J. Iran. Chem. Soc., Vol. 6, Suppl., November 2009, pp. S80-S102.

JOURNAL OF THE
Iranian
Chemical Society

Structure, Function and Metabolism of Biomolecules

P-10-97-1

The effect of cimetidine on renal alkaline phosphatase activity

*Somayeh Khodai, Somayeh Aminnaseri, Zahra Hassanpoor, Mahsa Damanafshan, Marzieh Nikoo, Aida Eyvazadeh, Dariush Minai-Tehrani**

BioResearch Lab, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran

Alkaline phosphatase (ALP) (EC 3.1.3.1) belongs to hydrolase group of enzymes. It is responsible for removing phosphate groups from many types of molecules, including nucleotides and proteins. In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney and, bone. The optimal pH for the enzyme activity is pH=10 in standard conditions. The primary importance of measuring alkaline phosphatase activity in blood samples is to check the possibility of bone disease or liver disease. All mammalian alkaline phosphatase isoenzymes except placental are inhibited by homoarginine and similarly all except the intestinal and placental ones are blocked by levamisole. Cimetidine (trade name Tagamet) is an antagonist of histamine H2-receptor that inhibits the production of gastric acid. Cimetidine is used for the treatment of duodenal ulcers, active gastric ulcers, and gastroesophageal reflux disease. It causes some side effects in body such as inhibition of P450 cytochrome and interfering with metabolism of the hormone estrogen. In this study the inhibitory effect of cimetidine on mouse renal ALP activity was investigated. Our results showed that cimetidine can inhibit ALP by un-competitive inhibition which indicated that the inhibitor could bind to ES complex. In the absence of inhibitor the Vmax and Km of enzyme were found to be 13.7mM/min and 28 μM, respectively. Double reciprocal plot showed that both Vmax and Km of enzyme were decreased by increasing cimetidine concentrations (0-1.2mM). The Ki of cimetidine was determined by Cornish-Dowsen method and was found to be 0.5 mM. The IC50 of inhibitor was determined to be about 0.82mM by drawing the plot of different cimetidine concentrations against the % ALP velocity.

Keywords: alkaline phosphatase, enzyme, inhibition, drug, cimetidine

---

P-10-150-1

Determination of the effect of some detergents and stabilizing agents on plasma membrane phosphatidate phosphohydrolase of rat liver

Esfandiar Heidarian [1], Esmat Jafari Dehkordi [2], Bahram Haghighi [3]*

1- Department of Biochemistry, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran, 2- Department of Biochemistry, Isfahan University of Medical Sciences, , Isfahan, Iran, 3- Health Center of Shahre-kord, Shahre-kord University of Medical Sciences, Shahre-kord, Iran

Phosphatidate phosphohydrolase (PAP) catalyzes the dephosphorylation of phosphatidic acid to yield Pi and diacylglycerol. In rat hepatocyte, two different forms of PAP have been reported. PAP1 is located in cytosolic and microsomal fractions and participates in the synthesis of phospholipids and triacylglycerols, whereas the other form of phosphatidate phosphohydrolase (PAP2) is primarily involved in lipid signaling pathways. PAP2 has two isoforms; one PAP2a and another PAP2b. In this study the effect of some detergents and stabilizing factors were investigated on the stability and activity of PAP2b. PAP2b was purified from rat liver plasma membrane by solubilizing with n-octyle glucoside and several chromatography steps. Gel electrophoresis (SDS-PAGE) in 10%gel slab was performed on purified enzyme in order to evaluate purity and to measure the molecular weight of the enzyme subunit. The effects of lubrol PX, CTAB and Tween 80 were examined on the stimulation of enzyme activity. The stability of solubilized PAP2b was determined in different concentrations of trehalose, sucrose, and albumin. The specific activity of purified enzyme was 7350mU/mg protein and the purified enzyme showed only a single band on SDS-PAGE with a MW of about 33.8 kDa. The enzyme was activated approximately 4 fold by lubrol PX and Tween 80 both at 3 mM. The activation by CTAB occurred at 1Mm. Trehalose, sucrose and albumin had stability effects on PAP2b at 3, 7 and 10 percentages, respectively. The enzyme was activated by lubrol PX, Tween 80 and CTAB. The nondetergent agents such as trehalose, sucrose and albumin had stability effect on the PAP2b and the trehalose showed the best stabilizing factor than other nondetergent agents such as sucrose and albumin. On the other hand, the lubrol PX had the high potential stimulatory effect with respect to other ionic and nonionic detergents on enzyme activity.

Keywords: detergent, trehalose, sucrose, albumin, membrane phosphatidate phosphohydrolase

---

P-10-97-2

Structural changes and inhibition of sucrase after binding of scopolamine (hyoscine)

Negar Fouladi [1], Zahra Sobhani-Damavandifa [1], Soraya Heydarzadeh [1], Tooka Aavani [1], Saeed Minoui [1], Habibollah Nazem [2], Dariush Minai-Tehrani [2]*

1- BioResearch Lab, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, 2- Payame Noor University, Tehran, Iran

Scopolamine (hyoscine) is a common anticholinergic drug. It is obtained from plants of the family Solanaceae (nightshades).

Scopolamine is a muscarinic antagonist structurally similar to the neurotransmitter acetylcholine and acts by blocking the muscarinic acetylcholine receptors and thus is classified as an anticholinergic. Scopolamine is used to relieve nausea, vomiting, and dizziness associated with motion sickness and recovery from anesthesia and surgery. Sucrase is a hydrolytic enzyme that breaks down sucrose to its monomers, glucose and fructose. Sucrase has various isoforms in both plants and yeast. The human gastrointestinal system has a kind of sucrose known as sucrase-isomaltase that resides in the apical surface of intestinal cells. In this study the effect of scopolamine hydrobromide on yeast sucrase activity was studied, on the other hand the effect of scopolamine on structural changes of enzyme was investigated by CD and fluorescence measurement method. Our results showed that binding of scopolamine to sucrase could inhibit the enzyme activity. Non-competitive inhibition was observed in different concentrations of scopolamine (0.6 to 3.6 mM). The Km (11.5mM) of enzyme was constant in the presence of scopolamine, while the Vmax was changed after binding of scopolamine. Study by CD measurement in far UV showed that the absolute enzyme exhibited a trough at 218 nm which is an indication of β-sheets in its secondary structure. Binding of substrate as well as inhibitor on enzyme made the trough deeper which proposed that more β-sheets were exposed to the medium. Study of fluorescence spectra with excitation at 295 nm and emission at 300-500 nm demonstrated that the absolute enzyme showed low intensity (Q) in 335 nm. This showed that the tryptophan residues may be in the surface of enzyme and in a polar environment, a quencher might be nearby. Binding of substrate increased the intensity (Q) of peak and also caused the red shift (350 nm). The binding of scopolamine to enzyme also increased the intensity of the peak and caused red shift in the peak which suggested that tryptophan residues were moved to the interior of the protein and away from the polar medium. The binding of inhibitor to the enzyme-substrate complex also increased the intensity of peak at 350 nm which indicates that the inhibitor could cause tryptophan delocalization in the complex. In conclusion, our results suggest that there is a separate binding site for scopolamine on sucrase molecule and its binding causes structural changes in the enzyme which inhibits the enzyme activity and affects its function.

Keywords: scopolamine, drug, inhibition, enzyme

P-10-137-1

Chaperone-like activity of heme group against amyloid-like fibril formation by hen egg ovalbumin: Possible mechanism of action

Reza Khodarahmi [1], Hosnieh Soori [1], Mehdi Beyrami [2]*

1- Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran, 2- Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

It is well accepted that porphyrins related to naturally occurring metabolite, heme, are effectively able to act as pharmaceuticals (drugs). For instance, they were found to interfere with the in vivo/in vitro aggregation of β-amyloid peptide. In present study, the influence of heme concentration on the amyloid-type aggregation of hen egg ovalbumin was investigated. We provided experimental evidence of heme's prevention of ovalbumin aggregation in the heat-denaturation process using turbidimetry, fluorescence and CD measurements. Additionally, different types of interactions were suggested to be involved in heme-ovalbumin amyloid communication. Since the consequence of heme-attenuated fibrillar aggregation and nature of its binding to amyloid fibrils have yet to be identified, additional data on chaperoning role of heme on amyloid-forming (model) systems may help us to manage amyloid aggregation processes, universally. We will discuss the importance of these observations.

Keywords: aggregation, amyloid, ovalbumin

P-10-138-1

Appraisal of casein's inhibitory effects on aggregation accompanying carbonic anhydrase refolding and heat-induced ovalbumin fibrillogenesis

Reza Khodarahmi [1], Mehdi Beyrami [1], Hosnieh Soori [2]*

1- Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran; Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran, 2- Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

It is well accepted that whole casein and its purified major components, due to their chaperone-like activity, are able to suppress the thermal and chemical aggregation of several substrate proteins. In this study, we set out to determine whether whole and β-casein are able to prevent (or attenuate) aggregation accompanying refolding of chemically denatured carbonic anhydrase or to recover lost biological activity after its denaturation. Additionally, we showed attenuated heat-induced fibrillar aggregation of egg white ovalbumin in the presence of these unfolded protein chaperones. Also, some parameters of aggregation kinetics such as order, extent and rate of aggregation, in the presence and absence of aggregation suppressors, were compared. Although β-casein did not prevent both aggregation types as strong as whole casein, they were effective not only in diminishing the aggregation extent of denatured carbonic anhydrase but also in delaying elongation process of amyloid fibril formation with no effect on nucleation phase. We will discuss the importance of these observations.

Keywords: aggregation, casein, carbonic anhydrase

P-10-191-1

Kinetic and thermodynamic analysis of Urmia-Water Buffalo lactoperoxidase activity in skim milk

Hossein Tayefi-Nasrabadi

Department of Biochemistry, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Lactoperoxidase catalyses the oxidation of thiocyanate by hydrogen peroxides, and generates the hypothiocyanite ion, which has proven antibacterial activity in milk. The purpose of this research was to investigate and characterize of kinetic and thermodynamic parameter of lactoperoxidase in skim milk of Urmia-Water Buffalo. The assay was performed in 0.1M phosphate buffer, pH 6.6. The H2O2-mediated oxidation of ABTS was used to assess the peroxidase activity. The mean skim milk lactoperoxidase activity was found 18.01 U.mL-1. Lactoperoxidase lost its enzymatic activity very slowly below 69°C, and at 71°C there was a remarkable increase in the rate of deactivation. The enzyme is more sensitive to increases of temperature above 71°C than to variations in the duration of treatment at lower temperatures. The results of this study confirm that buffalo milk lactoperoxidase

probably is more stable than bovine milk lactoperoxidase toward thermal processes, as suggested by higher value of activation energy.

Keywords: kinetic, lactoperoxidase, skim milk, Urmia-Water Buffalo

---

P-10-299-2

H2O2 and silver ion composition inhibits the growth of catalase positive bacteria

Mojtaba Davoudi [1], Abdorrahim Absalan [2], Parvaneh Talebi [2]*

1- Department of Environmental Health, Faculty of health, Yazd, 2- Department of Biochemistry, Faculty of Medicine, Yazd, Iran

H2O2 is an oxidative agent that functions by oxygen radical production and silver ion can reacts with bacterial DNA and proteins. Then both agents especially in composition together may inhibit bacterial growth. Here we determine the effect of H2O2 and silver ion composition on 3 types of bacteria, including E.coli, Klebsiella and Proteus, which all are catalase positive. We measure optical density (OD) of a bacterial suspension in peptone broth medium with modifications, in 450 nm in the presence or absorbance of H2O2 and silver for 3 days. We also determine bacterial growth during these 3 days by culturing on eosin methylene blue agar (EMB) medium. Here we showed that a composition of H2O2 and silver ion by a concentration of 30 ppm and 30 ppb respectively, reduces or inhibits all the bacterial suspension ODs and growth during the first 72h that we tested ($P<0.05$, ANOVA). It seems that H2O2 distraction by the catalase intensifies its inhibitory function on bacterial growth. We emphasize on the catalase role for inhibition of bacterial growth as we observed its intense reaction with H2O2 such that we could not be able to measure OD until first 24 hours. It should be noted that H2O2 is effective only until not reduces in bacterial environment and after its concentration reduction, mostly by degradation, bacteria, if be viable will have fortune to growth, as we observed in our experience. Also we observed that the most effective impact of noted solution was by this order: Proteus>Klebsiella> E.coli.

Keywords: bacterial growth, bacterial catalase, hydrogen peroxide, silver ion

---

P-10-166-1

In vitro study of Rutin, Taxifolin and Quercetin effect on low density lipoprotein carbamylation

Seyed Mohammadali Ghaffari, Mehrnoosh Shanaki Bavarsad*

Department of Clinical Biochemistry, Faculty of Medicine, Ahwaz Jundishapur University of Medical Science, Ahwaz, Iran

When decreased renal function occurs, the increased amount of urea undergoes spontaneous (chemical-non enzymatic) transformation to cyanate which accumulates in patients with kidney disease. Cyanate acts as a potential toxin, inducing a modification of proteins called carbamylation. In patients with kidney disease, carbamylation of low density lipoprotein (LDL) is a nontraditional risk factor for cardiovascular disease. Carbamylated LDL causes endothelial cell damage and would thus lead to accelerated atherosclerosis in patients with kidney disease. The inhibition of this process especially by natural and safe compounds like flavonoids is of major therapeutic relevance. The influence of three flovonoids on the carbamylation of LDL was studied under in vitro conditions. LDL was isolated from normolipidemic human plasma by sequential ultra-centrifugation. Isolation was confirmed by comparing electrophoretic movement of sample with plasma on 0.8% agarose gel. Protein content was determined by Bradford method. LDL (0.6 mg LDL protein/ml) was incubated with potassium cyanate (20 M) and different concentration of flavonoids for 4 hours at 35°C. Homocitrulline content was determined by colorimetric assay. Electerophoretic movement of samples on polyacrylamide gel compared with carbamylted LDL. Results showed that three flavonoids decreased LDL carbamylation, (as confirmed by electerophoretic movement of samples) in a dose dependent manner .The order of anticarbamylation activities of these flavonoids were: Rutin (69.3%)>Taxifolin (61.71%)>Quercetin (59.92%). According to data in this project we suggest using the flavonoids may decrease the LDL carbamylation related damage in patients with chronic kidney disease and consumption of flavonoids in foods or in dietary supplements can decrease cardiovascular complications in patients with chronic kidney disease.

