Journal of Fluorescence (2019) 29:827–835
https://doi.org/10.1007/s10895-019-02383-3

ORIGINAL ARTICLE

Probing the Interaction of Newly Synthesized Pt(II) Complex on Human Serum Albumin Using Competitive Binding Site Markers

Akram Najaran[1] · Adeleh Divsalar[2] · Ali Akbar Saboury[3] · Nasim Hayati Roodbari[1]

Received: 6 February 2019 / Accepted: 17 April 2019 / Published online: 19 June 2019

Abstract

Considering the importance of pharmacology and the influence of drugs on biological materials, the effects of a newly designed and synthesized platin complex (2,2′-Bipyridine-3,3′-dicarboxylic acid, oxalato Platinum(II), as an antitumor drug was tested on the structure of blood carrier protein of human serum albumin (HSA) using various spectroscopic techniques including UV-visible, fluorescence, and circular dichroism at 25 and 37 °C. Results of the fluorescence measurements revealed that adding the complex caused reduction in intrinsic fluorescence emission of HSA resulted from dynamic quenching of HSA. The number of binding sites and binding constants were calculated at both temperatures of 25 and 37 °C. In addition, in order to identify the complex's binding site on HSA employing spectroscopy, the competitive studies were followed using warfarin, digitoxin and ibuprofen as site markers of Sudlow sites I, II and III. Competitive binding test results have shown that Pt(II) complex bind on the warfarin binding site (or Sudlow sites I) on HSA. Besides, a reduction in thermal stability for HSA was observed in the presence of the newly designed Pt(II) complex.

Keywords Site markers · HSA · Sudlow sites · Competitive studies · Fluorescence quenching

Introduction

Human serum albumin (HSA), a negatively charged nonglycosylated serum protein, presents several functions [1]. HSA concentration is approximately 42 mg/ml (about 0.6 mM) in a normal person, which makes it an abundant plasma protein [2]. Owing to its high concentration in the blood plasma, HSA comprises nearly 80% of the colloid osmotic pressure of plasma [3]. Moreover, this protein transports various compounds in the blood [3]. Numerous drugs with a poor solubility in water are bound and carried in the blood by HSA [4]. According to the published reports, binding of drugs to HSA can change the free concentration, distribution, and metabolism of the drugs [5].

HSA is composed of a single polypeptide chain of 585 amino acids which is rich in α-helices and contains 17 disulfide bridges [6, 7]. The single HSA chain is formed from three similar domains, named I, II, and III. In each domain, two helical subdomains, called A and B, are connected by random coils [8]. Subdomains IIA and IIIA are two structurally selective binding pockets for drugs and correspond to Sudlow proposed sites I and II [8, 9]. Sudlow's site I is mainly preferred by heterocyclic anions like warfarin, while Sudlow's site II is favored by aromatic carboxylates possessing extended conformation such as ibuprofen [10–12].

Interaction of drugs with proteins influences both the apparent distribution volume and elimination rate of the drug. Furthermore, the drug displacement from HSA owing to the competitive binding of drugs administered simultaneously can result in higher levels of the free drug and consequently potential side effects. Therefore, in order to avoid the undesirable side effects, it is necessary to be aware of the impact of the drug displacement due to simultaneous administration of drug compounds [13].

Using metal compounds in the treatment of numerous diseases is prevalent such as bismuth, silver, platinum, iron, gold,

✉ Adeleh Divsalar
divsalar@khu.ac.ir

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Cell & Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

[3] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

and antimony as anti-ulcer, anti-microbial, anti-cancer, anti-malarial, anti-arthritic, and anti-protozoal compounds, respectively [14]. Numerous compounds of transition metals and also main group elements have been studied for anti-tumor activity [15]. The first reports on application of metals or metal-containing drugs in treatment of cancer dates back to sixteenth century [15].

Employing transition metal compounds as chemotherapeutic agents to treatment of cancer has attracted attention. Platinum-based drugs are considered among the most successful clinically administered anticancer chemotherapeutic agents. Improving efficiency and reducing the unwanted side effects of *cis*-diammine dichloroplatinum (II) (*cis*-platin) is the ultimate goal of many lines of researches in the field of metal-based drugs.

Emergence of *cis*-platin and other therapeutic Pt(II) compounds, as anticancer drugs have resulted in extensive research with the aim of developing novel compounds with both less toxicity and more selectivity towards cancer cells [16]. The undesirable side effects of applying *cis*-platin in treatment of cancer include nephrotoxicity which results from damage to renal tubular epithelial cells and can lead to renal electrolyte wasting [17, 18].

HSA is a flexible molecule and binding of one drug can change the binding of other drugs. Hence, studying the interactions between HSA and drug molecules is necessary and informative. The current study focused on biophysical characterization of interactions between a newly designed Pt(II) compound (2,2′-Bipyridine-3,3′-dicarboxylic acid, Oxalato Platinum(II), Fig. 1) and HSA using spectroscopic techniques. Also, the binding site of the Pt(II) complex on HSA was proposed using competitive study by applying various site markers of warfarin, digitoxin and ibuprofen.

