DNA/BSA interaction of platinum(II) complexes with phenothiazine and *N*-methylphenothiazine

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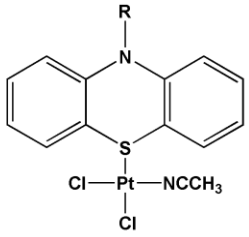
Abstract: In the present study, the interactions of two platinum(II) complexes of the general formula *cis*-[$PtCl_2$(L)(CH_3CN)], L is phenothiazine (phtz, **Pt1**) and *N*-methylphenothiazine (*N*-Mephtz, **Pt2**) with calf thymus DNA (ct-DNA) and bovine serum albumin (BSA) were studied by fluorescence emission spectroscopy to check their binding affinity towards these biomolecules for possible insights into the mode of their biological activity. A remarkable decrease in BSA fluorescence intensity after the addition of the complexes **Pt1** and **Pt2** and high values of the binding constants are in accordance with their high affinity toward this protein. On the other hand, the low binding affinity of the studied platinum(II) complexes to ct-DNA-EthBr system (EthBr is ethidium bromide, a well-known DNA intercalator) was observed. This has indicated that proteins could be more favorable binding sites for these platinum(II) complexes in comparison to the nucleic acids. Interestingly, **Pt1** complex has shown a higher binding affinity toward DNA than **Pt2**, while the latter complex is a more efficient BSA binder.

Keywords: platinum(II) complexes, phenothiazine, *N*-methylphenothiazine, DNA interactions, protein interactions

1. Introduction

In the last few decades, a large number of platinum(II) complexes have been synthesized and evaluated for their antitumor activity [1]. It is proposed that the interactions of platinum(II) complexes with DNA are responsible for their antitumor effects [2]. Besides that, the ability of platinum(II) complexes to bind serum proteins efficiently and to be transported to the target cells is an equally important factor for their mode of action [3]. Considering this, in the present study, we have investigated the interactions of two

platinum(II) complexes, *cis*-[$PtCl_2$(phtz)(CH_3CN)] (**Pt1**) and *cis*-[$PtCl_2$(*N*-Mephtz)(CH_3CN)] (**Pt2**) (phtz is phenothiazine and *N*-Mephtz is *N*-methylphenothiazine; Figure 1) with calf thymus DNA (ct-DNA) and bovine serum albumin (BSA) by fluorescence emission spectroscopy.



R = H (**Pt1**), CH_3 (**Pt2**)

Figure 1. Structural formula of the studied platinum(II) complexes **Pt1** and **Pt2.**

2. Material and Methods

2.1 Chemicals

Platinum(II) complexes, *cis*-[$PtCl_2$(phtz)(CH_3CN)] (**Pt1**) and *cis*-[$PtCl_2$(*N*-Mephtz)(CH_3CN)] (**Pt2**) were synthesized according to the method previously described in the literature [4]. Both complexes were pure based on elemental microanalysis and NMR spectroscopy. Dimethyl sulfoxide, phosphate-buffered saline (PBS), bovine serum albumin (BSA), calf thymus DNA (ct-DNA) and ethidium bromide (EthBr) were obtained from the commercial suppliers (Sigma-Aldrich and Acros Organics).

2.2 DNA/BSA interaction assay

Fluorescence spectra for BSA interactions were recorded on a Shimadzu RF-6000 spectrofluorometer at ambient temperature in the range of 285 – 500 nm, with an excitation wavelength of 280 nm. Fluorescence titration experiments were carried out in PBS (pH = 7.4) by keeping the concentration of BSA constant (17 μM), while varying the concentration of platinum(II) complexes (0 – 56 μM). The DNA binding experiments were also carried out in PBS by keeping [ct-DNA]/[EthBr] = 10, while increasing the concentration of the platinum(II) complexes. Measurements were performed in the wavelength range of 525 – 750 nm with an excitation wavelength of 520 nm.

3. Results and Discussion

3.1 BSA binding study

The fluorescence emission spectra of BSA of constant concentration were recorded in the presence of increasing concentrations of **Pt1** and **Pt2** complexes. As can be seen from Figure 2, the fluorescence intensity of BSA decreases by increasing the concentration of

the complexes, indicating their interactions with this protein. The values of binding constants (K_A) for **Pt1** and **Pt2** complexes (Table 1) are high enough to indicate their binding to BSA, which can transport them to the corresponding biological targets.

