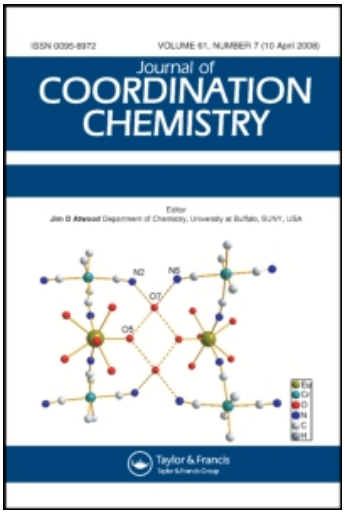


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Biljana V. Petrović[a]; Živadin D. Bugarčić[a]
[a] Faculty of Science, Department of Chemistry, University of Kragujevac, Kragujevac, Yugoslavia

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# KINETICS AND MECHANISM OF COMPLEX FORMATION BETWEEN $[PtCl(DIEN)]^+$ AND THIOLS AND THIOETHERS

BILJANA V. PETROVIĆ and ŽIVADIN D. BUGARČIĆ*

*University of Kragujevac, Faculty of Science, Department of Chemistry, P. O. Box 60, YU-34000 Kragujevac, Yugoslavia*

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Reactions of the monofunctional platinum(II) complex, $[PtCl(dien)]^+$, with different thiols and thioethers, including biologically important molecules, have been studied as a function of temperature (288.2–308.2 K) using conventional electronic spectrophotometry in 0.10 M aqueous hydrochloric acid and by $^1H$ NMR spectroscopy. The second-order rate constants, $k_2$, are similar, varying between $1.43 \times 10^{-3}$ and $46.1 \times 10^{-3}\,M^{-1}s^{-1}$ at 25°C. The reactivity follows the sequences: *D*-penicillamine < *L*-cysteine < glutathione < thiodiglycolic acid < thioglycolic acid < *L*-methionine < *S*-methylthioglycolic acid < glycyl-*D*,*L*-methionine. However, variation in size, bulkiness and solvation of the entering ligands reflect in their properties as nucleophiles. Large negative values of the entropy of activation ($\Delta S^{\neq}$), between $-140$ and $-190\,J\,K^{-1}\,mol^{-1}$, indicate that all thiols and thioethers react *via* the same associative mechanism. Results have been analyzed in relation to the antitumor activity and toxicity of platinum(II) complexes.

*Keywords*: Platinum(II); Reactivity; Thiols; Thioethers; Mechanism

## INTRODUCTION

Interactions between platinum complexes and sulfur-containing biomolecules are very important from a biological and medical point of view. For instance, *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) is routinely used in chemotherapy and has been particularly successful in the treatment of testicular and ovarian cancer [1,2]. Although platinum interactions with DNA are thought to

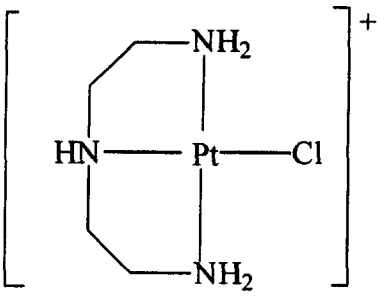
*Corresponding author.