Keywords: LDL, carbamylation, chronic Kidney disease, Rutin, Taxifolin, Quercetin

---

P-10-199-1

Spectroscopic study on the interaction of celecoxib with human carbonic anhydrase II: Thermodynamic characterization of the binding process

Masomeh Mehrabi [1], Reza Khodarahmi [2], Sirous Ghobadi [2]*

1- Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran, 2- Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

Human carbonic anhydrase II (hCAII, EC 4.2.1.1) is a metalloenzyme which catalyzes the reversible hydration of carbon dioxide to hydrogen and bicarbonate ions. Regarding to the importance of carbonic anhydrase inhibition in the treatment of some diseases such as cancer and glaucoma, in this study the effect of celecoxib (a sulfonamide drug) on the structure and function of hCAII was investigated by various spectroscopic techniques such as UV-Vis, intrinsic and extrinsic fluorescence, circular dichroism (CD) spectroscopy and differential scanning calorimetry (DSC), in 20 mM Tris buffer, pH 7.75. Kinetic results revealed that celecoxib inhibits the esterase activity of hCAII in a linear competitive manner. DSC data and the far- and near-UV CD results along with protein surface hydrophobicity measurements showed that in the presence of celecoxib a minor compactness occurs in the hCAII structure. Stern-Volmer analysis of the quenching data at different temperatures revealed that the intrinsic fluorescence of hCAII is quenched by celecoxib through a static quenching mechanism. The Job's plot indicated that binding of celecoxib to the enzyme occurs via a 1:1 stoichiometry. Analysis of the thermodynamic parameters of binding showed that hydrogen binding and hydrophobic interactions play major role in stabilization of the reversible complex. As the conclusion, celecoxib binding to the active site of hCAII is accompanied by linear competitive inhibition of the enzyme, quenching its fluorescence and inducing some compactness in its secondary and tertiary structures.

Keywords: binding study, Celecoxib, competitive inhibition, fluorescence quenching, human carbonic anhydrase II

P-10-385-1

Study of effect of some osmolytes on thermoinactivation of glutamate dehydrogenase

Elahe Alizadeh, Sirous Ghobadi, Mohammad Hossein Mirmomeni*

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

Thermoinactivation of bovine liver glutamate dehydrogenase (GDH) was studied using UV-Vis spectrophotometry. All the experiments were carried out in 50 mM phosphate buffer, pH 7.6. The effect of osmolytes, including sucrose, sorbitol and proline (0.1-1.5 M), raffinose (0.05-0.25 M) and arginine (0.1-0.8 M) on the remaining activity of the enzyme were measured. Sucrose, sorbitol, proline and raffinose were found to have a strong preserving effect on GDH activity and prevention of its thermoinactivation. Arrhenius analysis of the heat inactivation studies showed that these osmolytes (except arginine) increase the activation energy (Ea) of thermal unfolding of the enzyme. UV-Vis monitored unfolding curves indicated the raising of the midpoint of the unfolding transition temperature (Tm) of the enzyme in the presence of these osmolytes. Each of the possible mechanisms for irreversible thermoinactivation of the enzyme (aggregation & deamidation) was checked. All of the osmolytes in this research decreases the rate of deamidation and prevents the aggregation of the enzyme in a concentration dependent manner. Arginine has the same effect on deamidation and aggregation of the enzyme but with different effect on its thermoinactivation and Tm. The results show that sucrose, sorbitol, raffinose and proline reduce the GDH inactivation rate, increase the Tm of the enzyme and preserves GDH conformation against thermal unfolding and inactivation, whereas arginine acts as a weak protein destabilizer. All of the used osmolytes decrease the deamidation rate of the enzyme and have suppressive effect on the enzyme aggregation in a concentration dependent manner.

Keywords: Bovine liver glutamate dehydrogenase, osmolytes, stabilization, thermoinactivation

---

P-10-344-1

Binding of timolol to bovine carbonic anhydrase II: A spectroscopic study

Soraya Sharifighorveh [1], Sirous Ghobadi [1], Mohammad Joshaghani [2]*

1- Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran, 2- Department of Chemistry, Faculty of Science, Razi University, Kermanshah, Iran

The effects of timolol as an anti-glaucoma agent on the kinetic and structural properties of bovine carbonic anhydrase II (bCAII) was investigated using different spectroscopic techniques such as UV-Vis, intrinsic and extrinsic fluorescence and circular dichroism (CD) in 100 mM Tris, pH 7.5 at 25°°C. Kinetic results revealed that timolol inhibits the enzyme activity up to about 60% at 6 mM via the linear non-competitive manner. Intrinsic fluorescence data showed the quenching effect of timolol on bCAII fluorescence. Analysis of extrinsic fluorescence of 1-anilino-naphthalene 8-sulfonate (ANS) upon binding to BCAII indicated elevated surface hydrophobicity of the enzyme in the presence of the drug. Both the Stern-Volmer and drug mole ratio methods revealed the existence of 1 binding site in the enzyme for timolol. The distance between bCAII and timolol was obtained according to Förster non-radiation energy transfer theory as to be 3.06 nm. Far and near UV CD spectra of bCAII in the presence of timolol showed that the secondary structure content of enzyme is reduced upon interaction with timolol whereas its tertiary structure is relatively unaffected. Analysis of the thermodynamic parameters of binding in 25, 30 and 35°C, indicated the spontaneous nature of the binding process and suggests the involvement of both hydrogen bonding and hydrophobic interactions in timolol binding to bCAII. In conclusion, timolol binds to the enzyme via hydrogen bonding and hydrophobic interactions, inhibits its activity in the linear non-competitive fashion and reduces the enzyme secondary structure content with no considerable effect on its tertiary structure.

Keywords: carbonic anhydrase, Timolol, Quenching, binding study, glaucoma

---

P-10-347-1

Identification of chromatin proteins in Solanum tuberosum

Azra Rabbani chadegani, Asieh Aramvash*

Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Chromatin is a complex of DNA, RNA, histones and nonhistone chromosomal proteins. Identification of these proteins helps us for better understanding of different mechanisms of gene expression. There are several methods for extraction of histones and nonhistone proteins from animal cells, but less information of a comparable state is available for plants. In this study, potato leaf cells directly or 15 days cell suspensions made from the calli that was obtained from potato leaf cells, were extracted with different washing buffer, precipitation and extraction procedures using acids and salts. The results show that extracting with HCl and NaCl, using EDTA and NaCl as washing buffer and acetone precipitation was the best extraction procedure for the histones and HMG proteins in potato leaf cells. The proteins were analyzed on SDS gel and immunoblotted against histones and HMG proteins antisera. For identification of these proteins their calf thymus counterpart used as reference. The results show that histones H3 and H4 are relatively the same as their animal counterparts but H2A and H2B in potato are larger. Also HMG proteins exhibit four main proteins in total tissue extract and their electrophoretic pattern is different from mammalian HMG proteins. The potato leaf HMG proteins could not be detected by immunoblotting against thymus HMG antisera. It is therefore concluded that there is little homology between the chromatin proteins of the animals and potato leaf cells. Also comparison with other plant cells suggests considerable difference.

Keywords: extraction, histones, HMG, non-histone proteins, potato

---

P-10-94-2

Infraction frozen storage in biochemical composition changes of whitefish (RutilusFrisii Kutum) muscles

Zahra Bankehsaz Montazer

Department of Biotechnology, Ecological Academic of Caspian Sea, Iran

Changes in biochemical composition and quality control factors of frozen (-18°C) whitefish (Rutilus Frisii Kutum) muscles were investigated during 120 days of storage. Initial moisture and protein and lipid contents were 73.758%, 19.1%, and 6.01%, respectively, and changed to 71.991%, 18.44%, and 7.999% in the 120 days of storage. TVN and PV were determined during 120 days of storage. The

initial content of TVN and PV were 9.9 mg/100 and 0.513meq/kg, respectively, and changed to 20.2 mg/100 and 1.999meq/kg in the 120 days of frozen storage. All data were significant different ($P<0.05$). The nutritional value and quality control of muscles of whitefish after 120 days were safe for human consumption.

Keywords: whitefish, moisture, protein, lipid, biochemical composition

---

P-10-123-1

The effect of temperature on the amyloid fibril formation of destabilized κ-casein

Elham Shafii, Arezou Ghahghaei, Mohammad Hossein Sangtarash*

Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran

k-Casein is a protein of bovine milk, which has remarkable propensity to form amyloid fibril. It contains two cysteine residues, which joined together by interchain disulfide bond. These cysteines are also capable to form polymers by participating in interchain disulfide linkage. The reduction of disulfide bond in k-casein occurs in the presence of urea or SDS and moderate DTT concentration. Thus k-casein forms amyloid fibrils in a reducing environment. In this study the fibril formation of reduced k-casein at different temperatures (25, 37, 42, 45 and 48°C) were investigated by thioflavin T binding assay, visible absorption, near and far Circular Dichroism (CD) spectroscopy. An increase in the thioflavin T fluorescence intensity upon the increase of temperature reveals that the rate and extent of amyloid formation were significantly temperature dependent, which was verified by visible absorption spectroscopy. Temperature causes structural changes in reduced and non-reduced k-casein, which was supported by near, and far CD spectroscopy. Our findings demonstrated that reduced k-casein readily forms amyloid fibril, which increases by increasing temperature.

Keywords: amyloid, CD spectroscopy, thioflavin T

P-10-293-1

The effect of detergents on the stability of membrane proteins during solubilization and reconstitution: A comparative study

Mahdi Amiri, Mehran Habibi-Rezaie*

Protein Biotechnology Research Lab (PBRL), School of Biology, College of Science, University of Tehran, Iran

Detergents have unique properties that make them a powerful tool for solubilization and characterization of membrane proteins. Protein stability is one of the main challenges in the handling of detergent-solubilized membrane proteins. Whereas membrane proteins can be extremely stable in the lipid bilayer, many membrane proteins are poorly behaved in detergent solution and undergo rapid aggregation or inactivation. The resulting technical problems can make biochemical, biophysical, and structural characterization difficult if not impossible. In the present study, the effect of freezing temperature has been examined on the activity of the membrane protein Lactase-Phlorizin Hydrolase (LPH) in the detergents Triton X-100 and Triton X-114. Although the inactivation rate of LPH activity is rather permissive in these detergents at 37°C, freezing of the detergent extracts leads to a rapid loss of the most of LPH activity prior to completion of freezing. However, unlike Triton X-100, this effect is reversible in Triton X-114 and the lost activity is restored by reconstitution of the detergent extract in the phosphatidylcholine liposomes and subsequent detergent removal.

Keywords: liposomes, protein stability, reconstitution, Triton X-100, Triton X-114

P-10-384-1

Cloning, sequencing, expression and characterization of a Ca2+-independent α-amylase from Bacillus Sp. KRA2: Emphasis on the identification of C-terminal propeptide

Ali Salimi [1], Khosro khajeh [1], Sirous Ghobadi [2], Marzieh Ghollasi [2], Jahan Alikhajeh [2]*

1- Department of Biochemistry, Faculty of Science, Tarbiat Modares University, Tehran, Iran, 2- Department of Biology, Faculty of Science, Razi University of Kermanshah, Kermanshah, Iran

A Ca2+-independent α-amylase secreted from a Bacillus Sp. KRA2 was isolated. The nucleotide sequence of the amylase gene and the amino acid sequences of the gene products indicate that the α-amylase precursor is a preproenzyme composed of a signal peptide (33 residue), a short propeptide in N-terminal (11 residue), the mature α-amylase (422 residue, 48kDa) and a long C-terminal propeptide (193 residue, 21kDa). The complete amylase gene and the fragment encoding the mature α-amylase were cloned and expressed in Escherchia coli BL-21. The recombinant proteins were purified and used for biochemical characterization. While the optimum pH and the end products of starch hydrolysis for both mature and immature enzyme were the same, the kinetic parameters were different. Comparison of kinetic parameters between mature and immature α-amylase showed a lower Km and Vmax for starch and EPS in the later. The B. KRA2 α-amylase was not inhibited by EDTA, suggesting that it does not require CaCl2.

Keywords: amylase, Ca2+-independent, characterization, C-terminal propeptide

---

P-10-398-1

Partial Purification of Inulinase from Aspergillus awamori 16877

Maryam Vatani, Mehran Habibi-Rezaei*

School of Biology, University College of Sciences, University of Tehran, Tehran, Iran

Inulinase is a β-D-Fructofuranosidase (EC 3.2.1.7) which cleaves inulin and oligofructose to yield fructose. It can be used to produce High Fructose Syrups (HFSs) as an alternative sweetener to sucrose in the food industry. Inulinase has been produced in, and purified from various bacterial and fungal species. Aspergillus strains are one of the main inulinase producing microorganisms. Five Aspergillus strains were examined for the production of inulinase and A. awamori 16877 was chosen as the highest enzyme producing strain. The enzyme was partially purified from the culture medium of A. awamori by filtration, ammonium sulfate precipitation, and dialysis and affinity chromatography on ConA-Sepharose 4B. The enzyme was purified 74.38 folds with a specific activity of 278.96 U/mg. This high level of purification and specific activity achieved through a single chromatography step is a significant result compared to other reports in the literature which use multiple purification procedures. In this

study optimum pH and temperature of the enzyme were determined and functional stability of the enzyme was also investigated.

Keywords: inulinase, Aspergillus awamori, purification, affinity chromatography, High Fructose Syrups

---

P-10-422-1

Catalytic properties of Guaiacol peroxidase from Safflower (Carthamus tinctorious L.cv. M-CC-19)

Hossein Tayefi-Nasrabadi [1], Gholamreza Dehghan [2], Behrokh Daeihassani [2], Abbas Samadi [3], Ali Movafegi [3]*

1- Department of Biochemistry, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran, 2- Department of Plant Sciences, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran, 3- Department of Soil Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran

Guaiacol peroxidases are haem-containing enzymes participating in many physiological processes in plants. In this study, guaiacol peroxidase was extracted from Safflower (Carthamus tinctorious L.cv. M-CC-19) leaves using 0.1M phosphate buffer, pH 7.2. H2O2–mediated oxidation of guaiacol at 470nm was used to assess the peroxidase activity. Kinetic studies show that at least three isoenzymes of guaiacol peroxidase were present in safflower extract with pH optima at 4.5, 7 and 8, respectively. Between these isoenzymes of peroxidase, isoenzyme active at pH 7 had highest catalytic efficiency (Vmax/Km) value. The heat incubation study showed that temperature stability of three isoforms of guaiacol peroxidase, at three pH optima followed the pattern as: pH 8> pH 7> pH 4.5.

Keywords: Guaiacol peroxidase, kinetic, safflower, thermal stability

---

P-10-20-1

Hemoglobin investigation via electronic structure methods

Vajihe Sadat Farzadfar, Hamideh Sadat Farzadfarm*

Applied Science and Technology Educating Center of Kashan Worker House, Kashan, Iran

A hemoglobin molecule is composed of a protein group, known as globin, and four heme groups, each associated with an iron atom. In the lungs, each iron atom combines reversibly with a molecule of oxygen. Each hemoglobin molecule also has attached a single cysteine amino acid, which attracts nitric oxide from the lungs. We can calculate electronic energies and atom bonds in hemoglobin with Computational Chemistry (Gaussian software) and investigate spatial models for interaction with cells, iron and other drugs. The adsorption depends on certain properties of the hemoglobin molecule such as functional groups, electron density at donating atoms, aromaticity, and electronic structure of the molecule. Theoretical studies can predict molecular parameters related to the best interaction and adsorption.