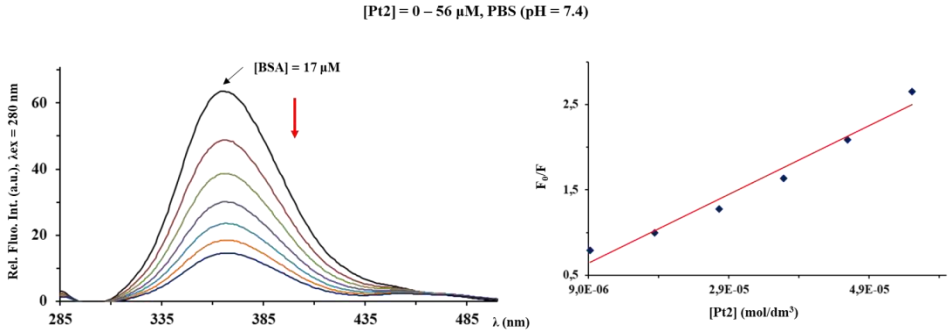


Figure 2. Fluorescence emission spectra of BSA in the presence of an increasing amount of **Pt2**. Inserted graph: Stern-Volmer plot of F_0/F *vs.* [**Pt2**].

Table 1. Values of the binding constants of **Pt1** and **Pt2** complexes with BSA.

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
Pt1	$(6.94 \pm 0.02) \cdot 10^4$	74.2	$6.94 \cdot 10^{12}$	$3.30 \cdot 10^5$	1.19
Pt2	$(1.44 \pm 0.02) \cdot 10^5$	77.1	$1.44 \cdot 10^{13}$	$1.58 \cdot 10^6$	1.34

3.2 DNA binding study

The emission spectra of the ct-DNA-EthBr system were recorded in the presence of an increasing amount of the investigated **Pt1** and **Pt2** complexes. After the addition of these complexes, no significant decrease in the fluorescence intensity of the EthBr-DNA system was observed (Figure 3). Moreover, the calculated values of the binding constants and the percentage of hypochromism for both complexes indicate that these complexes do not act as DNA intercalators (Table 2) [5].

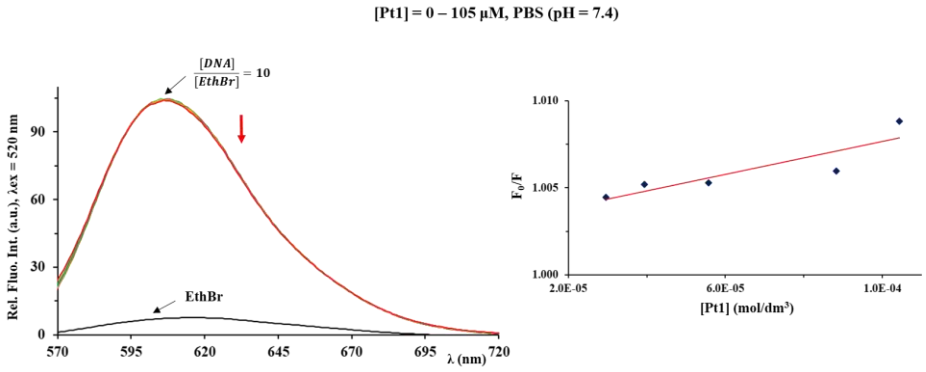


Figure 3. Fluorescence emission spectra of ct-DNA-EthBr system in the presence of an increasing amount of **Pt1** complex. Inserted graph: Stern-Volmer plot of F_0/F *vs.* [**Pt1**].

Table 2. Values of the binding constants of **Pt1** and **Pt2** complexes with ct-DNA.

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	n
Pt1	47.10 ± 0.10	0.7	$4.71 \cdot 10^9$	8.45	0.77
Pt2	32.90 ± 0.10	1.6	$3.29 \cdot 10^9$	1.10	0.42

4. Conclusions

The presented results have shown that platinum(II) complexes **Pt1** and **Pt2** interact with BSA, which can transport them to the target cells. On the other hand, their low affinity toward ct-DNA is observed, suggesting that this biomolecule is not the primary target for the mode of action of this type of platinum(II) complexes.

Acknowledgments

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