Materials and Methods

Materials

Human serum albumin (HSA) was obtained from Sigma. The Pt(II) complex was prepared in our laboratory based on a previously published procedure [19]. All other reagents and materials were of analytical grade. The site markers warfarin, ibuprofen and digitoxin were purchased from Danial Teb. The solutions were prepared using double-distilled water. Besides, 5 mM NaCl was employed as a solvent. HSA concentration was calculated using spectrophotometric methods and a molecular absorption coefficient of $\varepsilon^{10\%}$ at 278 nm = 5.3 M^{-1} cm^{-1} [20].

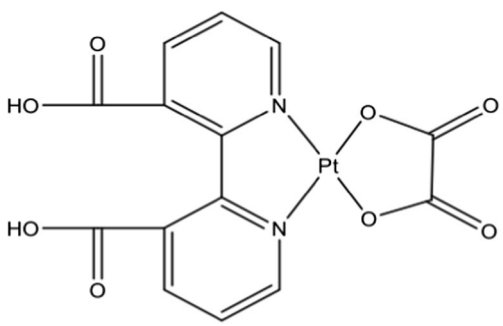


Fig. 1 The 2,2′-Bipyridine-3,3′-dicarboxylic acid, Oxalato Platinum(II)

Fluorescence Measurements

Fluorescence intensities were determined employing a Cary Spectrofluorometer. The excitation and emission wavelengths were 295 and 300–500 nm, respectively. The fluorescence intensities were measured at 25 and 37 °C using a fluorescence cuvette of 1 cm path length. The excitation and emission slits were set at 5 nm. The fluorescence intensity of the highest concentration of Pt(II) complex was measured at 295 nm, which was negligible. Furthermore, 5 μM HSA was applied in fluorescence studies. Moreover, competitive studies were performed in the presence of 5 μM warfarin, digitoxin, and ibuprofen as site markers.

CD Measurements

CD measurements were performed on an Aviv Spectropolarimeter model 215. Secondary structure of 5 μM HSA in the presence of 0, 49.3, and 147.9 μM of Pt(II) complex was monitored in the far UV region (200–260 nm) using a 0.1 cm path length cuvette. Far-UV CD results were converted to molar ellipticity [θ] (deg cm2 dmol−1) according to $[\theta]\lambda = (100 \times MRW \times \theta_{obs} / cl)$, where 113.7 is the mean amino acid residue weight (MRW) for HSA., and θ_{obs}, c, and l are the observed ellipticity (deg) at each wavelength, HSA concentration (mg/ml), and the length of the light path (cm), respectively. CDNN software was used to deconvolute the CD spectra and predict the content of HSA secondary structure elements upon interaction with Pt(II) complex at different concentrations.

Thermal Denaturation Study

The thermal denaturation of 16.4 μM HSA in the presence and absence of 106.6 μM Pt(II) complex was analyzed using a UV–Visible spectrophotometer CARY-100-Bio. Changes in absorbance at 280 nm of reference and sample cuvettes in the temperature range were recorded. The temperature was programmed to rise from 20 to 90 °C.

Results and Discussion

Fluorescence Studies

Trp-214 in the hydrophobic cavity of subdomain IIA, is responsible for HSA intrinsic fluorescence. Interaction of HSA with various ligands can alter the fluorescence emission of Trp-214. This also occurs during subunit association, conformational transition, or substrate binding [20]. Effect of adding various concentrations of Pt(II) complex on HSA fluorescence emission at different temperatures of 25 and 37 °C, has been shown in Fig. 2a, b.

Results showed a decrease on fluorescence intensity of Trp-214 after addition of the Pt(II) complex which suggests an interaction between Pt(II) complex and HSA (Fig. 2C). However, since the maximum emission wavelength presented no change in the constant concentration of HSA, it can be understood that the interaction with HSA may quench the HSA Trp-214 intrinsic fluorescence without changing the local dielectric environment [19–21].

A quenching effect occurs generally through a static or a dynamic mechanism, which are distinguishable by their distinct dependency on temperature. In the static mechanism, raising the temperature induces a reduction in quenching constants. Whereas, in dynamic quenching, a reversed phenomenon is observed. Raising temperature accelerates the diffusion of molecules and therefore induces the dissociation of weakly bound complexes. Consequently, greater numbers of molecules face collisional quenching and the quenching rate constant increases [21].

To discern the quenching mechanism type, the fluorescence quenching results are evaluated using the Stern–Volmer equation (Eq. (1)):