be responsible for antitumor activity, there are many other potential biomolecules which can react with the platinum complexes. Sulfur-containing molecules have a high affinity for platinum [3]. Likely reactive biomolecules are cysteine, methionine, peptides such as glutathione, *S*-methylglutathione, metallothionine and others. The nephrotoxicity of antitumor platinum drugs has been ascribed to their reactions with thiol groups of proteins [4–7]. In other words, nephrotoxicity is supposed to be the result of inactivation of certain enzymes due to binding of cisplatin to the thiol groups of cysteine residues [7]. The tripeptide glutathione (GSH or glut$H_5$) provides a model compound for a study of these interactions. Glutathione is a cysteine-containing tripeptide with the sequence $\gamma$-glutamylcysteinylglycine, and is frequently the most prevalent intracellular thiol with concentrations up to 8 mM [8, 9]. Reedijk *et al.* [10] have shown that the complex $[PtCl(dien)]^+$ reacts initially with glutathione to form a 1 : 1 complex with the ligand bound through sulfur. The reaction was pH-dependent. The product at pH > 7 was the mononuclear complex $[Pt(dien)(SG)]^+$, but at pH < 7, a dinuclear complex with a thiolate bridge, $[\{Pt(dien)\}_2(\mu\text{-SG})]$, was formed. A study of the reactions between $[PtCl(dien)]^+$ and $[Pt(dien)H_2O]^{2+}$ with glutathione (GSH) and *S*-methylglutathione (GS-Me) has shown that GS-Me is a stronger nucleophile than GSH, because of the positive inductive effect of the methyl group [11]. It has to be mentioned that glutathione may react with platinum compounds before they can reach DNA or may react with them after they are bound to DNA [12, 13]. These reactions to some extent protect the tumor cell from the action of the platinum drugs.

There is current interest in the antitumor activity of platinum(IV) diamine complexes [14]. However, platinum(IV) complexes undergo ligand substitution much more slowly than their platinum(II) analogues [15]. Therefore, biotransformation of platinum(IV) complexes is generally assumed to involve reduction to platinum(II) prior to reaction with DNA. Thiol-containing biomolecules and ascorbic acid seem to be the major cellular components responsible for that reduction [14, 16].

In recent years, there has been increasing interest in the interactions between the platinum drugs and sulphur-containing molecules [17–20]. Thioethers are efficient nucleophiles, forming strong complexes with soft metal centres [21]. A number of reactions between thiols and thioethers with square-planar complexes of palladium(II) and platinum(II) have been studied [22–37]. The inactive, monofunctional $[PtCl(dien)]^+$ complex (dien = 1,5-diamino-3-azapentane), shown below, is a very useful model for studying the binding of platinum compounds to sulfur-based nucleophiles

[38, 39]. Although $[PtCl(dien)]^+$ has been extensively used as a model for the first step in the binding of cisplatin to DNA and other biological targets, rate constants for complex formation with sulfur ligands are rarely reported [11, 40, 41].




The goal of this work was to determine and compare the second-order rate constants for complex formation of Pt(II) with thiols and thioethers, including biologically important molecules. We report detailed kinetic studies of reactions between $[PtCl(dien)]^+$ and *L*-cysteine, *L*-methionine, glutathione, *D*-penicillamine, glycyl-*D*,*L*-methionine, thioglycolic acid, thiodiglycolic acid and *S*-methylthioglycolic acid in aqueous solution (1).

