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Oxidation of 3,6-Dioxa-1,8-octanedithiol by Platinum(IV) Anticancer Prodrug and Model Complex: Kinetic and Mechanistic Studies

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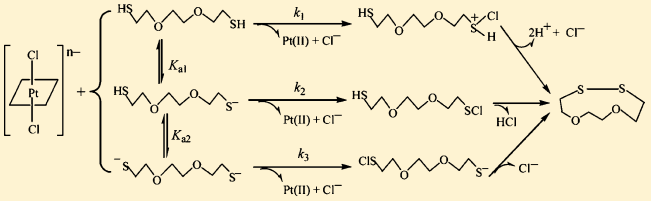
ABSTRACT: Thioredoxins are small redox proteins and have the active sites of Cys-Xaa-Yaa-Cys; they are overexpressed by many different cancer cells. Cisplatin and Pt(II) analogues could bind to the active sites and inhibit the activities of the proteins, as demonstrated by other researchers. Platinum(IV) anticancer drugs are often regarded as prodrugs, but their interactions with thioredoxins have not been studied. In this work, 3,6-dioxa-1,8-octanedithiol (dithiol) was chosen as a model compound for the active sites of thioredoxins, and its reactions with *cis*-$[Pt(NH_3)_2Cl_4]$ and *trans*-$[PtCl_2(CN)_4]^{2-}$ (cisplatin prodrug and a model complex) were studied. The pK_a values for the dithiol were characterized to be 8.7 ± 0.2 and 9.6 ± 0.2 at 25.0 °C and an ionic strength of 1.0 M. The reaction kinetics was followed by a stopped-flow spectrophotometer over a wide pH range. An overall second-order rate law was established, $-d[Pt(IV)]/dt = k'[Pt(IV)][dithiol]$, where k' stands for the observed second-order rate constants. Values of k' increased several orders of magnitude when the solution pH was increased from 3 to 9. A stoichiometry of $\Delta[Pt(IV)]/\Delta[dithiol] = 1:1$ derived for the reduction process and product analysis by mass spectrometry indicated that the dithiol was oxidized to form an intramolecular disulfide, coinciding with the nature of thioredoxin proteins. All of the reaction features are rationalized in terms of a reaction mechanism, involving three parallel rate-determining steps depending on the pH of the reaction medium. Rate constants for the rate-determining steps were evaluated. It can be concluded that Pt(IV) anticancer prodrugs can oxidize the reduced thioredoxins, and the oxidation mechanism is similar to those of the oxidations of biologically important reductants by some reactive oxygen species (ROS) such as hypochlorous acid/hypochlorite and chloramines.



For n =2, Pt(IV) = *trans*-$[PtCl_2(CN)_4]^{2-}$; For n = 0, Pt(IV) = *cis*-$[Pt(NH_3)_2Cl_4]$

1. INTRODUCTION

In order to develop a new generation of platinum-based anticancer drugs, numerous platinum complexes including many Pt(IV) compounds[1−5] have been synthesized and tested; the new generation of anticancer platinum anticancer drugs are anticipated to overcome the side effects and also to widen the curing spectra of the well-known and widely used anticancer drugs cisplatin and carboplatin. In fact, the syntheses and anticancer activity evaluations of the Pt(IV) compounds have been a big part of the effort in this development and are still of current interest.[1−26] As a consequence, several anticancer active Pt(IV) compounds entered into clinical trials with iproplatin, ormaplatin (tetraplatin), setraplatin (JM 216), and LA-12 (structures shown in Scheme 1) as representatives. While iproplatin and ormaplatin were given up for more trials, satraplatin and LA-12 are still under clinical development.[5−7]

Platinum(IV) anticancer active compounds, having an octahedral geometry around the metal center, are often regarded as prodrugs due to their readiness of reduction to their platinum(II) counterparts while the reduction usually results in the loss of their two axially coordinated ligands.[1−4,20−26] In the past few years, some new drug delivery techniques have been developed for some Pt(IV) anticancer compounds by use of the prodrug nature.[22−26] A recent work by Gibson et al. showed that there were more reduction modes for a particular anticancer prodrug.[17]

It has been assumed that biologically important small reductants such as L-glutathione and ascorbic acid are mainly responsible for the reductions of the Pt(IV) prodrugs.[1−4,20−26] Many investigations including some in vitro kinetic and mechanistic studies appear to substantiate this assumption.[27−30] However, Gibson and co-workers found in recent works that the reduction of *cis,trans,cis*-$[PtCl_2(OCOCH_3)(NH_3)_2]$ by the aqueous extracts of cancer cells was mainly by cellular components with MW > 3000 instead of the above small reductants.[4,31] This finding is in contrast with the above assumption and points to the importance of large biomolecules such as proteins for the reduction of the Pt(IV) prodrugs. Separately, McKeage et al. demonstrated that hemoglobin and cytochrome C could play an important role in the reductive activation of satraplatin.[32]

