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Binding Studies of Two Double Rollovers Cycloplatinated Compounds and Bovine Serum Albumin by Fluorescence Spectroscopy

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ABSTRACT

Platinum-containing compounds are among organometallic compounds that are considered promising drug candidates for cancer therapy. They have an important role in the treatment of several solid tumors. The interactions of small molecule drugs with serum albumin affect the pharmacokinetics of these therapeutic agents. In this study, the binding characteristics of two novel platinum complexes, named C1 and C2, and bovine serum albumin (BSA) were investigated by fluorescence and UV-Vis spectroscopies. The results showed that these compounds diminished the fluorescence emission intensity of BSA through ground state quenching mechanisms. The apparent binding constant (K_b) values for the association of these compounds and BSA were in the range of 10^3-10^4 M^{-1}, showing their moderate affinity to BSA. The K_b and the numbers of binding sites of BSA-complexes were increased by the elevation of temperature indicating that binding pockets were more accessible to these compounds at higher temperatures. Thermodynamic parameters showed that hydrophobic interactions are involved in C1-BSA complex formation and the interaction of C2 with BSA is through van der Waals interactions and hydrogen bonds.

Keywords: BSA, Platinum complexes, Fluorescence quenching, Thermodynamic parameter

INTRODUCTION

Organometallic compounds due to their structural diversity, ligand exchange, and catalytic properties are considered promising drug candidates for cancer therapy [1, 2]. Cisplatin, oxaliplatin, and carboplatin are platinum (Pt) containing compounds that are commonly used in the treatment of a variety of malignancies. They exert their antitumor effects by forming DNA adducts, and subsequent inhibition of DNA replication and transcription. Due to their side effects and the development of resistance against them, efforts have been intensively made to discovery of novel cytotoxic Pt-based complexes which have higher curative potential and less toxicity toward normal cells [3].

Binding of anticancer drugs to serum albumin has been shown to remarkably improve the pharmacokinetic profiles of the drugs. Albumin can bind to hydrophobic anticancer drugs through its hydrophobic pockets and enhance the circulatory half-lives of anticancer drugs and passively target the tumors by the enhanced permeability and retention (EPR) effect. Albumin-based drug delivery strategies have led to the development of potent antitumor agents that have been tested in various preclinical models, and several candidates have been evaluated clinically. Abraxane which is an exogenous human serum albumin bound paclitaxel formulation has benn approved by the FDA and used to treat locally advanced or metastatic tumors [4]. In this study, the binding of two novel double rollovers cycloplatinated compounds (C1and C2) to BSA was investigated. Cyclometalation has attracted much attention as a common method for transformation of non-activated hydrocarbons into value added products. In this reaction type the outcome of the reaction is highly predictable and provides a variety of intermediates for further transformations. The Roll-over cyclometalation is a common method of cyclometalation in which noncoordinated nitrogen atoms generated by the rollover provide additional catalytic sites. In this method a specific reversal of the coordination mode of a pyridyl moiety, from the expected metal-nitrogen bonding mode to a cyclometalated coordination mode occurs.

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A meta CH group in the same heterocyclic ring is deprotonated to generate a metal-carbon bond [5]. Earlier reports suggested rollover cyclometalation a promising strategy for design and synthesis of anticancer organometallic compounds [6]. Chemical structure of C1 and C2 complexes are presented in Fig. 1. In these compounds two Pt atoms are covalently connected to 2,2′-bipyridine (bpy). It has been reported that the presence of bpy ligand in Pt complexes increases their DNA and BSA binding [7-13]. Three phenyl groups in the structure of triphenylphosphine (PPh3) result in high lipophilicity of the compound and delocalization of the positive charges of phosphonium into the three aromatic rings which facilitate the passage of PPh3 across the lipid membranes[14]. Metal complexes having PPh3 have been shown to inhibit cell proliferation [15-17]. Presence of Iodine(I) halide leaving group in platinum complexes influences the intracellular distribution and cytotoxicity profile of compounds [18,19]. Herein, the binding of these compounds to BSA was investigated by spectroscopic methods.

