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Kinetics and mechanism for reduction of oral anticancer platinum(IV) dicarboxylate compounds by L-ascorbate ions†

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Ascorbate (Asc) reductions of the oral anticancer platinum(IV) prodrugs *cis*,*trans*,*cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216) and *cis*,*trans*,*cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (JM221) and of the isomers of JM216, *viz.* *trans*,*cis*,*cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM394) and *trans*,*trans*,*trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM576) (OAc = acetate, cha = cyclohexylamine) have been investigated in a 1.0 M aqueous perchlorate medium using stopped-flow and conventional UV/VIS spectrophotometry as a function of temperature and pH. JM216 and 221 are reduced to *cis*-$[PtCl_2(cha)(NH_3)]$ (JM118) and JM394 and 576 to *cis*- and *trans*-$[Pt(OAc)_2(cha)(NH_3)]$, respectively. The redox reactions follow the second-order rate law: $-d[Pt(IV)]/dt = k\,[Pt(IV)]\,[Asc]_{tot}$ where k is a pH dependent second-order overall rate constant and $[Asc]_{tot} = [Asc^{2-}] + [HAsc^-] + [H_2Asc]$. Reduction of JM216 and JM221 is slow (overall rate constants $k^{298} = 5.08 \pm 10^{-2}$ and 3.25×10^{-2} mol^{-1} dm^3 s^{-1} at pH 7.12, respectively) and is suggested to take place *via* an outer-sphere mechanism. Reductions of JM394 and JM576 are more than three orders of magnitude faster ($k^{298} = 230 \pm 6$ mol^{-1} dm^3 s^{-1} at pH 7.0 for JM394). They are suggested to take place by a mechanism involving a reductive attack on one of the mutually *trans* chloride ligands by Asc^{2-} and less efficiently by $HAsc^-$ leading to the formation of a chloride-bridged activated complex. The second-order rate constants for reduction of JM394 by $HAsc^-$ and Asc^{2-} at 25 °C are 0.548 ± 0.004 and (4.46 ± 0.01) × 10^6 mol^{-1} dm^3 s^{-1}, respectively. The rate constants for reduction of JM216 and JM221 by Asc^{2-} at 25 °C are calculated to be 672 ± 15 and 428 ± 10 mol^{-1} dm^3 s^{-1}, respectively and reduction by $HAsc^-$ was not observed under these conditions. Thus, Asc^{2-} is up to 7 orders of magnitude more efficient as a reductant than $HAsc^-$. H_2Asc is virtually inactive. The activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for reduction of JM216, JM221, JM394, and JM576 by Asc^{2-} are 52 ± 1, 46 ± 1, 56.2 ± 0.5, and 63 ± 2 kJ mol^{-1} and −97 ± 4, −120 ± 4, −24 ± 2, and −8 ± 5 J K^{-1} mol^{-1}, respectively. An isokinetic relationship gives further support to the mechanistic assignments.

Introduction

The current search for platinum-based anticancer active compounds aims at developing complexes which do not show cross-resistance with the widely used drugs *cis*-$[Pt(NH_3)_2Cl_2]$ (cisplatin) and *cis*-diammine(1,1-cyclobutanedicarboxylato)-platinum(II) (carboplatin), which are active against a broader range of tumours, and which can be taken orally. Oral drugs are simpler to use and less expensive than those requiring intravenous administration. A novel class of drugs, ammine/amine(dichloro)platinum(IV) dicarboxylates, with suitable properties for oral administration and antitumour activity in cisplatin-resistant human cancer cells has been developed.[1,2] Examples include *cis*,*trans*,*cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216) (cha = cyclohexylamine) and *cis*,*trans*,*cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (JM221) which are lipophilic and robust enough to survive the gastric environment.[3] In particular, they are effective in cells where resistance to other platinum drugs is due to a decreased uptake of platinum.[4] The precise mechanism whereby cisplatin enters the cells is not clear. Passive diffusion as well as carrier mediated mechanisms have been proposed.[5] The effectiveness of the Pt(IV) dicarboxylate compounds in cisplatin-resistant cells may be a consequence of their lipophilic nature facilitating transport into the cell.[4,6]

It is almost certain that cisplatin exerts its cytotoxicity through binding to DNA, producing both intra- and inter-strand cross-links which inhibit replication.[7] Since platinum(IV) complexes are generally substitution inert,[8] it is presumed that they are reduced to the more reactive platinum(II) analogues before interaction with DNA. Numerous experiments have shown that anticancer active and other platinum(IV) complexes are reduced by both extra- and intra-cellular reducing agents.[9–12] JM216, for instance, is reported to undergo bio-transformation in the plasma into at least six different metabolites amongst which *cis*-$[PtCl_2(cha)(NH_3)]$ (JM118) is a major product.[13] Similarly, JM221 displays a marked time dependent cytotoxicity in human ovarian carcinoma cells, implying that a slow reductive and hydrolytic activation of the drug might be required for activity.[14]

