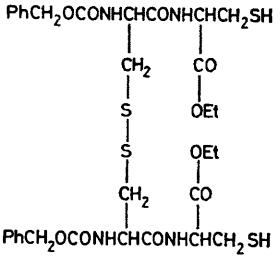
Mononuclear Molybdenum(v) Complexes of a Cysteinyl Peptide

By C. DAVID GARNER,* FRANK E. MABBS, and DAVID T. RICHENS
(*The Chemistry Department, Manchester University, Manchester* M13 9PL)

Summary Mononuclear molybdenum(v) complexes of a cysteinyl peptide have been prepared; e.s.r. characteristics and certain chemical behaviour observed resemble those of the molybdenum centres in the nitrate reductase and certain other molybdoenzymes.

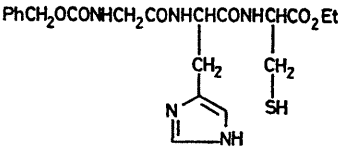
THE nature of the molybdenum sites in the various molybdoenzymes continues to attract attention.[1] Early studies[2] indicated similarities between the e.s.r. spectra of certain molybdenum(v) thiol complexes and the molybdenum e.s.r. signals observed[3] for the nitrate reductase, xanthine,

aldehyde, and sulphite oxidase enzymes. Such observations, together with the results of other investigations[4] led to the idea that the molybdenum was bound to cysteinyl sulphur atoms in the natural systems. This conclusion has now been substantiated by recent EXAFS studies accomplished for nitrogenase,[5] xanthine,[6] and sulphite oxidase[7] enzymes. In each case, the EXAFS data are consistent with the view that the molybdenum is bound to *ca.* 2 cysteinyl sulphur atoms; for nitrogenase, the remainder of the molybdenum's primary co-ordination sphere appears to consist of an iron-sulphur cluster, whilst in the oxidised state of sulphite and desulpho-xanthine oxidase the molybdenum seems to be attached to two oxo-groups and two N (or O) atoms.



(H_3L)

(1)



(H_3L'')

(2)

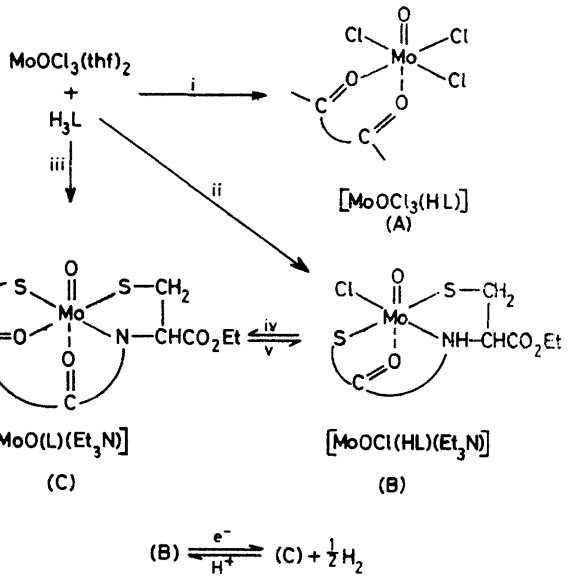
Although a large number of molybdenum–sulphur complexes have been reported,[1] little is known about the binding of this metal to cysteinyl-containing peptides. Therefore, we have undertaken such a study. The compound (**1**) was prepared and characterised.[8] The peptide was chosen since, in addition to the cysteinyl group, it contains a cystinyl group which, in close proximity to a molybdenum centre, could augment the metal's redox behaviour. The terminal amino and carboxylate groups were converted into *N*-benzyloxycarbonyl- and ethyl-ester functions, respectively, to reduce the number of possible ligating sites and to improve the solubility of the compound in the non-aqueous media used in the subsequent studies.

Compound (**1**) was treated with $MoOCl_3(thf)_2$ (thf = tetrahydrofuran) and the reactions were observed and the products obtained are summarised in the Scheme. These products were characterised analytically, and molecular weight, i.r., u.v.-visible, and e.s.r. data (Table) were recorded. The i.r. spectrum of (**A**) shows a band at 2,525 cm^{-1}, present in the spectrum of the free ligand and characteristic of

TABLE. Comparison of e.s.r. properties of complexes between H_nL and those for the nitrate reductases.[a]

Complex	g_1	g_2	g_3	$\bar{g}$	A/G
(**A**)	1·966	1·945	1·933	1·948	51
(**B**)	2·001	1·976	1·961	1·979	41
(**C**)	2·014	1·975		1·988	38
Nitrate reductase (low pH)	1·999	1·986	1·963	1·933	—
Nitrate reductase (high pH)	1·987	1·981	1·961	1·976	38

[a] Ref. 3.