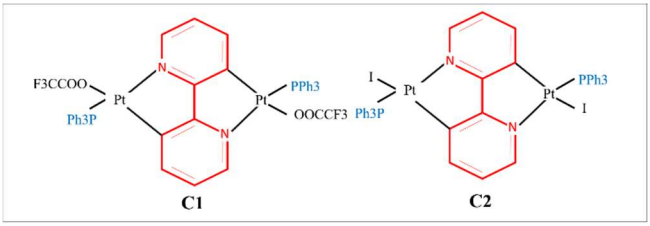

Fig. 1. The structures of C1 and C2 complexes. CF3COO=deprotonated trifluoroacetate, pph3= triphenylphosphine.

MATERIALS AND METHODS

UV-Visible and Fluorescence Spectroscopy Measurements

C1 and C2 complexes were synthetized with previously reported protocols [1]. Different concentrations of platinum compounds in DMSO (Sigma Aldrich, Germany) were added to BSA solution (2×10^{-5} M) and incubated for 5 min at room temperature followed by recording the absorption spectra of BSA in the rage of 240-320 nm using a T 90^{+} Spectrophotometer, (PG instruments Ltd, UK).

In fluorimetric analysis, different concentrations of C1 and C2 platinum compounds were added to BSA solution (2×10^{-5} M) and incubated for 5 min at 288 K and 310 K followed by spectroscopic measurements using a Cary-Eclipse spectrofluorimeter (Model Varian, Australia) upon excitation at 295 nm. Different concentrations of Pt compounds were tested until reaching the saturation state of the binding.

RESULTS AND DISCUSSION

Changes in Absorption Spectra of BSA by Pt Complexes

The BSA absorption spectra were recorded in the presence of an increasing amount of C1 and C2 compounds. As shown in Fig. 2, increase in the concentration of compounds resulted in a concentration-dependent hyperchromicity at 280 nm in BSA.

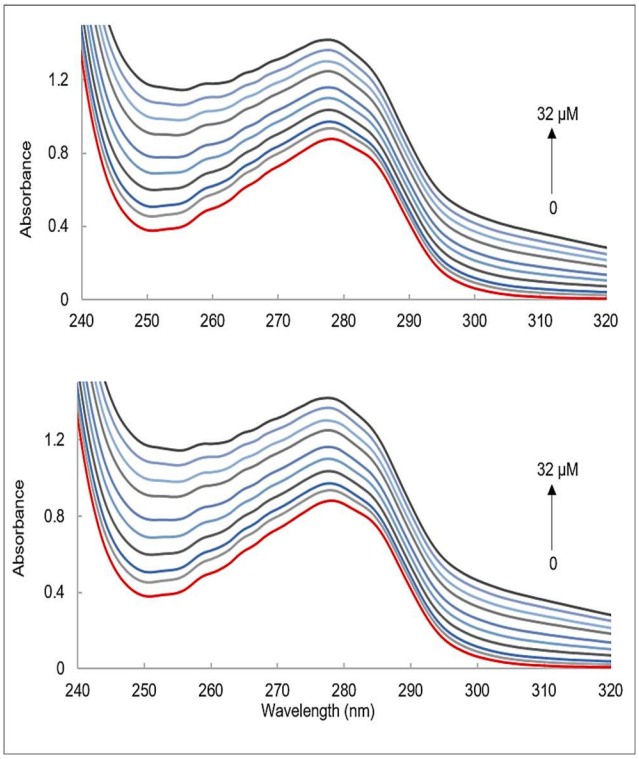

Fig. 2. The absorption spectra of BSA in the presence of increasing amount of C1 (Top), and C2 (bottom) complexes (0-32 µM). The absorption spectra of complexes in the absence of complexes were shown in red.

This observation is in favor of ground-state complex formation between BSA and the compounds resulting in static rather than dynamic quenching of BSA fluorescence. The mechanism of quenching is further discussed in Section 3-2. In static quenching, the absorption spectra of the protein molecules change in the presence of the quencher, which is not valid in the case of dynamic quenching, where the absorption spectra are not usually changed [20-22].

The Fluorescence Quenching of BSA by Pt Complexes

Fluorescence spectra of BSA in the presence of different amounts of C1 (Fig. 3) and C2 (Fig. 4) complexes were recorded at 288 K and 310 K. The intrinsic fluorescence of BSA originates from its aromatic residues. As the quantum yield of tryptophan is significantly higher than tyrosine, tryptophan is usually considered as an intrinsic fluorophore in protein studies [23,24]. The fluorescence emission of tryptophan residues is highly sensitive to perturbation in their local environment as a result of denaturation or conformational changes of the protein. By increasing the C1 and C2 concentration, a decrease in intrinsic emission of BSA was observed which reflected the structural changes in BSA as the result of complex formation with C1 and C2.

