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Coordination Chemistry Reviews
154 (1996) 5–11

COORDINATION CHEMISTRY REVIEWS

Aspects of the chemistry of oxo-molybdenum enzyme centers [1]

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Received 14 April 1995

Abstract

The influence of Professor Joseph Chatt upon some research in oxo-molybdenum chemistry is outlined.

Keywords: Oxo-molybdenum enzyme

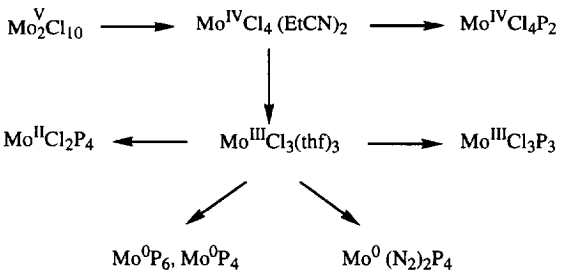
A holiday in southern Europe and northern Africa helps to relieve climate shock for many Australians working in Britain. Such a major event in a young life came for me in 1970–71 when on a working 'holiday' with Professor Joe Chatt at the ARC Unit of Nitrogen Fixation and the School of Chemical Sciences, University of Sussex. I have pleasant memories of both. I was transferred for a period from the University laboratories to the separately housed Unit, a reward for being caught with my feet up on the bench! In those relatively early days of the Unit, strong connections across the chemistry, biochemistry, microbiology and genetics of nitrogen fixation were just evolving. Nevertheless, Joe's breadth of view and chemical intuition aroused my interest in the molecular role of the trace metals in nutrition.

And there were tactical lessons to be learned: "It may not be efficient to attack a difficult problem head-on." It might be better to skirt the issue initially, to learn the terrain. This approach was advised for my project to synthesise dinitrogen complexes of molybdenum: starting with $Mo_2^{V}Cl_{10}(s)$, a general reductive route to oxidation states IV, III, II and 0 was developed (Scheme 1) [1,2]. This was a valuable experience in synthetic design and practice and led to efficient routes to $Mo^0(N_2)_2(PR_3)_4$ species. Subsequently, the N_2 ligands were shown to be reducible [3,4].

Joe's technique of daily discussions at the bench, the tossing around of ideas to be picked up, appealed to me. Of course, it was easier when you had results to talk

[1] Dedicated to the memory of Joseph Chatt.

SSDI 0010-8545(95)01213-3



Scheme 1. P = tertiary phosphine PR_3; the nature of R can determine the stoichiometry and isomeric form of the product. thf = tetrahydrofuran.

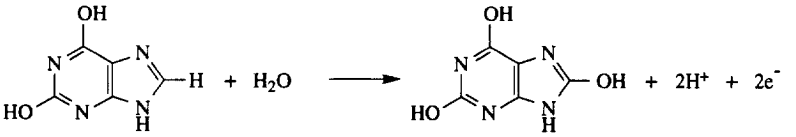
about! Fortunately, his walking stick on the wooden stairs at one end of the laboratory signalled his approach: escape at the other end was possible. The other senior chemists in the Unit, Jeff Leigh and Ray Richards, played crucial parts as both role models and translators. They were a key to the productivity of many of the younger chemists, teaching another valuable lesson: able collaborators with complementary interests make for interesting science.

A return to Australia provided a chance to establish an independent research program at La Trobe University in Melbourne. An opportunity to link an existing interest (early transition metal chemistry) with a burgeoning one (metals in biology) became apparent in the form of oxo-molybdenum and related chemistry. The molecular nature of the 'oxo-molybdenum' enzymes was just starting to be established, primarily through Bob Bray (also at Sussex) and his work on the EPR signals of xanthine oxidase [5]. These systems are now thought to be oxygen atom transfer catalysts (X = substrate) [6]:

$$X + H_2O \rightleftharpoons XO + 2H^+ + 2e^- \quad (1)$$

Enzymes which have been studied extensively to date include the following.

xanthine oxidase/dehydrogenase:



xanthine uric acid (2)

sulfite oxidase: $[SO_3]^{2-} + H_2O \rightarrow [SO_4]^{2-} + 2H^+ + 2e^-$ (3)

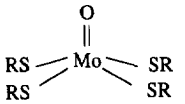
DMSO reductase: $Me_2SO + 2H^+ + 2e^- \rightarrow Me_2S + H_2O$ (4)

nitrate reductase: $[NO_3]^- + 2H^+ + 2e^- \rightarrow [NO_2]^- + H_2O$ (5)

The majority are complex enzymes with multiple prosthetic groups, and the value of fundamental chemistry in helping to identify the active sites was clear from the first significant modelling experiments in this area. EPR signals characteristic of Mo(V) appear upon reactions of $[Mo^{VI}O_4]^{2-}$ and glycolic acids $HECH_2CO_2H$

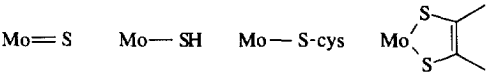
(E = O, S) [7]. Spectral parameters for the sulfur-based system were strikingly similar to the so-called Very Rapid and Rapid signals of xanthine oxidase [8].