On the other hand, thioredoxin family enzymes encompass several subfamilies of enzymes such as thioredoxins, glutaredoxins (thioltransferases), and protein disulfide iso-

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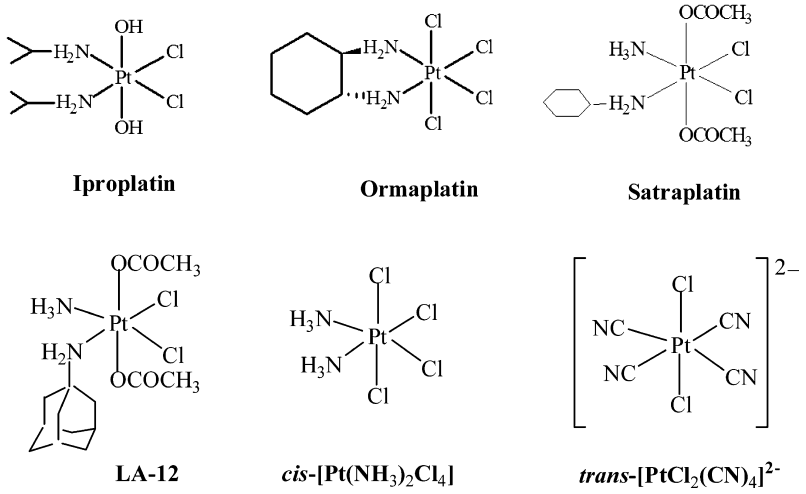
Scheme 1. Structures of Platinum(IV) Anticancer Drugs and Model Compounds



merases.[33−36] This class of enzymes, catalyzing the oxidation of protein thiols and reduction and isomerization of protein disulfide bonds, are found in all living cells and are characterized by a conservative active site Cys-Xaa-Xaa-Cys.[33−36] Thioredoxins are also redox-regulating proteins, playing critical roles in maintaining cellular redox homeostasis and cell survival.[37] Moreover, thioredoxins are highly expressed by several primary types of cancers.[37−40] It was delineated previously that cisplatin and Pt(II) analogues could bind to the active sites and inhibit the activities of the enzymes.[41−43] The interactions of the Pt(IV) prodrugs with this class of enzymes have, however, not been explored.

We chose 3,6-dioxa-1,8-octanedithiol (dithiol) as a model compound for the active sites of this class of enzymes and have undertaken a careful study of its reactions with the cisplatin prodrug *cis*-$[Pt(NH_3)_2Cl_4]$ and a model complex *trans*-$[PtCl_2(CN)_4]^{2-}$ (Scheme 1). Previously, a small dithiol[44] and a few small dicysteine peptides[45,46] were selected as model compounds for the enzymes but not for the purpose of reactions with Pt(IV) complexes. The aims of the present study are (i) to characterize some properties of the dithiol, (ii) to establish the rate law via kinetic measurements in a wide pH range, (iii) to derive a convincing reaction mechanism, and (iv) to discuss some biological and biomedical implications of the Pt(IV) prodrugs.

2. EXPERIMENTAL METHODS

2.1. Instrumentation. The pH values of buffer solutions were measured with an Accumet Basic AB15 Plus pH meter equipped with an Accumet AccutupH combination pH electrode (Fisher Scientific, Pittsburgh, PA). Standard buffers of pH 4.00, 7.00, and 10.00, also from Fisher Scientific, were used to calibrate the electrode. UV−vis spectra were recorded with a TU-1900 spectrophotometer (Beijing Puxi, Inc., Beijing, China) using 1.00 cm quartz cells. An Applied Photophysics SX-20 stopped-flow spectrometer (Applied Photophysics Ltd., Leatherhead, U.K.) was used for kinetic runs and for recording time-resolved spectra. Mass spectra for product analysis were recorded on an Agilent 1200/6310 ion trap mass spectrometer with the electrospray ionization (ESI).

