed Short Gold Nanorods

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Recent advances in nanotechnology have encouraged the development of novel techniques for synthesis and characterization of nanostructured materials. However, research on exploring and developing their applications is at its infancy. Gold nanorods appear to be a promising candidate in different fields of research; offering variety of applications in biomedicine and biosensing. Amongst numerous materials, gold nanorods with their LSPR property appear to be one of the most effective solutions for high contrast imaging systems, development of nanobiosensors and cancer therapy. Having ideal size for biological medium, they show high biocompatibility, long blood residency, and high efficiency at conversion of light energy into heat.
Seed mediated sequential growth process has been employed for synthesis of small sized gold nanorods. The seeds have been prepared through ultrasonication of a saturated cationic surfactant solution, followed by addition of $HAuCl_4$. Gold ions are reduced by rapid mixing of the solution with sodium borohydride. After keeping the seeds undisturbed for a couple of hours, formation of rods is started by addition of gold ions into the CTAB solution, followed by mixing with silver nitrate, ascorbic acid and the seed solution. Formation of Gold nanorods on the matrix of surfactant can be observed after few hours as the color of solution becomes intense purple. The pure product was isolated after centrifuging the solution several times, each time decanting the supernatant and diluting with deionized water. Different techniques have been utilized for characterization of gold nanorods. UV-Vis spectroscopy revealed a transverse and longitudinal plasmon resonance at wavelengths of 530 nm and 720 nm respectively. The purified gold nanorods were imaged by high resolution transmission electron microscopy (HRTEM). Stability and aggregation of the aged gold nanorods could be investigated from the spectral shifts and intensity changes observed in UV-Vis spectra of the samples recorded at different time intervals (1 week up to 1year).

Key words: gold nanostructures, LSPR, nanobiosensor, aging, HRTEM.

Abstract No.105

Selective Interaction of sea anemone toxin with voltage Gated Sodium Channels

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Voltage gated sodium channels (VGSCs) belong to the super-family of P-loop channels. Nine VGSC isoforms have been recognized in mammals with similar properties. They are responsible for action potential initiation and propagation in excitable cells. VGSCs contain four homologous domains (D_I-D_{IV}); each contains 6 trans-membrane α helices. These channels are target of several toxins which alter channel function by binding to several different receptor sites (1-6). Some groups of polypeptide toxins such as sea-anemone toxin occupy site 3 and inhibit sodium channel inactivation. The affinity of this toxin differs among various type of VGSCs. The specific effects of this toxin proposed it as the valuable target for drug design. Therefore, molecular study of this toxin and VGSCs-site 3 seems rational. Here, theoretical methods contain: sequence alignment, secondary structure prediction, trans-membrane structure prediction, molecular modeling, structure alignment and data analysis have been used to investigate the molecular properties of site 3 in different VGSCs. The results show that the sequence of site 3 is highly conserved between channels except Nav1.8 and Nav1.9 which have some special properties. Investigation on the model of channel demonstrated that all of them except Nav1.7 have the same architecture but there are small differences in segment 5, 6 and site 3. The loop structure in site 3 seems to be effective on channel-toxin interaction through surface complementary parameters. Structures investigation also shows considerable electrostatic potential differences between channels which can be effective on toxin-channel interactions. It seems that other sequences and structural parameters are also involved in toxin selectivity. Data also suggests that the mechanism of toxin interaction differ in various channel and it is probably the cause of different affinity and selectivity of toxin interactions.

Key words: VGSCs, polypeptide toxins, channel-toxin interaction.

Abstract No.106

Application of biomolecular markers and bioinformatics to assess fertilization scheme of Caspian trout in hatchery (*Salmo trutta caspius*)

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The advent of DNA biomarkers has simplified molecular based pedigree retention when mixed fertilization is performed in fish hatcheries. There is no information on how artificial propagation of the endangered Caspian trout (*Salmo trutta caspius*) impacts on the effective size of breeders (Ne), which is a critical parameter to maintain genetic diversity in progeny. Biomolecular markers (DNA microsatellites) and bioinformatic approaches served to determine if equalizing individual sperm volumes (ESV) in 2 different sperm pools each derived from four male Caspian trout breeders would reduce the variance in number of progeny compared to the current hatchery protocol (CHP) where 2 and 4 different male breeders had the opportunity to fertilize female gametes. Bioinformatic tools such as DNA sequencing using ABI PRISM® 3730 automatic sequencer and GeneMapper software were applied to assign progeny to the breeders through microsatellite profiling technique. One to one crosses of breeders were also performed and fertilized ova of different families were mixed to constitute balanced mixed families (BMF). Exclusion-based parentage programs like Cervus and FAP unambiguously assigned >93% of progeny to a single pair of parents using three polymorphic microsatellite markers in the 1490 progeny obtained. Ne was 4.69 and 4.25 compared to census size of 6 and 8 in CHPs, 6.72 and 4.11 compared to census size of 8 in ESVs and 3.99 compared to census size of 4 in BMF. Significant different contribution of breeders was observed in both CHPs and ESVs. The primary constraint on Ne in ESVs was the unbalanced contribution of male breeders to progeny. These results illustrate that minimizing loss of genetic diversity in this species largely depends on the management of fertilization scheme in hatchery and so, one male to one female crosses are the most appropriate scheme of fertilization for conservation of Caspian trout.

Key words: Biomolecular markers, Bioinformatic approaches, Effective size of breeders, Caspian trout.

Abstract No.107

Expression, purification and characterization of pyruvate kinase from thermophilic Geobacillus sp and coupled bioluminescent assay for ADP measurement

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ADP is involved in many biological reactions and ADP assay kit can be used for assaying some enzyme reactions universally by monitoring ADP formation or depletion. In previous studies, several methods for analysis of ADP have been developed. One of them depend on the stoichiometric conversion of ADP to ATP by the enzyme pyruvate kinase (PK), in the presence of variable amounts of ADP, and subsequent measurement of the ATP with a luciferin-luciferase preparation. Therefore The gene of PK (EC 2.7.1.40) from thermophilic bacterial has been cloned in expression vector pET28-a (+), sequenced and functionally expressed. Recombinant protein was purified using Ni-NTA column. The purified PK was used in a coupled bioluminescent assay for ADP measurement. Its structural and functional properties such as kinetic parameters, Circular Dichroism (CD) and fluorescence spectra are studied.

Key words: Geobacillus, Pyruvate kinase, Coupled assay, Bioluminescense, Adenosinediphosphate.

Abstract No.108

Effect of prolin on the Activity of *Pseudomonas fluorescens* lipase

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It has been determined that lipases; triacylglycerol hydrolayses; as an important enzyme group are able to catalyze hydrolysis or synthesis of esters. Lipases are notable enzyme because of their physiological and biotechnological applications. They are produced by microbes and other organisms. Nowadays, bacterial lipases play a vital role in commercial affairs. Lipase from *pseudomonas fluorescens* (E.C.3.1.1.3) is a thermophilic kind of lipases which is produced by pseudomonase strains (MW around 33 kD). This property causes scientists to conduct various researches to know more about the structure and function of PFL. In this study, we measured activity rate of PFL at visible wavelength (410 nm) and p-Nitrophenyl Palmitate acted as a substrate role. We investigated the effect of prolin on activity changes of PFL in the presence of prolin with different concentrations (i.e. 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 M). Results revealed that the activity is enhanced at 0.5M and reduced at 0.2M of prolin. This effect could be resulted from preferential hydration which may change PFL structure. We conclude that in case of prolin, 0.5 M is a critical concentration, which affects the PFL structure and enhances the enzyme activity rate. There is also a critical concentration of 0.02 M for prolin, which induces reduction of enzyme activity. This effect could be due to negative structural changes that perturb the active site coordination or lid. This phenomenon reduces PFL activity rate.

Key words: Pseudomonas fluorescens lipase, prolin, sorbitol, UV-spectroscopy.

Abstract No.109

Surface Arginine saturation effect on thermostability of firefly luciferase

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In life science, bioluminescence has become a vital tool for laboratory analysis and biomedical imaging both in academic research and industrial product development. Light emission from the North American firefly *Photinus pyralis*, is widely believed to be the most efficient bioluminescence system known. The bioluminescence reaction in firefly is catalysed by luciferase enzyme. Luciferase first converts the substrates firefly luciferin (LH2) and $Mg–ATP^{2+}$ into the corresponding luciferyl adenylate. This reactive intermediate combines with molecular oxygen to produce an electronically excited state product, which rapidly emits a photon of visible light. However, this enzyme is unstable and rapidly loses activity that leads to losses in sensitivity and precision in analytical applications. Our objective here is to produce mutants of luciferase with bioluminescent properties suitable for imaging techniques. Several strategies, including gene chimerization directed evolution, and random mutagenesis, have been implemented to produce thermostable luciferase reagents. A number of point mutations have previously been identified that significantly increase the thermostability of the *Photinus pyralis* enzyme. We have introduced

two different mutations including Q35R, I232R in a previously reported red-emitter mutant (E354R) of *L. turkestanicus* luciferase. For this, the QuikChange Site-Directed Mutagenesis procedure was used to create the mutants *L. turkestanicus*. Then we have overexpressed and purified luciferase mutants from L*. turkestanicus.* Thermostability, optimum pH and temperature and kinetic properties were determined and results show that these mutations have positive effects on the thermostability of enzyme.

Key words: *lampyris turkestanicus,* Firefly luciferase, thermostability, SDM.

Abstract No.110

Comparison of minor groove binding properties of four anticancer drugs: insights from Molecular Dynamic simulation

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Using DNA duplex of sequence (CGCGAATTCG CG)/(GCGCTTAAGC GC), we report the molecular dynamics (MD) simulations results for minor groove binding properties of four anticancer drugs including anthramycin, anhydrous form (IMI), distamycin A (DST), mithramycin A (MTA), and mithramycin SK (MSK). Simulation was done considering water solvation and adding sufficient number of Na+ counter ions for charge neutrality in the system. Our simulations are configured particularly so to characterize the latest MD models of DNA, and to provide a basis for examining the sensitivity of MD results to the treatment of boundary conditions, electrostatics, initial placement of solvent, and run lengths. Energy minimization was carried out using steep integrator, fmax, 1000 and 4000 step. All-bond constrained MD at 310K without pressure coupling was done for 100ps. Finally MD was done without constrain for up to 2ns at 310K and 1atm pressure. AMBER -03 force field on GROMACS software, version 3.3.3 was used in MD simulations. Our preliminary results show the difference in mode of binding and in molecular flexibility of DNA-drug complex for anti cancer drugs. Precise analysis of the DNA hydration in the presence of drugs shows that the hydration pattern differs cooperatively depending on the degree of drug intercalation. Our docking results shows MTA extends its binding in minor groove about 5-6 bp and determined that differences in binding preferences of MTA and MSK to DNA of different C+G-content. MTA don't bind to some sequences, for example G/A-rich. MTA and MSK contain the same tricyclic core moiety and oligosaccharide side chain, but they differ in the side chain at C-3. This chain is longer in MTA and bears a higher number, and different arrangement, of potential donors and acceptors of hydrogen bond. Hence, a higher (more negative) free binding energy (ΔG) for MTA is consistent with the formation of extra hydrogen bonds. The oligosaccharide moieties of the mithramycins are the same. They are involved in the binding within the DNA minor groove, forming equivalent intermolecular contacts with the sugar-phosphate backbone. The distamycin A, which is positively charged, quite flexible and highly selective towards AT-rich tracts, due to H-bonding between its amide nitrogens and N3(A) and O2(T) of DNA. The IMI is neutral, rather rigid with modest preference towards to alkylated guanine.

Key words: anticancer drug, groove binding, DNA-drug complex, MD models.

Abstract No.111

Molecular Dynamic Simulation of Aflatoxin B1 Binding to C/G-rich Sequence of DNA

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Aflatoxin B1 (AFB1) is a potent toxic, carcinogen, mutagenic chemical produced by Aspergillus flavus, acts as serious food contaminant. AFB1 is normally found in trace amount in food products. This carcinogenic chemical plays an important role in human liver cancer. A molecular lesion exerted by AFB1 is detected to be exclusively at guanines nucleotide with a preference towards GpG and methylated CpG sequences. Previous studies showed AFB1 adducts formed primarily at the N7 position of guanine via a non covalent binding through DNA minor groove. Molecular dynamic (MD) simulations were carried out on the AFB1-oligonucleotide (CGCGAATTCG CG)/ (GCGCTTAAGC GC) complex. The complex was made through inserting AFB1 instead of Mitramycin (MTA) in previously studied MTA-oligonucleotide complex. AFB1 molecule was prepared by Hyperchem software. Site directed dockings were done using Hex software with the following parameters: correlation type: Shape only, FFT Mode: 3D fast lite, Grid Dimension: 0.6, Receptor range: 180, Ligand Range: 180, Twist range: 360 and Distance Range: 40 and Arguslab software to

find out the best conformation for AFB1-oligonucleotide mimic the MTA, the minor-groove binder, complex. The constructed complex was solvated in cubic box, neutralized with counter ions, and subjected to MD-simulation with Amber-03 force field on Gromacs software version 3.3.3. The properties of the simulated systems were undisturbed and the errors were kept to a minimum. Energy minimization was carried out using steep integrator, fmax, 1000 and 4000 step. All-bond constrained MD at 310K without pressure coupling was done for 100ps. Finally MD was done without constrain for up to 2ns at 310K and 1atm pressure. RMSD analysis for complex throughout the simulation shows a stable complex between DNA and AFB1. The results show that AFB1 like MTA binds to DNA through minor groove. Hydrogen and radial distribution analysis of complex confirms thermodynamic stability for the complex.

Key words: molecular dynamic, carcinogen, MD-simulation, RMSD analysis.

Abstract No.112

Comparison of Trehalose and Sorbitol Effects on the kinetics and Thermodynamics Stability of *Pseudomonas cepacia* Lipase

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Lipase catalyzes the hydrolysis of triglycerides to produce free fatty acids and glycerols. This enzyme is widely used in the food, detergent and pharmaceutical industries. Active site of lipases has two conformations; i.e. the open conformation with the active state of enzyme and the close conformation with the inactive state. *Pseudomonas cepacia* lipase (PCL) is a microbial lipase, having variety of applications in organic solvents for stereo and regioselective modifications of pharmaceutical compounds and food products in the industry. We have compared the effects of trehalose and sorbitol osmolytes on the kinetics and thermodynamics stability of PCL and also on the active site conformation of the enzyme, using UV-Visible and Fluorescence spectroscopy. Osmolytes are capable of increasing the stability of macromolecules in the living organisms. They enhance the kinetics and thermodynamics stability of PCL. We noticed an enhancement of PCL activity in the presence of sobitol and trehalose, while each case was investigated individually. The solution containing both of the osmolytes, the activity increased synergistically compared to that of the individual sorbitol or trehalose. We conclude that osmolytes improve the effect of each other in a synergistic manner. Fluorescence results revealed that addition of osmolytes, led to the appearance of intense peaks in the presence of individial trehalose and sorbitol. There was no spectral shift in the fluorescence; whereas with both osmolytes, not only the intensity increased, but also the spectrum shifts to longer wavelengths (red shift). This indicates that in the presence of boths osmolytes, the tryptophan residues existing in the vicinity of active site are exposed to the solvent. Since PLC activity increases, it can be concluded that conformation of the PCL active site has more tendency to be in the open state. Therefore osmolytes play a key role in stabilizing the active site of PCL by enhancing its activity.

