A Homodinuclear $Cr^{V}-Cr^{V}$ Complex Forms from the Chromate−Glutathione Reaction in Water

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Although chromium was one of the first metals to be recognized as being toxic, its compounds being potent inorganic mutagens and carcinogens,[1] the molecular mechanisms underlying its uptake and metabolism are not presently well understood.

It is generally agreed that the mutagenic pathway is initiated by Cr^{VI}.[2] Chromate or dichromate may in fact easily enter the cell through the sulfate anionic channel.[3] Many potential reducing agents are located within the cell cytoplasm; however, only glutathione (GSH) and ascorbic acid were shown to reduce Cr^{VI} at a significant rate at pH 7.4.[4] Reduction of dichromate with GSH has been investigated, suggesting formation of a monodentate thiolate $chromium^{VI}$−GSH complex, $GSCrO_3^-$, at acidic pH.[4,5] This last species is not stable at higher pH, and it disappears within ca. 30 min, leaving only a strong absorption in the UV region at 206 nm, assigned to Cr^{V} or Cr^{IV} complexes.[6,7]

The ultimate fate of chromium is in the cell nucleus where, as Cr^{III}, it is found to cross-link DNA with GSH itself or other peptides and proteins, mainly actin.[8,9] A number of reports have appeared dealing with the identification of intermediates in the reductive pathway of chromate.[4−7,10,11] However, the relevance of the biological role of both chromium and GSH deserves further effort in delineating the precise structure of the most stable reaction product at physiological conditions.

Upon addition of sodium chromate at pH 7.4, the 600 MHz ^{1}H NMR spectra of a mixture of reduced:oxidized glutathione (GSH:GSSG) 5:1 in D_2O clearly demonstrate formation of GSSG at the expense of GSH. Lowering and raising, respectively, of the intensities of well-separated lines was indeed observed. However, the extensive broadening and the upfield shifts experienced by resonances belonging to GSH (Table 1) are indicative of the formation of a paramagnetic complex where GSH acts as a ligand.

Assignment of binding donors of GSH was based on analysis of NMR parameters yielding the following evidence: (i) Cys H_α, γGlu H_α, γGlu H_β and γGlu H_γ were the most upfield-shifted GSH signals upon addition of chromate (Table 1); (ii) all proton spin−lattice relaxation rates were consistently enhanced (Table 1), with protons within the Cys and γGlu moieties being more affected than those of Gly.

Table 1. Upfield Chemical Shifts ($\Delta\delta$, ppm) and Relaxation Rate Enhancements (ΔR_1, s^{-1}) Experienced by GSH 16.7 MM in Deuterium Oxide at pH 7.4 in the Presence of GSSG 3.3 mM and Sodium Chromate 5 mM and T = 300K[a]

	$\Delta\delta$	ΔR_1	r_M	r_S
γGlu-H_α	0.201	0.622	0.363	0.412
γGlu-H_β	0.119	0.469	0.498	0.432
γGlu-H_γ	0.089	0.472	0.533	0.406
Cys-H_α	0.216	0.164	0.559	0.484
Cys-H_β	0.003	0.172	0.492	0.481
Gly-H_α	0.001	0.021	0.634	0.682

[a] Chromium−proton distances (r_M, nm) calculated in the molecular model are also reported and compared to those calculated by the Solomon equation (r_S, nm).

Both the change in chemical shifts and the enhancement of relaxation rates are larger at closer distances from the metal center that provides dipolar and hyperfine local fields at the nearby nuclei.[12,13]

The dipolar contribution to the chemical shift relies on a large value of the anisotropy of magnetic susceptibility,[13] which is not manifest in EPR spectra. Only the contact contribution therefore needs to be considered, given by:[13]

$$\Delta\delta = -\frac{A}{\hbar}\frac{g\mu_B S(S+1)}{3\gamma_H kT}$$

where A is the contact coupling constant, $\hbar$ the reduced Planck's constant ($\equiv h/2\pi$), μ_B the electron Bohr magneton, S the total spin number, γ_H the proton magnetogyric ratio, k the Boltzmann constant, and T the temperature. The size of the shift experienced by any nuclear spin in a given metal complex is determined by A which, in turn, is proportional to the unpaired electron spin density at the nucleus.

