Journal of Applied Spectroscopy, Vol. 75, No. 4, 2008

SPECTROSCOPIC DETERMINATION OF BINDING BETWEEN HUMAN SERUM ALBUMIN AND A PLATINUM(II) DIMETHYLSULFOXIDE COMPLEX

K. R. Grigoryan,* **M. G. Aznauryan, N. A. Bagramyan, L. G. Gevorkyan, and Sh. A. Markaryan**

UDC 577.112.824

We have used electronic absorption and fluorescence spectroscopy to study binding between a platinum(II) dimethylsulfoxide complex (cis-[Pt(DMSO)$_2$Cl$_2$]) and human serum albumin (HSA), and the effect of complexation on the structure of the protein. We have calculated the binding parameters for binding between cis-[Pt(DMSO)$_2$Cl$_2$] and HSA. We have determined the binding constant $K_B = (1.2 \pm 0.1)\cdot 10^3\ M^{-1}$ and the Hill coefficient $h = 1.03 \pm 0.1$. We have determined that binding between cis-[Pt(DMSO)$_2$Cl$_2$] and the protein leads to a change in the internal packing of the macromolecule.

Key words: *human serum albumin, cisplatin, complexation, UV spectroscopy, fluorescence spectroscopy.*

Introduction. As we know [1, 2], *cis*platin (*cis*-[Pt(NH$_3$)$_2$Cl$_2$]) is used as an effective drug for treatment of cancers: ovarian tumors, sarcomas, breast cancer, and prostate cancer. However, the therapeutic action of the drug is accompanied by various side effects (vomiting, nephrotoxicity, neurotoxicity, alopecia, ototoxicity, etc.) which are due to inactivation of proteins. Biotransformation of *cis*platin in the body occurs by binding platinum to serum albumin [3]. This protein can reversibly bind to drugs and biologically active substances and is distinguished by rather low ligand-protein binding selectivity. It has been established that binding with the active centers of albumin occurs as a result of different types of interactions: hydrophobic, dipole, electrostatic, via van der Waals forces, hydrogen bonds. Complexes formed in this case are unstable and readily dissociated; the binding energy is no greater than 8–10 kcal/mol, which allows the ligands to easily interact with other substances [4]. The problem is complex because the structural diversity of drugs requires an individual approach to experimental assessment of these interactions, taking into account the spectral, physicochemical, and structural characteristics of the studied drug.

In order to reduce the toxicity of cancer drugs based on Pt(II), sulfur-containing ligands (thiols, thiocarbamates) are used in third-generation drugs [5, 6]. In this work, as the sulfur-containing compound we selected dimethylsulfoxide (DMSO), which has unique biomedical and pharmacological properties: it penetrates through biological membranes, improves transport properties of drugs, stimulates the immune system, and reduces the effects of chemotherapy and radiation therapy [7, 8].

Experimental Section. We used human serum albumin (HSA) from Sigma Chemical (USA), a solution of 0.9% sodium chloride (physiological saline) from the pharmaceutical company Likvor (Armenia). *Cis*-[Pt(DMSO)$_2$Cl$_2$] was synthesized as in [9]. In all the experiments, we used a 0.9% sodium chloride solution to prepare the protein solutions. The protein concentration (0.4 mg/mL) in solution was determined using electronic absorption spectra in the UV region (UV spectroscopy), where we used the molar absorption coefficient $\varepsilon = 36.500\ M^{-1}\cdot cm^{-1}$ for $\lambda = 280$ nm [10]. The concentration of *cis*-[Pt(DMSO)$_2$Cl$_2$] was varied within the range $(0–5)\cdot 10^{-5}$ M. The electronic absorption spectra of the solutions were measured on a Specord 50PC spectrophotometer.

The fluorescence studies were carried out on a Cary Eclipse spectrofluorimeter (Varian). The spectra were recorded in the range 310–450 nm for $\lambda_{ex} = 295$ nm. The synchronous fluorescence spectra were recorded with scanning ranges $\Delta\lambda = 60$ nm and 15 nm ($\Delta\lambda = \lambda_{det} - \lambda_{ex}$) in the absence of and in the presence of *cis*-[Pt(DMSO)$_2$Cl$_2$]. We

*To whom correspondence should be addressed.

Yerevan State University, 1 ul. Al. Manukyan, Erevan, 0025, Armenia; e-mail: kara@ysu.am. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 75, No. 4, pp. 573–576, July–August, 2008. Original article submitted February 28, 2008.

0021-9037/08/7504-0593 ©2008 Springer Science+Business Media, Inc.