Key words: *Pseudomonas cepacia* lipase, stability, osmolytes.

Abstract No.113

The Study of Osmolytes Effects on the Kinetics and Thermodynamic Stability of *Pseudomonas cepacia* Lipases

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Lipases belong to a family of enzymes which catalyze the hydrolysis of triglycerides. Being widely distributed in many organisms, lipases can be distinguished from esterases by their characteristic interfacial activation, exhibited at an oil–water interface. Microbial Lipases have been largely employed in organic solvents for stereo and regioselective modifications of pharmaceutical compounds and food products in the industry. Due to having lower activity in organic media compared to water (Klibanov, 1997), stability investigation and enhancing the catalytic activity of the *pseudomonas cepacia* lipase (PCL) is of high interest. We have studied the effect of osmolytes (trehalose and sorbitol) on the kinetics and thermodynamic stability of PCL using UV-

Visible, Stopped flow and Fluorescent spectroscopy. osmolytes are capable of increasing the stability of macromolecules in the living organisms. They enhance the kinetic and thermodynamic stability of PCL. We observed that in the presence of osmolytes, PCL activity is enhanced. It worth to mention that with higher concentrations of osmolytes, the activity of PCL increases in an almost linear trend. Stopped-Flow studies and measurement of unfolding rate constant in the presence of urea indicated that osmolytes induce changes in the rate of unfolding.The unfolding process consists of a two-step process, i.e. an intermediate denatured step (D), which can be refolded into the native step; and the final denatured step (FD), which is irreversible. We observed increase of fluorescence intensity in the presence of urea; whereas in Guanidine hydrochloride solution the intensity is decreased and shifts to longer wavelengths. Addition of osmolytes to the medium, led to the appearance of intense peaks in our studies; confirming the linear relationship between fluorescence intensity and concentration of the osmolytes. The fluorescence intensity is increased with higher concentrations of urea, in case both urea and osmolytes are present in the medium. Our thermodynamic studies give equilibrium denaturation curves, showing the various effects of additives on the stability of folding states.

Key words: *Pseudomonas cepacia* lipase, Stopped-Flow, osmolytes.

Abstract No.114

Effect of extremely low frequency electromagnetic fields on memory and learning

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Neural cholinergic synapses in central nervous system are important in learning and memory processes. In this study, the effect of extremely low frequency electromagnetic fields exposure to synaptic membrane was measured. Synaptosomes were exposed to a 0.3 mT electromagnetic field with frequencies ranging from 50 to 217 Hz for 30 minutes. The amount of acetylcholine release from presynaptic membrane was measured quantitatively by using High Performance Liquid Chromatography (HPLC) and UV-Visible spectrophotometery. Results show that amount of released acetylcholine was decreased by increasing the frequency. The non-linearity of acetylcholine decrease in the presence of various frequencies, results in a two-mechanism releasing process. We believe that opening and closing the ion gates and vesicle exocytosis are conducted during the period of exposure. So we suggest that exposing to extremely low frequency electromagnetic fields may decrease the ability of human brain for learning and memory processes.

Key words: Electromagnetic fields, learning and memory, acetylcholine, Synaptosome.

Abstract No.115

Vibrational Analysis of substituted 3-benzylidene-7-alkoxychroman-4-ones: ab-initio calculations

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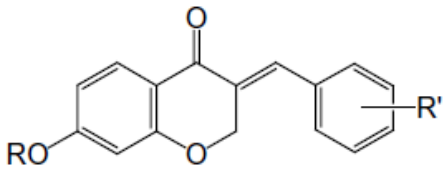
Antioxidative activity is a multifactorial potential. Propensity of radical formation and stabilization, ability of metal complexation, and lipophilicity are important factors for the antioxidant activity. The presence of ortho-electron donating hydroxy or methoxy substituent of the phenolic compounds is known to increase the stability of the radical and hence, the antioxidative activity. A series of 3-benzylidene-7-alkoxychroman-4-one derivatives were synthesized and evaluated for their antioxidant activities. In this research we studied the formation potential and analysis of 3-benzylidene-7-alkoxychroman-4-one component that contain catechol moieties, have been found the possible stabilization of the radical that is formed after hydrogen abstraction (Fig-1). The binding energy gradient was estimated to be 0.05 kcal using the conjugate gradient optimization method (Polad-Ribiere algorithm). In order to reach the Global Minimum, some structural parameters of the initially optimized molecules were changed manually and the molecular structures were re-optimized. This procedure was repeated until the most stable structure was obtained. B3LYP/6-311G (d, p) optimizations of three derivatives of 3-benzylidene-7-alkoxychroman-4-one was done by Gaussian 98. The final structure was done to include the effect of electron correlation and adding the polarization function as well as to reduce the basis sets superposition error (BSSE). To verify that the concluding structure wasn't in the local minimum point, the normal mode frequency calculation was carried out for the optimized molecules by using the HF/6-31G method.



R: Me, Et, n-Pr ; R`: 3,4 -(OH)2
Fig-1 Antioxidant activities of 3-benzylidene-7-alkoxychroman-4-ones

Key words: antioxidative activity, stability, catechol moieties.

Abstract No.116

Structure, ultra structure characteristics and protein assay and allergenicity of pollen in *Acacia saligna*

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Acacia (Fabales, Mimosaceae) is an anemophilous plant. Due to its resistance to dry climate, it is broadly dispersed in south regions of Iran. Pollens can cause allergic symptoms after their entrance into the respiratory tract. Allergenicity of pollen grains resides in the proteins of their envelope that ranged between 10 to 70 kDa. In the current work we aimed to study pollen structure and allergenicity of pollen in *A.saligna.* Pollen grains were collected from Ahwaz (Khuzestan) suburb. After isolation and microscopic control of grains, their structures were analyzed by Acetolysis technique and its ultra structures was determined by SEM. 15% extract of pollen grains were made in PBS (pH= 7.2, 0.1 M). Tests were done on Hartley male guinea pigs. Animals were sensitized by intraperitoneal injection of 100 µl pollen extract, during 3 weeks and two times per week. Eosinophils and Neutrophils' cells population were analyzed in blood samples taken from sensitized animals by smear slides and their IgE value were assayed by guinea pig Elisa kit. Subcutaneous injections were also used to evaluate flare diameter as another allergenicity marker. Total protein concentration of extract was measured based on Bradford method, then the proteins were subjected to SDS-PAGE (12%) to detect presumptive allergenic proteins. Our results showed that pollens of *A.saligna* are spherical and aggregated in the form of polyad. The outer surface was smooth and Psilate-Foveolate. Eosinophils, Neutrophils and IgE concentration in sera were significantly elevated in the sensitized animals in contrast to control ones ($p< 0.001$, $P< 0.01$, $P< 0.001$, respectively). Electrophoretic pattern of pollen proteins showed that 22 kDa, 32 kDa, 46 kDa, 55 kDa and 66 kDa bands are the most important and dominant bands that their corresponding proteins can proposed as allergenic proteins.

Key words: pollen, allergy, Electrophoresis, proteins, *Acacia saligna*, Acetolysis, SEM.

Abstract No.117

Study of Antioxidant activity, cytotoxic effects and apoptosis induction by Methoxy-Meso-VO-Salen on Hela and McCoy cell lines

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Vanadium is a rare mineral element that biochemical activity and anticancer effects of some its compounds were studied previously. The Methoxy-Meso-VO-Salen (MMVOS) complex was synthesized by aldol condensation of salicylaldehyde according to Bezaatpour, et al. (3). The radical scavenging avtivity of serial dilution of the MMVOS was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The results showed high antioxidant activity with RC_{50} of 88 µg/ml. Cell viability and cytotoxic effects of the complex were evaluated by the reduction of MTT and trypan blue exclusion assay, respectively. The cells in the exponential growth stage were dispensed into 24-well microplates at 3 x 10^5 cell/well. After being cultured for 24h, the cells were immediately treated with various doses of the MMVOS complex for another 8h and 16h. The results imply that the MMVOS complex inhibits the Hela cells viability with IC_{50} of 150 µg/ml and CC_{50} of 48 µg/ml. These values on McCoy cells were 33.6 µg/ml and 37 µg/ml, respectively. Morphological studies showed significant change of treated cell shape. The treated cells become round and condensed, inter cellular connection become loose, the proliferation inhibited and the granules is cytoplasm increased. DNA fragmentation was studied as a distinct sign of Apoptosis induction. After 16h of the total genomic DNA of cells was loaded on agarose gel electrophoresis. The results showed moderate DNA fragmentation. Treating for 72 h with low concentrations of the complex showed efficient fragmentation of DNA. These findings showed that the MMVOS is a very active complex that efficiently induces apoptosis on cancerous cell lines.

Key words: antioxidant activity, DNA fragmentation, apoptosis.

Abstract No.118

The Effects of auricular and Body Acupuncture on level of the serum hscrp in Iranian Obese and Overweight Subjects

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Auricular and Body acupuncture have been reported to reduce body weights of subjects in clinical practice. Also a few data is available on relationship between obesity and highly sensitive C-reactive protein (hsCRP) levels in Asian populations. hsCRP has been identified as a strong independent risk factor of cardiovascular events. In the present study we have evaluated the effects of auricular and body acupuncture on body weight and level of the serum hsCRP and the relationships between obesity and hsCRP levels in subjects of both genders, divided into 4 groups as following;

Auricular acupuncture:

1) case group: (n=98) subjects with low-calorie diet and auricular acupuncture. The auricular acupunoints on their ear include: CO1, CO4, CO13, CO14, CO17, CO18. TF4 and AT4. 2) Control group: (n=98) Subjects with low-calorie diet and Shame auricular acupuncture.

Body acupuncture:

1) Case Group: (n=90) subjects with low-calorie diet and body acupuncture. The acupoints on their bodies include: Tianshu(St25), Zasanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). 2) Control group: (n=92) Subjects with low-calorie diet and shame body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins.

Each patient passed three treatment sessions per week each 20-30 minutes for 6 weeks. Body weight and level of the serum hsCRP measured pre and post treatment for all subjects. A statistically significant reduction in body weight (p<0.05) and level of the serum hsCRP (p<0.05) observed before and after treatment in the subjects who received auricular acupuncture and there was no significant change in the subjects who received body acupuncture there was less reduction in the level of serum hsCRP (p<0.05) and body weights (p<0.05) in the case group.

Key words: Body acupuncture, auricular acupuncture, Needling, hsCRP, Body weight, Obesity, Overweight, cardiovascular events.

Abstract No.119

The Effects of Body Acupuncture on Body Composition in Iranian Obese and Overweight Subjects

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Body acupuncture has been reported to reduce body weight, BMI, body and trunk fat mass in subjects in clinical practice. In the present study, we have evaluated the effects of body acupuncture on body composition including body weight, BMI, body and trunk fat mass in subjects of both genders divided into 2 groups as following; Case Group: (n=90, Female=67, Male=23) subjects with low-calorie diet and body acupuncture. Subjects were recruited from Nutrition Clinic, Ghaem hospital, Mashhad, Iran.The acupoints on their bodies include: Tianshu(St25), Zasanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). Control group: (n=92, Femal=68, Male=24) subjects with low-calorie diet and unreal body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins. Each patient received three treatment sessions per week each 20-30 minutes for 6 weeks. Both groups investigated for 6 weeks. Body weight, BMI, body fat mass, trunk fat mass, percent of body and trunk fat measured pre and post treatment for all subjects. It was observed significant reduction in body weight (p<0.05), BMI (p<0.05), body fat mass (p<0.05), trunk fat mass (p<0.05), body and trunk fat percentage (p<0.05) in both the case and control groups. It appears that needling not body acupuncture has beneficial effects on body composition in obese and overweight subjects.

Key words: Body acupuncture, body weight, BMI, body fat mass, trunk fat mass, percent of body fat, percent of trunk fat.

Abstract No.120

Structural change on dextran-hrp complex to increase the number of poly-HRP, conjugated to antibody

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Increasing the number of dextran-HRP complex that is carried by antibody, is a process that leads to high sensitive detection systems. These systems have obvious advantages like detecting any antigen in nanogram or so in methods that involve antibodies as detectors.To achieve this goal, amount of biotin conjugated to dextran-polyHRP complex is increased. Biotin is a molecule that connects polyHRP complex to biotinylated antibody by using streptavidine as a bridge. Doing this process, the complex is synthesized in several steps.Then, it is treated with lysine amino acid to block aldehyde groups that used to attach HRP molecules and also raise the linkers for biotin connection. So that the probability of having biotin molecules on the complex increases and according to this, either the number of antibodies in a supercomplex raises, which results in high avidity, that plays very important role in antigen-antibody detection. The consequences was checked with ELISA. High sensitive detection systems are applicable and valuable that are used in different methods like ELISA, Immunohistochemistry, Immunocytochemistry and western bloting.

Key words: Dextran-HRP; biotin; High sensitivity.

Abstract No.121

Fabrication of Cauliflower-liked DNAs by LAMP Technology

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Fabrication of DNA-based nanostructures is a novel approach in Nanobiotechnology. The most essential nanostructures for DNA nanofabrication are stem-loop DNAs, DNA nanojunctions, sticky-end DNAs, and periodic long DNAs. Since the nanostructures have become candidate in DNA computing and nanoelectronics, construction of such nanoarchitectures need using suitable methods via nucleic acid engineering. Isothermal amplification of nucleic acids (RNA/DNA) offer potential applications in the mass synthesis of DNA nanostructures. Here, we have employed loop-mediated isothermal amplification (LAMP) for synthesis of multistructured nucleic acids, so-named "cauliflower-liked DNAs". These DNAs were shaped via loop and bumper primers with strand-displacement and polymerization properties of Bst DNA polymerase. Deposition of the DNAs on highly ordered pyrolitic graphite (HOPG) and their imaging by scanning tunneling microscopy (STM) confirmed the creation of key DNA nanostructures, such as DNA-templated nanowires, stem-looped DNAs, and three-way DNA nanojunctions. Also, the LAMP capability for synthesizing periodic/repetitive DNAs has been proved by gel electrophoresis. Further studies will be carried out by Nano DSC-III in order to determine thermodynamical and structural characteristics of these nanoarchitectures.