$$[PtCl(dien)]^+ + L \rightleftharpoons [Pt(dien)L]^{2+} + Cl^- \quad (1)$$

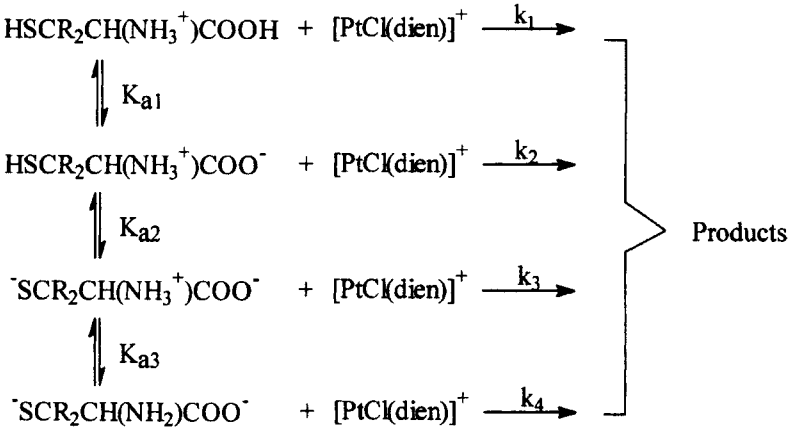
## EXPERIMENTAL

### Chemicals and Solutions

[PtCl(dien)]Cl was prepared according to the method of van Eldik *et al.* [42]. Pure white crystals of the complex were obtained by recrystallization from water. *Anal.* Calc. for $C_4H_{13}N_3Cl_2Pt$(%): C: 13.01; H: 3.54; N: 11.38. Found: C: 13.10; H: 3.48; N: 11.17. *L*-methionine, *L*-cysteine, glutathione, glycyl-*D*,*L*-methionine, *D*-penicillamine, thioglycolic, *S*-methylthioglycolic and thiodiglycolic acid were obtained from Sigma Chemical Co. and used without further purification. Ligand stock solutions were prepared shortly before use by dissolving these chemicals in 0.10 M hydrochloric acid, HCl, (Baker, p. a.) as supporting electrolyte. Under these experimental conditions, pH = 1.0, the complex $[PtCl(dien)]^+$ was stable and hydrolysis of the complex was negligible [38]. Water was doubly distilled from quartz. All solution were flushed with nitrogen to remove dissolved oxygen.

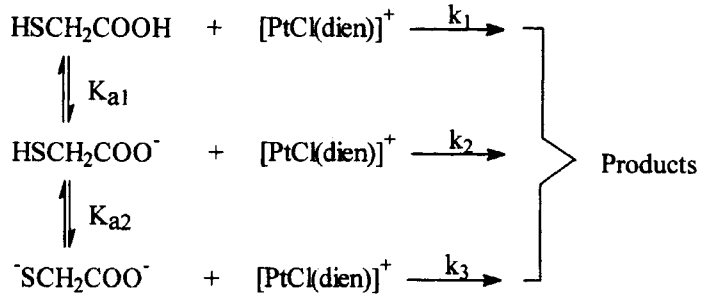
**Protonation Constants**

Protonation constants are defined in Schemes 1 and 2 below. At 25°C and $\mu = 1.0$, their values are as follows: for thioglycolic acid [43a] $pK_{a1} = 3.53$ and $pK_{a2} = 10.05$; for cysteine [43b] $pK_{a1} = 1.9$, $pK_{a2} = 8.10$ and $pK_{a3} = 10.1$; for penicillamine [40c] $pK_{a1} = 1.9$, $pK_{a2} = 7.92$ and $pK_{a3} = 10.5$. Protonation constants for glutathione at 25°C and ionic strength of 0.2–0.55 M have been reported as $pK_{a1} = 2.05$, $pK_{a2} = 3.40$, $pK_{a3} = 8.72$ and $pK_{a4} = 9.49$ [44]. However, under the experimental conditions, where $[H^+] \gg K_a$, all ligands were protonated, so the reactions




Cysteine, R = H; penicillamine, R = Me

SCHEME 1




Thioglycolic acid

SCHEME 2

pathways described by $k_3$, $k_4$, $k_5$ in Schemes 1 and 2 can be neglected at pH = 1.0, where all reported rate constants and activation parameters have been determined.

At low pH, reactions with rate constants $k_3$, $k_4$ and $k_5$ contribute less than 5% to the overall kinetics, an amount within the error limits of the kinetic measurements and the determinations of activation parameters.

## Kinetic Measurements

The reactions were sufficiently slow to be followed spectrophotometrically by measuring the change in absorbance at suitable wavelengths as a function of time. The kinetics were followed using a Varian Super-Scan 3 double-beam spectrophotometer equipped with thermostatted cells. In all cases the reactions were initiated by adding 0.30 $cm^3$ of a thermostatted solution of the platinum complex to 2.50 $cm^3$ of the ligand solution in the thermostatted spectrophotometric cell and recording changes of absorbance with time at 250 and 305 nm. The concentration of the entering thiols or thioethers was always large enough to provide *pseudo*-first-order conditions. Measurements were performed between 288 and 308 K. The *pseudo*-first-order rate constants, $k_{obsd}$, were determined graphically from plots of $\ln(A_\infty - A_t)$ against time [45] ($A_t$ and $A_\infty$ are the absorbances of the reaction mixture at time "$t$" and at the end of the reaction, respectively, usually after 10 half-lives) or from a non-linear least-squares fit of experimental data to (2)

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obsd}t) \quad (2)$$

with $A_0$, $A_\infty$ and $k_{obsd}$ the parameters to be optimized. Rate constants are accurate to within 5%.