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{1}$$

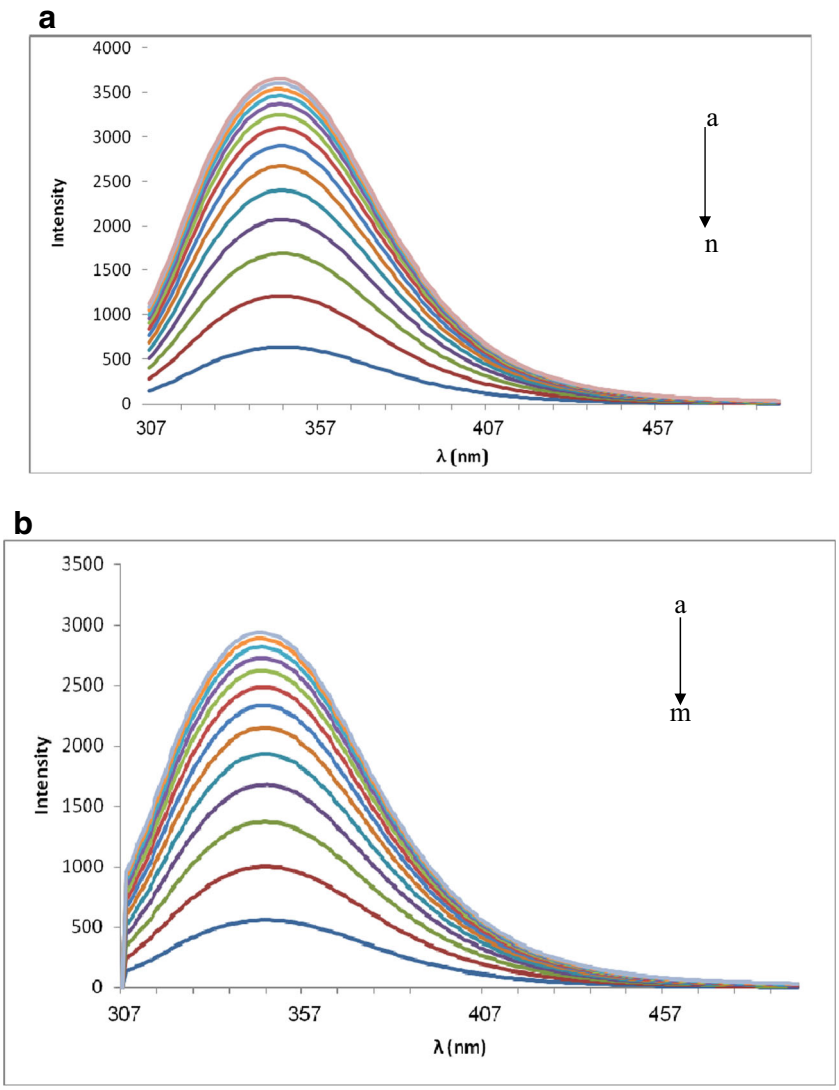


Fig. 2 **a** Fluorescence spectra of 5 μM HSA in the presence of various concentrations of Pt(II) complex: (a) 0, (b) 4.4, (c) 8.8, (d)13.1, (e) 17.5, (f) 21.8, (g) 26.2, (h) 30.5, (i) 39, (j) 47.5, (k) 55.9, (l) 64.2, (m) 72.4, and (n) 84.7 μM in 5 mM NaCl solution, pH 7.4, at 25 °C. **b** Fluorescence spectra of 2 μM HSA in the presence of various concentrations of Pt(II) complex: (a) 0, (b) 4.3, (c) 8.6, (d)12.9, (e) 17.2, (f) 21.4, (g) 25.7, (h) 29.9, (i) 34.1, (j) 38.3, (k) 50.7, (l) 63, and (m) 83 μM in 5 mM NaCl solution, pH 7.4, at 37 °C

where F_0 corresponds to the fluorescence emission intensity of HSA alone, and F is the fluorescence intensity of HSA in the presence of the quencher (Pt(II) complex). K_{sv}, and [Q] refer to the Stern–Volmer quenching constant and the quencher concentration, respectively.

As it can be seen in the Fig. 3, the F_0/F - [Q] plots demonstrate a positive deviation. The non-linear form of the Stern–Volmer plot may be due to either the higher concentration of the ligands surrounding the fluorophore or because of a combined form of static and dynamic quenching [21].

To have an estimation of the Stern–Volmer quenching constants values at the two tested temperatures, the Modified Stern–Volmer equation was employed (Eq. (2)) [21, 22]:

$$\frac{F_0}{\Delta F} = \frac{F_0}{F_0 - F} = \frac{1}{f_a K_{SV}} \frac{1}{[Q]} + \frac{1}{f_a} \tag{2}$$

where f_a refers to the quencher-accessible portion of the initial fluorescence, which is not necessarily equal to the portion of tryptophan residues accessible to the quenching.

According to Fig. 4, $F_0/(F_0 - F)$ and the reciprocal quencher concentration (1/ [Q]) have a linear relationship. Values of intercept and slope of the plot were used to calculate the f_a and K_{SV}, respectively.

The f_a values of HSA at both temperatures in the presence of the Pt(II) complex were calculated to be 1.54 (at 25 °C) and 1.17 (at 37 °C), respectively, meaning that the complex affects 99.2% (at 25 °C) and 98.4% (at 37 °C) of protein fluorophores (Fig. 4). The Stern-Volmer quenching constants values have been shown in Table 1. According to the Table 1, the values of K_{SV} have an obvious dependency on temperature. Furthermore, results show that raising the temperatures from 25 to 37 °C, led to a decrease in K_{SV} value. This phenomenon demonstrates that the static quenching mechanism has the highest contribution in quenching.

