Kinetics and Mechanism for Reduction of the Anticancer Prodrug *trans,trans,trans*-[$PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)$] (JM335) by Thiols

Kelemu Lemma,[†,‡] Tiesheng Shi,[§] and Lars I. Elding*[,†]

Inorganic Chemistry 1, Chemical Center, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden, Department of Chemistry, University of California, Riverside, California 92521, and Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Received November 22, 1999

The reduction of the platinum(IV) prodrug *trans,trans,trans*-[$PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)$] (JM335) by L-cysteine, DL-penicillamine, DL-homocysteine, *N*-acetyl-L-cysteine, 2-mercaptopropanoic acid, 2-mercaptosuccinic acid, and glutathione has been investigated at 25 °C in a 1.0 M aqueous perchlorate medium with $6.8 \leq pH \leq 11.2$ using stopped-flow spectrophotometry. The stoichiometry of Pt(IV):thiol is 1:2, and the redox reactions follow the second-order rate law $-d[Pt(IV)]/dt = k[Pt(IV)][RSH]_{tot}$, where k denotes the pH-dependent second-order rate constant and $[RSH]_{tot}$ the total concentration of thiol. The pH dependence of k is ascribed to parallel reductions of JM335 by the various protolytic species of the thiols, the relative contributions of which change with pH. Electron transfer from thiol (RSH) or thiolate (RS^-) to JM335 is suggested to take place as a reductive elimination process through an attack by sulfur at one of the mutually trans chloride ligands, yielding *trans*-[$Pt(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)$] and RSSR as the reaction products, as confirmed by ^1H NMR. Second-order rate constants for the reduction of JM335 by the various protolytic species of the thiols span more than 3 orders of magnitude. Reduction with RS^- is ~30−2000 times faster than with RSH. The linear correlation $\log(k_{RS^-}) = (0.52 \pm 0.06)pK_{RSH} - (2.8 \pm 0.5)$ is observed, where k_{RS^-} denotes the second-order rate constant for reduction of JM335 by a particular thiolate RS^- and K_{RSH} is the acid dissociation constant for the corresponding thiol RSH. The slope of the linear correlation indicates that the reactivity of the various thiolate species is governed by their proton basicity, and no significant steric effects are observed. The half-life for reduction of JM335 by 6 mM glutathione (40-fold excess) at physiologically relevant conditions of 37 °C and pH 7.30 is 23 s. This implies that JM335, in clinical use, is likely to undergo in vivo reduction by intracellular reducing agents such as glutathione prior to binding to DNA. Reduction results in the immediate formation of a highly reactive platinum(II) species, i.e., the bishydroxo complex in rapid protolytic equilibrium with its aqua form.

Introduction

The clinical efficacy of cisplatin, *cis*-[$PtCl_2(NH_3)_2$], is limited by toxic side effects, in particular a dose-limiting nephrotoxicity, by drug resistance in the tumor cells, and by a narrow range of activity.[1,2] With the second-generation drug carboplatin, *cis*-[Pt(cyclobutane-1,1-dicarboxylate)$(NH_3)_2$], toxicity is reduced, but drug resistance due to reduced platinum accumulation, increased cytoplasmic detoxification, and enhanced DNA−platinum adduct removal still presents limitations.[3] To circumvent these problems, there is a current interest in platinum complexes with a trans geometry,[4−9] in which ammonia is substituted by more sterically demanding ligands such as quinoline, cyclohexylamine, and iminoethers. The rationale behind the design of such complexes is that the structural features are expected to result in a binding to DNA distinct from that of cisplatin.[9,10]

Trans platinum(IV) dihydroxo complexes represent a new group of such drugs. The most widely studied compound of this type is *trans,trans,trans*-[$PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)$] (JM335), which exhibits antitumor activity in both murine and human subcutaneous cell tumor models.[8] The mechanism for the antitumor activity is not entirely known, but JM335 is reported to be efficient in forming interstrand cross-links to DNA and to be able to cause single-strand breaks.[11] In view of the fact that platinum(IV) complexes are coordinatively saturated and generally substitution-inert,[12] reduction of Pt(IV) by intracellular reducing agents, such as glutathione or similar reagents,

* To whom correspondence should be addressed: Professor Lars I. Elding, Inorganic Chemistry 1, Chemical Center, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden. Fax: +46-46-2224439. E-mail: LarsI.Elding@inorg.lu.se.

† Lund University.

‡ Addis Ababa University.

§ University of California.

(1) For recent general reviews on platinum-based antitumor drugs, see, for example: (a) Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451. (b) Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467. (c) Reedijk, J. *Chem. Rev.* **1999**, *99*, 2467.

(2) von Hoff, D. D.; Schilsky, R.; Reichert, C. M.; Reddick, R. L.; Rozencweig, M.; Young, R. C.; Muggia, F. M. *Cancer Treat. Rep.* **1979**, *63*, 1527.

(3) Kelland, L. R. *Crit. Rev. Oncol. Hematol.* **1993**, *15*, 191.

(4) Farrell, N.; Ha, T. T. B.; Souchard, J. P.; Wimmer, F. L.; Cros, S.; Johnson, N. P. *J. Med. Chem.* **1989**, *32*, 2240.

(5) Van Beusichem, M.; Farrell, N. *Inorg. Chem.* **1992**, *31*, 634.

(6) Farrell, N.; Kelland, L. R.; Roberts, J. D.; van Beusichem, M. *Cancer Res.* **1992**, *52*, 5065.

(7) Coluccia, M.; Nassi, A.; Loseto, F.; Boccareli, A.; Mariggio, M. A.; Giordano, D.; Intini, F. P.; Caputo, P.; Natile, G. A. *J. Med. Chem.* **1993**, *36*, 510.

