Electron-Transfer-Driven Trans-Ligand Labilization: A Novel Activation Mechanism for Pt(IV) Anticancer Complexes

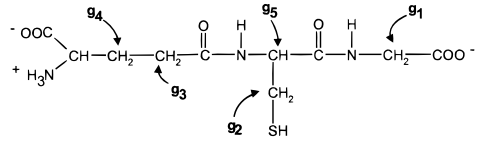
Nicole A. Kratochwil,† Zijian Guo,†
Piedad del Socorro Murdoch,† John A. Parkinson,†
Patrick J. Bednarski,‡ and Peter J. Sadler*,†

Department of Chemistry, The University of Edinburgh King's Buildings, West Mains Road, Edinburgh EH9 3JJ, U.K. The Institut für Pharmazie, Universität Regensburg 93040 Regensburg, Germany

Received February 4, 1998

The first orally active platinum drug, *cis,trans,cis*-[$PtCl_2(OAc)_2$-NH_3(c-$C_6H_{11}NH_2$)] (JM216), has now entered phase II clinical trials,[1] and octahedral Pt(IV) complexes in general offer new strategies for the design of platinum-based antitumor agents. Because of their inertness to substitution, Pt(IV) antitumor complexes are thought to be prodrugs activated in vivo by reduction to their Pt(II) analogues by biological reductants such as glutathione, with loss of two axial ligands.[2] However, there is only scant information in the literature concerning the mechanism of reduction of antitumor Pt(IV) diamines by biologically important thiols. We report here the unexpected detection of a long-lived chelate-ring-opened Pt(II) complex generated from a chelated cytotoxic Pt(IV) complex under biologically relevant conditions. Electron-transfer-driven trans-ligand labilization reactions may therefore provide novel activation mechanisms for Pt(IV) complexes in vivo.

We have studied the reduction of the Pt(IV) complex *trans,cis*-[$Pt(en)(OH)_2I_2$] (**1**) (en = ethylenediamine), a complex which is active against a wide variety of cancer cells in vitro and a member of a class of photoactivatable Pt(IV) complexes.[3] Reactions of **1** (100 μM) with glutathione (GSH, γ-Glu-Cys-Gly)



glutathione (γ-Glu-Cys-Gly)

or *N*-acetyl-L-cysteine (*N*-Ac-Cys) in a molar ratio of 1:2 were first investigated by ^{1}H NMR spectroscopy[4] at pHs 3 and 7 and 276 or 298 K for a period of 24 h. For the GSH reactions at pH 7 and 276 K (Figure 1A), signals for complex **1** (CH_2 2.776 ppm, **1a**) and those for the free thiol (e.g., **g2**) decreased in intensity within 5 min, and two new multiplets at 3.524 (peak **2a**) and 3.037 (peak **2a′**) ppm and resonances for free disulfide (GSSG, peaks **g2′**) were observed. Peaks **2a** and **2a′** reached their maximum intensity after 11 min and were detectable for over 6 h. At this pH, no release of ethylenediamine was observed during the reaction.[5] At pH 3 and 276 K (Figure 1, B), the multiplet at 3.494 ppm (peak **2a**) was clearly detectable after 11 min, together with signals assignable to free disulfide (peaks **g2′**) and free ethylenediamine (peak **b**, 3.380 ppm). The second multiplet (3.037 ppm at pH 7 (Figure 1A, peak **2a′**)) was overlapped with the β-CH_2 resonances of the cystine moiety at pH 3 and 276 and 298 K (Figure 1B,C). After 3 h at 276 K, only signals for free GSSG and free ethylenediamine were observed, suggesting that the disulfide product does not coordinate to Pt. At later stages, however, broad peaks appeared at 4.1, 3.9, 2.7, and 2.4 ppm. Analogous ^{1}H NMR spectral changes were observed for the reactions of *N*-Ac-Cys with complex **1**.[6]

The reduction of the labeled complex ([^{15}N]**1**) was investigated by 2D [^{1}H, ^{15}N] HSQC and 2D [^{1}H, ^{15}N] HSQC-TOCSY NMR.[4,7] The kinetic courses of the reactions with the reducing agents at a 1:2 molar ratio (1 mM [^{15}N]**1**) were studied only at pH 3 and 276 and 298 K because of the poor solubility of ([^{15}N]**1**) at pH 7. At 276 K the ^{15}N/^{1}H shifts for ([^{15}N]**1**) were 15.20/6.99 ppm (**1b**). A new cross-peak at −26.92/5.56 ppm (peak **2b**) was observed in the 2D [^{1}H, ^{15}N] HSQC NMR spectrum 9 min after the addition of GSH (or *N*-Ac-Cys). Cross-peak **2b** disappeared after 3 h. The 2D [^{1}H, ^{15}N] HSQC-TOCSY spectrum showed that the inequivalent CH_2 groups of the ethylenediamine moiety are part of the same spin system. They (**2a** and **2a′**) are coupled to the cross-peak **2b** and to one another (evidence from 2D COSY data, data not shown). At later stages of the reaction at 298 K, cross-peaks with ^{15}N/^{1}H shifts of −10.82/5.13 and −8.34/4.99 ppm, compatible with ethylenediamine NH_2 trans to S in Pt(II) complexes, and a cross-peak assignable to free [^{15}N]en (^{15}N/^{1}H shifts at 8.23/7.83 ppm) were observed in the 2D [^{1}H, ^{15}N] HSQC NMR spectrum.

