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Characterization of Long-lived Chromium(IV) Intermediates in Glutathione mediated Chromium(VI) Metabolites

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Glutathione mediated reduction of chromium(VI) yielded a long-lived chromium(IV) intermediate in acidic aqueous solution, the molar susceptibility and the magnetic moment of this hypervalent species are 3.4(± 0.2) × 10^{-3} cm^3 mol^{-1} and 2.8(± 0.1) μ_B at 25 °C; the first-order rate constants for the formation of the Cr^{IV} intermediate and its decay to Cr^{III} products were calculated to be 3.1 × 10^{-2} and 6.0 × 10^{-3} s^{-1} using excess tripeptide (25.0 mmol dm^{-3}).

The hypervalent chromium species formed during the reduction of Cr^{VI} by various cellular proteins, peptides and other biological reducing agents are believed to be responsible for the carcinogenic and mutagenic activities[1] of chromium(VI). The reduction of chromium(VI) by a number of biological reducing agents, including glutathione, has yielded long-lived EPR active chromium(V) intermediates[2–4] near neutral pH. The other less common chromium(IV) intermediates might have escaped detection by EPR spectroscopy at room temperature in aqueous solution.† We report here the characterization of long-lived chromium(IV) intermediates in acidic solution for the Cr^{VI}–glutathione reaction. The characterization of this rare oxidation state is based on time domained magnetic susceptibility measurements in solution using a nuclear magnetic resonance technique.[5] To the best of our knowledge this is the first report that establishes unequivocally the existence of Cr^{IV} species in aqueous acidic solution using a biological reducing agent.

The reaction of dichromate anion with glutathione (GSH) passes through an absorbing intermediate with λ_{max} at 460 nm. Both titrimetric analysis[6] and HPLC estimation‡ of the organic products reveal that each mole of Cr^{VI} reacts with three moles of the tripeptide to yield 1 mol Cr^{III} and 1.5 mol disulfide (GSSG). EPR measurements did not detect any signal during

† This triplet spin state is not detected by EPR spectroscopy at room temperature owing to a number of reasons, including larger zero-field splitting, faster relaxation and higher spin–orbit couplings.

‡ HPLC analyses were performed on a gradient ternary instrument (ISCO) utilizing H_3PO_3–$H_2PO_4^-$ buffer (pH = 3.0) as a mobile phase and a C-18 reversed column at 267 nm.

