Interaction of the $[PtCl_2(DMSO)_2]$ Complex with L-Cysteine

Dragana Vasić[a], Jasmina Savić[a], Živadin Bugarčić[b], Danijela Krstić[c], Nenad Tomić[d], Mirjana Čolović[a], Marijana Petković[a], and Vesna Vasić[a,*]

[a] Department of Physical Chemistry 050, "Vinča" Institute of Nuclear Sciences, PO Box 522, 11 000 Belgrade, Serbia. Fax: ++ 3 811 12 44 72 07. E-mail: evasic@vin.bg.ac.yu
[b] University of Kragujevac, Faculty of Science, Kragujevac, Serbia
[c] Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[d] Jacobs University Bremen, School of Engineering and Science, Biochemistry and Cell Biology, Bremen, Germany

* Author for correspondence and reprint requests

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The reaction between $[PtCl_2(DMSO)_2]$ and L-cysteine (L-Cys) has been investigated in the presence of micelles of sodium dodecyl sulfate (SDS) – as a model for biological membranes. Additionally, the inhibitory effect of $[PtCl_2(DMSO)_2]$ on the Na^+,K^+-ATPase activity and its partial prevention with 10 mM L-Cys were demonstrated. The interaction of L-Cys with $[PtCl_2(DMSO)_2]$ resulted in the formation of a $[Pt(DMSO)_2(L\text{-}Cys)_2]^{2+}$ $(DMSO)_2]$ complex, which most probably occurs through stepwise replacement of Cl^- with L-Cys. It has also been demonstrated that neither the pH value nor SDS affects the composition of the new complex. On the other hand, the pH value and SDS do affect the reaction rate, most probably due to electrostatic interactions with reactants. In summary, this study can be used as a simple model approach for the investigation of reaction mechanisms between platinum complexes and various biomolecules, and for the determination of potential toxicity and/or side effects of antitumour platinum drugs.

Key words: Platinum Complexes, L-Cysteine, Na^+,K^+-ATPase

Introduction

The interactions of Pt(II) complexes with sulfur-containing biomolecules are often associated with negative phenomena, such as nephrotoxicity and gastrointestinal toxicity (Reedijk, 1999). The nephrotoxicity of cisplatin is attributed to the inhibitory effect of by cisplatin on the renal activity of Na^+,K^+-ATPase (Nechay and Neldon, 1984; Daley-Yates and McBrien, 1982). Cardiotoxicity is also a side effect of platinum-based therapy (Reedijk, 1999). The mentioned toxic side effects of antitumour platinum complexes probably arise from the inactivation of certain enzymes due to binding of cysteine residues to thiol groups (Djuran *et al.*, 2002). Moreover, the interaction of platinum drugs with sulfur-containing molecules may be the reason for the resistance against this type of chemotherapy (Loh *et al.*, 1992). Therefore, it is of utmost importance to elucidate the interaction mechanisms between platinum(II) complexes and sulfur-containing agents.

Naturally occurring amino acids, such as L-cysteine (L-Cys), are strong sulfur-binding ligands containing a thiol group. Although the complexes of some metal ions with L-Cys have been synthesized and characterized (Chen *et al.*, 1998; Juranić *et al.*, 1995; Allain *et al.*, 1980), only a limited number of studies focused on their reactions with Pt(II) (Bugarčić *et al.*, 2002; Petrović and Bugarčić, 2005). Our previous studies dealt with the kinetics of complex formation of GSH and L-Cys with $PdCl_2$ and $[Pd(H_2O)_4]^{2+}$ and clarified the mechanism of the reaction (Vasić *et al.*, 2003; Tošić *et al.*, 1997). The effects of micelle-forming surfactants on the reaction mechanism have also been investigated by our research group (Vasić *et al.*, 2003).

