Electron Paramagnetic Resonance Spectroscopic Characterization of {2,2-Bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]ethanolato}oxochromate(v) †

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A new chromium(v) complex, {2,2-bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]ethanolato}-oxochromate(v), formed by the ligand-displacement reaction of bis(2-ethyl-2-hydroxybutanoato)-oxochromate(v) with 2,2-bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]ethanol, has been characterized by EPR spectroscopy; the reaction proceeded through a mixed-ligand chromium(v) intermediate.

Hypervalent chromium complexes exert severe toxic effects such as carcinogenesis and mutagenesis.[1,2] Usually, chromium(v) complexes are not stable near neutral pH. Lay and co-workers[3,4] have demonstrated that a circular plasmid DNA, pUC9, is nicked and subsequently cleaved by bis(2-ethyl-2-hydroxybutanoato)oxochromate(v) $[Cr(O)L^1{}_2]^-$ **1** and the corresponding oxochromate(IV) complexes in acidic solutions. The lack of stability[5] of compound **1** forced these workers to examine the DNA damage in acidic solutions.[3,4] In other cases, where metastable chromium(v) has been identified in biological reducing agents, coexistence of organic radicals is documented.[6–10] Therefore, it is not yet clear whether the DNA damage is accomplished by the hypervalent chromium complex or by the radicals.[11] Here we report the EPR characterization of {2,2-bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]-ethanolato}oxochromate(v) which is stable in aqueous solution at physiological pH.

Fig. 1 shows EPR‡ spectra of the reaction between chromium(v) complex **1** (1.0 mmol dm^{-3}) and 2,2-bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]ethanol (H_5L^2) (30 mmol dm^{-3}) at pH 7.6 recorded at various time intervals. The signal at $g = 1.978$ (linewidth 0.7 G) is for the starting chromium(v) complex. As the reaction proceeds, a weak signal at $g = 1.976$ develops, and this persists almost to the end of the reaction. In addition, a signal at $g = 1.965$ (linewidth = 4 G) grows continuously after an induction period with the concomitant decrease of the initial chromium(v) signal. After 20 min, only the signal at $g = 1.965$ was observed. Moreover, no appreciable decrease of the product signal at $g = 1.965$ was apparent 15 min later. This new signal persists for almost 6 h after mixing. Taking the integrated signal intensity of the starting chromium(v) complex at pH 3.3 as the reference,§ and assuming that the intermediate and the product are equally EPR sensitive, we conclude that >95% of the starting chromium(v) complex has been converted to the product. The reaction of complex **1** with H_5L^2 (20 mmol dm^{-3}) was also carried out in the presence of 2-ethyl-2-hydroxybutanoic acid (20 mmol dm^{-3}) at pH 7.6. This reaction yielded the same intermediate and product as before as judged by their EPR signals with $g = 1.976$ and 1.965.

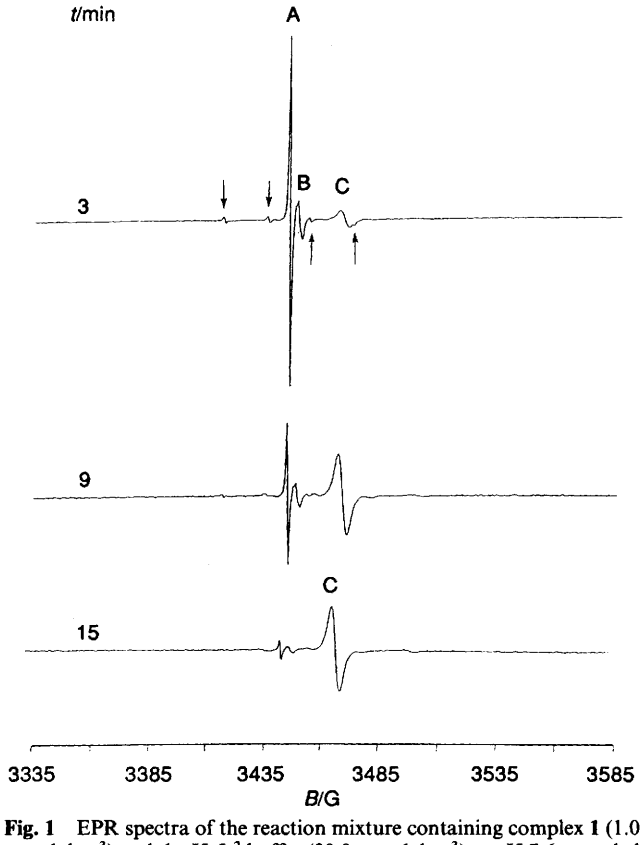


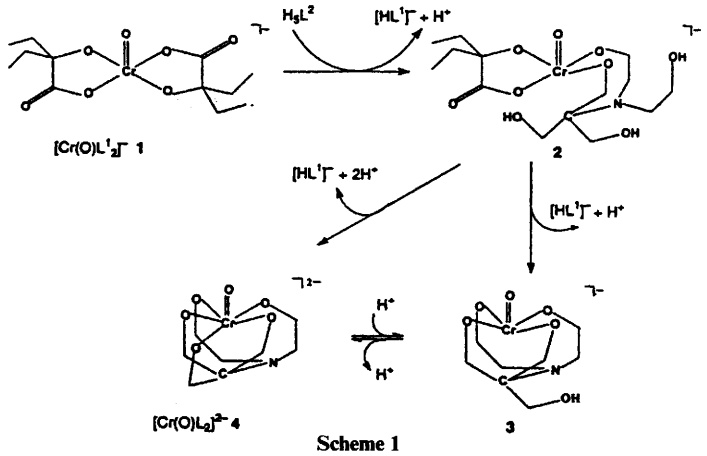
Fig. 1 EPR spectra of the reaction mixture containing complex **1** (1.0 mmol dm^{-3}) and the H_5L^2 buffer (30.0 mmol dm^{-3}) at pH 7.6 recorded at various time intervals. Signals A ($g = 1.978$), B ($g = 1.976$) and C ($g = 1.965$) are for complex **1**, an intermediate and $[Cr^V(O)(L^2)]^{2-}$. Signals due to hyperfine coupling ($A = 18.4$ G) with ^{53}Cr ($I = \frac{3}{2}$, natural abundance 9.8%) are also observed, indicated by arrows. The spectra were recorded with a modulation amplitude of 0.5 G

