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An Overview of Protein-DNA and Protein-RNA Interactions

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In this review the fundamental question of how does protein-DNA or protein-RNA interactions affect the structures and dynamics of DNA, RNA, and protein is addressed. Two models of human serum albumin (HSA) bindings to calf-thymus DNA and transfer RNA (tRNA) are presented here. In these models the binding sites, stability and structural aspects of DNA-protein and RNA-protein are discussed. Electrostatic binding of DNA or RNA *via* backbone phosphate group to the positively charged amino acids on the surface of protein is prevailing. Two binding sites with $K_1 = 4.8 \times 10^5$ M^{-1} and $K_2 = 6.1 \times 10^4$ M^{-1} for protein-DNA and one binding affinity with $K = 1.45 \times 10^4$ M^{-1} for protein-RNA are observed. A partial B to A-DNA transition is observed for protein-DNA complexes, while tRNA remains in A-family structure upon protein interaction.

Keywords: DNA, Protein, Binding mode, Binding constant, Secondary structure capillary electrophoresis, FTIR spectroscopy

INTRODUCTION

DNA-protein and RNA-protein interactions play important roles in a variety of biomolecular functions. Gene expression, transcription, replication, recombination, packaging and repairs all are controlled by DNA-protein interactions. Although the physical basis for these recognition processes is not fully understood, x-ray crystallography, NMR spectroscopy and molecular modeling provide us with a wealth of information on DNA recognitions [1-5]. The quantitative assessment of DNA-protein interaction is essential to understanding transcription, the beginning of biological processes including normal cellular function, development and many diseases [6]. RNA plays major role in diverse functions within the cell. Protein-RNA complexation is essential in many of these biological functions. Transfer RNAs bind to aminoacyl-tRNA synthetases for the translation of the genetic code during protein synthesis [7,8], while ribonucleoproteins bind RNA in post-transcriptional regulation of gene expression [9]. Although the biological significance of protein complexation with RNA has been well recognized, the specific mechanism of protein-RNA interaction is not fully understood [10]. Measurement of sequence-specific DNA-protein and RNA-protein interactions is a key experimental procedure in molecular biology of gene regulation. The most commonly used method is the electrophoretic gel mobility shift assay (EMSA), in which a radioactively labeled DNA probe is mixed with a solution of protein of interest and after a short reaction period, loaded on an electrophoretic gel [11]. Since there are some limitations using EMSA method, affinity capillary electrophoresis has been widely used to measure the mobility shift or zone electrophoresis to estimate the affinity of DNA-protein complexation [12-14].

Human serum albumin (Structure 1) is a principal extracellular protein with a high concentration in blood plasma (40 mg ml^{-1}) [15-18]. HSA is a globular protein composed of three structurally similar domains (I, II, and III), each containing two subdomains (A and B) and stabilized by 17 disulphide bridges [19-22]. Aromatic and heterocyclic ligands

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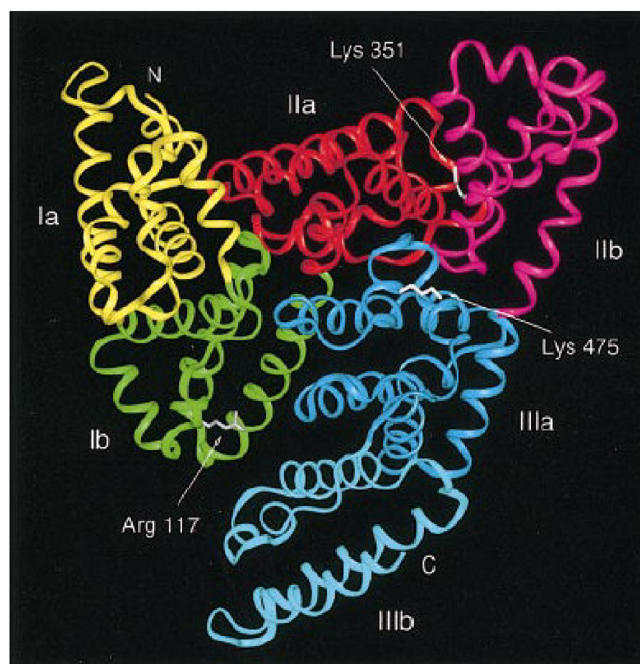
were found to bind within two hydrophobic pockets in subdomains IIA and IIIA, namely site I and site II [19-22]. Seven binding sites are localized for fatty acids in subdomains IB, IIIA, IIIB, and on the subdomain interfaces [16]. HSA has also high affinity metal binding site at the N-terminus [15]. The multiple binding sites underlie the exceptional ability of HSA to interact with many organic and inorganic molecules and make this protein an important regulator of intercellular fluxes, as well as the pharmacokinetic behavior of many drugs [15-22].

