Chromium(V) Complexes Can Generate Hydroxyl Radicals

PAUL O'BRIEN* and GUOFANG WANG

Department of Chemistry, Queen Mary College, University of London, Mile End Rd., London E1 4NS (U.K.)

(Received April 4, 1989)

Chromate is well known to be both carcinogenic and mutagenic [1]. However, chromium(VI) is itself unable to damage DNA *in vitro*, and it is generally believed that reactive intermediates produced during the reduction of chromium(VI) *in vitro* are responsible for the toxicity of chromate [1–3]. Glutathione (GSH) is one likely intra-cellular reductant and various workers have shown that chromium(V) complexes can be generated during the reduction of chromate by GSH at neutral pH [4–8]. Attention has recently focussed on other intermediates such as thionyl radicals ($RS^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$); two recent studies [7, 8] have used spin trapping experiments to provide evidence for the generation of $RS^{\bullet}$ during the reduction of Cr(VI) by GSH. There is evidence that in the presence of traces of iron (iron-(III) EDTA or iron(II) salts) GSH can generate $OH^{\bullet}$ [9], and $RSH/RS^{\bullet}$ could provide a route to $OH^{\bullet}$ *in vivo*.

There is controversy concerning the ability of chromium(VI) and glutathione to cause strand breaks in closed-circle DNA assays. Both Wetterhahn *et al.* [10] and Kawanashi *et al.* [11] have found no strand breaks in the absence of H_2O_2, whereas we have found both Cr(VI)/GSH mixtures and an intermediate chromium(V) complex of GSH able to cause such breaks [12]. The evidence presented to date [7, 8] does not rule out the formation of $OH^{\bullet}$ during the reduction of chromate *in vivo* or *in vitro*. The generation of radicals in the reduction of chromium(V) has been suggested (particularly $S^{\bullet}$, where S is a solvent molecule) as one route in organic oxidations [13, 14].

In this communication results are presented which demonstrate that chromium(V) can generate hydroxyl radicals. These results may be of relevance to a fuller understanding of the mechanisms of chromate toxicity.

Results and Discussion

In the present study three chromium(V) complexes have been investigated: $[N(Me)_4][CrOCl_4]$ [15], sodium bis(2-hydroxy-2-methylpropionato)-oxochromate(V) [16], and an intermediate, chromium(V) complex isolated from the reaction of GSH with chromate [17]. These solid compounds were mixed in silica EPR tubes with 0.1 mol dm^{-3} solutions of the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) [7, 8, 18]. The glutathione intermediate produced an EPR spectrum with the now well-known peaks at $g = 1.996$ and $g = 1.985$ due to chromium(V) species, together with a four-line spectrum of the kind typically associated with $RS^{\bullet}$ [4–8]. The hydroxy acid complex produced a typical chromium(V) spectrum with no evidence for any trapped radical species. The oxochromium(V) chloride complex [15], however, generated the spectrum shown in Fig. 1. The spectrum is charac-

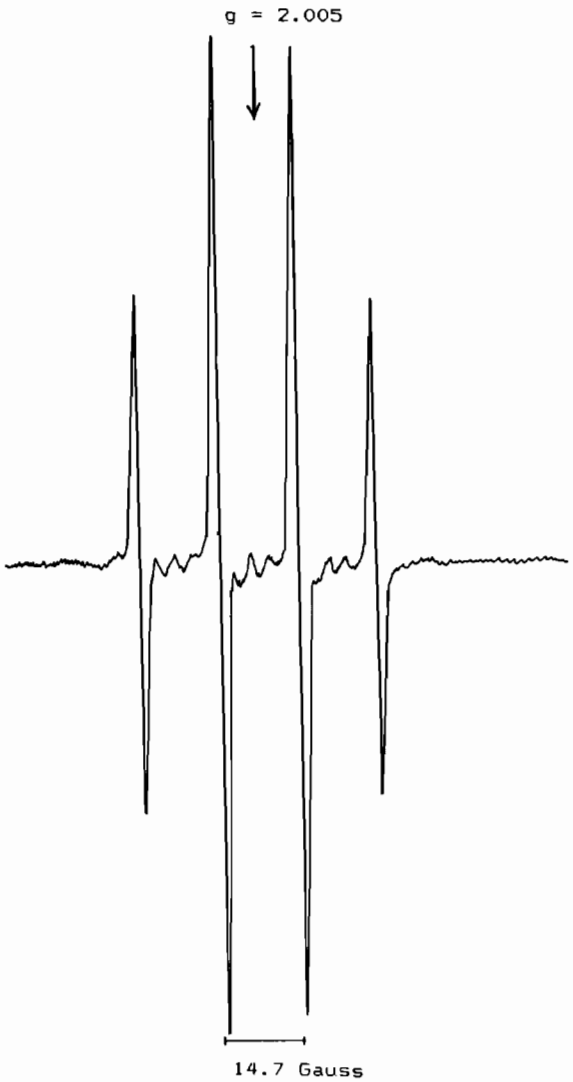

Fig. 1. Typical EPR spectrum observed on mixing $[N(Me)_4][CrOCl_4]$ and DMPO (5 min after mixing).

*Author to whom correspondence should be addressed.

0020-1693/89/$3.50

teristic of $OH^{\bullet}$, $a^N = a^H = 14.7$ G, and $g = 2.005$ [8, 17]. Presumably the chloride complex of chromium(V) aquates, and the resulting aquo complex decomposes to generate the hydroxyl radicals trapped by DMPO. The other chromium(V) complexes studied provide other substrates for oxidation, in the form of their ligands. The weakly coordinated chromium(V) centre in the chloro complex is hence, probably, for kinetic reasons, best able to generate concentrations of OH, sufficient to be detected by the spin trap.

Experimental

Potassium dichromate was BDH AnalaR grade, DMPO was purchased from Aldrich and glutathione from Sigma; all other chemicals were purchased from BDH Chemicals. Chromium(V) complexes were synthesised by literature methods [15–17]. EPR spectra were recorded with a Bruker ERD/2000/10 instrument, 5–10 min after mixing a 0.1 mol dm^{-3} solution (*c.* 5 ml) with 5–10 mg of the solid chromium(V) complex.

Conclusions

The results of this study clearly show that the reduction of chromium(V) in aqueous solution can generate hydroxyl radicals. Although hydroxyl radicals have not been observed in the reduction of chromate by glutathione, this observation could be related to the known ability of GSH to trap hydroxyl radicals [9]. The radicals trapped in any such experiments will be a complex function of the conditions of the experiment and it is difficult to exclude the possibility of hydroxide radical formation *in vivo* on the basis of spin trapping. We are at present investigating aromatic hydroxylation assays [9] as an alternative method of probing intermediates generated in such reactions.

Acknowledgements

This work has been supported by a grant from the Cancer Research Campaign with some additional support from The University of London Central Research Fund. We thank Dr D. Oduwole of this department for assistance in measuring the EPR spectra.

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