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Kinetics and mechanism for reduction of anticancer-active tetrachloroam(m)ine platinum(IV) compounds by glutathione

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Abstract Glutathione (GSH) reduction of the anticancer-active platinum(IV) compounds *trans*-[$PtCl_4(NH_3)$(thiazole)] (**1**), *trans*-[$PtCl_4$(cha)(NH_3)] (**2**), *cis*-[$PtCl_4$(cha)(NH_3)] (**3**) (cha = cyclohexylamine), and *cis*-[$PtCl_4(NH_3)_2$] (**4**) has been investigated at 25 °C in a 1.0 M aqueous medium at pH 2.0–5.0 (**1**) and 4.5–6.8 (**2–4**) using stopped-flow spectrophotometry. The redox reactions follow the second-order rate law $d[Pt(IV)]/dt = k[GSH]_{tot}[Pt(IV)]$, where k is a pH-dependent rate constant and $[GSH]_{tot}$ the total concentration of glutathione. The reduction takes place via parallel reactions between the platinum(IV) complexes and the various protolytic species of glutathione. The pH dependence of the redox kinetics is ascribed to displacement of these protolytic equilibria. The thiolate species GS^- is the major reductant under the reaction conditions used. The second-order rate constants for reduction of compounds **1–4** by GS^- are $(1.43 \pm 0.01) \times 10^7$, $(3.86 \pm 0.03) \times 10^6$, $(1.83 \pm 0.01) \times 10^6$, and $(1.18 \pm 0.01) \times 10^6\ M^{-1}\ s^{-1}$, respectively. Rate constants for reduction of **1** by the protonated species GSH are more than five orders of magnitude smaller. The mechanism for the reductive elimination reactions of the Pt(IV) compounds is proposed to involve an attack by glutathione on one of the mutually *trans* coordinated chloride ligands, leading to two-electron transfer via a chloride-bridged activated complex. The kinetics results together with literature data indicate that platinum(IV) complexes with a *trans* Cl-Pt-Cl axis are reduced rapidly by glutathione as well as by ascorbate. In agreement with this observation, cytotoxicity profiles for such complexes are very similar to those for the corresponding platinum(II) product complexes. The rapid reduction within 1 s of the platinum(IV) compounds with a *trans* Cl-Pt-Cl axis to their platinum(II) analogs does not seem to support the strategy of using kinetic inertness as a parameter to increase anticancer activity, at least for this class of compounds.

Key words Kinetics and mechanism · Reduction · Platinum(IV) compounds · Anticancer active · Glutathione

Abbreviations *cha*: cyclohexylamine · *dach*: ±-*trans*-1,2-diaminocyclohexane · *GSH*: glutathione · *RSH*: thiol · *Tz*: thiazole

Supplementary material. Tables S1–S7 are available in electronic form on Springer-Verlag's server at http://link.springer.de/journals/jbic/

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Introduction

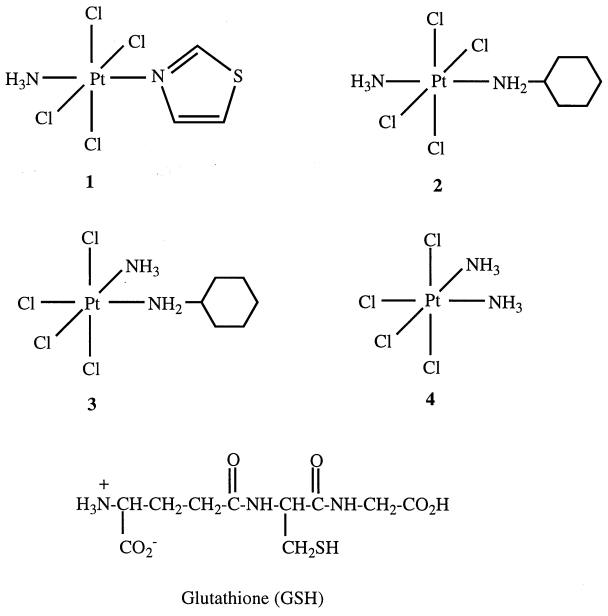
Platinum(IV) compounds have been known to be antitumour active since the discovery of cisplatin in 1969 ([1]; for general reviews on platinum-based antitumour drugs, see [2, 3, 4]). For instance, the second-generation drug *cis,trans,cis*-[$PtCl_2(OH)_2$(i-$PrNH_2)_2$] (iproplatin) is more active than the platinum(II) compounds cisplatin and carboplatin in lung, ovarian, breast, head, and neck cancers and it is not cross-resistant with these drugs, and [$PtCl_4$(dach)] (tetraplatin, dach = ±-*trans*-1,2-diaminocyclohexane) shows superior activity against leukemias [5, 6, 7]. During the last decade, platinum compounds with mixed amines, often

referred to as "third generation" drugs, have been developed. They include platinum(IV) derivatives which are robust enough to survive the gastric environment and which can therefore be administered orally [8]. One of these compounds *cis,trans,cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216, cha = cyclohexylamine) is currently in its phase III clinical trial [9, 10].