We now review the interaction of human serum albumin with calf-thymus DNA in aqueous solution at physiological conditions using protein concentration of 0.25%, 0.5%, 1%, and 2% with final DNA concentration of 12.5 mM (P). Capillary electrophoresis, UV-visible and FTIR spectroscopic methods were used to measure the protein binding mode, the apparent binding constant and the biopolymer secondary structure in the HSA-DNA complexes. To our knowledge, our spectroscopic results provide the first structural analysis regarding the HSA-DNA interaction, which helps elucidation of the nature of this biologically important complex formation *in vitro* and *in vivo*.



Structure 1. Human serum Albumin.

EXPERIMENTAL

Materials

Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical Co., and was deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. In order to check the protein content of DNA solution, the absorbance at 260 and 280 nm was recorded. The A_{260}/A_{280} ratio was 1.85, showing that the DNA was sufficiently free from protein. Human serum albumin (fatty acid free) fraction V was from Sigma Chemial Company. Other chemicals were of reagent grade and used without further purification.

Preparation of Protein-Polynucleotide Complexes

Sodium-DNA or sodium-tRNA was dissolved to 2% w/w (0.05 M DNA (phosphate)) in 0.1 M NaCl and 1 mM sodium cacodylate (pH 7.30) at 5 °C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The final concentration of the stock polynucleotide solution was determined spectrophotometrically at 260 nm by using molar extinction coefficient of 6600 cm^{-1} M^{-1} (expressed as molarity of phosphate group) [23,24]. The UV absorbance at 260 nm of a diluted solution (1/250) of calf-thymus DNA used in our experiments was measured to be 0.661 (path length was 1 cm) and the final concentration of the stock DNA solution was calculated to be 25 mM in DNA phosphate. The average length of the DNA molecules, estimated by gel electrophoresis was 9000 base pairs (molecular weight ~ 6 × 10^6 Da). The appropriate amount of HSA (0.08, 0.15, 0.3, and 0.6 mM) was prepared in phosphate buffer (pH 7.2). The protein solution then was added dropwise to DNA solution to attain desired protein concentration of 0.04, 0.08, 0.15, and 0.3 mM with a final DNA or RNA concentration of 12.5 mM polynucleotide(phosphate) for infrared measurements. For capillary electrophoresis, mixtures containing various concentrations of HSA and constant concentration of DNA (2.5 mM phosphate) with protein-polynucleotide molar ratios of 1/250 to 1/55. The pH solution was adjusted to 7.30-6.80, using NaOH solution. The infrared spectra were recorded 1h after incubation of protein with DNA or RNA solution.

