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Interaction of Pyrene with Human Serum Albumin (HSA): A Uv-Vis Spectroscopy Study

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ABSTRACT

In this research the interaction of Pyrene ($C_{16}H_{10}$) as a polycyclic aromatic hydrocarbon with human serum albumin (HSA) has been investigated. Variations of UV-Vis spectrum of Pyrene can help us to investigate the changes that are created in protein structure. Pyrene is insoluble in water and soluble in acetic acid, mixture of acetic acid and water and in organic solvents such as methanol. UV-Vis spectrum of Pyrene has three strong bands at 308, 348 and 433 nm. A series of UV-Vis titration experiments were carried out based on titration of a given amount of Pyrene with HSA at various pH, phosphate buffer and different temperatures. The titration spectrum were analyzed at each temperature using SQUAD program and based on 1:1, 1:2 and 2:1 models. Results indicated that formed complex between Pyrene and HSA is 1:1. All thermodynamic parameters of complex formation including ΔG°, ΔH°, ΔS° and formation constant of complex (K) were calculated and results showed that the process is endothermic and entropy driven. This issue shows the predominant role of hydrophobic forces in interaction between Pyrene and HSA. Investigating the effect of increasing the ionic strength on absorption spectrum of Pyrene-HSA complex also confirms the results of thermodynamic studies. Using the changes in the structure of absorption spectrum of Pyrene in water, plasma of human blood and in a buffer solution of HSA, we could indicate that Pyrene in blood plasma is concentrated in hydrophilic micro phases of plasma proteins and lipid.

Keywords: Pyrene ($C_{16}H_{10}$); Human serum albumin protein (HSA); Endothermic; ionic strength

INTRODUCTION

The interaction of small ions and molecules with special sites on the vital macromolecules is one of the most important issues in biophysical and biochemical investigations. Today the study of this kind of binding is the heart of molecular biology.

Titration of protein is one of the important information tools about titrable groups on protein. All proteins have various acidic and basic sites [1]. Since the proteins are different in kind and order of amino acids so each protein has special acidic and basic properties. According to Tanford model, each electrical charge occupy a special site on the protein so using the obtained equations from titration diagrams, the situation of ionizable sites are related to intrinsic properties of these sites.

The interaction between tracers such as Pyrene ($C_{16}H_{10}$) that is a polycyclic aromatic hydrocarbon and human serum albumin protein (HSA) is important because the UV-Vis absorption changes of bound tracer to protein can help us to trace the created changes in protein structure so the quality of binding of Pyrene as a proper absorption tracer to water soluble spherical protein (HSA) is investigated using UV-Vis absorption spectrum.

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The main purpose of this research is analyzing the interaction of Pyrene with HSA. In this research according to importance of Pyrene as an important tracer, we have investigated the physicochemical and thermodynamic properties and quality of interaction between Pyrene and HSA using the UV-Vis spectroscopic technique. We have determined the bonding constants by analyzing the spectral data of Pyrene at various concentration of HSA using the SQUAD program. We could determine all thermodynamic parameters of interaction with determining the binding constants at different temperatures and based on van't Hoff model. Calculated quantities give us valuable information about molecular mechanism of interaction.

EXPERIMENTAL

Materials

All materials containing Pyrene ($C_{16}H_{10}$), human serum albumin (HSA), methanol, acetic acid, ethanol, $Na_2HPO_4.12H_2O$, $NaH_2PO_4.2H_2O$, sodium chloride and sodium hydroxide were obtained from Fluka, Merck and Aldrich. All buffers were prepared using double distilled water. HSA and Pyrene solutions were prepared using these buffers. All solutions were freshly prepared and used.

Apparatuses

UV-Vis spectrophotometer

All absorption spectra were recorded on a Cary 100 Scan double beam UV-Vis-NIR spectrophotometer that was equipped by temperature regulation system.

Digital balance

A balance on the model of AE 160 from Mettler Company was used for weighing the materials that its precision was 0.0001 g.

pH-meter

Regulation of pH was carried out by a pH-meter on the model of F-12 from Metrohm Company.

Methods

Preparing the stoke solution of Pyrene

Pyrene with molecular mass of 202.26 g /mol is a yellow powder and is insoluble in water and should be crystallized in ethanol twice. In this regard at first Pyrene was dissolved in minimum ethanol by heating and then cooled to room temperature until the crystals formed. These stages were repeated once again. The obtained solution was filtered and obtained pure Pyrene was placed in oven with temperature of 60 °C for 10 minutes and then a 1 mM solution of Pyrene in alcohol was prepared. The 1 μM solution of Pyrene in water was prepared from this stock solution.