Analysis of the fluorescence quenching results was performed to determine the binding parameters of Pt(II) complex to HSA. Calculation of the number of binding sites (n) and the apparent association constant K was carried out employing the following equation (Eq. (3)) [22]:

$$\log\left[\frac{F_0 - F}{F}\right] = \log K + n\log[Q] \tag{3}$$

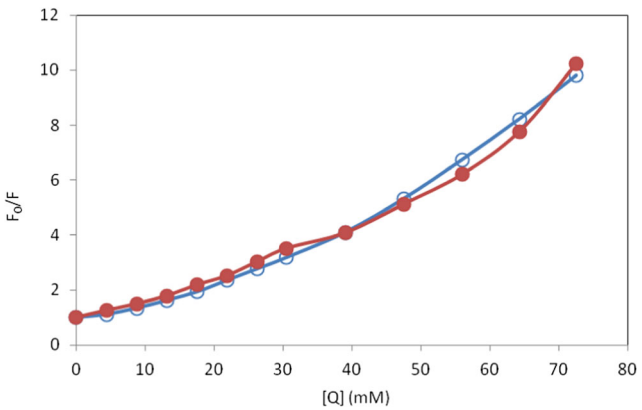


Fig. 3 Effect of different concentrations of the Pt(II) complex on HSA maximum fluorescence emission at 25 °C (●) and 37 °C (○)

As it can be seen in Fig. 5, the Log $[(F_0 - F) / F]$ as a function of log [Q] plot displayed a straight line. Therefore, K and n values were determined using the Y-axis intercept and the slope, respectively. K and n values for Pt(II) complex were calculated at both temperatures and shown in Table 1. It can be seen in the Table 1 that the n values were equal to 1.5 and 1.3 at 25 °C and 37 °C, respectively.

Furthermore, the reduction in binding constants upon increasing the temperature suggests that weak interacting forces were involved in the process of complexation between Pt(II) complex and HSA, which even weakens at higher temperatures [11]. Values of K obtained for the complex–HSA shows that in comparison with the other strong complexes of ligand-protein, the binding at Pt(II) complex–HSA has high affinity [23].

It has been reported that HSA has three homologous α-helical domains, namely domains I, II and III. Hydrophobic cavities of subdomains IIA and IIIA are the main ligand binding sites of albumin [4]. Albumin has 2 major binding sites for drug, named Sudlow's sites I and II. According to X-ray data, drug sites I and II should be in subdomains IIA and IIIA, respectively. Also, the hydrophilic cavity located in subdomain IB, which can be referred as site III, is considered as a possible protein pocket for small molecules. Warfarin bind specifically to serum albumin site I, whereas ibuprofen bind to site II and digitoxin bind to Site III [24].

In order to determining the binding site of Pt(II) complex on HSA, site marker competitive tests were performed using warfarin, ibuprofen and digitoxin as site markers of sites I, II and III, respectively. In this way, Pt(II) complex was added to a cuvette containing the site markers and HSA. Based on the results, addition of the Pt(II) complex to the cells holding HSA and warfarin, HSA and ibuprofen and HSA and digitoxin caused a decrease in the fluorescence intensity of HSA (data were not shown).

Furthermore, the fluorescence quenching data (Eq. (3)) was applied to determine the values of number of binding site and binding constants (K) of the Pt(II) complex on the protein in the presence of the site markers of warfarin, digtoxin, and ibuprofen (Fig. 6, Table 1).

Evaluating the changes in the complex binding constants on protein when the site markers were present, led to the following conclusion: K (HSA–Pt) >> K (HSA–war)–Pt, indicating that in the presence of warfarin, the binding affinity between HSA and Pt(II) complex significantly decreased (around 1990 fold). Whereas, in the presence of other site markers of ibuprofen and digiotoxin, the values of binding constants were changed and decreased; however, it can be

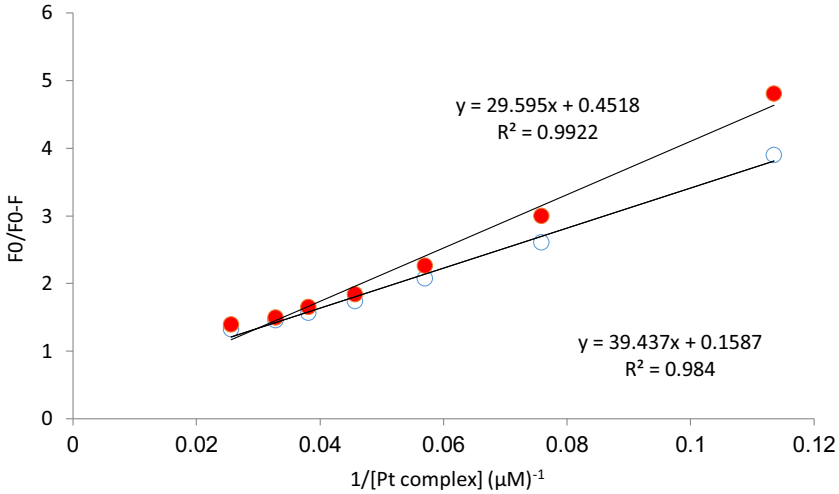


Fig. 4 The modified Stern–Volmer plot of HSA quenching by Pt (II) complex in 5 mM NaCl solution at 25 (●) and 37 °C (○). The best linear plots of $F_0 / F_0 - F$ against 1 / [Q] were drawn based on Eq. (2) at 25 (●) and 37 °C (○)

seen the highest decreasing in binding affinity of Pt(II) complex to the protein in the presence of warfarin (Table 1). So, it might be concluded that Pt(II) complex and warfarin competes for binding at the same binding site on the protein. Therefore, the binding site of the complex on HSA can be proposed as same as warfarin binding site or Sudlow's sites I, which is located in subdomain IIA.