FTIR Spectra

Infrared spectra were recorded on a Bomem DA3-0.02 FTIR spectrometer equipped with a nitrogen cooled HgCdTe detector and KBr beam splitter. The solution spectra are taken using AgBr windows with resolution of 2 to 4 cm^{-1} and 100-500 scans. Each set of infrared spectra were taken (three measurements) on three identical samples with the same DNA and protein concentrations. The water subtraction was carried out with 0.1 M NaCl solution used as a reference at pH 6.5-7.5 [25]. A good water subtraction is achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combination mode is located. This method is a rough estimate, but removes the water content in a satisfactory way [25]. The difference spectra [(polynucleotide solution + protein solution) - (polynucleotide solution)] are produced, using a sharp DNA band at 968 cm^{-1} and RNA band at 874 cm^{-1} as internal references. These bands, due to the sugar C-C and C-O stretching vibrations, exhibit no spectral changes (shifting or intensity variations) on protein-polynucleotide complexation and were cancelled upon spectral subtraction. The spectra are smoothed with a Savitzky-Golay procedure [25]. The intensity ratios of several DNA in-plane vibrations related to A-T and G-C and A-U base pairs and the PO_2 stretching are measured with respect to the reference band at 968 cm^{-1} or 874 cm^{-1} as a function of protein concentration with an error of ±3%. These intensity ratio measurements are used to quantify the amounts of protein binding to the backbone PO_2 group and DNA bases [26].

Capillary Electrophoresis

A P/ACE System MDQ (Beckman) with PDA (photodiode array) detector was used in this study. Uncoated fused silica capillary of 75 μm i.d. and 57 cm effective length was used. The capillary was initially conditioned by washing with 1 N sodium hydroxide for 30 min, followed by a 15-min wash with 0.1 M sodium hydroxide. Then it was extensively rinsed with deionized water and running buffer before use. Samples were injected using a voltage injection at 10 kV for 5 s. Electrophoresis was carried out at a voltage of 25 kV for 10 min using normal polarity. All runs were carried out at 25 ^{o}C. Stock solution of HSA (0.15 mM) was prepared in deionized water. The capillary inlet and outlet vials were replenished after every run. The HAS binding experiments were performed in a sample buffer containing 1.5 mM Tris-HCl, pH 7.2 and 3 mM NaCl, using constant concentration of DNA or RNA and various concentrations of HSA. Polynucleotide was dissolved 3 mM Tris-HCl, pH 7.2 and 6 mM NaCl, at a polynucleotide(P) concentration of 2.5 mM. The HSA solution prepared in deionized water was added to DNA or RNA solution to attain desired HSA/polynucleotide(P) molar ratios of 1/250 to 1/55. Each sample was allowed to equilibrate for 30 min and tested with two separate runs for the same stock solution. The electropherograms were monitored at 260 nm for HSA-polynucleotide complexation complexation.

Data Analysis

The binding constants for the protein-DNA and protein-tRNA complexes can be determined by capillary electrophoresis, using Scatchard analysis [27,28]. The saturation (R_f) of the DNA or RNA was determined from the change of the migration time of polynucleotides in the presence of various concentrations of HSA by the equation

$$R_f = (m_0 - m)/(m_0 - m_s) \quad (1)$$

where m is the change in the migration time of DNA or RNA measured for any added HSA concentration, while m_0 and m_s correspond to the migration times of free DNA or RNA and HSA saturated, respectively.
Using the equation for binding constant

$$K_b = [\text{HSA-polynucleotide}]/[\text{polynucleotide}][\text{HSA}] \quad (2)$$

The binding constant K_b was then computed by fitting the experimental values of R_f and HSA to the equation

$$R_f = K_b[\text{HSA}]/(1 + K_b[\text{HSA}]) \quad (3)$$

The last equation gives a convenient form for Scatchard analysis:

$$R_f/[\text{HSA}] = K_b - K_bR_f \quad (4)$$

RESULTS AND DISCUSSION

FTIR Spectra of Protein-DNA Adducts

In order to characterize the protein-DNA bindings the infrared spectra of HSA-DNA complexes were recorded using constant amount of DNA with various concentrations of protein and the results are presented in Figs. 1A and 1B. The DNA in-plane vibrations at 1750-1500 cm^{-1} related to the G-C and A-T base pairs and the back bone phosphate group at 1250-1000 cm^{-1} [25,29-34] were perturbed upon protein interaction. The mainly guanine carbonyl vibration at 1717 cm^{-1} of the free DNA gained intensity and shifted towards a lower frequency at 1713 cm^{-1} upon HSA complexation (Fig. 1A). Similarly, an increase in the intensity of the backbone PO_2 asymmetric stretching band at 1222 cm^{-1} was observed, which shifted towards a lower frequency at 1217 cm^{-1}, in the spectra of HSA-DNA complexes (Fig. 1A). The positive features at 1707 and 1217-1218 cm^{-1} in the difference spectra of HSA-DNA complexes are coming from an increase in the intensity of the guanine band at 1717 and the PO_2 band at 1222 cm^{-1}, respectively (Fig. 1B). In addition to a major spectral shifting of the PO_2 stretching at 1222 cm^{-1}, the relative intensities of the asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations of the backbone phosphate group were altered upon HSA interaction [25]. The ν_s PO_2 (1088 cm^{-1}) and ν_{as} PO_2 (1222 cm^{-1}) have changed, with the ratios ν_s/ν_{as} going from 1.75 (free DNA) to 1.55 (protein-DNA complexes). The observed spectral changes are due to the participation of the G-C bases (mainly guanine) and the backbone PO_2 group in the HSA-DNA interactions. Similar alterations of the phosphate group vibrational frequencies were observed in the infrared spectra of the calf-thymus DNA-polypeptide complexes, where the protein-nucleic acid interaction was mainly through the backbone phosphate group and the positively charged amino acids on the surface of protein [35,36]. Therefore, it can be assumed that the protein bindings are mainly through G-C bases and the backbone phosphate groups in the HSA-DNA adducts.

Additional evidence regarding HSA-DNA interaction comes from a major shifting of the protein amide I at 1656 and amide II band at 1541 cm^{-1} [37]. The amide I band at 1656 cm^{-1} shifted towards lower frequencies at 1651 (HSA-DNA), whereas the amide II band at 1541 cm^{-1} shifted towards higher frequencies at 1549 (HSA-DNA) upon DNA-protein interaction (Fig. 1A). Similarly, the protein amide A band at 3300 cm^{-1} (NH stretching) [37] shifted towards a lower frequency at 3290 cm^{-1} upon HSA-DNA adduct formation (spectra not shown). The observed spectral changes are indicative of protein-DNA interaction *via* polypeptide C=O, C-N and NH groups (H-bonding).

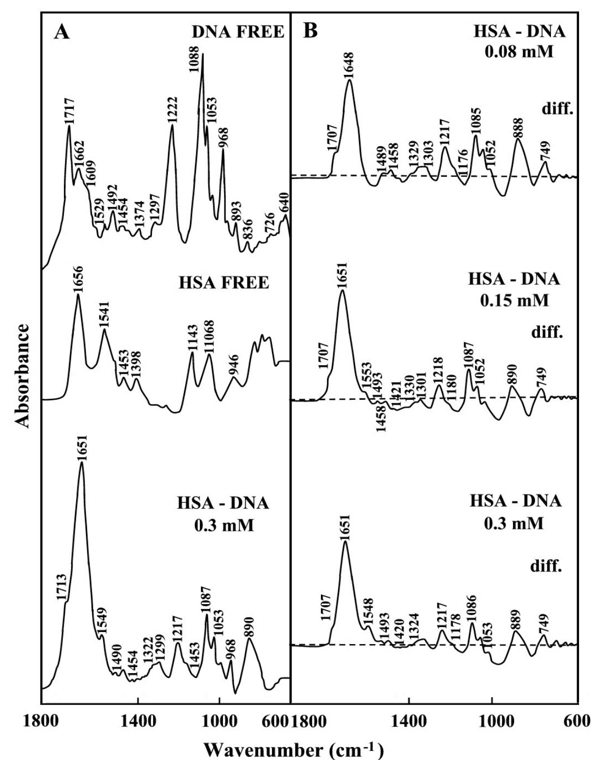


Fig. 1. FTIR spectra (A)) and difference spectra [(DNA solution + protein solution) - (DNA solution)] (B) in the region of 1800-1500 cm^{-1} for the free DNA and human serum albumin (HSA) and their complexes in aqueous solution at physiological pH with various protein concentrations.