This information encouraged us (and others) to look at the oxo-thiolate chemistry of molybdenum and led to the square pyramidal anions $[Mo^{V}O(SR)_4]^-$ [9–13]:



These are the species responsible for the EPR signals in the thioglycolate systems mentioned above [14]. The work was widened to include comparative tungsten and selenolate chemistry: isolation of $[MoO(SR)_4]^-$, $[MoO(SeR)_4]^-$, $[WO(SR)_4]^-$ and $[WO(SeR)_4]^-$ and the closely related binuclear systems $[M_2O_2(ER)_6L]^-$ (E = S, Se) permitted detailed spectroscopic and electrochemical examination. The role of the ligand disulfide/thiolate couple in the redox chemistry and the observation of ^{17}O coupling in EPR spectra were the most interesting aspects. Key collaborative contacts were made with Keith Murray, Alan Bond and John Pilbrow.

Since those 'early' days, the field has developed [2,15–26] through intensive examination of more enzyme systems (sulfite oxidase, nitrate reductase and dimethylsulfoxide reductase, in particular) and application of increasingly sophisticated techniques (X-ray absorption spectroscopy, electrochemistry). It seems that the different enzymes cycle through oxidation states VI [MoO_2, MoOS], V [MoO(OH), MoOS] and IV [MoO] and that a variety of Mo–S links (between 2 and 4, in all: Scheme 2) modulate enzyme properties at various stages of turnover. The 1,2-enedithiolate fragment is proposed as part of the molybdenum–pterin cofactor which characterises this class of enzyme [22].



Scheme 2.

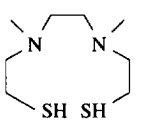
It became progressively apparent that the simpler model systems based upon fragments such as $[MoO(SR)_4]^-$ could not cope with the complexities demanded by the enzyme studies, despite initial success [27] with modelling oxo atom transfer reactions to various substrates X:

$$Mo^{VI}O_2L_n + X \rightarrow 'Mo^{IV}OL_n' + XO \quad (6)$$

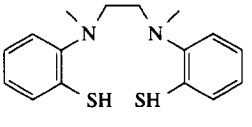
Access to oxidation states IV, V and VI in a single mononuclear system was a significant problem. In particular, one- or two-electron reduction of known *cis*-$Mo^{VI}O_2L_n$ species led to rapid elimination of ligand oxo as H_2O. This aspect, while related to substrate reactions (Eq. (1)), prevented access to intermediates along the reaction pathway. One possible tactic was to impose a ligand-based steric barrier (cf. porphyrin 'picket fences' [28]) to inhibit conversion of the pseudo-octahedral stereochemistry typical of *cis*-$[Mo^{VI}O_2]$ species to the five-coordinate square pyramidal arrangements favoured by $[Mo^{V,IV}O]$ species (cf. $[MoO(SR)_4]^-$). Importantly,

if properly designed, such a barrier would also inhibit condensation to binuclear $[Mo_2^{V}O_3]$ species, the bane of chemical modelling of these mononuclear enzymes.

Quadridentate ligands were plausible candidates. Initial success came with L^aH_2 [29] and then, in collaboration with Jack Spence, with L^bH_2 and related ligands:

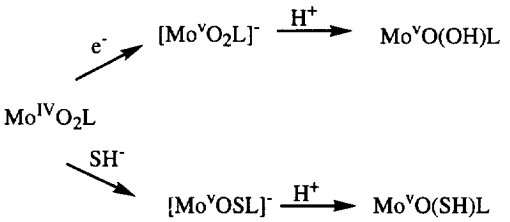


L^aH_2



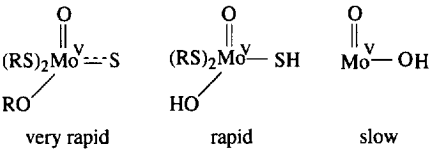
L^bH_2

Each *cis*-$[Mo^{VI}O_2L]$ complex exhibited reversible one-electron reductive chemistry which permitted access to $[Mo^{V}O_2]$, $[Mo^{V}O(OH)]$, $[Mo^{V}OS]$ and $[Mo^{V}O(SH)]$ centers in solution (Scheme 3).



Scheme 3.

