Coordination Chemistry Reviews, 100 (1990) 183–221
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE BIOLOGICALLY RELEVANT OXYGEN ATOM TRANSFER CHEMISTRY OF MOLYBDENUM: FROM SYNTHETIC ANALOGUE SYSTEMS TO ENZYMES *

R.H. HOLM

Department of Chemistry, Harvard University, Cambridge, MA 02138 (U.S.A.)

(Received 27 February 1989)

CONTENTS

ABBREVIATIONS

bipy	2,2′-bipyridyl
Cbz	carbobenzyloxy
co	cofactor

* Dedicated to that pioneer in the bioinorganic chemistry of molybdenum, Professor Jack T. Spence, on the occasion of his retirement.

0010-8545/90/$13.65

Cp*	pentamethylcyclopentadienide(1 −)
DMF	dimethylformamide
EXAFS	extended X-ray absorption fine structure
FAB	fast-atom bombardment
$HB(Me_2pz)_3$	hydrotris(3,5-dimethylpyrazolyl)borate(1 −)
L^1	*N*,*N*′-dimethyl-*N*,*N*′-bis(2-mercaptophenyl)ethylenediamine (2 −)
L^2	*N*,*N*′-dimethyl-*N*,*N*′-bis(2-mercaptoethyl)ethylenediamine (2 −)
L-NS_2	2,6-bis(2,2-diphenyl-2-sulfidoethyl)pyridine(2 −)
Me_3tacn	*N*,*N*′,*N*″-trimethyl-1,4,7-triazacyclononane
R_F	*p*-C_6H_4F
sap	2-(salicylideneamino)phenolate(2 −)
solv	solvent ligand
ssp	2-(salicylideneamino)benzenethiolate(2 −)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
X	reduced substrate (generalized)
XANES	X-ray absorption near-edge spectroscopy
XO	oxidized substrate (generalized)

A. INTRODUCTION

The fundamental chemistry of which transition element has been most rapidly and extensively advanced in the last decade? Such a question can provoke a number of answers, perhaps limited only by the number of transition elements and the subjectivity of the respondent! Highly viable candidates include technetium, rhenium, and ruthenium. Yet I would argue for molybdenum, and suggest that the case can be made by comparing the state of contemporary molybdenum chemistry today [1] with that summarized by Stiefel [2] in his 1977 treatment of the subject. With due recognition of the oft-desirable tendency of the inorganic chemist to expand his research activity into any perceived vacuum, there exist three clearly identifiable forces that have impelled research in molybdenum chemistry in recent times. Although discovered many years earlier, Mo–Mo bonds of different multiplicities have continued to present themselves both predictably and serendipitously in a rich variety of relatively small binuclear and trinuclear molecules and in larger clusters [3,4]. These interactions have stimulated an enormous growth of the chemistry of the element in its lower oxidation states in both molecular and solid state systems. A second force is industrial catalysis [5–7], particularly the hydrodesulfurization process [7] whereby organosulfur compounds in petroleum feedstocks are heteroge-

MOLYBDENUM-CONTAINING ENZYMES

● **Nitrogenase**

catalytic site: FeMo-cofactor ($MoFe_6S_{8\text{-}10}$)

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3 \quad (1)$$

● **Oxotransferases** (hydroxylases)

catalytic site: mononuclear Mo unit

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

Fig. 1. The two known classes of molybdoenzymes and the reactions catalyzed.

neously desulfurized with dihydrogen over a cobalt- or nickel-promoted molybdenum catalyst. A third force is the role of molybdenum in biology, which is summarized in Fig. 1. The element is present in two types of enzymes: nitrogenase [8,9], which is found in free-living and symbiotic microorganisms and catalyzes the reduction of dinitrogen to ammonia in reaction (1); and hydroxylases [9–13] or, as we have termed them, oxotransferases [14], which catalyze a variety of two-electron oxidation–reduction reactions (reaction (2)).