## $^1$H NMR Measurements

NMR spectra were obtained with a Varian Gemini 200 MHz NMR spectrometer equipped with a 5 mm probe for $^1$H NMR measurements. $^1$H spectra were measured in $D_2O$ (Matheson, USA company) and the chemical shifts ($\delta$) are reported in ppm relative to TSP. Equimolar amounts of the complex and the ligand were mixed in the NMR tube. The final concentration of the solution was 10 mM in each reactant. A drop of DCl was added to the ligand solution to prevent its dissociation and hydrolysis of the complex. After mixing the reactant solutions, spectra were immediately recorded as a function of time at 295 K.

## RESULTS AND DISCUSSION

The *pseudo*-first-order rate constants, $k_{obsd}$, from the conventional kinetic experiments were obtained from a linear least-squares analysis of the first three half-lives and represent the average of two to four experiments. Figures 1 and 2 show the concentration dependence of $k_{obsd}$.

As can be seen, the *pseudo*-first-order rate constants are linearly dependent on the concentration of excess ligand. The second-order rate constants, $k_2$, for formation of the complex (1) were derived by fitting $k_{obsd}$ values to (3).

$$k_{obsd} = k_1 + k_2[L] \tag{3}$$

One reaction involves direct nucleophilic attack by the entering ligand ($k_2$) and the other reaction involves the solvation of the complex ($k_1$) [46]. Second-order rate constants, are given in Table I.

The difference in $^1H$ NMR chemical shifts of $CH_3$ groups ($CH_2$ for thiodiglycolic acid) of free and coordinated ligands has been used to follow the reactions between Pt(II) complex and *S*-bonding ligands. Values of $k_2$ were determined by fitting the data from the early part of the reaction (1 h) to a second-order process [47], *i.e.*, by plotting $x/a_0(a_0-x)$ *vs.* time ($a_0$ = initial concentration of $[PtCl(dien)]^+$ and $x$ = concentration of $[Pt(dien)(RSR_1)]^{2+}$ at time $t$). The second-order rate constants obtained by $^1H$ NMR measurements are also given in Table I.

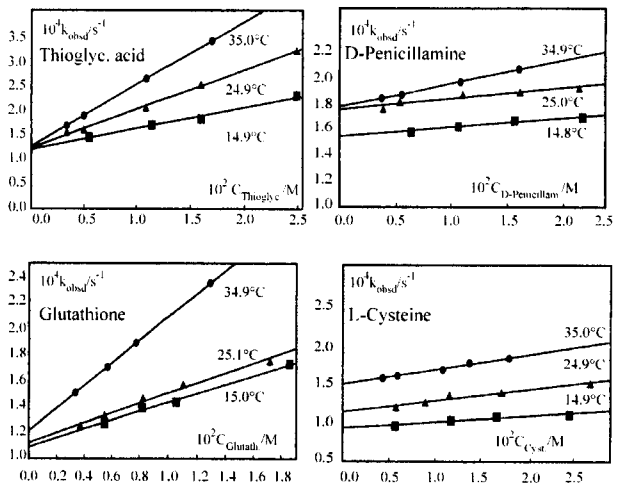



FIGURE 1 Observed *pseudo*-first-order rate constants as a function of excess thiol.

