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Delivery of drugs HSA protein with [Pd (bipy) Glycine] Cl complexes

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ABSTRACT

Human serum albumin (HSA) is the most abundant protein in the circulatory system; HSA is an abundant plasma protein that binds a wide variety interaction of [Pd (bipy) Glycine] Cl. The complex [Pd (bipy) Glycine] Cl has been synthesized and purified and the spectral characteristics of this complex were determined and a series of titration experiments were designed with respect to titration of complex with HAS solution. These experiments are done at pH=7, 1mM phosphate buffer and at 20, 25, 30, 37 and 40 ºC. The titration spectra were analyzed at each temperature based on binding models of 1:1, 1:2 and 2:1, using SQUAD software .The results represents the formation of 1:1 complex between drug and HSA .All of the thermodynamic parameters such as Δ G , Δ H , Δ S and formation constant (K) were calculated with their uncertainty. These results were interpreted on the basis of molecular viewpoint and represent the endothermic of process. Hence the process is entropy driven; this confirms the rule of hydrophobic interaction in the formation of drug-HSA complex. This evidence is also certified by studying the effect of ionic strength on the UV-Vis spectra of drug-HSA.

Keywords: [Pd (bipy) Glycine] Cl; HAS; Ionic strength; UV-Vis spectra; Drug delivery

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INTRODUCTION

Organometallic complexes have a special place in chemotherapeutic drugs. [Pd (bipy) Glycine]Cl is an organometallic compound which was discovered by Rosenberg and his colleagues [1]. He saw that platinum compounds control the division process. These compounds control DNA synthesis and bind to DNA through interstrand cross-links [2-3]. Like other chemotherapeutic drugs, they have some side effects such as nausea, vomiting, and renal poisoning and hearing nerve disorder; renal effects are due to the inactivating of enzymes like ATP and also binding to SH-groups of renal passages [4-6].

In order to design drugs with better quality, synthesis of complexes consisting of carrier molecules with low molecular weight was suggested [7]. In this regard complexes with general formula of [M (bipy) AA] Cl were synthesized in which M is Pd (II) or Pt (II), bipy is 2, 2'-bipyridyl amine or 2, 2'-bipyridine amine, and AA is the reagent of small amino acids like alanine and glycine.

There are two isobestic points in the interaction between these drugs to DNA. That indicates the equilibrium among free and bound complex to DNA. Metallic complexes bind to genetic substance through van der Waals and ionic interactions and also through hydrogen bond at minor – groove, and binding is not covalent [8-9]. Of course there are some complexes of platinum and palladium that bind to DNA, through covalent bond like [Pd (bipy) Glycine] Cl. Aromatic ligands are inseparable part of these complexes [10-11]. Binding parameters represent that amount of bound complexes to DNA are enough to change the natural structure of DNA but aren't too much to create mutation [12].

Pd complexes bind to DNA with a different mechanism with respect to [Pd (bipy) Glycine] Cl because strong binding of amino acids in these complexes is replaced by sulfidryle groups of renal passages hardly [13]. These compounds denaturize genetic substance after interaction, and change it's normal activity. On the other hand, distribution, free concentration and metabolism of different drugs, change the result of their binding to albumin

serum. So for better perception of the effect manner of drugs and improvement the existent drugs for making new drugs, investigation of the drug effect on the stability of stereo structure of carrier proteins is unavoidable.

In this report thermodynamic study on the interaction of 2, 2'-bipyridine glycinato palladium (II) chloride with HSA been done by UV-Vis spectroscopy. With analyzing the results of interaction, binding process of HSA-Complex HSA been investigated and binding information such as stoichiometry of drug-protein compound, mechanism of binding and changes of the thermodynamic functions of binding have been determined. Other purpose of this report is study and extending the binding concepts that is related to the binding of ligand, and representing better methods to achieve binding parameters.

MATERIALS AND METHODS

All materials containing: chloridric acid, sodium hydroxide, sodium chloride, phosphate buffer, human serum albumin (HSA), [Pd (bioy) Cl_2] and glycine were obtained from Merck and Sigma. Experiments were carried out at 20, 25, 30, 37, 40 and 45°C in phosphate buffer (5mM), pH=7 and I= 0.01. All solutions were prepared using deionized double distilled water. Sodium chloride was used to regulate the ionic strength. In all experiments, solutions were freshly prepared before using. (HSA) is considered with molecular weight of 66000 Dalton.