The EPR data indicate that $(L^2)^{5-}$ replaces the hydroxybutanoic acid and binds the chromium(v) centre through the alkoxy groups. The co-ordination by the polyalcohol proceeds through two distinct phases, as evidenced by the appearance of an intermediate. The first phase is most likely associated with the replacement of one unit of the hydroxybutanoic acid by H_3L^{2-}. The g value of the intermediate is close to that of the $[Cr(O)L^1(CH_2OH)_2]^-$ complex[12] ($g = 1.979$) in which ethane-1,2-diol is co-ordinated through the two alkoxy groups. A mixed-ligand complex **2** in which the polyalcohol co-ordinates through two alkoxy groups would be consistent with the EPR data (Scheme 1).

† *Non-SI unit employed:* G = 10^{-4} T.

‡ The EPR experiments were carried out on an IBM SRC 2000 instrument. Data acquisition and calculation of g values are described elsewhere.[6]

§ Complex **1** is stable at pH 3.3 and disproportionates rapidly in phosphate buffer[5b] near neutral pH. However, this disproportionation in unbuffered solution at neutral pH is slower than the formation of the $[Cr^V(O)(L^1)(H_3L^2)]$ intermediate. Therefore, the loss of Cr^V through the disproportionation reaction was minimal.



Scheme 1

The remaining hydroxybutanoate group is eliminated from the co-ordination sphere due to further chelation by the polyalcohol in the final phase of the reaction. The complete conversion of complex **1** to $[Cr^V(O)L^2]^{2-}$ may be due in part to a large chelate effect exerted by this polyalcoholic ligand. The product may either maintain a square-pyramidal or an octahedral geometry commonly observed for oxochromate(v) complexes.[13–15] In the square-pyramidal complex **3** only four of the five available alkoxy side-chains would be required to co-ordinate. Co-ordination through four alkoxy units, two from each of those bonded to carbon and nitrogen, would impose less steric constraint than that associated with the alternative of three from the carbon and one from nitrogen. Whereas, in the octahedral complex **4**, all available alkoxy groups should participate in bonding. The EPR data support a rapid equilibrium between the five- and six-co-ordinated species as discussed below.

The observed EPR signal of the product is six times broader than that for the starting chromium(v) complex. The square-pyramidal and octahedral complexes are expected to generate 9- and 11-line EPR spectra due to coupling with methylene protons. A hyperfine coupling constant of 0.6 G has been reported for coupling with methylene protons in $[Cr(O)L^1(CH_2OH)]^-$. We were unable to resolve any fine structure within the broad peak even when the spectrum was recorded with 0.2 G modulation amplitude indicating that the hyperfine coupling constant may be <0.2 G. The g values of five- and six-co-ordinated chromium(v) complexes are expected to be very close to each other, presumably due to the weak co-ordinating ability through the basal site of the square pyramid. For example, Srinivasan and Kochi[15] observed a small difference in the g values ($\Delta g = 0.003$) between $[Cr(O)(salen)]^+$ ($g = 1.978$) and $[Cr(O)(salen)(pyO)]^+$ ($g = 1.975$) [salen = *N*,*N*′-ethylenebis(salicylideneiminate)(2−), pyO = pyridine *N*-oxide]. Similarly, a small difference in the g values[16] (0.005) was also reported for $[Cr(O)(ox)_2]^-$ ($g = 1.9766$) and $[Cr(O)(ox)_2(H_2O)]^-$ ($g = 1.9716$) [ox = oxalate(2−)]. The absence of fine structure within the broad peak together with the closeness of the g values between the five- and six-co-ordinated complexes may reflect a rapid equilibrium between these two species. Such an equilibrium between complexes **3** and **4** involves the ligation and deligation of the fifth alkoxy group through the basal site of the square pyramid.

Metastable chromium(v) complexes co-ordinating through alcohols have been reported. For example, a transient EPR signal was observed during the reduction of Cr^{VI} with ascorbic acid in tris(hydroxymethyl)methylamine (tris) buffer. This signal was attributed to a chromium(v)–tris complex.[17] A partial displacement of the hydroxyacid ligand from complex **1** by methanol and ethanol is documented.[18] Furthermore, ethane-1,2-diol also replaces the ligand in **1** to form a bis(ethane-1,2-diol) complex. However, in these cases, the equilibrium constants were not sufficiently large[12] to deplete the starting complex **1**. In addition, polyalkoxy ligands, including sugars, are shown to intercept transient Cr^V species during the redox transformation of Cr^{VI} with biological reducing agents.[19]

Many biological reactions are carried out in the H_5L^2 buffer. The facile *in situ* formation of the chromium(v)–H_5L^2 complex may offer a unique opportunity to examine nucleotide oxidations at the molecular level under physiological conditions. In fact, significant cleavage of single-stranded calf thymus DNA has been observed[20] by this new complex specifically at guanine bases.

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