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Kinetics and mechanism of reactions of the drug tiopronin with platinum(IV) complexes

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ABSTRACT

Tiopronin, a synthetic thiol-containing drug being used in treatments of cystinuria and certain types of rare arthritis, is also a hepatoprotective and a detoxifying agent. Many analytical methods have been developed based on its redox chemistry with metal ions/complexes, but the kinetic and mechanistic aspects are poorly understood. In this work, the oxidation of tiopronin by cisplatin prodrug and a model compound, *cis*-$[Pt(NH_3)_2Cl_4]$ and *trans*-$[PtCl_2(CN)_4]^{2-}$, was investigated. The oxidation kinetics was followed by a stopped-flow spectrophotometer over a wide pH range under the pseudo first-order conditions of [Tiopronin]≫[Pt(IV)]. Time-resolved spectra were also recorded for both Pt(IV) complexes, enabling to establish an overall second-order rate law: $-d[Pt(IV)]/dt = k'[Tiopronin][Pt(IV)]$, where k' pertains to observed second-order rate constants. Under the kinetic conditions, tiopronin was oxidized to form the tiopronin-disulfide exclusively as identified by mass spectrometry. A reaction mechanism was proposed, involving parallel reductions of the Pt(IV) complexes by the three protolytic tiopronin species as rate-determining steps. The rate constants for the rate-determining steps were derived. The fully deprotonated tiopronin is about 4×10^4 more reactive than its corresponding thiol form for both Pt(IV) complexes; the huge reactivity difference orchestrates closely with the fact that the nucleophilicity of thiolate is much higher than the corresponding thiol. Hence, the attack of the sulfur atom in thiol/thiolate of tiopronin on the axially-coordinated chloride in the Pt(IV) complexes is nucleophilic in nature in the rate-determining steps, resulting in a bridge formation and a subsequent bridged electron-transfer.

1. Introduction

Tiopronin (also called thiola, *N*-(mercaptopropionyl)glycine, a racemic mixture around the single chiral center, cf. the structure in Fig. 1) is a synthetic thiol-containing drug, which has been used to treat cystinuria [1,2] and certain types of rare arthritis [3,4]. It has also been used as a hepatoprotective agent [5,6], and a detoxifying agent. For instance, tiopronin was found to have a function of protecting the nephtotoxicity and ototoxicity caused by cisplatin [7–9]. In addition, tiopronin protected gold clusters/nanoparticles have been found to possess some very attractive properties [10–12].

A number of analytical methods have been developed for the determinations of tiopronin in various kinds of samples due to its widely therapeutic applications as pointed out above; these methods encompass chromatographic [13–15], spectrophotometric [16–18], and chemiluminescent approaches [19–22]. Moreover, some of the analytical methods are based on the oxidation chemistry of tiopronin by metal ions/complexes involving iron(III) [17,18], thallium(III) [19], and cesium(IV) [20,21]. Kinetically or pharmacokinetically, studies on tiopronin are, in contrast, very scarce [23–26]. As a consequence, we have carried out a systematic investigation on kinetics and mechanisms of oxidation of tiopronin by some important metal ions/complexes; in this work we report the oxidation of tiopronin by two platinum(IV) complexes: *cis*-$[Pt(NH_3)_2Cl_4]$ and *trans*-$[PtCl_2(CN)_4]^{2-}$ (structures shown in Fig. 1).

Currently, there is a strong interest in the designs and syntheses of new type of platinum(IV) anticancer drugs and meanwhile, new drug delivery methods for some Pt(IV) anticancer prodrugs are vigorously pursued in order to find a new generation of platinum based anticancer drugs [27–37]. *cis*-$[Pt(NH_3)_2Cl_4]$ is the prodrug of cisplatin due to its readiness of reduction and to the formation of cisplatin [27–31]. *trans*-$[PtCl_2(CN)_4]^{2-}$ has been used as a model compound for the platinum(IV) anticancer drugs such as ormaplatin (cf. Fig. 1) as it has several characters very suitable to the kinetic studies [38–40]. In this work, the oxidations of tiopronin by the two Pt(IV) complexes are characterized kinetically over a wide pH range and a detailed mechanistic