UV-Vis spectra were recorded on a carry -100 double beam UV-Vis spectrometer. And also we used pH-meter on the model of Horiba F_{12} and thermostat with accuracy of 0.01°C.

Synthesis and purification of palladium complex

1mmol of [Pd (bipy) Cl_2] complex (333 mg) was added to water (40 ml) then the mixture was stirred magnetically to create a homogeneous suspension. 1mmol glycine (75 mg) and 1 ml NaOH (1 N) were added calmly to 10mL deionized double distilled water and were stirred completely. Then this solution was added to the suspension gradually. The mixture was stirred at 50-60°C to be dissolved completely and produce a yellow solution. The solution was concentrated at 40°C to attain the desired volume of 3 ml. The yellow precipitate was filtered and crystallized in distilled water twice to purify the product.

Spectrometry method

HSA maximum is wavelength at 278.9 nm. Of course there is a higher maximum at 204 nm. Solution of palladium complex in water have maximum absorptions at 308 , 242 and 208 nm and two shoulders at 318 and 298 nm, but palladium complex in phosphate buffer (pH=7), have four absorption bands at 208, 239, 305 and 314nm so a range of 300-340nm considered for binding studies.

Photospectrometry titration of solution of palladium- HSA complex for binding studies

HSA (1mg) was dissolved in 1 ml solution of palladium complex in order to eliminate the dilution effects of palladium complex due to the volume increase. At first, 2 ml buffer were placed in two quartz cells and correction of ground line was carried out, then we replaced contents of cells with 2 ml palladium complex with mentioned concentration. After two minutes temperature fixation, recording of total absorption spectra at range of 300-340 nm was determined. Solution of HSA (100µL) was added to the sample solution in each time, and previous stages were repeated. These experiments were designed in a manner in which absorptions settle in Beer region. Photospectrometry titration of solution of palladium- HSA complex was repeated in phosphate buffer (5mM), and constant ionic strength, pH=7 and at 20, 25, 35, 40 and 45°C and for each temperature, 50 titration were carried out and absorption of each titration was recorded in range of 300-340nm. In this research, effect of ionic strength (salt effect) of NaCl (5M) at 25°C was experimentally investigated.

RESULTS AND DISCUSSION

Empirical results of UV-Vis spectrometry were analyzed using SQUAD software, which HSA been designed for calculating the best amount of stability constant of equilibrium models of 1:1 and 1:2, and using the method of non-linear least square. The results indicate that, the most spectra of the mixture of free and bound complex contain isobestic points that show equilibrium of 1:1 between free and bound complex.

HSA+Drug⇔ HSA Drug

K= [HAS Drug]/ [HAS][Drug]

Where, K is stability constant. With analyzing the spectral data using SQUAD software, we can determine values of K at different temperatures. UV spectra of complex at various amounts of drug and at 25 are shown in Fig.1 and Fig.2 respectively.

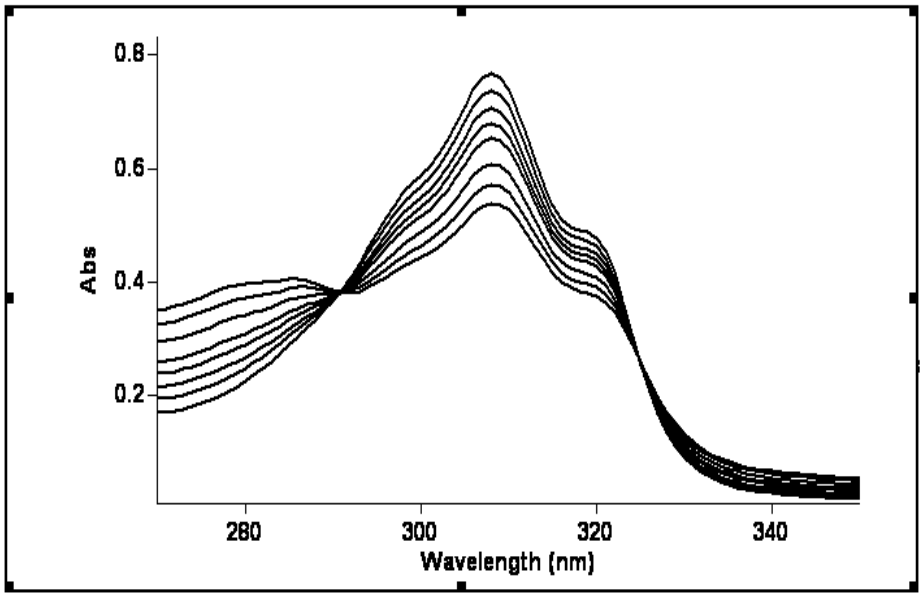

Fig.1 The absorption spectrum of complex drug-HSA in phosphate buffer 5 mM, (pH=7), I= 0.01 and in 25°C.