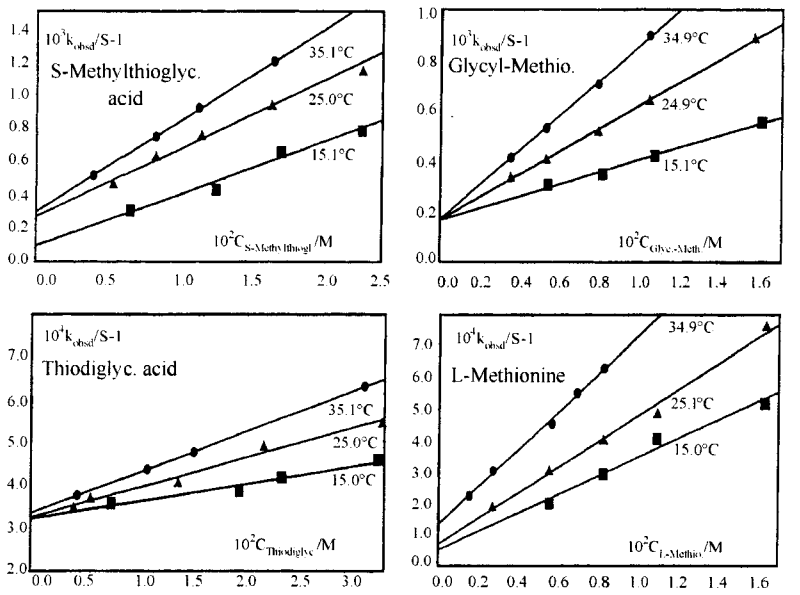



FIGURE 2 Observed *pseudo*-first-order rate constants as a function of excess thioethers.

TABLE I Rate constants and activation parameters for the reactions of $[PtCl(dien)]^+$ with thiols and thioethers in aqueous solution at 298 K

| *Ligand* | $10^3k_2/M^{-1}s^{-1}$ | $10^3k_2/M^{-1}s^{-1}$ [a] | $10^4k_1/s^{-1}$ | $\Delta H_2^{\neq}/kJ\,mol^{-1}$ | $\Delta S_2^{\neq}/JK^{-1}\,mol^{-1}$ |
|---|---|---|---|---|---|
| *L*-cysteine | 1.43 ± 0.2 | | 1.13 ± 0.1 | 31 ± 3 | −187 ± 5 |
| Thioglycolic acid | 7.86 ± 0.2 | | 1.22 ± 0.1 | 38 ± 3 | −148 ± 4 |
| Glutathione | 3.85 ± 0.2 | | 1.12 ± 0.1 | 33 ± 4 | −170 ± 4 |
| *D*-penicillamine | 0.80 ± 0.1 | 0.89 | 1.74 ± 0.1 | 34 ± 3 | −181 ± 5 |
| *L*-methionine | 40.90 ± 2.0 | 48.20 | 0.70 ± 0.2 | 24 ± 3 | −180 ± 4 |
| Glycyl-*D*,*L*-methionine | 46.10 ± 0.5 | 59.70 | 1.69 ± 0.1 | 37 ± 4 | −138 ± 5 |
| *S*-methylthioglycolic acid | 41.10 ± 2.0 | 33.30 | 2.71 ± 0.3 | 29 ± 2 | −166 ± 4 |
| Thiodiglycolic acid | 6.89 ± 0.5 | 8.86 | 2.25 ± 0.2 | 29 ± 3 | −178 ± 5 |

[a] Rate constants obtained by $^1H$ NMR spectroscopy at 296 K.

From Table I it could be concluded that the ligands are very good entering groups for the Pt(II) complex. However, the platinum(II) centre is sensitive to the detailed nature of the sulfur-bonding ligands. From the kinetic point of view, thiols and thioethers should react very similarly. In principle, undissociated thiols, HSR, may be expected to behave like thioethers, $RSR_1$, taking into account different electronic and steric peculiarities. The difference in nucleophilicity between thiols and thioethers towards platinum(II) complexes can be explained by different steric and

electronic structure at sulfur [28, 32]. Reactivity follows the sequence *D*-penicillamine < *L*-cysteine < glutathione < thiodiglycolic acid < thioglycolic acid < *L*-methionine < *S*-methylthioglycolic acid < glycyl-*D*, *L*-methionine.

