DALTON COMMUNICATION

A long-lived ascorbate radical in the platinum(II) catalysed reductions of platinum(IV) antitumor drugs

Rathindra N. Bose* and Evelyne L. Weaver

Department of Chemistry, Kent State University, Kent, OH 44242, USA

A long-lived ascorbate radical was characterized during the reduction of a series of platinum(IV) antitumor drugs; ascorbate reductions proceed through a platinum(IV)–ascorbate intermediate, the formation of which is catalysed by platinum(II).

A number of platinum(IV) complexes are considered to be prodrugs for the treatment of a variety of cancers.[1–3] It is generally believed that DNA is a cellular target for platinum(II) antitumor drugs.[4] Platinum(IV) complexes are more substitution inert than their platinum(II) analogues and therefore their direct reactions with DNA are extremely slow. It is generally accepted that platinum(IV) compounds are reduced to platinum(II) metabolites[2,3] and that they exert antineoplastic activities following a mechanism similar to that for platinum(II) drugs.[4–7] In fact, platinum(II) metabolites have been identified[8] in the urine and blood plasma of patients receiving iproplatin [*cis*-dichloro-*trans*-dihydroxy-*cis*-bis(isopropylamine)platinum(IV)]. Biological thiols and ascorbic acid are potential reductants in cellular milieu. We report here the detection of a long-lived ascorbate radical in the reaction between iproplatin and ascorbic acid and that the reduction is catalysed by Pt^{II}. This is the first reported example in which a long-lived ascorbate radical has been observed in the reduction of platinum(IV) complexes. Furthermore, the mechanism reported here is remarkably different from that reported in acidic solution[9] in which a straightforward reduction without platinum(II) catalysis and no radical participation was observed.

The reaction of iproplatin with ascorbic acid† afforded stoichiometric amounts of platinum(II) and dehydroascorbic acid, indicating that ascorbic acid functions as a two-electron reductant. The EPR spectra of iproplatin (5–10)–ascorbic acid (10–40 mM) reaction mixtures exhibited two signals separated by 1.76 G (Fig. 1) at pH 7.0. The *g* value, 2.005 and hyperfine coupling constant, 1.76 G are in excellent agreement with that reported for an ascorbate radical.[11] These signals grow within the first 3 min of the reaction and then slowly decay. The radical persists to the end of the reaction. No other signals were observed even at 77 K in a frozen glass (water : glycerol, 1 : 3 by volume). When the reactions were carried out in acidic solutions (pH < 4), no detectable EPR signals were observed. These intense signals were not observed in mixtures of ascorbic and dehydroascorbic acid under identical experimental conditions.

A typical absorbance *vs.* time trace‡ of the reaction with excess ascorbic acid exhibits an initial induction time followed by an accelerated decay in absorbance. Such features commonly characterize autocatalytic reactions. When a ten-fold excess of platinum(II) was added to the reaction mixture, the induction delay disappeared, but the kinetic curves did not exhibit pseudo-first-order features. These curves with excess $[Pt^{II}]$ can be described by a pair of consecutive reactions indicating a growth and decay of an intermediate.‡ The rate constants for the growth and decay of the intermediate were evaluated to be 2.1×10^{-3} (k_0) and 1.7×10^{-3} s^{-1} (k_3) by using 5.0 mM each of ascorbic acid and *cis*-diamminedichloroplatinum(II) and 0.50 mM ipropolatin. When the experiments were performed with

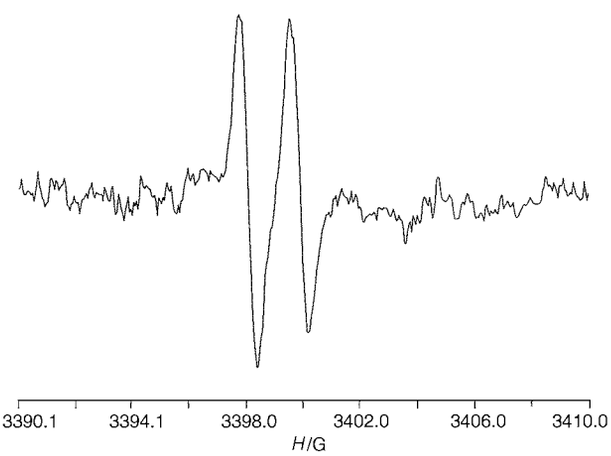


Fig. 1 The EPR spectrum of iproplatin (10.0 mM) and ascorbic acid (50.0 mM) in phosphate buffer at pH 7.0. The *g* value for the signals is 2.005 and the hyperfine coupling constant is 1.76 G. The spectrum was recorded with a modulation amplitude of 1.0 G