(8) Kelland, L. R.; Barnard, C. F. J.; Mellish, K. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Bryant, A.; Murrer, B. A.; Harrap, K. R. *Cancer Res.* **1994**, *54*, 5618.

(9) Natile, G.; Coluccia, M. *Top. Biol. Inorg. Chem.* **1999**, *1*, 73.

(10) Farrell, N. *Met. Ions Biol. Syst.* **1996**, *32*, 603.

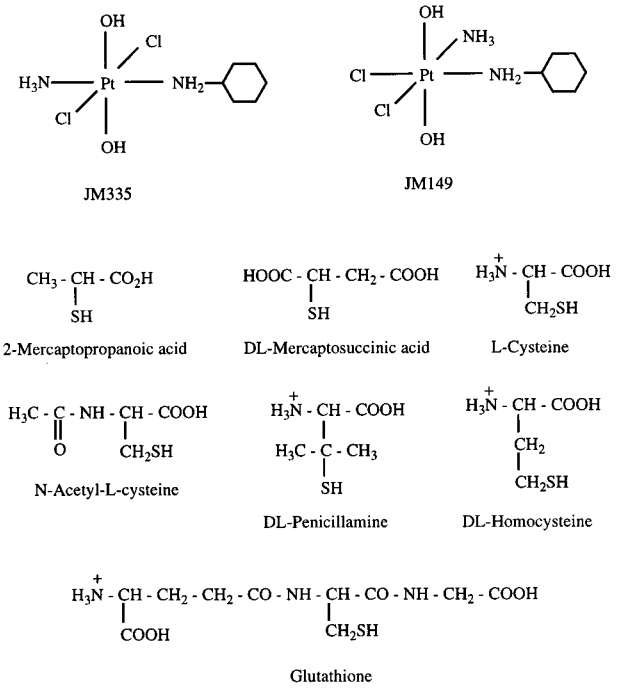
(11) Mellish, K. J.; Barnard, C. F. J.; Murrer, B. A.; Kelland, L. R. *Int. J. Cancer* **1995**, *62*, 717.

10.1021/ic991351l CCC: $19.00
Published on Web 03/25/2000

to the more reactive Pt(II) analogue is assumed to take place before binding to DNA. For example, reduction of JM335 by glutathione has been observed,[13] and *cis*-[$PtCl_2$(*c*-$C_6H_{11}NH_2$)-(NH_3)] (JM118) has been identified as the major metabolite in samples from patients treated with *cis*,*trans*,*cis*-[$PtCl_2(OAc)_2$-(*c*-$C_6H_{11}NH_2$)(NH_3)] (JM216).[14] Furthermore, it has been concluded from DNA unwinding experiments that Pt(IV) complexes do not form bifunctional adducts with DNA unless they are reduced to divalent species after addition of glutathione.[15,16]

A knowledge of the kinetics and mechanism for reduction of Pt(IV) compounds by biologically relevant reductants is thus important for an understanding of the mechanism of activity in vivo and for the design of new drugs. It has been shown that reduction of the model compound *trans*-$[PtCl_2(CN)_4]^{2-}$ by biologically relevant thiols is rapid already in acidic media,[17] and mechanisms for reduction of platinum(IV) complexes by cysteine and methionine,[18] ascorbate,[19,20] and glutathione[21] have been studied recently. We report here the kinetics and mechanism for reduction of JM335 by thiols of disparate electronic and steric properties. Reduction of the cis congener of JM335, viz., *cis*,*trans*,*cis*-[$PtCl_2(OH)_2$(*c*-$C_6H_{11}NH_2$)(NH_3)] (JM149), was not observed under the conditions used for JM335. This is an interesting observation as JM335 is reported to exhibit an in vitro cytotoxicity against human ovarian carcinoma cells greater than that of JM149.[8] Structures of JM335 and JM149 and the protonated forms of the thiols used are shown in Chart 1.

Chart 1



Experimental Section

Chemicals. JM335 and JM149 were kindly supplied as a loan by the Johnson Matthey Technology Centre (Reading, Berkshire, U.K.). JM334 was a generous gift from Dr. Nicholas Farrell. L-Cysteine (ICN Biomedicals Inc.), glutathione (Merck), 2-mercaptopropanoic acid (Acros), DL-homocysteine (Sigma), DL-penicillamine (Janssen), DL-mercaptosuccinic acid (Acros), and *N*-acetyl-L-cysteine (Janssen) were used as received. The quality of the thiols was assessed with Ellman's reagent.[22,23] All other chemicals used were of analytical grade. TRIS-HCl, HCO_3^-/CO_3^{2-}, and HPO_4^{2-}/PO_4^{3-} buffers containing 2−3 mM Na_2H_2(edta) and 100 mM sodium chloride were used to maintain constant pH in the studied region where $6.80 \le pH \le 11.22$. Water was doubly distilled from quartz.

Physical Measurements. The pH of the buffers was measured at 25 °C with a Metrohm 632 digital pH meter equipped with a combination glass electrode. Standard buffers of pH 7.0 and 9.0, obtained from Merck, were used to calibrate the electrode. Oxonium ion activities $a_H = 10^{-pH}$ were obtained directly from the pH meter readings. UV/Vis 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 on a Varian Unity 300 MHz spectrometer with D_2O as the solvent and with the residual solvent signal as the reference at constant temperature, pH, and ionic medium.

The compound *trans*-[$Pt(OD)_2$(*c*-$C_6H_{11}NH_2$)(NH_3)] was prepared by treating a solution of 2 mM *trans*-[$PtCl_2$(*c*-$C_6H_{11}NH_2$)(NH_3)] (JM334) in D_2O with 2 equiv of silver nitrate at room temperature for 24 h. After removal of the AgCl precipitate by centrifugation, the pH of the solution was raised to ∼7, and the proton NMR spectrum was recorded (Figure 2c below).