FTIR Spectra of Protein-RNA Adducts

No major protein-RNA interaction occurs at low protein concentration (0.04 mM). Evidence for this comes from lack of spectral changes for several RNA in-plane vibrations at 1698 (mainly guanine), 1654 (mainly uracil), 1608 (adenine), 1488 (mainly cytosine) and 1244 cm^{-1} (asymmetric PO_2 stretch) [25,29-34] upon HAS interaction. As protein concentration increased to 0.08 mM, minor intensity increases were observed for the PO_2 bands at 1244 and 1087 cm^{-1}, with

positive features at 1244 and 1088 cm^{-1} in the difference spectra of protein-RNA complexes (Fig. 2B). The observed spectral changes are indicative of some degree of protein-PO_2 interaction. As HSA content increased to 0.3 mM, the guanine band at 1698 cm^{-1} shifted to a lower frequency at 1695 cm^{-1} in the spectra of protein-RNA adducts (Fig. 2A). The shifting of the guanine band at 1698 cm^{-1} was also accompanied by major intensity variations of this band upon HSA interaction. The changes observed are due to protein interaction with guanine bases in HSA-RNA complexes. Further evidence regarding HSA-PO_2 interaction is also coming from the intensity ratio alterations of symmetric and asymmetric PO_2 bands at 1086/1244 cm^{-1} [17]. The ratio of ν_s/ν_{as} was changed from 1.55 (free RNA) to 1.40 (complexed RNA) upon HSA interaction (Fig. 1A, 0.3 mM). It has been suggested that many proteins bind DNA or RNA through positively charged amino acids on their surfaces [36]. Such positive charge can bind the negatively charged backbone PO_2 group through electrostatic interactions.

Additional evidence regarding protein-RNA interaction comes from shifting of the protein amide I at 1656 and amide II band at 1541 cm^{-1} [37,38]. The amide I band at 1656 cm^{-1} shifted towards lower frequencies at 1654 (HSA-RNA), whereas the amide II band at 1541 cm^{-1} shifted towards higher frequencies at 1544 cm^{-1} upon RNA-protein interaction (Fig. 2A, 0.3 mM). Similarly, the protein amide A band at 3300 cm^{-1} (NH stretching) [37] shifted towards a lower frequency at 3290 cm^{-1} upon HSA-RNA adduct formation (spectra not shown). The observed spectral changes are indicative of protein-RNA interaction *via* polypeptide C=O, C-N and NH groups (H-bonding).