2.2. Chemicals and Solutions. $K_2[Pt(CN)_4]\cdot 3H_2O$, *cis*-$[Pt(NH_3)_2Cl_4]$, 3,6-dioxa-1,8-octanedithiol (dithiol), and sodium perchlorate were obtained from Sigma-Aldrich (St. Louis, MO). Acetic acid, sodium acetate, sodium dihydrogenphosphate, disodium hydrogenphophate, sodium phosphate, sodium carbonate, and sodium bicarbonate were, all in analytical grade, purchased from Beijing Chemical Reagent Company (Beijing, China) and were used for preparations of buffer solutions without further purification. $K_2[PtCl_2(CN)_4]$ was synthesized according to the method reported in our previous work;[47] the UV−vis spectra of the solutions prepared from $K_2[PtCl_2(CN)_4]$ are in excellent agreement with that reported earlier for *trans*-$[PtCl_2(CN)_4]^{2-}$.[48] Doubly distilled water was used to prepare all of the solutions.

Buffers of the following combinations (with concentrations of 0.2−0.4 M) were prepared to cover a wide pH range: acetic acid/sodium acetate, NaH_2PO_4/Na_2HPO_4, $NaHCO_3/Na_2CO_3$, and Na_2HPO_4/Na_3PO_4. All of the buffers, which contained 2 mM EDTA and 0.10 M NaCl, were adjusted to 1.00 M ionic strength with sodium perchlorate. The role of EDTA is to eliminate the possible catalytic effect of traces of metal ions such as Cu(II) and Fe(III)[49−51] during the thiol autoxidation processes, while the addition of NaCl is to suppress the hydrolysis of the platinum(IV) complexes.

2.3. Kinetic Measurements. Stock solutions of 1.0 mM *trans*-$[PtCl_2(CN)_4]^{2-}$ and *cis*-$[Pt(NH_3)_2Cl_4]$ were prepared by dissolving an appropriate amount of the Pt(IV) complexes in solutions containing 0.90 M $NaClO_4$, 0.09 M NaCl, and 0.01 M HCl. The fresh stock solution of *cis*-$[Pt(NH_3)_2Cl_4]$ was used daily. For kinetic measurements, solutions of platinum(IV) complexes and dithiol were prepared, respectively, by adding an appropriate amount of the Pt(IV) stock solution and of dithiol to the buffer. Those solutions were flushed for 15 min with nitrogen before loading on the stopped-flow machine and were only used for a couple of hours. Reactions were initiated by mixing equal volumes of platinum(IV) and dithiol solutions directly in the stopped-flow machine and were followed under pseudo-first-order conditions with dithiol being at least 10-fold excess.

3. RESULTS

3.1. Reaction Stoichiometry. The stoichiometry was determined in 2 mM HCl in the case of *trans*-$[PtCl_2(CN)_4]^{2-}$, where the reduced product $[Pt(CN)_4]^{2-}$ is robust toward dithiol. In the determination, a series of solutions containing a 0.10 mM Pt(IV) complex, 2 mM HCl, and varying

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concentrations of dithiol ($0 < [\text{dithiol}] \leq 0.30$ mM) were prepared and aged for 7 h at 23 ± 1 °C. Absorbances measured at 255 nm (the typical absorption maximum of $[Pt(CN)_4]^{2-}$) as a function of [dithiol] are shown in Figure 1. The data points in Figure 1 essentially follow two straight lines, and from the intersection, a stoichiometry can be derived as $\Delta[Pt(CN)_4Cl_2^{2-}]/\Delta[\text{dithiol}] = 1: 0.90 \pm 0.05$.

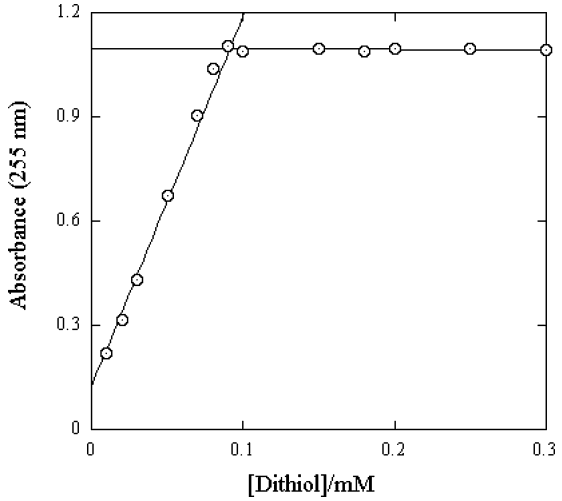
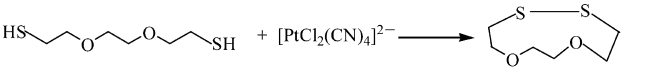


Figure 1. Absorbance at 255 nm for a series of reaction mixtures in which the concentrations of the dithiol were varied in the region of $0 < [\text{dithiol}] < 0.30$ mM and $[PtCl_2(CN)_4^{2-}] = 0.10$ mM was kept constant. Reaction medium: 2.0 mM HCl; temperature of 23 ± 1 °C.