Key words: DNA Nanotechnology, LAMP, Cauliflower-liked DNA, STM, Nano-DSCIII.

Abstract No.122

Harmalol-DNA Interaction Studied by Fourier Transform Infrared Spectroscopy

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DNA is a natural product, indeed the natural product of the paramount importance in understanding the mechanism of genetic processes, of cell growth and diffrentiation, of ageing and senescence. It is also a logical target for chemotherapy. Binding of peptides, small organic and inorganic molecules to DNA can interfere with the numerous processes, including transcription and replication in which DNA participates. Such interference can retard or prevent cell growth. Extensive chemical and biochemical studies have characterised a variety of molecules of this type and numerous antiviral, antibiotical. antiprotozoal and antitumour agents have been identified.

Small molecules that bind to DNA are extremely useful as biochemical tools for the visualization of DNA both in vitro and inside the cell. Additionally, the clinical significance of DNA-binding

compounds can hardly be overstated, as many anticancer regimens include a compound that binds to and/or modifies DNA.

Alkaloids are nitrogen-containing, low-molecular-weight compounds found in approximately 20% of all plants. Peganum harmala is a poisonous plant that grows in Central Asia, North Africa and Middle East Peganum harmala contains several alkaloids. called β-carbolines; harmine (7-methoxy-1-methyl-9H -pyrido[3,4-b] indole) harmaline (4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-b]indole), harmalol (1-methyl-4,9-dihydro-3H-b-carbolin-7-ol), and harman (1-methyl-9H-b-carboline). Peganum harmala is used as medicine for digestive system cancers. Harmine shows cytotoxicity against different kinds of cell lines.

This study was designed to examine the interactions of harmalol with calf thymus DNA in aqueous solution at physiological conditions, using constant DNA concentration (12.5mM) and various drug/DNA (phosphate) ratios of 1/240 to 1/5. FTIR spectroscopic method was used to determine the ligand binding modes. Spectroscopic evidence shows intercalation binding of harmine to DNA. Structural analysis showed major intercalation of harmalol into polynucleotides A-T
Ab initio calculations to study the π-stacking interactions between base-pair and ligand also to obtain oscillator strength (f) are used according to the results of UV-Visible spectroscopy.

Key words: alkaloid, β-carbolines, DNA, FTIR, intercalation, Peganum harmala, *ab initio* calculations.

Abstract No.123

Study of thermo-stable amylase from *Bacillus* isolate

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The α-amylases constitute a very diverse family of glycosyl hydrolases that cleave α1 4 linkages in amylose and related polymers. The 16 *Bacillus* strains were isolated from mud samples of warm springs in Sabalan Mountain. The isolates were inoculated on screening HII agar medium containing 1% starch. Flooding the overnight plates in iodine solution was carried out to visualizing starch hydrolysis. In order to preliminary evaluation of thermo-stability of the enzyme, the same media were incubated at 50°C for 72 h in a humidified condition. The amylolytic potential was estimated using the amylolytic ratio defined as the diameter of the hydrolyzation zone divided by the diameter of the producing colony. The 16 isolated strains were screened and the most productive strain showed significant amylolytic property as 4.88 mm. the Enzyme production was performed using HII liquid media and the activity of α-amylase was assayed spectrophotometically at OD_{575} by incubating of crude enzyme with soluble starch in glycine buffer. One unit of amylase activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar per minute. Optimum condition of enzyme activity was investigated using a range of pH from 7 to 11 and a range of temperature from 30 to 94 ℃ (Boiling temperature). The results imply that the Optimum pH and temperature of the enzyme activity were 10.5 and 70 ℃, respectively. The optimum production time was 72h and the optimum time of enzyme stability at 70℃ was 30 minutes. SDS-page analysis and the zymogram examinations were performed for more characterization of the enzyme. The K_m and V_{max} of the enzymes were determined. The enzyme purification and the protein structure identification are in progress.

Key words: α-amylase, thermostability, amylolytic potential, enzyme activity.

Abstract No.124

Molecular dynamics simulation fibrinogen in vigorous salt conditions:

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Fibrinogen is a 340 kDa soluble plasma glycoprotein, synthesized by the liver and converted to fibrin by a serine protease enzyme called thrombin in coagulation cascade. Fibrinogen, the principal protein of vertebrate blood clotting is a hexamer containing two sets of three different chains (α, β, and γ), linked to each other by disulfide bonds. The N-terminal sections of these three chains contain the cysteines that participate in the cross-linking of the chains. The C-terminal parts of the α, β and γ chains contain a domain of about 225 amino-acid residues, which can function as a molecular recognition unit. In fibrinogen this domain is implicated in protein-protein interactions. In the present work, we used molecular dynamic software to simulate the dynamic structure of fibrinogen to study the structural determinants in temperature induced conformation changes. The software used for MD calculations is Gromacs package *version 4.0.3*, both single and double precision variety, installed over UBUNTU Linux version 9.04 on a Intel® Core™2 Duo based PC at 2.53 GHz (P8700) with 2.8 GiB RAM. The base coordinate for fibrinogen is obtained from RCSB Protein Data

Bank, with PDB ID: 2ZZU. The protein were equilibrated in a cubic box with 7.42nm x 5.14nm x 9.072nm Energy minimization was carried out using steep integrator and F_{max} were chosen 1000 for 1000 step. Molecular dynamics with all-bond constrain for 200ps and finally no constrain were used to simulate done for up to 4ns. A careful analysis of fibrinogen denaturing trajectories revealed two transition states in fibrinogen denaturation. The first transition is appeared at 50°C and the second at 95°C. Solvent accessible surface area (SAS) and gyration radius changes at these two transition points are in accordance with previously postulated mechanism for fibrinogen behavior on denaturation. The principal findings of these experiments is that deferent portions of fibrinogen undergo thermal denaturation independently, and that repulsive electrostatic forces between positively charged groups are responsible to facilitate fibrinogen denaturation. However decrease in protein-protein and protein-solvent hydrogen bond after fluctuation points is in complete accordance with formation of hydrophobic denatured structure.

Key words: molecular dynamics, Fibrinogen, thermal denaturation.

Abstract No.125

Gold Nanoparticles Competitive ALT Immunosensor Based on Fluorescence Spectrometric Analysis

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Development of the immunosensors with variety of formats is increasingly finding applications in clinical diagnostics and biological researches. It this research study we developed a novel technique for the fabrication of fluorescence-based nanosensors with improved properties. The level of antigen in serum samples was determined based on a fluorescence absorption changes that resulted from aggregation of antibody-coated gold nanoparticles in the presence of antigen molecules in the analyte sample. Antiprotein ALT was used in these experiments as a model target analyte. Horseradish peroxidase (HRP) was used as the florescence immunelabel. The aggregation of commercially available 40-nm-sized Alt coated gold nanoparticles in the presence of ALT antigen has been studied. Aggregation of the gold nanoparticles results in an absorption change at 650 nm that is used to calibrate the amount of antigens. The effects of ALT protein-coated gold nanoparticles concentration on the sensitivity of the assay were investigated. The present study includes general considerations of the competitive immunoreaction protocols. Alanine aminotransferase (ALT) monoclonal antibody (anti-ALT-mAb) was successfully coated on gold nano particles. ALT antigen was detected by competitive immunoreactions based on microarrays of anti-ALT-mAb or antigen immobilized on nano Gold particles. For the Anti-ALT-mAb immobilized microarray the dynamic range is 1 pg/mL - 100 µg/ mL, and the detection limit is 1 pg/mL. The Gold Nanoparticles Competitive ALT immunosensor microarray provided much better technical performance when compared to other self-assembled monolayers (SAMs) sensor with immobilized-anti-ALT-mAb.

Key words: Gold nanoparticles, Immunosensor, Fluorescense spectroscopy.

Abstract No.126

Study of alkalin protease activity and characteristics from four native *Bacillus* sp

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Protease constitutes one of the most important groups of industrial enzymes. Among them, alkaline proteases are the largely studied group of enzymes because of their wide use in many industrial applications such as food, pharmaceutical, leather and detergent industries. In the present study, isolation, optimization of production and characterization of alkaline proteases were performed using several alkalophilic *Bacillus* which were isolated from hot mineral waters of Sabalan (North-west of Iran). Bacterial protease was directly determined using a skim milk agar plate during cultivation after a critical heat shock treatment. In order to enzyme production, the bacteria were inoculated in a production media containing 0.5% casein and supernatants of media used as crude enzyme. The total protein contents of the supernatants were evaluated by Bradford method. Enzyme activity was determined spectrophotometrically at 660nm using casein as a substrate. Protease activity was determined as released tyrosine from the supernatants according to Takami et al (1989). One unit of enzyme activity was defined as the amount of the enzyme resulting in the release of 1 µg of tyrosine per min under the reaction conditions. Optimum condition of enzyme activity was investigated using a range of pH from 7 to 11 and a range of temperature from 40 to 90 °C. The results imply that the Optimum pH and temperature of the enzyme activity were 9 and 70 °C, respectively.

A time course of enzyme activity assays, SDS-page analysis and K_m and V_{max} determination, the zymogram examinations were performed for more characterization of the enzyme.The enzyme purification and the protein structure identification are in progress.

Key words: Protease, enzyme activity, tyrosine.

Abstract No.127

Molecular dynamics study of lysozyme C in various conditions: temperature, pressure, salts, alcohol,

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Lysozyme with 129 residues and 14.7 KDa Molecular weight is an enzyme, EC 3.2.1.17 with hydrolase activity acts as antibacterial in human saliva. It is widely distributed in the human body including: tissues, exocrine secretions, and circulating cells and considered as an important component of innate immune system against bacteria. Lysozyme has a total of four intra molecular disulfide bonds, take part in tertiary structure stability. We have used molecular dynamics simulation to study the mechanism of thermal stability in lysozyme focusing on secondary structures elements. In the present work Gromacs Version 3.3.3, installed on ubuntu linux Version 8.10 package under ffgmx force field was used as simulation media. Lysozyme coordinate was obtained from RCSB protein data bank with PDB ID: 2W1M the protein was equilibrated in a cubic box with (4.94*4.32*5.07) nm dimensions. Energy minimization was carried out using steep integrator and fmax were chosen 1000 for 1000 step. Molecular dynamics with all-bond constrain for 200ps and then with no constrain were done for up to 4ns. Our findings show that lysozyme has transition temperature, 47-77°C. There is about 10% increase in gyration radius in this transition state, which is swelling like state of lysozyme. produced by thermal denaturation. The change in hydrodynamic radius is deduced from direct salt bridge analysis shows thermal denaturation promotes by increased positive-positive repulsive forces during with temperature. A solvent-protein and protein-protein hydrogen bond alteration caused by heating is not determinately, in protein denaturation. Hydrogen bonds break down take place upon melting temperature (67°C). The results show that the four disulfide bonds of lysozyme remain unchanged during simulation and even at higher temperatures over melting point and resist structural distortion.

Key words: molecular dynamics, Lysozyme, denaturation, structural distortion.

Abstract No.128

The interaction mode between DNA and salen-Co(III) N,N′-dipyridoxyl (1,4-butanediamine) Shiff-base complex

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Interaction of cationic metal complexes of Schiff bases, as a new agent in order to study anticancer characteristics and analytical application with DNA has been developed. The cationic metalocomplexes bind to DNA through a series of following interactions: (i) electrostatic forces, (ii) hydrophobic interactions with minor and major grooves, (iii) hydrogen bonding, and (iv) π-stacking interactions associated with the interaction of aromatic heterocyclic groups between the base pairs. It is valuable to understand the type of interactions involved between the complex and DNA sites.

In this work, we studied the interaction of new salen-co(III) of N,N′-dipyridoxyl (1,4-butanediamine) Schiff-base complex with DNA by melthing temperature, fluorescence spectrometry and gel electrophoresis techniques. This salen-Co(III) complex shows increase in melting temperature when bound to native calf thymus-DNA (CT-DNA). The intersection point of the binding isotherm indicated a binding site size of 3 bp per bound complex molecule in Tris–HCl buffer. Upon adding the new salen-Co(III), the electrophoretic mobility of pTZ57R DNA plasmid becomes slower for both super coiled and open circular forms without any structural changes in DNA. The experimental results showed that the salen-Co(III) complex bound to DNA by intercalative mode.

Key words: Schiff bases, anticancer, melting temperature, structural change.

Abstract No.129

A Survey on the Features influencing Cysteine Binding State

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Regarding the fact that the protein structure is principally encoded in its sequence, investigating the bonding state of cysteine has gained a great deal of attention due to its significance in the formation of protein structure. Due to lack of evident influence of free cysteines on the protein structure, it may be expected that only half-cystines convey encoded information. Nonetheless, the results obtained from the analysis of amino acid distribution in proximity of both states of cysteines explicitly indicated that perquisite information for inducing cysteine bonding state is present even in the flanking amino acid sequences of free cysteines.

Key words: Sequence analysis; Singlet local propensity; Doublet local propensity.

Abstract No.130

Wavelet Analysis of Features in Classification of Membrane Protein Types

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As a study with structural bioinformatics implications, the present research has been devoted to theoretically classify and discriminate the membrane proteins of different structural classes using the information hidden in their sequences. Due to experimental difficulties in determining the structure of membrane proteins by standard experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR), obtaining relationships between protein sequence and structure through theoretical approach is crucial. This is of great significance in the process of data mining in biological context, in which the basic data covert in protein sequences is scrutinized with the purpose of acquiring valuable sequence information with contribution to and related to structural features. In the present study, the mentioned information was acquired by applying the wavelet analysis to the sequences and consequently extracting several features, each of them revealing a proportion of the information content present in the sequence. The resultant features were made normalized and subsequently fed into a cascaded model developed in order to reduce the effect of the existing bias in the dataset, rising from the difference in size of the membrane protein classes. The devised model compensates the stated difficulty as much as possible and the results indicate an improvement in prediction accuracy of the model in comparison with similar works. This encourages us to further improve such models with the purpose of making use of them as complementary tools along with experimental studies.

Key words: Wavelet, membrane protein, structural bioinformatics.