Keywords: computational chemistry, Gaussian hemoglobin, interaction, iron

---

P-11-469-1

A thermodynamic study on the binding of zinc with human growth hormone

Elham Tazikeh [1], Ali Akbar Saboury [2], Gholamreza Rezaie Behbehani [2], Majid Monajjemi [2]*

1- University of Gorgan, Gorgan, Iran, 2- Institute of Biochemistry and Biophysics, University of Tehran, Tehran,

A thermodynamic study on the interaction between Zn2+ ion and human growth hormone (hGH) was studied in NaCl solution using isothermal titration calorimetric technique. A new solvation model was used to reproduce the enthalpies of Zn^{2+}-hGH interaction over the whole range of Zn2+ concentrations. It was found that there is a set of three identical and noninteracting binding sites for Zn2+ ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 1.540mM, -17.6kJ mol-1 and 1.930mM, -17.1kJ mol-1, respectively. Zinc binding to the growth hormone leads to the protein destabilization. The extended solvation method is able to correlate the solvation parameters to the effect of metals on the stability of protein in a very simple way according to the equation 1. Values for hGH+Zn^{2+} interactions are negative, indicating that the hGH structure is destabilized, resulting in an decrease in its biological activity. In the Zn2+-rich region values are positive, indicating that Zn2+ stabilizes the hGH structure in Zn2+-rich domain, leading to sustaining of its native or original characteristic, as would be expected. There is no significant aggregation and p values are less than one.

Keywords: human growth hormone (hGH), solvation model

P-10-137-2

Protein surface hydrophobicity, as prerequisite of TFE-induced fibrillogenesis by α-chymotrypsin, triggers early stages of protein aggregation

Reza Khodarahmi [1], Hosnieh Soori [2], Mojtaba Amani [3]*

1- Department of Pharmacognosy and Biotechnology and Medical Biology Research Center, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran, 2- Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran, 3- Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

The misfolding of specific proteins is often associated with their assembly into fibrillar aggregates, commonly termed amyloid fibrils. Despite the many efforts expended to characterize amyloid formation in vitro, there is no deep knowledge about the environment (in which aggregation occurs) as well as mechanism of this type of protein aggregation. Alpha-chymotrypsin was recently driven toward amyloid aggregation by the addition of intermediate concentrations of trifluoroethanol. In the present study, successful approaches such as intrinsic fluorescence and quenching studies as well as chemical modification have been used to elucidate the underlying role of hydrophobic interactions (involved in early stages of amyloid formation) in α-chymotrypsin-based experimental system. We will discuss the importance of these observations.

Keywords: α-chymotrypsin, Aggregation, hydrophobicity, TFE

P-10-523-1

Study of nonenzymatic glycation of transferrin and its effect on iron-binding antioxidant capacity

Mojtaba Rashidi, Mohamad Taghi Goodarzi, Mohsen Rezaei*

Hamadan University of Medical Sciences, Hamadan, Iran

Nonenzymatic glycosylation (glycation) occurs in many of macromolecules. This phenomenon takes place in aging and diabetes because of exposure of biomolecules with high level of glucose. Glycation can change functions, activities and structure of many biomolecules, such as hemoglobin (Hb), albumin, Igs, LDL and DNA. Considering the important role of transferrin in iron transport and antioxidant activity in plasma, this study was carried out to investigate the effect of glycation in these processes. In this study, first human apotransferrin at a concentration of 5 mg/ml was dissolved in sodium phosphate buffer (PBS), and then this solution was incubated with different concentrations of glucose in different time periods (10days and 20days). The rate of glycation was measured using thiobarbituric acid method. The effect of glycation on antioxidant capacity of apotransferrin with two methods (RBC hemolysis and fluorescent) was also investigated. Result showed that rate of glycation of apotransferrin increased with the rise in glucose concentration and with increasing incubation time ($p<0.05$). In present study we showed that antioxidant capacity of apotransferrin decreased as a result of glycation ($p<0.05$). According to these results, a relationship can be suggested to exist between glycation of apotransferrin and oxidative stress that occurs due to hyperglycemia in diabetic patients.

Keywords: transferrin, diabetes mellitus, glycosylation, oxidative stress

---

P-11-529-1

Cytochrome c in sodium dodecyl sulfate reverse micelles: Spectroscopic and structural investigations

*Farzaneh Farivar [1], Yahya Sefidbakht [1], Khodadad Nazari [2], Ali Akbar Moosavi-Movahedi [2]**

1- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, 2- Research Institute of Petroleum Industry, N.I.O.C., Tehran, Iran

The reverse micelles composed of n-dodecane, aqueous phosphate buffer, sodium dodecyl sulfate (SDS) and 1-hexanol were prepared and cytochrome c was directly encapsulated into the aqueous cores of reverse micelles through the self-assembly formation mechanism. Consistent with the replacement of Methionim-80 by another strong field ligand at the sixth heme iron coordination position, cytochrome c exhibited 1-nm soret bond blue shift and ε enhancement in electronic absorption maximum accompanied by disappearance of the 695-nm charge-transfer bond. Circular dichroism (CD) spectrum of cytochrome c exhibited significant changes suggestive of alterations in heme iron microenvironment and conformation. Disappearance of the negative peak in soret region and broadening and ε enhancement of the positive peak (406 nm), consistent with electronic absorption results were indicative of different low spin state, probably alternative low spin state of heme iron. Far-UV CD results showed that a significant amount of secondary structure of cytochrome c remains folded. The fluorescence intensity increased significantly, suggestive of opening of heme crevice. Unfolding of cytochrome c with denaturants usually causes the red shift of fluorescence peak because of exposure of trp-59 to polar solvent but here florescence peak showed blue shift which may be due to the presence of organized water with low dielectric constant in reverse micelles. Small changes in the helical structure, increasing in fluorescence intensity based on tryptophan residue and disappearance of charge-transfer bond in electronic absorption curves imply partial unfolding with unchanged secondary structure suggestive of reaching cytochrome c to a molten globule-like state.

Keywords: alternative low spin state, molten globule, nano encapsulation, anionic surfactant

---

O-10-565-1

Oligomerization status directs overall activity regulation of the Escherichia coli class Ia ribonucleotide reductase

Reza Rofougaran [1], Mikael Crona [2], Munender Vodnala [3], Britt-Marie Sjöberg [3], Anders Hofer [3]*

1- Department of Medical Biochemistry & Biophysics, Umeå University, Umeå, Sweden and Department of Biochemistry, Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran, 2- Department of Molecular Biology and Functional Genomics, Stockholm University, Stockholm, Sweden, 3- Department of Medical Biochemistry & Biophysics, Umeå University, Umeå, Sweden

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.

Keywords: allosteric regulation, Escherichia coli, Gas-Phase Electrophoretic Mobility Macromolecule Analysis (GEMMA), oligomerization, ribonucleotide reductase

---

P-10-567-1

Determination of the beta-casein cleavage sites of the Deg proteases in the cyanobacterium Synechocystis sp. PCC 6803

Ava Hossein Zadeh [1], Helder Miranda [2], Lam Xuan Tam [2], Christiane Funk [2]*

1- Department of Chemistry and Department of Molecular Biology, Umeå University, Umeå, Sweden, 2- Department of Chemistry, Umeå University, Umeå, Sweden

The family of Deg/HtrA proteases is represented in all known groups of organisms, Archaea, bacteria and eukaryotes. It consists of ATP-independent serine type proteases, which are important in protein quality control and they have great impact in degrading misfolded proteins. The aim of this study was to reveal the cleavage sites of the members of this protease family (HhoA, HhoB and HtrA) in the model organism *Synechocystis* sp. PCC 6803 by applying biochemical and molecular techniques. Gene copies coding for *Synechocystis* 6803 wild type as well as inactive Deg proteases were cloned into the pET151/D-TOPO vector, introducing a C-terminal His-tag. After over expression in *E. coli* the recombinant proteins were purified by affinity chromatography and analyzed by SDS-PAGE to determine their purity. To examine the endopeptidase activity of the proteases, purified beta-casein was obtained by gel filtration (FPLC). After degradation, the beta-casein fragments obtained from the PVDF membrane were analyzed using N- and C-terminal sequencing. These findings hopefully will help to elucidate the cleavage sites of the cyanobacterial proteases. The results will be discussed.

Keywords: beta-casein, Deg/HtrA protease,

P-10-478-1

Design of disulfide bridge in Firefly Luciferase

Mehdi Imani [1], Saman Hosseinkhani [2], Shahin Ahmadian [2]*

1- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, 2- Department of Biochemistry, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran

Bioluminescence is a natural phenomenon that occurs in many different living organisms. Firefly Luciferase (Fluc) is the enzyme that catalyses the reaction of light emission in lampyridea family. In the presence of Mg2+, the Fluc catalyses the binding of ATP to D-luciferin followed by a reaction with oxygen molecule to generate oxyluciferin, CO2 and light. Fluc has been used in a variety of applications including rapid detection of pathogens, imaging of cancer cell growth and detection of gene expression. Because of its different applications many efforts have been made to create Fluc with improved properties to achieve these purposes. Thus many mutations have been created on Fluc gene and some of the generated enzymes have higher stability and catalytic activity than native Fluc. Disulfide bonds have been observed in many extracellular and stable proteins and involved in protein stability. Therefore, generation of disulfide bonds in proteins that naturally lacks disulfide bridge is an interesting strategy in protein engineering for improving stability and catalytic activity of an enzyme. In the current work, two disulfide bridges are introduced in photinus pyralis luciferase using site directed mutagenesis. The bacterial strain BL21 (DE3) was used as host for protein overexpression. The structural and functional properties of mutant proteins will be reported.

Keywords: bioluminescence, firefly luciferase, site directed mutagenesis

---

P-10-211-1

Targeting acetate kinase: Inhibitors as potential bacteriostatics

Saeme Asgari [1], Azadeh Ebrahim Habibi [2], Parvin Shariati [3]*

1- Department of Biology, School of Basic Science, Islamic Azad University-Science and Research Branch Tehran, Iran, 2- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran, 3- Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Acetate kinase is a conserved enzyme that is widespread in bacteria and Archaea. Despite the importance of this enzyme in the metabolism of bacteria, limited structural studies have been carried out in this regard. A three-dimensional structure of Escherichia coli acetate kinase was created by molecular modeling methods. By considering the structure of the catalytic intermediate, trifluoroethanol and trifluoroethyl butyrate were proposed as potential inhibiting compounds of the enzyme. Use of the Autodock program revealed that they can be well fitted in the acetate binding pocket. To study the role of these potential enzyme inhibitors in the metabolic pathway of E. coli, their effects on the growth of this bacterium were studied. Escherichia coli were cultured aerobically and anaerobically in M9 medium containing 0.4% glucose as carbon source, at 37°C with shaking at 150 rpm. The results showed that bacterial growth was considerably reduced in the presence of inhibitors, under both aerobic and anaerobic conditions. In order to further study the products of this metabolism, bacterial cells were incubated in the same medium containing either of the above mentioned inhibitors for 4-5 hours at 37°C. Changes in metabolic products were studied by in vivo proton nuclear magnetic resonance (NMR) spectroscopy. Remarkable changes were observed in the quantity of acetate; yet other products were less altered. In this study, an inhibition of growth by the two inhibitors as reflected by a change in the metabolism of E. coli suggests the potential use of these compounds as bacteriostatics.

Keywords: acetate kinase, Autodock program, bacteriostatic, inhibitor, metabolic pathway, molecular modeling

---

O-10-176-1

Interaction of calf thymus DNA with a Diimine Palladium (II) Complex of hexyldithiocarbamato ligand: Thermodynamic, cytotoxic and spectroscopic studies

Hassan Mansouri-Torshizi [1], Maryam Saeidifar [1], Adeleh Divsalar [2], Ali Akbar Saboury [3]*

1- Department of Chemistry, University of Sistan & Baluchestan, Zahedan, Iran, 2- Department of Biological Sciences, Tarbiat Moallem University, Tehran, Iran, 3- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Cisplatin (cis-diamminedichloroplatinum(II)) is currently used clinically and is one of the most effective anticancer drugs in the treatment of a variety of human tumors. Unfortunately, its usefulness is limited by the development of resistance in tumor cells and the significant side effects exhibited by cisplatin have generated new areas of research, which mainly focus on the search for new metal-based complexes with low toxicity and improved therapeutic properties. Recently, palladium(II) complexes have attracted significant attention due to their biological activities. Moreover, palladium complexes having chelating ligands such as N–N-diamines, O–S-donor, N–S-amino-

thioether, diaminoacids, dicarboxylic acids, and the most important dithiocarbamates are the recent advances of palladium(II) complexes. 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)(hex-dtc)]NO3 (where Phen =1,10-Phenanthroline and hex-dtc=hexyldithiocarbamate) in 10 mmol/L of Tris-HCl buffer of pH=7.0. 50% cytotoxic concentration (Cc50) value of this complex against chronic myelogenous leukemia cell line, K562, was much lower than that of cisplatin. The interaction of DNA by this antitumor complex has been investigated in aqueous solution using fluorescence, electronic absorption titration and gel filtration methods. In these interaction studies, binding parameters, thermodynamic parameters, and the types of bindings between this agent and DNA are described as follow: There is a set of 4 binding sites (g) for the complex on the DNA with positive cooperativity in binding. n, the Hill coefficient (as a criterion of cooperativity) was found to be 1.898 at 300K and 2.29 at 310K, respectively. Kapp, the apparent equilibrium constants, are 44.78 mM-1 and 37.21mM-1 at 300K and 310K, respectively. 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 0.012mmol/L at 300K to 0.011mmol/L at 310K. The conformational stability of DNA in the interaction with ligand (ΔG0H2O) was determined to be 7.691kJ/mol and 8.058kJ/mol at 300K and 310K, respectively. Thus DNA is more stable at 310K; i.e., the presence of ligand led to less stability of DNA. Values for m, (a measure of ligand strength for DNA denaturation) are 0.533and 0.603 (kJ/mol).(mol/L)-1 at 300K and 310K, respectively . Also the enthalpy of DNA denaturation by the complex (ΔH0 conformation or ΔH0 denaturation) in the range of 300K and 310K was found out to be 0.44kJ/mol. In addition, the calculated entropy (ΔS0H2O) of DNA denaturation by complex was -0.024 at 300K. The negative value of entropy change is related to the less disorder of denatured DNA with respect to the native DNA. The results of fluorescence titration suggest that the above metal complex presumably intercalates 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 does not break readily.

Keywords: palladium(II) complex, DNA binding, fluorescence, cytotoxicity

---

P-10-576-1

Investigation on the interaction between two different drugs (lomefloxacin and colchicines) and human serum albumin: A spectroscopic description

Masoud Homayoni [1], Azade Habibi [2], Jamshid Chamani [3]*

1- Department of Biology, Faculty of Science, Islamic Azad University-Science and Research Branch, Tehran, Iran, 2- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran, 3- Department of Biology, Faculty of Science, Islamic Azad University-Mashhad Branch, Mashhad, Iran

The interaction of colchicine and lomefloxacin with human serum albumin (HSA) has been investigated using fluorescence spectroscopic approach for the first time. Participation of tyrosine and tryptophan groups in drug–serum albumin complexes is assessed using different excitation wavelengths. At 280 nm the tryptophanyl and tyrosyl residues in serum albumin are excited, whereas 295 nm excites only tryptophanyl residues. Comparison of fluorescence quenching of protein excited at 280 nm and 295 nm allows estimating the participation of tryptophan and tyrosine groups in the complex. Fluorescence data revealed the number of binding sites, n and the binding constant values, K, as 1.5 and 1.22×10^6 $L.mol^{-1}$ for colchicine and 0.57 and 3.75×10^3 $L.mol^{-1}$ for lomefloxacin, respectively. The binding affinities (Ksv) were noticed to be 1.442×10^5 $L.mol^{-1}$ and 2.86×10^5 $L.mol^{-1}$ for colchicine and lomefloxacin, respectively. Florescence quenching was caused by a specific interaction between HSA and lomefloxacin and colchicines, and the quenching arose mainly from static quenching by complex formation. It can also be noticed from the spectra that the interaction of HSA with colchicine led to significant blue shift of the protein fluorescence emission band, especially at high drug concentration, which indicated that trp214 in the binding pocket has been brought to a more hydrophobic environment. The competition of lomefloxacin and colchicines in binding to serum albumin should be taken in to account in the multi-drug therapy.