The oxidation product of the dithiol was analyzed by ESI-mass; a reaction mixture of 1.0 mM dithiol with 1.5 mM $[PtCl_2(CN)_4]^{2-}$ in 10 mM HCl was subjected to the analysis. The mass spectrum for the above reaction mixture showed a dominant peak at $m/z = 181$, corresponding to MH^+, where M stands for the cyclic disulfide compound in eq 1 below. On the other hand, no peaks around $m/z = 362$ corresponding to a linear intermolecular disulfide were observed. In comparison, a mass spectrum recorded for the standard dithiol in the same medium gave rise to a major peak at $m/z = 205$ (dithiol·Na^+) and a minor peak at $m/z = 183$ (dithiol·H^+). Hence, the derived stoichiometry and the oxidation product can be described by eq 1.



$$+ [Pt(CN)_4]^{2-} + 2Cl^- + 2H^+ \qquad (1)$$

For reduction of *cis*-$[Pt(NH_3)_2Cl_4]$ by the dithiol, a similar stoichiometric reaction to eq 1 was assumed.

3.2. Determination of the Thiol pK_a Values. A series of buffer solutions containing 6.08×10^{-5} M dithiol at 1.0 M ionic strength were prepared, covering a pH range of 7.35−12.34. Those solutions were flushed by nitrogen gas and concurrently thermo-equilibated at 25.0 °C for 10 min. For each pH, absorbance at 235 nm was measured with the corresponding buffer without dithiol as a reference. A plot of absorbance as a function of pH is displayed in Figure 2. Equation 2, describing the correlation between the absorbance and pH for dithiol compounds, was derived by other researchers,[44−46] where

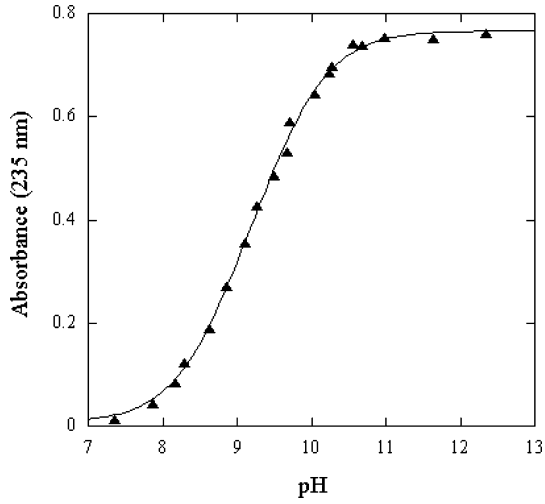


Figure 2. Absorbance (at 235 nm) of 6.08×10^{-5} M dithiol as a function of pH (data points) at 25.0 °C and an ionic strength of 1.0 M. The solid curve resulted from the simulation of eq 2 to the experimental data by a nonlinear least-squares method.

$$\text{Abs}(235\text{ nm}) = \frac{C_0\{\varepsilon_2 \times 10^{(\text{pH}-\text{p}K_{a2})} + \varepsilon_1 + \varepsilon_0 \times 10^{(\text{p}K_{a1}-\text{pH})}\}}{10^{(\text{pH}-\text{p}K_{a2})} + 1 + 10^{(\text{p}K_{a1}-\text{pH})}} \qquad (2)$$

pK_{a1} and pK_{a2} denote the deprotonation constants of the two thiol groups, and ε_0, ε_1, and ε_2 are the molar absorptivities of the dithiol, monothiol-monothiolate, and the fully deprotonated species, respectively. The $\varepsilon_0 = 112 \pm 10$ M^{-1} cm^{-1} at 235 nm was derived from six measurements in the region of $3.90 \leq \text{pH} \leq 6.10$ using much higher dithiol concentrations. Equation 2 was used to fit the absorbance−pH data in Figure 2 by a nonlinear least-squares routine with $\varepsilon_0 = 112$ M^{-1} cm^{-1} fixed, rendering a nice fit. Values of p$K_{a1} = 8.7 \pm 0.2$ and p$K_{a2} = 9.6 \pm 0.2$ and values of ε_1 (235 nm) = $(6.4 \pm 2.2) \times 10^3$ M^{-1} cm^{-1} and ε_2 (235 nm) = $(1.26 \pm 0.02) \times 10^4$ M^{-1} cm^{-1} at 25. 0 °C and ionic strength 1.0 M were obtained from the fit.