Electronic effects of the substituents have an obvious influence on reactivity. Comparison of the reactivity of thioglycolic acid with that of *S*-methylthioglycolic and thiodiglycolic acid support this observation. The large sensitivity of the reaction rate to the $\sigma$-donor properties of the entering ligands is as expected for an associative mode of activation. On the other hand, steric effects are very important as well. For example, *D*-penicillamine has the lowest reactivity of the thiols used, which can be explained by steric effects involving the two methyl groups on carbon near to the sulfur atom. $[PtCl(dien)]^+$ is somewhat more sensitive to the stereochemistry of the entering thiols and thioethers than $[Pd(H_2O)_4]^{2+}$ and $[Pt(H_2O)_4]^{2+}$ [32, 48]. The dien ligand hinders to some extent the configurational changes occurring during the activation process. Moreover, strong steric interactions between the bound dien and the entering thioethers are expected.

At the same time the nucleophiles containing an $NH_3^+$ group, for instance *L*-methionine or glycyl-*D*,*L*-methionine, are considerably more reactive than expected. This anomaly seems to suggest an appreciable anchimeric effect capable of reducing the activation energy of the substitution, arising from hydrogen bonding interactions between the acidic group located in a suitable position of the nucleophile and the leaving chloride in the transition state. The anchimeric effect has been reported for other reactions at Pt(II) complexes and is well known for organic reactions [49].

The $k_1$ term (Tab. I), which refers to the solvolytic reaction pathway, is independent of the nature of the entering ligands, is small (ca. $10^{-4}s^{-1}$) and contributes little to the observed rates; $k_1$ values are comparable to those reported previously by spectrophotometry [50] and by $^1H$ NMR spectroscopy [11]. Although the first-order pathway was attributed to hydrolysis of $[PtCl(dien)]^+$, no peaks for an aqua or hydroxo intermediate were observed by two-dimensional [$^1H$, $^{15}N$] NMR spectroscopy [51].

The rate constants obtained at the three temperatures allow calculation of the corresponding enthalpies and entropies of activation through a fit to the Eyring equation [45]. The activation entropies are also summarized in Table I. Large negative values of entropy of activation are compatible with an associative $I_a$ or *A* mechanism. This indicates that bond-making with the entering ligand is important in the activation processes and that the leaving group is still tightly bound to the metal centre in the transition state.

## Implications for Platinum(II) Antitumor Drugs

The present results explicitly demonstrate that platinum(II) complexes have a high affinity for sulfur-bonding ligands like thiols and thioethers, is in agreement with results cited earlier. Thioether amino acids such as methionine and its derivatives play an important role in the metabolism of platinum anticancer drugs. Recent studies on the interactions of the drug carboplatin with sulfur-containing biomolecules suggest that long-lived Pt(II)-methionine adducts may be important metabolites *in vivo* [19]. The complex [Pt(*L*-met-S,N)$_2$] has been isolated from the urine of patients treated with cisplatin [52]. Also, the remarkably stable ring-opened complex [Pt(cbdca-O)(NH$_3$)$_2$(*L*-Hmet-S)], cbdca = cyclobutane-1,1-dicarboxylate, has been detected during the reaction of carboplatin and *L*-methionine. Moreover, a similar species was found in the urine of animals treated with carboplatin [17, 19]. These data suggest that methionine adducts are not inert species devoid of cytotoxic activity. It has been already mentioned that thiols react slower than thioethers with [PtCl(dien)]$^+$. This is in agreement with previous results concerning the anticancer drug and amino acids with thiol groups [17–19]. In these cases thiols react very slowly and form a dinuclear sulfur-bridged complex containing a $Pt_2S_2$ four-membered ring [16]. It has been suggested that *in vivo* the Pt-thiolate complex is a very stable product which does not undergo intermolecular displacement reactions with nitrogenous nucleophiles [18, 19].

Finally, it may be concluded that sulfur-containing biomolecules are very reactive species and they have a high affinity for Pt(II) complexes. Moreover, they may react with Pt(II) before the complex can reach DNA. Certainly, intracellular thiol peptides, such as glutathione and metallothionine, may react with antitumor Pt(II) complexes thus preventing or reducing the extent of platinum binding to DNA. Reaction with SH groups of protein side chains (*e.g.*, in metallothionein and glutathione, GSH) is thought to trap and deactivate the drug before it reaches its cellular target, DNA, to form intrastrand cross-linked guanine bases, the likely cytotoxic adduct [53].

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