Kinetic Measurements. Reduction of JM335 by the thiols was investigated by UV/VIS spectrophotometry using an Applied Photophysics Bio-Sequential SX-18MV stopped-flow ASVD spectrophotometer. Kinetic measurements were made at 25 °C over the region $6.80 \le pH \le 11.22$. The pH of stock solutions of the thiols was tested and adjusted by addition of a few drops of strong base when different from the desired value. Na_2H_2(edta) (2−3 mM) was present in all buffers in order to sequester trace transition metal ions such as Cu(II) and Fe(III) that could catalyze autoxidation of the thiols.[24−27] Buffer solutions were flushed with argon for ∼30 min before use in order to remove dissolved oxygen. Fresh stock solutions of the thiols (5−40 mM) prepared in buffer were used for each kinetic run. Sample solutions of JM335 were prepared by diluting 1.0−1.5 mL of stock solutions (2 mM JM335 in 100 mM sodium chloride) with buffer to 10 mL. The ionic strength of all solutions was adjusted to 1.00 M with $NaClO_4$. All kinetic measurements were performed under pseudo-first-order conditions with excess thiol by monitoring the decrease in absorbance at 272 nm where the thiols and reaction products are practically transparent. Reactions were followed for at least 4 half-lives with 4−6 repetitive runs. Single-exponential kinetic traces were obtained in all cases. Pseudo-first-order rate constants k_{obsd} were obtained from an online nonlinear least-squares analysis of the absorbance−time data using an Applied Photophysics software package.[28]

(12) Roundhill, D. M. *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon Press: Oxford, U.K., 1987; Vol. 5, p 351.
(13) Talman, E. G.; Bruning, W.; Reedijk, J.; Spek, A. L.; Veldman, N. *Inorg. Chem.* **1997**, *36*, 854.
(14) Poon, G. K.; Raynaud, F. I.; Mistry, P.; Odell, D. E.; Kelland, L. R.; Harrap, K. R.; Barnard, C. F. J.; Murrer, B. A. *J. Chromatogr., A* **1995**, *712*, 61.
(15) Keck, M. V.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 3386.
(16) Ellis, L. T.; Er, H. M.; Hambley, T. W. *Aust. J. Chem.* **1995**, *48*, 793.
(17) Shi, T.; Berglund, J.; Elding, L. I. *Inorg. Chem.* **1996**, *35*, 3498.
(18) (a) Chen, L.; Lee, P. F.; Ranford, J. D.; Vittal, J. J.; Wong, S. Y. *J. Chem. Soc., Dalton Trans.* **1999**, 1209. (b) Shi, T.; Berglund, J.; Elding, L. I. *J. Chem. Soc., Dalton Trans.* **1997**, 2073.
(19) Choi, S.; Filotto, C.; Bisanzo, M.; Delaney, S.; Lagasee, D.; Witworth, J. L.; Jusko, A.; Li, C.; Wood, N. A.; Willingham, J.; Schwenker, A.; Spaulding, K. *Inorg. Chem.* **1998**, *37*, 2500.
(20) Lemma, K.; Sargeson, A. M.; Elding, L. I. *J. Chem. Soc., Dalton Trans.* **2000**, 1167.
(21) Lemma, K.; Berglund, J.; Farrell, N.; Elding, L. I. *J. Biol. Inorg. Chem.* **2000**, *5*, in press.
(22) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.
(23) Riddles, P. W.; Blakeley, R. L.; Zerner, B. *Anal. Biochem.* **1979**, *94*, 75.
(24) Taylor, J. E.; Yan, J. F.; Wang, J.-L. *J. Am. Chem. Soc.* **1966**, *88*, 1663.
(25) Bridgart, G. J.; Wilson, I. R. *J. Chem. Soc., Dalton Trans.* **1973**, 1281.
(26) York, M. J.; Beilharz, G. R.; Kuchel, P. W. *Int. J. Pept. Protein Res.* **1987**, *29*, 638.
(27) Ehrenberg, L.; Harms-Ringdahl, M.; Fedorcak, I.; Granath, F. *Acta Chem. Scand.* **1989**, *43*, 177.

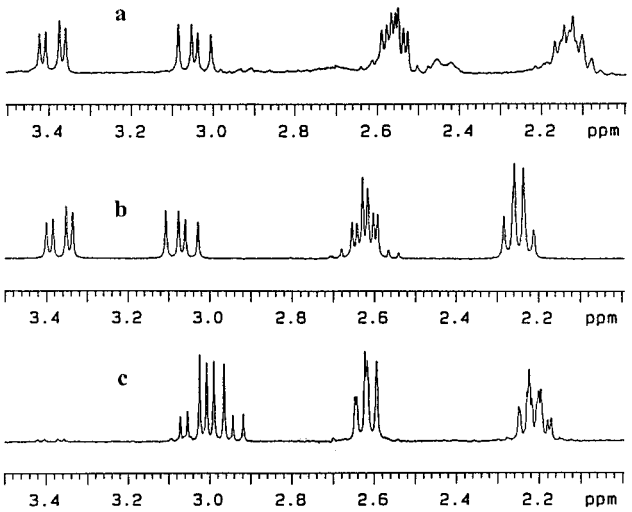


Figure 1. Proton NMR spectra at 25 °C of (a) the oxidation product for the reaction between 2 mM JM335 and 4 mM glutathione (GSH) in a 1:2 JM335:GSH molar mixture at pH ~9, (b) 2 mM GSSG at pH ~5, and (c) 4 mM glutathione at pH ~9. Oxidation of GSH to GSSH is confirmed by a disappearance of the multiplet peak at 3 ppm assigned to the Cys-βCH_2 group of GSH (c), and the apperance of the doublet of doublets at 3.06 and 3.36 ppm for GSSG (a and b).