3.3. Time-Resolved Spectra. Time-resolved spectra were recorded on the stopped-flow spectrometer in the case of *trans*-$[PtCl_2(CN)_4]^{2-}$ under a set of reaction conditions (cf. Figure 3). The large increase in the absorption peak at around 255 nm is ascribed to the formation of the reaction product of $[Pt(CN)_4]^{2-}$. Two clear isosbestic points at 242.8 and 286.4 nm (an inset in Figure 3 shows an enlarged scale at one isosbestic point) can be noticed from the figure. These isosbestic points indicate that the reduction process is the conversion of the two absorbing species *trans*-$[PtCl_2(CN)_4]^{2-}$ and $[Pt(CN)_4]^{2-}$, while the absorption variation from the dithiol to the disulfide form (the small fraction of dithiol oxidized by the Pt(IV) complex) does not make a significant contribution to the overall spectral changes displayed in Figure 3.

3.4. Kinetics and Rate Law. Reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ was followed at 255 nm (cf. Figure 3), while that of *cis*-$[Pt(NH_3)_2Cl_4]$ was monitored at 240 nm where the absorbance decreased. Pseudo-first-order conditions were fulfilled by using $[\text{dithiol}] \geq 10 \times [\text{Pt(IV)}]$ and constant pHs controlled by buffers. Under such conditions, the kinetic traces at 255 nm could be simulated very well by single exponentials, demonstrating that the reduction is indeed first-order in $[PtCl_2(CN)_4^{2-}]$.

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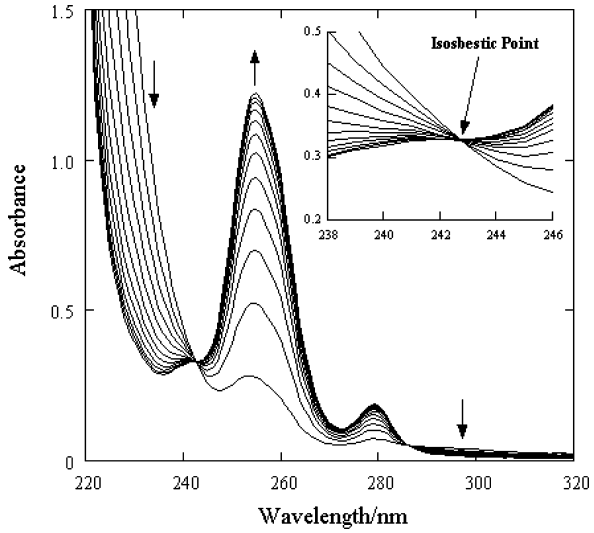

Figure 3. Time-resolved spectra for the reaction between *trans*-$[PtCl_2(CN)_4]^{2-}$ and dithiol under the reaction conditions [Pt(IV)] = 0.10 mM, [dithiol] = 1.00 mM, [HCl] = 0.050 M, ionic strength of 1.0 M, and 25.0 °C. The time between two scans was 30 s.

Absorbance−-time profiles at 240 nm for reaction between *cis*-$[Pt(NH_3)_2Cl_4]$ and the dithiol displayed a relatively quick decrease followed by a sluggish increase. The decrease part can be assigned to the reduction of *cis*-$[Pt(NH_3)_2Cl_4]$ to *cis*-$[Pt(NH_3)_2Cl_2]$ (cisplatin), whereas the sluggish increase is likely the substitution of chloride(s) in cisplatin by the dithiol. Moreover, the reduction reaction was well-separated from the substitution reactions on the time scale and could also be well-simulated by single exponentials. Pseudo-first-order rate constants k_{obsd}, derived from the simulations, are reported as the average values from 5 to 7 runs; standard deviations are usually much less than 5%.

Effects of varying [dithiol] on the reduction rates were studied in different buffer solutions covering a wide range of pHs. Plots of k_{obsd} versus [dithiol] are displayed in Figure 4; the plots are clearly linear, with no significant intercepts, showing that the reductions are also first-order in [dithiol]. Therefore, an overall second-order rate law is established as eq 3, where k' denotes observed second-order rate constants.

$$d[Pt(CN)_4^{2-}]/dt = k_{obsd}[PtCl_2(CN)_4^{2-}] = k'[dithiol][PtCl_2(CN)_4^{2-}] \quad (3a)$$

or

$$-d[Pt(NH_3)_2Cl_4]/dt = k_{obsd}[Pt(NH_3)_2Cl_4] = k'[dithiol][Pt(NH_3)_2Cl_4] \quad (3b)$$

and

$$k_{obsd} = k'[dithiol] \quad (4)$$

Values of k' were calculated from the linear plots of k_{obsd} versus [dithiol] and are summarized in Table 1.