According to the results of Table 1, ibuprofen and digitoxin did not occupy all the protein binding sites. Therefore, the Pt(II) complex is also able attach to the protein indicating that Pt(II) complex, digitoxin, and ibuprofen bind to various positions on the HSA (Fig. 6). These findings could be important throughout chemotherapy in obtaining the proper arrangement of using multiple drugs.

The n values for all three binary and ternary systems were calculated to be around 1, which indicates that the number of binding site of Pt(II) complex on the protein did not changed in the presence of various site markers (Table 1 and Fig. 6).

Considering the dependency of binding constant on the temperature, analysis of thermodynamic parameters at various temperatures was carried out to determine the active forces dominating the interaction. Generally, van der Waals, hydrogen bond, hydrophobic, and electrostatic interactions take part as forces establishing the protein interactions with small substrates [25]. The principal evidences to determine binding mode are the thermodynamic parameters including the standard enthalpy (ΔH°) and entropy (ΔS°) changes of binding reaction. The standard Gibbs free energy change (ΔG°), ΔH° and ΔS° were calculated using the van't Hoff equation (Eqs. (4–5)) and listed in Table 1 [26]:

$$lnK = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \tag{4}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT\ lnK \tag{5}$$

where R is the gas constant.

Ross and Subramanian [27] performed the characterization of the magnitude and sign of the thermodynamic parameters corresponded to different types of interactions that are contributing in protein association processes. For a hydrophobic interaction, usually both ΔS° and ΔH° have positive signs, whereas in the case of Van der Waals forces and hydrogen-bonds in low dielectric media, they have

Table 1 Calculated binding parameters of Pt(II) complex to HSA with and without site markers of digitoxin (dig), ibuprofen (ibu), and warfarin (war) at 25 and 37 °C

[dig] (μM)	[ibu] (μM)	[war] (μM)	T (°C)	n	K ($\times 10^6$ M^{-1})	K_{SV} (μM^{-1})	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (kJ/mol.K)
0	0	0	25	1.5	19.9	0.01	−41.7	−249.1	−0.6
0	0	0	37	1.3	1.2	0.004	−34.8		
5	0	0	25	1.1	0.2	0.0767	−30.1	–	–
0	5	0	25	1.3	0.9	0.0554	−32.4	–	–
0	0	5	235	1	0.01	0.026	−23.7	–	–

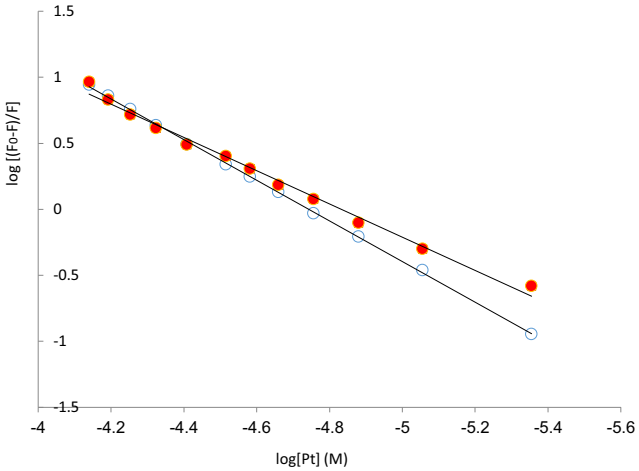


Fig. 5 The best linear plot of log F_0-F/F against log [Pt] based on the Eq. (3) at 25 (●) and 37 °C (○). The slope and the vertical intercepts can be applied to calculate the values of K and *n* related to binding of the Pt(II) complex to HSA

negative signs [28]. Furthermore, formation of the specific electrostatic forces in an aqueous solution between ionic species has a positive ΔS° value and a small (almost zero) negative ΔH° value [29].

ΔG° with negative value demonstrates that the process of forming interaction between Pt(II) complex and HSA is spontaneous (Table 1). Furthermore, positive ΔS° along with positive ΔH° are indicators for an entropy-driven and endothermic process. This data indicates that there is a hydrophobic interaction between HSA and Pt(II) complex [30]. Also, The negative values of ΔG° for all reactions (HSA-Pt(II) complex) in the absence and presence of various site markers of warfarin, digitoxin and ibuprofen, implies that all processes are spontaneous. Moreover, the value for both ΔS° and ΔH° van der Waals forces and hydrogen –bonds.

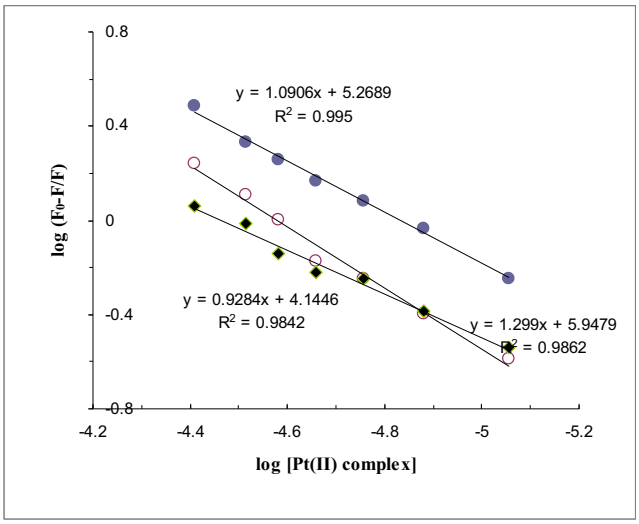


Fig. 6 The best linear plots of log F_0− F / F against log [Pt(II) complex] based on Eq. (3) in the presence of site markers of warfarin (♦), ibuprofen (○) and digitoxin (●) at 25 °C

Circular Dichroism Studies

CD analysis can be used for sensitive monitoring of conformation alterations in the protein interacting with ligands [31]. Results in Fig. 7 implies α-helical structure of HSA by presenting two negative bands in the UV region at 208 nm ($\pi \rightarrow \pi^*$) and 222 nm ($n \rightarrow \pi^*$). According to the published reports, HSA mostly comprises of α-helical structures (roughly 60%) and no β-sheet content [32].