More recently, unconventional platinum(II) complexes which seem to violate earlier structure-activity relationships have been investigated [11]. Of special interest is the demonstration that compounds of the type *trans*-$[PtCl_2(NH_3)L]$, where L denotes bulky or sterically demanding amine ligands such as pyridine, quinoline, thiazole, or cyclohexylamine in place of NH_3 in a *trans* geometry, have a cytotoxicity equivalent to their *cis* isomers and indeed in some cases comparable to cisplatin itself [12, 13, 14]. Part of the rationale behind this development is that sterically demanding amine ligands may retard the kinetics of substitution compared to the more rapidly substituted *trans* diammine compounds, giving the platinum complexes sufficient time to enter the cells and interact with DNA. Some factors affecting the DNA binding of these novel agents such as *trans*-$[PtCl_2(NH_3)$(thiazole)] have been discussed recently [15].

A further strategy to retard the kinetics of substitution would be the use of the related platinum(IV) compounds, provided that their redox reactions are slow. Indeed, some *trans* platinum(IV) complexes such as *trans,trans,trans*-$[PtCl_2(OH)_2(cha)(NH_3]$ (JM335) exhibit in vivo activity [14], and substitution reactions of nucleobases at platinum(IV) complexes have been studied [16, 17]. However, many platinum(IV) compounds, including JM335 and JM216, are reduced by biological reductants, in agreement with the assumption that these compounds act as prodrugs to their platinum(II) analogs [18–28]. Direct evidence comes from the detection of platinum(II) compounds as metabolites of iproplatin [29] and the rapid reduction of tetraplatin in tissue culture medium [30]. The reduction potentials of the platinum(IV) complexes depend on the nature of the coordinated ligands [31].

Although most evidence to date indicates that platinum(IV) compounds are reduced to platinum(II) by potential cellular reductants, the reaction mechanism, in particular the relation between the structures of the platinum(IV) complexes and the rates and mechanisms of their reduction, is poorly understood. The kinetics for reduction of a model compound, *trans*-$[PtCl_2(CN)_4]^{2-}$, by thioglycolic acid, cysteine, penicillamine, glutathione, or methionine has been investigated in an acidic medium [23, 24], as well as reduction of *trans,trans,trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (JM335) and tetraplatin by thiols ([25]; T. Shi, L. I. Elding, in preparation), and *cis,trans,cis*-$[PtCl_2(OCOMe)_2(NH_3)_2]$ by cysteine and methionine [26]. Reduction of anticancer-active platinum(IV) compounds by ascorbate has been observed [27] and recent mechanistic studies show that both outer-sphere and bridged reductive elimination processes are possible ([28]; K. Lemma, D. A. House, N. Retta, L. I. Elding, in preparation).



Scheme 1 Structures of compounds used

The principal objective of the work reported here has been to investigate the kinetics and mechanism for reduction of some anticancer-active *cis*- and *trans*-tetrachloroam(m)ine platinum(IV) compounds by glutathione, in order to elucidate the relation between the structure of the complexes and the redox activity. Glutathione, which is present in the cytoplasm at concentrations of 0.5–10 mM [32], is known to affect the biological activity of platinum complexes, including sequestering of DNA adducts [33, 34] and reduction of platinum(IV) species [23, 25]. Structures of the platinum(IV) compounds used and glutathione are shown in Scheme 1.

Materials and methods

Reagents

Compounds **1–4**, prepared by literature methods [14], were received as generous gifts from R. Roat, W. Barklage, and U. Bierbach (Department of Chemistry, Virginia Commonwealth University, Richmond, USA). Glutathione (Merck) was used directly. All other chemicals used were of analytical grade. Sodium chloroacetate, acetate, and phosphate buffers were used to maintain constant pH. They contained 0.2–0.8 M chloride to suppress hydrolysis of the platinum(IV) complexes and 0.5–2 mM $Na_2(H_2edta)$ sufficient to scavenge trace concentrations of transition metals (vide infra). Buffer solutions were deoxygenated by flushing for ca. 30 min with argon. Stock solutions of compounds **2–4** (0.5–1 mM) containing 0.2–0.8 M NaCl were stable to hydrolysis and could be stored for a week in the dark without observable changes. Compound **2** required several hours of sonication to dissolve to a stock solution concentration of ca. 1 mM. Solutions of the platinum(IV) compounds **2–4** used

for kinetic measurements were prepared by diluting 1–2 mL stock solution to 10 mL with buffer and those of **1** by dissolving weighed samples of the solid compound in a minimum amount of water (1 mL) and diluting with buffer to 10 mL. Fresh stock solutions of glutathione (4–30 mM) were used for each kinetic run. A total ionic strength of 1.0 M adjusted with $NaClO_4$ was maintained throughout the kinetics measurements. Water was doubly distilled from quartz.

