Platinum(II) complexes with epoxide derivative of 1,10-phenanthroline in interaction with human serum albumin

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Abstract: In order to investigate the nature of binding drugs to HSA interaction of mononuclear [Pt(5,6-epoxy-1,10-phen)Cl_2] and [Pt(mal)(5,6-epoxy-1,10-phen)] complexes with HSA were followed by fluorescence emission and UV-Vis spectroscopy. To determine the preferred site of the complex binding to the HSA competitive experiments with site markers eosin Y and ibuprofen were performed. Obtained results showed that the examined complexes bind to HSA and primary interaction is at site II.

Keywords: Platinum(II) complexes, HSA, Eosin Y, Ibuprofen

1. Introduction

Due to the toxic effects exhibited by platinum(II) drugs in chemotherapy, exponential growth has been observed in the development of alternative platinum(II) complexes with other inorganic and organic ligands. 1,10-Phenanthroline (phen), with its rigid structure of three fused aromatic rings with the two nitrogen donors is one of the most commonly used ligands in modern coordination chemistry [1]. We investigated the influence of 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (5,6-epoxy-1,10-phen) as the derivative of 1,10-phenanthroline and malonic acids on the nature of mononuclear platinum(II) complexes [2]. These complexes showed good anticancer activity on human and murine cancer cell lines and good antitumor activity on breast and colorectal cancer in *in vivo* and *in vitro* experiments. In this work, we investigated the interaction of a representative of the previously investigated mononuclear complexes [Pt(5,6-epoxy-1,10-phen)Cl_2] (**Pt1**) and [Pt(mal)(5,6-epoxy-1,10-phen)] (**Pt2**) with HSA. Fluorescence measurement experiments as well as UV-Vis spectroscopy were used to investigate these interactions and to determine the influence of malonic acid on binding to HSA as one of the most important biomolecules.

2. Experimental

Mononuclear complexes [Pt(5,6-epoxy-1,10-phen)Cl_2] (**Pt1**) and [Pt(mal)(5,6-epoxy-1,10-phen)] (**Pt2**) were synthesized by the procedure previously published [2]. Phosphate

buffered saline (PBS, 10 mM, pH = 7,4), human serum albumin (HSA), ibuprofen and eosin Y were purchased from the Aldrich Chemical Co (Merck).

Fluorescence measurements were carried out on a RF-1501 PC spectrofluorometer (Shimadzu, Japan). The experiments involving the interaction of **Pt1** and **Pt2** complexes with HSA were performed at room temperature in 10 mM PBS buffer solution (pH = 7.40) to keep the physiological conditions. The concentration of HSA was kept fixed at 8 μM while the concentration of investigation Pt(II) complexes increased up to 160 μM so that the molar ratio (r) was from 0 to 20. The fluorescence emission spectra of HSA were recorded in the scanning range 300–500 nm at an excitation wavelength of 295 nm in the absence and presence of different concentrations of **Pt1** and **Pt2** complexes. The fluorescence quenching data were analyzed using the Stern–Volmer equation:

$$F_0/F = 1 + K_{sv}[complex] = 1 + k_q\tau_0[complex] \quad (1)$$

where F and F_0 are the fluorescence intensities of HSA with and without complex, respectively, k_q is the quenching rate constants of the biomolecule, K_{sv} is the dynamic quenching constant, τ_0 is the average lifetime of the biomolecule without a quencher (around 10^{-8} s) and [complex] is the concentration of **Pt1** and **Pt2** complexes.

The fluorescence intensities in this were corrected for absorption of the exciting light and reabsorption of the emitted light to decrease the inner filter effect using the following equation:

$$F_{cor} = F_{obs}\, e^{(A_{ex}+A_{em})/2} \quad (2)$$

The binding constant of the complex with BSA was calculated by the Scatchard equation:

$$\log[(F_0 - F)/F] = \log K_a + n \cdot \log[complex] \quad (3)$$

where K_a is the association binding constant and n is the number of binding sites which have been obtained from the plot of log[(F_0-F)/F] *versus* log[complex].

Competitive experiments were carried out using two site markers (eosin Y and ibuprofen) by keeping the concentration of HSA and the site markers constant at 8 μM. Then **Pt1** and **Pt2** complexes were gradually added to HSA/marker mixtures so that r was from 0 to 20. Fluorescence spectra of the above systems were recorded from 300 to 500 nm at an excitation wavelength of 295 nm.

The UV–Vis spectra were recorded on a Shimadzu double-beam spectrophotometer equipped with thermostated 1.00 cm quartz Suprasil cells over the wavelength range of 200–500 nm. Electronic absorption spectra were recorded in the presence and absence of **Pt1** and **Pt2** complexes at room temperature. The concentration of HSA was constant (8 μM) while the concentration of investigated Pt(II) complexes increased from 0 - 120 μM.