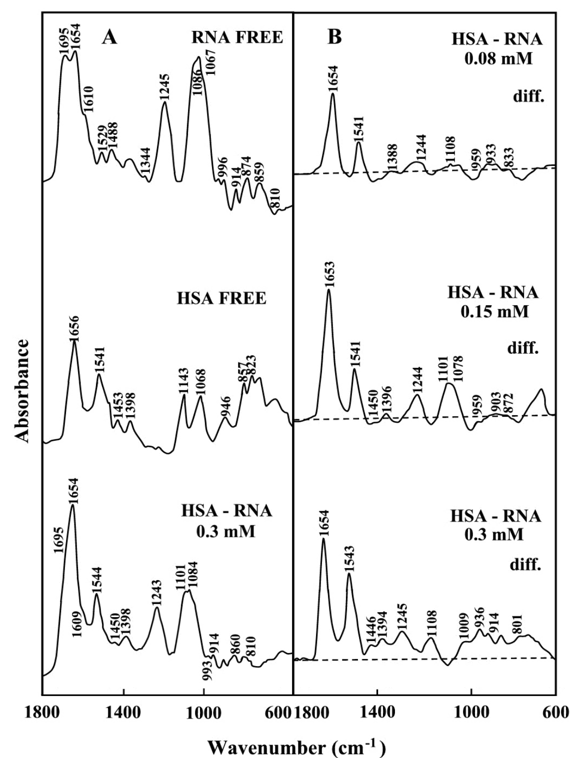


Fig. 2. FTIR spectra (A) and difference spectra [(tRNA solution + protein solution) - tRNA solution)] (B) in the region of 1800-1500 cm^{-1} for the free tRNA and human serum albumin (HSA) and their complexes in aqueous solution at physiological pH with various protein concentrations.

Protein Conformation

DNA and RNA interactions with HSA induced no major alterations of the protein secondary structure. Conformational analysis of the free HSA in H_2O solution shows æ-helix 55% (1656 cm^{-1}), ß-sheet 22% (1616-1635 cm^{-1}), ß-anti 12% (1684 cm^{-1}) and 11% turn 11% (1673 cm^{-1}) (Fig. 3 A) [39]. The random structure was estimated 12% from D_2O solution (1640-1645 cm^{-1}). The second derivative and curve-fitting procedures [37,38] were applied to the protein amide I band (at 1651 cm^{-1} of the difference spectra of HSA-DNA and RNA adducts). The results showed no major alterations of the HSA secondary structure, on DNA and RNA complexation (Figs. 3B and 3C). This is indicative of some degree of stabilization of the protein secondary structure upon polynucleotide interaction.

DNA and RNA Conformations

Minor alterations of B-DNA structure were observed upon HSA complexation. Evidence for this comes from the spectral change for B-DNA marker bands at 1222 cm^{-1} (PO_2 stretch) and 1717 cm^{-1} (mainly guanine) [31,32], upon protein complexation (Fig. 1A). Other B-DNA indicator at 836 cm^{-1} (phosphodiester mode), was overlapped by the several protein bands centered at about 800 cm^{-1} (Fig. 1A). In a B to A transition, the marker band at 837 cm^{-1} shifts towards a lower

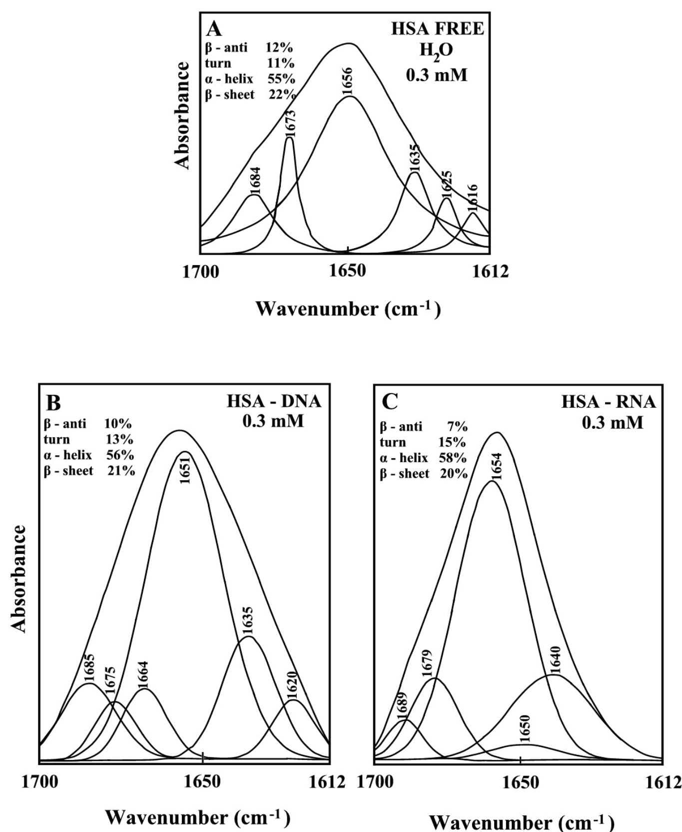


Fig. 3. Curve-fitted amide I region (1700-1612 cm^{-1}) and secondary structure determination of the free human serum albumin (A) and its HSA-DNA (B) and HSA-tRNA (C) in aqueous solution with 0.3 mM protein and 12.5 mM polynucleotide concentrations.