Mendeleev Commun., 1991, 1(3), 108–110

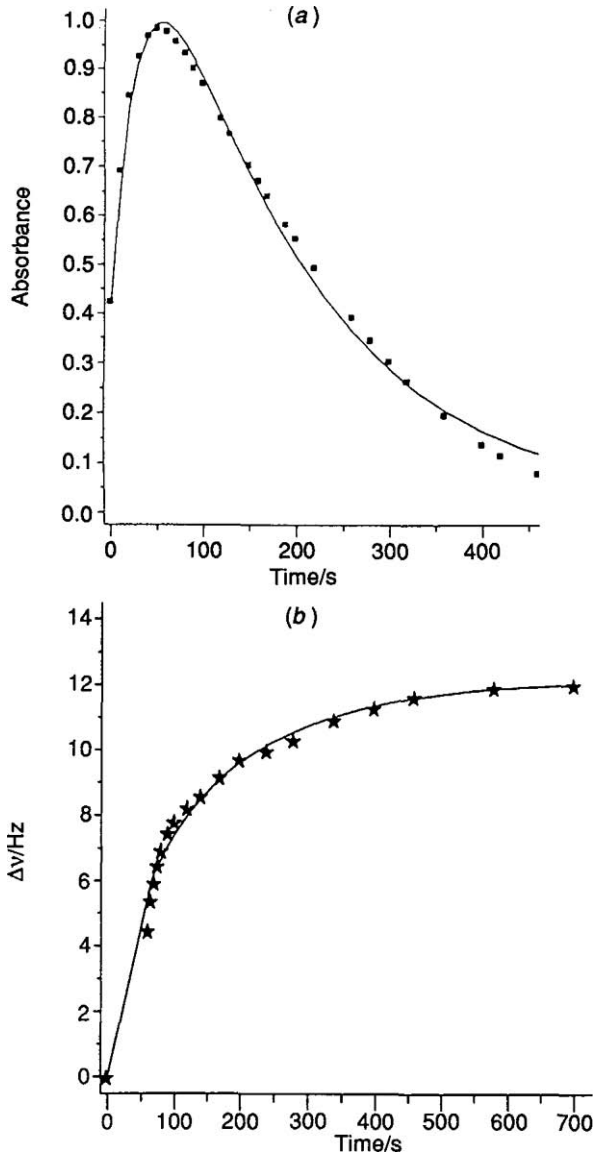

Fig. 1 Observed (solid rectangles and asterisks) and simulated (solid line) absorbance–time (*a*) and $\Delta\nu$–time curves (*b*) for the redox reaction between glutathione (25.0 mmol dm^{-3}) and chromium(VI) [1.0 mmol dm^{-3} and 1.25 mmol dm^{-3} for (*a*) and (*b*)] at pH 2.70, $T = 25$ °C. The simulated curves correspond to $k_1 = 3.0 \times 10^{-2}$, $k_2 = 6.2 \times 10^{-3}$ s^{-1}, $\epsilon_I = 1390$ cm^{-1} mol^{-1} dm^3, and $D_\infty = 0.02$ for curve (*a*), and $k_1 = 3.1 \times 10^{-2}$, $k_2 = 5.8 \times 10^{-3}$ s^{-1}, $a_1 = -4.08$, $a_2 = -8.18$, and $c = 11.52$ for curve (*b*).

the experiments even utilizing higher concentrations of chromium(VI) (10 mmol dm^{-3}) and glutathione (10–50 mmol dm^{-3}). Fig. 1(*a*) shows the absorbance–time trace§ for the reaction at pH 2.70 using excess tripeptide. Fig. 1(*b*) exhibits the change in the resonance frequency of the H–O–D signal§ during the same reaction in aqueous solution containing 5% D_2O. The change in frequency $\Delta\nu$ is related to the rate of formation of the intermediates and products along with the molar susceptibility of the paramagnetic species involved in eqn. (1),[5] where k_1 and k_2 are the rate constants for the formation of the intermediate and for its decay, respectively. The parameters a_1, a_2 and c are defined in eqns. (2)–(4),[5] where M is the initial concentration of

$$\Delta\nu = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} + c \quad (1)$$

$$a_1 = \frac{4\pi\nu M}{3(k_2 - k_1)} (\chi'_I k_1 - \chi'_P k_2) \quad (2)$$

$$a_2 = \frac{4\pi\nu k_1 M}{3(k_2 - k_1)} (\chi'_P - \chi'_I) \quad (3)$$

$$c = \frac{4\pi\nu M \chi'_P}{3} \quad (4)$$

Cr^{VI} (mol ml^{-1}), ν is the spectrometer frequency, and χ'_I and χ'_P are the uncorrected molar susceptibilities of intermediate and products.

A nonlinear least-squares iterative computer fit[7] of the frequency–time data yielded values of a_1 and a_2 of -4.08 and -8.18 along with the rate constants for the formation (k_1) of the intermediate and its decay (k_2) of 3.1×10^{-2} and 5.8×10^{-3} s^{-1}. These rate constants are in excellent agreement with the corresponding values, 3.0×10^{-2} and 6.2×10^{-3} s^{-1}, obtained from the absorbance–time profile.[8] The rate of decomposition of the intermediate is a straightforward second-order process, first order with respect to each reactant. However, the first-order rate constant for the formation of the intermediate exhibits complex dependence on the glutathione concentrations. By using the values of the preexponential parameters, we obtained uncorrected molar susceptibilities¶ for the intermediate and products of $3.4(\pm 0.2) \times 10^{-3}$ and $7.3(\pm 0.3) \times 10^{-3}$ cm^3 mol^{-1}. The magnetic moments of the intermediate and products can then be calculated as $2.8(\pm 0.1)$ and $4.2(\pm 0.1)$ μ_B. These values are within the range of expected magnetic moments for Cr^{IV} and Cr^{III} species containing two and three unpaired electrons.