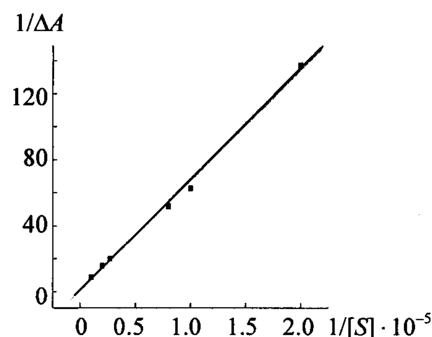

Fig. 1. $1/\Delta A$ vs. $1/[S]$ for the system human serum albumin — *cis*-$[Pt(DMSO)_2Cl_2]$.

used cuvets with l = 1 cm. The widths of the entrance and exit slits were 5 nm. All the measurements were made at pH 5.5.

Results and Discussion. *UV studies.* Complexation of *cis*-$[Pt(DMSO)_2Cl_2]$ and HSA was studied by UV spectroscopy. The UV spectra of HSA are characterized by a broad absorption band at λ = 280 nm. With an increase in the concentration of *cis*-$[Pt(DMSO)_2Cl_2]$, the absorption increases in the UV spectrum. From these data, we determined the dissociation constant (K_D) for the protein–*cis*-$[Pt(DMSO)_2Cl_2]$ complex formed, according to the equation [11]:

$$1/\Delta A = K_D/\Delta A_\infty [S] + 1/\Delta A_\infty , \quad (1)$$

where $\Delta A = A - A_0$, A_0 and A are the absorption of HSA at 280 nm in the absence of and in the presence of *cis*-$[Pt(DMSO)_2Cl_2]$; $[S]$ is the concentration of *cis*-$[Pt(DMSO)_2Cl_2]$; ΔA_∞ is the change in the absorption when the adduct is completely formed. The dissociation constant $K_D = (8.2 \pm 1.0)\cdot 10^{-4}$ M was determined from the dependence of $1/\Delta A$ on $1/[S]$ (Fig. 1). Using this value, we calculated the binding constant for binding between *cis*-$[Pt(DMSO)_2Cl_2]$ and HSA $K_B = (1.2 \pm 0.1)\cdot 10^3$ M^{-1}. As shown in [12], the complexation constants for strongly bound ligand–protein complexes vary within the range 10^6 to 10^8 M^{-1}. From the data obtained, it follows that the complex formed is unstable and readily dissociated.

The degree of cooperativity for the binding (the Hill coefficient h) is found from the slope of the straight line plotted in coordinates log $[\Delta A/\Delta A_\infty - \Delta A]$ and log $[S]$ [11]:

$$\log \left[\Delta A/(\Delta A_\infty - \Delta A)\right] = h \log [S] + \log K_D , \quad (2)$$

where ΔA_∞ and K_D are calculated from formula (1); h = 1.03 means that no cooperative binding is observed.

Since in the *cis*-$[Pt(DMSO)_2Cl_2]$ complex, binding of DMSO to the metal occurs through the sulfur atom [13], formation of additional hydrogen bonds and hydrophobic interactions with the protein are possible. However, as shown in [14], hydrophobic interactions and/or hydrogen bonds, arising upon interaction of globular proteins with DMSO, are not sufficient to form a stable complex. The major binding site for binding between *cis*platin and albumin is cysteine-34 (Cys-34) [15], while the binding constant for binding between albumin and *cis*platin is an order of magnitude lower ($1.2\cdot 10^2$ M^{-1}) [16] than for binding with *cis*-$[Pt(DMSO)_2Cl_2]$. Probably the binding site remains Cys-34 in the case of cis-$[Pt(DMSO)_2Cl_2]$, but all kinds of van der Waals interactions affect the stability of the complex.

Intrinsic fluorescence spectra of HSA. Fluorescence spectroscopy methods are widely used to study binding between ligands and proteins. Proteins contain three amino acid residues which are responsible for intrinsic fluorescence of the protein (tyrosine (Tyr), phenylalanine (Phe), and tryptophan (Trp)). Fluorescence of proteins is observed for excitation at λ_{ex} = 280 nm (general fluorescence) and 295 nm (tryptophan fluorescence). Absorption of proteins for λ_{ex} = 280 nm is connected with tyrosine and tryptophan residues. Phenylalanine is not excited in most experimental cases. Furthermore, the quantum yield is small for phenylalanine, and emission by this residue is not observed. The intrinsic fluorescence of HSA for λ_{ex} = 295 nm is due to fluorescence of the Trp 214 residue (the only tryptophan

