Chromium(V) can be Generated in the Reduction of Chromium(VI) by Glutathione

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The carcinogenicity and mutagenicity of chromium(VI) are well established [1]. This toxicity is usually considered in terms of the uptake/reduction model [1]: chromium(VI) readily passing into the cell, via anion channels and once within the cell being eventually reduced by cellular components to chromium(III) species. These complexes are trapped within the cell and may be the agents responsible for the toxic effects of chromate. The systems which reduce chromium(VI) are as yet unknown, as are the final products of the reaction. However microsomes [2] are capable of reducing chromium(VI) as are various nucleotides [3], in both of these cases chromium(V) species of considerable stability have been observed using EPR spectroscopy. Very recently chromium(V) has been observed in the EPR spectrum of frozen glasses obtained from reaction mixtures of chromium(VI) and glutathione [4]. In this letter we report that chromium(V) species of considerable stability can be generated in the reduction of chromium(VI) by glutathione.

Glutathione (γ-glutamyl-cysteinyl-glycine) is an intra-cellular peptide important in the maintenance of redox status [5], and it is found at millimolar concentrations in typical mammalian cells. It is implicated, in general, in the defence of cells challenged with oxidising agents. However, in the case of chromate toxicity cells with elevated levels of GSH have been shown to have an increased susceptibility to damage [6], perhaps indicating that a chromium complex of glutathione is an active intra-cellular toxin. We have studied the reaction of GSH with chromate at neutral pH and have directly observed the formation of chromium(V) complexes in aqueous solution at room temperature. Our results are compared with a recent study [7] of the reaction of L-cysteine with chromium(VI) at neutral pH and the relevance of these observations to chromate toxicity is discussed.

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The reaction of GSH with chromium(VI) has previously been studied in acidic solutions [8], in which the complete reduction of chromate to chromium(III) is rapid. Mechanisms involving thiolate esters of chromium(VI) which react with either protons or GSH have been suggested to be important. We observed that at neutral values of pH, solutions of chromium(VI) and glutathione (1:10, see Table I for details) rapidly develop a green colour, which is characterized by a single λ_{max} 650 nm peak in the visible region of the electronic spectrum. The peak slowly decays and a purple solution typical of chromium(III) complexes is the final product. Concurrent with the green colour the typical EPR spectrum of a chromium(V) species is observed. The first order decay of the EPR signal parallels the decrease in absorbance at 650 nm, strongly suggesting that both are characteristic of the same complex. Results are reported in Table I and the decay of the solution EPR spectrum approximately 10 min after mixing is illustrated in Fig. 1. The EPR spectrum is typical of those reported for chromium(V) complexes [9]: a characteristically narrow line and some evidence for hyper-fine structure due to ^{53}Cr (9.55% abundance) which confirms that the peak is due to chromium. In some later experiments we have clearly observed the four satellite lines due to ^{53}Cr ($I = 3/2$), however the time course was not followed in these cases. Solid complexes with similar spectroscopic properties can be isolated from such solutions, these may be stable chromium(V) species.

TABLE I. Summary of Typical Observations.

Observation	Comment
2.39×10^{-3}	Disappearance of EPR signal (k_{obs}/s^{-1})[a]
2.14×10^{-3}	Disappearance of λ_{max} 650 nm peak (k_{obs}/s^{-1})[b]
1.996	g_{av}

[a] 21 °C, pH = 7.00, GSH = 1.00 m dm^{-3}, [GSH] : [Cr(VI)] = 10:1. [b] 21 °C, pH = 7.00, GSH = 0.1–0.025 m dm^{-3}, [GSH] : [Cr(VI)] = 10:1.

The reaction of L-cysteine with Cr(VI) at neutral pH has been studied in detail [7], the product of the reaction was suggested to be the bis-*trans*-S complex of cysteine characterized crystallographically by DeMeester *et al.* [10], no evidence for Cr(V) species could be obtained, and the rate determining step was suggested to involve two-electron transfer. It has been reported [11] that initial one-electron reduction of chromium(VI) leads to chromium(III) complexes in which the oxidised form of substrate is coordinated. Our results, which clearly show the presence of

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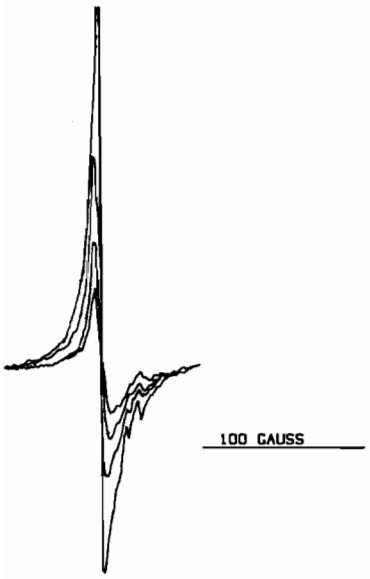

Fig. 1. EPR spectrum of Cr(V). g = 1.995 (9.270 MHz). Spectra recorded at *ca.* 10 min after mixing and at 280, 479 and 668 s. (first order rate constant $k = 2.39 \times 10^{-3}$ s^{-1}). pH = 7.0. [GSH] : [Cr(VI)] = 10:1. [GSH] = 1.0 m dm^{-3}.

chromium(V), hence suggest that GSSG may be captured in the reaction of GSH with chromium(VI). This is in marked contrast to the results of Pennington [7] with L-cysteine, but Wetterhahn [1] has some evidence for Cr(III)/GSSG complexes from such reactions. The substantial stability of the chromium-(V) complexes we have generated in solution ($t_{0.5}$ *ca.* 300 s, 21 °C) suggests that if similar complexes are formed *in vivo* they would have time to reach many intracellular compartments and could hence be active intermediates in the toxicity of chromate. A detailed study of the reduction of chromate by various biological reducing agents (in neutral, buffered solutions, at lower GSH concentrations) indicates that chromium(V) species are not generated [12], however the conditions studied were somewhat different from the ones used in the present study. The way in which the dominant reduction mechanism for chromate changes with the reaction conditions is not as yet clear. As outlined above the products of the reaction may depend on the mechanism of reduction and these, as yet, unidentified chromium complexes are probably the agents responsible for the mutagenicity of chromate.

The present work indicates that chromium(V) complexes of considerable stability can be generated by the reaction of GSH with chromate. Preliminary results indicate that these complexes are isolable. Our present studies are aimed at the characterization of both the isolable intermediates and the final products of the reaction. The isolated complexes will then be tested for biological activity.

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