4. DISCUSSION

4.1. Reaction Mechanism. Platinum(IV) complexes are essentially substitution inert, as characterized by rather slow substitution reactions. On the other hand, the present reduction reactions are fast; thus, formation of short-lived Pt(IV) species through substitution by the dithiol is unlikely. This is coherent with the observations of two isosbestic points in the time-resolved spectra of Figure 3.

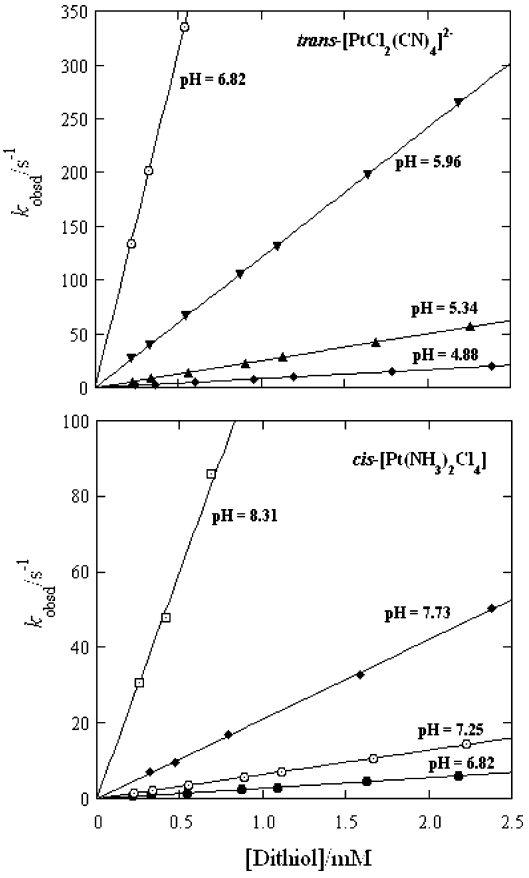

Figure 4. Plots of k_{obsd} versus [dithiol] at 25.0 °C.

Table 1. Observed Second-Order Rate Constants k' as a Function of pH at 25.0 °C and 1.0 M Ionic Strength

Pt(IV) complex	pH	$k'/M^{-1}\ s^{-1}$
trans-$[Pt(Cl_2CN)_4]^{2-}$	2.90	116 ± 2
	3.57	520 ± 10
	4.21	(2.09 ± 0.02) × 10^3
	4.88	(8.32 ± 0.08) × 10^3
	5.34	(2.50 ± 0.02) × 10^4
	5.96	(1.21 ± 0.01) × 10^5
	6.82	(6.23 ± 0.04) × 10^5
	7.25	(1.47 ± 0.02) × 10^6
cis-$[Pt(NH_3)_2Cl_4]$	3.57	4.7 ± 0.1
	4.24	15.0 ± 0.2
	4.88	46.7 ± 0.4
	5.34	127 ± 2
	5.96	593 ± 3
	6.82	(2.76 ± 0.02) × 10^3
	7.25	(6.41 ± 0.03) × 10^3
	7.73	(1.99 ± 0.05) × 10^4
	8.31	(1.20 ± 0.03) × 10^5
	8.60	(1.79 ± 0.11) × 10^5
	9.29	(4.39 ± 0.08) × 10^5

Observed second-order rate constants k' listed in Table 1 increase several orders of magnitude when the reaction medium is changed from acidic buffers to slightly basic ones. The large

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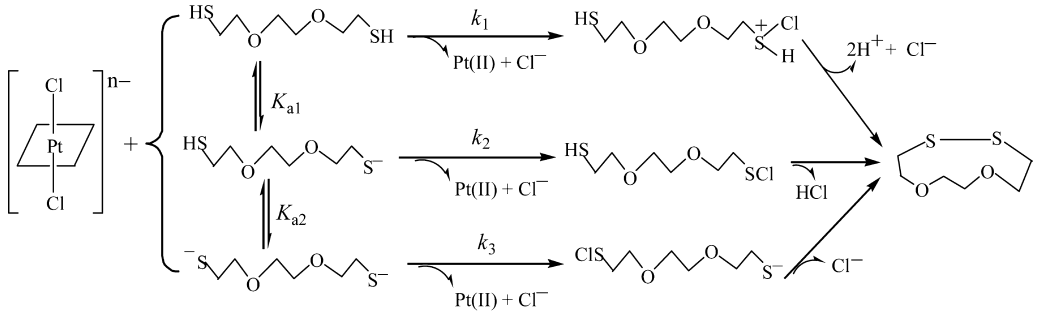
Scheme 2. Proposed Reaction Mechanism