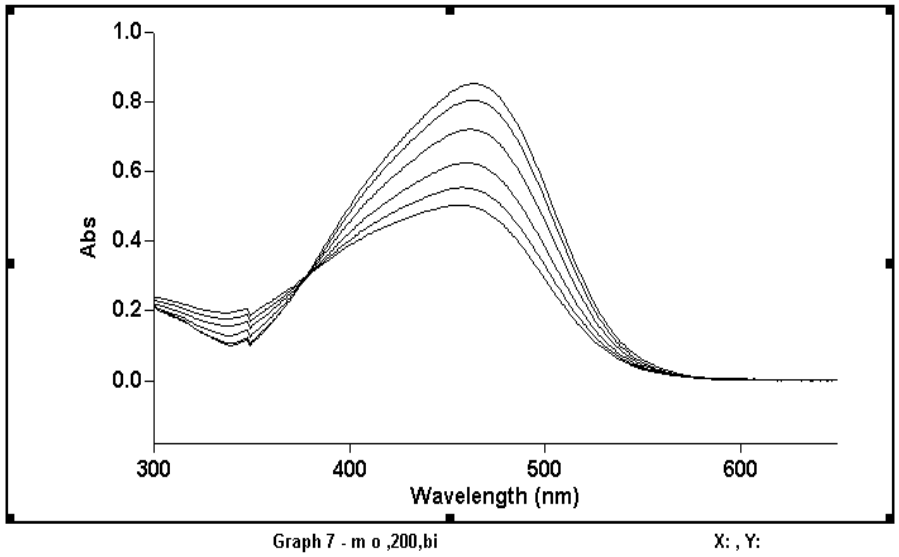

Fig.2 The absorption spectrum of complex drug-HSA in phosphate buffer 5 mM, (pH=7), I= 0.01 and in 37°C.

The molar ratio of drug to HSA is changeable, between 0.5–3.5 in each titration experiment. With selecting 50 wavelength and 15 spectra, 750 absorption data were extracted from titration experiments and were used for estimating the stability constants of HSA-drug complex using SQUAD software. The data were analyzed on the basis of 1:1, 2:1 and 1:2 models, and results indicated that the formation of 1:1 complex HSA the least uncertainty. This issue confirms isobestic points in the spectra of titration experiments, so we can conclude that there is just one special site for complex at HSA. Based on studies on the other drugs we can calculate free energy change of binding:

$\Delta G^{o} = -2.303\ RT \log K$

With determining the log K at various temperatures and Van't Hoff relation we can determine enthalpy of binding (ΔH^o):

(d log K /d 1/T)=-(Δ H/2.303R)

High linear correlation coefficient of this curve confirms the correctness of model and analysis method. Changes of binding entropy (ΔS^o) can be calculated based on Gibb's equation:

$\Delta G^o = \Delta H^o - T\Delta S^o$

All thermodynamic parameters of interaction of the drug with HSA with amount of absolute uncertainty, which HSA been calculated using statistical software, are shown in table 1.

Table1. Thermodynamic parameters of interaction of the drug with HSA

T(K)	log K	ΔG^o(kJ/mol)	ΔH^o(kJ/mol)	ΔS^o(J/mol)
293	4.27	-23.97	20.25	150.84
298	4.39	-25.06	20.25	151.97
303	4.40	-25.54	20.25	151.05
310	4.49	-26.49	20.25	151.67
313	4.52	-27.10	20.25	151.20
318	4.54	-27.83	20.25	151.12

Ionic strength increases with increasing the salt concentration that causes to decrease the absorption intense of complex in all spectral regions, while the total scheme of spectrum is nearly constant. This issue again indicates the low effect of electrostatic interactions at process of binding of drug to HSA and confirms the thermodynamic results.

CONCLUSIONS

Results show that process is endothermic and interaction between drug and HSA increases upon increasing the temperature. Positive amounts of ΔS^o indicate that process is entropy driven so we can claim that hydrophobic interactions play basic role in the binding of drug to HSA. The relatively high amounts of binding constants indicate that HSA can act as a proper carrier for drug, so we can use this property for calculating the releasing of drug.

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