The anticancer activity of the platinum(IV) dicarboxylate compounds is likely to be due to effective platinum transport into the cell followed by reduction to the more reactive platinum(II) compounds. Knowledge of the reactivity of the platinum(IV) compounds towards reduction by potential bio-reductants such as ascorbic acid and glutathione may be important for the understanding of the mechanism of their antitumour activity as well as for the design of new compounds with suitable pharmacokinetic properties.[15] Choi and co-workers recently studied ascorbate reduction of a series of

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra of JM576, JM216 and their reduction products with ascorbic acid and ^{1}H NMR spectra of 2 mmol dm^{-3} JM576 with 200 mmol dm^{-3} chloride recorded after 1.5 and 72 h. See http://www.rsc.org/suppdata/dt/a9/a909484i/

Pt(IV) anticancer active complexes, investigating the effects of axial and carrier ligands on the relative reduction and cytotoxicity,[16] and Ranford and co-workers have investigated the reduction of a bis(carboxylato) complex by methionine and cysteine.[17]

The study by Choi *et al.*[16] did not focus on elucidating the details of the molecular redox mechanism (*vide infra*). In view of this limitation and the significance of JM216 and JM221 as oral anticancer prodrugs, it was considered worthwhile to undertake a kinetic and mechanistic investigation on the reduction of JM216 and JM221 by ascorbic acid. In order to obtain further insight into the mechanism, the reduction of *trans*,*cis*,*cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM394) and *trans*,*trans*,*trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM576) have also been studied. The structures of the platinum(IV) compounds and L-ascorbic acid are shown in Chart 1. Attempts to study the reduction of

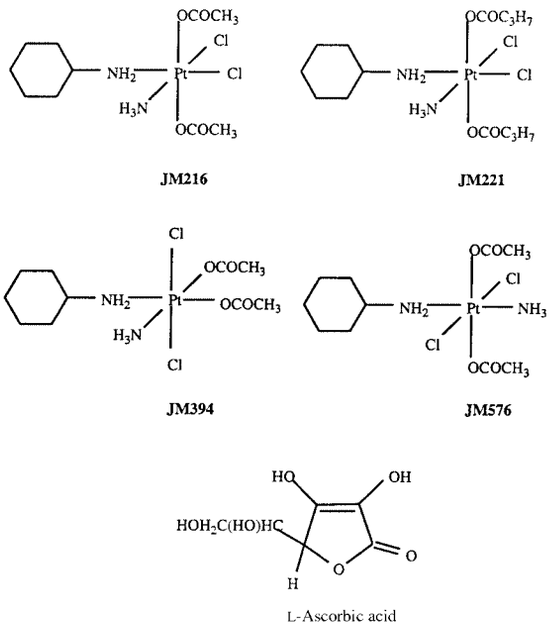


Chart 1

JM216 and JM221 with glutathione were hampered by early interference from substitution reactions at the platinum(II) product. In keeping with this observation, del Socorro Murdoch *et al.* recently described substitution reactions between glutathione/glutathione disulfide and $[Pt(en)_2Cl_2]$.[18] Qualitatively, however, the glutathione reductions of the Pt(IV) carboxylate complexes studied in the present work proceed at similar rates to those of ascorbate.

Experimental

Materials and solutions

The compounds JM216, JM221, JM394, and JM576 were kindly loaned by the Johnson Matthey Technology Centre (Reading, Berkshire, UK). L-Ascorbic acid (Merck) and sodium hydrogenascorbate (NaHAsc) (ICN Biochemicals Inc.) were used as received. All other chemicals used were of analytical grade. Deionized (Millipore) water was boiled and flushed with argon for *ca.* 30 min to remove dissolved dioxygen. Acetate, phosphate, and TRIS buffers containing 3×10^{-3} mol dm^{-3} Na_2H_2(edta) were prepared using oxygen-free water. The Na_2H_2(edta) was added to the buffers to sequester trace concentrations of transition metal ions that might catalyse the autoxidation of ascorbic acid.[19] Deoxygenated solutions of hydrogenascorbate (3–50) $\times 10^{-3}$ mol dm^{-3}, JM394, and JM576 (0.3–1) $\times 10^{-3}$ mol dm^{-3} were prepared immediately before use by dissolving weighed solid samples directly in buffer. Weighed samples of JM216 and JM221 were dissolved in water to give (0.4–1.0) $\times 10^{-3}$ mol dm^{-3} solutions.

Physical measurements

The pH of the buffer solutions was measured with a Metrohm 632 digital pH meter equipped with a combination Metrohm glass electrode and activities of oxonium ions $a_H = 10^{-pH}$ were calculated from direct pH meter readings. UV/VIS spectra were recorded with a CARY 300 Bio UV/VIS spectrophotometer using 1.0 cm Quartz Suprasil cells. Proton NMR spectra were recorded with a Varian Unity 300 spectrometer operating at a frequency of 299.779 MHz with D_2O as solvent and the residual solvent signal as a reference at constant pH, ionic medium and temperature.