The purpose of the present study has been to investigate the interaction of $[PtCl_2(DMSO)_2]$ with the amino acid L-Cys and to elucidate the effects of pH value and sodium dodecyl sulfate (SDS) – as a model system for biological membranes – on the rate of complex formation. These studies are important not only in terms of reaction mechanisms, but also from a biochemical point of view – as models for electron-transfer and ligand-exchange reactions on the surface of a biomembrane or at the interface of a globular

protein. Finally, the biological significance of this kind of study is directly demonstrated by the investigation of $[PtCl_2(DMSO)_2]$ inhibition of brain Na^+,K^+-ATPase activity and its partial prevention by L-Cys.

Materials and Methods

Chemicals and solutions

All commercial chemicals were of analytical grade. L-Cys (99.5%), was obtained from Fluka (Germany) and was used without further purification. $[PtCl_2(DMSO)_2]$ was synthesized according to the procedure described by Breet and van Eldik (1983). Chemical analyses and UV/VIS spectral data were in good agreement with those obtained for the previous preparation. Na^+,K^+-ATPase from porcine brain (specific activity of 2.75 μmol Pi h^{-1} mg $protein^{-1}$) as well as some chemicals for the medium assay (ATP, NaCl, KCl, $MgCl_2$, Tris-hydroxymethyl aminomethane-HCl), Britton-Robinson buffer components (H_3BO_3, H_3PO_4, CH_3COOH, NaOH), SDS, and $HClO_4$ were purchased from Sigma Aldrich (Germany). Chemicals for the determination of the activity of Na^+,K^+-ATPase (stannous chloride and ammonium molybdate) were obtained from Merck (Darmstadt, Germany).

Stock solutions of L-Cys and $[PtCl_2(DMSO)_2]$ were prepared shortly before use by dissolving the appropriate chemical in 0.1 M $HClO_4$ (Merck, p.a.) as a supporting electrolyte. the concentration of the standard solution of $[PtCl_2(DMSO)_2]$ was $2.43 \cdot 10^{-3}$ M in 0.1 M $HClO_4$ in order to ensure the stability of the complex and eliminate hydrolysis to the greatest possible extent (Breet and van Eldik, 1983; Mahal and van Eldik, 1985; Bugarčić *et al.*, 2001). Triply distilled water was used throughout. Finally, pH values were controlled by Britton-Robinson buffer (Lurie, 1975).

The SDS stock solution (0.1 M in water) was prepared. The concentration of SDS in all experiments (0.01 M) was higher than the critical micellar concentration (CMC).

Methods

All absorption spectra were measured using GBC Cintra 10 and Perkin Elmer Lambda 35 spectrophotometers with thermostated 1.00-cm quartz cells. The pH values of the solutions were measured by a Metrohm pH meter, Model 713.

Reactions between $[PtCl_2(DMSO)_2]$ and L-Cys in the absence or presence of 10 mM SDS were initiated by mixing the appropriate volumes of the stock solutions, and the absorption spectra were recorded in the wavelength range 220–500 nm. The reaction rate was measured by monitoring the changes in the absorbancies either at 300 or 350 nm during a 25-min period. The pH value of the reaction medium was adjusted by HCl prior to mixing of the reactants. Rate constants k were calculated from the dependence of the absorbance A on the time t at 300 nm, which fitted very well the equation $A = A_\infty(1 - e^{-kt})$, where A_∞ is the absorbance at the plateau of kinetic curve. All kinetic measurements were carried out at 25 °C and the quoted values are the averages of at least three runs under identical experimental conditions.

The activity of brain Na^+,K^+-ATPase in the presence of various concentrations of $[PtCl_2(DMSO)_2]$ was determined using a modified spectrophotometric procedure based on the measurement of liberated inorganic orthophosphate (Pi) from ATP (Vasić *et al.*, 1999). The results are expressed as the mean percentage of enzyme activity relative to the corresponding control value, from at least three independent experiments performed in triplicate.