Preparing the 1 mM buffer solution of phosphate

The pH was regulated at 7.0 by concentrated solution of hydrochloric acid and sodium hydroxide and then 0.675 g of dipotassium hydrogen phosphate and 0.0414 g of potassium dihydrogen phosphate were reached to volume of 1000 ml.

Preparing the 5 mM buffer solution of phosphate with a specified ionic strength

0.4675 g of NaCl was dissolved in double distilled water and 0.04758 g $NaH_2PO_4.2H_2O$ and 0.2023 g $Na_2HPO_4.12H_2O$ were added to it. The pH of solution was regulated on 7.0 and volume of solution was reached to 100 mL.

Preparing the stoke solution of sodium chloride

73.125 g NaCl was dissolved in phosphate buffer with pH=7 and volume of solution was reached to 250 mL. The concentration of this solution would be 5.0 M. This solution was used as stoke solution of salt for titration experiments of HSA and Pyrene with salt.

The Beer - Lambert experiment

The molar absorption coefficient of Pyrene solutions in methanol in concentration range of 10^{-7}-10^{-5} M at 25°C and using 1 mM phosphate buffer and at pH=7.00 were determined according to obtained results of absorption spectra of Pyrene at different temperatures and wavelengths and its amount was obtained 5×10^4 $M^{-1}cm^{-1}$.

Preparing the stoke solution of HSA

20 mL of 3% solution of HSA (4.4×10^{-4}M) in phosphate buffer (0.3 M) at different pH was prepared. This solution was used for preparing more dilute solutions of HSA in next stages of research.

Titration experiment of HSA on the absorption spectrum of Pyrene

HSA has an absorption maximum wavelength at 261 nm and also there is a higher maximum at 204 nm. The solution of Pyrene in methanol has maximum wavelengths at 305, 318 and 333 nm

so the range of 280-400 nm was considered for banding studies. 1 mg of HSA was dissolved in 1 mL of Pyrene solution in water and methanol (6.7×10^{-5}). Dissolving of HSA in solution of Pyrene in water and methanol was carried out in order to eliminating the dilution effects of Pyrene in water and methanol due to increasing the volume.

Two quartz cells were charged with 2 mL buffer and baseline correction was carried out. With replacing the contents of sample cell with 2 mL of Pyrene solution in water and methanol with mentioned concentration and after two minutes temperature fixation, the total absorption spectrum in range of 280-400 nm was recorded. Then each time 100 µL of HSA solution was added to sample solution and previous stages were repeated. The optical spectrometric titration of Pyrene solution in water and methanol and in phosphate buffer (5mM) at constant ionic strength at pH 7 with HSA at 20, 25, 30, 35, 40 and 45 °C was repeated. At each temperature about 50 titrations were carried out and absorption of each titration was recorded in range of 300-350 nm. The measured absorptions obey Beer Lambert law.

Titration experiment of salt effect on the pyrene-HAS

The sample cell was charged with 500 µL of a solution with a ratio of 1:2 of Pyrene-HSA and the volumes of 50 µL of NaCl solution (5.0 M) were added to it consecutively. After each adding, the solution of cell was stirred for 1 minute and then its spectrum was recorded. The effect of dilution on the obtained spectrum was done and corrected spectrum was obtained. This experiment was carried out at pH=7 and 25 °C.

RESULTS AND DISCUSSION

Analyzing the UV-Vis absorption spectra of HSA and Pyrene

In absorption spectrum of HSA there is a maximum absorption at 261 nm and a higher maximum at 205 nm (Fig 1). The band of 261 nm is related to electron transition ($\pi\rightarrow\pi^*$) of π bonds of aromatic amino acids of tyrosine, phenyl alanine and tryptophan and absorption at 205 nm is related to transition ($\pi\rightarrow n^*$) of amid groups of peptide bonds. In absorption spectrum of Pyrene there are 10 absorption bands at different wavelengths (Table 1 and Fig. 2).