Keywords: HSA, multi-drug therapy, fluorescence spectroscopy

P-10-531-1

Cholecystokinin inhibition of intestinal glucose transport in vitro

Mojtaba Beheshti Tabar

Department of Biochemistry, Yazd University of Medical Sciences, Yazd, Iran

Gastrointestinal hormones affect glucose metabolism and energy expenditure. In this research the effects of cholecystokinin peptides on intestinal monosaccharide transport were investigated, largely using in-vitro techniques. Measurement of transmucosal flux over 45 minutes with an established mini-Ussing chamber technique indicated that CCK3 and CCK4 had no inhibitory effect when added to the serosal compartment of paired mucosal sheets of guinea pig small intestine. CCK8 (the C-terminal octapeptide of CCK33) sulphated and non-sulphated and an analogue (FPL 1429 KF from Fisons) inhibited glucose and 3H-3-o-methyl-glucose transport from the luminal to the serosal chamber by up to 45% through the concentrations required to do so were high (i.e. greater then 1micromolar). The results indicated that there is a minimum amino acid sequence within the CCK octapeptide that is necessary for transport inhibition.

Keywords: cholecystokinin, glucose, intestine

P-10-577-1

Synchronize fluorescence study of interaction between human lactoferrin as a carrier and lomefloxacin

Neda Tafrishi [1], Azade Habibi [2], Jamshid Chamani [3]*

1- Department of Biology, Faculty of Science, Islamic Azad University-Science and Research Branch, Tehran, Iran, 2- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran, 3- Department of Biology, Faculty of Science, Islamic Azad University-Mashhad Branch, Mashhad, Iran

Lactoferrin is a 673 amino acid Fe3+ binding glycoprotein. Its molecular weight varies between76-80 kDa depending on the number of glycosyl side chain. Lomefloxacin (LMF) is one of the synthetic antibacterial in the third generation which exhibit high activity against a broad spectrum of gram-negative through inhibition of their DNA gyrase. It is used to treat respiratory tract, urinary tract and skin structure infections. Synchronous fluorescence is used to evaluate the conformational changes of lactoferrin. Synchronous method can

provide information about the molecular environment surrounding the chromosphere molecules. The shift in position at maximum emission corresponds to changes of polarity around the chromophore molecule. The effect of (LMF) on the synchronous fluorescence spectra of Lac has been studied in different pH. When wavelength interval is 15 nm, the spectrum characteristic of tyrosine residues in Lac was observed. When $\Delta\lambda$=60nm, the spectrum characteristic of tryptophan residues was manifested. It is apparent that the maximum emission wavelength of tyrosine residues does not have a significant shift. In contrast, an obvious red shift of tryptophan residues was observed, which indicated that the polarity around the tryptophan residues was increased and the hydrophobicity was decreased, yet the microenvironment around the tyrosine residues has no discernable change during the binding process. Three dimensional fluorescence spectrum and their characteristics of Lac and Lac-LMF were analyzed. Obviously, the fluorescence of Lac has been quenched by LMF indicating that a complex between Lac and LMF has formed.

Keywords: lactoferrin, lomefloxacin, synchronize fluorescence, three dimensional

---

P-11-530-1

A thermodynamic study on the binding of, Magnesium and Cobalt ions with Myelin Basic Protein

Azadeh Fallahbagheri, Ali Akbar Saboury*

Institute of Biochemistry and biophysics, University of Tehran, Tehran, Iran

The interaction of myelin basic protein (MBP) from bovine central nervous system with two divalent metal ions (Mg^{+2} and Co^{+2}) was studied by isothermal titration calorimetry (ITC) at 300 K in aqueous solution. The extended solvation model was used to reproduce the enthalpies of metal ion-MBP interaction. The solvation parameters recovered from the solvation model were attributed to the structural change of MBP due to the metal ion interaction. It was found that there is a set of two and three identical and noninteracting binding sites for Mg^{+2} and Co^{+2} ions, respectively. The molar enthalpy of binding for Mg^{+2} and Co^{+2} is 15.4 and 14.5 kJ mol^{-1}, respectively. The association equilibrium constant for Mg^{+2} and Co^{+2} is 48, 42 and 63 μM^{-1}, respectively. We have previously developed a theory to account for the solvation of solutes in mixed solvent systems. Studies within our group are aimed at developing an understanding of how the metal ions and other ligands binding to proteins affect the stability of the biomolecules. One of the unique aspects of our approach is studying the stability of proteins by using the extended solvation model. As a clear understanding of operational stability constitutes an important goal in protein technology, our efforts aimed at elucidation of the structure-stability using the extended solvation model. This model is able to correlate the solvation parameters to the effect of metals on the stability of a protein in a very simple way. Deimination limits MBP ability to maintain a compact myelin sheath by disrupting both its tertiary structure and its interaction with lipids. Mg^{2+}-MBP and Co^{2+}-MBP interactions increase the stability and the biological activity of MBP.

Keywords: Myelin Basic Protein, calcium, magnesium, cobalt, Isothermal Titration Calorimetry, solvation parameters

---

P-11-661-1

Thermotolerance enhancement of Escherichia coli through expression of artemin

Shirin Shahangian, Reza H. Sajedi, Majid Taghdir, Behnam Rasti*

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran

Artemin, a thermostable abundant protein found in *Artemia* cysts, is thought to help encysted *Artemia* embryos survive severe physiological stresses. Artemin is extremely heat-stable, confers stability to transfected mammalian cells against heat and oxidative stresses and inhibits heat-induced aggregation of citrate synthase *in vitro*. Artemin also binds RNA at high temperature *in vitro*, suggesting a role in RNA protection. In the present study, a gene encoding artemin from *Artemia Urmiana* was introduced into *E.coli* using the pET28a expression vector to analyze the possible function of this protein under heat stress. Artemin was purified by affinity chromatography and subsequently evaluated by SDS-PAGE and dot blotting. The transformants were then subjected to heat shock treatment at lethal temperatures. To investigate the artemin chaperoning activity *in vitro*, the refolding of chemically denatured horse radish peroxidase was monitored in the presence and absence of purified artemin. Artemin significantly promoted survival of transformed bacterial cells upon exposure to thermal stress. Cells producing artemin (induced with IPTG) demonstrated much more thermotolerance compared to control cells (non-induced with IPTG). After exposing cells to 47.5°C for 1 h, the survival of induced and non-induced cells was 72% and 16%, respectively. The purified artemin enhanced the efficiency of refolding and activity recovery of denatured horse radish peroxidase in a concentration–dependant manner as well. Based on these *in vivo* and *in vitro* studies, it can be concluded that artemin acts as a molecular chaperone.

Keywords: Artemin, expression, thermotolerance, chaperone

P-10-658-1

Isolation and purification of PSA complex by various chromatographic procedures

Haleh Foroutan, Mohammadhossein Babaei*

Nuclear Biomolecule Laboratory, Radioisotope Department, Nuclear Science Research School, Nuclear Science & Technology Institute, Atomic Energy Organization of Iran

We have explored various chromatographic procedures with the intention of establishing an isolation procedure that would allow us to isolate the amount of prostate specific antigen–α-antichymotrypsin complex from human serum. We used men serum that contained the PSA-ACT complex and free PSA as the first sample. DEAE Sephacel chromatography was carried out at with a linear gradient of NaCl from 0 to 0.5 M. The next step was carried out using Con A Sepharose chromatography. The elution was performed using a linear gradient of methyl-α-D-glucopyranoside from 0 to 5%W/V in the equilibrium buffer. A column containing Sephacryl S-200 was used to separate free PSA from the PSA-ACT complex based on their differences in size. The protein in the eluate was analyzed by electrophoresis method (SDS-PAGE). The total PSA and free PSA were determined by IRMA method. We found that at pH=7.2, both free PSA and PSA-ACT molecules are negatively charged and bound to the DEAE-Sephacyl column. They could be separated from other proteins using a linear gradient of NaCl at pH=7.2. A large quantity of albumin in serum was separated from

free PSA and PSA-ACT molecules by Con A Sepharose column. These two molecules were not separable by Con A chromatography. The large difference in molecular size between free PSA and PSA-ACT complex allowed their separation by gel filtration chromatography on a column containing Sephacryl S-200 appeared to be the best for the separation of free PSA from PSA-ACT and for the removal of other contaminating serum proteins. Finally it is appeared that the combined use of these chromatographic procedures would permit the isolation of pure PSA-ACT complex from human serum that could be used in the preparation of more specific antibodies and a suitable calibrator for a new generation of immunoassay for PSA analysis.

Keywords: α-antichymotrypsin, prostate specific antigen, chromatographic procedures, immunoassay

---

O-10-423-1

Design of new alpha-glucosidase inhibitors based on molecular modeling studies

Azadeh Ebrahim-Habibi [1], Monika Höltje [2], Birte Schmitz [2], Hans-Dieter Höltje [2]*

1- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Dr. Shariati Hospital, Tehran, Iran, 2- Department of Pharmacy, Heinrich-Heine Universität, Düsseldorf, Germany

Glucosidase inhibitors are potential antidiabetic medications, which are able to act on the postprandial increase of blood glucose. Acarbose, voglibose, and miglitol are the three main marketed drugs possessing this mechanism of action. However, due to their side effects, there is an interest to design other specific inhibitors of this enzyme. Thus, a computer-aided study has been performed to propose new inhibitors of alpha-glucosidase. As there is no available crystal structure for this enzyme, a previously designed computational model was used for the docking of known inhibitors into the active site. Docking was performed using Auto dock and moe, and results were cross-checked with GRID. The set of ligands included acarbose, voglibose, miglitol and a tetrahydropyran triol (BOG). Simulations were then run with Gromacs with voglibose and BOG in interaction with the enzyme in aqueous environment. Important residues in these interactions were followed during the time of simulation, and hydrogen bonding and electrostatic interactions were found to be the main interactions type. Based on BOG structure, a pharmacophore was defined and the moe molecules database was searched against this pharmacophore. Of the retrieved results, three compounds were retained and successfully docked into the active site of the enzyme. In summary, new BOG-based structures are proposed here as potential inhibitors of human alpha-glucosidase, with possible antidiabetic effect.

Keywords: alpha-glucosidase, inhibitor, antidiabetic

P-10-670-1

Investigation on the effect of static magnetic field and natural radioactivity on the peroxidation of membrane lipids in Vicia faba

Fatemeh Javani, Parviz Abdolmaleki, Faezeh Ghanati*

Department of Biology, Faculty of Science, Tarbiat Modares University, Tehran, Iran

This investigation was performed to evaluate the influence of the static magnetic field and natural radiation on the peroxidation of membrane lipids in Vicia faba seeds. Fourteen soil samples were collected from Ramsar area, in north Iran, where the annual radiation absorbed dose from background radiation is substantially higher than the 20 mSv/year. The specific activity of the radionuclides of 232Th, 236Ra and 40K were measured by gamma spectrometry. The soil that had the highest radioactivity was selected and ten seeds cultured. Ten other seeds were cultured in control soil. Seeds cultured in high radioactivity soil were once affected by static magnetic field and once without static magnetic field. The level of damage of membranes was determined by measuring malonyldialdehyde as the end product of peroxidation of membrane lipids. The level of the peroxidation of membrane lipids in those plants which were exposed to static magnetic field as well as those cultivated in soils with natural radiation was significantly higher than that of the control groups in control soils. The results suggested SMF and natural rays caused oxidative stress in Vicia faba and the effects of static magnetic field and natural rays together were even higher.

Keywords: oxidative stress, peroxidation of membrane lipids, static magnetic field, Vicia faba

---

P-10-635-1

Partial characterization of anti-human IgG antibodies from egg yolk

Shahla Korani [1], Ali Mostafaie [1], Mitra Korani [2]*

1- Medical Biology Research Center, Kermanshah University of Medical Science, 2- Tehran University of Medical Sciences

Egg yolk is an accessible rich source of immunoglobulin Y (IgY) that is suitable for medical diagnosis and therapy of microbial agents. In the present study human IgG was used to immunize the chicken in order to purify the produced anti IgG antibody from the yolk. The specific IgG was extracted from the yolk using acidic distilled water solution method and purified by absorption chromatography. Isoelectric points of the purified antibody were estimated using isoelectric focusing. In addition, the specific activity of the purified product was assayed in presence of detergent, urea and heat treatment, respectively. The isoelectric points of the heavy and light chains of IgY were estimated to be 5.2–5.4 and 4.8-5.1, respectively, for the first time by the present investigation. The results of our study indicated that in high temperatures of 80˚C and 100˚C, the activity of IgY is lost. It was found that urea and anionic detergents (SDS) destabilize IgY. This product can be used for diagnosis of IgG in different diagnostic tests.

Keywords: human IgG, IgY, affinity chromatography

P-10-655-1

Purification and characterization of alpha-amylase from Bacillus sp. GHA1

Adel Ahmadi, Khosro Khajeh, Sirous Ghobadi*

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

Amylases are among the most important industrial enzymes in which the enzymes from microbial sources such as Bacillus species generally meet industrial demands. Programs for finding the new microorganisms for enzyme production are increasing around the world. In this work, mineral springs in Kermanshah and Ilam provinces in the west of Iran have been surveyed for finding the new

microorganisms producing amylase. Bacillus sp. GHA1 (has been registered in Genbank, Accession Number EU109536) was selected for the production of amylase. Maximum production of the enzyme by this strain occurs at 42°C, pH 6.5 and 72 h after cultivation in production medium. The enzyme was purified using ammonium sulfate precipitation, ion exchange and hydrophobic interaction chromatography, resulting in a single band with an apparent molecular weight of 66 kDa, as judged by SDS-PAGE. The new extracellular alpha-amylase is active in a wide range of pH with its maximum activity at pH values 5.5-8.0. The optimum temperature for enzyme activity is 57°C and the presence of calcium has relatively low influence on its activity and thermostability. These findings may be valuable in that the Bacillus sp. GHA1 alpha-amylase may find some applications in detergent and food industries.