Contrariwise, the relatively long electron relaxation times expected for $S = {}^1/_2$ metal ions make the contact contribution to nuclear spin−lattice relaxation negligible.[12,13] The longitudinal relaxation rate of a nucleus sensing the electron magnetic moment can be therefore approximated by the dipole−dipole interaction as described by Solomon:[14]

$$R_{1M} = \frac{1}{15}\left(\frac{\mu_o}{4\pi}\right)^2\frac{\gamma_H{}^2 g^2 \mu_B{}^2 S(S+1)}{r^6} \otimes \left\{\frac{3\tau_c}{1+\omega_H{}^2\tau_c{}^2} + \frac{7\tau_c}{1+\omega_e{}^2\tau_c{}^2}\right\}$$

where μ_o is vacuum permeability, ω_H and ω_e are the proton and electron Larmor frequencies, τ_c is the motional correlation time, and r is the proton-metal distance. The isotropic hyperfine coupling of the unpaired electron with the metal nuclear spin modifies the Solomon equation,[15] and moreover, the Curie mechanism is likely to contribute the nuclear relaxation pathway at the high magnetic field used.[15] However the dependence upon r^{-6} is maintained in any case. As a consequence, a large relaxation rate enhancement indicates spatial proximity of the paramagnetic center to the corresponding proton.

NMR data therefore support the exclusion of Cys-SH and Gly carboxylate from the possible donors to the paramagnetic chromium ion.

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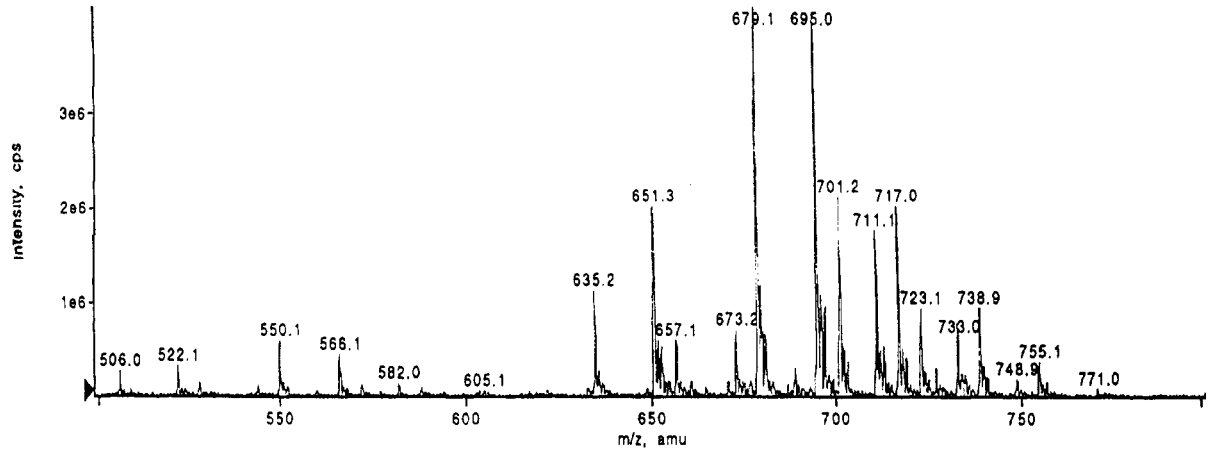

Figure 1. Selected regions of the electrospray mass spectrum taken on GSH:GSSG:chromate 5:1:1 in deuterium oxide at pH 7.4.

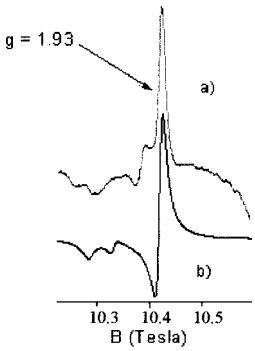

Figure 2. Experimental (a) and simulated (b) 282 GHz EPR spectrum of GSH:GSSG:chromate 5:1:1 in deuterium oxide at pH 7.4 and $T = 10$ K.