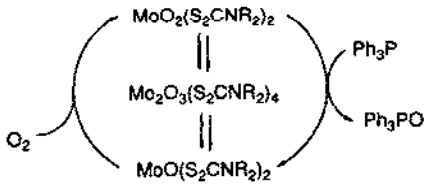
Here attention is directed toward a particular type of reactivity exhibited by molybdenum, usually when in its higher oxidation states. The process in question is oxygen (oxo) atom transfer, as defined by reaction (3):

$$Mo^zO_aL_n + XO \rightleftharpoons Mo^{z+2}O_{a+1}L_n + X \quad (3)$$

This is an example of primary oxo transfer [15] wherein XO is an oxygen atom donor and X is an oxygen atom acceptor in a reaction that results in a change of two units in the molybdenum oxidation state. An entirely common circumstance in such systems is the occurrence of the reversible or, more frequently, irreversible electron transfer reaction (4) with concomitant formation of a μ_2-oxo bridge:

$$Mo^zO_aL_n + Mo^{z+2}O_{a+1}L_n \rightleftharpoons 2\left[L_nMo^{z+1}O_a\right]_2O \quad (4)$$

In the great majority of such reaction systems, $z = 4+$ and $a = 1$. The first clear indication of molybdenum-mediated oxo transfer was found in the work of Barral et al. [16] in 1972, who demonstrated the catalytic aerial oxidation of tertiary phosphines in cycle (5):



(5)

As will be seen, the steps in this sequence have a more general significance. In this article, molybdenum oxo transfer chemistry is summarized in an attempt to relate the chemistry demonstrated in synthetic systems to enzyme function.

Emphasis is placed on work from this laboratory, but other important contributions related to models of other states in catalytic cycles are included. An earlier version [14] of some of the considerations offered here has appeared, as has a more general treatment of metal-centered oxo transfer which includes a comprehensive summary of the oxo transfer reactions of molybdenum [15].

B. OXOTRANSFERASES

(i) Reactions catalyzed

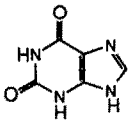
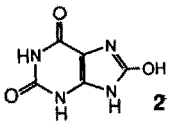
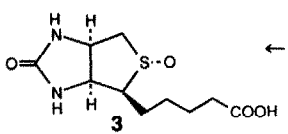
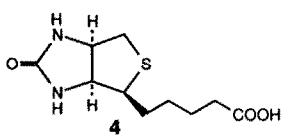
Over a dozen enzymes have been identified which catalyze the two-electron reaction (2) in which water is the source and sink of oxygen. This is an overall reaction and does not imply direct transfer of oxygen from water to substrate or the reverse. In an operational sense, reaction (2) is equivalent to $X + [O] = XO$. Substrates and products of selected enzymes are included in Table 1. Among the enzymes of particular interest here are xanthine oxidase, which converts xanthine (**1**) to uric acid (**2**), and D-biotin *S*-oxide reductase, which reduces the biologically inactive oxide (**3**) to the coenzyme sulfide (**4**). A listing of most known enzymes is available [17], as are detailed accounts of those in Table 1 [9–13,18–32]. Suffice it to say that these molybdoenzymes are usually multisubunit assemblies which contain, in addition to a mononuclear catalytic site, one or more electron transfer units (flavin, cytochrome, Fe–S cluster). Certain enzymes have broad substrate specificity, especially xanthine and liver aldehyde oxidases [33]. For example, in addition to their oxidase function, aldehyde oxidases will reduce *S*-oxides to sulfides and tertiary and aromatic *N*-oxides to the corresponding amines. This profligate behavior will be of interest later.