The inequivalence of the two CH_2 groups (**2a** and **2a′**) of coordinated ethylenediamine in the 1D ^{1}H and 2D [^{1}H, ^{15}N] HSQC-TOCSY NMR spectra and the observation of only one ^{15}N/^{1}H cross-peak **2b** (−26.92/5.56 ppm) in the 2D spectra of both reactions of GSH and *N*-Ac-Cys suggest that ring-opened Pt(II) ethylenediamine complexes[8] are formed during the above reduction reactions. This is also argued on the basis of chemical shifts in the 1D ^{1}H spectrum (Figure 1), in which **2a** is similar to **b** for free ethylenediamine and **2a′** is similar to **1a** for complex **1**. The ^{1}H/^{15}N cross-peak for the NH_3^+ group of monodentate [^{15}N]enH^+ is likely to be broadened beyond detection due to NH exchange with the solvent. Siebert and Sheldrick[9] have reported that a ring-opened Pt(II) species is formed during reaction of [Pt(en)$(H_2O)_2$]$^{2+}$ with methionine-containing di-and tripeptides and at pH 2.4 gave rise to two ^{1}H NMR CH_2 multiplets for monodentate ethylenediamine, which have shifts similar to those observed here. The observation of only free disulfide in reactions of either GSH or *N*-Ac-Cys with complex **1** indicates that the ring-opened intermediate **2** does not contain bound disulfide. At later stages, the ring-opened Pt(II) species undergoes further substitution reactions. In the case of *N*-Ac-Cys and pH 7, the final products of the reaction of complex **1** with *N*-Ac-Cys were

† The University of Edinburgh.
‡ Universität Regensburg.
* To whom correspondence should be addressed.

(1) Beale, P. J.; Kelland, L. R.; Judson, I. R. *Expert. Opin. Invest. Drugs* **1996**, *5* (6), 681−693.

(2) (a) Bose, R. N.; Weaver, E. L. *J. Chem. Soc., Dalton Trans.* **1997**, 1797−1799. (b) Talman, E. G.; Brüning, W.; Reedijk, J.; Spek, A. L.; Veldman, N. *Inorg. Chem.* **1997**, *36*, 854−861. (c) Shi, T.; Berglund, J.; Elding, L. I. *Inorg. Chem.* **1996**, *35*, 3498−3503. (d) Gibbons, G. R.; Wyrick, S.; Chaney, S. G. *Cancer Res.* **1989**, *49*, 1402−1407.

(3) Kratochwil, N. A.; Zabel, M.; Range, K. J.; Bednarski, P. J. *J. Med. Chem.* **1996**, *39*, 2499−2507.

(4) ^{1}H, 2D [^{1}H, ^{15}N] HSQC, and 2D [^{1}H, ^{15}N] HSQC-TOCSY NMR spectra (TOCSY mixing time of 50 ms) were recorded on a Bruker DMX 500 (^{1}H, 500 MHz; ^{15}N, 50.7 MHz) NMR spectrometer using procedures similar to those described previously: Berners-Price, S. J.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 8649−8659. All experiments described in the present paper were carried out in the absence of light.

(5) At pH 7, en CH_2 peak at 3.263 ppm (298 K), 3.260 ppm (276 K).

(6) Reactions of [$Pt(en)I_2$] (**3**) and *N*-Ac-Cys gave rise to Pt(II)−(*N*-Ac-Cys) complexes, which have ^{1}H (Figure S3) and 2D [^{1}H, ^{15}N] NMR spectra (Figure S4) comparable to those of the end products observed for the reaction of complex **1** with *N*-Ac-Cys (Figures S1 and S2A,B).

(7) The ^{15}N chemical shifts of square-planar Pt−am(m)ine are diagnostic of the trans ligand: Berners-Price, S. J.; Sadler, P. J. *Coord. Chem. Rev.* **1996**, *151*, 1−40.

(8) The possibility that the intermediate is still a Pt(IV) species could be ruled out because of the ^{15}N shift in the 2D spectra indicating a Pt(II) species and the immediate observation of disulfide ($2e^-$ oxidation step).