Kinetic measurements

The redox reactions were investigated in a 1.0 mol dm^{-3} aqueous perchlorate medium using stopped-flow and conventional UV/VIS spectrophotometry. All kinetic measurements were performed under pseudo-first-order conditions with at least a 10-fold excess of ascorbic acid by monitoring the absorbance decrease at 310 (JM394 and JM576) and 318 nm (JM216 and JM221). Three to six repetitive measurements were made and reactions were followed for at least 4 half-lives. The reduction of JM394 and JM576 was investigated in the region $4.0 \leq pH \leq 7.0$ at 25 °C in acetate or phosphate buffers using an Applied Photophysics SX-18MV Stopped-Flow ASVD spectrophotometer, and that of JM216 and JM221 at 35 °C with $7.0 \leq pH \leq 7.5$ in TRIS buffer using the Cary 300 Bio UV/VIS spectrophotometer. For JM-216 and -221, 1.00 cm^3 of metal solution in water was mixed with 1.00 cm^3 of NaHAsc dissolved in TRIS buffer of total ionic strength 2.0 mol dm^{-3} ($NaClO_4$) directly in a 1.00 cm spectrophotometer cell. No reactions between the buffers and the platinum complexes were observed during the time of the kinetics experiments. Data for JM-216 and -221 were not collected above pH 7.5 since biphasic kinetic traces, attributed to hydrolysis of the platinum(IV) compounds, hampered the data analysis. Constant temperature (±0.1 °C) was maintained by an external RM6 LAUDA circulating-water bath for stopped-flow measurements and a CARY Peltier thermostat (±0.01 °C) coupled with a circulating-water temperature control unit for measurements using the Cary UV/VIS spectrophotometer. The temperature dependence of the second-order overall rate constants k was investigated at pH 6.28 for JM394 and JM576 and 7.12 for JM216 and JM221. At these pH, Asc^{2-} dominates the reaction completely (*vide infra*).

Single-exponential kinetic traces were collected in all cases and the pseudo-first-order rate constants k_{obs} were derived from an on-line non-linear least squares fit of the absorbance–time data using Applied Photophysics[20] and Cary WinUV Bio[21] software packages. A spectrophotometric investigation of the reduction of JM216 and JM221 by glutathione (GSH) was not feasible because of interference from slow subsequent processes presumed to be substitution of chloride by GSH/GS^- in the Pt(II) reduction product.[18,22,23]

Results and discussion

Stoichiometry and reaction products

The stoichiometry $[Pt(IV)]:[Asc]_{tot}$ for ascorbate reduction of platinum(IV) halide complexes has been observed to be 1:1.[24] By analogy, equimolar quantities of the reactants are assumed to be consumed in the present reductions. Ascorbate is also known to be oxidised to dehydroascorbate (DHA) by some transition metal ions and complexes.[25] Proton NMR spectra of the starting materials and the product mixture at 25 °C showed that *cis*-$[PtCl_2(cha)(NH_3)]$ (JM118), *cis*-$[Pt(OAc)_2(cha)(NH_3)]$, and *trans*-$[Pt(OAc)_2(cha)(NH_3)]$ were the sole reduction products of JM216, JM394, and JM576, respectively, along with DHA. The two acetate ligands were released to solution when

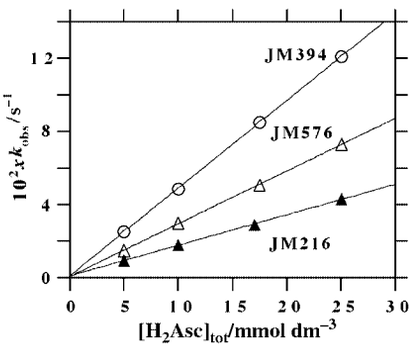


Fig. 1 Dependence of pseudo-first-order rate constants k_{obs} for reduction of JM394 (○, $x = 1$), JM576 (△, $x = 1.5$), and JM216 (▲, $x = 10$) on $[H_2Asc]_{tot}$. Conditions: pH 7.38, 35 °C (▲); pH 5.26, $[Cl^-] = 10$ mM, 25 °C (O, △). For clarity the rate constants for JM216 and JM576 have been multiplied by $x = 10$ and 1.5, respectively.

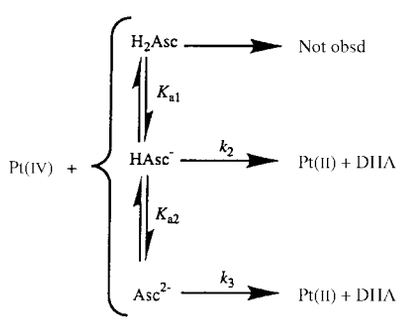
JM216 was reduced; a singlet at δ 2.20 assigned to the coordinated acetates in JM216 disappeared and a new peak at δ 2.00 due to free acetate was observed. Reduction of JM221 is assumed to give the same product as that of JM216. No acetate ligands were released to solution when JM394 and JM576 were reduced. Two peaks at δ 2.231 and 2.218 assigned to the non-equivalent acetate groups in JM394 disappeared and a singlet at δ 2.052 appeared upon reduction. A singlet at δ 2.063 observed upon reduction of JM576 is assigned to the *trans* acetate ligands in the platinum(II) product formed. Proton NMR spectra of JM-216 and -576 and their reduction products are available as ESI Fig. S1.