The electrospray mass spectrum, obtained from the GSH:GSSG:chromate 5:1:1 aqueous solution at pH 7.4, is shown in Figure 1. Several peaks were detected in the high *m*/*z* region. Among them, quite unexpectedly to us, peaks corresponding to the occurrence of dimeric $Cr_2(GSH)_2$ species were apparent. The peak at *m*/*z* 711 corresponds to the dimer $C_{20}H_{27}O_{12}N_6S_2Cr_2$, and the fingerprint agrees with isotopic abundance's. The peak at *m*/*z* 717 is originated by the deuterated $C_{20}H_{21}D_6O_{12}N_6S_2Cr_2$ species; whereas the peaks at higher *m*/*z* derive from the uptake of sodium atoms. At $m/z < 711$, the peaks at 635 and 651 may arise from fragmentation of the complex that may be hydrolyzed at level of the Cys-Gly peptide bond.

Delineation of the main species was obtained from EPR spectra recorded at 282 GHz and $T = 10$ K (Figure 2). The simulated spectrum was calculated by considering the dipole−dipole interaction between two Cr^V ions ($S = {}^1/_2$) removing the degeneracy of the triplet state. The parameters for the best fitting were $r_{Cr-Cr} = 0.414$ nm, $g_{iso} = 1.93$, $D = -0.0811$ cm^{-1}, $E = 0$. It was therefore concluded that a dimeric complex having the two metal ions in cubic symmetry is formed, and it is very likely to be the predominant species in solution. Bridging of the metal ions can be easily accomplished by GSH through the γGlu carboxylate, as verified by NMR data and by the structure of several polynuclear Cr complexes.[16]

The hypothesis on the structure of the complex was verified by performing some molecular mechanics and dynamics calculations. Two hydrated Cr^V ions were bridged by the γGlu carboxylate of two GSH molecular models as obtained by the HYPERCHEM molecular graphics package.[17] The model was then subjected to 25 ps restrained molecular dynamics at 300 K with the MM+ force field. Five structures/ps were sampled over the MD run. The two Cr ions were found at binding distances from the γGlu amide and the γGlu-NH_3^+ nitrogens. These groups were therefore deprotonated, the four bonds were built, and the MD run was repeated. One of the minimum energy structures is shown in Figure 3. The Cr−proton distances calculated in the model agree with either chemical shifts or relaxation rates, as also shown in Table 1. The last column of the table reports the Cr−proton distances calculated by NMR relaxation rates under the following assumptions: (i) exclusive contribution from the dipole−dipole interaction, that is, consideration of the Solomon equation only; (ii) assumption of the reorientational correlation time measured for GSH in water solution; (iii) negligible effects from the coupling of electron spins on nuclear relaxation rates.

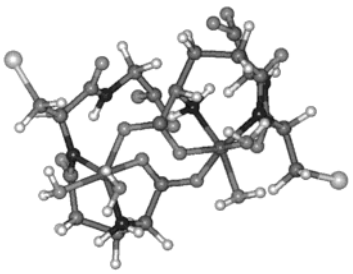

Figure 3. Stick model of one of the low-energy structures of the [Cr^V−GSH]$_2$ complex.

Although the measured distances are not completely reliable, the comparison with distances calculated in the model is anyway not very far from being satisfactory.

It was therefore concluded that glutathione not only reduces chromate but also acts as a ligand effective in clustering Cr^V ions resulting from the reaction. The biological relevance of the obtained results is supported by the isolated chromium-binding factor of unspecified composition that has been reported to hold four clustered chromium ions.[18] Moreover, it has been assessed that GSH plays a relevant role in vivo on Cr^{VI}-induced carcinogenesis.[19]

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Supporting Information Available: NMR spectra of GSH/GSSG at increasing amounts of chromate at pH 7.4, experimental details of EPR spectra, interpretation of the ES-MS spectrum, experimental and theoretical fingerprints of the peak at *m*/*z* 711 in the ES-MS spectrum, interpretation of the EPR spectrum (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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