Results and Discussion

Stoichiometry. The stoichiometry of [Pt(IV)]:[RSH] for reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ by a series of thiols has been established to be 1:2.[17] An analogous spectrophotometric determination is not feasible in the present cases because of interference from slow subsequent substitution processes at the Pt(II) product.[29−32] However, glutathione was quantitatively oxidized to the disulfide GSSG in a JM335:GSH 1:2 molar mixture at 25 °C and pH ~9, consistent with the assumed 1:2 stoichiometry in the present cases (cf. spectra in Figure 1). Proton NMR spectra of reactants and products (Figure 2) show that JM335 was reduced to *trans*-$[Pt(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$.

Kinetics. Observed rate constants are independent of the initial concentration of Pt(IV), and the plots of k_{obsd} vs $[RSH]_{tot}$ at constant pH are linear with zero intercept. Figure 3 shows four examples of such plots. Thus, the redox reactions are first-order with respect to both JM335 and thiol, according to the experimental rate law defined by eq 1.

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

The pH-dependent second-order overall rate constants k, obtained as slopes of plots of k_{obsd} vs $[RSH]_{tot}$, are summarized in Supporting Information Table S1. The fact that the redox reactions are first-order with respect to $[RSH]_{tot}$ implies that the second molecule/ion of the thiols reacts in a subsequent rapid step (vide infra). The pH dependence of k is attributed to the displacement of protolytic equilibria involving the various anionic species of the thiols, cf. Schemes 1−4. JM335 is not expected to be involved in such equilibria in the pH region studied.

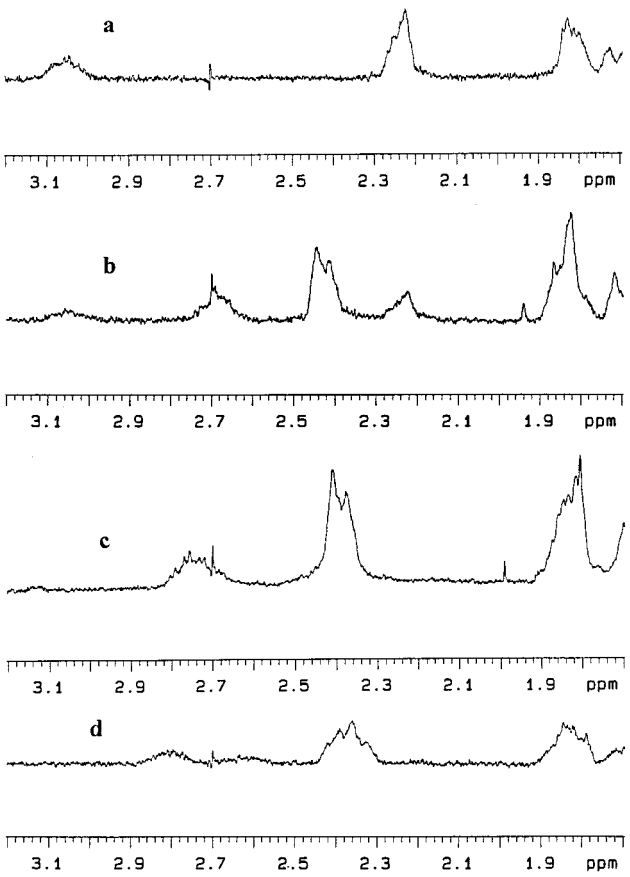


Figure 2. Proton NMR spectra at 25 °C of (a) JM335, (b) reaction product for the redox reaction between JM335 (in slight excess) and penicillamine at pH ~9, (c) *trans*-$[Pt(OD)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ at pH ~7, and (d) *trans*-$[PtCl_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$. The two broad bands at ~2.4 and 1.8 ppm in spectra b−d due to the protons of the cyclohexylamine moiety are largely different for the bishydroxo (c) and bischloro (d) complexes. The peak in the spectrum of the product mixture (b) at ~2.22 ppm is due to residual Pt(IV). The similarity between spectra b and c indicates that the bishydroxo complex is the reaction product.

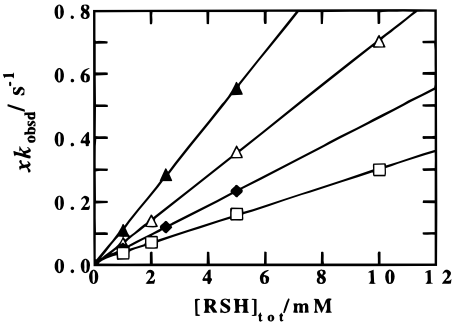


Figure 3. Plots of xk_{obsd} as a function of $[RSH]_{tot}$ for the reduction of JM335 by *N*-acetyl-L-cysteine (▲, $x = 1.25$), L-cysteine (△, $x = 1$), 2-mercaptopropanoic acid (◆, $x = 0.6$), and DL-penicillamine (□, $x = 1$) at pH 9.52 and 25 °C.

The anionic species of the thiols reduce JM335 in parallel reactions in which the contribution of each pathway to the overall reduction depends on the relative concentration and reducing power of the various thiol species. Only those protolytic species whose concentrations are significant in the pH region 6.8−11.2 are included in the schemes. Second-order rate constants k_i ($i = 2$, 3, 4, or 5) were calculated by least-squares fitting of equations derived from Schemes 1−4 to the experimental data using acid dissociation constants from the

(28) Applied Photophysics Bio Sequential SX-18 MV, Sequential Stopped-Flow ASVD Spectrofluorimeter software, Applied Photophysics Ltd., Leatherhead, Surrey, U.K.