Results of far-UV CD analysis of HSA in the absence and presence of 0, 49.3 and 147.9 μM Pt(II) complex at 25 °C are shown in Fig. 7. According to the results, native HSA is composed of 50.6% α-helix, 25.8% β-sheet, and 22.8% random coil. Based on the results presented in Fig. 7, the interaction with Pt(II) complex did not significantly change in HSA secondary structure content. As it can be seen from CD results, addition of Pt(II) complex with concentrations of 49.3 and 147.9 μM at 25 °C, induced no significant changes in the secondary structure of the protein.

It is known that the formation of HSA-drug complexes caused this phenomenon. Nevertheless, similar shapes of the CD spectra from HSA and HSA interacting with drugs in all systems suggested that HSA structure was still mostly α-helical.

Thermal Denaturation Analysis

The effect of Pt(II) complex on the thermal stability of HSA was investigated by the thermal denaturation analysis of HSA using temperature scanning spectroscopy (Fig. 8a). Pace analysis of thermal denaturation plots can be used to calculate the values of ΔG°_{25} (the standard Gibbs free energy of protein at 25 °C) and transition temperature (T_m) [14]. The standard Gibbs free energy of denaturation (ΔG°) is a standard of conformational stability of a globular protein and can be determined using two-state theory:

$$\text{Native(N)} \Leftrightarrow \text{Denatured(D)}.$$

The process was defined as a one-step denaturant-dependent based on the two-step theory.

It is possible to discern the process by measuring the changes in the absorbance, considering the two-state mechanism for denaturation of protein via temperature. Thus, calculation of the fraction of denatured protein (F_d) and also the equilibrium constant (K_D) is performed using the following equations (Eqs. 6 and 7):

$$F_d = \frac{Y_N - Y_{obs}}{Y_N - Y_D} \quad (6)$$

$$K_D = \frac{F_d}{1 - F_d} \quad (7)$$

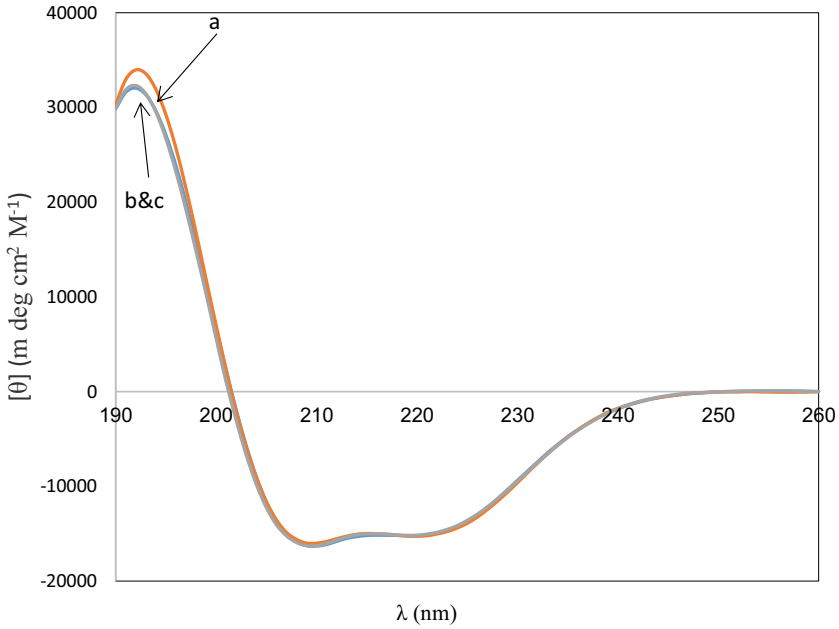
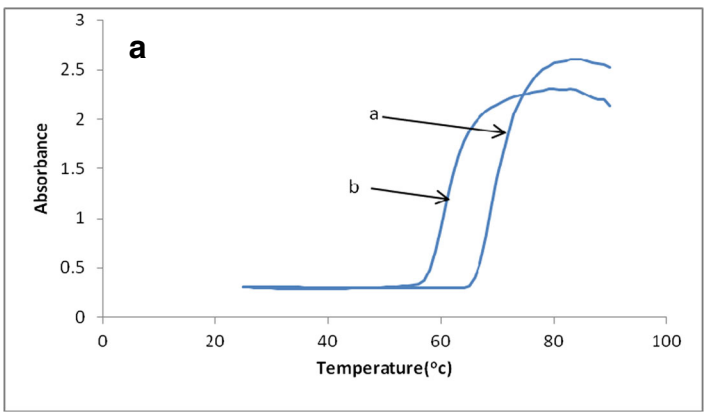