Abstract No.131

FT-IR Vibrational Analysis of the Complexes composed of p-t-Calix[4]arene and Hydrophilic α-Amino Acids: A Comparative Study of Theoretical and Experimental Approaches

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Recent progress in computational ability facilitates the study of the relatively large and complicated supramolecular systems. The ability of calixarene-based molecules to form complexes with amino acids has been the central topic of numerous studies. In the present research, we studied the potential complex formation between calix[4]arene-based molecules and hydrophilic amino acids, including Arginine, Aspartic acid, Glutamic acid, Histidine, and Lysine using Fourier transform infrared (FT-IR) spectroscopy. Band frequencies and intensities in the IR spectra of the above mentioned amino acids and calix[4]arene complexes have been calculated. *Ab initio* calculations at HF/6-311G (d, p) level gave vibrational frequencies and infrared intensities for hydrophilic amino acids and calixarene. The geometry optimization has been performed on the basis of C2 symmetry frame of p-tert-butyl-calix[4]arene as a calculation input since it contains no imaginary vibrational frequencies. Furthermore, the experimental IR spectra of the p-tert-butyl-calix[4]arene were measured and assigned to the theoretical geometric parameters and force constants. The obtained characteristic bands indicate desirable concordance between theoretical and practical results.

Key words: supramolecular systems, potential complex formation.

Abstract No.132

Investigation of structural properties of firefly luciferase upon mutations in flexible regions

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Firefly Luciferase (EC. 1.13.12.7) from *Photinus pyralis* is a single polypeptide chain, which catalyzes the light emission reaction, in the presence of luciferin, Mg^{+2}-ATP and molecular oxygen to produce yellow-green light. Firefly luciferase is widely used as a reporter gene in cell and molecular biology. Crystal structure of luciferase reveals that protein folded into two compact large N-terminal and C-terminal domains. Its pronounced susceptibility to proteolytic degradation is one of its properties which reduce its intracellular half-life. Previous studies, using limited trypsinolysis of *P. pyralis* luciferase indicated six cleavage sites on two flexible regions ; 206-220 including (K^{206}, R^{213} and R^{218}) and 329-341 including (K^{329}, R^{330} and R^{337}) on N-terminal domain. In order to generate more stable luciferase against trypsin digestion, we substituted R^{213} with M and E and R^{337} with Q. In general, all mutations show resistance against trypsin hydrolysis which accompanied by structural changes which revealed Spectroscopic studies.

Key words: Luciferase, Protease stability, Structural properties, trypsin.

Abstract No.133

Characterization of two forms of an α-amylase isolated from *Bacillus* sp. KRA2.

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Bacillus sp. KRA2, an aerobic bacterium producing an α-amylase, has been isolated. The complete amylase gene and the fragment which encodes the mature enzyme (amylase gene without the N- and C-terminal fragments) were cloned and expressed in *Escherichia coli* BL-21. The recombinant proteins were purified by Ni-NTA column chromatography and used for primary biochemical characterization. The kinetic parameters such as K_m, V_{max}, k_{cat} and k_{cat}/K_m of the mentioned enzymes were determined in presence of both starch and EPS as substrates. Both enzymes followed Michaelis-Menten kinetics for these substrates. When starch was used as substrate, the immature enzyme showed lower K_m or higher affinity for substrates. The results also showed that the catalytic efficiency of both forms of the enzyme is similar. Using starch as substrate, the effect of various pH values on α-amylase activity was studied at 50 °C. The purified mature and immature BKA were active between pH values 4 and 8 with an optimum around pH 6. The thermal stability of the purified enzyme was examined by incubation of BKA at six times up to 30 min in absence of Ca^{2+} at different temperatures (50- 70 °C). Loss of activity was observed at 60, 65, and 70 °C. At 60 °C the enzyme retained 60% of its original activity. Calculation of K_{inact} for mature and immature enzyme showed the same values. The product content of both forms of BKA on starch and pullulan has been analyzed. When starch was used as substrate, the major hydrolysis product of mature and immature BKA was Glucose and maltose while it was not able to hydrolyze pullulan.

Key words: α-amylase, kinetic parameters, stability, hydrolysis product.

Abstract No.134

Characterization of a mutagenised thermal- resistant α-amylase from *Bacillus megaterium* WHO

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Site-directed mutagenesis of an α-amylase isolated from *Bacillus megaterium* WHO has been performed to evaluate the roles of the calcium binding site residues in enzyme thermostability. Mutations of Asn-75, Ser-76, and His-77 that were identical to their thermophilic counterpart residues in the second calcium binding site resulted in an increase in thermostability. All mutants retained their hydrolytic activity. In S76P and H77E, the K_m for starch was reduced and the overall activity (k_{cat}/K_m) was increased. In the presence of calcium, conversion of His-77 to Glu resulted in a 4-fold enhancement in enzyme half life as well as an upward shift of 9 °C in T_{50}, when compared to the wild type. Further analysis suggested that H77E as the most stable mutant increased the affinity of the enzyme for calcium ion and the optimum temperature was 5 °C higher than the wild type. In this study, we present and discuss about a point mutation

that converts a mesophilic α-amylase into a stable enzyme without losing its catalytic power at moderate temperatures.

Key words: α-amylase, *Bacillus megaterium*, calcium binding site, thermostability, site-directed mutagenesis.

Abstract No.135

Improved activity and stability in organic solvents by increased active site polarity of a metalloprotease

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Proteases can be utilized to synthesize amino acid esters and peptides in hydrophilic organic solvents. Salinivibrio zinc-metalloprotease (SVP) is an enzyme which was isolated from *Salinivibrio proteolyticus*, a moderately halophilic species from a hypersaline lake in Iran. A195E and G203D variants were constructed to increase polarity near the active site of SVP to preserve the hydration layer against organic solvents (DMF, methanol, isopropanol and n-propanol). In aqueous media, K_m of these mutants decreased in while, k_{cat} of these mutants increased. Results showed that, C_{50} values of A195E variant not only increased about 5 and 6% in the presence of DMF and methanol but also increased about 3% in the presence of isopropanol and n-propanol. In the case of G203D variant, C_{50} Values were slightly lower than A195E in the presence of these organic solvents. The irreversible thermoinactivation rate (k_i) for A195E is about 60 and 130 (10^{-3} min^{-1}) in the presence of DMF and n-propanol, respectively**,** in while k_i of SVP is about 90 and 190 (10^{-3} min^{-1}) in the same condition. Although, G203D variant show the same k_i as A195E in the presence of methanol and isopropanol, but it shows 70 and 160 (10^{-3} min^{-1}) in the presence of DMF and n-propanol. In overall, these results indicate that increase active site polarity can improve activity and stability of SVP in the presence of organic solvents.

Key words: organic solvent, site directed mutagenesis, zinc-metalloprotease, activity and stability.

Abstract No.136

The study of biological effects of 5-Br-meso-VO-Salen complex on McCoy cell line

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The presence of vanadium in biological systems, its possible physiological roles, insulin-enhancing action and anticancer activity have driven a considerable amount of research. During the last few years, several studies on vanadium have shown its promising ability to inhibit cancers of liver, Lung, breast, gastrointestinal tract and meny human cancer cell line. The mineral complex of 5-Br-meso-VO-Salen was synthesized and antioxidant activity of the complex was evaluated using DPPH reduction. The effects of the concentration gradients of the complex were evaluated on McCoy cells for 8h, 16h and 24h. The cytotoxicity and viability were evaluted by tripan blue staining and MTT reduction assay, respectively. These results depicted that this complex with RC_{50} of 0.829 mg/ml is a significant antioxidant. Study of survival fraction for 8h, 16h and 24h showed IC_{50} of 336.394, 274.704 and 197.260 µg/ml and tripan blue result for 8h and 16h showed CC_{50} of 259.87 and 179.229 µg/ml respect to incubation time. The complex inhibits the prolifiration of McCoy cells in a time and concentration dependent manner. Treatmet of the cells using 100 and 400 µg/ml of the complex was efficiently led to DNA fragmentation. The DNA fragmentation is a crucial sign of apoptosis induction. Morphological studies showed that the treated cells became condensed, round and the cell membrane was irregular.

Key words: anticancer, antioxidant, DNA fragmnetation, apoptosis.

Abstract No.137

Taken a Soluble Luciferin-Regenerating Enzyme, Strategies and Failures

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During the cold light emission in fireflies, luciferin-regenerating enzyme (LRE) – a physico-chemical extremely insoluble protein- plays an important role in the recycling of oxyluciferin into luciferin. Although LRE is interested both for its industrial potential and biochemical

features, high yield expression of LRE has not been reported. Here we report different strategies for improving the solubility of LRE in *E. coli* including; high throughput optimization of LRE expression, chemical and biological foldase co-expression and fusion-technology based methods. The successful soluble expression was obtained with a construct containing a NusA tag which clearly alters the solubility physico-chemical index (*CV-CV*) of LRE.

Key words: firefly, Luciferase, luciferin-regenerating enzyme (LRE), Over expression.

Abstract No.138

Inhibition and Activation Effect of Metal Co-factors on Human Inosine Triphosphate Pyrophosphatase

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Many enzymes require additional co-factors to catalyse reactions effectively. The human catalyzing enzyme of inosine triphosphate is named human inosine triphosphate pyrophosphatase (hITPase). In the presence of divalent ions e.g., Mg^{2+}, Mn^{2+} and Ni^{2+}, this enzyme shows activity. However, previous studies have shown that hITPase activity is inhibited by other bivalent cations such as Cd^{2+}, Co^{2+} and Ca^{2+}. On the other hand, activity of an enzyme is affected by conformational changes of its structure; hence, hITPase is studied by circular dichroism spectroscopy and fluorescence method under activating and inhibiting conditions. In a case study, we selected magnesium as activator, and calcium as inhibitor. Far UV-CD spectra exhibit increase of hITPase compactness in the presence of magnesium solution, whilst with calcium ions a decrease is noticed. Intrinsic fluorescence results revealed decreasing intensity in the presence of two cations, and ANS fluorescence presented an increase in both cations. It can be concluded that Mg^{2+} induces a molten-globule like intermediate in hITPase; while calcium ions might play role in the denaturation of hITPase.

Key words: hITPase, inhibator, activator, molten globule like intermediate, fluorescence, CD.

Abstract No.139

The First Study of the Secondary Structure of Hepatitis C Virus F Protein Using Circular Dichroism Spectroscopy

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Hepatitis C virus (HCV) is a highly mutable RNA virus with a high propensity for chronic infection. Recently, different groups of investigators have reported that there is another protein with a different open reading frame from HCV core protein. Until now, there is no report on the secondary structure of HCV-F protein, therefore determining the structure of this protein is of high interest. Circular dichroism (CD) is being increasingly recognised as a valuable technique for examining the structure of proteins. The aim of this study was to investigate the secondary structure of HCV-F protein for obtaining relationship between structure and stability.F gene was produce from Core gene by introducing a deletion mutation. F gene was cloned in pET28a(+) expression vector and verified by sequencing. The constructed plasmid was transfected into Ecoli BL21.The expression of F protein was induced by IPTG and detected by SDS-PAGE. Qiagen Ni-nitrilotriacetic acid agarose was used for protein purification. Freshly purified protein samples were used for CD analyses. We have studied the secondary structure of F protein using far-UV CD and the stability of the protein was estimated for the first time in the world.With introduction of a deletion in the designed primers, we were able to produce F construct and then express it into BL21 by pET28a(+). Expression of F protein was confirmed by SDS-PAGE and purification was done by Qiagen Ni-nitrilotriacetic acid agarose. CD spectroscopy indicated the secondary structure of HCV-F protein. It seems that structure of this protein is relatively unstable. With introduction of a deletion in the designed primers, we were able to produce F construct form core gene of HCV and then express it into BL21 by pET28a(+). Estimation of the secondary structure of F protein by CD showed that determination of F protein structure would be valuable to obtain appropriate method for stabilization of this proein.

Key words: Hepatitis C, Circular Dichroism, protein stabilization.

Abstract No.140

Preparation and characterization of a novel protein conjugated complex as an anticancer agent

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Oxovanadium shift base complex has an anticancer and catalytic activity. To improve its solubility, we attached it to BSA and cytochrome C. In this study, we covalently attached an anticancer oxovanadium shift base complex to BSA and cytochrome C, which has been verified by FTIR spectroscopy. Furthermore, secondary structures of products were evaluated by analysis of CD and IR spectra. different concentrations of anticancer protein–complex conjugates were delivered to cancerous cell lines. Cytotoxicity of oxovanadium shift base complex, BSA-oxovanadium shift base complex and cytochrome C–oxovanadium shift base complex were determined. For determining cell death ratio, we performed MTT assay. Cytochrome C naturally presents in the space between outer membrane and inter membrane of mitochondria, which during programmed cell death is released into the cytosol. Presence of cytochrome C in the cytosol initiates formation of apoptosome, which causes cysteine proteases activation and results in programmed cell death. According to our results and above statements, cytochrome C has a key role in apoptosis initiation and we anticipate that cytochrome C-oxovanadium shift base complex conjugate leads to more cell death, rather than other conjugates.

Key words: oxovanadium, cytochrome C, anticancer.

Abstract No.141

Prediction of Protein Thermostability using Neural Networks fed by sequential and secondary structural factors

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Sequential and secondary structural factors were used to develop quantitative structure-property relationship models and predict the Tm of relatively small globular proteins. Many quantitative descriptors (n=43) were generated for a set of 83 proteins with known sequence, crystal structure and Tm. The descriptors were chosen through feature selection methods such as Genetic Algorithms and systematic search. Different sets of sequential and secondary structural factors were tested using neural networks to obtain the best model with the optimum predictive capability. The best model was found to be a 5-3-1 Neural Network topology which was fed with five secondary structural descriptors. This model resulted in training-set a root mean square error (RMSE) equal of 7.26 and prediction-set RMSE of 16.26 and coefficient of determination of R^2 equal of 0.83 which showed a good fitness for our data to the established model.

Key words: Thermostability, Genetic algorithm, Neural Network, Secondary structure.

Abstract No.142

Recognition Protein-RNA Interaction interfaces via the Voronoi Diagram

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Ribonucleic acid (RNA) molecules perform essential and diverse functions in the cell. RNA–protein interactions are vitally important in a wide range of biological processes, including regulation of gene expression, protein synthesis, replication and assembly of many viruses. RNA–protein interactions studies are useful to design and production of drugs.All macromolecule consist of thousands atoms. The atoms of macromolecule can be shown as three-dimensional balls by Van der Waals radius from a geometric point of view. A geometric model of a protein is the union of spherical balls. In 1995, Varshney et al. defined an interface between macromolecules geometrically. The surface is as the set of points that the distance between a given point of the surface and each macromolecule will be equal or less than the van der Waals radius of a solvent molecule. In this study, we compute interaction interface using the weighted Voronoi faces of atoms which is dual to a Delaunay edge. Also it enables filtering on Delaunay edge. Adding this filtering, have been used for keep only those Voronoi faces that is less distance from radius of a solvent molecule .The algorithm is implemented to a data bank of RNA–protein complexes in five different

groups, taken from the protein Data Bank (PDB, Berman et al. 2002) and provide interface surface properties and compared between five these different groups. Obtained results of this study is in agreement to the results from experiments.