(ii) Nature of the molybdenum site

Both types of molybdoenzyme contain dissociable cofactors, but these are quite different. The nitrogenase cofactor is a cluster of the approximate composition $MoFe_6S_{8-10}$, whereas the oxotransferase cofactor [34] is a mononuclear complex with oxo and sulfur ligation. Each contains the full molybdenum content of its enzyme. The minimal structure **5** of Mo–co, as deduced by the Duke group [35] in a singularly important contribution, is presented in Fig. 2. The ligand, molybdopterin, contains a pterin nucleus

TABLE 1

Substrates and products of selected molybdoenzymes

X		XO	Enzyme	Ref.
 1	→	 2	Xanthine oxidase/dehydrogenase	11, 13, 18
RCHO	→	RCOOH	Aldehyde oxidase	18
CO	→	HCO_3^-	Carbon monoxide oxidase	19–21
SO_3^{2-}	→	SO_4^{2-}	Sulfite oxidase	18, 22
HCO_2^-	→	HCO_3^-	Formate dehydrogenase	23
NO_2^-	←	NO_3^-	Nitrate reductase	23, 24
 3	←	 4	D-Biotin *S*-oxide reductase	25
RSR	←	R_2SO	*S*-Oxide reductase	26–28
R_3N	←	R_3NO	*N*-Oxide reductase	28–32
ArN		ArNO		

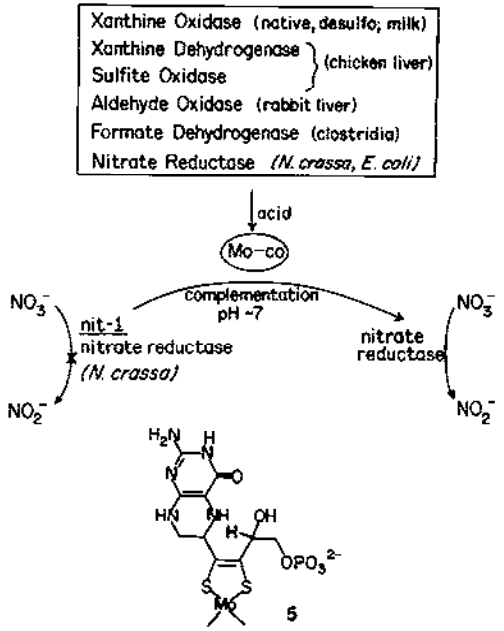


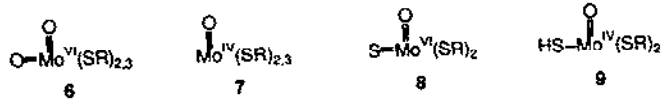
Fig. 2. Schematic representation of the complementation reaction of *N. crassa nit*-1 nitrate reductase to full activity by the molybdenum cofactor (5, Mo–co) obtained from the indicated enzymes. Also shown is the structure of the molybdopterin (reduced) form in the cofactor.

(shown in the fully reduced form) and a side-chain carrying an enedithiolate chelate functionality, a chiral center, and a terminal phosphate group. Mo–co is an oxygen-labile species and has not yet been isolated in substance.

On the basis of the evidence presented schematically in Fig. 2, Mo–co is probably a "universal" cofactor [36]. When extracted from various enzymes and incubated with the inactive assimilatory nitrate reductase isolated from the *nit-1* mutant of *Neurospora crassa*, Mo–co complements the enzyme, which otherwise lacks molybdenum, to full activity [36–38]. In every molybdoenzyme in which it has been sought, molybdopterin has been found, its detection being aided by its characteristic fluorescence spectrum. For enzymes other than those in Fig. 2, cofactors have recently been detected in, for example, formate dehydrogenase from a methanogen [39] and in carbon monoxide dehydrogenase from *Pseudomonas* [40]. The cofactor from the latter source appears to have a molecular mass more than twice that of cofactors from others enzymes and has been named "bactopterin" to emphasize the difference [40]. Its structure is unknown. Complementation of cofactor-deficient nitrate reductase has succeeded in all cases except for

Mo–co from formate dehydrogenase. This anomalous behavior has not yet been explained.