3. Results and discussion

In order to investigate the structural changes in HSA caused by the addition of Pt(II) complex and determine the binding constant (K_a) and the number of binding sites (n) for the complex formed between platinum(II) complexes and HSA, fluorescence spectra

were measured (**Figure 1)**. As can be seen from **Fig 1**. the addition of the complexes **Pt1** and **Pt2** to a solution of HSA provoked a significant decrease in the fluorescence intensity of HSA. The changes in the emission spectra of HSA protein indicate the occurrence of the binding of each complex to albumin. The fluorescence of HSA comes from tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. Trp214, located in the depth of subdomain IIA is largely responsible for the intrinsic fluorescence of HSA, because the fluorescence of tyrosine is almost completely quenched if it is ionized or in the vicinity of an amino group, a carbonyl group, or a tryptophan residue while phenylalanine quantum yield is very low quantum yield [3]. It can be concluded that the addition of **Pt1** and **Pt2** complexes resulted in concentration-dependent quenching of Trp214 fluorescence.

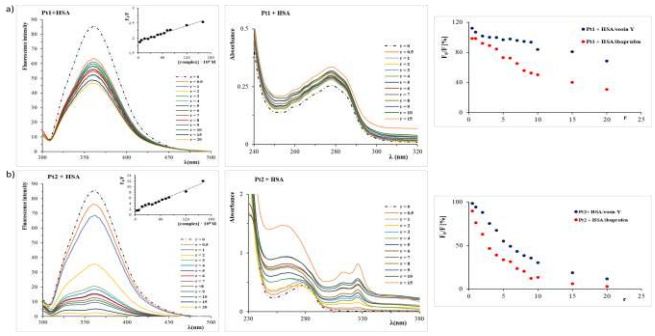

Figure 1. Emission, UV-Vis absorption spectra of the HSA and the relative intensity of the fluorescence emission of HSA/eosin Y or HSA/ibuprofen in the absence and in the presence of **Pt1 (a)** and **Pt2 (b)** complexes.

The values of K_{sv} ($4.3 \cdot 10^4$ M^{-1} for **Pt1** and $1.9 \cdot 10^5$ M^{-1} for **Pt2**) and k_q ($4.3 \cdot 10^{12}$ kJ/mol for **Pt1** and $1.9 \cdot 10^{13}$ kJ/mol for **Pt2**) for the interaction of **Pt1** and **Pt2** complexes with HSA indicate a good HSA-binding tendency of the complexes. The **Pt2** complex showed a higher value for K_{sv} in relation to **Pt1** complex which can be attributed to the presence of malonic acid as the bidentate coordinated ligand. Moreover, the k_q values for investigated complexes are higher than for different kinds of quenchers for biopolymer and point evidently to the presence of static quenching. The calculated K_a value ($5.4 \cdot 10^5$ M^{-1} for **Pt1** and $7.4 \cdot 10^6$ M^{-1} for **Pt2**) indicated that a strong affinity existed between HSA and platinum(II) complexes. The value of n (0.9 for **Pt1** and 1.0 for **Pt2**) suggested that there was one independent class of binding site on HSA for investigated platinum(II) complexes. The fluorescence quenching titration was used to determine the binding constants of **Pt1** and **Pt2** complexes interaction with HSA in the presence of eosin Y and ibuprofen as site probes for sites I and II (**Figure 1**).

Table 1. The binding constants of the interaction between HSA and platinum(II) complexes in the presence of site markers.

System		K_{sv} (1/M)	K_a (1/M)	n
HSA/eosinY	**Pt1**	$6.9 \cdot 10^3$	$2.4 \cdot 10^3$	1.1
	Pt2	$4.7 \cdot 10^4$	$1.8 \cdot 10^5$	0.6
HSA/ibuprofen	**Pt1**	$2.4 \cdot 10^3$	$2.7 \cdot 10^3$	0.9
	Pt2	$1.8 \cdot 10^5$	$6.1 \cdot 10^4$	0.7

With the increasing concentration of investigated platinum(II) complexes fluorescence intensity of HSA/eosinY or HSA/ibuprofen system decreases. From **Table 1** it is obvious that there was a competitive interaction between complexes and both site markers, which means that complexes can bind at both sites [4]. Accordingly, complexes displace ibuprofen more easily than eosin Y, so it can be concluded that the primary interaction is at site II. From **Fig. 1**, it can be observed that with increasing amounts of **Pt1** and **Pt2** complexes added to the HSA solution, the intensity of the absorption peak of HSA at 278 nm increases. In the UV-Vis spectra of HSA and **Pt1** complex, there are no changes so it can be concluded that the interaction of the formation ground state complex is done via static process. On the other hand, in the UV-Vis spectra of HSA and **Pt2** complex there is an evident red shift of peak at 278 nm so it can be concluded that the interaction between complex and HSA is a dynamic process [5].

3. Conclusions

In this work we investigated the interaction of two mononuclear [Pt(5,6-epoxy-1,10-phen)Cl_2] and [Pt(mal)(5,6-epoxy-1,10-phen)] complexes with HSA using different spectra techniques. The complex **Pt2** showed a higher value for K_{sv} and K_a constants in relation to the **Pt1** complex which is a consequence of presence of malonic acids in the coordination sphere in the Pt2 complex. Both investigated complexes bind to one place of HSA. Also, competitive experiments with two site markers eosin Y and ibuprofen as site probes for sites I and II showed that investigated platinum(II) complexes bind to HSA at both sites but the primary interaction is at site II. Absorption measurements confirm the binding of mononuclear platinum(II) complexes to HSA. From these experiment results we can conclude that the Pt1 complex binds to HSA through a static process while the Pt2 complex prefers a dynamic process.

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