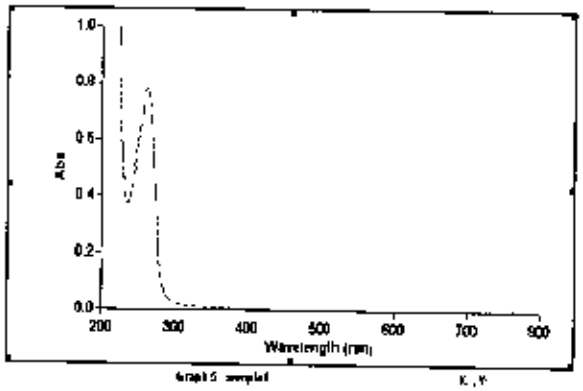

Fig.1. Absorption spectrum of HSA in 1 mM buffer, pH=7, NaCl and at 25°C

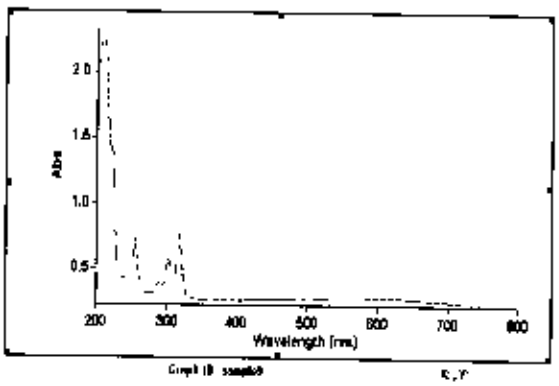

Fig.2. Absorption spectrum of Pyrene in methanol at 25 °C

Binding of Pyrene to HSA

After investigating the absorption spectra of HSA and Pyrene, the best spectral region for investigating the interaction of Pyrene and HSA was chose between 300-400 nm because in this region there is no absorption for HSA but Pyrene has three strong bands at 305, 318 and 333 nm so the created changes in UV-Vis spectrum of Pyrene and HSA mixture in range of 300-400 nm is related to Pyrene-HSA complex. Fig. 3 is absorption spectrum of Pyrene with different concentrations of HSA at 25 °C. Increasing of HSA causes to decreasing of absorption intensity at all wavelengths. The isobestic points indicate a 1:1 simple equilibrium between Pyrene and HSA. In other word it seems that there is only one binding site for Pyrene on the HSA. Fig. 4 shows changes of complex spectrum in presence of HSA in different ionic strength and at 25 °C. The molar ratio of Pyrene to HSA is about 5.

After investigating the absorption spectra at different pH, the 1 mM phosphate buffer and pH=7 was chosen as the best buffer and pH. Fig. 5 shows the effect of temperature on the absorption spectrum of Pyrene with different concentration of HSA. The obtained spectral data can be analyzed in order to extracting the equilibrium constant using SQUAD program.

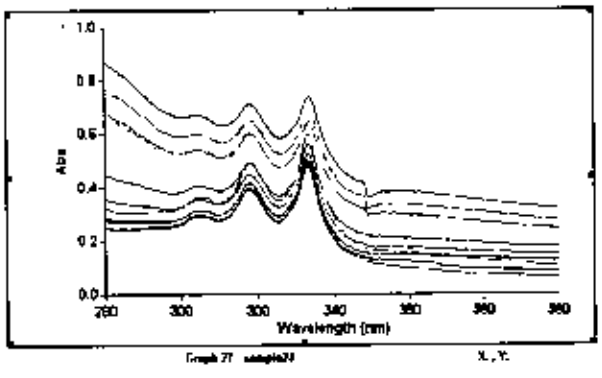

Fig.3. Absorption spectrum of Pyrene in methanol at different concentrations of HSA in phosphate buffer (5 mM), pH=7 and at 25 °C

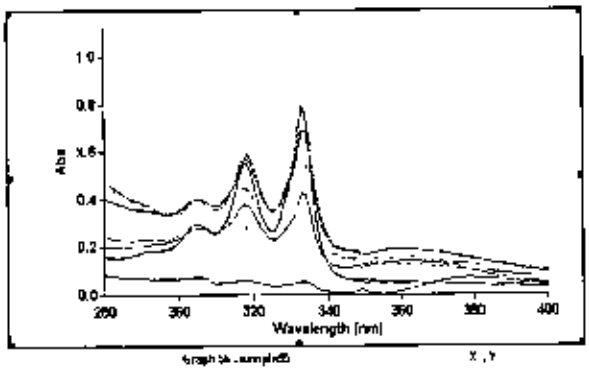

Fig.4. Absorption spectrum of Pyrene in methanol in different concentrations of HSA in phosphate buffer (1 mM), pH=7 and 25 °C and in high concentration of NaCl

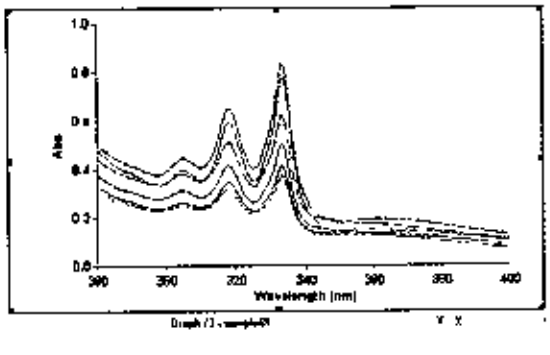

Fig.5. Absorption spectrum of Pyrene in methanol in different concentrations of HSA in phosphate buffer (1 mM), pH=7 and different temperatures.