Kinetics

Plots of the pseudo-first-order rate constants k_{obs} *versus* $[Asc]_{tot}$ at constant pH are linear with zero intercept. Such plots are illustrated in Fig. 1 for JM216, JM394, and JM576. JM221 is assumed to behave similarly. Thus, the redox reactions follow the second-order rate law defined by eqn. (1) where k denotes a

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

pH-dependent second-order overall rate constant. Since there are no protolytic equilibria associated with the Pt(IV) compounds, under the experimental conditions, the pH dependence of k is attributed to displacement of equilibria involving the three protolytic species of ascorbic acid. We then arrive at the reaction shown in Scheme 1.



Scheme 1

The second-order overall rate constants k for reduction of JM216 and JM221 calculated using eqn. (1) and those of JM394 and JM576 obtained as slopes of the plots of k_{obs} *vs.* $[Asc]_{tot}$ are collected in Table 1 together with their pH variation. In deriving eqn. (2) which describes the pH-dependence of k,

$$k = \frac{k_2K_{a1}a_H + k_3K_{a1}K_{a2}}{a_H^2 + K_{a1}a_H + K_{a1}K_{a2}} \quad (2)$$

Table 1 Second-order overall rate constants k as a function of pH for reduction of JM216, JM221, JM394, and JM576[a]

		k/mol^{-1} dm^3 s^{-1}	
Compound	pH	Measured	Calculated
JM216	7.00	0.083 ± 0.005	0.078 ± 0.001
	7.12	0.100 ± 0.003	0.102 ± 0.002
	7.25	0.142 ± 0.005	0.138 ± 0.002
	7.38	0.18 ± 0.01	0.186 ± 0.003
	7.52	0.25 ± 0.01	0.257 ± 0.004
JM221	7.00	0.047 ± 0.003	0.0460 ± 0.0003
	7.12	0.059 ± 0.001	0.0600 ± 0.0004
	7.25	0.083 ± 0.001	0.0810 ± 0.0005
	7.38	0.109 ± 0.003	0.109 ± 0.001
	7.52	0.17 ± 0.01	0.151 ± 0.001
JM394	4.00	0.420 ± 0.004	
	4.25	0.657 ± 0.003	
	4.50	1.13 ± 0.01	
	4.77	1.69 ± 0.02	
	5.00	3.00 ± 0.01	
	5.26	4.79 ± 0.02	
	5.50	8.27 ± 0.04	
	5.98	28.1 ± 0.4	
	6.28	48.5 ± 0.6	
	6.5	90 ± 1	
	6.66	118 ± 2	
	6.86	186 ± 4	
	7.00	230 ± 6	
JM576	4.50	0.532 ± 0.011	
	5.26	1.92 ± 0.02	
	6.28	21.8 ± 0.2	

[a] Reaction conditions: 25 °C, $[Cl^-] = 10$–20 mmol dm^{-3}, 3 mmol dm^{-3} Na_2H_2(edta) for JM394 and JM576; 35 °C, 3 mmol dm^{-3} Na_2H_2(edta) for JM216 and JM221. Errors are given as one standard deviation.

Table 2 Second-order rate constants for reduction of JM216, JM221, JM394, and JM576 by hydrogenascorbate $HAsc^-$ (k_2) and ascorbate Asc^{2-} (k_3) at 25 °C and 1.0 mol dm^{-3} ionic strength

Compound	k_2/mol^{-1} dm^3 s^{-1}	k_3/mol^{-1} dm^3 s^{-1}
JM216	Not observed	672 ± 15
JM221	Not observed	428 ± 10
JM394	0.548 ± 0.004	(4.46 ± 0.01) × 10^6
JM576	*ca.* 0.3[a]	*ca.* 2 × 10^6[a]

[a] Estimated based on the observation that JM576 is about 2 times less reactive than JM394 (*cf.* Table 1).

hydrogenascorbate ($HAsc^-$) and ascorbate (Asc^{2-}) are considered to be the redox active species (Scheme 1).

In eqn. (2), K_{a1} and K_{a2} are the acid dissociation constants of ascorbic acid with the values: p$K_{a1} = 3.96$ (25 °C, 1 mol dm^{-3}),[26] 3.90 (35 °C, 1 mol dm^{-3})[27] and p$K_{a2} = 11.24$ (25 °C, 1 mol dm^{-3}),[28] 11.06 (35 °C, 1 mol dm^{-3}, extrapolated).[27] The second-order rate constants for reduction of JM394 by hydrogen ascorbate (k_2) and by ascorbate (k_3) derived from the fit of eqn. (2) to the experimental data are given in Table 2.