Keywords: alpha-amylase, Bacillus sp. GHA1, pH profile, Broad range of pH, Industrial applications

---

P-10-101-1

The comparison of non-Michaelis-Menten behavior of adenosine deaminase at physiological and pathological temperatures

Shiva Kalantari [1], Seyedeh Zahra Moosavi-Nejad [2], Mostafa Rezaei-Tavirani [2], Amin Rostami [3], Minoo Shahani [4], Mohammad Hossein Heidari [5], Abdo Reza Ardeshiri-Lajimi [5]*

1- Clinical Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University (M.C.), Tehran, Iran, 2- Department of Biology, Faculty of Science, Alzahra University, Tehran, Iran, 3- Novin Institute of Research and Industrial Services, Tehran, Iran, 4- Science and Researches Branch, Islamic Azad University, Tehran, Iran, 5- Department of Basic Sciences, Faculty of Paramedical Sciences, Shahid Beheshti University (M.C.), Tehran, Iran

Adenosine deaminase (ADA) is a (β/α)8 enzyme, which deaminates (deoxy)adenosine to (deoxy)inosine irreversibly. ADA is widely distributed in mammalian tissues and its role is critical in proliferation, maturation and function of lymphoid cells. The previous studies showed that structure and activity of ADA are sensitive to changing of temperature. In this study we have demonstrated that ADA has more than one binding site for its substrate (adenosine) with a complex allosteric effects at two different temperatures (37 and 42ºC) by studying the changes in activity of ADA in physiological and pathological temperatures and a regulatory role for adenosine (substrate) was described. The enzyme assay in Tris buffer 50 mM, pH 7.5 in different concentrations of adenosine showed that saturation curve of the enzyme has non-Michaelis-Menten behavior at both 37 and 42ºC, but the enzyme activity is decreased at 42ºC. Moreover the saturation curves have several phases that makes the data analysis more complicated. Therefore, the data was analyzed using some theoretical tools via drawing Eadi-Hofstee and Clearance Plots as well as Hill Plot. It seems that ADA has more than one site for binding of adenosine which has allosteric effect on the enzyme activity. Three sequential autoactivation (positive cooperation) can be observed at 37ºC, but one positive and two negative cooperative phases at 42ºC. All in all the decrease of efficiency of enzyme in fever condition might influence some processes that adenosine is involved in them such as inflammation, signal transduction and so on.

Keywords: allosteric effect, autoactivation, non-Michaelis-Menten behavior

---

P-11-705-1

Determining features contributing to pH activity of enzymes by using neural networks modeling

Mansour Ebrahimi [1], Esmaeil Ebrahimi [2], Mahdi Ebrahimi [3]*

1- Green Research Center, University of Qom, Iran, 2- Department of Crop Production & Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran, 3- Department of Information Technology, International University in Germany, Bruchsal, Germany

Most enzymes are active in limited pH range but some industries need enzymes active in both alkaline and acidic pH so understanding the main factors contributing to pH resistance of theses enzymes are very important. Here we looked at more than seventy properties of 30 xylanase enzymes – as an example- (active in different pH) by applying a feature selection algorithm which assigned a p value to each attribute based on the asymptotic distribution of a transformation on the Pearson correlation coefficient. The attributes were then sorted in a descending order of their importance to xylanase pH resistance based on calculated p values. The results showed that the frequency of Arg, Ser, Pro, Tyr, the count of Arg, Pro, Trp, Gly, Leu, Gln, the frequency of positively charged residues, the count of hydrophobic residues, the count of positively charged residues, non-reduced cysteine extinction coefficient and reduced cysteine extinction coefficient were the most important features contributing to the resistance of xylanases at different pH, and thirteen other attributes were considered to have a marginal contribution to this function. The results confirmed that this method may be used for determining other enzymes' features.

Keywords: bioinformatics, neural Networks, modeling, xylanase, pH

O-11-705-2

Application of neural networks methods to define the most important features contributing to xylanase enzyme thermostability

Mansour Ebrahimi [1], Esmaeil Ebrahimi [2], Mahdi Ebrahimi [3]*

1- Green Research Center, University of Qom, Ira n, 2- Department of Crop Production & Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran, 3- Department of Information Technology, International University in Germany, 76646 Bruchsal, Germany

Thermostable enzymes are in high demands in many industries and understanding the features involved in enzyme thermostability is inevitable task. Here we have looked at features contributing to Endo-1,4,β-xylanase (EC 3.2.1.8) thermostability, the key enzyme with possible applications in waste treatment, fuel and chemical production and paper industries. We trained different neural networks with/without feature selection and classification modeling on all available xylanase enzymes amino acids sequences to find features contributing to enzyme thermal stability. The count of Lie (0.326) and Glu (0.324) showed the strongest direct correlation while the count of oxygen (-0.38) and frequency of Gln (-0.299) reversely correlated to xylanase enzyme thermostability. In 6 out of 24 neural networks generated here, the frequency of Gln was the most important feature contributing to optimum xylanase temperature. Considering the analytical and performance evaluation, multiple models generated in modeling without feature selection and validation set showed to be a good candidate for testing thermostability in 7030 virtually generated Bacillus halodurans mutants. We applied this model on those mutants

and in some of them up to 10ºC thermal stability improvement were observed.

Keywords: bioinformatics, neural networks, modeling, xylanase, thermostability

---

P-10-632-1

Determination effective factors of lipid metabolism at rest and during exercise

Arash Saadatnia, Amir Rashid Lamir*

Department of Physical Sciences, Faculty of Physical Education, Ferdowsi University, Mashhad, Iran

The purpose of this review study was to determine effective factors of fat mobilization and metabolism. With considering 23 Latin articles, authors proceeded to collect these factors and concluded the results. Increased rate of physical activity causes an increased energy expenditure and fat oxidization capacity. Also age, sex, exercise intensity, and nutritional manner are other effective factors in these lines. Uptaking blood triacylglycerols by muscles is regulated by insulin in interaction with glucagon. During exercise, muscle contraction upregulates insulin and compensates its diminished levels within blood. Furthermore, exercise in 63%vo2 maximum intensity is likely to maximize fat oxidization rate. In this study the roles of chatecholamines and lipolytic enzymes has been reviewed. And finally adaptation which occurs during fat metabolism has been explained. Importance of this study has two aspects, first showing instructions to athletes for developing their performance and as second guidelines for preventing metabolic diseases.

Keywords: fat metabolism, fat mobilization, lipolysis, exercise

---

P-10-557-2

Increased stability of luciferase towards proteolysis by DMSO

Farangis Ataei, Saman Hosseinkhani, Khosro Khajeh*

Department of Biochemistry, Faculty of Basic Science, Tarbiat Modares University, Tehran, Iran

Luciferase is the enzyme that catalyzes the light-emitting reaction in bioluminescent organisms and has been used extensively for sensitive applications in biotechnological processes. This enzyme is unstable against environmental proteolytic contaminations. In this study, the effect of Dimethyl Sulfoxide (DMSO) on the stability of luciferase against protease degradation was investigated. The purified recombinant luciferase was incubated by various amounts of DMSO, and then trypsin or chymotrypsin was added. Reactions were terminated by adding PMSF and samples were subjected to SDS-PAGE gel. As a main result, our data revealed that DMSO protects luciferase against proteolysis in a concentration-dependent manner. Remaining activity measurements, intensity of luciferase bioluminescence, intrinsic fluorescent and ANS binding were carried out to elucidate the effect of DMSO on the structure of the luciferase. Results indicated perturbation of native structure. It is proposed that DMSO has an important effect to stabilizing the conformation of the luciferase, prevent binding and adaptation of the protein substrate at the active site of the proteases, thereby the extent of proteolysis is reduced and its active conformation kept.

Keywords: luciferase, proteolysis, trypsin, chymotrypsin, DMSO

P-10-712-1

Enhanced heme degradation of hemoglobin in diabetes

Fatemeh Rezaie [1], Mahzad Sharifahmadian [1], Mehran Habibi-Rezaei [1], Ali Akbar Moosavi Movahedi [2]*

1- School of Biology, College of Science, University of Tehran,
2- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Protein glycation and radical formation are the most important complications of diabetes. Glycated proteins lose their normal functions. Intact hemoglobin in diabetic situation at first oxidize to methemoglobin by free radical species and then degrade during glycation process. Hemoglobin from human was purified. Samples with different percentage of methemoglobin were prepared and glycation situation was simulated. Heme degradation products were identified by spectroscopic and representative fluorescence species. By increasing the level of methemoglobin in glycated samples, fluorescence products of heme degradation were increased. During glycation, the presence of FeIII makes metHb more susceptible toward heme oxidation and degradation.

Keywords: methemoglobin, heme, glycation, spectroscopy

P-11-678-1

Thermodynamic study of intermediate state of papain at different pH condition induced by n-alkyl sulfates: a spectroscopic description

Masome Heshmati, Jamshid Chamani*

Department of Biology, Faculty of Science, Islamic Azad University-Mashhad Branch, Mashhad, Iran

Papain assumes a native conformation at pH 5, while the conformation of pH 3.2 is partially unfolded state. Here we report the presence of intermediate state under acidic and native condition in the presence of n-alkyl sulfates including sodium octyl sulfate, sodium decyl sulfate, and sodium dodecyl sulfate. A systematic investigation of n-alkyl sulfates induced conformational alteration in acid and native unfold state of papain was examined by tryptophan fluorescence and 1-anilino 8-sulfonic acid binding and UV absorbance. Addition of increasing concentrations of n-alkyl sulfates at acidic pH, to partially unfolded state shows decrease in tryptophan fluorescence and in quenched ANS fluorescence but to native state leads to enhancement in tryptophan fluorescence and increase in ANS fluorescence. In the presence of n-alkyl sulfates two different intermediate state I(1) and I(2) were obtained at acidic and native pH, respectively. These results altogether imply that the n-alkyl sulfates induced intermediate state at acidic pH lie between the partially unfolded state and like-native state and at native state lies between the native state and unfold. The addition of n-alkyl sulfates to the partially unfolded state and native state of papain in different pH condition appears to support the stabilized form of intermediate state. Based on the results obtained, the merits of two models of the protein-surfactant structure are discussed for various n-alkyl sulfates concentration in inducing the intermediate state at two different pH conditions.

Keywords: papain, intermediate state, spectroscopy, stability

---

O-11-677-1

Spectroscopic and calorimetric studies of the interaction between paclitaxel and human serum albumin in the presence of tamoxifen

Jamshid Chamani

Department of Biology, Faculty of Science, Islamic Azad University-Mashhad Branch, Mashhad, Iran

The interaction of paclitaxel with human serum albumin (HSA) in the presence of tamoxifen HSA been investigated by using different spectroscopic and calorimetric approaches for the first time. Fluorescence data revealed the presence of a specific binding site on HSA for paclitaxel and the binding affinity was 2.41 x 10^{-5} L mol^{-1} in the physiological condition. The HSA-paclitaxel interaction caused an obvious red shift of the UV absorption band I of paclitaxel and paclitaxel bound to the protein as an anionic species with deprotonation of one hydroxyl group on B ring in the physiological condition. It was also noticed that the level of protonation of the hydroxyl groups played an important role during the drug-protein. At lower concentrations of tamoxifen, most of the tamoxifen reacted with the paclitaxel-HSA, but free tamoxifen was present at higher concentrations. The results of synchronous fluorescence spectra and three-dimensional fluorescence spectra showed that binding of paclitaxel to HSA can induced conformational changes in HSA. The interaction between paclitaxel and HSA in the presence of tamoxifen induced an obvious reduction of the protein alpha helix and beta-sheet structures. The second derivative of HSA fluorescence spectra in the presence of paclitaxel were characterized in order to describe changes in the tryptophan environments of proteins. The relative peak composition of the HSA derivative spectra excited at 295 nm is indicative of the rather hydrophobic environment of its tryptophan residue (H = 0.95), as expected from HSA structures.

Keywords: Anti-cancer drugs, HSA, Spectroscopic methods, Calorimetry

---

P-10-727-1

Saturated and unsaturated fatty acids as tyrosinase effectors!

Reyhaneh Sariri, Fatemeh Shabani, Vahab Jafarian*

Department of Biology, University of Guilan, Rasht, Iran

Tyrosinase (monophenol, dihydroxy-L-phenylalanine: oxygen oxidoreductase EC 1.14.18.1) is a monooxygenase that catalyzes both, o-hydroxylation of monophenols (cresolase activity) and their subsequent oxidation to o-quinones (catecholase activity). The o-quinones polymerize to synthesize melanin or other pigments. The hair bulb is the only site of pigment production for the hair shaft. Loss of the hair shaft melanin is associated with decrease of tyrosinase activity in the bulb of melanocytes. In this study, the effect of various concentrations of saturated and unsaturated fatty acids on tyrosinase activity was investigated. Dopamine hydrochloride was used as tyrosinase substrate and the progress of enzymatic reaction was followed spectrophotometrically. The results showed that, while unsaturated fatty acids were negative effectors, saturated fatty acids could react as positive tyrosinase effectors, i.e. activators. Kinetic studies showed that activator effect of saturated fatty acids depends directly on both their concentration and chain length. Therefore, stearic acid was more potent than palmitic and lauric acid as tyrosinase activator. This was a surprising, interesting and promising result as activators of tyrosinase, although rarely studied, can be of interest for prevention of hair graying, a dream of almost every body! These types of effects could be thought as natural alternatives for hair dying.

Keywords: tyrosinase, activator, fatty acid, saturated, unsaturated

---

P-10-317-1

The effect of amino acid substitution of pro 473 on thermostability and structural properties of firefly luciferase

Zahra Aminibayat, Mahboobeh Nazari, Saman Hosseinkhani*

Department of Biochemistry, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran

Luciferases are the enzymes that catalyze the light-emitting reactions in bioluminescent organisms. Nowadays luciferases have found a wide range of bioanalytical applications that include monitoring gene expression, protein localization and protein–protein interactions, as well as detection of infections, tumor growth and metastasis in whole animals, reporter gene assays and detection of bacteria and environmental contamination. One of the most important issues in the application of luciferase is low thermostability. For this reason, engineering of this enzyme for thermostability is a particularly exciting and challenging field, as it is crucial for broadening its industrial application. In this study thermostability prediction servers were used for appropriate amino acid substitution. After theoretical investigation, pro 473 in Lampyris turkestanicus Luciferase is mutated and its effect on thermostability and structural properties are investigated.

Keywords: luciferase, Lampyris turkestanicus, mutagenesis, thermostability

---

O-11-757-1

Effect of calcium binding on the functional properties of camel alpha-Lactalbumin

Maryam Salami [1], Reza Yousefi [2], Mohammad Reza Ehsani [2], Ali Akbar Saboury [2], Ali Akbar Moosavi-Movahedi [3]*

1- Department of Food Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, 2- Institute of Biochemistry and Biophysics, University of Tehran, 3- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Alpha-Lactalbumin (α-La) is a small, acidic and hydrophilic metalo-protein, which is present in milk of all mammals. As the principal protein in human and camel milk, alpha-lactalbumin (α-La) possesses attractive nutritional value with potential applications for food products, especially infant formula. The tightly bound Ca+2 ion of α-La has a large influence on tertiary structure, molecular stability and biological properties of this protein. Among the important bioactivities is the antioxidant activity. The present in vitro study was carried out in order to investigate the effect of calcium depletion on the functional property (anti-oxidant activity) of camel α-La. The calcium depleted species (molten globule) of α -La was generated after dissolving the purified protein in phosphate buffer (20 mM, pH 7.8) including 1mM ethylene glycol tetra acetic acid (EGTA). The antioxidant activity of α-La was determined by a 2, 20-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid (ABTS)-based method. The results revealed a significantly greater anti-oxidant activity of calcium depleted species than those of native α-La. The antioxidant activity of a protein depends on both the amino acid composition and accessibility of the amino acids therefore it can be concluded that the more opened structure of the protein in the

absence of calcium leads to higher antioxidant activity. Since the acidic environment of stomach easily releases the calcium bound of α-La, thus calcium depleted species of this protein is suggested as a new example of a naturally occurring molecule with the improved functional properties.