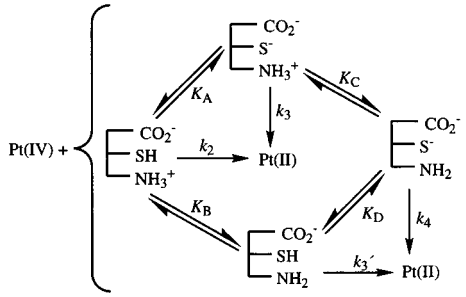
(29) *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ are known to undergo substitution reactions with cysteine and glutathione.[30−32] The reaction conditions chosen here (i.e., pseudo-first-order with excess of thiol) suggest that similar reactions will occur.

(30) Appleton, T. G.; Connor, J. W.; Hall, J. R.; Prenzler, P. D. *Inorg. Chem.* **1989**, *28*, 2030.

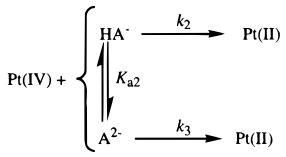
(31) Berners-Price, S. J.; Kuchel, P. W. *J. Inorg. Biochem.* **1990**, *38*, 305.

(32) Odenheimer, B.; Wolf, W. *Inorg. Chim. Acta* **1982**, L41−L43.

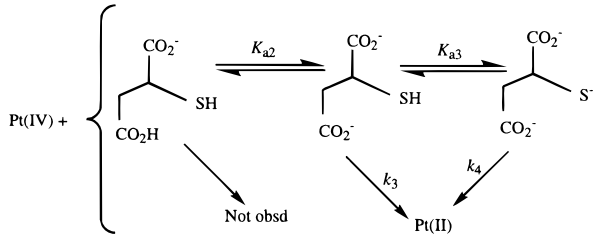
Scheme 1. Model for Reduction of JM335 by Cysteine, Penicillamine, and Homocysteine



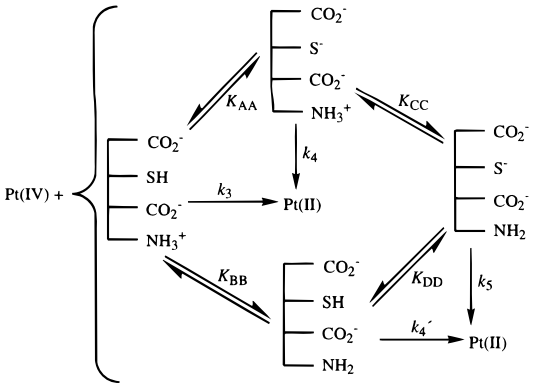
Scheme 2. Model for Reduction of JM335 by *N*-Acetyl-L-cysteine and 2-Mercaptopropanoic Acid



Scheme 3. Model for Reduction of JM335 by Mercaptosuccinic Acid



Scheme 4. Model for Reduction of JM335 by Glutathione



literature as constants (cf. Appendix).[33−37] The pH profiles, i.e., the plots of k vs pH, for all of the thiols used are displayed in Figures 4−6, and the derived second-order rate constants k_i are summarized in Table 1.

Reaction Mechanism. Because platinum(IV) compounds are generally substitution-inert,[38] electron transfer by a substitution-controlled inner-sphere mechanism is unlikely. Previous mechanistic studies on reductions of platinum(IV) halide complexes by inorganic[39−43] and biological[17−21] reductants have shown that

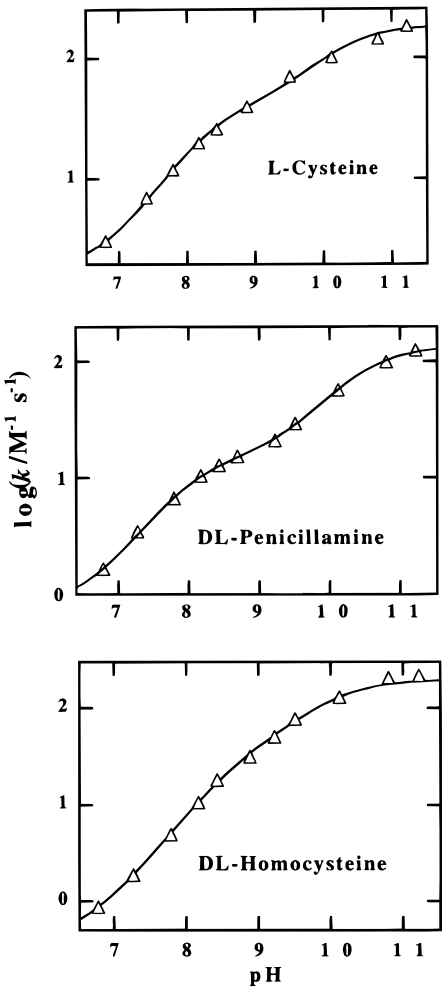


Figure 4. Plots of the second-order rate constants k as a function of pH for the reduction of JM335 with L-cysteine, DL-penicillamine, and DL-homocysteine. The solid lines represent the fits of eq 6 to experimental data.

electron transfer involves reductive elimination through nucleophilic attack by the reductant on a halide coordinated trans to a good leaving group. Reductive elimination reactions of platinum(IV) compounds via the halide-bridged activated complex are formally equivalent to a transfer of X^+ (X = Cl, Br) from the oxidizing Pt(IV) center to the reducing nucleophile, followed by loss of the trans ligand.[17,18b,39−43] The detection of a BrCN intermediate for reduction of *trans*-$[PtBr(CN)_4(OH)]^{2-}$ by CN^- has been presented as strong support for such a mechanism.[39] Reduction of biscarboxylato platinum(IV) complexes by methionine and cysteine was recently proposed to take place by a similar reductive elimination mechanism.[18a] For these trans biscarboxylato complexes, however, an outer-sphere reaction might also be feasible.[20]

An important question regarding the detailed mechanism for reduction of JM335 is whether the reaction takes place via attack