In view of the observation that ascorbate Asc^{2-} is about 7 orders of magnitude more reactive than hydrogenascorbate $HAsc^-$ it is assumed that the former is the dominant reductant for JM216 and JM221 in the near neutral pH region studied. Accordingly, eqn. (2) is reduced to eqn. (3) and fitted to the

$$k = \frac{k_3K_{a2}}{a_H + K_{a2}} \quad (3)$$

experimental data. The rate constants k_3 derived from the curve fitting are 886 ± 14 and 524 ± 4 mol^{-1} dm^3 s^{-1} for reduction at 35 °C of JM216 and JM221, respectively. Values recalculated to 25 °C are given in Table 2.

Table 3 Second-order overall rate constants and activation parameters for the reduction of JM216, JM221, JM394, and JM576 by ascorbate, Asc^{2-} [a]

Compound	T/°C	k/mol^{-1} dm^3 s^{-1}	$\Delta H^{\ddagger}$ [b]/kJ mol^{-1}	$\Delta S^{\ddagger}$ [b]/J K^{-1} mol^{-1}
JM216	20	$(3.34 \pm 0.09) \times 10^{-2}$	52 ± 1	−97 ± 4
	25	$(5.08 \pm 0.02) \times 10^{-2}$		
	30	$(7.23 \pm 0.01) \times 10^{-2}$		
	35	0.100 ± 0.003		
	40	0.140 ± 0.007		
JM221	25	$(3.25 \pm 0.05) \times 10^{-2}$	46 ± 1	−120 ± 4
	30	$(4.4 \pm 0.1) \times 10^{-2}$		
	35	$(5.94 \pm 0.05) \times 10^{-2}$		
	40	$(8.3 \pm 0.2) \times 10^{-2}$		
JM394	10	14.0 ± 0.2	56.2 ± 0.5	−24 ± 2
	15	21.8 ± 0.1		
	20	32.7 ± 0.6		
	25	48.5 ± 0.6		
	30	73.2 ± 0.2		
JM576	15	8.4 ± 0.2	63 ± 2	−8 ± 5
	20	14.4 ± 0.3		
	25	21.8 ± 0.2		
	30	34.3 ± 0.6		
	35	50.1 ± 0.6		

[a] Conditions: pH 7.12, 3 mmol dm^{-3} Na_2H_2(edta) for JM216 and JM221; pH 6.28, [Cl^-] = 10 mmol dm^{-3}, 3 mmol dm^{-3} Na_2H_2(edta) for JM394 and JM576. Errors are given as one standard deviation. [b] Activation parameters refer to reduction with ascorbate, Asc^{2-}, *i.e.* k_3 pathway in Scheme 1.

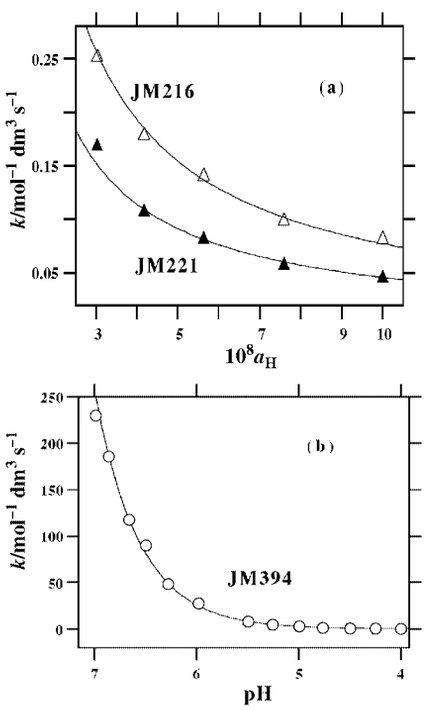


Fig. 2 Plots of the second-order overall rate constants k as a function of activity of oxonium ions for JM216 (△) and JM221 (▲) (a) and as a function of pH for JM394 (○) (b). The solid lines represent the fits of eqn. (2) and (3) to the experimental data.

The second-order overall rate constants k calculated from eqn. (3) are in a good agreement with those obtained experimentally (*cf.* Table 1). This observation supports the assumption that ascorbate is the primary reductant despite the fact that hydrogenascorbate constitutes more than 99% of the total concentration of ascorbic acid in the region $7.0 \leq pH \leq 7.5$. The fits of eqn. (2) and (3) to the experimental data are depicted in Fig. 2.

The contribution of the k_2 pathway to the overall reduction of JM394 at pH 6.28 is only 1%. Hence, the activation parameters presented in Table 3 are associated with the reductions of JM394 and JM576 by Asc^{2-}. Similarly, the activation parameters determined for reductions of JM216 and JM221 refer to the k_3 pathway.