Keywords: camel alpha-lactalbumin, calcium-depleted species, functional properties

---

P-10-574-1

Structure-function analysis of brevinin 18 analogue

Hanieh Hosseinnejad Ariani, Hossein Naderimanesh, Safeih Soofian*

Tarbiat Modares University,

Antibacterial peptides from different sources have opened new horizon to medicine science due to their strong capabilities. Brevinin 18, a truncated form of Brevinin-1E, is extracted from frog skin of Rana genus (Rana Ridibunda) which is native of north of Iran. This peptide with 18 amino acids and one intra-disulfide bridge has been investigated and its antibacterial feature in amide form has been reported in 1998. For improving peptides capabilities, their sequences can be manipulated in designs and they can be synthesized chemically. Brevinin 18 analogues were designed according to peptidomietic strategies and they were synthesized by solid phase peptide synthesis (SPPS) method. Minimum inhibitory concentration (MIC) has been assayed and antibacterial effect was determined for the analogues. Structural studies on these peptides were carried out by circular dichroism (CD). The influence of disulfide bond and D-amino acids on structure and activity of peptides has been discussed. The hemolytic assay revealed no lyses in red blood cells in determined concentration.

Keywords: antibacterial assay, Brevinin 18, circular dichroism, disulfide bond, peptidomimetics

---

P-11-777-1

A comparative study on the stability of bovine and camel α-Lactalbumin: Effects of temperature and N-Dodecyl Trimethylammonium Bromide

Maliheh Sadat Atri, Ali Akbar Saboury, Reza Yousefi, Ali Akbar Moosavi-Movahedi*

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Alpha-Lactalbumin (α-La), a small acidic globular protein, participates in lactose biosynthesis as the regulatory subunit of the lactose synthase complex. A folding variant of α-La induces cell death in tumor cells. Thermal denaturations of bovine and camel α-La in the calcium-saturated states were compared using differential scanning calorimetry. Also denaturation of bovine and camel α-La has been studied using n-dodecyl trimethylammonium bromide (DTAB) as a denaturant by fluorescence spectroscopy. Melting points obtained from differential scanning calorimetry showed that camel α-La is more resistant against thermal denaturation. Two state transitions of both α-La monitored by red shift of intrinsic fluorescence maxima, which is accompanied with Trp exposure to the solvent and loss of tertiary structure. The concentration of DTAB in the midpoint of transition, [DTAB]1/2, was higher for camel α-La than that of bovine counterpart. We used the Pace theory to evaluate the Gibbs free energy in the absence of denaturant (ΔG o(H2O)) as a parameter for determination of protein stability. The results show that camel α-La is more stable, by 12kJ mol-1, than that of bovine α-La. Hence, camel α-La is more stable against thermal denaturation as well as against denaturation by DTAB as a surfactant denaturant. The difference in stability between two α-La proteins may be related to the greater contribution of hydrophobic interactions to the stability of camel α-La than in its bovine counterpart.

Keywords: Alpha-Lactalbumin, camel milk, DTAB, protein stability

---

O-10-778-1

Substrate brownian dynamics nearby active site of enzyme entrapped inside reverse micelle

E. A. Ermakova [1], N. L. Zakharchenko [1], O.S. Zueva [2], Yu. F. Zuev [2]*

1- Institute of Biochemistry and Biophysics of Russian Academy of Sciences, Kazan, Russian Federation, 2- Kazan State Energetic University, Kazan, Russian Federation

Surfactant-based reverse micelles are widely used to study enzyme action in environment which from on side is close to natural one and from other one brings new peculiar properties to enzymatic action. Brownian Dynamics simulation has been applied to analyze the influence of electrostatic field of reverse micelle on the process of enzyme-substrate complex formation inside reverse micelle. The probability to form the enzyme-substrate complex by serine protease (trypsin) and specific hydrophilic cationic substrate Nα-benzoyl-L-arginine ethyl ester have been studied within the framework of the encounter complex (EC) formation theory. It has been shown that the surfactant charge, dipole moments formed by charged surfactant molecules and counterions, permittivity of the inner core of reverse micelle can be used as regulatory parameters to make alterations in substrate orientation nearby the enzyme active site and to change probability of the enzyme-substrate complex formation inside reverse micelle.

Keywords: enzyme-substrate complex, reverse micelle, brownian dynamics simulation

---

P-10-631-1

Interaction studies of 2,2'-bipyridineoctyldithiocarbamatoplatinum(II) nitrate with DNA

Hassan Mansouri-Torshizi, Fatemeh Khosravi, Maryam Saeidifar*

Department of Chemistry, University of Sistan & Baluchestan, Zahedan, Iran

For long dithiocarbamates have been evaluated for their efficacy as inhibitors of cisplatin induced nephrotoxicity; they were shown to protect against cisplatin renal injure in several animal models by reversing cellular damage. In this work an antitumor complex of formula [pt(bpy)(oct-dtc)] NO3 (where bpy is 2,2'-bipyridine, and oct-dtc is octyldithiocarbamate has been interacted with DNA using UV-Visible isothermal titration method in Tris-HCl buffer solution containing 25mmol/L sodium chloride (pH=7.0) at 300K and 310K. There is a set of 6 binding sites (g) for the complex on DNA (per 1000 nucleotides) with positive cooperativity in binding. n, the Hill coefficient was found to be 2.47 at 300K and 2.40 at 310K, respectively. Kapp, the apparent equilibrium constants are 51.16 and 146.74 (mmol/L)-1 at 300K and 310K, 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 0.061mmol/L at 300K to 0.055mmol/L at 310K. The conformational stability of DNA in the interaction with complex ($\Delta G°H2O$) was determined to be 8.945 and 8.298 kJ/mol at 300K and 310K, respectively. Thus DNA is more stable at 310K; i.e., presence of complex led to decrease the stability of the DNA. Values for m, (a measure of complex strength for DNA denaturation) are 133.1 and 150.89 (kJ/mol).(mol/L)-1 at 300K and 310K, respectively. Also the molar enthalpy of DNA denaturation by the complex ($\Delta H°$confromation or $\Delta H°$denaturation) in the range of 300K and 310K was found out to be 271.05kJ/mol. In addition, the calculated molar entropy ($\Delta S°H2O$) of DNA denaturation by the complex is 0.87kJ/mol at 300K. The positive value of entropy change is related to the less disorder of denatured DNA with respect to the native DNA. Gel chromatogram obtained from experiment gel filtration has shown two peaks at two wavelengths 321 and 258 nm, which indicate that metal complex have not separated from DNA and their bindings with DNA are strong enough and do not break readily.

Keywords: dithiocarbamate, Pt(II) complex, gel filtration, DNA binding, spectrophotometer

---

P-10-828-1

New method for reducing intercellular flux measurements in metabolic flux analysis

Mahdi Pesaran, Valiollah Babaeipour*

Biochemical Engineering Group, Biotechnology Research Center, Malek Ashtar University of Technology, Tehran

Metabolic fluxes are the most fundamental measure of cell physiology. Metabolic flux analysis (MFA) methods use external flux and isotopic measurements to quantify the magnitude of flows in metabolic networks. Whenever, sufficient fluxes are measured, the best solution can be resulted by over-determine systems. In this study, a new method is described for modifying under-determine systems to determinate or over-determine systems. Some rigid points are recognized from proposed metabolic model and then, they are considered as measured fluxes. More to the point, for suggested procedure validating, experimental data from other authors is examined. Thus, by applying this procedure, number of intercellular enzyme reaction rate assay and isotope labeling will be reduced.

Keywords: metabolic flux analysis, over-determine system, determinate systems, rigid point value, under-determine system

---

P-10-849-1

Optimizing the purification of lipid transfer proteins from rice seeds

Fakhroddin Yousefi [1], Ali Mostafaie [2], Sirous Ghobadi [2]*

1- Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran, 2- Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Plant non-specific lipid-transfer proteins (nsLTPs) are small basic proteins, which transport phospholipids between membranes. The nsLTPs found in plants have important roles in plant systems such as antimicrobial activity and have a potential for drug binding and delivery. In order to purify the nsLTPs, rice paddy flour was stirred in sulfuric acid (50 mM) for 4 h and centrifuged to remove particulate matter. The pH of the supernatant was adjusted to 7.8 and incubated at 4°C for 12 h. The precipitate was equilibrated with 50 mM sodium acetate buffer, pH 4, and after adding actinidin (for eliminating the interfering proteins), it was incubated at 37°C for 3 h. The sample was dialyzed against 50 mM sodium phosphate buffer, pH 7.2, and was loaded onto a CM-Sepharose column which had been pre-equilibrated with the phosphate buffer. Bound proteins were separated by a discontinuous gradient of 0-0.3 M NaCl. The eluted fractions of ion-exchange column were analyzed by SDS-PAGE whose results confirmed the homogeneity of the purified protein at 0.2 M NaCl. Buffer tests using sodium acetate (pH 4), sodium citrate (pH 5.5) and phosphate buffer (pH 7.2) show that actinidin acts very effective in sodium acetate buffer rather than other buffers. The results suggested that ion-exchange chromatography on CM-sepharose column along with actinidin under the above conditions may be a convenient method for purification of nsLTPs.

Keywords: nsLTP, purification, actinidin, CM-Sepharose, SDS-PAGE

P-10-668-1

The effect of vitamin C on 3,4-methylenedioxymethamphetamine (MDMA)-induced-nephrotoxicity toxicity in mice

Massumeh Ahmadizadeh

Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

The substance, 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy, is an amphetamine derivate. Increasing evidence indicated that this agent produced nephrotoxicity. The aim of the present study was to investigate the effect of vitamin C on ecstasy induced renal damage. Adult male mice were pretreated with 300 mg/kg vitamin C (vitC). Control group received vehicle only. 30 min later, animals were given MDMA at doses 0, 10, 20 or 30 mg/kg. 24 h later, animals were killed with over dose of sodium pentobarbital. Blood was collected for determination of blood urea nitrogen (BUN) and creatinine (CR). Kidney tissues were removed, fixed and processed for light microscopy Results of the present study showed that MDMA produced dose-dependent manner injury in kidney cells. Vit C diminished cells against MDMA-induced toxicity. These findings further support the view that ecstasy and/or its metabolite(s) may cause oxidative stress and potential free radical damage in kidney. Vit C has ability to reduce kidney nephrotoxicity.

Keywords: kidney, MDMA, mouse, vitamin C

O-10-883-1

Bacterial dihydroxyacetone kinases: Structure and function in metabolism and transcription control

Christoph Bachler, Ulrich Baumann, Sandra Christen, Bernhard Erni, Luis Garcia-Alles, Jean-Marc Jeckelmann, Christian Siebold, Annapurna Srinivas, Andreas Zurbriggen*

Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

Dihydroxyacetone (Dha) kinases are a family of structurally conserved enzymes, which utilize either ATP (in animals, plants and eubacteria) or phosphoenolpyruvate (PEP; in eubacteria) as phosphate source. Bacterial dihydroxyacetone (Dha) kinases have signaling in addition to

their metabolic functions. X-ray crystallography, biochemical and reporter-gene assays were used to characterize the ATP-dependent Dha kinase of *Citrobacter freundii* and the PEP-dependent kinases of *Escherichia coli* and *Lactococcus lactis*. ATP-dependent kinases are homodimers of subunits with swapped domains. The DhaK domain binds Dha covalently in hemiaminal linkage to N 2 of an invariant histidine; the DhaL domain assumes a novel eight-helix barrel fold that contains the nucleotide binding site. PEP-dependent kinases consist of three subunits: DhaK, DhaL, and DhaM. DhaL contains ADP as a tightly bound cofactor which is rephosphorylated *in situ* by DhaM. DhaM mediates phosphate transfer from the bacterial PEP: sugar phosphotransferase system (PTS) to the Dha kinase in the following sequence: PEP=>EI=>HPr=>DhaM=>DhaL::ADP=>Dha. The DhaL and DhaK subunits of the PEP-dependent *Escherichia coli* kinase act as coactivator and corepressor of DhaR, a transcription factor from the AAA^+ family of enhancer binding proteins. In *Lactococcus lactis* DhaQ, a homolog of DhaK acts as transcriptional coactivator of the transcription factor DhaS of the tetracyclin resistance (TetR) family.

Keywords: AAA+-ATPase, enhancer binding protein, nucleotide binding, phosphortransferase, PTS

P-10-890-1

We have several chemicals that inhibit fibril formation. One of them appears to effect the oligomer formation, a step before formation of mature fibrils.

Mostafa Kamizi, Hamid Reza Kalhor*

Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran

Formation of amyloid fibril causes a number of neurodegenerative diseases in human including Parkinson, Alzheimer and Huntington. Understanding the mechanism of amyloid formation can be helpful in finding cure for those debilitating diseases. We have been investigating the effect of various chemical compounds including ionic and ionic liquid compounds on the formation of amyloid fibril in vitro using hen egg white lysozyme (HEWL) as a model protein. In our procedure, we incubated HEWL under low pH and high temperature in the presence and the absence of various chemical compounds. Subsequently we measured the amount of amyloid fibril formed in our in vitro assay by various techniques such as amyloid specific dye binding assays and transmission electron microscopy (TEM). We also examine the unfolded states of the protein oligomers using Circular Dichroism (CD), ANS binding assay, and etc. Our preliminary results indicate that few of the compounds tested have an inhibitory effect on the amyloid formation in vitro.

Keywords: amyloid formation, oligomers, ionic liquids

O-10-60-1

NEMO binding to linear ubiquitin chains is essential for NF-κB activation

Simin Rahighi [1], Fumiyo Ikeda [2], Masato Kawasaki [2], Masato Akutsu [2], Nobuhiro Suzuki [2], Ryuichi Kato [2], Tobias Kensche [2], Tamami Uejima [2], Stuart Bloor [3], David Komander [3], Felix Randow [3], Soichi Wakatsuki [4], Ivan Dikic [4]*

1- SBRC, PF, IMSS, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan, 2- Institute of Biochemistry II, Goethe University School of Medicine, Frankfurt, Germany, 3- MRC Laboratory of Molecular Biology, Cambridge, UK, 4- Graduate University for Advanced Studies, Hayama, Japan

Nuclear factor-κB (NF-κB), as a family of inducible transcription factors, has essential roles in regulation of the gene expression in innate or adaptive immune responses. In the inactive state, NF-κB is sequestered in the cytoplasm by the binding of inhibitory molecules (IκBs). Activation is tightly regulated by a signaling network which integrates stimuli from various sources and requires binding of NF-κB essential modulator (NEMO) to ubiquitylated substrates. Here we report that the UBAN (Ubiquitin Binding in ABIN and NEMO) motif of NEMO selectively binds linear (head-to-tail), rather than Lys63-linked ubiquitin chains. Crystal structures of the UBAN motif revealed a parallel coiled-coil dimer that formed a heterotetrameric complex with two linear diubiquitin molecules. Binding occurred via a novel surface on the proximal ubiquitin moiety and the canonical Ile44 surface on the distal one, thereby providing specificity for linear chain recognition. Residues of NEMO involved in binding linear ubiquitin chains are essential for NF-κB activation by TNF-α and other agonists, which explain the detrimental effect of NEMO mutations in patients suffering from X-linked ectodermal dysplasia and immunodeficiency.