For n =2, Pt(IV) = *trans*-$[PtCl_2(CN)_4]^{2-}$; For n = 0, Pt(IV) = *cis*-$[Pt(NH_3)_2Cl_4]$

increase of k' reflects that the deprotonated forms, that is, thiolates, are much more reactive than the protonated dithiol. A reaction mechanism, described in Scheme 2, is suggested to account for the experimental observations. The mechanism involves three parallel rate-determining steps depending on the pH of the reaction medium; each step takes place through the attack at one of the two axially coordinated chlorides by the sulfur atom in thiol/thiolate group, leading to formation of transient chlorothiol and/or chlorothiolate (sulfenylchloride) species. This is followed by a rapid intramolecular substitution reaction of the transient species, producing the cyclic disulfide product.

4.2. Rate Constants of the Rate-Determining Steps. On the basis of the reaction mechanism described in Scheme 2, a rate law can be derived as eq 5

$$-\mathrm{d[Pt(IV)]}/\mathrm{d}t = \mathrm{d[Pt(II)]}/\mathrm{d}t = \frac{k_1 a_H^{\,2} + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^{\,2} + K_{a1} a_H + K_{a1} K_{a2}} [\mathrm{Pt(IV)}] [\mathrm{dithiol}] \quad (5)$$

where a_H pertains to the proton activity, corresponding to the pH measurements. A comparison of eq 5 with eq 3 renders

$$k' = \frac{k_1 a_H^{\,2} + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^{\,2} + K_{a1} a_H + K_{a1} K_{a2}} \quad (6)$$

Equation 6 was used to simulate the k'–pH data in Table 1 with k_1, k_2, and k_3 as adjustable parameters, whereas the values K_{a1} and K_{a2} determined above were utilized. A weighted nonlinear least-squares simulation in the case of *cis*-$[Pt(NH_3)_2Cl_4]$ gave a nice fitting, as displayed in Figure 5. Values of k_1–k_3 obtained from the simulation are listed in Table 2. In the case of *trans*-$[PtCl_2(CN)_4]^{2-}$, a similar simulation showed that the values of k_3 could not be determined. This is ascribed to the fact that we were able to collect the kinetic data only up to pH 7; in the region of 2.3 < pH < 7, the reaction path described by k_3 in Scheme 1 makes a negligible contribution to the overall reduction process. When the k_3 term in eq 6 is eliminated, the simulation also generated a nice curve fitting (also shown in Figure 5). The resulting values of k_1 and k_2 are also listed in Table 2.

4.3. Mechanistic Insights. The huge reactivity difference between the thiolate and the protonated thiol observed in present work for both Pt(IV) complexes (Table 2) resembles the nature of nucleophilic substitution reactions[52] because the nucleophilicity of the thiolate is much higher than that of the corresponding thiol. When the sulfur atom in thiol/thiolate

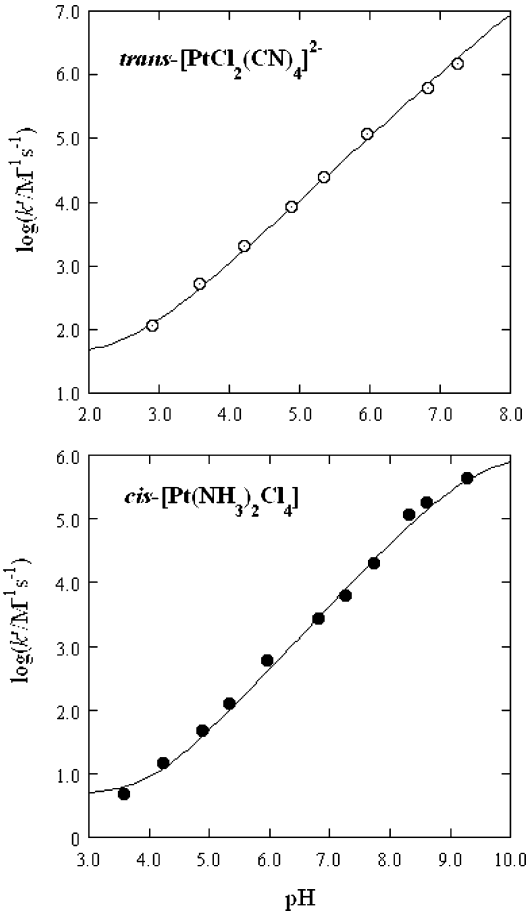


Figure 5. Observed second-order rate constants, k', as a function of pH at 25.0 °C (data points). The solid curves represent the best fits of eq 6 to the experimental data by a weighed nonlinear least-squares routine.