Reaction mechanism

Most studies on ascorbic acid reduction of platinum(IV) complexes have involved chloro and hydroxo compounds where outer-sphere and platinum(II) catalysed inner-sphere mechanisms have been proposed.[29] However, to date there has been no detailed mechanistic investigation on the reduction of platinum(IV) dicarboxylate compounds with ascorbic acid. Since platinum(IV) compounds are generally substitution inert,[8] a substitution-controlled inner-sphere mechanism is unlikely. Based on the results of the kinetic studies and on the nature of the reduction products, an outer-sphere mechanism is suggested for reduction of compounds JM216 and JM221 and a chloride-bridged reductive elimination mechanism for JM394 and JM576. The latter involves a reductive attack by the ascorbate anion Asc^{2-} on co-ordinated chloride leading to the formation of a bridged activated complex of the type formulated for JM576 (*cf.* Chart 2).[24] Despite the electronegativity of chloride,

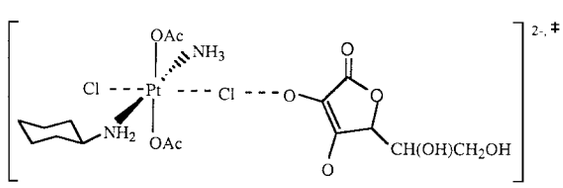


Chart 2

this entity bound to the highly oxidising Pt(IV) centre could have appreciable Cl^+ character and therefore be susceptible to a reductant. This inner-sphere mechanism, which is well-known for reductions of platinum(IV) complexes with other reductants,[10–12,15] leads in the present case simply to a concerted two-electron transfer from ascorbate to the Pt(IV) centre and delivers the Pt(II) product, dehydroascorbate and two eliminated chloride ions as products. Such intramolecular paths are inherently faster than outer-sphere bimolecular paths in keeping with the present observations.[30]

For JM216 and JM221, the chloride-bridged pathway is energetically unfavourable, since the chloride ligands are co-ordinated *trans* to ammonia and cyclohexylamine which are firmly bound to the metal centre. In addition, the carboxylate ligands in these complexes are not efficient bridging groups in a reductive *trans* elimination process since Pt(IV) is less able to generate CO_2^+ character in the carboxylate for the ascorbate ion to access.

JM394 and JM576 are more than 3 orders of magnitude more reactive than JM216 which is attributed to the fact that inner-sphere electron transfer reactions are generally faster than outer-sphere ones.[30] Further compelling evidence for the proposed chloride-bridged reductive elimination mechanism for reduction of JM394 and JM576 where the chloride ligands are co-ordinated *trans* to each other is the identification of *trans*-$[Pt(OAc)_2(cha)(NH_3)]$ as the product of reduction of JM576. A reductive elimination reaction of JM576 *via* an outer-sphere mechanism would be expected to result in the release of the inherently more labile acetate ligands instead of chloride. This property is gauged from the fact that dissociation of acetate from JM576 takes place slowly in the presence of chloride at room temperature (*cf.* ESI NMR spectra, Fig. S2).

In this context it might be interesting to compare the present results with those recently reported by Ranford *et al*. for reduction with cysteine and methionine of *cis*,*trans*,*cis*-$[PtCl_2(OAc)_2(NH_3)_2]$ as a model for JM216.[17] In these cases also, reduction results in release of two acetates per platinum, and the reduction is much slower than in our previous model systems.[10,11] A reductive elimination *via* oxygen-bridged electron transfer is suggested in these two cases,[17] but in view of the present results, an outer-sphere reduction might also be operative.

Our results pertaining to the reductions of JM216 and JM221 do not support some of those reported by Choi *et al.*[16] who effectively did not control their pH conditions. Our experimental conditions allow us to determine the kinetic and thermodynamic parameters with very good accuracy. We have maintained a constant pH using buffers with no observable interference with the reacting systems. Since the apparent reducing agent for JM216 and JM221 is ascorbate Asc^{2-}, which is about seven orders of magnitude more reactive than hydrogen ascorbate $HAsc^-$, failure to control pH will introduce quite large errors in the kinetic measurements. Moreover, the activation parameters reported by Choi *et al.*[16] are unrealistic with extraordinary and unusual uncertainties.[31] Further, their conclusion that JM221 is reduced twice as fast as JM216 does not agree with common experience that steric blocking usually retards the rates of bimolecular processes.[32] Nor is it compatible with our finding that JM216 reacts faster than the sterically more hindered JM221. This result, on the other hand, does agree with the report that the rate of reduction of 1,2-diaminocyclohexanedicarboxylato(oxalato)platinum(IV) complexes to give oxaliplatin decreases as the carboxylate chain length increases.[33]

Further support for our mechanistic assignments is given by the isokinetic relationship shown in Fig. 3.[34] The data for JM394 and JM576 agree very well with those derived from a series of model platinum(IV) complexes, which are reduced by Asc^{2-} in reductive elimination processes involving attack on co-ordinated halide, *viz. cis*-$[PtCl_4(NH_3)_2]$ (**1**), *trans*-$[PtCl_4(NH_3)_2]$ (**2**), *trans*-$[PtCl_2(en)_2]^{2+}$ (**3**), $[PtCl_6]^{2-}$ (**4**), and $[PtBr_6]^{2-}$ (**5**).[24] The present data for JM216 and JM221, on the other hand, deviate significantly from the isokinetic plot, implying reduction by a different mechanism, *i.e.* in this case an outer-sphere process. The unfavourable entropies of activation for JM216 and JM221 (Table 3) might reflect the requirement for substantial solvation of the leaving carboxylate groups in the activated complex in these instances.