frequency at about 810 cm^{-1} and the guanine band at 1717 cm^{-1} appears at 1700 cm^{-1}, while the phosphate band at 1222 cm^{-1} shifts towards a higher frequency at 1240 cm^{-1} [31,32]. In a B to Z conformational changes, the sugar-phosphate band at 836 cm^{-1} appears at 800-780 cm^{-1}, and the guanine band displaces to 1690 cm^{-1}, while the phosphate band shift to 1216 cm^{-1} [31,40]. The shifting of the bands at 1717 (G) to 1713 and 1222 (PO_2 stretch) to 1217 cm^{-1} are due to some degree of DNA conformational changes upon protein interaction. The changes are due to minor perturbations of B-DNA structure towards Z-conformation. However, it is not a complete Z-formation, while the band at 1717 cm^{-1} appeared at 1713 cm^{-1} (in a complete Z structure this band appeared at 1690 cm^{-1}), while the shift of the PO_2 band at 1222 to 1217 cm^{-1} is consistent with Z-conformation (Fig. 1A)). However, no conformational changes occurred for tRNA upon HSA adduct formation. Evidence for this comes from no major alterations of A-RNA marker bands at 1698-1695 cm^{-1} (guanine), 1244-1243 (phosphate) and 810 cm^{-1} (ribose-phosphate) in both free tRNA and its protein complexes (Fig. 2A).

Stability of Protein-DNA and Protein-RNA Adducts by Capillary Electrophoresis

The stability of the HSA-DNA and HSA-tRNA complexes was studied by capillary electrophoresis. Mixtures containing various concentrations of HSA and constant concentration of polynucleotide (2.5 mM phosphate) with molar ratios of 1/250 to 1/55 were prepared and subjected to the electrophoresis, using uncoated silica capillary (57 cm effective length) at 25 kV. The electropherogram was monitored at 260 nm for DNA and RNA in a run buffer (20 mM Tris-HCl pH 7.2) at 25 ^{o}C. The DNA, RNA, DNA-HAS, and RNA-HAS were separated

