Electron Spin Resonance Studies of Monomer–Dimer Equilibria involving Molybdenum(V) Complexes with Cysteine and Glutathione

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Summary Cysteine and glutathione ligands maintain dimeric Mo^V complexes in weakly alkaline solution, labilizing the dioxo-bridge, and forming small concentrations of paramagnetic monomers.

ELECTRON SPIN RESONANCE signals from Mo^V species in molybdoflavoenzymes[1–3] suggest that in the catalytic site monomeric molybdenum(v) is co-ordinated with the sulphur of the cysteine residue. However, most studies of simple cysteine with Mo^V have revealed diamagnetic species[4–7] which appear[5,6] to contain dimeric species $Mo_2O_4^{2+}$ with ligands co-ordinated to each Mo atom. Recently Martin and Spence[8] reported time dependent e.s.r. signals from 10^{-2}M-Mo^V and an excess of cysteine in 1M-phosphate buffer at pH 6, but details of the experiment are lacking. Under the conditions employed in this study, no well-defined e.s.r. signal was detected at pH 6. A time-independent, clearly defined e.s.r. signal was observed at higher pH. No previous study of a Mo^V–glutathione complex has been reported.

The paramagnetic molybdenum(v) complexes with cysteine and glutathione have been studied over the pH range 6—10 in 0·2M-phosphate buffer. The concentrations of Mo^V and ligand are of the order of 10^{-3} and 10^{-2}M, respectively. The signal develops fairly slowly, during what appears to the eye to be a second colour change following the mixing of reagents. Strength of signals increase with pH over the range 6—10 and fall off and disappear as pH is further increased to 12. The e.s.r. parameters are listed in the Table. Mo^V alone gives no signal in this pH range forming polymeric hydroxides in alkaline media.

Electron spin resonance parameters of Mo^V *complexes*

Ligand	Solvent	g	a (gauss)	g_3	g_2	g_1
Cysteine[a]	pH 9	1·969	35	2·029	1·972	1·914
Glutathione[a]	pH 8	1·951	32			
Xanthine oxidase[b]						
Γ, δ form	pH 10	1·977	34	2·025	1·956	1·951
α, β form	pH 10	1·977	41	1·990	1·971	1·971
"Slowly developing signal"	pH 8·2	1·967		1·975	1·970	1·957

[a] $[Mo^V] = 10^{-3}$M, [ligand] $= 10^{-2}$M.
[b] Refs. 1 and 12.

Further, isotopically enriched $^{95}Mo^V$–cysteine complex in solution gives six lines in the e.s.r. spectrum consistent with splitting expected for a paramagnetic monomer. $^{95}Mo^V$–glutathione complex in solution gives eleven lines indicating the paramagnetic species contains two molybdenum nuclei.

Integrated e.s.r. signals for cysteine complexes, however, were proportional to the square-root of $(Mo^V)_2$ concentration and independent of the excess of ligand concentration consistent with the equilibrium

$$(MoL)_2 \rightleftarrows 2\,MoL \qquad (1)$$

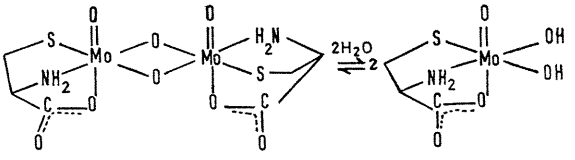
Quantitative e.s.r. measurements revealed the amount of monomer is less than 2%. Signal intensities increase reversibly with increasing temperature, indicating that reaction (1) is endothermic and that the dimers are quite labile.

Spectrophotometric observations indicate a very weak transition at 580 nm. (ϵ 120 at pH 10) in both cysteine and glutathione complexes not observed in complexes of Mo^V with ethanedithiol, 2-aminoethanethiol and β-mercaptopropionic acid, but observed in xanthine oxidase. The 580 nm. band is presumably due to the paramagnetic monomer since it was observed only during the second colour change. However, analysis of the weak band was not accurate enough to confirm or deny this. The nature of this band is still being investigated. Spectrophotometric determination (Job's method) indicates a 1:1 complex of Mo^V–cysteine, and the microanalytical result of the isolated compound indicates a 2:1 complex of $(Mo^V)_2$–glutathione.

We have compared solutions prepared by mixing Mo^V prepared and stored in 3M-HCl with buffered cysteine solutions and by direct dissolution of $Na_2Mo_2O_4(cys)_2,5H_2O$ kindly supplied by Dr. P. C. H. Mitchell[5] with identical results.

Structure determination of Mitchell's compound[6] enables us to speculate on the structure of monomeric products for the cysteine complex.

Stoicheiometry in OH^- has not been determined. However, one may suppose that base attack on Mo–OH groups will shift the equilibrium to the right. Strong base, however, destroys the paramagnetic complex at pH >10. Presumably glutathione, a cysteine containing peptide, co-ordinates to both Mo^V atoms in the $-Mo_2O_4-$ unit,



increasing the lability of the dioxo-bridge, since signals are stronger in this case. Our additional finding that cysteine-containing apoenzyme of putidaredoxin gives a much stronger signal with Mo^V supports this.

Similarities between properties of the cysteine complex

and properties of Mo^V in xanthine oxidase are intriguing.[1,5,6,10–12] (i) It forms a dinuclear complex, which is in equilibrium with a paramagnetic monomer. This meets the requirements of two molydenum(v) atoms co-ordinated by sulphur-donor ligands and of having a paramagnetic monomer for xanthine oxidase. (ii) A weak *d–d* transition at about 580 nm. was observed in both cases. (iii) The similarity of the spectral line shapes and the near equal values of the e.s.r. parameters (see Table) suggest that Mo^V may exist in the same electronic environment in both cases.

It appears that thiol containing ligands labilize the $\mu\mu$-dioxodimolybdenum(v) bridge, and that increasingly complex polypeptides containing thiol groups enhance this lability.

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