The effect of 10 mM L-Cys on the prevention of the $[PtCl_2(DMSO)_2]$-induced inhibition was

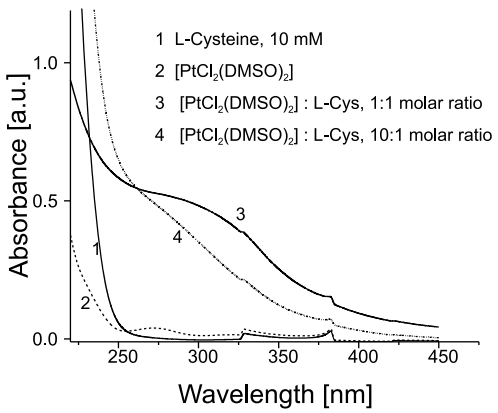

Fig. 1. UV/VIS spectra of 10 mM L-Cys (1), 10^{-4} M $[PtCl_2(DMSO)_2]$ (2), a mixture of 10^{-4} M L-Cys and 10^{-4} M $[PtCl_2(DMSO)_2]$ (3), and of 10^{-3} M L-Cys and 10^{-4} M $[PtCl_2(DMSO)_2]$ (4). All spectra were recorded at pH 1.82, and the reaction mixtures were left at room temperature for 30 min prior to recording. The wavelength range 220–450 nm is presented.

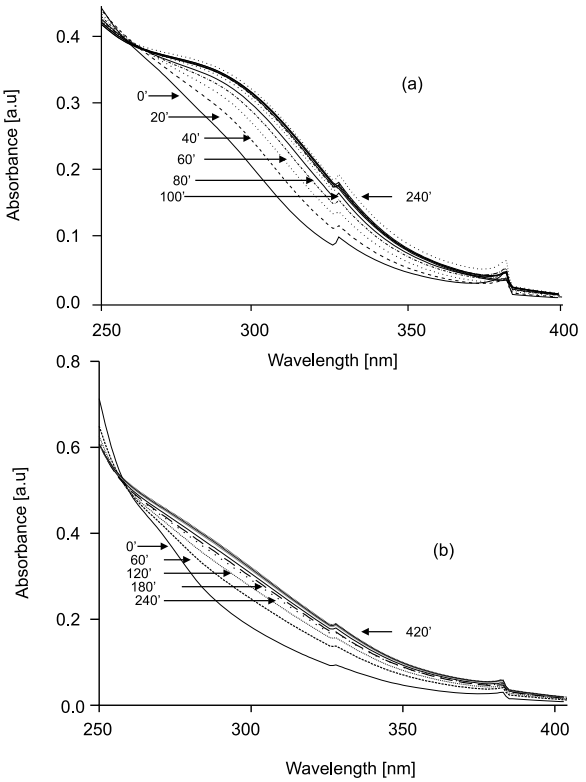

Fig. 2. Changes in the absorption spectra of (a) an equimolar mixture of $[PtCl_2(DMSO)_2]$ and L-Cys recorded at 20-s intervals after mixing the reactants and (b) of the mixture in the ratio 1:10 recorded at 60-s intervals. Concentrations of the reactants were (a) 10 mM and (b) 10 mM for the Pt(II) complex and 1 mM for L-Cys. The pH value of the reaction mixture was 1.82. Arrows indicate the times after the beginning of the reaction, as well as at the end of reaction.