The coordination unit of molybdenum in the enzyme is at present not completely defined, and thus it is not known if the entire unit is conserved. Partial ligand identification has come from EPR [41,42] and EXAFS [43–47]. The combined information, much of which has been summarized by Cramer [48], is consistent with the representations **6** and **7** for the fully oxidized and dithionite-reduced forms respectively of sulfite oxidase, nitrate reductase from *Chlorella*, and the cyanolyzed form of xanthine oxidase/dehydrogenase. The unit $MoO(OH)(SR)_{2,3}$ is indicated for the molybdenum(V) state of sulfite oxidase. Similarly, **8** is the minimal coordination unit in oxidized xanthine oxidase/dehydrogenase and **9** is that in the reduced form. The presence of (at least) two sulfur atoms at 2.35–2.50 Å is in agreement with cofactor structure **5**. The other type of sulfur ligand is sulfido, in view of the Mo=S distance near 2.15 Å. Given the common prosthetic group composition of $2Mo + 4Fe_2S_2 + 2FAD$ per dimeric molecule, it is probable that liver aldehyde oxidase and carbon monoxide oxidase, as xanthine oxidase/dehydrogenase, contain the $Mo^{VI}OS$ group when oxidized. Units **6–9** are coordinatively unsaturated, making it likely that Mo–co is held within the enzyme by additional coordination to the protein.



6 **7** **8** **9**

C. SYNTHETIC ANALOGUE APPROACH

The methodology of this approach as practised in this laboratory has been described at some length previously [49,50] and is summarized in Fig. 3. There are three types of metal sites in metallobiomolecules. These are subject to examination by spectroscopy or, ideally, are structurally defined by crystallography. Many of the structures recently determined by metalloprotein crystallography have been collected [51]; regrettably, no molybdoenzyme is among them. Utilizing the information from these techniques, a target molecule is designed and synthesized. Ideally, this molecule is obtainable in crystalline form and approaches or duplicates the metal site in the attributes of a structural model. If the site in question is catalytic, then a functional model—a model capable of executing stoichiometric or, better, catalytic transformation of an enzyme substrate—may also have been achieved. The advantage of this approach is the susceptibility of

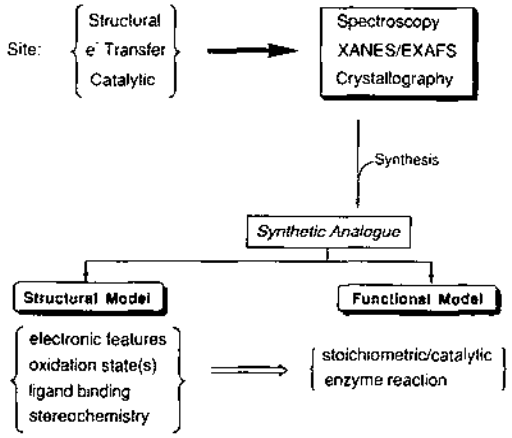


Fig. 3. Summary of the synthetic analogue approach showing the three types of metal-containing sites and certain defining features of synthetic analogues that are structural and functional models of these sites.

analogues to characterization of structural, electronic, and reactivity properties at the small-molecule level of detail.

With regard to catalysis, it is usual that far more is known about a given reaction, namely, its binding and kinetics parameters, substrate specificity, stereochemistry, inhibition, and related matters, than is known in detail about the active site itself. Consequently, if in such a case a functional model is desired, only a crude representation of the catalytic site can be expected at the outset. Bray in 1988 observed that "Until a few years ago, the impact of low molecular weight model compound work on understanding molybdenum centers in enzymes was small. The situation has now changed dramatically, however" [13]. Despite the imperfections of structural, and especially functional models, the synthetic analogue approach has made, and will continue to make, effective contributions to the key matters of active site structure and function. What follows is an account of the progress made in applying this approach to the sites in molybdenum oxotransferases, which draws largely on the experimental findings of this laboratory [14,52–60].

