# A Potentially Significant One-electron Pathway in the Reduction of Chromate by Glutathione Under Physiological Conditions

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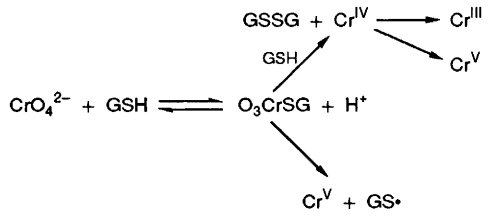
A one-electron path is shown to be a potentially important route in the reduction of chromate by glutathione at neutral pH; this pathway may be significant in the generation of reactive intermediates important in understanding chromate toxocity.

The toxicity of chromate has been extensively studied in recent years.[1] Chromium(VI), predominantly chromate $[CrO_4]^{2-}$ at neutral pH, can enter cells *via* non-specific anion carriers[2] and once within the cell reduction to chromium(III) occurs.[3] The absence of any *in vitro* effects of chromate on DNA has led to the hypothesis that either reactive intermediates generated during the reduction[4] (causing for example DNA strand breaks), or the binding of chromium(III) complexes to DNA[5] (leading to cross links) are ultimately responsible for chromate toxicity.

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is likely to account for much of the cytoplasmic reduction of chromate.[1] Among the reactive intermediates observed, at room temperature, during the reduction of chromate by GSH are: chromium(V) complexes,[6] thiyl radicals[1] and active oxygen species[7] (especially after the addition of hydrogen peroxide). There have been a number of studies of the reduction of chromate by thiols at neutral pH.[8,9] The accepted mechanism for glutathione[8] involves a thiolate ester, formed in a rapid preequilibrium step, which is reduced by a second mole of GSH, leading to an initial two-electron reduction of chromate.

The major evidence for the predominance of the two-




**Scheme 1**

**Table 1** Pseudo-first-order rate constants for the reduction of chromate by GSH[a]

| [GSH]/$10^{-2}$ mol $dm^{-3}$ | $k_{obs}$/$10^{-2}$ $s^{-1}$[b] |
|---|---|
| 2.0 | 0.527 |
| 4.0 | 1.21 |
| 6.0 | 1.95 |
| 8.0 | 2.52 |
| 10.0 | 3.38 |

[a] The pseudo-first-order rate constants ($k_{obs}$, 370 nm) for the disappearance of chromate in the presence of GSH in HEPES 0.05, [$CrO_4^{2-}$] 1 × $10^{-4}$ mol $dm^{-3}$, the reported values are the average of duplicate runs. (pH = 7.0, at 25 °C.) [b] $k_{obs}$ = 0.358(±0.015) × 131(±59)[GSH]²/(1 + 131(±59)[GSH]).

**Table 2** Initial rate of disappearance of chromate[a,b]

| [GSH]/$10^{-5}$ mol $dm^{-3}$ | Rate/$10^{-10}$ mol $dm^{-3}$ $s^{-1}$ |
|---|---|
| 5 | 1.25 |
| 7 | 1.53 |
| 10 | 2.39 |
| 12 | 3.05 |
| 15 | 3.61 |

[a] d[$CrO_4^{2-}$]/d$t$ = 2.5(±0.2)[GSH] × $10^{-6}$ mol $s^{-1}$. [b] Measured at 370 nm. [$CrO_4^{2-}$] = 1 × $10^{-4}$ mol $dm^3$, 25.0 °C, pH 7, HEPES (0.05 mol $dm^{-3}$).

electron route is the direct observation of the thiolate ester intermediate, and the dependence of the pseudo-first-order rate constant (for chromate disapperance) on the GSH concentration. The rate law, eqn. (1), is obeyed. However, a one-electron, proton-mediated ([$H^+$]²) pathway is observed for the reduction of chromate by GSH in acidic solution[10] this route is unlikely to be significant at neutral pH.

$$k_{obs} = Kk_2[GSH]^2/(1 + K[GSH]) \quad (1)$$

It is hard to account for the observation of the thiyl radical at neutral pH on the basis of an initial two-electron reduction. Indeed, if the chromium(v) species generated by disproportionation (see Scheme 1) undergoes a clean two-electron reduction, toxic, reactive intermediates are not likely to be generated. But, much of our work suggests that species capable of causing oxidative damage can be generated during the reduction of chromate by glutathione. Consequently, we decided to investigate the possibility that a minor one-electron pathway operates at neutral pH (see Scheme 1).

All studies were carried out by following changes in the absorbance of chromate at 370 nm [25 °C, pH 7.0, 0.05 mol $dm^{-3}$ HEPES buffer, HEPES = *N'*-(2-hydroxyethyl)-piperazine-*N*-ethanesulfonic acid)]; chromate solutions were found to be stable for up to 5 h in this buffer. An initial series of experiments served to confirm rate law (1) ([GSH] > 0.01 mol $cm^{-3}$) and results are summarized in Table 1. Using eqn. (1) values for $k_2$ and $K$ may be determined, by non-linear least-squares analysis, as 0.358(±0.015) $dm^3$ $mol^{-1}$ $s^{-1}$ and 131(±59), respectively; values which are in reasonable agreement with the values reported previously, 0.2 $dm^3$ $mol^{-1}$ $s^{-1}$ and 20, respectively[8] (25 °C, pH 7.4, 1 mol $dm^{-3}$ Tris). Stopped-flow experiments confirmed that there was an initial rapid step, with an increase in absorbance at 450 and a decrease at 370 nm, corresponding to the formation of the thiolate ester.