Physical measurements

The pH of buffer solutions was measured with an Orion Research Expandable Ion Analyzer EA 920 digital pH meter equipped with an Orion combination glass electrode. Standard buffers of pH 4.0 and 7.0 (Merck) were used to calibrate the electrode. Activities of oxonium ions $a_H=10^{-pH}$ were calculated directly from pH meter readings. Spectra were recorded with Milton Roy 3000 diode array and Cary 300 Bio UV/VIS spectrophotometers using 1.00 cm quartz Suprasil cells. Proton NMR spectra were recorded with a Varian Unity 300 spectrometer operating at 299.779 MHz with D_2O used as solvent and the residual solvent signal as the reference at constant pH, ionic medium, and temperature.

Kinetics measurements

The redox reactions were studied at 25 °C by monitoring the absorbance decrease at 280 (**1**) and 240 nm (**2–4**) under pseudo-first-order conditions with at least a 10-fold excess of glutathione using an Applied Photophysics Bio Sequential SX-18MV stopped-flow ASVD spectrofluorimeter. The redox reactions were monitored in the presence of 0.5–2 mM $Na_2(H_2edta)$ in order to avoid autoxidation of glutathione [35, 36, 37] and 0.2–0.8 M chloride to suppress hydrolysis of the platinum(IV) compounds. Under these conditions, single exponential kinetic traces were obtained and the observed pseudo-first-order rate constants k_{obsd} were calculated by an on-line nonlinear least-squares analysis of the absorbance-time data using an Applied Photophysics software package. Reactions were followed for at least 4 half-lives with 4–6 repetitive runs. All kinetics data are summarized in the Supplementary material (Tables S1–S7).

Results and discussion

Stoichiometry

The stoichiometry [Pt(IV)]:[RSH] for reduction of the model compound *trans*-$[PtCl_2(CN)_4]^{2-}$ by a series of thiols has been reported to be 1:2 [23]. A similar spectrophotometric study was not feasible for the present redox reactions because of interference from subsequent slow processes [38, 39, 40]. Glutathione (GSH) is known to react readily with *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ [38, 39]. Unlike reactions with nucleobases, chloride hydrolysis is not a prerequisite and direct substitution of Cl^- by GSH could occur [32, 40]. The reaction conditions chosen here (i.e. pseudo-first-order with excess glutathione) suggest that similar reactions will occur. Proton NMR spectra showed that glutathione is quantitatively oxidized to the disulfide GSSG in a 1:2 Pt(IV):GSH mixture at 25 °C and pH ca. 7, confirming the assumed 1:2 stoichiometry for the present reactions; see Fig. 1.

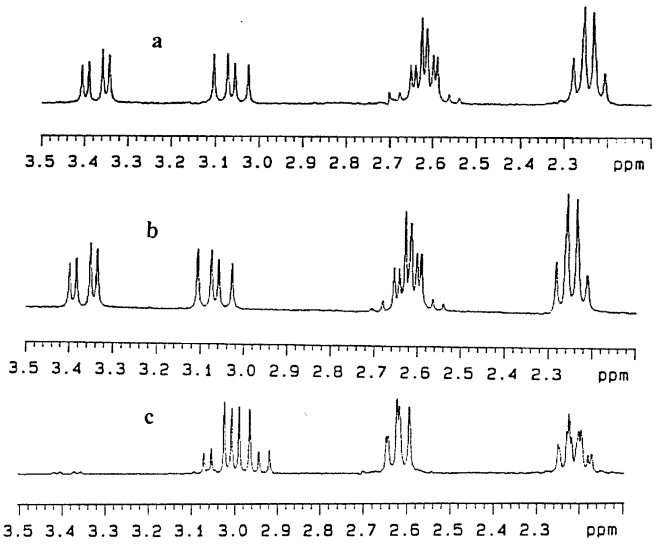


Fig. 1 Proton NMR spectra of **a** 4 mM GSH, **b** 2 mM GSSG, and **c** a mixture of 2 mM *cis*-$[PtCl_4(NH_3)_2]$ (**4**) with 4 mM GSH at 25 °C and pH ca. 7

Kinetics

The pseudo-first-order rate constants are independent of the initial concentrations of the platinum(IV) compounds (Supplementary material, Table S1), which is consistent with a first-order dependence in the platinum(IV) complexes. Plots of k_{obsd} versus $[GSH]_{tot}$ are linear and pass through the origin, indicating that the redox reactions are first order in glutathione as well. Small intercepts were observed for reactions carried out in the absence of $Na_2(H_2edta)$.These intercepts are caused by autoxidation of glutathione which might be catalyzed by trace concentrations of transition metal ions such as Cu(II) and Fe(III) [35, 36, 37]. Addition of Cu^{2+} to reaction mixtures has the effect of increasing the intercept, and addition of $Na_2(H_2edta)$ removes it (Fig. 2). From these observations it is concluded that the reactions follow the second-order