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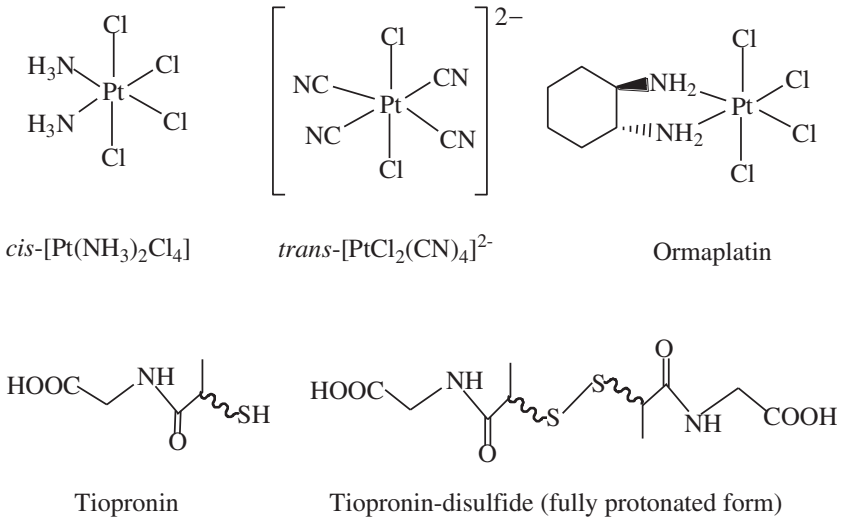


Fig. 1. Structures of *cis*-$[Pt(NH_3)_2Cl_4]$, *trans*-$[PtCl_2(CN)_4]^{2-}$, ormaplatin, tiopronin (a racemic mixture at the chiral center), and tiopronin-disulfide.

picture is delineated. Moreover, the oxidation products are well characterized by mass spectrometry.

2. Materials and methods

2.1. Materials

Tiopronin, L-glutathione, $K_2[Pt(CN)_4]\cdot 3H_2O$ and *cis*-$[Pt(NH_3)_2Cl_4]$ were obtained from Sigma-Aldrich (St. Louis, MO). Acetic acid, sodium acetate, sodium dihydrogenphosphate, disodium hydrogenphosphate, sodium carbonate, and sodium bicarbonate, sodium perchlorate, and perchloric acid were, all in analytical grade, purchased either from Fisher Scientific (in Beijing, China) or from Alfa Aesar (in Tianjin, China) and were used for preparations of buffer solutions without further purification. The synthesis of $K_2[PtCl_2(CN)_4]$ was carried out according to the method reported in a previous work [41]; the UV-Visible (UV-vis) spectra of the solutions prepared from $K_2[PtCl_2(CN)_4]$ are in excellent agreement with that reported earlier for *trans*-$[PtCl_2(CN)_4]^{2-}$ [42]. Doubly distilled water was used to prepare all the solutions.

2.2. Buffer solutions

The following combinations (with concentrations of 0.2–0.3 M) were prepared to cover a wide pH range: acetic acid/sodium acetate, NaH_2PO_4/Na_2HPO_4 and $NaHCO_3/Na_2CO_3$ and Na_2HPO_4/Na_3PO_4. All 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 was to eliminate the possible catalytic effect of traces of metal ions such as Cu(II) and Fe(III) [43–45] during the thiol autooxidation processes, while the addition of NaCl is to suppress the hydrolysis of the platinum(IV) complexes. 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 just before the pH measurements.

2.3. Kinetic experiments

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. Stock solutions of 1.0 mM *cis*-$[Pt(NH_3)_2Cl_4]$ and *trans*-$[PtCl_2(CN)_4]^{2-}$ were prepared by dissolving 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 stock solution of *cis*-$[Pt(NH_3)_2Cl_4]$ was used daily in fresh. Solutions of platinum(IV) complexes and tiopronin were prepared, respectively, by adding an appropriate amount of the Pt(IV) stock solution and of tiopronin to the buffer. Those solutions were flushed for 10 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 tiopronin solutions directly in the stopped-flow machine and were followed under pseudo-first-order conditions with tiopronin being at least 10-fold excess.

2.4. Time-resolved spectra

UV–vis spectra were recorded with a TU-1900 spectrophotometer (Beijing Puxi, Inc., Beijing, China) using 1.00 cm quartz cells. The spectrophotometer was equipped with a cell compartment which could be thermostated by circulation of water from a thermostate (BG-chiller E10, Beijing Biotech Inc., Beijing). Time-resolved spectra were recorded on the above spectrophotometer for the reaction between *cis*-$[Pt(NH_3)_2Cl_4]$ and tiopronin in an acetic acid/acetate buffer of pH 3.68. For the reaction between *trans*-$[PtCl_2(CN)_4]^{2-}$ and tiopronin, the time-resolved spectra were recorded on the stopped-flow spectrometer in 0.020 M perchloric acid solutions.

2.5. Mass spectrometric analysis

Mass spectra were recorded on an Agilent 1200/6310 ion trap mass spectrometer with electrospray ionization (ESI); both positive and negative ionization modes were employed. For each of the Pt(IV) complexes, a reaction mixture containing 8 mM tiopronin, 1 mM Pt(IV) and 20 mM HCl was subjected to the mass analysis. In addition, mass spectra were also recorded for pure tiopronin in dilute HCl; they were used as a reference for mass analysis.