(i) The oxo transfer hypothesis

The difference between substrates and products in Table 1 is one oxygen atom. This is also the case for the substrate and metal in cycle (5) and in

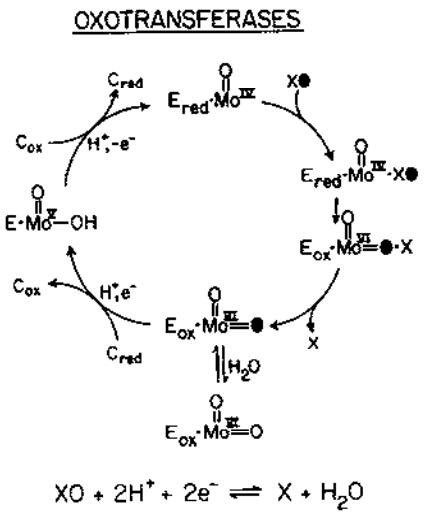


Fig. 4. Representation of the oxo-transfer hypothesis in the form of a catalytic cycle for substrate reduction whose starting events are substrate binding, atom transfer, and product dissociation, followed by electron transfer and protonation steps that recover the initial state of the enzyme. A possible hydroxo ligand is not shown in the molybdenum(IV) state. An analogous cycle may apply to an enzyme with sulfur ligands, there being an Mo=S bond in the molybdenum(VI, V) state and an Mo–SH bond in the molybdenum(V, IV) states (see Fig. 8).

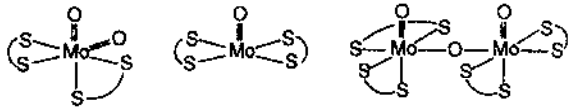
several other apparent molybdenum-mediated atom transfer reactions reported through 1980 [15]. The ^{18}O tracer work of Halperin and Taube [61] in the early 1950s demonstrated that sulfite and chlorate, later identified as enzyme substrates, formed sulfate and chlorite by direct atom transfer, albeit not involving a metal. These observations led J.M. Berg and myself to consider the possibility that some or all of the enzymes in Table 1 operate by an oxygen atom transfer mechanism and to design a reaction system that might examine this proposition. After the initial demonstrations of atom transfer reactions in that system, we offered the term "oxotransferases" for these enzymes, without, at the time, the results of experiments designed to test the mechanism. This was to follow later for one enzyme.

The oxotransferase hypothesis is illustrated in schematic form in Fig. 4, where the reaction cycle is written (arbitrarily) for substrate reduction. The scheme is initiated by formation of an enzyme–substrate complex, followed by oxo transfer to molybdenum, and dissociation of a possible enzyme–product complex. Re-reduction to the $Mo^{IV}O$ state by an electron carrier C_{red} (e.g. a cytochrome or ferredoxin) occurs in two one-electron steps with accompanying protonations. In this way, an EPR-active molybdenum(V) state is developed, which under this hypothesis is a compo-

nent of that leg of the cycle which restores the initial catalytic form. When applied to the mechanism of action of sulfite oxidase suggested by Rajagopalan [12], the cycle in Fig. 5 is obtained. Here the $Mo^{VI}O_2$ group is reduced by atom transfer to sulfite in the initial phase of catalysis. The remaining steps are intraprotein electron transfer between molybdenum and cytochrome *b*, and intermolecular electron transfer between an external cytochrome *c* and the enzyme. In both cycles, the bond-breaking and bond-making steps of substrate and product are the early events in the sequence and are those of primary interest here.

(ii) An analogue reaction system

Until about 1980, those molybdenum complexes that had been the most thoroughly scrutinized in oxo transfer and closely related chemistry were the dithiocarbamates. The pertinent complexes are $MoO_2(S_2CNR_2)_2$ (**10**), $MoO(S_2CNR_2)_2$ (**11**), and $Mo_2O_3(S_2CNR_2)_4$ (**12**), whose structures have been crystallographically established [62,63] and which are otherwise very well characterized. In addition to cycle (5), the early observations by Mitchell and Scarle [64], although not entirely correct, provided examples of oxo transfer reactions with **10** and **11**. The substrates known to be oxidized or reduced in reaction (6) are indicated below:



10 **11** **12**

$$MoO_2(S_2CNR_2)_2 + X \rightleftharpoons MoO(S_2CNR_2)_2 + XO \qquad (6)$$

$X = R_3P$ [15,53]	$XO = R_2SO$ [53,64–66]
	RCO_3H [67]
	N-oxides [53,64,65]
	Ph_3AsO [65]
	Ph_3SbO [65]
	NO_2^- [68]
	epoxides [69]