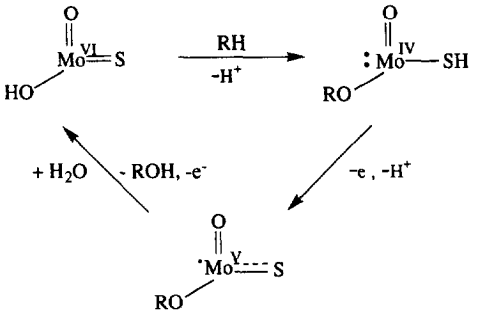
They were characterised by EPR studies on isotope-substituted (^2H, ^{17}O, ^{33}S, ^{95}Mo and ^{98}Mo) species [30–34]. Detailed comparisons with enzyme data [5,23] were possible. For example, partial structures can be assigned to the centers responsible for the characteristic EPR signals of xanthine oxidase, named for their relative rates of appearance (OR is the uric acid anion: see Eq. (1)):



A minimal catalytic cycle [33] for xanthine oxidase is provided in Scheme 4. Upon reduction, the thio ligand becomes strongly basic and capable of activating C–H bonds for oxygen atom insertion. A detailed mechanism is still evolving [6,35].

Xanthine oxidase and the other 'hydroxylase' enzymes which act upon C–H bonds feature $[Mo^{VI}OS]$ centers in their resting oxidised forms. However, the majority of the enzymes exhibit $[Mo^{VI}O_2]$ sites. A significant problem with studying these enzymes is that no intermediates have been detected positively during turnover. Various resting forms ($[Mo^{VI}O_2]$, $[Mo^{V}O(OH)]$, $[Mo^{IV}O]$) provide the available structural and redox information.

A promising synthetic model has been developed recently in which catalytic intermediates can be detected [36,37]. It is based upon the species $LMo^{VI}O_2X$ (L=



Scheme 4.

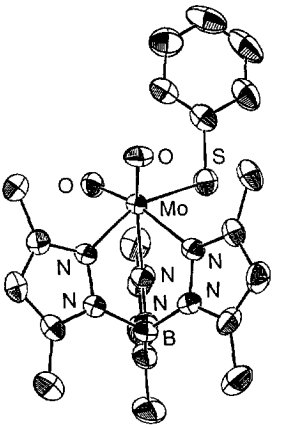


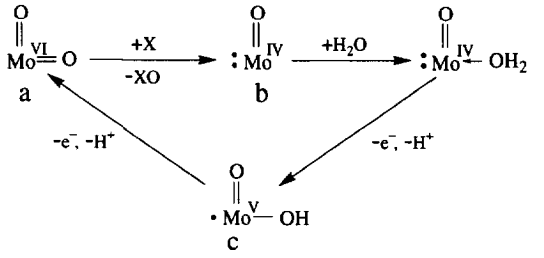
Fig. 1. Molecular Structure of $L^cMo^{VI}O_2(SPh)$.

trispyrazolylborate ligand; X = anionic ligand). Ligands L, first introduced to this chemistry by Enemark and co-workers [38], restricts reaction chemistry to three facial sites of a distorted octahedral center (Fig. 1, for example).

Variation of L and X permits fine tuning of structural and redox properties [39,40]. In particular, $L^cMo^{VI}O_2(SPh)$ (L^c = hydrotris(3,5-dimethyl-1-pyrazolyl) borate; Fig. 1) catalyses Eq. (1) ($X = PPh_3$) with detection of centers a, b and c of Scheme 5 [36,37].

Scheme 5 provides a basis for a mechanism of action for $[Mo^{VI}O_2]$ enzymes (e.g. reactions (3)–(5)). A 'spectator' oxo ligand controls the electronic structure while a second ligand position participates in oxygen atom transfer (the oxidase reaction (3)). The catalytic site is regenerated by coupled electron–proton transfer steps. Reductase enzymes (reactions (4) and (5)) cycle in the opposite direction.

This model system also provides, through the work of Young and co-workers [41], the sole structurally characterised example of a *cis*-$[Mo^{VI}OS]$ center of the type proposed for xanthine oxidase. The complex $L^cMoOS(S_2P^iPr_2)$ features a significant S·S interaction between the Mo=S and S=P functions, apparently stabilising the $[Mo^{VI}OS]$ center. The existence of an equivalent stabilising mechanism



Scheme 5.

in the enzymes would reconcile the extreme reactivity of [$Mo^{VI}OS$] centers with their natural occurrence.

To a significant extent, the approach to chemistry described in this brief account arose from my experience with Joe Chatt and his people. I thank particularly Jeff Leigh and Ray Richards for good advice, generously given.

Acknowledgement

The work carried out in Australia was funded by generous grants from the Australian Research Council.

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