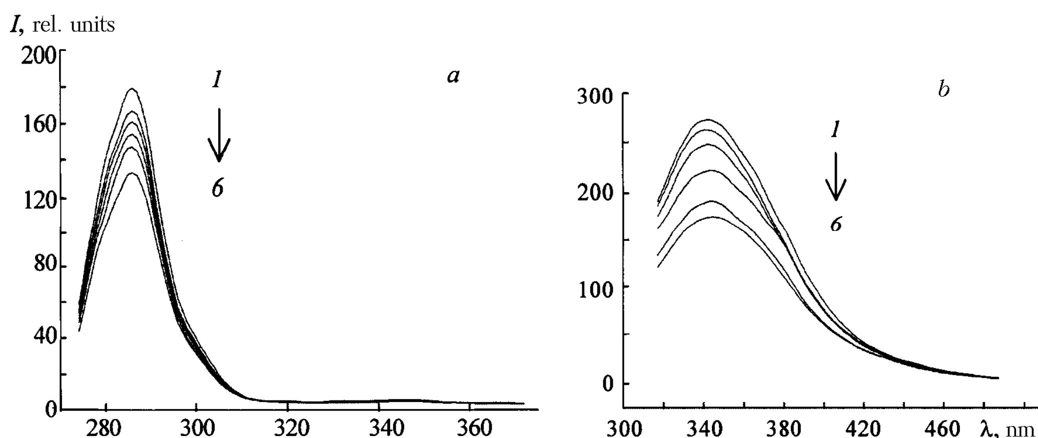

Fig. 2. Synchronous fluorescence spectra of human serum albumin in the presence of *cis*-$[Pt(DMSO)_2Cl_2]$; [HSA] = 0.8 mg/mL, [*cis*-$[Pt(DMSO)_2Cl_2]$] (in 10^{-5} mg/mL): 0 (1), 0.33 (2), 0.82 (3), 1.66 (4), 2.49 (5), 5.00 (6); $\Delta\lambda$ = 15 nm (a) and 60 nm (b).

residue). As shown by analysis of the position of the maximum (345 nm) and the width of the spectra (≈55 nm), Trp 214 belongs to class II in the Burstein model [17]. This suggests that the indole chromophore is located on the surface of the protein.

We have studied the interaction between HSA and *cis*-$[Pt(DMSO)_2Cl_2]$ by quenching the intrinsic fluorescence of the protein. Fluorescent titration data were treated according to the Stern-Vollmer relation [18]:

$$F_0/F = 1 + K_{SV}\,[Q]\,, \tag{3}$$

where F_0 and F are the intensities of fluorescence in the absence of and in the presence of *cis*-$[Pt(DMSO)_2Cl_2]$; $[Q]$ is the concentration of *cis*-$[Pt(DMSO)_2Cl_2]$; K_{SV} is the Stern–Vollmer constant. The spectra were recorded in the range λ = 310–450 nm for λ_{ex} = 295 nm, which corresponds to excitation of the tryptophan residue. With an increase in the concentration of *cis*-$[Pt(DMSO)_2Cl_2]$, the intensity of fluorescence for tryptophan decreases. Interaction between HSA and *cis*-$[Pt(DMSO)_2Cl_2]$ leads to quenching of the tryptophan fluorescence; no shift of the signal is observed. The linear dependence in Stern–Vollmer coordinates indicates homogeneous quenching (dynamic or static) in this system. From this dependence, we calculate the Stern–Vollmer quenching constant $K_{SV} = 8.2\cdot10^3$. Assuming that dynamic quenching occurs, this constant can be represented as $K_{SV} = K_q\tau_0$, where K_q is the quenching constant; τ_0 is the average lifetime of the molecule in the absence of the quenching agent. The fluorescence lifetime for a biopolymer is 10^{-8} sec [19]. Consequently, $K_q = 8.2\cdot10^{11}$ $M^{-1}\cdot sec^{-1}$. As shown in [20], the quenching constant for biopolymers using different quenching agents is $2.0\cdot10^{10}$ $M^{-1}\cdot sec^{-1}$. Obviously the assumption of dynamic quenching is incorrect, and the decrease in tryptophan fluorescence can be considered as the result of static quenching.