Keywords: NF-κB, ubiquitin, NF-κB essential modulator

P-10-905-1

Effect of various stresses on human proIslet amyloid polypeptide aggregation

Maryam Hassanlou, Hamid Reza Kalhor*

Department of Biophysics, Tarbiat Modares University, Tehran, Iran

Protein aggregation in the form of amyloid deposit is a prominent feature of many neurodegenerative diseases such as Alzheimer, Huntington, Parkinson and other disease including systemic amyloidosis and type 2 diabetes mellitus. Type 2 diabetes mellitus (T2DM) is a progressive disease that can cause pancreatic beta cells degeneration. There are more than 200 million people in the world who are identified with this disease. Formation of amyloid deposits in the islets of Langerhans is a characteristic pathophysiological hallmark of T2DM. The main protein that forms amyloid in pancearatic β cell is a 37 amino acid polypeptide named islet amyloid polypeptide (IAPP) or amylin. To evaluate the effects of static magnetic field on IAPP amyloid formation we transfected Chinese Hamster Ovary (CHO) cells with the construct expressing human proIAPP-EGFP fusion. The cells were then exposed to static magnetic field and the amount of amyloid formation in the samples was examined by the intensity and morphology of EGFP as well as apple-green birefringence of Congo red staining. The amyloid fibril in both exposed and control samples were also quantitatively measured by Thioflavine T and congo red binding assay. CHO cells under static magnetic field showed differences in the amount of amyloid formation when compared to control. Various stresses can alter the rate of amyloid formation and these stresses must be considered perhaps as therapeutic tools in amyloidosis.

Keywords: type 2 diabetes mellitus, IAPP, aggregation, static magnetic field

---

P-10-872-1

Binding studies of 1,10-Phenanthrolinepiperidine-dithiocarbamatopalladium(II) Nitrat with calf thymus DNA

*Hassan Mansouri-Torshizi, Zahra Yekke-GHasemi, Maryam Saeidifar**

Department of Chemistry, University of Sistan & Baluchestan, Zahedan, Iran

The development of pd(II) antitumor complexes is mainly based on the structural activity relationship used for platinum(II) anticancer drugs as well as good models for the analogous pt(II) complexes in solution. The use of dithiocarbamate in combination with cisplatin has protected a variety animal species from renal, gastrointestinal and bone marrow toxicity induced by cisplatin. Thus, in this study, we have prepared two water soluble new palladium (II) complexes of formula [pd(phen)(pip-dtc)]No3 (where phen=1,10-phenanthroline and pip-dtc=piperidine-dithiocarbamate) with calf thymus DNA was studied by isothermal titration UV-Visible spectroscopy in Tris-HCl buffer of pH=7.0 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 cooperativity in binding. The above complex can denature DNA and the concentration of this ligand in the midpoint of transition, [L1/2], is decreased by improving temperature, from 0.021mmol/L at 27°C to 0.016mmol/L at 37°C. The conformational stability of DNA in the interaction with complex (ΔG°H2o) was determined to be 18.90kJ/mol and 10.58kJ/mol at 27°C and 37°C, respectively. Thus, DNA is more stable at 37°C; i.e., presence of complex led to decrease the stability of the DNA. Values for m (a measure of complex strength for DNA denaturation) are 799.2 and 759.8(kJ/mol).(mol/L)-1 at 27°C and 37°C, respectively. Also the molar enthalpy of DNA denaturation by the complex (ΔH°confromation or ΔH°denaturation) in the range of 27°C and 37°C is found out to be 275.74KJ/mol. In addition, the calculated molar entropy (ΔS°H2O) of DNA denaturation by the complex is 0.85kJ/mol at 27°C. The positive value of entropy change is related to the more disorder of denatured DNA with respect to the native DNA. In addition, gel filtration results had shown the two peaks obtained at two wavelengths 300 and 258, which indicate that complexes have not separated from DNA and their binding with DNA is strong enough that not readily break.

Keywords: DNA binding, dithiocarbamate, gel filtration, Pd(II) complex, spectrophotometry

---

P-10-67-1

In silico antibody-protein interaction: Comparative evaluation of discontinuous epitope prediction tools

Mohammad Hossein Zabeh Jazi, Ali Malekshahi*

Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

A B-cell epitope is defined as a part of protein antigen recognized by either a part of particular antibody or the B-cell of immune system. Reliable prediction of these binding sites for a given protein is highly important for design of immunodiagnostics and vaccines. Many immunoinformatic software's and databases have developed to facilate vaccine design and would allow the immunologist to greatly reduce the experimental work, providing a valuable starting point for potential binding sites. As crystallographic studies of antibody-protein complexes have shown most B-cell epitopes are discontinuous, here we used some structure-based tools and software, to allow a prediction and visualization of antibody epitopes in a given protein structure or sequence. In comparison with three structure based methods, ElliPro performed the best, when the most significant prediction was considered for each protein. It is an open question as to whether discriminatory features to distinguish epitope from non-epitope can be found.

Keywords: antibody epitope, antibody-protein complex, discontinuous, vaccine, immunoinformatics

---

P-11-915-2

Synthesis of new Cd (II) complexes, and study of interaction of these compounds with ctDNA

Ali Reza Rezvani [1], S. Zahra Bathaie [2], Masoud Memar [2], Javad Farzanfar [2]*

1- Department of Chemistry, University of Sistan and Baluchestan, Zahedan, Iran, 2- Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran

Minor groove-binding ligands are of great interest due to their extraordinary importance as transcription-controlling drugs. Metal complexes of the type [M(A)m]n+ where A is multidentat hetrocycle aromatic are particularly attractive species to recognize and cleavage DNA. Ligands or the metal ion in these complexes can be varied in an easily controlled manner to facilitate an individual application. The change in the metal ion or ligand would lead to changes in the binding mode and affinity. In this study, new complexes of [Cd(phen-dione)(DPA)](NO3)2 and [Cd(phen-dione)(dipic)] for intraction with ctDNA. All experiments on ligand-DNA interaction were performed in 0.06 M tris buffer, pH =7.4, at 25oC. Addition of increasing amounts of ctDNA to all the complexes shows a decrease in molar absorptivity of the π π* absorption band as well as a red-shift of a few nm (6 nm) and hyperchromic indicating the binding of the complexes to ctDNA in different modes and to different extents. The CD spectra of ctDNA were obtained in the presence of complexes. Changes CD spectrum of ctDNA include the negative in the peak at 275nm, the increase in the presence value at 248nm. Some changes were observed in the CD plots of ctDNA in the presence of different concentrations of these compounds. Analysis of the resulting complexes reveals a base-specific metal ion binding to the minor groove site N3, with coordination observed only with adenine, and formation of a polynucleotide analogue in the case of guanine.

Keywords: Cd (II) complexes, base-specific, minor groove

P-10-972-1

Identification and purification of the three toxins from Odotobuthus bidentatus, Lourenco & pezier 2002, venom

Behzad Masihipour [1], A. M. Zare [2], SH Navidpour [3], Ahmad Taghavi Moghadam [3], A. Akbari [3], H Rabiei [3]*

1- Razi Reference Lab of scorpion Research(RRLS), Razi Vaccine and Serum Research Institute, Ahwaz, Iran, 2- Venomus Animals and Antivenin production Department , Razi Vaccine and Serum Research Institute, Karaj, Iran, 3- Razi Reference Lab of Scorpion Research(RRLS),Razi Vaccine and serum Research Institute, Ahvaz, Iran

The scorpion venom contains insect and mammal selective toxins. Odonthobuthus scorpion belongs to Buthidae family from which 2 species; i.e., O. doriea and O. bidentatus have been collected and

reported from Iran. In this study we investigated the venom of O. bidentatus scorpion to identify mammal toxic fractions. After collecting scorpions, they were milked with electroshock technique and then the venoms were lyophilized. The LD50 of the venom was determined by injecting the venom to 18-20 grams mice via tail vein. In order to separate different fractions, the freeze dried venom was solubilized in distilled water and centrifuged at 15000 rpm for 20 minutes , to separate insoluble material and the clear supernatant containing soluble venom was loaded on a chromatography column packed with sephadex G50 gel and the fractions were collected according to U.V absorption at 280 nm wavelength . Second fraction was toxic in Lab animal and in order to study sub fractions, fraction II (O2) was loaded first on an anionic ion exchange resin (DEAE sephacel) out of which we obtained 2 toxic fractions (fraction21 and 23). These 2 fractions were then loaded on a cationic resin (CM sephadex C25) out of which, from fraction 21, we got 2 toxic fractions (O211, O213) and from fraction O23 we obtained one toxic fraction (O232). SDS-PAGE electrophoretic studies on these toxins showed single band appearance that indicates purified toxins with molecular weight of O211 9.2kDa, O213 8.6kDa and O233 6.8 kDa.

Keywords: scorpion, venom, fraction, odontobuthus bidentatus, gel

---

P-10-980-1

The investigation of tertiary conformational changes of immobilized glucose oxidase on silver nanoparticles

Nasrin Hashemifard [1], Afshin Mohsenifar [2], Bijan Ranjbar [3], Abdolamir Allameh [4], Abbas Sahebghadam Lotfi [4], Batool Etemadikia [4]*

1- Department of Biochemistry, Sciences-Research Branch of Azad University, Tehran, Iran, 2- Department of Toxicology, Tarbiat Modares University, Tehran, Iran, 3- Department of Biophysics, Tarbiat Modares University, Tehran, Iran, 4- Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran

The aim of this study was characterization of tertiary structure of immobilized glucose oxidase on silver nanoparticles. In this study, glucose oxidase was immobilized on silver nanoparticles synthesized by reducing $AgNO_3$ with $NaBH_4$. Tertiary structure of glucose oxidase was studied by spectrofluorometer in pH range 3-11. In order to investigate if and how the tertiary structure of glucose oxidase is influenced by the adsorption onto the silver nanoparticles, the glucose oxidase spectrum in the far uv region was recorded with and without nanoparticles. Tertiary structural changes of immobilized glucose oxidase were measured using spectrofluorometer. All measurements were made with excitation and emission band widths of 5 nm and 10 nm, respectively. The samples excited at 280 nm and fluorescence intensity were recorded between 300–420 nm. The results of our research showed that immobilization of glucose oxidase on the silver nanoparticles had no remarkable effect on tertiary structure of the immobilized enzyme. The results from kinetic studies also confirmed that tertiary structure of immobilized glucose oxidase was not considerably influenced. So here we introduce a method of immobilization of enzymes on the surface of nanoparticles in order to its application in medicine, biosensors, etc.

Keywords: silver nanoparticles, immobilized glucose oxidase, tertiary structure

---

P-11-986-1

Potent inhibitory effects of benzyl and p-xylidine-bis dithiocarbamate sodium salts on catecholase activity of mushroom tyrosinase

Ehsan Amin [1], Ali Akbar Saboury [1], Hassan Mansouri-Torshizi [2], Ali Akbar Moosavi- Movahedi [3]*

1- Institute of Biochemistry and Biophysics, University of Tehran, 2- Department of Chemistry, University of Sistan and Baluchestan, Zahedan, Iran, 3- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

A novel synthesized monofunctional benzyldithiocarbamate, C6H5CH2NHCSSNa (I) and a bifunctional p-xylidine-bis(dithiocarbamate) NaSSCNHCH2C6H4CH2NHCSSNa (II) as sodium salts, were examined for their inhibition of catecholase activity of mushroom tyrosinase (MT) from a commercial source of Agricus bisporus. L-3,4- dihydroxyphenylalanine (L-DOPA), was used as natural substrate for the catecholase enzyme reaction. Kinetic studies showed noncompetitive inhibition of I and mixed type of II on activity of MT. Inhibition constant (KI) of II is smaller than of I. Raising temperature from 27 to 37°C caused decreasing KI values of I and increasing those of II. The binding process for inhibition of I is only entropy driven, which means that the predominant interaction in the active site of the enzyme is hydrophobic, meanwhile the electrostatic interaction can be important for inhibition of II due to the enthalpy driven binding process. Fluorescence studies showed a decrease of emission intensity without shift of emission maximum in the presence of different concentrations of compounds. Extrinsic fluorescence study did not show any considerable change of tertiary structure of MT. Probably inhibitor binding causes molecular oxygen to penetrate to the active site and so quench intrinsic fluorescence.

Keywords: mushroom tyrosinase, benzyldithiocarbamate, p-xylidine-bis (dithiocarbamate), noncompetitive inhibition, mixed inhibition

---

O-10-968-1

Molecular cloning, expression and purification of pyruvate kinase from thermophilic Geobacill

Soheila Mohammadi, Saman Hosseinkhani, Mahboobeh Nazari*

Department of Biochemistry, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran

Pyruvate kinase (EC 2.7.1.40) catalyses an essentially irreversible step in glycolysis, the phosphoryl group of phosphoenolpyruvate being transferred to ADP to yield pyruvate and ATP. It can be used in ADP assay kits by luciferase that has valuable applications in different fields. We describe the isolation and characterization of a PYK from the thermophilic Geobacillus. This protein appears to be a tetramer composed of four subunits. The gene for the enzyme was cloned in Escherchia Coli and its entire nucleotide sequence was determined and was expressed in E.coli (BL21) cells. Then the PYK was purified by His-Tag affinity chromatography and then analyzed by SDS-PAGE to estimate the molecular mass of the enzyme and subunits. The purified PYK was used in a coupled bioluminescent assay for ADP measurement. Kinetic and structural properties will be reported.

Keywords: Geobacillus, phosphoenolpyruvate, pyruvate kinase, coupling assay, bioluminescent, ADP, luciferase

---

P-10-1009-1

Spectroscopic investigation of cationic porphyrin complexes with double-stranded calf thymus DNA

*Abbas Eslami, Kayhaneh Berijani**

Department of Chemistry, University of Mazandaran, Babolsar, Iran

The binding of water-soluble, cationic porphyrins and metalloporphyrins with DNA have been intensively studied because these compounds are known as anti-tumor drugs, photosensitizers in photodynamic therapy of cancer, and inhibitors of HIV-1. The two basic modes of porphyrin binding to duplex DNA are intercalation and outside groove binding. In this work, a asymmetric porphyrin 5-(1-nonyl pyridinum-4-yl)-10,15,20-tris(methyl pyridinum-4-yl) porphyrin (MNTM), its Cu (MNTM(Cu)) and Ni (MNTM(Ni)) complexes have been synthesized and their interaction with the increasing amount of stock solution of calf thymus DNA in phosphate buffer (pH 7.6) investigated by uv–vis spectra and resonance light scattering (RLS). The concentration of calf thymus DNA was determined by uv absorbance at 260 nm with the molar absorption coefficient (1.32×104 M-1 cm-1). According to the experimental results, the binding of certain porphyrins to DNA produces hypochromism, a broadening and red-shifts of the Soret band of porphyrin. These results and RLS experiments corroborate that MNTM and MNTMCu intercalate into the CT-DNA helix but MNTMNi outside binds to along CT-DNA matrix.

Keywords: water-soluble porphyrin, calf thymus DNA, intercalation, outside-binding

---

P-10-909-1

The critical structural role of a highly conserved Aspartate residue in Luciferin-regenerating enzymes: a bioinformatics study

Mahsa Farazandeh Mehr [1], Rahman Emamzadeh [2], Saman Hosseinkhani [2], Golshan Bagheri Sadeghi [2]*

1- Department of Biology, Faculty of Science, Science & Research Unit, Azad University, Tehran, 2- Department of Biochemistry, Faculty of Basic science, Tarbiat Modares University, Tehran, Iran

In the firefly light organ, luciferin-regenerating enzyme (LRE) plays an important role in the recycling of oxyluciferin into luciferin. Homology modeling of this enzyme, combined by analysis of the structural thermodynamic parameters, shows that an Asp residue buried in protein core is critical for correct folding. This Asp is strictly conserved in the SGL family which includes the LREs. Moreover a similar role is proposed for Thr38, which is physico-chemically conserved in this group of enzymes, and it provides part of the stabilizing interactions for Asp.