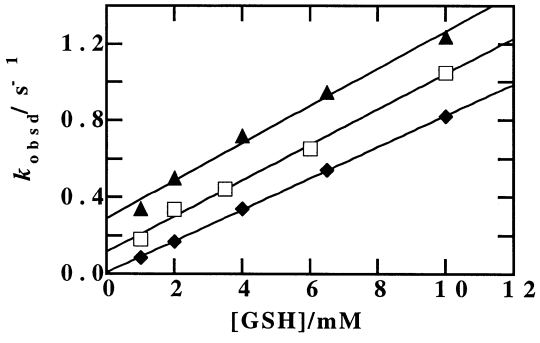
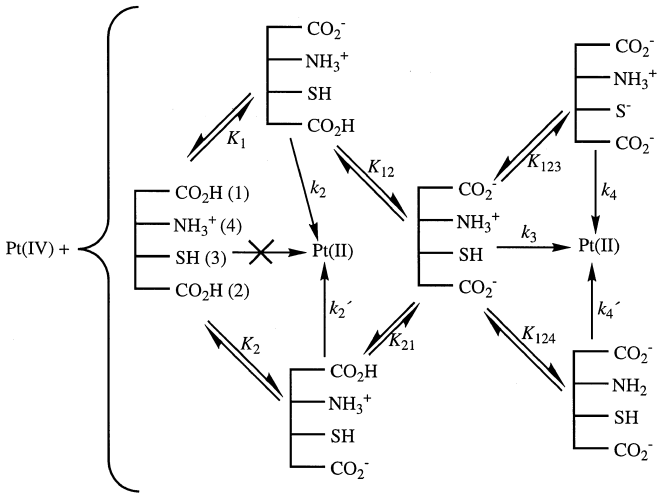


Fig. 2 Effect of Cu^{2+} on the pseudo-first-order rate constant k_{obsd} for the reduction of *cis*-$[PtCl_4(NH_3)_2]$ by glutathione at pH 5.04 and 25 °C. Conditions: no added Cu^{2+}, aerobic (□); $[Cu^{2+}]=1\times10^{-5}$ M, aerobic (▲); $[Cu^{2+}]=1\times10^{-5}$ M, $[Na_2(H_2edta)]=3\times10^{-3}$ M, anaerobic (◆); $[Pt(IV)]_{tot}=1\times10^{-4}$ M in all cases



Scheme 2 Reaction model for reduction of platinum(IV) compounds with glutathione (k rate constants, K protolysis constants)

rate law defined by Eq. 1, where k denotes a pH-dependent second-order overall rate constant and $[GSH]_{tot}$ the total concentration of glutathione:

$$-d[Pt(IV)]/dt = k_{obs}[Pt(IV)] = k[GSH]_{tot}[Pt(IV)] \quad (1)$$

The first-order dependence on $[GSH]_{tot}$ suggests that only one molecule of GSH is involved in the rate-determining step, the second one thus reacting in subsequent rapid steps (vide infra). Since there are no protolytic equilibria associated with the platinum(IV) compounds, the pH dependence of k is ascribed to the displacement of protolytic equilibria involving the various species of glutathione which reduce the platinum(IV) complexes in parallel pathways (see Scheme 2). The second-order rate constants k (Supplementary material, Table S7) at constant pH were obtained from slopes of the plots of k_{obsd} versus $[GSH]_{tot}$. These rate constants increase significantly with increasing pH, indicating that the deprotonated species of glutathione are more reactive than the protonated ones.

Equation 2, which describes the pH dependence of k, was derived from Scheme 2 by assuming that the contribution of the rate term containing k_4' to the overall rate of reduction of the platinum(IV) complexes is insignificant. This assumption is based on the fact that there is a large difference between the reactivity of a protonated thiol (RSH) and thiolate (RS^-) species [23]:

$$k = \frac{(k_2K_1 + k_2'K_2)a_H^2 + k_3K_1K_{12}a_H + k_4K_1K_{12}K_{123}}{a_H^3 + (K_1 + K_2)a_H^2 + K_1K_{12}a_H + K_1K_{12}(K_{123} + K_{124})} \quad (2)$$

Equation 2 was fitted to the experimental data obtained for compound **1** by the method of weighted non-linear least-squares analysis with a_H taken as an independent variable, $(k_2K_1+k_2'K_2)$, k_3, and k_4 as adjustable, and K_1, K_2, K_{12}, K_{21}, K_{123}, and K_{124} as constant parameters. The microscopic acid dissociation constants of glutathione used in Eq. 2 are: $pK_1 = 2.19$; $pK_2 = 3.22$; $pK_{12} = 3.45$; $pK_{21} = 2.42$; $pK_{123} = 8.97$; $pK_{124} = 9.17$ at 25 °C and ionic strength of 0.2–0.55 M [41]. The second-order rate constants k_2, k_2', k_3, and k_4 refer to reduction of the platinum(IV) compounds by the protolytic species of glutathione shown in Scheme 2. The value of $(k_2K_1+k_2'K_2)$, obtained from the curve fitting, is $(4.02 \pm 0.04) \times 10^{-2}$, but k_2 and k_2' cannot be obtained directly. However, since K_1 is more than ten times larger than K_2, the sum $k_2K_1+k_2'K_2$ can be approximated to $k_2K_1 \approx 4.0 \times 10^{-2}$ s^{-1} to yield an approximate value of $k_2 \approx 6$ M^{-1} s^{-1}.