Conclusion

Ascorbic acid reduction of the oral anticancer compounds JM216 and JM221 in a near neutral aqueous perchlorate medium follows an outer-sphere mechanism where ascorbate Asc^{2-} is the predominant reductant. These two compounds are reduced at comparable rates but more than 1000 times slower than JM394 and JM576, whose reduction is proposed to take place by the usual halide bridged reductive elimination mechanism.[10–12,15] Using the data in Tables 1–3, the half-life for reduction of JM216 with 5 mmol dm^{-3} total concentration of ascorbic acid (15-fold excess) at pH 7.40 and 35 °C is calculated to be *ca.* 12 min, and that of JM221 *ca*. 20 min. Thus, reduction of JM216 and JM221 in a biological medium is fairly rapid compared to hydrolytic bio-transformation pathways. Reductions of JM216 and JM221 with glutathione at pH 7.40 also take place at similar rates to those of ascorbate reduction, but the kinetics are complicated by parallel substitution processes in the platinum(II) products.

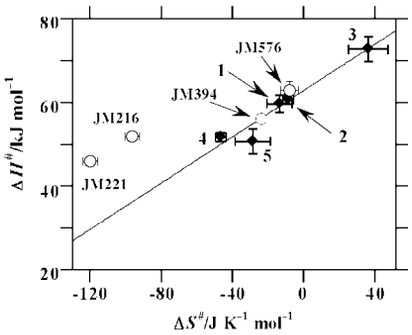


Fig. 3 Isokinetic relationship[34] for JM216, JM221, JM394, and JM576 including data for the model platinum(IV) complexes **1**–**5** (see text) from ref. 24. Complexes on the line are subject to reductive elimination by Asc^{2-} attack on co-ordinated halide, whereas JM216 and JM221 which deviate from the relationship are suggested to react by an outer-sphere mechanism.

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References

1 L. R. Kelland, B. A. Murrer, G. Abel, C. M. Giandomenico, P. Mistry and K. R. Harrap, *Cancer Res.*, 1992, **52**, 822.
2 C. F. J. Barnard, F. I. Raynaud and L. R. Kelland, in *Topics in Biological Inorganic Chemistry*, ed. M. J. Clarke and P. J. Sadler, Springer-Verlag, Heidelberg, 1999, vol. 1, pp. 45–71.
3 K. R. Harrap, *Cancer Res.*, 1995, **55**, 2761.
4 M. J. McKeage, M. Jones, P. M. Goddard, M. Valenti, B. A. Murrer and K. R. Harrap, *Cancer Res.*, 1993, **53**, 2581.
5 P. A. Andrews and S. B. Howell, *Cancer Cells*, 1990, **2**, 35.
6 P. Mistry, L. R. Kelland, S. Y. Loh, G. Abel, B. A. Murrer and K. R. Harrap, *Cancer Res.*, 1992, **52**, 6188.
7 J. J. Roberts, R. J. Knox, F. Friendlos and D. A. Lydall, in *Biochemical Mechanisms of Platinum Antitumor Drugs*, ed. D. C. H. McBrien and T. F. Slater, IRL Press Ltd., Oxford, 1986, pp. 29–64.
8 W. R. Mason, *Coord. Chem. Rev.*, 1972, **7**, 241.
9 E. Rotondo, V. Fimiani, A. Cavallaro and T. Ainis, *Tumori*, 1983, **69**, 31; E. E. Blatter, J. F. Vollano, B. S. Krishnan and J. C. Dabrowiak, *Biochemistry*, 1984, **23**, 4817; A. Eastman, *Biochem. Pharmacol.*, 1987, **36**, 4177; L. Pendyala, A. V. Arakali, P. Sansone, J. W. Cowens and P. J. Creaven, *Cancer Chemother. Pharmacol.*, 1990, **27**, 248; J. L. van der Veer, A. R. Peters and J. Reedijk, *J. Inorg. Biochem.*, 1986, **26**, 137.
10 T. Shi, J. Berglund and L. I. Elding, *Inorg. Chem.*, 1996, **35**, 3498.
11 T. Shi, J. Berglund and L. I. Elding, *J. Chem. Soc., Dalton Trans.*, 1997, 2703.
12 K. Lemma, T. Shi and L. I. Elding, *Inorg. Chem.*, 2000, **39**, in the press.
13 G. K. Poon, F. I. Raynaud, P. Mistry, D. E. Odell, L. R. Kelland, K. R. Harrap, C. F. J. Barnard and B. A. Murrer, *J. Chromatogr. A*, 1995, **712**, 61; F. I. Raynaud, P. Mistry, A. Donaghue, G. K. Poon, L. R. Kelland, C. F. J. Barnard, B. A. Murrer and K. R. Harrap, *Cancer Chemother. Pharmacol.*, 1996, **38**, 155.