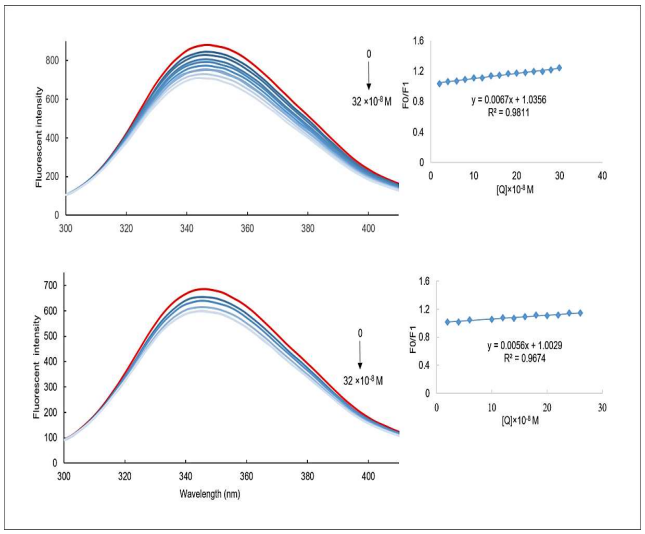

Fig. 3. Intrinsic emission spectra of BSA in the presence of increasing amount of C1 at 288 K (top) and 310 K (bottom). The fluorescence spectra of BSA in the absence of C1 were shown in red. The insets indicate the [F0/F1] *versus* [Q].

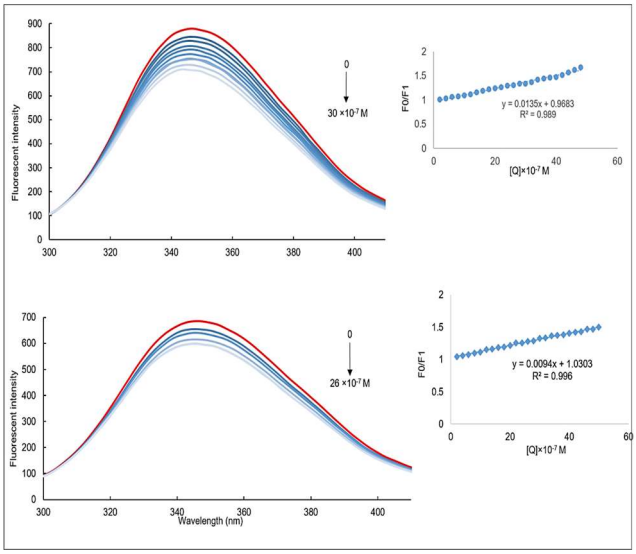

Fig. 4. Intrinsic emission spectra of BSA in the presence of increasing amount of C2 at 288 K (top) and 310 K (bottom). The fluorescence spectra of BSA in the absence of complex were shown in red. The insets indicate the [F0/F1] *versus* [Q].

The process of fluorescence quenching can be originated from two different mechanisms, dynamic and static quenching. In the case of dynamic quenching the quencher molecule collides with the fluorophore in the excited state resulting in its relaxation by nonradioactive pathways. In static quenching, the fluorophore and the quencher form a non-fluorescent complex resulting in a reduction in its steady state fluorescence intensity [25]. To investigate the quenching mechanism of C1 and C2 complexes, Stern-Volmer equation was applied [26]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = Kq\tau 0 \ + \ 1 \qquad (1)$$

Where F_0 and F are fluorescence intensities of BSA in the absence and presence of quencher, respectively. K_{sv} is the Stern-Volmer quenching constant and [Q] is the molar concentration of quencher. K_q denotes the rate constant or the bimolecular quenching constant for the fluorescence quenching reaction, τ_0 represents an average integral lifetime of the tryptophan residue that is equal to $\sim 10^{-8}$ s. Quenching constant shows positive and negative correlation with temperature in dynamic and static quenching mechanisms, respectively [27,28]. The calculated K_{sv} ($l\ M^{-1}$) are listed in

Table 1. In the case of C1 and C2, K_{sv} values decreased by increasing the temperature; providing a clue that the quenching is static and is governed by ground-state complex formation. The bimolecular quenching constant (K_q) was calculated by employing Eq. (1). The values of K_q obtained at 288 K and 310 K are higher than limiting diffusion rate constant of the biomolecule (2×10^{10} l M^{-1} s^{-1}) [29]. This provides another clue that fluorescence quenching is governed by ground-state BSA-Pt complex formation and is static in nature.