Key words: Interaction interface, RNA–protein interaction, Voronoi diagram, Protein Data Bank.

Abstract No.143

Molecular dynamic and docking of deoxyguanosine kinase inhibitors:

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Deoxyguanosine kinase (EC 2.7.1.113) is a transferases enzyme that catalyzes the following reaction:

ATP + deoxyguanosine $\rightarrow$ ADP + dGMP

This is the transfer of phosphate group from ATP to deoxyguanosine compound. The systematic name of this enzyme class is ATP: deoxyguanosine 5'-phosphotransferase. This enzyme participates in purine metabolism and is constitutively expressed in mitochondria. Deficiency of deoxyguanosine kinase causes early-onset; hepato-cerebral mitochondrial DNA (mtDNA) depletion syndrome. Deoxy nucleotides e.g. dGTP dITP dGDP dGMP dGDP dIMP dTDP, with previously determind inhibitory constants, inhibit the enzyme activity and cause the same consequences as enzyme deficiency. In the present work using molecular dynamic and docking methods we decided to study the inhibition mechanism for these inhibitors. Hyperchem software and Dundee PRODRG2 Server were used for constructing inhibitors coordinates and topologies. Hex software with the following parameters: correlation type: Shape only, FFT Mode: 3D fast lite, Grid Dimension: 0.6, Receptor range: 180, Ligand Range: 180, Twist range: 360 and Distance Range: 40 and Arguslab software were used for docking purposes. The docked complexes were solvated in cubic box, neutralized with counter ions, and subjected to MD-simulation with Amber-03 force field on Gromacs software version 4.0.3. Energy minimization was carried out using steep integrator, fmax, 1000 and 1000 step. All-bond constrained MD at 310K without pressure coupling was done for 100ps. Our results show first: that some inhibitors e.g. dGTP, dIMP, dGMP, and dTMP act by competitive mechanisms, and their inhibitory potency could be simply interpreted by their binding energies. Second: two other inhibitors i.e. dITP and dGDP act as by uncompetitive manner and bind to an inhibitory binding site comprises of LYS-2, LYS-3, LYS-79, GLU-129, and ASP-131 amino acid residues. However their inhibitory potency also interpreted by their binding energy.

Key words: molecular dynamics, docking, inhibitory potency.

Abstract No.144

Molecular dynamics study of coagulation factor VII - tissue factor complex in various conditions

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Factor VII (FVII) is one of coagulation cascade proteins. It is a serine protease enzyme with EC 3.4.21.21. FVII is synthesized in the liver and circulates in the blood as inactive zymogens. The main physiological role of factor VII is to initiate the process of coagulation in conjunction with tissue factor (TF). Upon vascular injury and the presence of TF, FVII is complexed to TF and is cleaved to its active form, FVIIa. The FVIIa/TF complex then cleaves and activates both factors X and IX to initiate the coagulation process. The active form of the enzyme, comprising a light chain (152 residues) and a heavy chain (254 residues) linked by a disulfide bond. In the present work, Gromacs version 4.0.3, installed on UBUNTU linux version 9.04 packages was used as simulation media. FVIIa coordinate was obtained from RCSB Protein Data Bank with PDB ID: 2ZZU. The protein were equilibrated in a cubic box with $5.21\times4.25\times9.35$ nm. Energy minimization was carried out using steep integrator and Fmax were chosen 1000 for 1000 step. Molecular dynamics with all-bond constrain for 200ps and finally no constrain have been applied to simulate done for up to 4ns. Our result show the melting temperature of FVII is about 60°C with good approximation with experimental melting temperature of 58.1°C. Unlike pervious reports our studies show a simple transition state FVII unfolding. Protein-protein hydrogen bond changes during denaturation shows a linear decrease in hydrogen bond count and is concomitant with same changes in protein-solvent hydrogen bond. Solvent accessible area and gyration radius of transition state also show a simple two state unfolding pattern for FVII.

Key words: molecular dynamics, coagulation cascade protein, two state unfolding.

Abstract No.145

Antibacterial and Antifungal Properties of Several Cyclic and Linear Dithiocarbamato Zn(II) Complexes

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Bacterial and fungal infections have increased in the last years affecting mainly those patients immuno-compromised. The complexes and dithiocarbamates ligands are known for their biological interest as antifungal, antibacterial and biocide agents. It has been established that dithiocarbamates already play an important role in medicine. For example, the diethyldithiocarbamate anion ($-S_2CNEt_2$) has had extensive clinical use in the treatment of microbial diseases. Therefore, the coordination of Zn(II) with dithiocarbamates would enhance such biological aspects.

In this study, a series of eight dithiocarbamate as sodium salts of formula R-NHCSSNa, LNa (where R is methyl-, ethyl-, propyl-, nonyl- and L is diphenyl-, piperidine- and morpholine-dithiocarbamate) and piperazine-bis(dithiocarbamate) sodium salts were assayed for their antibacterial (S.aureus, E.fecalis, B.cereus, E.coli, P.aeroginosa, S.thyphi, Y.entroculitica, P.mirabilis) and antifungal (A.niger, C.albicans) activities by Paper Disk Diffusion method. The microorganisms *tested* in this study, can infect the oral and vaginal cavities, skin and more seriously essential organs. Standard drugs such as Amoxicillin, Chloramphenicol and ketoconazole were used for comparison purpose. In addition to, activity dependence of different compound's structure, concentration and type of microbs were studied. The studies indicated the antifungal and antibacterial activities of Zinc complexes are better than dithiocarbamate sodium salts. The highest antibacterial activity was found for Bis(nonyldithiocarbamato)zinc(II), Bis(piperidinedithiocarbamato)zinc(II) and Bis(morpholinedithiocarbamato)zinc(II) against E.coli, Y.entroculitica and P.mirabilis bacteries.

The antifungal activities against A.niger and C.albicans were investigated. The results showed that Ethyldithiocarbamate sodium salt showed the most favorable antifungal activity against C.albicans and among dithiocarbamate Zn(II) complexes tested, Bis(ethyldithiocarbamato)zinc(II), Bis(piperidinedithiocarbamato)zinc(II) and Bis(morpholinedithiocarbamato)zinc(II) showed very strong antifungal activity against C.albicans fungi.

In comparison, the above two series of compounds showed better antifungal activity than antibacterial.

Key words: dithiocarbamate Zn(II) complexes, antibacterial, antifungal, diseases.

Abstract No.146

Interactions of sodium selenat and sodium selenite with DNA

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Selenium has long been of interest for possible cancer chemo preventive effects, largely assumed to be due to its role in the anti-oxidant enzyme glutathione peroxidase. Recent studies suggest that selenium compounds may increase oxidative stress as well as relieve it. Sodium selenite has been found to be an effective prophylactic treatment for erysipelas (a Streptococcal infection of the skin) in patients with secondary lymphoedema after cancer surgery. The anticancer and antiviral effects of these natural products are attributed to their potential biomedical applications. Sodium selenate and sodium selenite bindings to DNA and RNA are not fully investigated. This study was designed to examine the interactions of one of the most important kinds of antioxidants, called sodium selenate and sodium selenite with calf-thymus DNA in aqueous solution at physiological conditions, using constant DNA concentration (0.51 mM) and various concentration of drudge. sodium selenate and sodium selenite can stimulate the central nervous system by inhibiting the metabolisms of amine neurotransmitters or by direct interaction with specific receptor. UV-visible spectroscopic methods were used to determine the ligand binding modes, the binding constant and the stability of sodium selenate and sodium selenite-DNA complexes in aqueous solution. Spectroscopic showed major binding of sodium selenate and sodium selenite to DNA with overall binding constants of

$K_{\text{sodium selenate}} = 5.2 \times 10^4\ M^{-1}$, where K is the binding constant.

$K_{\text{sodium selenite}} = 1.5 \times 10^3\ M^{-1}$, where K is the binding constant

Key words: DNA, sodium selenate, sodium selenite, UV-visible spectroscopic.

Abstract No.147

Ethanol biosensor based on the alcohol dehydrogenase immobilized on carbon nanotubes adsorbed on methylene green nano-layer

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Alcohols are inducible factors in various diseases such as cardiovascular disorders and liver diseases. Here we have developed a novel biosensor by immobilization of yeast alcohol dehydrogenase (ADH) on the specific nano-substrate. At first a poly methylene green (PMG) nanolayer was electropolymerized on the glassy carbon electrode by applying potentials between 1.0 to -0.6 V for 15 cycles (PMG/GC electrode). Then a mixture (1:1) of carboxylic-functionalized multi wall carbon nanotubes (MWCNT) and poly (dimethyldiallylammonium chloride) (PDDA) was adsorbed onto the PMG/GC electrode. In the next step ADH was assembled on the PDDA/MWCNT/PMG/GC electrode via electrostatic interaction between enzyme and PDDA. Finally, Nafion polymer as a holder was dropped onto modified electrode surface and permitted to dry at room temperature.

The activity of immobilized ADH was investigated in presence of ethanol by cyclic voltammetery and amperometry methods. As an electrochemical property, resulted operational stability was more than 97% after 90 cycles in cyclic voltammetery method. Besed on amperometry experiment, detection limit and linear range response of ADH modified electrode toward ethanol was 10^{-4} and 10^{-7}-10^{-2} M, respectively. These results showed that this immobilization procedure for ADH onto nano-substrate modified electrode leads to construct an excellent biosensor toward ethanol detection.

Key words: alcohol dehydrogenase, carbon nanotubes, methylene green, ethanol.

Abstract No.148

An Illustration of the dynamical similarities in the catalytic site of the two analogues of Serine Protease families

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Each enzymatic reaction on average consists of 4.2 stages and 2.7 intermediates. The structure of catalytic site, Appropriate for each stage, is necessary to change for stabilizing transition state intermediate. This Structural plasticity is result of the internal motion in the catalytic site. By Using molecular dynamics simulation methods, the ensemble of Consecutive structures is produced which useful for tracking and understanding the effect of internal motion in active site .mechanistically convergent enzymes have same active site but Completely different structure. By accepting the role of internal motions in the enzymatic reaction, it is expected to observe similar active site dynamics for these enzymes group.

To clarify this assumption, two mechanistically analogues from serine protease family, Chymotrypsin and Prolyl Oligopeptidase, were studied. Hydrogen bond network in the active site of serine protease family plays an important role in reaction and stabilization of the transition state intermediates .we performed MD simulation in two different conditions, with and without substrate for each enzyme. Analysing the change of the distance between acceptor and donor, in hydrogen bond network of catalytic site, shows a similar meaningful pattern between two enzymes in condition that both have the substrate and neither have the substrate. This investigation provides an approach to drive similar patterns of correlated motions in the active site of other functionally related enzymes.

Key words: protease, structural plasticity, stabilization, MD simulation.

Abstract No.149

Rational domain-engineering strategy; an approach to achieve more applicable enzymes

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Preferential chemical modification of non-catalytic domain of endoinulinase (EC 3.2.1.7) is presented as a novel chemical modification strategy. This approach has been used to improve the thermostability of this model enzyme without any adverse effects on the enzyme activity as an important task in the industrial application. We used pyridoxal 5′-phosphate (PLP) to modify the more accessible lysine residues at the surface of endoinulinase followed by a necessary reduction step by ascorbate. Melting temperature (T_m) of the modified enzyme was increased from 64.1°C to 72.2°C and comparative thermal stability studies have confirmed stabilization through increasing of the half-life ($t_{1/2}$) of the enzyme after doing PLP-modification/ascorbate reduction.

Key words: endoinulinase, pyridoxal 5′-phosphate, accessible surface area, ascorbic acid, chemical modification.

Abstract No.150

Enhanced expression of a recombinant bacterial laccase at low temperature and microaerobic conditions: purification and biochemical characterization

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The existence of environmental problems caused by industrial oxidation reactions has persuaded researchers to employ alternative biological systems, such as enzymatic oxidation to avoid such problems. Laccases (benzenediol oxygen oxidoreductase; EC 1.10.3.2) are copper-containing enzymes that oxidize a variety of aromatic compounds are highly interesting for biotechnological and industrial applications. Laccases are widespread among fungi, plants, insects and bacteria. Among them, fungal laccases have been currently used in biotechnological applications. But the presence of laccases also in bacterial sources has interested researchers to isolate and characterize other members of this family. This work focused on cloning, expression and characterization bacterial laccase, isolated from *Bacillus* sp. HR03 which had been identified from the Iranian micro flora. Laccase gene expressed under microaerobic condition and decreased temperature in order to obtain high amounts of soluble protein. The laccase was purified and its biochemical properties were investigated using three common laccase substrates,
2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), syringaldazine (SGZ) and 2, 6-dimethoxyphenol (2, 6-DMP). K_M and k_{cat} were calculated 252 μM and 1.3×10^{-2} $s^{-1}$for ABTS, 108 μM and $2.7\times10^{-4}s^{-1}$ for 2, 6-DMP and 3 μM and $2\times10^{-2}s^{-1}$ for SGZ. The laccase have shown thermal activation with increasing temperature. We also determined the tyrosinase activity using L-dopa as a substrate. The K_M and k_{cat} of cathecolase activity were measured 5.7 mM and 194 s^{-1}, respectively.

Key words: bacterial Laccase, tyrosine activity, thermal activation.

Abstract No.151

Preparation, Characterization and in vitro Evaluation of a Novel Peptidic antitumor delivery system based on PLGA Nanoparticles

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In this presentation our aim was development of a drug delivery system as a peptidic antitumor agent based on PLGA nanoparticles. Surfactin is a very powerful nonribosomal bacterial acidic cyclic lipoheptapeptide containing a beta-hydroxy fatty acid and L, D amino acid residues. Although various biological activities such as antitumor, antimicrobial, antifungal, antiviral, inhibition of fibrin clot formation and hemolytic functions have been reported for surfactin but mechanisms of its actions are unknown. The antitumor activity of surfactin was investigated in conjugate with PLGA, a biodegradable and biocompatible polyester, on MKN-45 (Human gastric carcinoma cell

line), Hela and McCoy cell lines. At first we conjugated surfactin with PLGA which confirmed by FTIR spectroscopy. Furthermore, conformational biocompatibility verified via deconvolution of vibrational spectra of surfactin-PLGA nanoparticles and surfatin in amideI region. Comparison of them shows negligible change in secondary structure for surfactin in conjugated state. Treatment of cell lines by surfactin-PLGA conjugate induced apoptotis. The typical apoptotic morphological change was confirmed via Acridin orange/Ethediom bromide staining and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetraziolium Bromide (MTT) assay. Surfactin-PLGA conjugate exhibited significant antiproliferative effects on cancerous cell lines.