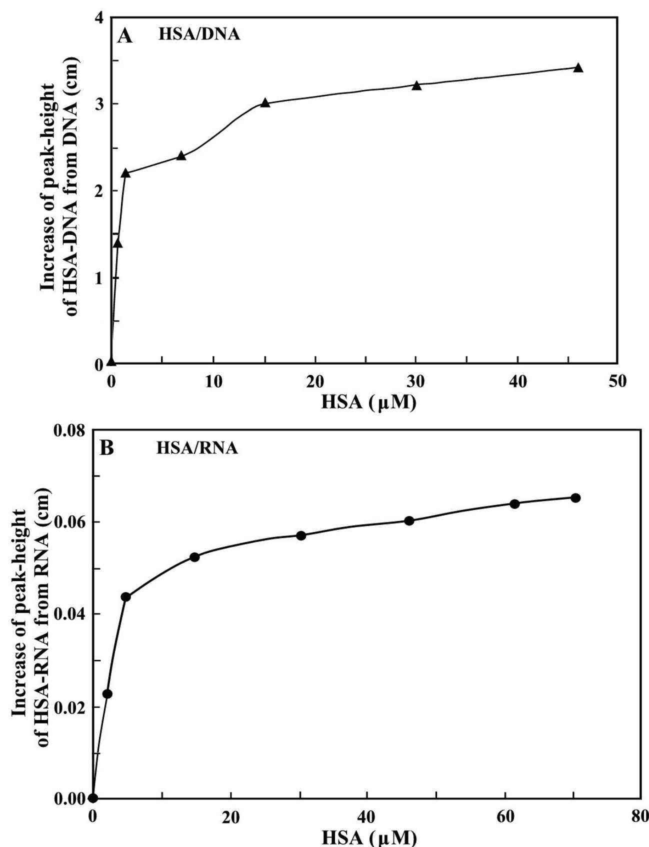


Fig. 4. Plots (A) for protein conentrations *vs*. increase of mobility shift of the HSA-DNA complexes from free DNA and HSA-tRNA from free RNA (B). Incubation of constant concentration of DNA or tRNA (1.25 mM of phosphate) with various concentrations of HSA were carried out in 15 mM Tris-HCl pH 6.5 and 15 mM NaCl, and the mixtures were subjected to the capillary electrophoresis. The increase of mobility shift in protein-polynucleotide was determined by subtracting mobility shift of the free DNA or free tRNA from each protein adduct.

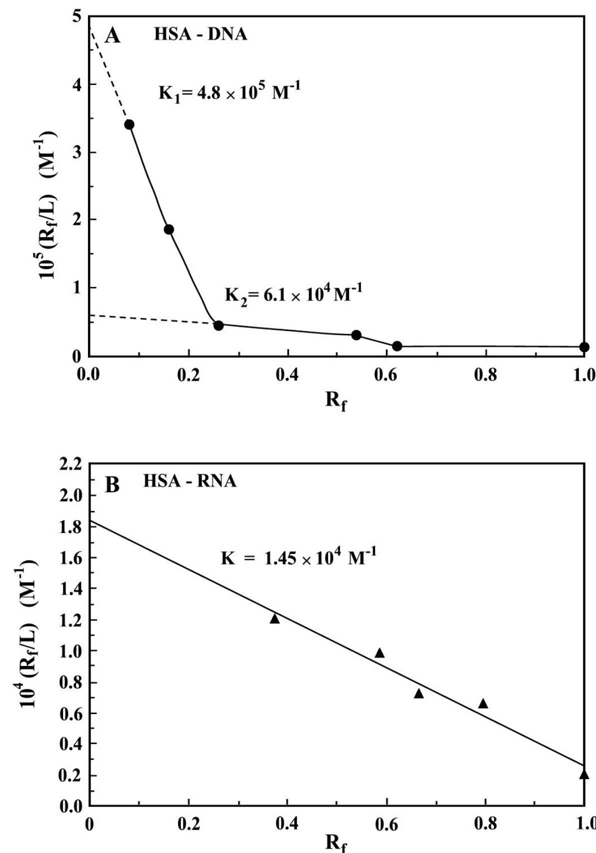


Fig. 5. Scatchard plots and binding constants for HSA-DNA (A) and HSA-tRNA (B).

and their migration times were 3.16 min, 3.83 min and 4.58 min, respectively. It was observed that the migration time of polynucleotide gradually increased as the HSA concentration increased. Based on these results, binding constant of HSA-DNA and HSA-RNA complexes were determined by Scatchard analysis as described in Materials and Methods. As shown in Fig. 4, the slope for HSA-DNA is biphasic (Fig. 4 A) and monophasic for HSA-RNA (Fig. 4B) suggesting the presence of low affinity and high affinity binding sites for HSA-DNA and one binding affinity for HSA-RNA adducts.

The apparent binding constants for HSA-DNA for the high and low affinity sites were estimated were $K_1 = 4.8 \times 10^5$ M^{-1} and $K_2 = 6.1 \times 10^4$ M^{-1} (Fig. 5A) respectively, while HSA-RNA showed one binding constant $K = 1.45 \times 10^4$ M^{-1} (Fig. 5B). This is indicative of protein binding to DNA bases and the backbone phosphate group, while phosphate binding is more prevalent for protein-RNA complexes. Similar bindings were observed for AZT-DNA and AZT-RNA adducts [41].

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