3. Results and discussions

3.1. Time-resolved spectra and kinetic data

Time-resolved spectra for the reaction between *trans*-$[PtCl_2(CN)_4]^{2-}$ and tiopronin under a set of reaction conditions are displayed in Fig. 2. The large increase in the absorption peak around 255 nm can be assigned to the formation of the reaction product of $[Pt(CN)_4]^{2-}$ [42]. Moreover, two clear isosbestic points at 241.1 nm and 285.7 nm (an inset in Fig. 2 shows an enlarged scale at one isosbestic point) are found from the figure.

These isosbestic points suggest that the reduction process is the transformation between the two absorbing species *trans*-$[PtCl_2(CN)_4]^{2-}$ and $[Pt(CN)_4]^{2-}$, while the absorption variation from the tiopronin oxidation does not make a significant contribution to the overall spectral changes displayed in Fig. 2. Reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ was thus followed at 255 nm. By use of pseudo first-order conditions ([tiopronin] ≥ 10·[Pt(IV)] and constant pHs controlled by buffers), the kinetic traces at 255 nm could be simulated very well by single exponentials, indicating that the reduction is indeed first-order in $[PtCl_2(CN)_4^{2-}]$. 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%.

Time-resolved spectra for the reaction between *cis*-$[Pt(NH_3)_2Cl_4]$ and tiopronin in an acetic acid/acetate buffer are shown in Fig. 3A. The absorbance decrease was simulated by Eq. (1), where A_t, A_0, and A_∞ stand for absorbances at time t, zero and

$$A_t = (A_0 - A_\infty)\exp(-k_{obsd}t) + A_\infty \quad (1)$$

infinity, respectively. The simulations around two major bands gave rise to very good fittings (shown in Fig. 3B) and further, the values of k_{obsd} obtained from the fittings at different wavelengths were in excellent agreement within the experimental errors. These attributes manifest that the absorbance decrease in Fig. 3 corresponds to the reduction of *cis*-$[Pt(NH_3)_2Cl_4]$ without other complications. Hence, the reduction kinetics was followed around 230 nm, and similarly, the obtained k_{obsd} values were from 3 to 5 duplicate runs. Values of k_{obsd} as functions of [Tiopronin] and pH at 25.0 °C and ionic strength of 1.00 M are summarized in Supporting Tables S1 and S2 in the supplementary materials.

3.2. Rate law

The influences of varying [Tiopronin] on the reduction rates were studied in different buffer solutions covering a wide range of pH. Values of k_{obsd} as a function of [Tiopronin] at several pHs are shown in Figs. 4 and 5. Clearly, all the plots of k_{obsd} versus [Tiopronin] are of straight lines with no significant intercepts, demonstrating that the reductions are also first-order in [Tiopronin]. Therefore, the

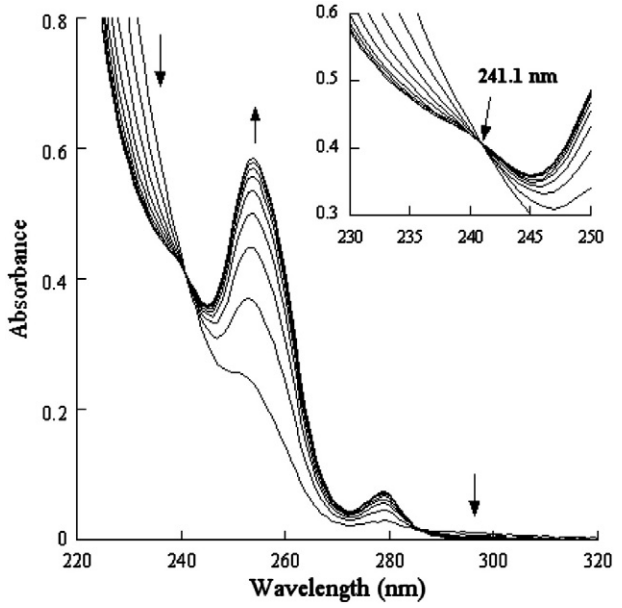


Fig. 2. Time-resolved spectra for reaction between *trans*-$[PtCl_2(CN)_4]^{2-}$ and tiopronin under the reaction conditions: [Pt(IV)] = 0.05 mM, [Tiopronin] = 1.00 mM, $[H^+]$ = 0.020 M, $[Cl^-]$ = 0.10 M, μ = 1.0 M and 25.0 °C. The time between scans was 17 s.