Binding Constant and Number of Binding Sites

In static quenching process, when small molecules are bound independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by Eq. (2) [30,31]:

$$Log\frac{F0-F}{F} = LogK_b + nLog[Q] \quad (2)$$

In this equation, the F_0 and F are the fluorescence intensities of the fluorophore in the absence and presence of quencher, respectively. [Q] is the concentration of the quencher, K_b is the binding constant and n is the number of binding sites. The obtained K_b and n values are presented in Table 2. The binding constants of BSA-complexes were increased by increasing temperature. The increase in K_b values with increasing temperature indicates that the protein can better accommodate the Pt-compounds at 310 K than at 288 K, which may be due to BSA binding pocket being more accessible to these compounds at higher temperature. The number of binding sites (n) is also increased by temperature elevation indicating that either the additional binding sites are occupied at higher temperatures or the stoichiometry of the binding step is changed [32]. The calculated K_b values suggested that the C1 had higher tendency for BSA binding than the other compound.

Thermodynamic Parameters Determination

To determine the involved forces in protein interaction with complexes, thermodynamics parameters including enthalpy (ΔH°) and entropy (ΔS°) were calculated using van't Hoff equation (Eq. (3)) [33]:

$$lnK_b = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (3)$$

Table 1. The K_{sv} and k_q Values of BSA Quenching by Pt Containing Compounds at 288 K and 310 K

Complexes	Temperature	K_{sv}	K_q
C1	288 K	0.67×10^6	0.67×10^{14}
	310 K	0.56×10^6	0.56×10^{14}
C2	288 K	0.13×10^6	0.13×10^{14}
	310 K	0.094×10^6	0.094×10^{14}

Table 2. The Number of Binding Sites (n) and Binding Constants (K_b) of C1 and C2 Complexes with BSA at 288 K and 310 K

Complexes	Temperature	n	K_b
C1	288 K	0.66	0.49×10^4
	310 K	0.82	4.07×10^4
C2	288 K	0.513	0.065×10^4
	310 K	0.80	0.91×10^4

R is the gas constant and T is the temperature in Kelvin. The values of Gibbs free energy change (ΔG°) were obtained from Eq. (4):

$$\Delta G^\circ = -RTLnK_b \quad (4)$$

The obtained thermodynamic parameters are reported in Table 3. Negative values of ΔG mean the spontaneous and favorable binding between C1 and C2 complexes and BSA. In hydrophobic interactions the values of ΔH° and ΔS° are positive, but in van der Waals interactions and hydrogen bonding both the values are less than zero. Negative values of ΔH° and positive values of ΔS° represent electrostatic interactions [34,35]. The thermodynamic parameters showed that C1 was interacting with BSA by hydrophobic interaction. The obtained negative values of ΔH° and ΔS° for C2 suggest that hydrogen bonding and van der Waals interactions were major forces playing essential roles in stabilizing the C2-BSA complex.

Table 3. The Thermodynamic Parameters of Binding C1 and C2 Complexes with BSA

Complexes	ΔH° (kJ mol^{-1})	ΔS° (kJ mol^{-1})	ΔG° (kJ mol^{-1})	
			288 K	310 K
C1	72.39	0.319	-35.50	-34.11
C2	-155.7	-0.486	-15.53	-23.5

CONCLUSION

Theoretical and experimental studies of drug binding to serum albumin are critical for better understanding of pharmacokinetic and pharmacodynamics of the drugs. In this research, the interactions of two novel platinum containing compounds with BSA have been investigated using spectrometric methods. The fluorescence of BSA was diminished by increasing concentrations of complexes through static quenching. The compounds showed moderate affinity for BSA binding which increased by temperature elevation. Based on calculated thermodynamic parameters, hydrophobic interaction played significant role in C1 interaction with BSA, while the van der Waals and hydrogen interactions were involved in the C2 binding to the protein.

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