Equation 2 does not fit the experimental data obtained for compounds **2–4**. For these compounds, very good curve fittings are obtained using Eq. 3, which is derived by considering GS^- as the predominant reductant:

$$k = \frac{k_4K_1K_{12}K_{123}}{a_H^3 + (K_1 + K_2)a_H^2 + K_1K_{12}a_H + K_1K_{12}(K_{123} + K_{124})} \quad (3)$$

The curve fittings are displayed in Fig. 3 and the second-order rate constants k_2, k_3, and k_4 derived from them are summarized in Table 1. It can be seen from this table that reductions of *trans*-$[PtCl_4(NH_3)(thiazole)]$ and *trans*-$[PtCl_2(CN)_4]^{2-}$ by GS^- are five orders of magnitude faster than reductions by the protonated species, GSH.

Reaction mechanism

Since platinum(IV) compounds are generally substitution inert [42] and the pseudo-first-order rate constants are independent of added chloride (Supplementary material, Table S2), a substitution-controlled inner-sphere electron transfer reaction is unlikely. Reduction of platinum(IV) halide compounds by anionic reductants is well known to take place through reductive elimination reactions via halide-bridged activated complexes [42–53]. This mechanism involves a reductive attack by the reducing agent on a halide coordinated *trans* to a good leaving group such as halide, and it is formally equivalent to transfer of X^+ (X = Cl, Br) from Pt(IV) to the reducing agent [42–45, 48–53]. The detection of a BrCN intermediate for reduction of *trans*-$[PtBr(CN)_4(OH)]$ by CN^- supports this halide-bridged two-electron transfer mechanism [43]. Electron transfer via reductive attack on chloride ligands coordinated *trans* to the am(m)ine ligands in compounds **3** and **4** does not take place since the am(m)ines are not good leaving groups. The much larger reactivity of GS^- compared to GSH indicates that reduction is initiated by attack of sulfur on a coordinated chloride. This interaction results in for-

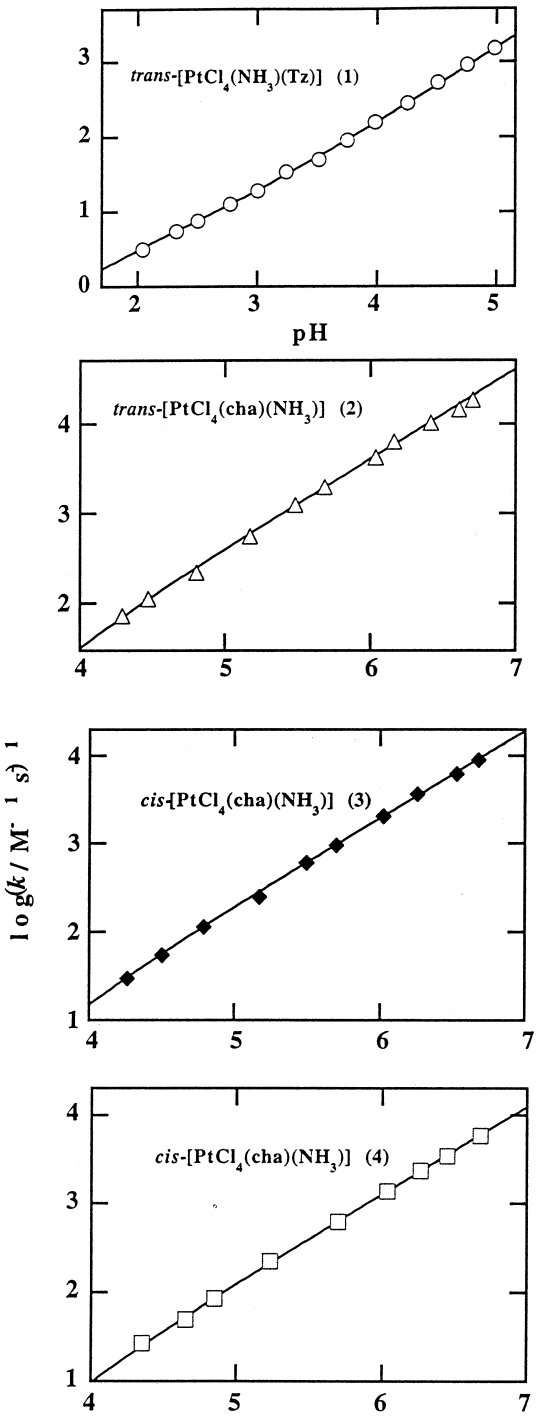


Fig. 3 pH-dependent second-order overall rate constants k plotted against pH. The *solid lines* represent the fits of Eqs. 3 (O) and 4 (▽, ◆, □) to the experimental data

mation of an intermediate GSCl, which is assumed to undergo rapid subsequent hydrolysis and reaction with GS^- and/or GSH as given by Eqs. 4, 5, 6 [54]:

$$GSCl+2H_2O \rightarrow GSOH+Cl^-+H_3O^+ \quad (4)$$

$$GSOH+GS^- \rightarrow GSSG+OH^- \quad (5)$$

$$GSOH+GSH \rightarrow GSSG+H_2O \quad (6)$$

Thus, glutathione reduction of the platinum(IV) compounds used in the present investigation is suggested to follow a reductive elimination mechanism, in which a chloride coordinated *trans* to another chloride is attacked by GS^- and/or GSH. Thiol-induced reduction of the model compound *trans*-$[PtCl_2(CN)_4]^{2-}$ and of JM335 has been proposed to take place by a similar mechanism [23, 25]. Reduction of compounds **2**–**4** by the glutathione species in which both carboxylic groups are deprotonated was not observed. Likewise, protonated thiols, RSH, are not reactive in thiolate-disulfide exchange reactions [55].