SCHEME. All reactions proceed smoothly at *ca.* 20 °C. i, CH_2Cl_2; ii, CH_2Cl_2, $3Et_3N$; iii, CH_2Cl_2, $5Et_3N$; iv, dmf, Et_3N; v, dmf, *p*-$MeC_6H_4SO_3H$.

ν(S–H) which is not evident in the i.r. spectra of (**B**) and (**C**), and a shoulder at *ca.* 1640 cm^{-1} on the low energy side of the ν(C=O) stretching absorptions; this latter feature is not present in the i.r. spectrum of the free ligand or in that of the complex (**B**) but is apparent for (**C**). The profile of the broad i.r. absorption band centred at *ca.* 1540 cm^{-1}, attributed to the N–H bending modes is displaced some 10 cm^{-1} to lower energy for (**B**) as compared to (**A**) or (**C**). All of the complexes exhibit a strong i.r. absorption characteristic of a ν(Mo=O) stretching mode at 985, 965, and 960, respectively. These data, together with the e.s.r. characteristics of the complexes and the usual co-ordination geometry obtained for monomeric oxo-molybdenum(v) centres, lead to the suggested co-ordination geometries included in the Scheme. Co-ordination of the C–O and N–H groups *cis* to the oxo-group is thus suggested to lower their respective stretching and bending modes and the attachment of sulphur to the molybdenum(v) is manifest by the increase in *g*-values and the reduction of ν(Mo=O).[8]

The similarity of the e.s.r. characteristics of the complexes (**B**) and (**C**) to those recorded for the nitrate reductases and the xanthine, aldehyde, and sulphite oxidases is gratifying. Furthermore, the reversible deprotonation–protonation equilibrium established between (**B**) and (**C**) resembles that characterised for a group adjacent to the molybdenum centre in each of the above enzymes. However, no proton hyperfine splitting of the molybdenum(v) resonance could be detected. Therefore, the nature of the third site of deprotonation is not clear but we suggest that, as has been established[9] for copper–peptide complexes, this could involve loss of the proton on a peptidic nitrogen.

The reduction of (**B**) (1 mol) by one reducing equiv. of Na–Hg (in 1,2-dichloroethane), $NaBH_4$ [in dimethylformamide (dmf)], Na(acenaphthylenide) (in 1,2-dimethoxyethane), or $Cr_2(O_2CMe)_4$ (in dmf) in each case led to the evolution of a gas with the characteristics of dihydrogen

and the production of a molybdenum complex with e.s.r. characteristics identical to those of (**C**). This complex, however generated, underwent reversible protonation to a species with e.s.r. characteristics identical to those of (**B**). Therefore, it appears that, rather than the reduction leading to a Mo^{IV} or cleavage of the persulphide bond, it results in the third deprotonation and release of H_2. Addition of an excess ($\leqslant 4$ equiv.) of reductant to (**C**) produced a significant loss in the intensity of the e.s.r. signal, consistent with the formation of a $Mo^{IV}O$ complex. (Although further reduction to Mo^{III} is possible, these centres typically exhibit strong e.s.r. signals[8]).

Complex (**A**) reacts slowly (3—4 h) with Et_4NNO_3 in CH_2Cl_2, in the manner typical[10] of other labile $Mo^{V}OCl_3L_2'$ complexes, to produce NO_2 and *cis*-$MoO_2Cl_2(H_3L)$. Complexes (**B**) and (**C**) are not oxidised by Et_4NNO_3, suggesting that the chelation of the molybdenum(V) centre by the peptide leads to a substantial inertness at the centre. However, in the presence of an excess of $NaBH_4$, (**C**) catalyses the reduction of NO_3^- (to NO_2^-) with a turnover number of $\leqslant 4$ per Mo atom. The data presented above suggest that reduction of (**C**) to the molybdenum(IV) level is likely before oxidation by NO_3^- proceeds, a sequence which appears to parallel that which occurs in the nitrate reductase.[11]

The peptide (**2**) (H_3L'') has also been prepared.[8] 1 : 1 reaction between (**2**) and $MoOCl_3(thf)_2$ in CH_2Cl_2–hexane containing $\geqslant 3$ equiv. of Et_3N, leads to the formation of MoOL''. This is a mononuclear complex and has e.s.r. characteristics, $g_1 = 1{\cdot}985$, $g_2 = 1{\cdot}978$, $g_3 = 1{\cdot}966$, $\bar{g} = 1{\cdot}976$, and $\bar{A} = 36$ G, which bear an even closer resemblance to those characteristic of the molybdoenzymes than those of (**B**) and (**C**).

We thank the S.R.C. for financial support.

(*Received*, 22*nd January* 1979; *Com.* 065.)

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