(33) Connet, P. H.; Wetterhahn, K. E. *J. Am. Chem. Soc.* **1986**, *108*, 1842.
(34) Reid, R. S.; Rabenstein, D. L. *Can. J. Chem.* **1981**, *59*, 1505.
(35) Reuben, D. M. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 114.
(36) Smith, R. M.; Martell, A. E. *Critical Stability Constants − Other Organic Ligands*; Plenum Press: New York, 1977.
(37) Rabenstein, D. L. *J. Am. Chem. Soc.* **1973**, *95*, 2797.
(38) Mason, W. R. *Coord. Chem. Rev.* **1972**, *7*, 241.
(39) Wilmarth, W. K.; Fanchiang, Y.-T.; Byrd, J. E. *Coord. Chem. Rev.* **1983**, *51*, 141.
(40) Wilmarth, W. K.; Dooly, M. M.; Byrd, J. E. *Coord. Chem. Rev.* **1983**, *51*, 125.
(41) Chandayot, P.; Fanchiang, Y.-T. *Inorg. Chem.* **1985**, *24*, 3532.
(42) Chandayot, P.; Fanchiang, Y.-T. *Inorg. Chem.* **1985**, *24*, 3535.
(43) (a) Elding, L. I.; Gustafson, L. *Inorg. Chim. Acta* **1976**, *19*, 165. (b) Elding, L. I.; Gustafson, L. *Inorg. Chim. Acta* **1977**, *24*, 239. (c) Drougge, L.; Elding, L. I. *Inorg. Chim. Acta* **1986**, *121*, 175. (d) Berglund, J.; Voigt, R.; Fronaeus, S.; Elding, L. I. *Inorg. Chem.* **1994**, *33*, 3346. (e) Shi, T.; Elding, L. I. *Inorg. Chim. Acta* **1998**, *282*, 55.

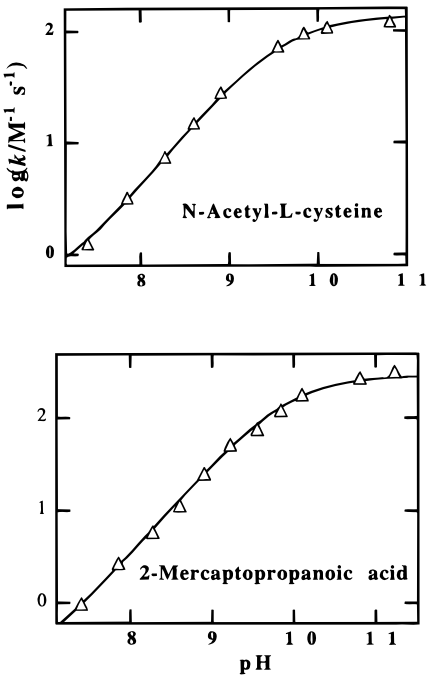


Figure 5. Plots of the second-order rate constants k as a function of pH for the reduction of JM335 with *N*-acetyl-L-cysteine and 2-mercaptopropanoic acid. The solid lines represent the fits of eq 7 to the experimental data.

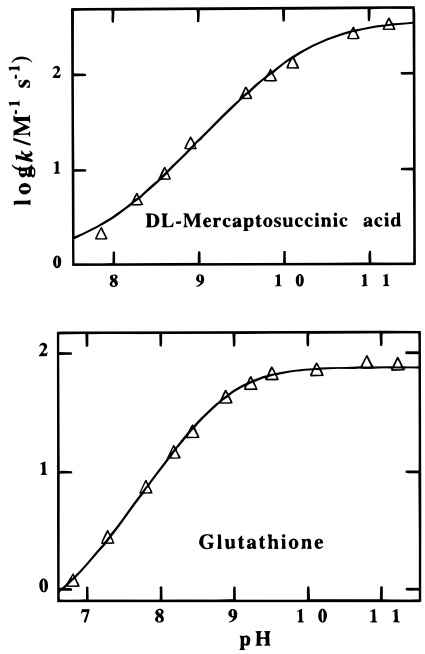


Figure 6. Plots of the second-order rate constants k as a function of pH for the reduction of JM335 with DL-mercaptosuccinic acid and glutathione. The solid lines represent the fits of eqs 8 and 9 to the experimental data for DL-mercaptosuccinic acid and glutathione, respectively.

on chloride or on hydroxide. The ammonia and the cyclohexylamine are not good bridging or leaving ligands.[38] Although hydroxide as well as chloride is capable of bridging, electron transfer is expected to take place by a reductive attack by thiol or thiolate on the chloride, as it is much easier to produce Cl^+ than OH^+ thermodynamically. Thus, reduction of JM335 is expected to take place via a chloride-bridged activated complex of the type shown in Chart 2. This conclusion is confirmed by

Chart 2

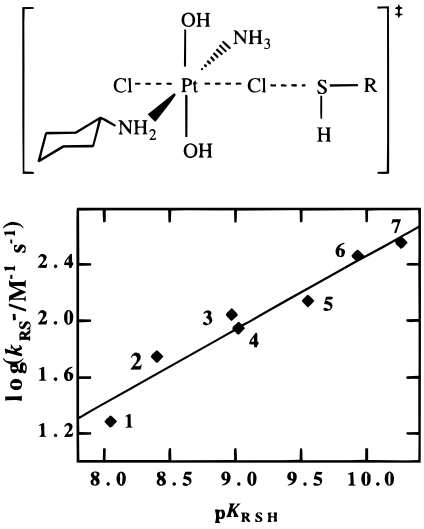


Figure 7. Correlation between the second-order rate constants k_{RS^-} for the reduction of JM335 by thiolates RS^- and the acid dissociation constants K_{RSH}. The numbers 1−7 denote the thiolates of DL-penicillamine, L-cysteine, glutathione, DL-homocysteine, *N*-acetyl-L-cysteine, 2-mercaptopropionic acid, and DL-mercaptosuccinic acid, respectively.

the product analysis, which shows that the trans bishydroxo complex is the reduction product. Further characterization of the product by ^{195}Pt NMR is not possible because of interference from subsequent processes, presumably substitution of the hydroxide/aqua ligands of the platinum(II) product by the excess thiol.