The variation of the rate for reduction of the platinum(IV) compounds in Table 1 which have a Cl-Pt-Cl axis demonstrates that the spectator ligands influence the redox properties [31]. Compound **2** is more reactive than **3** only by a factor of two, which reflects the fact that statistically there are four chances for attack of GS^- on a coordinated chloride in **2** and only two in compound **3**. The reduction of compounds **2–4** by GS^- is about two orders of magnitude slower than the reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ by the same species (Table 1). The higher reactivity of the latter complex may be attributed to the π-acceptor property of the cyanide ligands which stabilize the Pt(II) product through backbonding. Compound **1** is less reactive than *trans*-$[PtCl_2(CN)_4]^{2-}$ only by a factor of 10 since the thiazole ligand plays the same role of stabilizing the platinum(II) oxidation state. Noteworthy, reductions of compounds **1–4** by GS^- are up to four orders of magnitude faster than reduction of *trans,trans,trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (JM335) [25], which is reported to be anticancer active in vivo [14]. This large difference in reactivity might be due to stabilization of JM335 by the hydroxide ligands. The in vivo anticancer activity of JM335 may be related to this relatively low rate of reduction. On the other hand, compounds without a Cl-Pt-Cl axis, such as *cis,trans,cis*-$[PtCl_2(OCOMe)_2(cha)(NH_3)]$ (JM216) and *cis,trans,cis*-$[PtCl_2(OCOPr)_2(cha)(NH_3)]$ (JM221), which are also in vivo anticancer active, are reduced relatively slowly by ascorbate (Asc^{2-}), in that case by an outer-sphere mechanism [28]. The reduction of these compounds by glutathione is also relatively slow, but it is complicated by parallel substitution processes in the platinum(II) products [28].

Implication to in vivo reduction

The half-lives for reduction of compounds **1–4** by thiolate GS^- at physiological conditions, i.e. pH 7.4, 37 °C, and a concentration of glutathione of ca. 4 mM, can be roughly estimated from the rate constants in Table 1 to be much shorter than 1 s. This rapid reduction has the implication that the cytotoxicities of the platinum(IV) compounds should be

Table 1 Second-order rate constants for reduction of platinum(IV) compounds by protolytic species of glutathione (see Scheme 2) and ascorbate (Asc^{2-}) at 25 °C and $I = 1.0$ M

Pt(IV) compound	Pt(II) product	k_2 ($M^{-1} s^{-1}$)	k_3 ($M^{-1} s^{-1}$)	k_4 ($M^{-1} s^{-1}$)	k_{Asc}^{2-} ($M^{-1} s^{-1}$)	Ref
trans-$[PtCl_4(NH_3)Tz]$ (**1**)	*trans*-$[PtCl_2(NH_3)Tz]$	6[a]	56.3 ± 0.4	$(1.43 \pm 0.01) \times 10^7$	–	this work
trans-$[PtCl_4(cha)(NH_3)]$ (**2**)	*trans*-$[PtCl_2(cha)(NH_3)]$	not obsd	not obsd	$(3.86 \pm 0.03) \times 10^6$	–	this work
cis-$[PtCl_4(cha)(NH_3)]$ (**3**)	*cis*-$[PtCl_2(cha)(NH_3)]$	not obsd	not obsd	$(1.83 \pm 0.01) \times 10^6$	–	this work
cis-$[PtCl_4(NH_3)_2]$ (**4**)	*cis*-$[PtCl_2(NH_3)_2]$	not obsd	not obsd	$(1.18 \pm 0.01) \times 10^6$	–	this work
	–	–	–	–	$(9.85 \pm 0.01) \times 10^6$	–[d]
trans-$[PtCl_2(CN)_4]^{2-}$	$[Pt(CN)_4]^{2-}$	23.4 ± 0.3	655 ± 4	$(1.10 \pm 0.01) \times 10^8$	–	[23]
JM335[c]	*trans*-$[Pt(OH)_2(cha)(NH_3)]$	–	0.48 ± 0.01	111 ± 1	–	[25]
$[PtCl_4(dach)]$	$[PtCl_2(dach)]$	–	–	$(2.47 \pm 0.03) \times 10^5$		–[e]
JM216[c]	*cis*-$[PtCl_2(cha)(NH_3)]$	–	–	–[b]	672 ± 15	[28]
JM221[c]	*cis*-$[PtCl_2(cha)(NH_3)]$	–	–	–[b]	428 ± 10	[28]