Analyzing the binding process of Pyrene to HSA

Thermodynamic analysis of a process is based on three basic quantities of Gibbs free energy (ΔG°), enthalpy changes (ΔH°) and entropy changes (ΔS°). With determining the value of ΔH° based on diagram slope of ln K versus 1/T, all thermodynamic parameters of Pyrene-HSA complex formation were determined. These parameters are shown in tables 2 and 3.

The results indicated that the binding process is endothermic and with increasing the temperature, the binding affinity increased and it seems that process is basically entropy driven that represent the special role of hydrophobic interactions in binding process. The values of K are about 10^4 M^{-1} that confirms the correctness of calculations. According to SQUAD calculations and isobestic points, the formation of 1:1 complex was confirmed and also the amounts of binding constants were determined.

Doing experiments at six different temperatures and analysis of data made the possibility of thermodynamic analysis according to van't Hoff equation. Results showed that process is endothermic and entropy driven. The binding process indicated that there is one binding site on the surface of HSA for Pyrene. This issue indicates the predominant and comparable role of hydrophobic interactions in comparison with electrostatic forces. The high amounts of binding constants (10^4 M^{-1}) indicate that HSA can act as a proper tracer. Studying the effect of increasing the ionic strength on the absorption spectrum of Pyrene-HSA complex shows that increasing the concentration of salt cause to decreasing the absorption intense of complex in all spectral regions but total scheme of spectrum is nearly constant. This issue also indicates the low effect of electrostatic interactions in the binding process of Pyrene and HSA and confirms the thermodynamic results.

Changes in structure of absorption spectrum of Pyrene in water, blood plasma of human and in a buffer solution of HSA showed that Pyrene in blood plasma is concentrated in hydrophilic micro phases of plasma proteins and lipid.

Table1. The properties af absorption spectrum of Pyrene solution at different wavelengths

λ (nm)	Abs
437.00	0.2110
333 00	1.8436
318.00	1.5036
305.00	0.6595
271.00	1.9744
261 00	1.3359
251.00	0.6533
238.00	2.4864
231.00	2.1826
205.00	0 8566

Table2 Thermodynamic parameters for binding of Pyrene ta HSA in phosphate buffer (1 mM), pH=7 and different temperatures

t °C	$(K \pm \Delta K)\times 10$ (M^{-1})	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H\ kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	0 974 ± 1.026	-16.770 ± 0.061	44.528 ± 0 117	209.101 ± 0.208
25	1.432 ± 1.023	-18.014 ± 0.057	44.528 ± 0.117	209.765 ± 0.191
30	1.876 ± 1.030	-18.993 ± 0.076	44.528 ± 0.117	209.536 ± 0.254
35	2.432 ± 1.028	-20.008 ± 0.072	44.528 ± 0.117	209.430 ± 0.117
40	2.899 ± 1.023	-20.751 ± 0.060	44.528 ± 0.117	208.456 ± 0.138
45	4.109 ± 1.030	-22.009 ± 0.079	44.528 ± 0.117	209.137 ± 0.254

Table3. Thermodynamic parameters for binding of Pyrene to HSA in phosphate buffer (1 mM), pH=6 and different temperatures

t °C	$(K \pm \Delta K)\times 10^{-}$ (M^{-1})	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-}$	$\Delta H^{\circ} \pm \Delta\Delta H\ kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	0 2879 ± 1.020	-13.797 ± 0.021	57 197 ± 0.010	242.176 ± 0.101
25	0.4507 ± 1.011	-15.148 ± 0.010	57.197 ± 0.010	242.646 ± 0 101
30	0 6239 ± 1 021	-16.206 ± 0 021	57.197 ± 0.013	242.134 ± 0.011
35	0.9086 ± 1.021	-17.452 ± 0.021	57.197 ± 0 012	242 248 ± 0 012
40	1 279 ± 1 0310	-18 625 ± 0 011	57.197 ± 0.011	242.126 ± 0 101
45	1 887 ± 1 011	-19.951 ± 0 014	57.197 ± 0.012	242.489 ± 0.111

CONCLUSION

The results of studies show that Pyrene is a proper absorption probe for binding to HSA and can be used in order to following the created structural changes in this protein under different environmental conditinns. Binding of Pyrene to HSA indicates the special role of hydrophobic forces in interaction of this probe with protein. About studied protein there was no aggregation phenomenon and this issue is one of the advantages of this probe because aggregation can create great changes in protein.

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