Key words: Surfactin, Amide I, PLGA, Nanoparticles, Antitumor.

Abstract No.152

Remarkable Improvements of a Neutral Protease Activity and Stability Share the Same Structural Origins

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Thermostabilizing an enzyme while improving its activity may be difficult with respect to general trade off relation between stability and function. Therefore, understanding a mechanism for parallel improvements of stability and function of proteins would be a great interest in biotechnology. Thermolysin-like proteases are special targets in protein engineering studies because they are widely used for transesterification of sucrose, synthesis of peptides and utilized for the synthesis of aspartame. Here, we designed surface located mutations that individually and combinedly are able to promote the activity and stability of a neutral protease from *Salinovibrio proteolyticus*. Combination of four beneficial mutations resulted in 25-fold more activity at 60°C and 50-fold at their optimum temperatures. Quadruple-mutant Ca-dependently acquired 15°C higher temperature optimum while it increased low temperature activity in the absence of calcium. As a result of higher affinity toward Ca^{2+}, half-life of quadruple mutant increased 20, 24, 6 and 5-fold at 60, 65, 70 and 75°C, respectively. Theoretical and experimental studies have provided evidences that the hinge-bending angle is determinant for the efficiency of substrate binding in neutral proteases. Finally we conclude that the extended surface region between residues 187-228, which involves three out of four beneficial mutations, influences the hinge angle which is determinant for catalysis and also involves the structural calcium which is critical for stability.

Key words: Neutral Proteases, surface regions, hinge angle, *Salinovibrio proteolyticus* protease, thermostability.

Abstract No.153

Cloning, gene expression and hinge-bending analysis of the Elastase of *Pseudomonas aeruginosa*

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In the present work, enzymatic activity of the recombinant Elastase of *Pseudomonas aeruginosa* (PAE) was studied in both kinetic and bioinformatics aspects compared to the other well known neutral protease, thermolysin of *Bacillus thermoproteolyticus* (TLN). Following the biochemical tests on bacterium (strain PTCC 1430), genomic DNA was extracted, the nucleotide sequence of PAE gene was extracted by PCR, and then the full length gene encoding preproelastase was subcloned into pET-21a(+) and transformed into *Escherichia coli* BL21 (DE3). The nucleotide sequence analysis of the gene revealed a single open reading frame (ORF) of 1494 bp encoding 498 amino acids. Following induction by IPTG, active enzyme was found within cells. Catalytic constants (K_m and k_{cat}) of purified PAE have shown meaningful differences compared to that of TLN. Previous investigations revealed that neutral proteases undergo hinge-bending during catalysis, and also it was found that catalytic activity might strikingly improve by the reduction of hinge angle i.e. closure of the active site cleft. To address kinetic variations from a structural point of view, hinge-bending angle between two domains were compared between PAE and TLN. Relative to TLN, PAE has a hinge-bending displacement of 11º. The extended flexible surface region between amino acids 179-221 in PAE correspond to 181-229 in TLN occurs in proximity of the active site. In this region, PAE contains single bound calcium but TLN binds three calcium ions. Our results propose that kinetic variations between PAE and TLN are, at least in part, a result of hinge-angle displacement between N- and C-terminal domains.

Key words: Elastase, hinge bending, cloning, gene expression.

Abstract No.154

Involvement of intermolecular disulfide bonds on artemin structure and function

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Encysted embryos of *Artemia urmiana* are among the most stress-resistant of multicellular eukaryotes due in part to massive amount of Cys rich protein artemin. The abundance of Cysteines and their intramolecular spatial distribution suggest that artemin protects embryos against oxidative damage and/or its function is redox regulated. Thermal stability of artemin allowed its purification using ammonium sulfate fractionation followed by incubation at 70°C for 14 min and ion-exchange chromatography. The purified artemin was present primarily in the oligomeric form with a little amount of monomeric form. In this study, free thiols and disulfide bonds in artemin was counted by Ellman's method. The data showed 9 free thiols (7 buried and 2 exposed free SH groups) while only one of them is involved in disulfide bond formation per a monomer of artemin. This spectroscopic result was confirmed by theoretical analysis on structural model of artemin, and appearance of an additional band about 50 kDa in non-reducing SDS-PAGE further verified the presence of inter-subunit disulfide bond, maybe between every two neighbour monomers. Our previous study revealed that artemin enhanced the efficiency of refolding and reactivation of denatured horse radish peroxidase and chaperone-like activity of artemin decrease in the presence of reducing agents, DTE and GSH/GSSG. Intrinsic and extrinsic fluorescence measurements in reducing and non-reducing conditions indicated indicated that tertiary structure of artemin altered and its surface hydrophobicity diminished in the presence of reducing agents.

Key words: Artemin, Cysteine, Disulfide bond, Chaperone, Fluorescence.

Abstract No.155

Cloning and Sequencing of the Calcium Activated Photoprotein, mnemiopsin: Sequence Comparison and Structural Analysis by Molecular Modeling

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Six photoproteins have so far been isolated and characterized from organisms in the phyla Cnidaria and Ctenophora. These include aequorin, halistaurin, obelin, mnemiopsin, berovin, and phialidin. All of these photoproteins are known to emit light on reacting calcium. Furthermore, the photoproteins are relatively small in size (21.4-27.5 kDa) and they are thought to contain a common organic substrate, coelenterazine, and molecular oxygen bound in the form of a complex. The luminescent system of the ctenophore *Mnemiopsis leidyi* involves a photoprotein called mnemiopsin. Similarly to aequorin, mnemiopsin emits light in the presence of calcium (λmax=485nm). Mnemiopsin was initially extracted in 1974 and two forms of it, M1 and M2, were characterized. However, no sequence for the cDNA encoding this protein has so far been reported. In the present work, after collecting *Mnemiopsis leidyi* at Caspian Sea, the samples were used for total RNA extraction and cDNA library construction. The Mnemiopsin cDNA was then cloned and sequenced. The deduced amino acid sequence of mnemiopsin (consists of 206 amino acids) revealed a very high sequence homology to bolinopsin (86.4% identity, 93.2 % similarity) and berovin (88.5 % identity, 93.8 % similarity). Based on berovin three-dimentional structure, a structural model of the mnemiopsin was constructed by homology modeling. Mnemiopsin amino acid sequence analysis indicated three putative calcium binding sites (EF hands) found in photoproteins. Expression of mnemiopsin encoding gene in a prokaryotic cell, measurement of bioluminescence spectra and some biochemical properties of recombinant mnemiopsin are still under investigation.

Key words: *Mnemiopsis leidyi,* mnemiopsin, photoprotein, Homology modeling.

Abstract No.156

Iron complexes of bithiazole, as new suggested antitumor agents

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In following the studies on bithiazoles as bio-active ligands and the investigation of effective parameters of Fe-bithiazole complexes, we considered 4,4'-bithiazole (**4bt**) and a branched one, 2,2'-dimethyl-4,4'-bithiazole (**dm4bt**) as ligands and treated them with FeX_3 salts, where X= NO_3, Cl and Br.

There has been considerable interest in the synthesis and characterizes and properties of iron complexes with aromatic nitrogen heterocycles. These studies are performed to understand biomimetic processes, spin transition phenomena, mixed valent complexes and magnetic properties.

To our surprise, octahedral Fe(II) complexes were prepared by interaction of the ligands with Fe(III) salts where the oxidation number of Fe center is reduced to +2 in main tris(N-N) complex but remained +3 in counterion, as we received to $[Fe(4bt)_3](NO_3)_2$ (**1**), $[Fe(4bt)_3][FeBr_4].Br$ (**2**), $[Fe(dm4bt)_3][FeCl_4]_2$ (**3**) and $[Fe(dm4bt)_3][FeBr_4]_2$ (**4**). The complexes were thoroughly characterized. The complexes **1** and **2** show LS characteristics but **3** and **4** are HS. The compound **3** shows dynamic mechanochromic properties upon grinding and dissolving. The cytotoxicity of the compounds was compared with cis-platin drug in normal and cancer cell-lines.

Key words: antitumor agent, dynamic mechanochromic properties, cytotoxicity.

Abstract No.157

Application of DSC in detection of protein Domains

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Differential Scanning calorimetry is a powerful tool in study of protein stability and its thermodynamics since it directly measures enthalpy of protein denaturation. By the structural view Protein domain is a part of protein sequence that can evolve, function, and exist independently of the rest of the protein chain. Each domain forms a compact three-dimensional structure and often can be independently stable and folded. Each domain exerts a special function to the protein, such as an enzyme's active site, which binds the substrate to the enzyme. In Biophysical chemistry we consider protein domain as "Independently folding structural unit". Gaining the information about the protein domains has great value in biotechnology and protein stability.

Depending on protein properties and environment conditions, protein thermal denaturation can be reversible or irreversible. More than 90% of proteins undergo the irreversible denaturation process. Protein domain detection is based on its behavior under thermal unfolding. Mainly for proteins which unfold reversibly, protein domain detection carried out by deconvolution of excess molar heat capacity profile. We successfully could determine the structural domains of Euphorbia Latex Amine oxidase using DSC profiles of modified ELAO. The major method for domain detection of proteins undergoing irreversible denaturation is the successive annealing method. In this method the sample heated repeatedly 1-2°C above the predicted transitions. This method has been used for the calorimetric analysis of proteins like sub-fragment1 of myosin Zn2+-complex of α-Lactalbumin. But the relations between the energetic and structural domains have not been clarified yet and needs more investigation.

Key words: DSC, stability, protein domains, calorimetric analysis.

Abstract No.158

Analysis of Thermal Denaturation of Pepsin on Basis of DSC, UV, PAGE and MALDI-TOF MS Experimental Data

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The thermal stability of pepsin in a strong acid media as a function of pH has been investigated using differential scanning calorimetry (DCS), UV absorbance, Polyacrylamide gel electrophoresis (PAGE) and MALDI-

TOF MS methods. The "two independent two-state transitions with subunits dissociation model" with view of physiological function of pepsin was discussed. The thermodynamic parameters derived from DSC profiles and novel introduced theoretical model, that is based on modified Gibss-Helmholtz equation are similar and in good agreement. The transition temperature (T_m) values ranging from 32 to 49°C for the first transition and from 59 to 76°C for the second transition in the examined pH range implicating the higher stability at pH 4 are in good agreement with MALDI-TOF MS results. DSC method gave transitions curves adequately fitted to a "two independent two-state transitions with subunits dissociation model" as judged by the cooperative ratio between the van't Hoff and the calorimetric enthalpy energies close to unity in all of the pH conditions analyzed, except at pH 1 and pH 2 for the first transition. Thermodynamic analysis using experimental and theoretical models reveals that pepsin molecule is thermally stable over the analysed pH range. The corresponding maximum stabilities, $\Delta G^0_{(25)}$, was obtained at pH 4 with values of 15.63 kcal mol^{-1}.

Key words: Oligomeric proteins, Pepsin thermal denaturation, Thermal stability.

Abstract No.159

Effect of Co^{2+} on the kinetic, structure and stability of mushroom tyrosinase

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Tyrosinase is a widespread enzyme with great promising capabilities. It's outstanding role have to be addressed with considering kinetic, structure and stability issues. Comprehensive kinetics studies on both cresolase and catecholase activities of mushroom tyrosinase (MT) resulted in elucidation of complicated enzyme kinetics. Yet some important aspects of the enzyme structure, mechanism, and behavior have been unresolved. This study devoted to explore the MT structure through circular dichroism (CD) and spectroflourimetric techniques. Its true kinetics assessed in the presence of caffeic as substrate and Co^{2+} in different pHs 6, 7, 8 and 9.

Kinetic of MT activation with this metal ion was obeyed from non-essential activation of an enzyme by an activator molecule. Then, the slope and Y-intercept changes in the Lineweaver-Burk plot due to the presence of metal at different concentration are obtained and replotted their inverse *vs.* inverse concentration of the activator as the secondary plot to find α, β and K_A values. The α value (α < 1) obtained herein suggests that the binding of the ion to the enzyme can increase the binding affinity of the substrate. The β value (β > 1) obtained also suggests that the binding of metal ion to the enzyme can increase the maximum velocity of the enzyme due to the increase of the enzyme catalytic constant (k_{cat}). The optimum activity of the enzyme without the presence of metal ions was carried out in pH=7. Conformational changes of the enzyme through the measurements of its secondary and tertiary structures by CD and fluorescence spectroscopic studies, respectively showed enzyme stability due to the structural changes for Co^{2+} only in pH 9. It is concluded that with this transitional metal ion kinetic pathway of activation follows the general non-essential activation system and its binding may be close enough to the binuclear centre to interact allosterically with the substrate binding site. Besides, the enzyme optimum activity not occurred in its optimum stability.

Key words: Mushroom Tyrosinase, Co^{2+}, Kinetic, Structure.

Abstract No.160

The buffer specificity of two-way activity of sorbitol dehydrogenase: a comparative study

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Sorbitol dehydrogenase (SDH, EC.1,1,1,14) is the second enzyme in polyol pathway. This enzyme catalyzes the interconversion of sorbitol and fructose in the presence of NAD or NADH as coenzymes.
In vitro investigation of this enzyme in conditions similar to the physiologic ones can improve our knowledge on the basis of its operation in the pathogenic situations, providing clues to overcome the related complications in diabetes. In the present study, the effect of some of the most common buffer on the catalytic activity of SDH has been investigated at pH 7.4. The results indicate that both in sorbitol oxidation and fructose reduction, the enzyme show its best activity in Tris-HCL buffer. Appropriate interpretation have been discussed.

Key words: sorbitol dehydrogenase, polyol pathway, buffer, enzyme activity.

Abstract No.161

Preparation and investigation on Nano-sized Cross-linked Horseradish peroxidase

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Horse radish peroxidase is a useful enzyme for applied purposes such as renovation of oil contaminations. However, there are various criteria limiting the applications for the soluble enzyme which can be relieved in immobilized preparations. In this study, carrier free immobilization of Horseradish peroxidase (HRP EC 1.11.1.7) is used and the cross-linked enzyme aggregates (CLEA) are prepared. Optimization of the nano-scaled CLEA preparation was carried out upon stoichimeteric considerations on the safe enzyme cross-linking using glutaraldehyde as a bifunctional cross-linking agent. The products of immobilization have resulted with kinetic improved kinetic features and stability. For instance, the catalytic performance of Horseradish peroxidase was improved and the thermal stability of nano-sized CLEAs was increased. However, the pH profile of the cross-linked enzyme aggregates did not show any observable differences compared to the free one. The Size and the morphological characteristics of the CLEAs nanoparticles were determined respectively with dynamic light scattering (DLS) and transmission electron microscopy.