† Reactions between Pt^{IV} complexes and ascorbic were monitored by following the change in absorbance at 305, 280 and 260 nm on a UV/VIS or stopped-flow spectrophotometer. Reactions were carried out in 'Tris' [2-amino-2-(hydroxymethyl)propane-1,3-diol] or phosphate buffers at pH 7.0. The EPR measurements were performed on an IBM spectrometer operating at 9.34 GHz at ambient temperature (21 °C) utilizing reaction mixtures containing 5.0–10 mM platinum(IV), with ascorbic acid concentrations 10–40 mM. The signal intensity of the derivative spectra was calculated upon double integration. The magnetic susceptibility of the reaction mixture was measured at various time intervals utilizing a newly developed NMR method.[10] Owing to a small change in bulk susceptibility, 15 mM $[Pt^{IV}]$ and 30–100 mM ascorbic acid were mixed in Tris buffer in D_2O (98% atom). The ^{195}Pt NMR spectra were recorded on a 300 MHz instrument and the chemical shifts are with reference to H_2PtCl_6. Isocratic HPLC separation was accomplished on a C-18 reverse phase column utilizing water–acetonitrile (1 : 1 by volume).

‡ The absorbance *vs.* time data in the presence of Pt^{II} were fitted to equation (1) where A_0, A and A_∞ are absorbance values at $t = 0$, at time t

$$A = A_0 e^{-k_0 t} + k_0 \frac{[Pt^{IV}]\varepsilon_I}{k_3 - k_0}(e^{-k_0 t} - e^{-k_3 t}) + \frac{A_\infty}{k_3 - k_0}(k_0 e^{-k_3 t} - k_{33} e^{-k_0 t}) + A_\infty \quad (1)$$

and at infinite time. The two rate constants k_0 and k_3 relate to the formation and decay of the intermediate, and ε_I is the molar absorptivity of the intermediate. In the absence of added Pt^{II} these traces were subjected to a fitting procedure utilizing the numerical integration of differential equations resulting from the reaction sequence shown in equations (2)–(4) by using Kin.Sim computer programs. The computer simulations yielded concentration profiles which are then converted to absorbance *vs.* time data by introducing the molar absorptivities of all the species.

different ascorbic acid (5.0–50.0) and platinum(II) concentrations (3.0–7.0 mM), k_0 exhibited linear dependence on each of [asc] (asc = ascorbic acid) and [Pt^{II}] while the value of k_3 remained unchanged within the experimental uncertainty (<10%). These data indicate that the formation of the intermediate is a third-order process whereas its decay is mainly a first-order process. In the absence of added Pt^{II}, an additional feature, an induction time was observed. These curves can be satisfactorily simulated‡ by a numerical integration technique using the reaction sequence shown in equations (2)–(4) where

$$Pt^{IV} + asc \xrightarrow{k_1} Pt^{II} + dasc \quad (2)$$

$$Pt^{IV} + asc + Pt^{II} \xrightarrow{k_2} Intermediate + Pt^{II} \quad (3)$$

$$Intermediate \xrightarrow{k_3} Pt^{II} + dasc \quad (4)$$

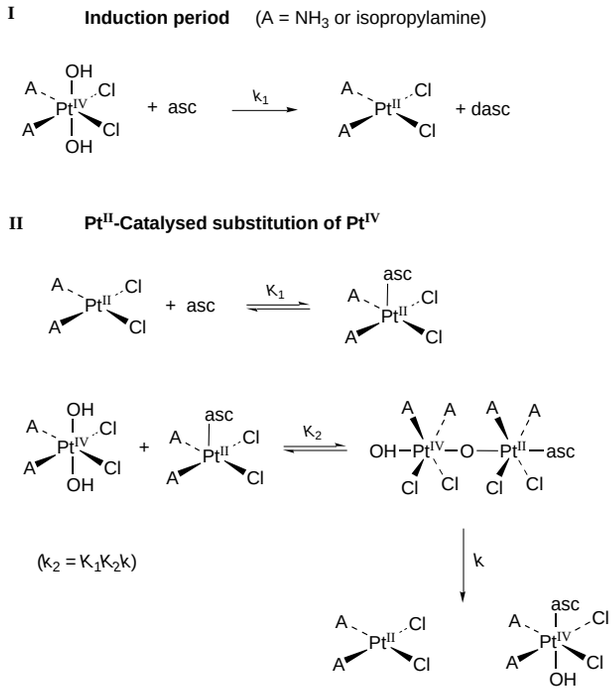
dasc is dehydroascorbic acid. The ligands from the platinum co-ordination sphere are omitted.