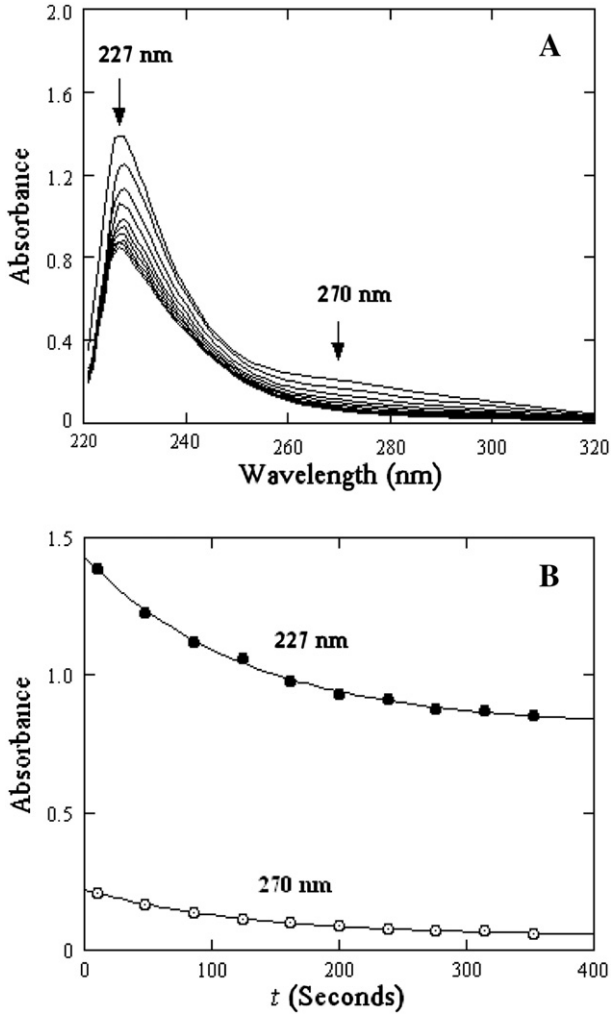


Fig. 3. (A) Time-resolved spectra for reaction between *cis*-$[Pt(NH_3)_2Cl_4]$ and tiopronin under the reaction conditions: [Pt(IV)] = 0.05 mM, [Tiopronin] = 1.00 mM, $[Cl^-]$ = 0.10 M, pH = 3.68 acetic acid/acetate buffer, μ = 1.0 M and 25.0 °C. The first spectrum was obtained ca. 10 s after reaction and time between scans was 38 s. (B) Kinetic traces at 227 nm and 270 nm for reaction between *cis*-$[Pt(NH_3)_2Cl_4]$ and tiopronin; the data points are from the absorbance readings in Fig. 3A. The solid curves were obtained by fitting Eq. (1) the experimental data by use of a nonlinear least-squares method.

reductions can be generally described by an overall second-order rate law (2), where k' denotes observed second-order rate constants.

$$\begin{aligned} d\left[Pt(CN)_4^{2-}\right]/dt &= k_{obsd}\left[PtCl_2(CN)_4^{2-}\right] \\ &= k'[\text{Tiopronin}]\left[PtCl_2(CN)_4^{2-}\right] \end{aligned} \quad (2a)$$

or

$$\begin{aligned} -d\left[Pt(NH_3)_2Cl_4\right]/dt &= k_{obsd}\left[Pt(NH_3)_2Cl_4\right] \\ &= k'[\text{Tiopronin}]\left[Pt(NH_3)_2Cl_4\right] \end{aligned} \quad (2b)$$

and

$$k_{obsd} = k'[\text{Tiopronin}]. \quad (3)$$

Values of k' as a function of pH are listed in Table 1; they were calculated from the linear plots of k_{obsd} versus [Tiopronin].