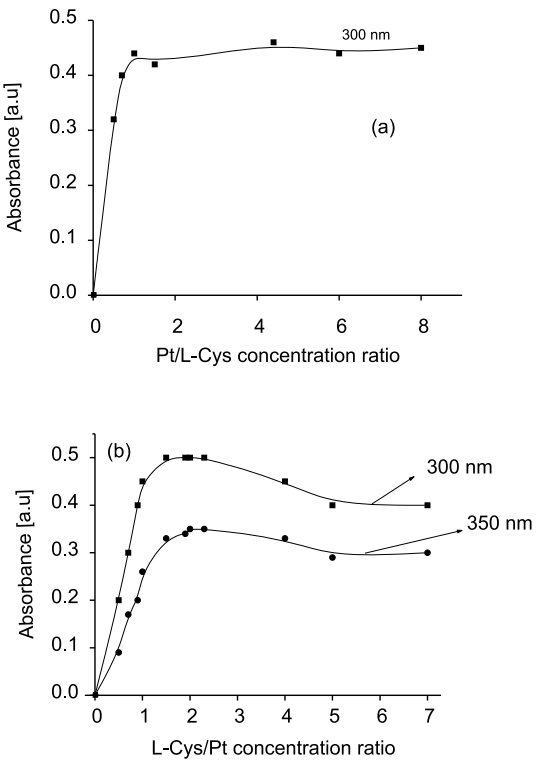

Fig. 3. (a) Dependence of the absorbance intensity at 300 nm on the $[PtCl_2(DMSO)_2]$/L-Cys concentration ratio. The concentration of L-Cys was kept constant, whereas the concentration of $[PtCl_2DMSO_2]$ varied from $5 \cdot 10^{-5}$ M up to $7 \cdot 10^{-4}$ M. (b) Dependence of A_{300} and A_{350} on the L-Cys/$[PtCl_2(DMSO)_2]$ concentration ratio. The concentration of the Pt(II) complex was kept constant and the concentration of L-Cys varied from $5 \cdot 10^{-5}$ M up to $7 \cdot 10^{-4}$ M. In all cases, the pH value of the reaction mixture was 1.82.

measured under the same conditions as described above, with the ligand added to the medium before exposure to the metal complex.

Results and Discussion

The UV/VIS absorption spectra of $[PtCl_2(DMSO)_2]$, L-Cys, and their mixtures are given in Fig. 1. $[PtCl_2(DMSO)_2]$ and L-Cys were mixed at 1:1 and 1:10 molar ratios, as indicated in the figure. The spectrum of $[PtCl_2(DMSO)_2]$ shows a broad low-intensity band with a maximum at about 275 nm. However, the absorption spectra of the solution containing $[PtCl_2(DMSO)_2]$ and L-Cys show a new absorption band with the maximum at 300 nm, indicating that a new complex was formed at both concentration ratios.

Fig. 2 presents the absorption spectra of the reaction mixture recorded at 20-s intervals for 1:1 (Fig. 2a) and 60-s intervals for 1:10 (Fig. 2b) Pt:L-Cys molar ratio, respectively. Obviously, the intensity of the absorbance increases with time and a well defined isosbestic point at 260 nm is observed in both cases.

The stoichiometry of the complex was determined by the molar ratio method, keeping the L-Cys or $[PtCl_2(DMSO)_2]$ concentration constant (10^{-4} M), whereas the concentration of the other component varied from $5 \cdot 10^{-5}$ to $7 \cdot 10^{-4}$ M. Fig. 3 presents the dependence of the absorbance at 300

nm and 350 nm on the concentration ratio of the reactants. The A_{300} in Fig. 3a increases linearly up to the equimolar ratio of the reactants. It achieves the plateau at an excess of $[PtCl_2(DMSO)_2]$. On the other hand, in the case of the constant concentration of $[PtCl_2(DMSO)_2]$ (Fig. 3b), both A_{300} and A_{350} increase linearly up to the concentration ratio of 1:1 and further remained constant until the concentration of L-Cys became 2.5-fold higher. This is followed by a decrease in A_{300} and A_{350}.

Based on the above presented results, it may be assumed that the reaction occurs according to

$$[PtCl_2(DMSO)_2] + \text{L-Cys} \rightleftharpoons [PtCl(DMSO)_2(\text{L-Cys})]^+ + Cl^- \quad (1)$$

due to the nucleophile attack of the sulfur donor from the thiol group of L-Cys followed by the replacement of one Cl^- ligand in the Pt(II) complex. With further increase in the L-Cys concentration, a chelate complex is formed due to the attack of the nitrogen atom of the amino group of L-Cys and displacement of another Cl^- ligand:

$$[PtCl(DMSO)_2(\text{L-Cys})]^+ + \text{L-Cys} \rightleftharpoons [Pt(DMSO)_2(\text{L-Cys})_2]^{2+} + Cl^-. \quad (2)$$

Finally, L-Cys acts as a bidentate ligand, forming a bond between Pt(II) and both S and N donors from its thiol and amino groups, respectively. The resulting complex formed in the excess of L-Cys is a chelate square planar complex, the spectrum of which slightly differs from the spectrum of $[PtCl(DMSO)_2(\text{L-Cys})]^+$.