Key words: Immobilization, CLEAs, Horseradish peroxidise.

Abstract No.162

Comparison of two Isoperoxidases purified from Turnip root (*Brassica napus* Var. Okapi)

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Peroxidases (E.C. 1.11.1.7) have been found in a wide range of plant species. Horseradish (*Armoracia sp.*) roots represent the traditional source for commercial production of peroxidase (Krell 1991), but it is possible that other plants could provide some isoperoxidases that exhibit similar or better characters. Comparison of isoenzymes from different plant sources and cultivars revealed significant differences between them. Turnip (*Brassica napus*) is an annual herb cultivated in widespread regions of the world mainly for its valuable seed oil but its root, a byproduct in agriculture, is a rich source of peroxidases. In the present work Two Isoperoxidases were purified from the turnip root by using ammonium sulfate precipitation and DEAE-Sephadex column chromatography. Peroxidase activity determined following the formation of tetraguaiacol from guaiacol. Two purified Isoperoxidases named TP1 and TP2 have some different properties. It seems that TP1 is an acidic peroxidase and TP2 is an alkali one. K_m for two peroxidases was estimated about 0.055 mM in a fixed concentration of 5 mM guaiacol. Substrate inhibition was not observed on both TP1 and TP2 by using high concentrations of H_2O_2. TP1 is most active in pH 6 and 6.5 but the activity of TP2 is highest in a pH range from 4.5 to 7. The inhibition effect of NaN_3 and NaCN was studied on TP1 and TP2 activities. Both Isoenzymes were sensitive to various concentrations of NaCN rather than NaN_3. It's shown that phenol-AAP can be a more suitable substrate for TP1 than the other substrates.

Key words: peroxidase, Horseradish roots, substrate inhibition.

Abstract No.163

Reactivation of the purified lactase-phlorizin hydrolase upon reconstitution

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The Structure and function of membrane proteins depends on the membrane bilayer. However, due to the complexity of the biological membranes, extraction and purification of these biocatalysts from their native environment is an inevitable step for many experiments.
In the present study, the intestinal brush border membrane (IBBM) of suckling rabbit was extracted in 1% (v/v) Triton X-100 and the lactase-phlorizin hydrolase (LPH, EC: 3.2.1.23/62) was purified using an anti-LPH immunoaffinity column. Although the purification process was successful and a single band was detected by denaturing gel, the purified enzyme was found to be drastically inactivated. Following that, the purified enzyme was reconstituted in phosphatidylcholine liposomes which was associated with re-activation of the enzyme. This procedure declares the unique function of the lipid bilayer on the organization and the functional properties of the membrane proteins.

Key words: lactase-phlorizin hydrolase, purification, immunoaffinity chromatography, reconstitution, liposome.

Abstract No.164

A review on the scientific documents of Iran during the last decade: A more emphasis on Biosciences

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The total number of scientific documents of Iran, indexed in the Thomson **I**nstitute for **S**cientific **I**nformation (**ISI**), during the last decade (2000-2009) is 64,977 from total world of 13,886,858 shows 0.47% contribution of our country. From those total documents, 63820 indexes are related to the science, 2554 indexes are related to the social sciences and 189 indexes are related to the art and humanity sciences. The contribution of Iran in scientific documents indexed by ISI has been increased year by year so that it has been reached to more than one percent at the end of the last decade, 2009. The number of documents related to biosciences has been increase from 226 in 2000 to 2078 (nine-fold) in 2009. The total number of documents was 10492 during the last decade, which is 16% of the contribution of total Iranian documents. Pharmacology & Pharmacy by 2450, Biochemistry & Molecular Biology by 1467, Immunology by 1378, Plant Sciences by 1268, Neurosciences by 1115, Biotechnology & Applied Microbiology by 897 documents have had the most contributions. The contributions of other fields related to biosciences are also reported. There are many young and active scientists graduated in bioscience departments of our universities; however, old and timeworn equipments for bioscience research can not produce a good situation to present a fantastic work to be published in a prestigious international journal.

Key words: ISI, Scientific Documents, Bioscience, Iran, Scientific Contribution.

Abstract No.165

New method for urease immobilization on alkylated macro porous silica

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There are several methods for immobilization of urease, such as covalent bonding, entrapment, physical adsorption and micro-encapsulation. In this study, urease was immobilized on macro porous silica with an average pore size 60 nm that was alkylated by octadecyltrichlosilane (OTS). Alkylated macro porous silica creates a hydrophobic support for enzyme. Urease was immobilized on it by denaturation- renaturation in the organic solvent. Urease enzyme was unfolded by dioxane and the effect of different concentration of dioxin on the structure of enzyme was investigated by UV-spectrophotometer, intrinsic and extrinsic fluorescence and ANS bonding. Protein tertiary structure was studied by circular dichroism (CD) spectra in the near-UV range. The effect of dioxin on the activity of enzyme was considered by berthelot reaction. According to these results, the best concentration of dioxane for denaturation of urease enzyme was 32 %(v/v).

Urease does not normally show high affinity for binding to hydrophobic supports but when urease is unfolded by organic solvent it is exposed hydrophobic residues. Hydrophobic-hydrophobic interaction is formed between enzyme and alkyl groups. Results indicate that enzyme will be immobilized on the alkylated macro porous silica with high efficiency.

Key words: urease, immobilization, macro porous silica, dioxane.

Abstract No.166

NMR study on protonated 8OG:C base pairs: DFT calculation

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8-Oxoguanine (8OG) is a mutagenic oxidative product of guanine. The protonation of 8OG at N3 site affect individual hydrogen bonds in the syn and anti conformations of 8-oxoguanine:cytosine (8OG:C). Pervious studies show that anti is more stable than syn conformer. In this paper, Two-bond spin-spin coupling constants ($^{2h}J_{X\text{-}Y}$) have been investigated across X-H···Y hydrogen bonds in anti conformer. All structures were optimized by Gaussian03 program package at PBE1KCIS level with 6-311++G (d,p) basis set. In addition, $^{2h}J_{X\text{-}Y}$, isotropic values of the proton shielding tensor, and isotropic chemical shift (δ_{iso}^{H}) have been calculated at mentioned level using SPINSPIN keyword.

Anti conformer has three hydrogen bonds (**A**, **B**, **C**). Geometrical parameters show that hydrogen bond length of **B** and **C** decrease and of **A** increases by protonation of N3 site. The second-order perturbation energies $E^{(2)}$ of $n_X \rightarrow \sigma^*_{H\text{-}Y}$ interactions obtained from NBO analysis and the most important topological properties of electron charge density ρ(r) calculated by AIM method show that the hydrogen

bond strengths of **B** and **C** increase and of **A** decreases by protonation. Total $^{2h}J_{O\text{-}N}$ value of **A** decreases and of **B** and **C** increases by protonation. The total spin-spin coupling constant (^{2h}J) is the sum of four components: the paramagnetic spin-orbit (PSO), diamagnetic spin-orbit (DSO), Fermi-contact (FC), and spin-dipole (SD) terms. All these terms are in agreement with mentioned trend. After protonation, the isotropic chemical shifts (δ_{iso}^{H}) of **B** and **C** increases and of **A** decreases in mentioned process. Also, the trend of isotropic value of proton shielding tensor (protonic IS) in agreement with other calculated NMR data. This trend is reversed for anisotropy of the proton tensor. All NMR data are in accord with the results of AIM and NBO analyses, and geometrical parameters.

Key words: 8-Oxoguanine, anti conformer, NMR, spin-spin coupling constant.

Abstract No.167

Experimental detection of signal peptid in ppic and trmt1 with yeast secretion trap

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Secreted and cell surface proteins play essential roles in the biological process and cell communications in different organisms. There are few methods for the Identification of these proteins. Yesat secretion trap method is well established one of them that could detect signal peptid in many organisms from fungi to human cell. There also some softwares used to predict the secretory status of a given protein sequence. Here we intend to compare precision of these softwares as well as investigate the secretory status of some human genes in the Yeast.

We have PCR amplified trmt1(GeneID: 55621), PPic(GeneID: 5480), tfam(GeneID: 7019), and Pi16(GeneID: 221476) first exon genes and cloned in pYST0, pYST1 and pYST2. Pi16 is already known as secretory protein and has been considered as positive control in these investigation. tfam has been considered secretory in some of softwares while it has been shown to be localized in mitochondria and we used it as negative control. Currently, we are investigating first exon of ppic and trmt1 in the yeats to confirm their signal peptid functionality in the yeast.

Key words: cell surface protein, signal peptid, trmt1, ppic.

Abstract No.168

Identification of *Pseudomonas syringae* using structural and functional homology of *Hrcv* protein

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Pseudomonas syringae is one of important plant pathogenic bacteria that causes many important plant diseases that lead to sever epidemics. To control this pathogen, appropriate diagnostic methods must be available. We detect the bacteria in the plant, soil, water and other resources using serological techniques, but due to limitations of this method, the cost and time, many methods based on DNA which are stable, fast, specific and reliable have been developed. In this study for specific detection of bacteria, amplification of genes coding for specific proteins, including the *Hrpr*, *Hrcv* which are specific to this pathogen were used. A pair of primers for consensus region of *Hrcv* were designed which amplified a fragment of 400bp in some *P. syringae* pathovars. Due to lack of this protein in some pvs. of *P. syringae*, which was revealed bioinformatically, structurally and functionally similar to *Hrcv* was identified and the primers were designed and the pathogen was detected.

Key words: protein *Hrcv*, bacteria *Pseudomonas syringae,* homology structure and function.

Abstract No.169

Using of a Minisatellite Marker located in MdMYB10 gene for Recognition of Iranian Red-Flesh Apple Genotypes

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Anthocyanins are antioxidant component that reduce the incidence of cancers and some diseases. There are some rare Iranian apple (*Malus domestica*) genotypes with red-flesh and foliage in which anthocyanins

are accumulated in those tissues and causes red color. At the molecular level it has been shown that high level of specific MYB transcription factors induce anthocianin accumulation in red tissues of apple. Reports shows the presence of a minisattelite in the promoter of a MdMyb10 gene of red flesh apple cultivars while such a satellite is absent in the same gene of white flesh appls. It seems the number of repeat of the satellite correlates with the intensity of red color of apple flesh. Here we intend to investigate some Iranian red flesh apple cultivars to examine the presence of such a gene and also the number of repeat in its promoter satellite. PCR amplification and sequencing results on Iranian red flesh cultivar showed all tested red apples are heterozygote for such a repeat. The number of repeat at the satellite seemd equal to what have been reported befor.

Key words: Apple, Myb, Red-Flesh, Anthocyanin.

Abstract No.170

Small Molecules: Application as Therapeutics (in Diabetes & Cancer), Protein Folding and DNA Structural Changes Studies

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Small molecules are low molecular weight organic compounds that bind to a biomacromolecule, such as protein or nucleic acid, and change its folding (structure) and thus its function. It is also possible that after binding of small molecules to biomacromolecules, the structure and folding of protein or DNA is protected from the attack of other harmful ligands. The small molecules can be natural or synthetic. They may have some beneficial effects against some diseases. We studied various small molecules from 10 years ago in our lab, not only for investigation of protein and DNA structural alterations but also for induction of some diseases in animal models and then, treatment of them. We induced breast cancer, gastric cancer and diabetes type I and II in rat using NMU, MNNG and STZ; then the beneficial effect of some natural anticancer compounds such as carotenoids (Crocin and crocetin) and monoterpene aldehydes (picrocrocin and safranal) of saffron, chemical chaperones from different classes (including: amino acids, poly amines and polyols) and some other compounds (some known drugs, e.g. aspirin and celecoxib) were studied on these animal models. In addition, mechanisms of the action of these compounds were investigated. Here I will present a summary of the obtained results in our lab during these years.

Key words: small molecules, therapeutics, protein folding, DNA structural changes.

Abstract No.171

Heme degradation and platelet aggregation in diabetics

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Increased fructose concentration as moderately reducing sugar in erythrocytes of diabetics subject hemoglobin (Hb) to be glycated by fructose. Hemoglobin glycation results in early and advanced glycation end products which are known as HbA1c and Hb-AGE, respectively. Under such conditions, heme is more prone to be oxidized to hemin which consequently turns it to be released from its pocket in glycated-globin structure. Based on evidences, it is presumed that fructation-induced hemin release makes it to be more accessible for oxidation and as a result degradation via a complicated reactive oxygen species but non-hydrogen peroxide involved mechanisms. Moreover, pre-incubation of platelets with glycated Hbs leads to an increase in ADP induced platelet aggregation which increases along with the extension of glycation process. It seems that the interaction between AGE structures and promising receptors in platelet surface leads to these cells hyperactivity and aggregation.

Key words: Hemoglobin glycation, reactive oxygen species, platelet aggregation, cells hyperactivity.

Abstract No.172

Polymerization Mechanism of Mesophilic Alcohol Dehydrogenases into Nanoaggregates

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The shelf-life of biotechnological potent enzymes is limited by self-assembly of proteins into nanoaggregates such as nanoensembles or nanofilaments. We have evaluated the thermal stability and the mechanism of alcohol dehydrogenases aggregation, trying to improve the shelf-life of ADH by fluorescence, circular dichroism, UV-Vis

spectrophotometry, dynamic light scattering (DLS) technique, enzymatic activity assay, molecular dynamics and molecular docking methods. Assaying thermal polymerization of horse liver alcohol dehydrogenase (HLADH; dimeric) and yeast alcohol dehydrogenase (YADH; tetrameric), as biotechnological ADH representative enzymes, indicated reliable correlation between the variation in scattering and λ^{-2} which was related to the amorphousness of the nanoaggregated ADHs, shown by electron microscopy (EM) images. Enzymes quaternary structural changes delocalization of subunits lead to enzymes polymerization without unfolding. Constructed ADHs nuclei, which grew to larger amorphous nanoaggregates, were prevented via high repulsion strain of the net charge values. Based on the experimental findings we have proposed a new model of self-assembly for ADH enzymes that construction of nuclei and growing to formless nanoaggregates without enzymes denaturation and unfolding.

Key words: self-assembly, thermal stability, alcohol dehydrogenases, nanoaggregates, denaturation.