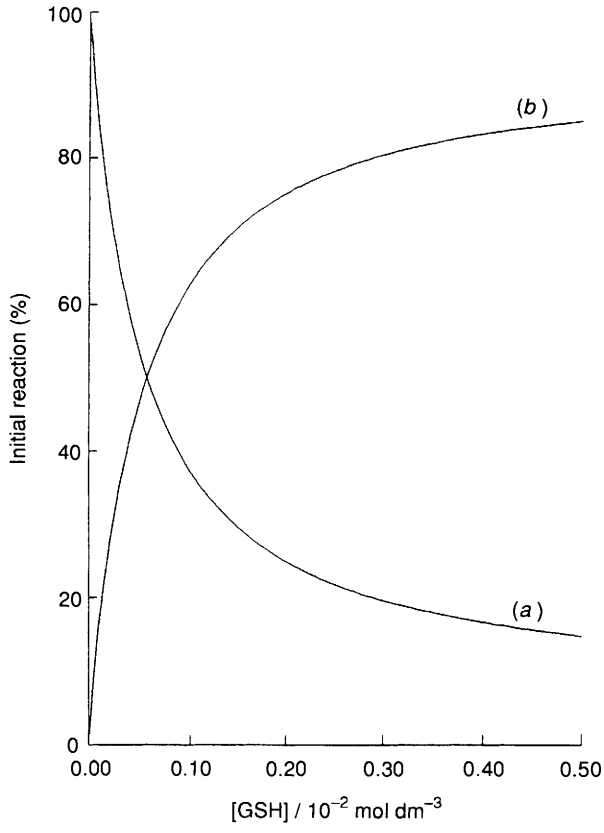



**Fig. 1** Relative importance of one- (*a*) and two-electron (*b*) routes in the initial reduction of chromate in the physiological range of GSH concentration (extrapolated from the results in Tables 1 and 2)

We reasoned that any minor one-electron path to reduction might best be detected at low GSH concentrations and have studied the rate of disappearance of chromate under rather different conditions (1 × $10^{-4}$ mol $dm^{-3}$) at GSH (5–15 × $10^{-5}$ mol $dm^{-3}$); results are summarized in Table 2 and in Fig. 1. The reaction was characterized by an initial rapid, small, change in absorbance, thiolate ester formation (typically in the region of 1% of the chromate), followed by a slower disappearance of chromate. The loss of chromate was followed for 1–2 h and the initial rate of chromate disappearance determined (*ca.* 1–2.5% of reaction). The results clearly show that, under these conditions, the disappearance of chromate is first order in glutathione (rate = 2.5 × $10^{-6}$ [GSH] mol $dm^{-3}$ $s^{-1}$).† However, we would expect eqn. (1) to collapse into a quadratic at low concentrations of GSH. In the absence of a pseudo-first-order excess of either reactant it is hard to establish formally, the order of the reaction. However, if we assume a first-order process, following an initial preequilibrium step ($K$ = 131), the first-order rate constant for the disappearance of the thiolate ester would be 1.9 × $10^{-4}$ $s^{-1}$.

In related work, we have sought evidence for a one-electron path at higher concentrations of GSH and chromate in

† Similar results have been obtained in Tris buffer. Although as presented the results in Tables 1 and 2 seem to indicate that both the one- and two-electron routes should be observed at low concentration of GSH. It must be appreciated that extrapolation from results at much higher concentrations of GSH is difficult and the equilibrium constant is both poorly determined, and sensitive to the precise reaction conditions. The linearity of the initial rate, and colinearity with the origin, at low GSH concentrations strongly suggests that the one electron route is predominating under these conditions.

spin-trapping experiments with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). In the concentration range 0.005–0.025 mol $dm^{-3}$ GSH (pH = 7.0, $[CrO_4^{2-}]$ $1 \times 10^{-3}$ mol $dm^{-3}$, [DMPO] 0.05 mol $dm^{-3}$) the yield of the spin adduct characteristic of the thiyl radical is linear in the GSH concentration; further, indirect, evidence for a one-electron route.

These results indicate that the one-electron path for the reduction of chromate by GSH may be significant at physiological concentrations of GSH. Fig. 1 shows the percentage contribution of the two paths to the initial rate of chromate reduction. One-electron reduction could result in the release of thiyl radicals into the solution, these species represent one possible route to reactive oxygen species and strand breaks.[7] The chromium(v), thus generated, is likely to be free of ligands and more reactive than the relatively stable chromium(v) GSH complexes have been identified in solution.[7]

Chromate can cause a wide range of DNA lesions *in vitro* including: strand breaks, hydroxylations and cross links (of various kinds). Cross linking may be particularly important as a marker for chromate exposure but which, if any, of these routes leads to the mutagenicity/carcinogenicity of chromate is far from clear. Reactive intermediates may have a role in oxidative damage and initiate and/or be involved in both Fenton and pseudo-Fenton chemistry, direct oxidative damage by high oxidation state chromium species may also be important. In order to properly understand the system we need to appreciate the routes for chromate reduction by likely reductants such as ascorbate and glutathione. The present communication suggests that the reduction of chromate, under physiological conditions of pH may be more complicated than at present appreciated.

We conclude that a one-electron route may be a significant source of reactive intermediates in the reduction of chromate by GSH at neutral pH. Highly oxidizing intermediates are the most likely explanation for the formation strand breaks, which have been observed in a wide range of assays of chromate toxicity.

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