14 M. J. McKeage, G. Abel, L. R. Kelland and K. R. Harrap, *Br. J. Cancer*, 1994, **69**, 1.
15 K. Lemma, J. Berglund, N. Farrell and L. I. Elding, *J. Biol. Inorg. Chem.*, 2000, **5**, in the press.
16 S. Choi, C. Filotto, M. Bisanzo, S. Delaney, D. Lagasee, J. L. Whitworth, A. Jusko, C. Li, N. A. Wood, J. Willingham, A. Schwenker and K. Spaulding, *Inorg. Chem.*, 1998, **37**, 2500.
17 L. Chen, P. F. Lee, J. D. Ranford, J. J. Vittal and S. Y. Wong, *J. Chem. Soc.*, *Dalton Trans.*, 1999, 1209.
18 P. del Socorro Murdoch, N. A. Kratochwil, J. A. Parkinson, M. Patriarca and P. J. Sadler, *Angew. Chem.*, *Int. Ed.*, 1999, **19**, 2949.
19 R. F. Jameson and N. J. Blackburn, *J. Chem. Soc.*, *Dalton Trans.*, 1982, 9; A. J. Holmes and D. L. H. Williams, *Chem. Commun.*, 1998, 1711; R. A. Rickman, R. L. Sorensen, K. D. Watkins and G. Davies, *Inorg. Chem.*, 1977, **16**, 1570; P. Martinez, J. Zuluaga, J. Kraft and R. van Eldik, *Inorg. Chim. Acta*, 1988, **146**, 9.
20 Applied Photophysics SX-18MV, Sequential Stopped-Flow ASVD Spectrofluorimeter, Software manual, Applied Photophysics Ltd., Kingston Road, Leatherhead, UK.
21 Cary WinUV Bio Package, Varian Australia, 679 Springvale Road, Mulgrave, Victoria, Australia 3170.
22 T. G. Appleton, J. W. Connor, J. R. Hall and P. D. Prenzler, *Inorg. Chem.*, 1989, **28**, 2030.
23 S. J. Berners-Price and P. W. Kuchel, *J. Inorg. Biochem.*, 1990, **38**, 305.
24 K. Lemma, D. A. House, N. Retta and L. I. Elding, unpublished work.
25 See, for example: C. Creutz, *Inorg. Chem.*, 1981, **20**, 4449; B. Bänsch, P. Martinez, D. Uribe, J. Zuluaga and R. van Eldik, *Inorg. Chem.*, 1991, **30**, 4555; M. J. Sisely and R. B. Jordan, *Inorg. Chem.*, 1992, **31**, 2137; N. Kagayama, M. Sekiguchi, Y. Inada, H. D. Takagi and S. Funahashi, *Inorg. Chem.*, 1994, **33**, 1881.
26 M. Kimura, M. Yamamoto and S. Yamabe, *J. Chem. Soc.*, *Dalton Trans.*, 1982, 423.
27 D. H. Macartney and A. McAuley, *Can. J. Chem.*, 1981, **59**, 132.
28 D. H. Macartney and N. Sutin, *Inorg. Chim. Acta*, 1983, **74**, 221.
29 U. S. Mehrotra, M. C. Agrawal and S. P. Mushran, *J. Inorg. Nucl. Chem.*, 1970, **32**, 2325; D. J. Evans and M. Green, *Inorg. Chim. Acta*, 1987, 183; R. N. Bose and E. L. Weaver, *J. Chem. Soc.*, *Dalton Trans.*, 1997, 1797; K. Hindmarsh, D. A. House and R. van Eldik, *Inorg. Chim. Acta*, 1998, **278**, 32.
30 H. Taube, *Electron Transfer Reactions of Complex Ions in Solution*, Academic Press, New York 1970, ch. 2.
31 Experimental values for enthalpies of activation are generally more accurate than those for entropies of activation since the former are obtained from slopes and the latter from a long extrapolation to $1/T = 0$ in the Eyring plots.
32 R. G. Wilkins, *Kinetics and Mechanisms of Reactions of Transition Metal Complexes*, VCH Verlag, Weinheim, 2nd edn., 1991, pp. 237–238.
33 R. Kuzu, T. Nakanishi, M. Miyazaki, T. Tashiro, M. Noji, A. Matsuzawa, M. Eriguchi, Y. Takeda, N. Akiyama and Y. Kidani, *Anti-Cancer Drugs*, 1996, **7**, 248.
34 For a summary of the significance of such plots, see for instance: J. H. Espenson, *Chemical Kinetics and Reaction Mechanisms*, McGraw-Hill Inc., New York, 2nd edn., 1995, pp. 164–165.

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