(9) Siebert, A. F. M.; Sheldrick, W. S. *J. Chem. Soc., Dalton Trans.* **1997**, 385−393. [Pt(Hen-κN)(gly-met-$\kappa^3 N,N',S$)]$^{2+}$ at pH 2.4 has ^{1}H NMR en CH_2 signals at 2.92 and 3.15 ppm.

S0002-7863(98)00393-X CCC: $15.00
Published on Web 08/01/1998

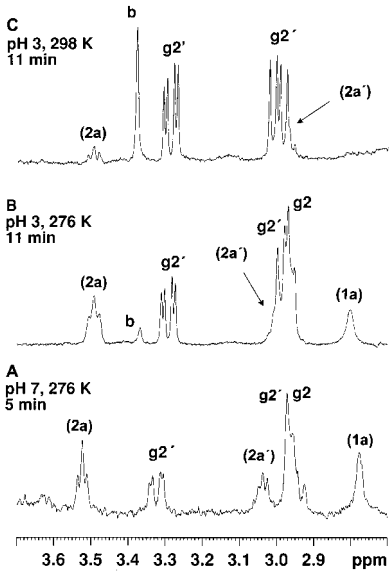
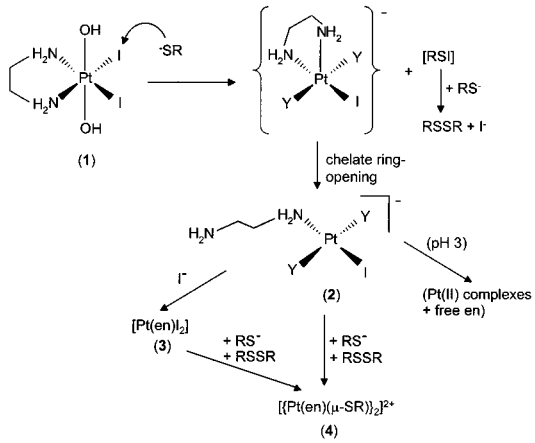


Figure 1. 500-MHz ^{1}H NMR spectra recorded during the reduction of *trans,cis*-[Pt(en)(OH)$_2$I$_2$] (**1**) by GSH in a 1:2 molar ratio: (A) pH 7, 276 K, after 5 min (200 μM GSH); (B) pH 3, 276 K, after 11 min (2 mM GSH); (C) pH 3, 298 K, after 11 min (2 mM GSH). The two multiplets at 3.037 (peak **2a′**) and 3.524 ppm (peak **2a**) in part A are assignable to the en CH$_2$ of a ring-opened intermediate (**2**). In B and C peak **2a′** is overlapped with **g2′**; free en is labeled **b**, and the peak for **1** is at 2.8 ppm (peak **1a**).

Scheme 1. Proposed Mechanism for the Reduction of *trans,cis*-[Pt(en)(OH)$_2$I$_2$] (**1**) by Biologically Important Thiols[a]



[a] The nature of the ligands Y in complex **2** is unknown, but strong candidates are H$_2$O/OH.

characterized by HPLC, 2D [^{1}H, ^{15}N] HSQC NMR, and APCI mass spectroscopy, giving rise to complex **3** and the Pt(II) dimer, [{Pt(en)(μ-SCys-*N*-Ac)}$_2$]$^{2+}$ (**4**) (Figures S1−S4).[6] At pH 3, however, ethylenediamine is easily released during the reduction reaction and the later substitution products were not further characterized, being of no biological relevance.

On the basis of these results, a mechanism for the reduction of complex **1** by GSH or *N*-Ac-Cys at neutral pH can be proposed (Scheme 1), consisting of **initial attack of the thiol on an iodide ligand of the Pt(IV) complex** forming a five-coordinated transition state,[10,11] which then undergoes a ring-opening reaction. Attack on the highly reactive sulfenyliodide by another glutathione molecule would give rise to the disulfide. The ring-opened Pt(II) species **2** can then undergo further ring-closure reactions and react with the released I$^-$, forming [Pt(en)I$_2$] (**3**), and with RSH or RSSR,[12] giving [{Pt(en)(μ-SR)}$_2$]$^{2+}$(**4**).[13] At pH 3, ethylenediamine is released, but at pH 7, no free ethylenediamine is observed.