The systems are complicated to an extent by μ-oxo molybdenum(V) dimer formation (reaction (7)):

$$MoO_2(S_2CNR_2)_2 + MoO(S_2CNR_2)_2 \rightleftharpoons Mo_2O_3(S_2CNR_2)_4 \qquad (7)$$

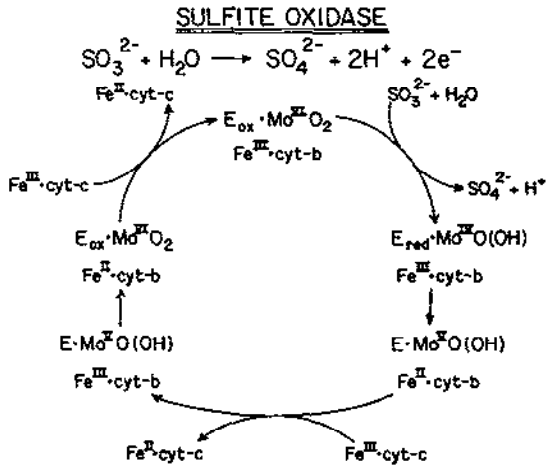
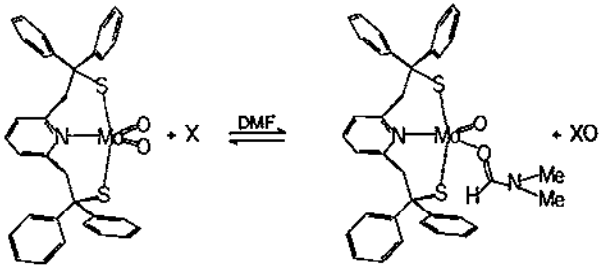


Fig. 5. The mechanism of action of sulfite oxidase (adapted from ref. 12).

However, this reaction is a reversible equilibrium ($K_{eq} \approx 500\ M^{-1}$ in 1,2-dichloroethane at 298 K) with known rate constants [70] and it does not hinder stoichiometric or catalytic product formation. Another favorable feature of reaction (6) is the availability of reaction enthalpies for several substrates [67], a matter to which we will return.

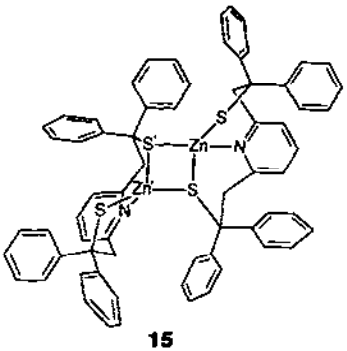
The occurrence of reactions (6) and (7) in the same system considerably complicates the kinetics analysis of the oxo transfer reaction. We have provided a formal solution to this problem and applied it to the determination of the reduction of **10** with tertiary phosphines and the oxidation of **11** with Me_2SO [53]. Neither the occurrence of reaction (7) nor the dithiocarbamate ligand system is biologically realistic. We have sought a system in which ligation corresponds to an extent found in minimal sites **6** and **7**, and μ-oxo dimer formation is prevented. Toward that end, the tridentate ligand L-NS_2 was designed and synthesized and from it the complexes MoO_2(L-NS_2) (**13**) and MoO(L-NS_2)(DMF) (**14**) were prepared [55]:



(8)

13 **14**

The X-ray structure of MoO_2(L-NS_2) reveals that one of the phenyl rings in each *gem*-diphenyl group tends to overlie an Mo=O bond, thereby providing a steric barrier to oxo bridge formation. Diffraction-quality crystals