Fig. 7 Results of far-UV-CD analysis of (a) 5 μM pure HSA; in the presence of (b) 49.3 μM and (c) 147.9 μM Pt(II) complex.at 25 °C

where Y_N and Y_D correspond to values of Y characteristics of a completely native and denatured protein conformations, respectively, and Y_{obs} is the observed variable parameter. The standard Gibbs free energy change for denaturation process ($\Delta G°_D$) for HSA denaturation is obtained using the equation below (Eq. (8)) [33]:

$$\Delta G^0{}_D = -RT\ln K \tag{8}$$



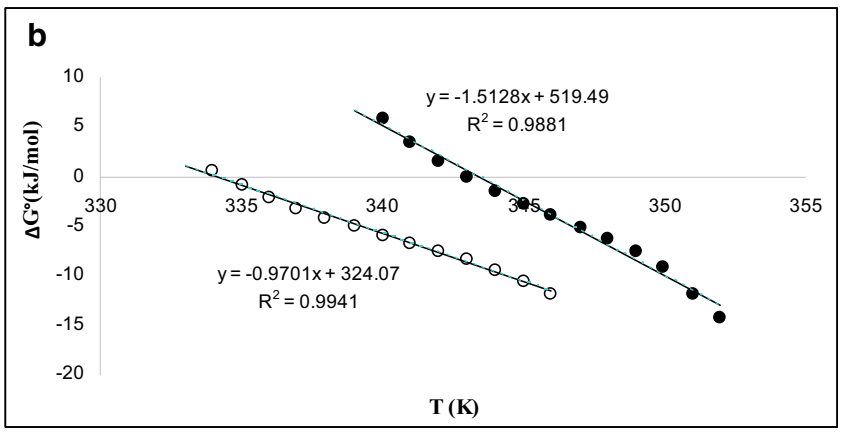


Fig. 8 **a** Profiles of thermal denaturation of (a) 16.43 μM pure HSA and (b) HSA interacting with of 106.6 μM Pt(II) complex. **b** Standard Gibbs energy versus temperature of HSA in the absence (●) and presence (○) of Pt(II) complex

Table 2 The values of $\Delta G°_{25}$ and T_m for 16.4 μM non interacting HSA and HSA interacting with Pt(II) complex

	$\Delta G°_{25}$ (kJ/mol)	T_m (°C)
HSA	68.7	69
HSA + Pt	34.9	60

The $\Delta G°_D$ values change in a linear way with T in a restricted region (Fig. 8b). Presumption that this linear dependency continues to 25 °C and employing a least-square analysis is the most straightforward way to estimate the conformational stability of protein at room temperature ($\Delta G°_{25}$). $\Delta G°_{25}$ was calculated and presented in Table 2. Values in the table also shows that interacting with Pt(II) complex leas to a change in T_m of HSA. It is evident that $\Delta G°_{25}$ values are reduced like T_m values of the protein. Therefore, it may be concluded that thermal stability of the protein may reduce in the presence of the Pt(II) complex.

Conclusion

Studying the quenching of relative fluorescence of HSA was demonstrated to be an important strategy to obtain improved insights about the critical and specific changes in the protein. Fluorescence measurement analysis proved the potent capability of Pt(II) complex in quenching the intrinsic fluorescence of HSA. The obtained results indicated that interaction of the newly designed drug (Pt(II) complex) with HSA led to substantial changes in the thermal stability of the protein and minor reductions in the protein α-helical content. Here, properties of Pt(II) complex-HSA binding in the absence and presence of three site markers (warfarin, digitoxin and ibuprofen) was investigated using multiple spectroscopic techniques. Results indicated that using easy spectroscopic approaches, we could detect the binding sites of a platinum complex on HSA without using costly or lengthy crystallographic work.

Because drug (designed ligand) binding could cause damaging effects on the protein through conformational changes, results and information achieved regarding the interaction of the Pt(II) complex with HSA can provide valuable information for developing enhanced metal anticancer complexes with less side effects in the future.

Acknowledgments Authors thank the Research Council of Kharazmi University for financial support of this work.