It is noteworthy that the cis, trans, cis isomer, JM149, which has been reported to have a cytotoxicity lower than that of JM335,[8] does not undergo observable reduction under the conditions used for reduction of JM335. The chloride ligands in JM149 are coordinated trans to the strongly bound ammonia and cyclohexylamine ligands (cf. Chart 1), and therefore, the reductive elimination pathway via attack on chloride is energetically unfavorable. However, thiol reduction of JM149 may occur on a much longer time scale than that observed for JM335. Thus, Sadler and co-workers recently observed thiol reduction of *cis,trans,cis*-$[Pt(en)(OH)_2I_2]$.[44,45]

The intermediate oxidation products, RSCl, undergo the rapid subsequent reactions 2−4, leading to the final products RSSR (cf. Figure 1).[46]

$$RSCl + H_2O \rightarrow RSOH + Cl^- + H^+ \quad (2)$$

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (3)$$

$$RSOH + RS^- \rightarrow RSSR + OH^- \quad (4)$$

The fact that the thiols attack coordinated chloride through the sulfur atom is inferred from the large difference between the reactivity of the thiols (RSH) and that of the thiolates (RS^-); deprotonation of the thiol group −SH increases the reduction rate by a factor between ∼30 and more than 2000 (Table 1). Other substituents affecting the electron distribution at the sulfur

(44) Kratochwil, N. A.; Guo, Z.; del Socco Murdoch, P.; Parkinson, J. A.; Bednarski, P. J.; Sadler, P. J. *J. Am. Chem. Soc.* **1998**, *120*, 8253.
(45) Kratochwil, N. A.; Ivanov, A. I.; Patriarca, M.; Parkinson, J. A.; Gouldsworthy, A. M.; del Socco Murdoch, P.; Sadler, P. J. *J. Am. Chem. Soc.* **1999**, *121*, 8193.
(46) Allison, W. S. *Acc. Chem. Res.* **1976**, *9*, 293.

Table 1. Second-Order Rate Constants k_i for Reduction of JM335 by Protolytic Species of the Thiols at 25 °C and an Ionic Strength of 1.00 M

thiol species	k_i, M^{-1} s^{-1}
$HSCH_2CH(NH_3^+)CO_2^-$	k_2, 1.77 ± 0.03
$^-SCH_2CH(NH_3^+)CO_2^-$	k_3, 56.4 ± 0.3
$^-SCH_2CH(NH_2)CO_2^-$	k_4, 184 ± 1
$HSCH_2CH_2CH(NH_3^+)CO_2^-$	k_2, 0.37 ± 0.03
$^-SCH_2CH_2CH(NH_3^+)CO_2^-$	k_3, 88.5 ± 0.4
$^-SCH_2CH_2CH(NH_2)CO_2^-$	k_4, 197 ± 1
$HSC(CH_3)_2CH(NH_3^+)CO_2^-$	k_2, 0.74 ± 0.01
$^-SC(CH_3)_2CH(NH_3^+)CO_2^-$	k_3, 19.3 ± 0.1
$^-SC(CH_3)_2CH(NH_2)CO_2^-$	k_4, 136 ± 1
$HSCH_2CH(CO_2^-)NHCOCH_3$	k_2, 0.40 ± 0.02
$^-SCH_2CH(CO_2^-)NHCOCH_3$	k_3, 138.6 ± 0.7
$HS(CH_3)CHCO_2^-$	k_2, 0.122 ± 0.006
$^-S(CH_3)CHCO_2^-$	k_3, 290 ± 1
$^-O_2CCH_2CH(SH)CO_2^-$	k_3, 1.283 ± 0.013
$^-O_2CCH_2CH(S^-)CO_2^-$	k_4, 362 ± 1
$^-O_2CCH(NH_3^+)(CH_2)_2CONHCH(CH_2SH)CONHCH_2CO_2^-$	k_3, 0.48 ± 0.01
$^-O_2CCH(NH_3^+)(CH_2)_2CONHCH(CH_2S^-)CONHCH_2CO_2^-$	k_4, 111 ± 1
$^-O_2CCH(NH_2)(CH_2)_2CONHCH(CH_2S^-)CONHCH_2CO_2^-$	k_5, 76.0 ± 0.4

atom are also expected to have a large influence on the reactivities. On the other hand, steric factors and deprotonation of the carboxylic acid groups appear to have little effect on the rates.

The kinetics data in Table 1 fit well to the Brønsted correlation defined by eq 5, where k_{RS^-} denotes the second-order rate constant for reduction of JM335 by a thiolate species RS^- and K_{RSH} is the acid dissociation constant for the corresponding thiol. It can be seen from Figure 7 that the reactivity of the thiolate species is directly related to their proton basicities

$$\log k_{RS^-} = (0.52 \pm 0.06)\mathrm{p}K_{RSH} - (2.8 \pm 0.5) \qquad (5)$$

and that glutathione, which is a bulky thiol, and penicillamine, which has a much larger cone angle,[47] do not deviate significantly from the correlation. This is in line with a mechanism in which the thiols attack a coordinated ligand rather the Pt(IV) center where steric effects are expected to be much more significant.

It is also noteworthy that the reduction of JM335 by thiols is several orders of magnitude slower than that of *trans*-$[PtCl_2(CN)_4]^{2-}$.[17] This large difference can be attributed to stabilization of JM335 in the tetravalent state by the hydroxo ligands,[48] as well as to stabilization of the platinum(II) product formed from the platinum(IV) cyanide complex by the π-acceptor properties of the cyanide ligands.