[a]Estimated value (see text)
[b]Reduction by glutathione of JM216 and JM221 has been estimated to occur with a rate similar to ascorbate reduction [28]
[c]JM335: *trans,trans,trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$;
JM216: *cis,trans,cis*-$[PtCl_2(OCOMe)_2(cha)(NH_3)]$;
JM221: *cis,trans,cis*-$[PtCl_2(OCOPr)_2(cha)(NH_3)]$
[d]K. Lemma, D. A. House, N. Retta, L. I. Elding, in preparation
[e]T. Shi, L. I. Elding, in preparation

expected to be very similar to those of the product platinum(II) complexes. In agreement with this conclusion, the cytotoxicity profiles of *trans*-$[PtCl_4(NH_3)(Tz)]$ (**1**, Tz = thiazole) and its platinum(II) analog *trans*-$[PtCl_2(NH_3)(Tz)]$ are similar (U. Bierbach, H. M. Nguyen, M. J. Doedee, T. W. Hambley, N. Farrell, in preparation). The cytotoxicity of these compounds is also similar to those of the cyclohexylamine analogs [14]. Furthermore, the *pattern* of cytotoxicity of the thiazole platinum(IV) and platinum(II) derivatives is very similar to that found for *trans*-$[PtCl_2(py)_2]$, confirming the classification of these new *trans* platinum compounds with planar amine ligands as a discrete group of cytotoxic agents distinct from cisplatin and its analogs [56]. The overall results suggest that the incorporation of planar amines into the Pt(IV) coordination sphere is not likely to retard greatly the substitution reactions in the biological milieu since reduction of all these compounds is rapid anyhow and hence they are expected to behave very similar to their Pt(II) analogs. This is also true for the rapidly reduced tetraplatin, the cytotoxicity of which is reported to be very similar to that of its Pt(II) analogue in the presence of glutathione [30, 57].

The rapid reduction of the platinum(IV) compounds with a *trans* Cl-Pt-Cl axis observed in this study seem not to support the strategy of using kinetic inertness of platinum(IV) as a parameter to increase the anticancer activity, at least for these compounds. Of interest in this context is the fact that there is a wide difference in anticancer activity between *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**) and *trans,trans,trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (JM335), with the latter compound showing some in vivo antitumor activity [14]. The compounds *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**) and *cis*-$[PtCl_4(NH_3)_2]$ (**4**), which are reduced rapidly to *cis*-$[PtCl_2(cha)(NH_3)]$ (JM118) and *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin), respectively, are also active in vivo [11]. Platinum(IV) compounds with two hydroxide ligands *trans* to each other such as in iproplatin and JM335, on the other hand, are reduced much more slowly by ascorbic acid and thiols than the tetraplatin analogs, indicating that hydroxide ligands stabilize the Pt(IV) state for these complexes [58].

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References

1. Rosenberg B, VanCamp L, Trosko JE, Mansour VH (1969) Nature 222:385–386
2. Wong E, Giandomenico CM (1999) Chem Rev 99:2451–2466
3. Reedijk J (1999) Chem Rev 99:2499–2510
4. Gelasco A, Lippard SJ (1999) In: Clarke MJ, Sadler PJ (eds) Topics in biological inorganic chemistry, vol 1. Springer, Berlin Heidelberg New York, pp 73–98
5. Bramwell VHC, Crowther D, O'Malley S, Swindell R, Johnson R, Cooper EH, Thatcher N, Howell A (1985) Cancer Treat Rep 69:409–416
6. Pendyala L, Cowens JW, Chedda GB, Dutta SP, Creaven PJ (1988) Cancer Res 48:3533–3536
7. Rahman A, Roh JK, Wolpert-DeFilippes MK, Goldin A, Venditti JM, Woolley PV (1988) Cancer Res 48:1745–1752
8. Giandomenico CM, Abrams MJ, Murrer BA, Vollano JF, Rheinheimer MI, Wyer SB, Bossard GE, Higgs III JD (1995) Inorg Chem 34:1015–1021
9. Barnard CFJ, Raynaud FI, Kelland LR (1999) In: Clarke MJ, Sadler PJ (eds) Topics in biological inorganic chemistry, vol 1. Springer, Berlin Heidelberg New York, pp 45–71
10. Ferrante K, Winograd B, Canetta R (1999) Cancer Chemother Pharmacol 43:S61–68
11. Farrell N (1996) In: Sigel A, Sigel H (eds) Metal ions in biological systems, vol 32. Dekker, New York, pp 603–639
12. Natile G, Coluccia M (1999) In: Clarke MJ, Sadler PJ (eds) Topics in biological inorganic chemistry, vol 1. Springer, Berlin Heidelberg New York, pp 45–71