Synchronous fluorescence spectra. Conformational changes in a protein molecule can be assessed by measuring the synchronous fluorescence intensity for the amino acid residues. This method involves measuring the fluorescence spectrum while simultaneously changing the wavelengths of the exciting and detected radiation. We used the synchronous fluorescence method for scanning ranges $\Delta\lambda$ = 60 nm and 15 nm in the absence of and in the presence of *cis*-$[Pt(Me_2SO)_2Cl_2]$ $(0–5)\cdot10^{-5}$ M). For $\Delta\lambda$ = 60 nm, the synchronous fluorescence signal for HSA belongs to the tryptophan residues, while for $\Delta\lambda$ = 15 nm we also observe fluorescence of the tyrosine residues. Figure 2 shows the synchronous fluorescence spectra of HSA in the presence of *cis*-$[Pt(DMSO)_2Cl_2]$ additives for $\Delta\lambda$ = 60 nm and 15 nm. As we see, in the presence of *cis*-$[Pt(DMSO)_2Cl_2]$, the intensity of the fluorescence of tryptophan and tyrosine decreases; no shift is observed in the signals. This indicates that binding between *cis*-$[Pt(DMSO)_2Cl_2]$ and the protein does not lead to a change in the polarity of the microenvironment of the tryptophan and tyrosine residues, but the internal packing of the protein changes.

Conclusion. Using electronic absorption spectroscopy and fluorescence spectroscopy, we have studied binding between *cis*-$[Pt(DMSO)_2Cl_2]$ and human serum albumin. We have determined the binding parameters for binding of *cis*-$[Pt(DMSO)_2Cl_2]$ with HSA: the binding constant $K_B = (1.2 \pm 0.1)\cdot 10^3$ M^{-1} and the Hill coefficient $h = 1.03 \pm 0.1$. From the data obtained, we conclude that the complex formed is unstable and readily dissociated. Based on fluorescence studies, we have determined that binding between *cis*-$[Pt(DMSO)_2Cl_2]$ and the protein does not affect the polarity of the microenvironment of the chromophores, but changes the internal packing of the protein.

REFERENCES

1. F. Parnis, I. Olver, T. Sandeman, J. Bishop, and C. Laidlaw, *Am. J. Clin. Oncol.*, **20**, 319–321 (1997).
2. A. Polyzos, N. Tsavaris, M. Veslemes, K. Dardoufas, T. Dosios, and C. Kosmas, *J. Chemother.*, **9**, 102–105 (1997).
3. H. Zeng, Z. Xu, and K. Wang, *Intern. J. Biol. Macromol.*, **20**, 107–113 (1997).
4. T. Peters, *All About Albumin*, Academic Press, San Diego (1995), p. 76.
5. J. Galbraith, K. Menzel, E. Ratilla, and N. Kostic, *Inorg. Chem.*, **26**, 2073–2078 (1987).
6. J. Reedijk, *Platinum Metals Rev.*, **52**, 2–11 (2008).
7. S. Bonora, S. Markarian, A. Trinchero, and K. Grigorian, *Thermochim. Acta*, **433**, 19–26 (2005).
8. Z. Yu and P. Quinn, *Biosci. Rep.*, **14**, 259–280 (1994).
9. F. Rochon, P. Kong, and L. Girard, *Can. J. Chem.*, 1897–1902 (1986).
10. A. Singh and M. Darshi, *New J. Chem.*, **28**, 120–126 (2004).
11. K. Bryan, S. Yeung, W. Xiaotang, J. Sigman, P. Petillo, and Y. Lu, *Chemistry and Biology*, **4**, 215–221 (1997).
12. U. Kragh-Hansen, *Dan. Med. Bull.*, **37**, 57–84 (1990).
13. Yu. N. Kukushkin, *Zh. Koord. Khim.*, **23**, 163–174 (1997).
14. H. Johannesson, V. Denisov, and B. Halle, *Protein Sci.*, **6**, 1756–1763 (1997).
15. A. Ivanov, J. Christodoulov, A. Parkinson, K. Barnham, A. Tuker, J. Woodrow, and P. Sadler, *J. Biochem. Chem.*, **273**, 14721–14730 (1998).
16. A. Ahmed-Quameur, S. Diamantoglou, M. Sedaghat-Herati, Sh. Nafisi, R. Carpenrier, and R. Tajimir, *Cell Biochem. Biophys.*, **45**, 203–213 (2006).
17. A. Ladokhin, *Fluorescence Spectroscopy in Peptide and Protein Analysis (Encyclopedia of Analytical Chemistry)*, Wiley, New York (2000), pp. 5762–5799.
18. B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim (2002), pp. 72–90.
19. J. Lakowics and G. Weber, *Biochemistry*, **12**, 4161–4170 (1973).
20. W. Ware, *J. Phys. Chem.*, **66**, 455–458 (1962).