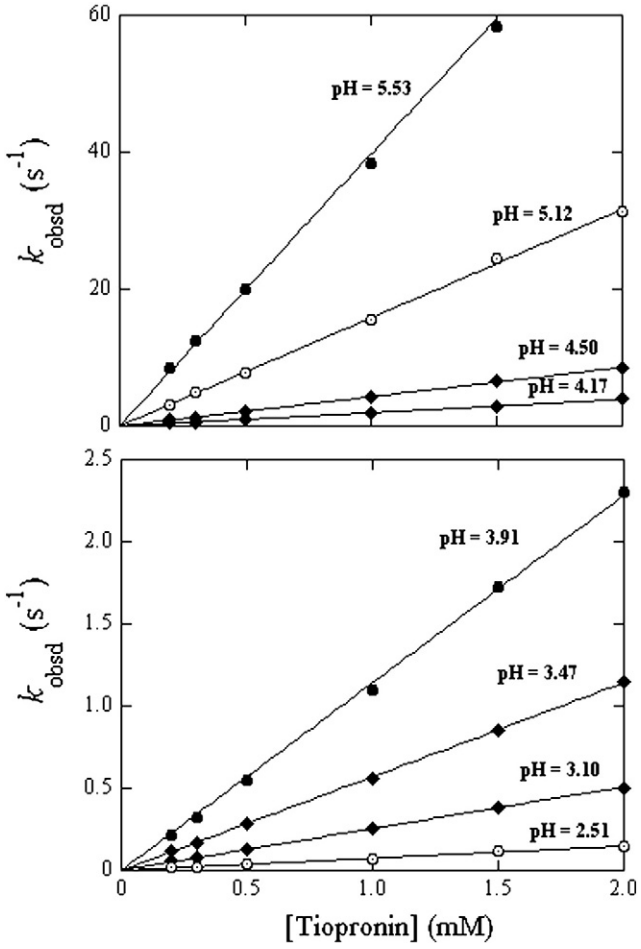


Fig. 4. Pseudo first-order rate constants, k_{obsd}, as a function of [Tiopronin] for oxidation of tiopronin by *trans*-$[PtCl_2(CN)_4]^{2-}$ at 25.0 °C and ionic strength of 1.0 M.

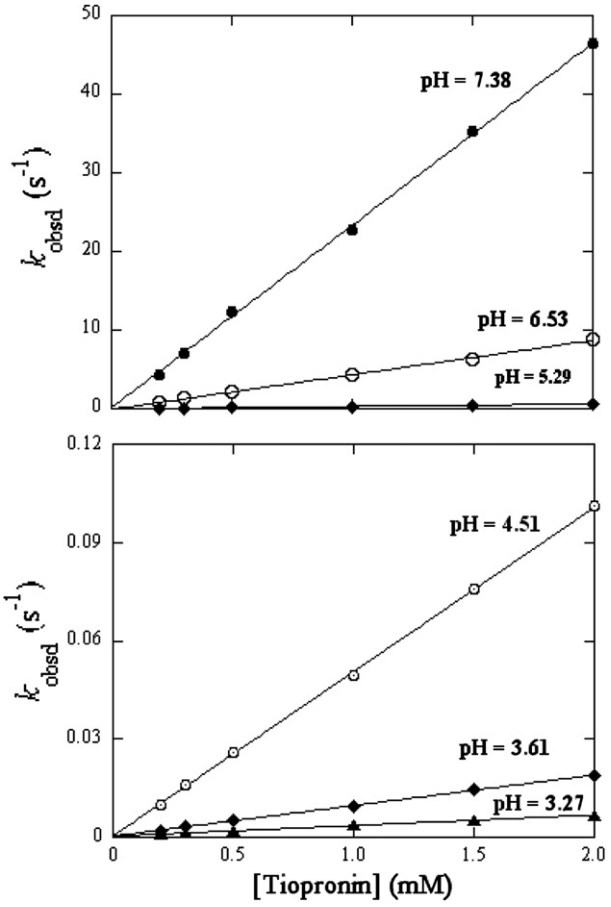


Fig. 5. k_{obsd} as a function of [Tiopronin] for oxidation of tiopronin by *cis*-$[Pt(NH_3)_2Cl_4]$ at 25.0 °C and ionic strength of 1.0 M.

3.3. Reaction products

The mass spectra for pure tiopronin, for the reaction mixtures of tiopronin with *trans*-$[PtCl_2(CN)_4]^{2-}$and with *cis*-$[Pt(NH_3)_2Cl_4]$ are given in supporting Figs. S1–S3 in supplementary materials. Assignments of the major peaks are also provided in each of the figures. Under the conditions used which are similar to those employed in the kinetic experiments, tiopronin was found to be oxidized almost exclusively to form tiopronin-disulfide (structure also shown in Fig. 1) by both the Pt(IV) complexes; some other conceivable products from deeper oxidations of tiopronin were not noticeable. Previously, *trans*-$[PtCl_2(CN)_4]^{2-}$ was utilized, as a very efficient reagent, for formation of *intramolecular disulfides* in dithiol-containing peptides [46,47] and small dithiol compound [40]. In this work, we found that formation of *intermolecular disulfide* bond by *trans*-$[PtCl_2(CN)_4]^{2-}$ was also efficient under the kinetic conditions used.

The mass spectra do not, however, provide any meaningful information about the platinum complexes after reaction; this is ascribed to their much lower sensitivity compared to tiopronin disulfide. On the other hand, the UV–vis spectra assisted us to clarify this issue. The UV–vis spectra for 0.08 mM $[Pt(CN)_4]^{2-}$ in 5 mM HCl and for a reaction mixture of 0.08 mM *trans*-$[PtCl_2(CN)_4]^{2-}$ with 0.30 mM tiopronin in 5 mM HCl are shown in the supporting Fig. S4. Virtually, the two spectra give rise to two identical absorption peaks around 255 nm and 279 nm, while the minor spectral difference in the region 225–235 nm is caused by the tiopronin background contribution. Therefore, it can be safely concluded that the reduced product of *trans*-$[PtCl_2(CN)_4]^{2-}$ is $[Pt(CN)_4]^{2-}$.