15

of $MoO(L\text{-}NS_2)(DMF)$ have yet to be obtained. The structure indicated is derivative of **13** with a DMF solvate molecule occupying the position of an oxo atom, but it has not been demonstrated. When prepared by reduction of $MoO_2(L\text{-}NS_2)$ with Ph_3P in DMF, a purple solid analyzing as $MoO(L\text{-}NS_2)(DMF)$ is obtained in good yield and is slightly soluble in DMF. When the reduction is carried out in $CHCl_3$–DMF (5 : 1 v/v), another modification is obtained which contains, and is more soluble in, DMF. Both forms give virtually the same absorption spectrum. It is possible that one or both of them are not mononuclear, at least in the solid state. Recently, Kellogg and coworkers [71] have prepared "$Zn(L\text{-}NS_2)$" from the reaction of the ligand and $Zn(NO_3)_2$ in acetonitrile. Despite the steric bulk of the ligand, it is not surprising, given the relatively weak coordinating abilities of nitrate and the solvent, that coordination is completed by a thiolate bridge at each zinc(II) atom, to afford the dimer **15**. A similar bridged structure with five- or six-coordinate molybdenum(IV) cannot be excluded for **14**. Monomer or not, the compound reacts smoothly with a number of oxo donors in the reaction system (8). In this system, there has been no indication of the formation of a μ-oxo molybdenum(V) dimer. We will retain, for simplicity, the monomeric formulation of the molybdenum(IV) complex.

(iii) Oxo transfer reactions

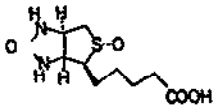
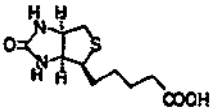
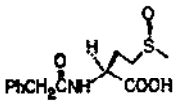
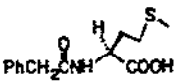
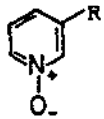
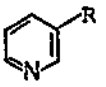
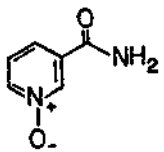
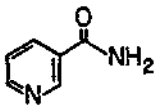
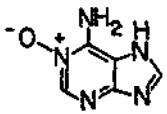
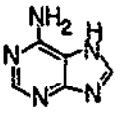
Reaction system (8) can proceed in either direction depending upon the substrate. Listed in Table 2 are substrates that have been transformed in this reaction system. Kinetics data for reactions of certain of these substrates are collected in Table 3.

Substrate oxidation

Oxo transfer to substrate has been studied in detail for two tertiary phosphines; certain others react similarly. The reaction is bimolecular and is

TABLE 2

Substrates and products of analogue reaction system (8) [a]

Substrate (X/XO)	Product (XO/X)
Ph_3P	Ph_3PO
R_2SO (R = Me, Ph, p-C_6H_4F)	R_2S
 3	 4
	
 (R = H, F)	
	
	
$(PhCH_2)_3NO$	$(PhCH_2)_3N$
NO_3^-	NO_2^-
Ph_3AsO	Ph_3As

[a] Results in DMF solutions from refs. 55–60.

TABLE 3

Kinetics of oxo transfer in reaction system (8) at 298 K

Substrate X/XO	$k_1 \times 10^3$ (s^{-1}, $M^{-1}\ s^{-1}$)	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (e.u.)	Ref.
Ph_3P	7(1) [a]	–	–	56
$(p\text{-}C_6H_4F)_3P$	9.7(4) [a]	11.7(6)	−28.4(1.6)	59
Me_2SO	1.50(3)	–	–	56
Ph_2SO	1.43(3)	–	–	56
$(p\text{-}C_6H_4F)_2SO$	1.40(7)	23.4(1.4)	7.2(2.0)	59
D-Biotin *S*-oxide	1.36(3)	–	–	56
Cbz-(*S*)-Met l-(S–O)	1.63(3)	–	–	56
Cbz-(*S*)-Met d-(S–O)	1.70(3)	–	–	56
$3\text{-}FC_6H_4NO$	1.60(8)	22.1(1.3)	2.6(1.6)	59
NO_3^-	1.49(5)	23.7(6)	8.0(2.0)	60