Conclusion

The present results show that reduction of JM335 by biologically relevant thiols in a moderately alkaline aqueous perchlorate medium is fairly rapid. The half-life for reduction by 6 mM glutathione (40-fold excess) at physiologically relevant conditions of pH 7.30 and 37 °C was determined to be 23 s (Supporting Information Table S1). Thus, it is likely that JM335 undergoes rapid in vivo reduction by intracellular reducing agents before binding to DNA. Glutathione appears to be the most likely reductant in the cytoplasm as its concentration ranges from 5 to 10 mM.[49] JM335 exhibits in vivo anticancer activity,[8] whereas *trans*-$[PtCl_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM334), which has been presumed to be the reduction product, is inactive.[11,50] Because the present experiments show that reduction of JM335 results in formation of *trans*-$[Pt(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$, and not formation of the trans bischloro complex as assumed,[11,50] a study of the clinical properties of the trans bishydroxo platinum(II) complex is highly desirable, in particular because this compound is in rapid protolytic equilibrium with highly reactive aqua complexes (pK_a values for the analogous *trans*-$[Pt(H_2O)_2(NH_3)_2]$ complex are 4.35 and 4.70 at 298 K).[51]

Acknowledgment. Financial support from the Swedish National Science Research Council (NFR) and a grant for K.L. from the Swedish International Development Cooperation Agency (SIDA) within the SAERC sandwich program are gratefully acknowledged. We thank Johnson Matthey Technology Centre and Dr. C. F. J. Barnard for the generous loan of the compounds JM149 and JM335, Professor N. Farrell for a loan of *trans*-$[PtCl_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$, and Professor A. M. Sargeson for constructive criticism of the manuscript.

Supporting Information Available: Second-order rate constants k as a function of pH for reduction of JM335 by thiols at 25 °C and an ionic strength of 1.00 M (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

Appendix: Calculation of Rate Constants

Equation 6, derived from Scheme 1, was fit to the experimental data for reduction of JM335 with cysteine, penicillamine, and homocysteine. The contribution of the term containing k_3' to the overall rate of reduction is assumed to be insignificant compared to that of the term containing k_3 on the basis of the fact that the thiolate (RS^-) is much more reactive than the protonated species (RSH).[17]

$$k = \frac{k_2 a_H^{\,2} + k_3 K_A a_H + k_4 K_A K_C}{a_H^{\,2} + (K_A + K_B) a_H + K_A K_C} \qquad (6)$$

In eq 6, K_A, K_B, and K_C are the microscopic acid dissociation constants of the thiols corresponding to the reported values of $pK_A = 8.40$, $pK_B = 8.85$, and $pK_C = 10.05$ at 25 °C for cysteine;[33,34] $pK_A = 8.05$, $pK_B = 8.61$, and $pK_C = 10.29$ at 25 °C for penicillamine;[33,34] and $pK_A = 9.02$, $pK_B = 9.04$, and $pK_C = 9.71$ at 30 °C for homocysteine.[35]

Equation 7, derived from Scheme 2, was fit to the data obtained for reduction with the diprotic thiols *N*-acetyl-L-cysteine and 2-mercaptopropanoic acid. The parameter K_{a2} denotes the acid dissociation constant for the sulfhydryl protons of the thiols. The values for K_{a2} used in eq 7

$$k = \frac{k_2 a_H + k_3 K_{a2}}{a_H + K_{a2}} \qquad (7)$$

are 9.55 at 25 °C for *N*-acetyl-L-cysteine[33] and 9.93 at 25 °C for 2-meracptopropanoic acid.[36]

Equation 8, derived from Scheme 3, was fit to the data obtained for reduction with mercaptosuccinic acid, which is a triprotic thiol with the acid dissociation constants corresponding to $pK_{a1} = 3.13$, $pK_{a2} = 4.63$, and $pK_{a3} = 10.26$ (SH) at 25 °C.[34] The fully deprotonated trianionic species and the dianionic species in which only the carboxylic groups are deprotonated are the redox active species; reduction by the monoanionic

(47) Shi, T.; Elding, L. I. *Inorg. Chem.* **1996**, *35*, 5941.
(48) Beattie, J. K.; Basolo, F. *Inorg. Chem.* **1967**, *6*, 2069.
(49) Lempers, E. L. M.; Inagaki, K.; Reedijk, J. *Inorg. Chim. Acta* **1988**, *152*, 201.
(50) Goddard, P. M.; Orr, R. M.; Valenti, M. R.; Barnard, C. F. J.; Murrer, B. A.; Kelland, L. R.; Harrap, K. R. *Anticancer Res.* **1996**, *16*, 33.
(51) Appleton, T. G.; Bailey, A. J.; Barnham, K. J.; Hall, J. R. *Inorg. Chem.* **1992**, *31*, 3077.

species was not observed in the pH region used.

$$k = \frac{k_3 K_{a2} a_H + k_4 K_{a2} K_{a3}}{a_H{}^2 + K_{a2} a_H + K_{a2} K_{a3}} \quad (8)$$

Equation 9, derived from Scheme 4, was fit to the data obtained for reduction of JM335 by glutathione. In the derivation of this equation, the term containing k_4' can be neglected because of the large difference between the reactivities of the species RSH and RS^-.[17] The microscopic acid dissociation constants used in eq 9 correspond to $pK_{AA} = 8.97$, $pK_{BB} = 9.17$, and $pK_{CC} = 9.35$ at 25 °C.[37]

$$k = \frac{k_3 a_H{}^2 + k_4 K_{AA} a_H + k_5 K_{AA} K_{CC}}{a_H{}^2 + (K_{AA} + K_{BB}) a_H + K_{AA} K_{CC}} \quad (9)$$

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