Table 2. Values of the Rate-Determining Steps Derived from Curve Fittings at 25.0 °C and 1.0 M Ionic Strength

Pt(IV) complex	k_m	value/$M^{-1}s^{-1}$
trans-$[PtCl_2(CN)_4]^{2-}$	k_1	38 ± 2
	k_2	(5.37 ± 0.02) × 10^7
	k_3	*a*
cis-$[Pt(NH_3)_2Cl_4]$	k_1	4.6 ± 0.1
	k_2	(2.19 ± 0.01) × 10^5
	k_3	(1.01 ± 0.03) × 10^6

[a]Could not be derived from the kinetic data collected.

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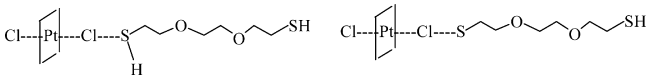
attacks one of the two axially coordinated chlorides in the platinum(IV) complexes, partial bond formation (or bridge formation) between Cl and S atoms takes place, whereas the bonds of Cl−Pt−Cl are substantially weakened simultaneously. In the present reaction systems, the bridge formation is illustrated by the following transitional species



By the end of the bridge formation, the bonds of Cl−Pt−Cl are totally broken, resulting in a Cl^+ transfer to the attacking sulfur atom[27−30,53,54] and formation of two intermediates described in Scheme 2. Virtually, halide-bridged electron transfer has been known for a long time.[55]

4.4. Biological Implications and Conclusions. Although iproplatin was abandoned for more clinical trials, its analogue oxoplatin (*cis*,*cis*,*trans*-diamminedichloridodihydroxidoplatinum(IV)) is still under preclinical development.[13,19] When oxoplatin was exposed to 0.10 M HCl mimicking the gastric acid, it could be converted to *cis*-$[Pt(NH_3)_2Cl_4]$.[13] Thus, the reduction process of oxoplatin may involve the reduction route of cisplatin prodrug *cis*-$[Pt(NH_3)_2Cl_4]$. On the other hand, ormaplatin possesses a configuration similar to *cis*-$[Pt(NH_3)_2Cl_4]$, and their reduction mechanisms are expected to resemble each other.

To mimic the active sites of the thioredoxin enzymes by a model dithiol compound, two important requirements are (1) the dithiol compound should be converted to an intramolecular disulfide loop when it is oxidized and (2) the loop size is close to those of the oxidized active sites of the enzymes (14-membered rings).[33−36] The dithiol that we chose in the present work is oxidized to form a 10-membered loop, fulfilling these requirements. Moreover, the pK_a values for the dithiol determined in this work are about the same to those derived for other model compounds[44−46] for the thioredoxin family enzymes. These attributes confer the dithiol chosen in this work as a suitable model compound for the active sites of the enzymes.

The second-order rate constants for reduction of *cis*-$[Pt(NH_3)_2Cl_4]$ by the dithiol are in fact very close to those by L-glutathione.[28] This manifests that the Pt(IV) prodrugs can oxidize the reduced forms of the enzymes in a competitive process to the oxidations of small thiols like L-glutathione and L-cysteine. Thus, the thiol−disulfide equilibria of these enzymes can be disturbed or shifted by the Pt(IV) prodrugs, at least locally where the concentrations of these enzymes are relatively high.

When a human body is under oxidative stress, a number of reactive oxygen species (ROS) encompassing superoxides, hydrogen peroxide, peroxynitrite, HOCl, HOBr, chloramines, and bromamines could be generated.[56] These ROS are very active and will oxidize some small reductants such as L-glutathione, L-cysteine, and ascorbic acid and some big molecules like proteins and DNA.[56−58] Among these ROS, HOCl and chloramines are particularly relevant to the present reaction systems because their reduction mechanisms are similar to that proposed in this work, namely, a Cl^+ transfer from the oxidants to the reductants is the key step.[59,60] In this regard, the toxic effects of HOCl and chloramines that stemmed from the oxidation processes in human bodies might be a reason for the toxic effects of the Pt(IV) prodrugs.

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Notes

The authors declare no competing financial interest.

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