The values of k_1, k_2 and k_3 obtained from the numerical integration technique‡ are $(5 \pm 1) \times 10^{-2}$ M^{-1} s^{-1}, $(1.2 \pm 0.7) \times 10^{2}$ M^{-2} s^{-1} and $(1.3 \pm 0.1) \times 10^{-3}$ s^{-1} respectively. Note that the values of third-order ($k_2 = k_0$/[asc]) and first-order (k_3) rate constants are in good agreement with those obtained in the presence of platinum(II). The participation of Pt^{IV}, Pt^{II} and ascorbate in the formation of the intermediate reminds us of the established Pt^{IV} substitution mechanism catalysed by Pt^{II}. Kinetic data indicate that the intermediate is a new platinum(IV) complex co-ordinated to an ascorbate ligand as with the mechanism of Pt^{II}-catalysed Pt^{IV} substitution by Basolo and co-workers.[12] Subsequently, the intermediate decays to products through a first-order process which most likely is an internal ligand (ascorbate) to metal electron-transfer process.

Although ascorbic acid functions as a two-electron reductant, a sequential one-electron transfer process is evident from the detection of the radical. Several reactions might be responsible for the generation of radicals which include reductions of platinum(IV), Pt^{III} and the internal electron-transfer process indicated earlier. However, an accumulation of Pt^{III} can be ruled out based on the low-temperature EPR experiments, implying that platinum(III) generated by a one-electron reduction must be quickly depleted by further reaction with the reducing agent. A mechanism consistent with our data is shown in Scheme 1. By utilizing the available rate data for the disproportionation reaction,[13] we estimate the highest concentration of the radical to be 1–2% of initial platinum(IV) concentrations. In fact, we have attempted to estimate the concentration of the radical by measuring the bulk susceptibility of the reaction mixture assuming that the radical contains one unpaired electron.[10] A small change in bulk susceptibility was observed for the reaction using 15.0 [Pt^{IV}] and 30.0 mM [asc], at pH 7.0 in phosphate buffer. An upper limit of the radical concentration can be placed at less than 3% of the initial platinum(IV) complex which is also consistent with the proposed mechanism.

The formation of the radical is not only limited to the reduction of iproplatin. Reductions of *cis*-diammine-*cis*-dichloro-*trans*-dihydroxyplatinum(IV), tetrachloro-*trans*-dihydroxyplatinum(IV), and *cis*-diamminetetrachloroplatinum(IV) with ascorbic acid are also accompanied by the formation of the same radical (data not shown). Likewise, a sequential one-electron transfer is also encountered during the reaction of iproplatin with glutathione. This thiol reducing agent afforded a thiyl radical§ when a radical capturing agent, DMPO (5,5-dimethyl-3,4-dihydropyrrole *N*-oxide) was added to the reaction mixture.

§ The DMPO–thiyl radical exhibits four-line EPR spectra with an average hyperfine coupling constant of 14.6 G as observed in other redox reactions.[14–17]



Scheme 1

The presence of ascorbate radicals in intra-cellular milieu may provide an additional mechanism for DNA damage if ascorbic acid were to compete effectively with other biological reductants. Apart from the fate of the ascorbate radical, platinum(II)-catalysed ligation of platinum(IV) drugs may lead to unanticipated metabolites depending on the position of the bridging ligand. In iproplatin, hydroxide and chloride bridges are expected to produce different Pt^{II} products. Cheney and co-workers[3] have observed several metabolites in the biotransformation of tetraplatin in L1210 cell lines, some of which were produced during the redox process. The HPLC data on hand also point to a pair of platinum(II) compounds generated during the ascorbate reduction of *cis*-diamminetetrachloroplatinum(IV). Evidence indicates that these metabolites were not derived from subsequent substitution onto the platinum(II) centre. Unfortunately, owing to the limited solubility of platinum(II) products, further characterization by ^{195}Pt NMR spectroscopy was not feasible.

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