These magnetic susceptibility results unequivocally establish that the long-lived intermediate is a chromium(IV) species. Although the detailed mechanism of formation of the chromium(IV) intermediate in acidic solutions is yet to be elucidated,[9] the failure to detect any EPR signal suggests that the rate of disproportionation of the Cr^{IV} complex is small compared to that for its further reduction by the tripeptide. A rapid disproportionation of the Cr^{IV} species has been attributed for the formation of chromium(V) intermediates at neutral pH by O'Brien and Ozolins.[10] The former oxidation state is formed by a rate-limiting two electron transfer reaction between a rapidly equilibrating Cr^{VI}–thioester complex and a glutathione molecule.[10] Although the influence of pH on the rate of disproportionation of Cr^{IV} complexes is not known, the rates of the same reaction for the Cr^{V} complexes[11] are markedly accelerated at higher pH. Connett and Wetterhahn[12] proposed a general mechanism for the reduction of chromium(VI) by a number of thiol ligands in which parallel one- and two-electron transactions within a Cr^{VI}–thioester complex directly led to the formation of Cr^{V} and Cr^{IV} intermediates. If such a mechanism is operative, subsequent reduction of Cr^{V} with a second molecule of glutathione must be rapid in acidic solutions since we do not observe any detectable Cr^{V} species. Furthermore, the long-lived intermediate is not a Cr^{IV}–thioester complex in acidic solution as proposed by McAuley and Olatunji.[9]

Finally the mutagenic and carcinogenic activities of Cr^{IV} metabolites are not known although extensive DNA damage has been reported for Cr^{V} complexes.[1,2] Careful selection of experimental conditions may lead to the isolation of stable Cr^{IV} complexes and their interaction with nucleic acids can be explored further. Since triplet chromium(IV) states have so far escaped detection in ESR experiments, it remains to be seen whether both Cr^{IV} and Cr^{V} intermediates are formed at physiological pH in cellular milieu. Our NMR-based kinetic method should be valuable in unveiling the compositions of such mixtures of paramagnetic intermediates.

§ UV-VIS spectroscopic measurements were carried out on a computer interfaced Perkin-Elmer spectrophotometer (Lambda 600). The NMR experiments were performed on a 300 MHz GE instrument (GN 300) equipped with variable temperature probe heads.

¶ We have shown (ref. 6) that these uncorrected molar susceptibility and magnetic moments are within 5% of the actual values and should not impose any problem in distinguishing alternative oxidation states.

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References

1 H. H. Sky-Peck, *Clin. Physiol. Biochem.*, 1986, **4**, 99; S. C. Rossi and K. E. Wetterhahn, *Carcinogenesis*, 1989, **10**, 913; A. Aiyar, H. J. Berkovits, R. A. Floyd and K. E. Wetterhahn, *Chem. Res. Toxicol.*, 1990, **3**, 595.
2. K. E. Wetterhahn, D. Y. Cupo and P. H. Connett, *Trace Subst. Environ. Health*, 1984, **18**, 154.
3 D. M. L. Goodgame and A. M. J. Joy, *Inorg. Biochem.*, 1986, **26**, 219.
4 S. Kitagawa, H. Seki, F. Kametani and H. Sakurai, *Inorg. Chim. Acta*, 1988, **152**,251.
5 R. N. Bose, D. Li and S. Moghaddas, *Anal. Chem.*, submitted for publication. This method is based on the Evans method for magnetic susceptibility measurements in solution: D. F. Evans, *J. Chem. Soc.*, 1959, 2003.
6 G. E. Woodward and E. G. Fry, *J. Biol. Chem.*, 1932, **97**, 465.
7 K. A. Easom and R. N. Bose, *Inorg. Chem.*, 1988, **27**, 2331.
8 The rate expression for absorbance–time traces is similar to eqn. 1 except that the a_1 and a_2 term contain molar absorptivities of the reactants, intermediates and products. See, for example: R. N. Bose, R. E. Viola and R. D. Cornelius, *J. Am. Chem. Soc.*, 1984, **106**, 3336.
9 McAuley and Olatunji (A. McAuley and M. A. Olatunji, *Can. J. Chem.*, 1977, **55**, 3335) proposed a mechanism in acidic solution in which the long-lived intermediate is assigned to a Cr^{IV}–thioester complex and that the second phase of the reaction is the reduction of this thioester complex to Cr^{III}. Our data do not support such a mechanism.
10 P. O'Brien and Z. Ozolins, *Inorg. Chim. Acta*, 1989, **161**, 261.
11 M. Krumpolc and J. Rocek, *Inorg. Chem.*, 1985, **24**, 617.
12 P. H. Connett and K. E. Wetterhahn, *J. Am. Chem. Soc.*, 1985, **107**, 4282.