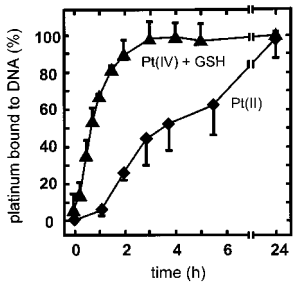


Figure 2. Platination of calf thymus DNA with *trans,cis*-[Pt(en)(OH)$_2$I$_2$] (**1**, 7.5 μM) in the presence of glutathione (15 μM) (▲). Conditions: 10 mM PIPES (pH 6.8), 0.250 mg L^{-1} calf thymus DNA, at 310 K in the dark. Platinum bound to DNA was determined by AAS.[3] No platination was observed in the absence of GSH. In contrast, platination by [Pt(en)I$_2$] (**3**) (◆) is significantly slower.

In conclusion, our data enable us to postulate the existence of a new mechanism for the reduction of complex **1** by biologically important thiols such as GSH involving the formation of an unexpected chelate ring-opened Pt(II) complex, even at pH 7. Such a reduction produces Pt species which are capable of forming DNA−Pt adducts in the presence of GSH much more rapidly than [Pt(en)I$_2$] (**3**) (Figure 2), which might be expected to be the major product from reduction of complex **1** if the reaction simply involved the loss of two axial ligands. Moreover, the Pt(IV) complex **1** is highly cytotoxic to tumor cells in contrast to [Pt(en)I$_2$] (**3**).[3] The only report of a related reaction appears to be that of Beattie et al.,[14] who postulated that the ring-opened complex [Pt(pn)(pnH)Cl]$^{2+}$ (pn = propylenediamine) was a product from inner-sphere two-electron reduction of the Pt(IV) complex *cis*-[Pt(pn)$_2$Cl$_2$]$^{2+}$ by [Cr(H$_2$O)$_6$]$^{2+}$, although the nature of the product was investigated only by ion-exchange chromatography and titration studies. Vorob'ev-Desyatovskii and Kukushkin[15] reported that during the reverse reaction (oxidation of Pt(II) to Pt(IV)) certain electrophiles can oxidize a coordinated ligand giving rise to a large trans effect and expulsion of the trans ligand. The introduction of electron-transfer-driven trans effects into Pt complexes should allow the generation in vivo of Pt(II) complexes which are not simple analogues of the parent Pt(IV) prodrugs and provide a novel concept for metallodrug design.

Acknowledgment. We thank the EC (Marie Curie Research Training Grant No. ERB4001GT963865 to N.A.K. and COST Actions D1 and D8), BBSRC, EPSRC, and DFG (Grant Be 1287/1-2) for their support for this work.

Supporting Information Available: 500-MHz ^{1}H and 2D [^{1}H, ^{15}N] HSQC NMR spectra of reactions of complex **1** and [Pt(en)I$_2$] (**3**) with *N*-Ac-Cys and HPLC chromatogram and 2D NMR spectra of the final products (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980393Q

(10) (a) Sulfenyliodides are very reactive and well-known in organic sulfur chemistry: Field, L.; Vanhorne, J. L.; Cunningham, L. W. *J. Org. Chem.* **1970**, *35*, 3267−3273. (b) The complex {[(en)$_2$Co(SCH$_2$CH$_2$NH$_2$)]$_2$I}$^{5+}$ has been characterized by X-ray analysis, can be viewed as containing a derivative of a coordinated sulfenyliodide, and reacts with thiols to form disulfides: Nosco, D. L.; Heeg, M. J.; Glick, M. D.; Elder, R. C.; Deutsch, E. *J. Am. Chem. Soc.* **1980**, *102*, 7786−7787.

(11) (a) Goldberg, K. I.; Yan J.; Breitung, E. M. *J. Am. Chem. Soc.* **1995**, *117*, 6889−6896. (b) van Beek, J. A. M.; van Koten, G., Smeets, W. J. J.; Spek, A. L. *J. Am. Chem. Soc.* **1986**, *108*, 5010−5011. (c) Byers, P. K.; Canty, A. J., Crespo, M.; Puddephatt, R. J.; Scott, J. D. *Organometallics* **1988**, *7*, 1363−1367. Chelate ring opening on Pt(IV) prior to the reduction by the thiol could also be proposed as an alternative mechanism to the Scheme 1 but seems rather unlikely.[11a]

(12) There is precedent for the reduction of disulfide bonds by Pt(II): Lempers, E. L. M.; Inagaki, K.; Reedijk, J. *Inorg. Chim. Acta* **1980**, *152*, 201−207. Ohta, N.; Inagaki, K., Muta, H.; Yotsuyanagi, T.; Matsuo, T. *Int. J. Pharmaceutics* **1998**, *161* (1), 15−21.

(13) Appleton, T. G. *Coord. Chem. Rev.* **1997**, *166*, 313−359.

(14) Beattie, J. K.; Starink, J. *Inorg. Chem.* **1975**, *14*, 996−999.

(15) Vorob'ev-Desyatovskii, N. V.; Kukushkin, Yu. N. *Zh. Obshch. Khim.* **1991**, *61*, 2613−2623.