The effect of the surface-active compound SDS on the absorption spectra and the reaction rate of the complex formed in excess of L-Cys was studied at pH 3–5. In all experiments, 10 mM SDS was used, which is the lowest concentration required for micelle formation (Tošić *et al.*, 1997). Negligible changes in the intensity, as well as in the shape of the absorption spectra were observed at various pH values and in the presence of SDS (data not shown). These variations most probably arise from the difference in the ionic strength caused by the presence of SDS and from changes of the L-Cys ionic state due to the dissociation of the non-complexing –COOH group within the complex (pK_a = 1.9; Smith and Martel, 1989) at various pH values.

The influence of acidity on the reaction rate of the complex formation was investigated under pseudo-first-order conditions (*i.e.* in the 10-fold molar excess of L-Cys) at pH 2–5. The dependence of the rate constants on the pH values in the absence and presence of 10 mM SDS is presented in Fig. 4. Both rate constants *vs.* pH curves are bell-shaped, with the maxima at pH 3.5 in the absence and at pH 4.0 in the presence of SDS. The bell-shaped pH profile is typical for complex formation between species which undergo to the protolytic equilibria (Vasić *et al.,* 2003; Tošić *et al.,* 1997). At pH < 2.0 L-Cys is positively charged, since $[PtCl_2(DMSO)_2]$ is neutral. As the pH value increases, the complex becomes positively charged (Hartley *et al.*, 1980) due to its hydrolysis, *i.e.* substitution of one Cl^- by H_2O. Besides, the dissociation of the –COOH group of L-Cys occurs (Smith and Martel, 1989). Although this group does not participate in complex formation, it can affect the reaction rate due to the electrostatic attractions between ions; a result of which is the acceleration of complex formation. Above pH 3.5 the complex formation is slowed down, since OH^- replaces H_2O and the resulting complex is neutral $[PtCl(OH)(DMSO)_2]$.

The shift of the pH maximum towards higher pH values in the presence of SDS is most likely caused by electrostatic interactions of the negatively charged surface of SDS with reactants. The anionic micelles provide a dispersed negatively charged surface in solution, which attracts positively charged L-Cys (at pH values between 2.0 and 3.5), resulting in a decrease of their concentration in the bulk aqueous phase. The conse-

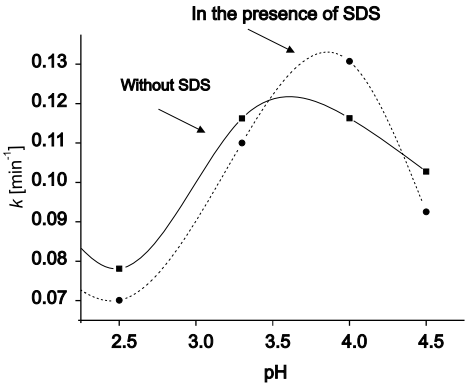

Fig. 4. pH profile of the reaction rate constant (k) for the formation of the $[PtCl_2(DMSO)_2]$-L-Cys complex in the presence and absence of 10 mM SDS. The concentration of L-Cys was $5 \cdot 10^{-4}$ M, and of $[PtCl_2(DMSO)_2]$ it was $5 \cdot 10^{-5}$ M.

Table I. The rate constants k_1 and k_2 determined for the reaction between $[PtCl_2(DMSO)_2]$ and L-Cys carried out without and in the presence of 10 mM SDS.