3.4. Reaction mechanism

As discussed above, the time-resolved spectra shown in Figs. 2 and 3 suggest that the reductions of the two Pt(IV) complexes follow a simple process. This coincides with the nature of platinum(IV) complexes, namely, substitution inert as characterized by rather slow substitution reactions. The present reduction reactions are fast in general and in

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-$[PtCl_2(CN)_4]^{2-}$	1.98	22.6 ± 0.7
	2.51	73.6 ± 2.5
	3.10	250 ± 2
	3.47	571 ± 20
	3.91	(1.14 ± 0.01) × 10^3
	4.17	(1.93 ± 0.02) × 10^3
	4.50	(4.28 ± 0.03) × 10^3
	5.12	(1.58 ± 0.02) × 10^4
	5.53	(3.97 ± 0.04) × 10^4
	6.22	(1.73 ± 0.02) × 10^5
	6.82	(6.9 ± 0.1) × 10^5
	7.24	(1.5 ± 0.1) × 10^6
cis-$[Pt(NH_3)_2Cl_4]$	3.27	3.24 ± 0.05
	3.61	9.3 ± 0.1
	4.51	50.1 ± 0.4
	5.29	304 ± 4
	6.53	(4.39 ± 0.07) × 10^3
	7.38	(2.32 ± 0.03) × 10^4

contrast; it is therefore anticipated that formation of short-lived Pt(IV) species through substitution by the tiopronin has no time to occur.

The second-order pH-dependent rate constants k' listed in Table 1 display a similar trend for the Pt(IV) complexes, increasing several orders of magnitude when the reaction media are changed from acidic solutions to slightly basic ones. This trend clearly indicates that the thiolate form of tiopronin is much more reactive than its thiol forms. By taking the three protolytic forms of tiopronin into account, a reaction mechanism is proposed and depicted in Scheme 1. In this mechanism, all the protolytic species of tiopronin reduce the Pt(IV) complexes in parallel relying on the solution pH. In addition, all the reactions described by $k_1 - k_3$ are rate-determining steps, generating transient chlorothiol and sulfenylchloride species (cf. more discussions on the rate determining steps below). From the product analysis, it can be concluded that the chlorothiol and sulfenylchloride species are rapidly and mainly trapped intermolecularly by the excess thiol/thiolate forms of tiopronin, leading to formation of tiopronin-disulfide.

3.5. Calculation of rate constants

According to Scheme 1, Eq. (4) was derived as the rate law, where a_H denotes to

$$-\mathrm{d[Pt(IV)]}/\mathrm{dt} = \mathrm{d[Pt(II)]}/\mathrm{dt} = \frac{k_1 a_H^{\,2} + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^{\,2} + K_{a1} a_H + K_{a1} K_{a2}} [\mathrm{Pt(IV)}][\mathrm{Tiopronin}] \quad (4)$$

the proton activity, corresponding to the pH measurements. When Eq. (4) is compared to Eq. (3), Eq. (5) can be obtained:

$$k' = \frac{k_1 a_H^{\,2} + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^{\,2} + K_{a1} a_H + K_{a1} K_{a2}} \quad (5)$$

The k'–pH data in Table 1 were analyzed by Eq. (5) with k_1, k_2 and k_3 as adjustable parameters whereas $pK_{a1} = 3.42$ and $pK_{a2} = 8.33$ determined at 25.0 °C by other researchers were utilized as fixed values [48]. A weighted nonlinear least-squares analysis in the case of *trans*-$[PtCl_2(CN)_4]^{2-}$ resulted in a good fitting shown in Fig. 6; meanwhile, the fitting affords $k_1 = 0.11 \pm 0.79\ M^{-1}\ s^{-1}$, $k_2 = 630 \pm 6\ M^{-1}\ s^{-1}$, and $k_3 = (2.45 \pm 0.02) \times 10^7\ M^{-1}\ s^{-1}$. Apparently, k_1 has no meaning because it is indeterminate within the data collected. Eq. (5) is reduced to Eq. (6) when the k_1-term is neglected:

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

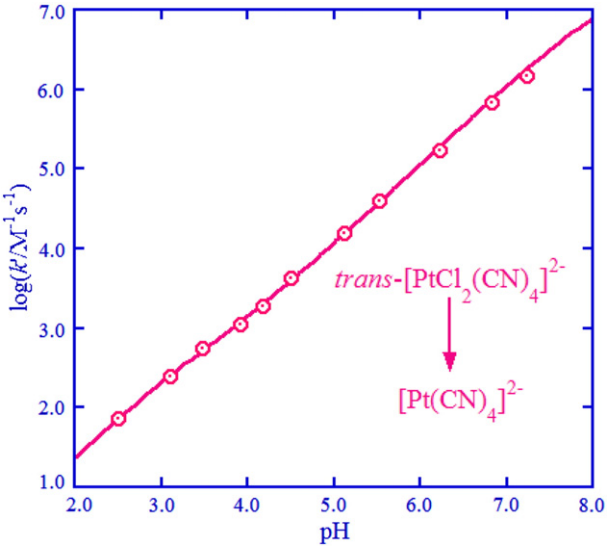
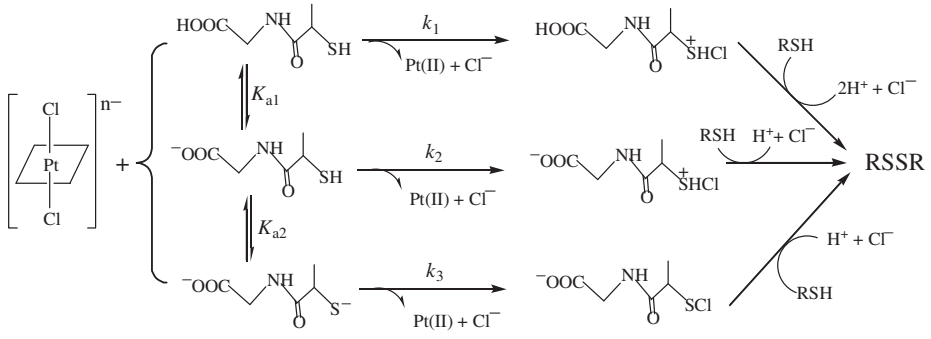


Fig. 6. Second-order rate constants k' as a function of pH at 25.0 °C and ionic strength of 1.0 M for oxidation of tiopronin by *trans*-$[PtCl_2(CN)_4]^{2-}$ (data points). The solid curve was obtained by fitting Eq. (5) the experimental data by use of a weighed nonlinear least-squares method.

Eq. (6) was also used to simulate the data in Fig. 6, giving rise to exactly the same values of k_2 and k_3 when Eq. (5) was used, confirming that the k_1-term in Eq. (5) has indeed a negligible contribution. In the case of *cis*-$[Pt(NH_3)_2Cl_4]$, Eq. (6) was directly employed to simulate the k'–pH data, and the simulation also rendered a good fitting shown in Fig. 7. The derived rate constants for the rate-determining steps are summarized in Table 2.

3.6. Rate-determining steps

It has been interpreted that the reductive elimination of the *trans*-dichoro-platinum(IV) complexes takes place through a chloride-bridged electron-transfer process [38–40,49–53]. By analog, the rate-determining steps in Scheme 1 proceed conceivably through a similar transition state where a bridge is formed between the axially coordinated chloride of the Pt(IV) complex and the sulfur atom from the thiol/thiolate group of tiopronin [38–40,49–53]. It follows by the collapse of the bridge, resulting in a Cl^+ transfer from the Pt(IV) complexes to tiopronin species (to form chlorothiol and/or sulfenylchloride); concurrent to the collapse of the bridge, the Pt(IV) complexes are acquiring two electrons in one-step from the bridged chloride (inner-sphere electron transfer),



For n =2, Pt(IV) = *trans*-$[PtCl_2(CN)_4]^{2-}$; For n = 0, Pt(IV) = *cis*-$[Pt(NH_3)_2Cl_4]$
RSH denotes any of the three protolytic species of tiopronin indicated above

Scheme 1. Suggested reaction mechanism.

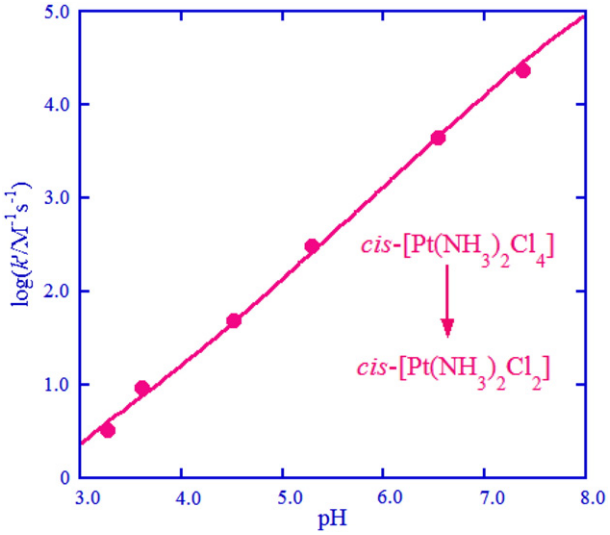


Fig. 7. Second-order rate constants k' as a function of pH at 25.0 °C and ionic strength of 1.0 M for oxidation of tiopronin by *cis*-$[Pt(NH_3)_2Cl_4]$ (data points). The solid curve was obtained by fitting Eq. (6) the experimental data by use of a weighed nonlinear least-squares method.