Keywords: firefly luciferin-regenerating enzyme (LRE), homology modeling, oxyluciferin, SGL family

---

P-10-143-1

Antifatty streaks effect of tryptophan in cholesterol-fed rabbits

Ahmad Movahedian [1], Gholam Reza Dashti [2], Marjan Khademie Zadeh [3]*

1- Department of Clinical Biochemistry, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran, 2- Department of Anatomy, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, 3- Department of Clinical Biochemistry, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

There is experimental evidence that low density lipoprotein (LDL) oxidation is a potential atherogenic agent. Therefore protecting LDL from oxidation prevents atherogenesis. It has been shown that tryptophan (Trp) decreases lipid peroxidation and protects LDL from atherogenic modification. The aim of this study was evaluating the effect of the dietary administration of Trp on fatty streaks formation in cholesterol-fed rabbits. Male New Zealand white rabbits were fed for 12 weeks with either a high-cholesterol diet (control group) or the same diet supplemented with Trp in drinking water. Plasma lipids, malondialdehyde (MDA), conjugated diens (CDs), antioxidant capacity (AC) and atherogenic index (AI) were determined after the end of treatment. The coronary arteries were used to quantify the extent of fatty streaks formation by histological evaluation. Some plasma lipids were significantly changed in Trp treated group in comparison with control group. Plasma MDA, AC and AI decreased in a group treated with Trp compared to control. The mean size of produced fatty streaks also showed a significant reduction in treated group compared to control. Our results suggest that dietary supplementation with Trp seems to protect the arterial wall from atherogenesis in an experimental animal.

Keywords: tryptophan, fatty streaks, low density lipoprotein, Atherogenesis

---

P-10-1053-1

Isolation of a psychrotolerant Exiguobacterium sp. from soils and characterization of its amylase

Fateme Hoseini [1], Sara Abolhassani Rad [2], Azam Safari [3], Gholam Reza Bakhshi Khaniki [3], Hossein Shahbani Zahiri [3]*

1- Molecular Genetic Department, National Institute of Genetic Engineering and Biotechnology Payame Noor University, Tehran branch, 2- National Institute of Genetic Engineering & Biotechnology, Tehran, Iran, 3- Payame Noor University, Tehran branch, Tehran, Iran

The genus Exiguobacterium is an interesting bacterium with an impressive diversity in terms of geographic distribution. This bacterium has been isolated form hot springs in Yellowstone National Park as well as glacial ice core samples in Greenland and the Siberian permafrost environment. Most reported Exiguobacterium strains are known for their enzyme production and extremophilic abilities. In this study we isolated a psychrotolerant bacterium which was identified as Exiguobacterium sp. after amplification of 16s rDNA and sequencing. The maximum growth of the bacterium was at 30 ºC above which growth was severely decreases. This bacterium was able to produce a cold adapted amylase with interesting features. The maximum activity of the enzyme was achieved at 30°C; however, the enzyme retained about 60% of its maximum activity at cold (4°C). The optimum pH for enzyme activity was 7; however, the enzyme was active in various pH

conditions ranging from 5 to 9. Although this enzyme was psychrotolerant, it was able to retain about 50% of its maximum activity after 7 hour incubation at 80˚C. Amylases are among mostly used enzymes with applications in various industries from bakeries and sugar processing to detergents manufacturing. Efforts to find novel enzymes are still underway because these enzymes are interesting not only form industrial but also from enzymology point of view. The enzyme characterized here shows interesting features demanding more studies in terms of molecular and biochemical characteristics.

Keywords: Exiguobacterium, psychrotolerant, amylase, enzyme, cold, stability

---

P-10-940-1

Production of vinegar by Kombucha culture

Mohammad Balvardi [1], Mehran Habibi-Rezaei [2], Mohammad Safari [2], Ali Akbar Moosavi-Movahedi [3]*

1- Department of Food Science and Engineering, Faculty of Biosystem Engineering, University of Tehran, Karaj, Iran, 2- School of Biology, College of Science, University of Tehran, Tehran, Iran, 3- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Kombucha is a traditional beverage obtained by the fermentation of sweetened tea, involving a symbiotic culture of yeast species and acetic acid bacteria. For the kombucha production, sucrose has been used as the main carbon source whereas tea acts as a nutrient broth for the microorganisms. The main metabolites identified in the kombucha fermented beverage are: acetic, lactic, gluconic and glucuronic acids, ethanol and glycerol. Kombucha can be used for production of vinegar. For this case the content of acetic acid must be increased while, the contents of lactic acid and ethanol must be decreased. In this study, different concentrations of sucrose were used for kombucha fermentation and contents of acetic and lactic acids and ethanol were determined by High Performance Liquid Chromatography (HPLC) simultaneously after 14 days of fermentation. Our results indicate that the content of acetic acid in 4% sucrose as substrate was at the highest level and reached to 2.33%, at higher substrate concentrations the acetic acid concentration decreased with increasing of substrate concentration. The content of lactic acid increased with access of substrate, but in 4% substrate was negligible (0.12%) also, ethanol was not detected in this substrate concentration. Therefore, kombucha culture in low concentration of sucrose as substrate can be used for vinegar production. Since kombucha is useful for metabolic disorders, arthritis, psoriasis, constipation, indigestion and hypertension, vinegar produced with kombucha culture can be used as a functional product.

Keywords: Kombucha, vinegar, sucrose, fermentation

P-10-948-1

Effect of different organic solvents on structure of firefly luciferase

Sohameh Mohebbi, Saman Hosseinkhani, Bijan Ranjbar, Farangis Ataei*

Department of Biochemistry, Faculty of Basic science, Tarbiat Modares University, Tehran Iran

The use of enzymes in organic solvents has been one of the most exciting facets of enzymology in recent time. Enzymes loose their activity in organic solvents which correlate with conformational rigidity, denaturation and enzyme inhibition. Enzymes are less stable in organic solvents and strategies such as protein engineering, chemical modification and additives are used for stabilization. Firefly luciferase is a hydrophobic enzyme and its activity and structure depends on the type of solvent type. Solvents with different log P (from -1.93 to -0.32) and ε were used: ε increasing (formamide), ε decreasing (ethanol and ethylene glycol) and non-effective on ε (dimethyl sulfoxide). The influence of four organic solvents on the enzyme's structure was evaluated through the intrinsic fluorescence and circular dichroism. Fluorescence spectroscopy shows that, although the presence of all the organic cosolvents cause conformational changes in the enzyme molecule at different concentrations, some of these changes lead to better activity and stability. With increasing concentrations of ethanol and DMSO up to 15% (v/v) the fluorescence spectra decrease, but by increasing the concentrations up to 25% (v/v) the spectra go near the control. Increasing formamide concentrations, caused fluorescence decrease. Formamide is an aprotic solvent, so it cause decline of fluorescence. The fluorescence of ethylene glycol was increased by increasing the concentrations. Fluorescence spectroscopy shows that ethylene glycol causes the enzyme to become more rigid.

Keywords: firefly luciferase, organic solvents, structure

P-10-920-1

Purification and characterization of amylase from a native strain of Xanthomonas campestris 0307 isolated from soil

Fereshteh Rahmati [1], Sara Gharavi [1], Mohammad Reza Soudi [1], Khosro Khajeh [2]*

1- Department of Biology, Alzahra University, Tehran, 2- Department of Biochemistry, Faculty of Sciences, Tarbiat Modares University, Tehran, Iran

Amylases are starch-degrading enzymes important for their use in textile and detergent industries as well as food and pharmaceutical industries where the required purity of the enzymes is higher. Xanthomonas campestris species predicted to produce amylases were isolated by screening soil from Karaj, Iran. The isolated species were compared by a semi-quantitative method for amylase production capability. Xanthomonas campestris0307 strain was finally selected. Amylase was purified by precipitation with ammonium sulfate and by anion exchange chromatography. The homogeneity of the purified fraction was tested by polyacrylamide gel electrophoresis and the molecular weight was subsequently found to be 65 kDa. The optimum temperature and pH were determined to be 47˚C and 6.1, respectively. Calcium cations enhanced the thermal stability of the enzyme which remained stable at 47˚C, but lost about 90% of its activity at 60°C after 30 min (pH 6.1).

Keywords: amylase, characterization, purification, Xanthomonas campestris

---

P-11-1064-1

Optimization PHAs production by Cupriavidus necator from molasses and acetate as substrate

M. Sharifzadeh Baei [1], F. Tabandeh [2], M. Yunesi [3], G. Najafpour [4], H. Issazadeh [4], M. Khodabandeh [4]*

1- Islamic Azad University, Ayatollah Amoli Branch, 2- Biotechnology Department, National Institute of Genetic Industrial Engineering and Biotechnology, 3- Department of Environmental Science, Faculty of Natural Resources & Marine Sciences, Tarbiat Modares University, 4- Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran

In present research we used molasses and acetate as combinational substrate. Molasses permeate from sugar industry which was hydrolyzed to cleave its main carbon source, to glucose. The hydrolysis products were chosen as carbon sources or the production of poly-3-hydroxybutyric acid (PHB) by Cupriavidus necator. The application of hydrolyzed molasses permeate turned out to be advantageous compared with the utilization of pure sugars. Therefore, fermentation under controlled conditions (250 rpm shaking rate, 15 hr for inoculum age, 60 hr fermentation time and temperature at 30°C) was performed and as a result, maximum polymer concentration was 2.86 g/l.

Keywords: poly-3-hydroxybutyric acid, molasses permeate

P-10-974-1

Use of agar as an additive to increase the removal of the phenolic compounds from synthetic wastewater treated with soybean peroxidase and hydrogen peroxide

Sara Hejri, Azra Saboora*

Department of Biology, Alzahra University, Tehran, Iran

Many industries generate phenolic pollutants during their manufacturing processes. Most of these compounds are toxic and have been classified as hazardous pollutants. Soybean peroxidase (SBP) catalyzes the oxidation and polymerization of phenolic compounds in the presence of hydrogen peroxide. The polymerized products can easily precipitate and be filtered from the solution. Peroxidase is inactivated during the reaction. The catalytic lifetime of the peroxidase can be significantly extended in the presence of protective additives. In this study phenolic solutions as synthetic wastewater containing phenol, o-cresol, m-cresol, 2-chlorophenol and 4-chlorophenol were treated with soybean peroxidase (SBP) and the effect of Agar as an additive on the removal of phenols was studied. Phenols concentrations were read with spectrophotometer. The results showed that, use of Agar layers with different thicknesses but similar concentrations could increase the removal efficiency. After 24 hrs, increasing in the thickness resulted in significant increasing in the reduction of phenolic compounds. Application of Agar layers with similar thickness, in spite of different concentrations, led to similar removal. Agar solutions with different concentrations did not appear the effect on the elimination of phenolic compounds.

Keywords: agar, phenolic compounds, soybean peroxidase

P-10-1065-1

Characterization of two catechol 2,3-dioxygenase enzymes from indigenous pseudomonas SA01 and SA07

Iman Rad, K. Akbari Noghabi*

Molecular Genetic Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Pseudomonas strains are known as one of the most abundant microorganisms that make physiological response to phenolic pollutant, due to their loci that are located on plasmid or chromosome. Regarding this fact that almost all of monocyclic aromatics convert to different derivatives of catechol, catechol 2,3-dioxygenase is the key enzyme in the meta-cleavage pathway of phenol degradation. The isolated bacteria were identified as pseudomonas via 16srDNA sequencing. In enzyme assay stage, the cytoplasmic crude extracts were prepared and enzyme activity was calculated spectrophotometerically by measuring the enzyme product absorbance (2-hydroxymuconic semialdehyde or 2-HMS) at 375nm after addition of 10mM of catechol as substrate. Effect of different ions on enzyme activity was studied and the optimum pH and temperature were specified too. The catalytic characteristics of dioxygenases enable these enzymes for regio- and stereo-selective introduction of oxygen in a wide variety of organic substrates. This ability could be applied in the conversion of compounds that are not attainable by conventional chemical synthesis.

Keywords: Pseudomonas, phenolic pollutant, 2-hydroxymuconic semialdehyde, 16srDNA

P-10-940-2

Antioxidant activity of bovine whey-based kombucha beverage

Mohammad Balvardi [1], Maryam Salami [1], Reza Yousefi [1], Mohammad Safari [1], Mehran Habibi-Rezaei [1], Ali Akbar Moosavi-Movahedi [2]*

1- Institute of Biochemistry and Biophysics, University of Tehran,
2- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Kombucha is a traditional beverage known to be associated with a number of health Benefits. Although sweetened tea is the preferred substrate for Kombucha fermentation, other beverages have also been tested as substrates with fair results. Cheese whey is the liquid remaining following the precipitation and removal of milk casein during cheese-making. Besides the presence of valuable macro- and micronutrients of this byproduct, possible antioxidant activity of milk whey has also been suggested. In this study, The total antioxidant activity of bovine whey before and during fermentation by kombucha culture up to 6 days were investigated using spectrophotometry ABTS-based method (reduction of the cation radical of 2,20-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid)), result were compared with kombucha prepared with sweetened tea . Our results indicate that antioxidant activity of kombucha prepared with whey as substrate increased during the 6 day fermentation but in sweetened tea substrate antioxidant activity decreased during the fermentation because of degradation of tea polyphenols by kombucha microorganisms. Increasing in antioxidant activity in fermented whey can be due to production of bioactive peptides. Therefore, fermentation of whey with kombucha culture can result in production

of a beverage with high functional properties that can be used for preventing many diseases.

Keywords: Kombucha, whey, antioxidant activity, bioactive peptides

---

O-10-433-8

Effect of various ligands on DNA structural transition

S. Zahra Bathaie

Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

DNA structural changes induced by various ligands are the new area of study that has been attracted by some investigators. Many ligands such as drugs, pollutants, various chemicals, and even proteins and peptides can bind to DNA and induce its conformational change which in turn can affect the biological functions such as replication, transcription, recombination and repair. Thus, some of the side effects of these ligands should be considered from this respect. This area of research has been followed from several years ago in our Lab. and some papers were published or are under publication. Various techniques including circular dichroism (CD), fluorescence and UV-Vis spectroscopy, electrochemical methods, biocalorimetry (ITC), etc. were used. Our results indicated that the side effect of some drugs by their long- term using, e.g. 8-MOP, is due to their binding to the DNA minor groove. In addition, some mild to harsh conformational changes of DNA after interaction with ligands were detected. For example the B- to A- DNA induction by anionic detergents, B- to C- DNA transition due to binding of crocin or crocetin, B- to φ-DNA conversion in the presence of cations, B- to H-DNA transition or triplex formation after interaction with safranal were reported. Triplex formation is may be a reason for co-mutagenic effect of safranal. The new DNA-nano biosensors were also designed and made that not only is able to detect various drugs in samples, but also can distinguish between the mechanisms of their DNA-binding. These mechanisms were reviewed and classified here.

Keywords: DNA, ligand, conformational change