References

1. Varshney A, Sen P, Ahmad E, Rehan M, Subbarao N, Khan RH (2010) Ligand binding strategies of human serum albumin: how can the cargo be utilized? Chirality 22(1):77–87
2. Keppler BK (1993) Metal complexes in cancer chemotherapy. Wiley-VCH, Weinheim & New York
3. Kragh-Hansen U, Watanabe H, Nakajou K, Iwao Y, Otagiri M (2006) Chain length-dependent binding of fatty acid anions to human serum albumin studied by site-directed mutagenesis. J Mol Biol 363(3):702–712
4. Divsalar A, Bagheri MJ, Saboury AA, Mansoori-Torshizi H, Amani M (2009) Investigation on the interaction of newly designed anticancer Pd (II) complexes with different aliphatic tails and human serum albumin. J Phys Chem B 113(42):14035–14042
5. Yang F, Bian C, Zhu L, Zhao G, Huang Z, Huang M (2007) Effect of human serum albumin on drug metabolism: structural evidence of esterase activity of human serum albumin. J Struct Biol 157(2):348–355
6. Zunszain PA, Ghuman J, Komatsu T, Tsuchida E, Curry S (2003) Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. BMC Struct Biol 3(1):6
7. Yamasaki K, Maruyama T, Kragh-Hansen U, Otagiri M (1996) Characterization of site I on human serum albumin: concept about the structure of a drug binding site. Biochim Biophys Acta 1295(2):147–157
8. Ascenzi P, Fasano M (2010) Allostery in a monomeric protein: the case of human serum albumin. Biophys Chem 148(1–3):16–22
9. Espósito BP, Najjar R (2002) Interactions of antitumoral platinum-group metallodrugs with albumin. Coord Chem Rev 232(1–2):137–149
10. Curry S, Mandelkow H, Brick P, Franks N (1998) Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. Nat Struct Mol Biol 5(9): 827–835
11. Petitpas I, Bhattacharya AA, Twine S, East M, Curry S (2001) Crystal structure analysis of warfarin binding to human serum albumin anatomy of drug site I. J Biol Chem 276(25):22804–22809
12. Sudlow G, Birkett D, Wade D (1976) Further characterization of specific drug binding sites on human serum albumin. Mol Pharmacol 12(6):1052–1061
13. Liu X, Li S, Zhang J, Chen X (2009) Flow injection-capillary electrophoresis frontal analysis method for the study of the interactions of a series of drugs with human serum albumin. J Chromatogr B 877(27):3144–3150
14. Divsalar A, Khodabakhshian S (2015) Probing the binding site of a new synthesized anti-cancer compound to HSA via competitive ligand binding method. J Mol Liq 206:82–88
15. DESOIZE B (2004) Metals and metal compounds in cancer treatment. Anticancer Res 24(3A):1529–1544
16. Tušek-Božić L, Juribašić M, Traldi P, Scarcia V, Furlani A (2008) Synthesis, characterization and antitumor activity of palladium (II) complexes of monoethyl 8-quinolylmethylphosphonate. Polyhedron 27(4):1317–1328
17. Polyanskaya TV, Kazhdan I, Motley DM, Walmsley JA (2010) Synthesis, characterization and cytotoxicity studies of palladium (II)–proflavine complexes. J Inorg Biochem 104(11):1205–1213
18. Pourgonabadi S, Reza Saberi M, Khan Chamani J (2011) Investigating the antagonistic action between aspirin and tamoxifen with hsa: identification of binding sites in binary and ternary drug-protein systems by spectroscopic and molecular modeling approaches. Protein Pept Lett 18(3):305–317
19. Ghalandari B, Divsalar A, Saboury AA, Haertlé T, Parivar K, Bazl R, Eslami-Moghadam M, Amanlou M (2014) Spectroscopic and theoretical investigation of oxali-palladium interactions with β-lactoglobulin. Spectrochim Acta A Mol Biomol Spectrosc 118:1038–1046
20. Saeidifar M, Mansouri-Torshizi H, Divsalar A, Saboury AA (2013) Spectroscopic investigation on the binding of the antitumoral Pd (II) complex to human serum albumin. J Chin Chem Soc 60(2):133–139
21. Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Springer, New York

22. Abbasi-Tajarag K, Divsalar A, Saboury AA, Ghalandari B, Ghourchian H (2015) Destructive effect of anticancer oxaliplatin palladium on heme degradation through the generation of endogenous hydrogen peroxide. J Biomol Struct Dyn 34(11):2493–2504
23. Song M, Liu S, Yin J, Wang H (2011) Interaction of human serum album and C60 aggregates in solution. Int J Mol Sci 12(8):4964–4974
24. Curry S (2002) Beyond expansion: structural studies on the transport roles of human serum albumin. Vox Sang 83:315–319
25. Divsalar A, Saboury AA, Mansoori-Torshizi H, Ahmad F (2010) Design, synthesis, and biological evaluation of a new palladium (II) complex: β-lactoglobulin and K562 as targets. J Phys Chem B 114(10):3639–3647
26. Kratochwil NA, Huber W, Müller F, Kansy M, Gerber PR (2002) Predicting plasma protein binding of drugs: a new approach. Biochem Pharmacol 64(9):1355–1374
27. Ross PD, Subramanian S (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20(11):3096–3102
28. Mallick A, Haldar B, Chattopadhyay N (2005) Spectroscopic investigation on the interaction of ICT probe 3-acetyl-4-oxo-6, 7-dihydro-12H indolo-[2, 3-a] quinolizine with serum albumins. J Phys Chem B 109(30):14683–14690
29. Yue Y, Chen X, Qin J, Yao X (2009) Spectroscopic investigation on the binding of antineoplastic drug oxaliplatin to human serum albumin and molecular modeling. Colloids Surf B: Biointerfaces 69(1): 51–57
30. Divsalar A, Saboury AA, Ahadi L, Zemanatiyar E, Mansouri-Torshizi H (2010) Investigation of effects of newly synthesized Pt (II) complex against human serum albumin and leukemia cell line of K562. BMB Rep 43(11):766–771
31. Singha Roy A, Ghosh KS, Dasgupta S (2013) An investigation into the altered binding mode of green tea polyphenols with human serum albumin on complexation with copper. J Biomol Struct Dyn 31(10):1191–1206
32. Curry S (2009) Lessons from the crystallographic analysis of small molecule binding to human serum albumin. Drug Metab Pharmacokinet 24(4):342–357
33. Sarraf N et al (2005) Thermodynamic studies on the interaction of copper ions with carbonic anhydrase. Bull Kor Chem Soc 26(7): 1051–1056