13. Van Beusichem M, Farrell N (1992) Inorg Chem 31:634–639
14. Kelland LR, Barnard CFJ, Evans IG, Murrer BA, Theobald BRC, Wyer SB, Goddard PM, Jones M, Valenti M, Bryant A, Rogers PM, Harrap KR (1995) J Med Chem 38:3016–3024
15. Bierbach U, Qu Y, Hambley TW, Peroutka J, Nguyen HL, Doedee M, Farrell N (1999) Inorg Chem 38:3535–3542
16. Roat R, Reedijk J (1993) J Inorg Biochem 52:263–274
17. Roat R, Jerardi MJ, Kopai CB, Heath DC, Clark JA, DeMars JA, Weaver JM, Bezemer E, Reedijk J (1997) J Chem Soc Dalton Trans 3615–3621
18. Rotondo E, Fimiani V, Cavallaro A, Ainis T (1983) Tumori 69:31–36
19. Blatter EE, Vollano JF, Krishnan BS, Dabrowiak JC (1984) Biochemistry 23:4817–4820
20. Eastman A (1987) Biochem Pharmacol 36:4177–4178
21. Pendyala L, Arakali AV, Sansone P, Cowens JW, Creaven PJ (1990) Cancer Chemother Pharmacol 27:248–250
22. Talman EG, Brüning W, Reedijk J, Spek AL, Veldman N (1997) Inorg Chem 36:854–861
23. Shi T, Berglund J, Elding LI (1996) Inorg Chem 35:3498–3503
24. Shi T, Berglund J, Elding LI (1997) J Chem Soc Dalton Trans 2073–2077
25. Lemma K, Shi T, Elding LI (2000) Inorg Chem 39: (in press)
26. Chen L, Lee PF, Ranford JD, Vittal JJ, Wong SY (1999) J Chem Soc Dalton Trans 1209–1212
27. Choi S, Filotto C, Bisanzo M, Delaney S, Lagasee D, Witworth JL, Jusko A, Li C, Wood NA, Willingham J, Schwenker A, Spaulding K (1998) Inorg Chem 37:2500–2504
28. Lemma K, Sargeson AM, Elding LI (2000) J Chem Soc Dalton Trans 1167–1172
29. Pendyala L, Krishnan BS, Walsh JR, Arakali AV, Cowens JW, Creaven PJ (1988) Cancer Chemother Pharmacol 25:10–14
30. Chaney SG, Wyrick S, Till GK (1990) Cancer Res 50:4539–4545
31. Ellis LT, Er HM, Hambley TW (1995) Aust J Chem 48:793–806
32. Lempers ELM, Inagaki K, Reedijk J (1988) Inorg Chim Acta 152:201–207
33. Bancroft DP, Lepre CA, Lippard SJ (1990) J Am Chem Soc 112:6860–6870
34. Eastman A, Barry MA (1987) Biochemistry 26:3303–3307
35. Taylor JE, Yan JF, Wang JI (1966) J Am Chem Soc 88:1663–1667
36. Bridgart GJ, Wilson IR (1973) J Chem Soc Dalton Trans 1281–1284
37. Ehrenberg L, Harms-Ringdahl M, Fedorcak I, Granath F (1989) Acta Chem Scand 43:177–187
38. Appleton TG, Connor JW, Hall JR, Prenzler PD (1989) Inorg Chem 28:2030–2037
39. Berners-Price SJ, Kuchel PW (1990) J Inorg Biochem 38:305–326
40. Djuran MI, Lempers ELM, Reedijk J (1991) Inorg Chem 30:2648–2652
41. Rabenstein DL (1973) J Am Chem Soc 95:2797–2803
42. Mason WR (1972) Coord Chem Rev 7:241–255
43. Wilmarth WK, Fanchiang Y-T, Byrd JE (1983) Coord Chem Rev 51:141–153
44. Chandayot P, Fanchiang Y-T (1985) Inorg Chem 24:3532–3534
45. Chandayot P, Fanchiang Y-T (1985) Inorg Chem 24:3535–3537
46. Peloso A, Dolcetti G, Ettorre R (1967) Inorg Chim Acta 1:403–406
47. Peloso A, Ettorre R, Dolcetti G (1967) Inorg Chim Acta 1:307–310
48. Elding LI, Gustafson L (1976) Inorg Chim Acta 19:165–171
49. Elding LI, Gustafson L (1977) Inorg Chim Acta 24:239–246
50. Drougge L, Elding LI (1986) Inorg Chim Acta 121:175–183
51. Berglund J, Voigt R, Fronaeus S, Elding LI (1994) Inorg Chem 33:3346–3353
52. Shi T, Elding LI (1998) Inorg Chim Acta 282:55–60
53. Wilmarth WK, Dooley MM, Byrd JE (1983) Coord Chem Rev 51:125–139
54. Allison WS (1976) Acc Chem Res 9:293–299
55. Szajewski RP, Whitesides GM (1980) J Am Chem Soc 102:2011–2026
56. Farrell N, Kelland LR, Roberts JD, van Beusichem M (1992) Cancer Res 52:5065–5072
57. Gibbons GR, Wyrick S, Chaney SG (1989) Cancer Res 49:1402–1407
58. Evans DJ, Green M (1987) Inorg Chim Acta 130:183–184