	In the presence of SDS	Without SDS
k_1 [s^{-1}]	$(2.4 \pm 0.01) \cdot 10^{-2}$	$(4.8 \pm 0.01) \cdot 10^{-2}$
k_2 [M^{-1} s^{-1}]	$(4.0 \pm 0.4) \cdot 10^{-3}$	$(4.3 \pm 0.2) \cdot 10^{-3}$

quence is a decrease of the reaction rate at the same pH value compared to the reaction carried out without SDS. With increase of the pH value, the formation of $[PtCl(H_2O)(DMSO)_2]^+$ leads to its adsorption on the surface of SDS. The result is a higher concentration of reactants on the micelle surface and a faster reaction rate. At pH > 4, OH^- replaces H_2O and the resulting complex $[PtCl(OH)(DMSO)_2]$ is mostly located in the bulk solution; and the complex formation process is slowed down.

The dependence of the reaction rate on L-Cys concentration was studied at pH 4.5 in the absence or presence of SDS. Linear plots were obtained, typical for the ligand substitution of square planar complexes. The results obey the equation $k = k_1 + k_2 C_{L\text{-}Cys}$, where k_1 is the solvolysis rate constant, which is independent on L-Cys, and k_2 is the second-order reaction constant, characteristic for direct nucleophilic attack and the formation of a new complex. These parameters are given in Table I.

The influence of anionic micelles on the reaction mechanism can be easily approximated to the physiological conditions, *i.e.* when Pt(II) complexes are applied in antitumour therapy. The phospholipid milieu with negatively charged groups on the surface might affect the reaction rate between Pt(II) complexes and any membranous protein in a similar manner. For this reason, the effect of $[PtCl_2(DMSO)_2]$ on the activity of Na^+,K^+-ATPase from porcine cortex brain was tested. The experiments were performed with or without 10 mM L-Cys, and the corresponding inhibition curves are given in Fig. 5. The IC_{50} value

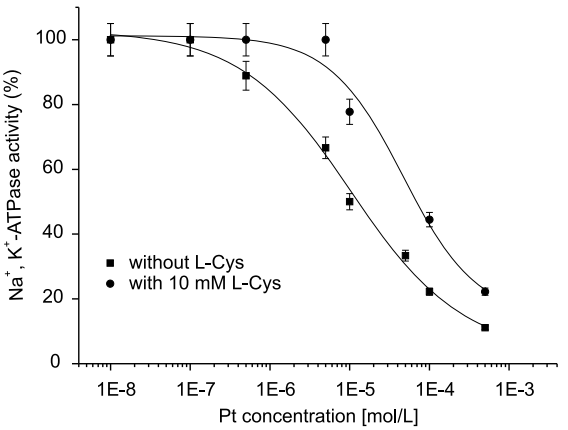

Fig. 5. Dependence of the Na^+,K^+-ATPase activity on the $[PtCl_2(DMSO)_2]$ concentration in the absence and in the presence of 10 mM L-Cys. $[PtCl_2(DMSO)_2]$ was added in the concentration range $1 \cdot 10^{-8}$ to $1 \cdot 10^{-3}$ M. The results are expressed as the percentage of the activity of the enzyme. The activity of Na^+,K^+-ATPase without the Pt-complex and SDS was set as 100%. Mean values and standard deviations from three measurements are presented.

determined from the inhibition curve without L-Cys was $1.2 \cdot 10^{-5}$ M. After addition of 10 mM L-Cys to the reaction mixture the IC_{50} value was $6.8 \cdot 10^{-5}$ M. Most likely, the $[PtCl_2(DMSO)_2]$ complex reacted with L-Cys forming a stable complex, and in, similar manner to the behaviour of Pd(II) complexes (Krinulović *et al.*, 2006), partial prevention of the enzyme inhibition was achieved. These results once more confirm that the administration of SH-containing molecules, such as L-Cys or glutathione, to an organism might be used for the prevention of toxic effects of a wide range of metal complexes.

Acknowledgements

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