and are being reduced to their Pt(II) counterparts. For the two Pt(IV) complexes, the ratios of k_3/k_2 are similar and about 4×10^4, highlighting the huge reactivity difference between thiolate form of tiopronin and its corresponding thiol form. This huge reactivity difference coincides with the fact that the nucleophilicity of thiolate is much higher than the corresponding thiol observed by other researchers [54,55]. In other words, the chloride bridged electron-transfer process resembles, in nature, to a nucleophilic substitution.

3.7. Biological/biomedical relevance

A series of Pt(IV) anticancer active compounds including ormaplatin and some recently developed ones [56,57] have a configuration similar to that of *cis*-$[Pt(NH_3)_2Cl_4]$; reduction of this class of Pt(IV) agents by tiopronin will be expected to follow the reaction mechanism delineated above. Observed second-order rate constants k' for reductions of *cis*-$[Pt(NH_3)_2Cl_4]$ by ascorbate and glutathione at biological relevant pH are listed in Table 3; all these reductions proceed via the inner-sphere electron transfer mode. Reduction of the orally active Pt(IV) drug satraplatin by ascorbate is about 10,000 times slower than that of *cis*-$[Pt(NH_3)_2Cl_4]$ (Table 3). Due to the poor bridging effect of the coordinated acetate in satraplatin, the much slower reduction was believed to take place via an outer-sphere electron transfer [52]. On the other hand, reduction of satraplatin by glutathione appeared to be more complex, not following the simple second-order kinetics [58]. Ascorbic acid or glutathione or both are often supposed to be responsible for in vivo reductions of Pt(IV) prodrugs, but large biomolecules may also play a big role for these reductions as pointed out by Gibson [29].

Table 2
Values of rate constants 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	–[a]
	k_2	630 ± 6
	k_3	(2.45 ± 0.02) × 10^7
cis-$[Pt(NH_3)_2Cl_4]$	k_1	–[a]
	k_2	7.3 ± 0.3
	k_3	(3.01 ± 0.03) × 10^5

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

Table 3
Observed second-order rate constants k' for reduction of *cis*-$[Pt(NH_3)_2Cl_4]$ and satraplatin at 25.0 °C and 1.0 M ionic strength.

Pt(IV) Complex	Reductant	pH	$k'/M^{-1}s^{-1}$
cis-$[Pt(NH_3)_2Cl_4]$	Ascorbate	7.16	675[a]
	Glutathione	7.36	1.31×10^4 [b]
	Tiopronin	7.38	2.32×10^4 [c]
Satraplatin	Ascorbate	7.12	5.08×10^{-2} [d]
	Glutathione		NA

NA, not available.
[a] Ref [53].
[b] Measured in this work, cf. Fig. S5 in the supporting material.
[c] This work.
[d] Ref. [52].

Tiopronin was reported to have a nephroprotective property for cisplatin [7,8] while glutathione has not. This differentiation may stem from their interactions with cisplatin in some different ways. It is thus reasonable that tiopronin may also have a nephroprotective effect for Pt(IV) prodrugs of cisplatin although tiopronin and glutathione reduce *cis*-$[Pt(NH_3)_2Cl_4]$ with reaction rates differing not too much each other.

4. Conclusions

We have carried out a careful and detailed study on the oxidations of tiopronin by cisplatin prodrug *cis*-$[Pt(NH_3)_2Cl_4]$ and a model complex *trans*-$[PtCl_2(CN)_4]^{2-}$ in a wide pH range. A rate law is established through the stopped-flow spectrometric and kinetic measurements. The mass spectrometric analysis of the oxidations products, together with the rate law, enables us to suggest a convincing reaction mechanism and to gain some insights into the oxidative degradation of tiopronin. It appears to be the first detailed kinetic and mechanistic study for the oxidation of tiopronin by metal ions/complexes, although some of the oxidation processes have been widely used in determinations of tiopronin in various samples. Since *cis*-$[Pt(NH_3)_2Cl_4]$ is rapidly reduced to cisplatin by tiopronin and by other thiol-containing compounds, it is anticipated that tiopronin may also protect the nephtotoxicity and ototoxicity caused by Pt(IV) prodrugs of cisplatin [7–9].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jinorgbio.2013.04.003.

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