Abstract No.173

Design and development of novel firefly luciferases by site-directed mutagenesis: structure-function relationship

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Firefly luciferase (EC.1.13.12.7) is a single polypeptide chain (62 kDa), responsible for emission of yellow-green (557 nm) light, known to be most efficient bioluminescence system which make it an excellent tool for reporter in nano-system biology. The bioluminescence color of firefly luciferases is changed due to alterations in the luciferase structure and assay conditions. Upon sequence identification and cloning of two Iranian firefly luciferase they were modified by site-directed mutagenesis in order to design novel luciferases. Insertion of Arg356 in a green-emitter luciferase (*Lampyris turkestanicus*), that is corresponding to Arg353 in *P. hirtus*, changed the emitted light from green to red. In order to further clarification of the effect of this position in the light shift, residues with positive side-chain (Arg356 and Lys356) were inserted in *Photinus pyralis* luciferase which changed the light color to red, while insertion of a residue with negative side-chain (Glu356) had little effect on color. On the other hand, by insertion and substitution of positive-charged residues, in the same loop of *Lampyris turkestanicus* luciferase, different specific mutation (E354R/Arg356, E354k/Arg356, E354R, E354K) lead to changes of the bioluminescence color. In order to generate more stable luciferases against protease digestion, we substituted two tryptic sites; R213, R337 and also next residue to it (Q338) with another amino acids. Structural alterations of firefly luciferase upon mutation were monitored by spectroscopy and homology modeling. Moreover, further related studies will be discussed.

Key words: Firefly luciferase, bioluminescence, protease digestion, homology modeling.

Abstract No.174

Distance-dependent atomic knowledge-based potential

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The current approaches to protein structure prediction are based on the thermodynamic hypothesis according to which native state of protein is at the lowest free energy state under physiological condition. Thus, using energy function to detect a correct protein fold from incorrect ones is very important for protein structure prediction and protein folding. Commonly, two different types of potential energy functions have been used either for the identification of native protein models from a large set of decoys, or protein fold recognition and threading studies. The first types of potentials are based on the fundamental analysis of the forces between the particles referred to as physical energy function. The second types are knowledge-based energy function and are based on information from known protein structures. Although physical energy function is widely used in molecular dynamic simulation of proteins, these functions have been out of favor in protein structure prediction because of their greater computational costs. To reduce computational complexity of the protein folding problem, knowledge-based or empirical mean force potential is widely used. The structure of folded proteins reflects the energy of the interaction of all their components, including all enthalpic and entropic contributions, as well as solvent effects. Such potentials provide an excellent shortcut toward a powerful objective function. It can be used to coarse grain the system to obtain potential between groups of atoms by the use of experimentally determined structures. In this approach, statistical thermodynamics is used in an analysis of the frequency of observed state in order to approximate the underlying free energy.

Key words: protein structure, thermodynamic hypothesis, free energy state, molecular dynamics.

Abstract No.175

Pilot-scale Peroxidative phenol removal from aqueous solutions

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Wastewaters of oil refineries, plastics, resins, textiles, iron and steel industries have phenolic compounds (phenol, phenol derivatives, quinolines, naphthols, …) at levels of several hundred of ppms. Traditional treatment procedures like adsorption, solvent extraction and oxidation can not remove phenolic compounds below 50 ppm, efficiently. Enzymatic phenol removal is one of the alternatives to biocatalyze the removal of these compounds from wastewater. The main challenges of this process are enzyme deactivation during the reaction due to a precipitation with the reaction products (oligomers and polymers) and peroxide inactivation of the enzyme by excess hydrogen peroxide. Previously, we reported the kinetic model and methods for lab-scale controlling and minimizing the inactivation effects of a successful phenol removal. In the present work, optimization of phenol removal conditions was performed in 800 ml stirred-flow reactor equipped with temperature, stirrer rate and viscosity controller using suitable interfaces and software. Hydrogen peroxide was gradually added to the reaction mixture of phenols and peroxidase extract (RZ~0.8). A peristaltic pump was used for circulation of mixture and filtration of the produced polymers through a centrifugal filter system. The influence of operational variables, both enzyme and substrate concentrations and spatial time, on the removal efficiency was studied also in a small-size pilot-scale (10 L) for homogeneous and heterogeneous processes. Kinetics of the process based on the bisubstrate compulsory kinetic equation was used for the used reactor and optimization pathway of the removal process.

Optimization of phenol removal conditions was carried out using the Taguchi method, which uses the orthogonal arrays for design of experiments. Results showed that crude enzyme samples with an enzyme concentrations of ~300 nM, at temperature of 15 °C, stirrer rate of 200 rpm and in the presence of hydrogen peroxide (kept constant at about 1 mM by a dosing pump), provides a removal efficiency around 100% (no ppm detection of phenols by HPLC method) for treatment of 300 ppm of phenolic compounds in aqueous media.

Key words: enzyme deactivation, kinetic model, peristaltic pump, Taguchi method.

Abstract No.176

Biogenesis silver nanoparticles by plant extract versus chemical methods

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The development of environmentally benign processes for synthesis of nano scale materials is an important aspect of nanotechnology. Biosynthesis of nanoparticles by plant extracts is currently under explotation. Plant extracts are very cost effective and eco-friendly and can be an economic and suitable rout for the large-scale synthesis of nanoparticles. Many achievements have been reported in the field of silver nanoparticles production using plant extracts. And now we show a facile bottom-up green synthetic method using *Rhus coriaria L.* extract as redusing agent produced silver nanoparticles in aqueos solution at ambient conditions. We are working on the optimizing of process of nanoparticles synthesis as well.

Key words: Nanoparticle production, Plant extracts, Nanosilver, *Rhus coriaria L.*

Abstract No.177

Oligomerization Status Directs Overall Activity Regulation of the *Escherichia coli* Class Ia Ribonucleotide Reductase

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Ribonucleotide reductase (RNR) is a key enzyme for the synthesis of the four DNA building blocks. Class Ia RNRs contain two subunits, denoted R1 (α) and R2 (β). These enzymes are regulated via two nucleotide-binding allosteric sites on the R1 subunit, termed the specificity and overall activity sites. The specificity site binds ATP, dATP, dTTP or dGTP and determines the substrate to be reduced, whereas the overall activity site binds dATP (inhibitor) or ATP. By using Gas-Phase Electrophoretic Mobility Macromolecule Analysis (GEMMA) and enzyme assays, we found that the *Escherichia coli* class Ia RNR formed an inhibited $\alpha_4\beta_4$ complex in the presence of dATP and an active $\alpha_2\beta_2$ complex in the presence of ATP (main substrate: CDP), dTTP (substrate: GDP) or dGTP (substrate: ADP). The R1-R2 interaction was 30-50 times stronger in the $\alpha_4\beta_4$ complex than in the $\alpha_2\beta_2$ complex, which was in equilibrium with free α_2 and β_2 subunits. Studies of a known *E. coli* R1 mutant (H59A) showed that deficient dATP inhibition correlated with reduced ability to form $\alpha_4\beta_4$ complexes. ATP could also induce the formation of a generally inhibited $\alpha_4\beta_4$ complex in the *E. coli* RNR but only when used in combination with high concentrations of the specificity site effectors, dTTP/dGTP. Both allosteric sites are therefore important for $\alpha_4\beta_4$ formation and overall activity regulation. The *E. coli* RNR differs from the mammalian enzyme which is stimulated by ATP also in combination with dGTP/dTTP, and forms active and inactive $\alpha_6\beta_2$ complexes.

Key words: Oligomerization Status; Ribonucleotide reductase; *E. coli.*

Abstract No.178

Hydrophobic interactions: a "lifetime's" experiences

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Hydrophobic interactions are attractive forces between nonpolar entities in water. They constitute the most important non-covalent interactions in nature, decisively involved in almost all important biologically-relevant systems. They are entropy-driven, have the same range as, but are about an order of magnitude stronger than, the van der Waals dispersion forces. These interactions are mainly responsible for stabilization of biological macromolecules and contribute to some of their most important biological functions. Along with other non-covalent interactions, they determine the three dimensional shape of biologically important structures such as proteins and play a central role in micelle formation and biological membranes.

In this talk, I will try to demonstrate how one's own earlier observations may be developed into, and form some of the foundations of, later findings. I will start by describing my first resarch experiences as a graduate student by outlining some of my earlier work on involvement of hydrophobic interactions in biological membranes. I will then describe some of my earlier studies related to adsorptive immobilization of proteins on hydrophobic supports and interaction of "soluble" enzymes with mitochondrial membranes. Induction of molten-globular intermediate structures in protein molecules leading to enhanced affinities for hydrophobic supports, and use of reversible denaturation for protein immobilization will then be described. The presentation will be continued with studies on prediction of protein orientation for its immobilization on biological and non-biological surfaces, followed by a discussion on the involvement of hydrophobic sites in thermal aggregation of protein molecules, and comparative studies on protein stability using mesophilic and thermophilic proteins. Some of the more recent studies on involvement of hydrophobic interactions in amorphous and amyloid aggregate formation in protein molecules related to neurodegenerative disorders will then be presented.

Key words: Hydrophobic interactions, aggregation, attractive forces, immobilization, neurodegenerative disorders.

Abstract No.179

Structural analysis of carbonated hydroxyapatite nanoparticles containing Magnesium

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In recent years magnesium (Mg^{2+}), as a dopant in both HAp and β-TCP structures, has been the subject of specific interest owing to its essential role in biological process upon implantation. In this study, pure and Mg-doped hydroxyapatite [$(Ca, Mg)_{10}(PO_4)_6(OH)_2$] nanoparticles were synthesized via sol-gel method. Triethyl phosphite, calcium nitrate tetrahydrate and ***Magnesium Nitrate Hexa-hydrate*** were used as P, Ca and Mg precursors. The ratio of (Ca+Mg)/P and the amount of magnesium (x) as defined in $Ca_{10-x}Mg_x(PO_4)_6(OH)_2$ were kept constant at 1.67 and 1.5, respectively. Phase percentages, crystallite size, degree of crystallinity and lattice parameters of Mg-doped HA and Pure HA(control) were investigated by XRD method. The control and Mg-doped sample were composed of low carbonated HA and Mg-doped high carbonated HA phases, respectively. A very small

trace of b-TCMP has been identified in the latter sample. Based on the results of this research, degree of crystallinity and crystallite size decreased with Magnesium content. The specific peaks ((002), (211), (112) and (300)) gradually shifted in the Mg-doped sample. A decrease of a-axis length has been found taking into account the characteristic peaks of (300) and (211). Increasing of c-axis length estimated from the shift of (002) and (112) can be attributed to the substitution of carbonated ions in HA crystals. The calculated values for lattice parameters confirm the stabilization role played by Mg.

Key words: Mg-Doped Carbonated Hydroxyapatite, Sol gel, Nanoparticles, X ray Diffraction

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Abstract No.180

Simulation of protein adsorption in a typical body cell

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Finite Element Method has been used to solve equilibrium time-dependent relations governing the adsorption of proteins to an ion-exchanger medium. In our model, the fluid phase contains three components: two proteins (A&B) and one salt (S). The adsorption/desorption kinetics are described by a set of equilibrium reactions where proteins displace ions adsorbed at the surface and vice versa:

$$zSa+A \leftrightarrow z\,S+Aa \;\&\; zSa+B \leftrightarrow z\,S+Ba$$

Where Aa, Ba and Sa are in the adsorbed phase and z is the binding charge. Setting up the mass balance equations results in a system of six time dependent partial differential equations. The unknown reaction rates have been removed by using the mass action law (assuming zero steric factor for both reactions).The resulting time-dependent equations have been solved simultaneously in 1D using finite element method (assumed length is 10^{-5} m, typical of body cell). We have used two typical proteins to illustrate the feasibility of solving this system of highly involved dependant partial differential equations (PDES). We have obtained time-dependant concentration profiles of the species, in good agreement with that of the observed experimental data reported elsewhere.

Key words: Protein adsorption, Ion exchange, Finite Element Method.

Abstract No.181

A study on structural, chemical, thermal and morphological changes in magnesium hydroxyapatite nanoparticles synthesized using biomimetic method

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Magnesium hydroxyapatite nanopowder was synthesized by wet chemical method using calcium nitrate tetra hydrate, magnesium nitrate hexa hydrate and diammonium hydrogen phosphate in biomimetic environment using glutamic acid. It had been calcinated at temperatures which is determined by thermal analysis and characterized by XRD, FTIR and SEM. XRD results showed the formation of β-TCP ((Ca1-xMgx)3(PO4)2) above 650°C but below this temperature only HA(with low crystalinity) was observed in biomimetic samples but samples synthesized without presence of amino acid showed a biphasic mixture of Hydroxyapatite and β-TCP.In addition These findings had been compared to FTIR results and became confirmed by results. By increasing the temperature from synthesis temperature (90°C) to 650°C there were no major changes on degree of crystalinity. presence of glutamic acid had great influence on particles size, morphology and degree of crystallinity in the range of 650 to 920°C so that needle like β-TCP nanoparticles had been formed .Observation by SEM and calculation using scherrer formula confirmed the formation of particles sizes below 100nm.

Key words: Wet chemical method, degree of crystallinity, biomimetic method, thermal analysis.

Abstract No.182

Trifluoroethanol effect on protease-sensitive regions of luciferase enzyme

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Bioluminescence enzyme, 'luciferase', is a family of photo-proteins that can be isolated from a large variety of prokaryotes, marine organisms, and insects. Firefly luciferase is a α/β protein that consists of two distinct domains connected by a disordered loop with two regions that

were found to be much susceptible to proteolytic degradation. Limited proteolysis is performing in peptide bond fissions at exposed and flexible loops of the proteins, not at the level of regular secondary structure elements such as helices. Trifluoroethanol (TFE) is known to induce the helical content of the proteins. Peptides with a predisposition for helical secondary structure can often be induced to undergo a transition from random coil to α-helix by addition of TFE to aqueous solutions. The aim of the current research was investigation of conformational aspects and helical propensity of *Photinus pyralis* luciferase in aqueous TFE. Insights into the structure and dynamics of the firefly luciferase in aqueous TFE were obtained through proteolysis studies, spectroscopy and activity measurements in the presence of various concentration of TFE. With addition of TFE, protein conformation changes considerably. Our results point to the extension of helical state at high TFE concentrations. It seems that under such conditions, even with increasing the α-helical content or disrupting tertiary structure, *P. pyralis* luciferase retains two flexible regions. In fact, in spite of large conformational changes of *P. pyralis* luciferase induced by TFE, sites of proteolytic attack were still located at the same chain segments.

Key words: Bioluminescence, Luciferase, Trifluoroethanol, Structure, Proteolysis.