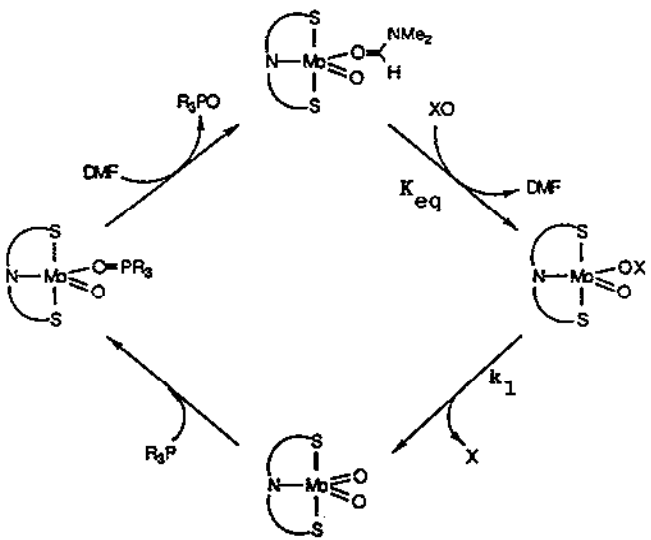
[a] Second-order reaction, $M^{-1}\ s^{-1}$; all other rate constants in s^{-1}.

about 10 times slower than the corresponding reduction of $MoO_2(S_2CNEt_2)_2$ with Ph_3P [53], indicating that **10** is easier to reduce than **13**, a point that will be of interest subsequently.

Substrate reduction

The remaining substrates in Table 2 have been reduced by oxo atom transfer to MoO(L-NS_2)(DMF). Comparison with Table 1 reveals that all but Ph_3AsO are compound types (*N*-oxide, *S*-oxide, nitrate) that are enzyme substrates. More specifically, Me_2SO, Ph_2SO, D-biotin *S*-oxide, and nitrate are known substrates of oxotransferases, and nicotinamide *N*-oxide and adenine 1-oxide are reduced with rat liver or milk xanthine oxidase [29,72] (vide infra). The diastereoisomeric pair of N-protected methionine *S*-oxides was investigated on the chance that one or more of the methionine *S*-oxide reducing enzymes [73,74] is a molybdoenzyme. At present, it is still not known if any of these enzymes is molybdenum dependent. These reactions can be monitored spectrophotometrically because of the favorable spectral differences between MoO_2(L-NS_2) and MoO(L-NS_2)(DMF) [55]. Thus the course of reduction of nitrate in DMF solution is readily examined, as illustrated in Fig. 6. As the reaction proceeds, the bands of the molybdenum(IV) complex at 365, 528 and 734 nm are diminished in intensity and are finally replaced by those of MoO_2(L-NS_2) at 385 and 449 nm. The tight isosbestic points at 386 and 473 nm indicate the presence of only two chromophores. This behavior is found with all reducible substrates in Table 2. Reactions are clean and uncomplicated and follow the same kinetics scheme.

The reduction of nitrate is typical of that of other substrates XO in that the reaction velocity becomes independent of substrate concentration above a certain level. This is a case of saturation kinetics, a familiar property of enzymes. The substrate saturation behavior has been successfully analyzed in terms of the first two steps in scheme (9). These are a reversible substrate binding equilibrium followed by a rate-limiting intramolecular atom transfer with rate constant k_1, and departure of the reduced substrate. Substrate binding is assumed to be fast compared with oxo transfer. To the extent to which this assumption does not apply, the rate constants are lower limits of the true values. Saturation behavior is reached when all molybdenum(IV) species are complexed by substrate. In the case of *S*-oxide substrates, some of which present rather different extents of hindrance around the sulfur atom, the near-invariance of k_1 values suggests that substrate binds at the oxygen atom of the sulfoxide group. The quantity k_1/K_m where $K_m = [DMF]/K_{eq}$ is in effect the second-order rate constant for substrate reduction. For Me_2SO, the value is 0.5 M^{-1} s^{-1}. This is about 10^3 higher than the rate constant for the reduction of the same substrate with $MoO(S_2CNEt_2)_2$ [53], a result consistent with the larger rate of reduction of **10** with phosphine. Further, it would seem unlikely that this rate difference would exist if $MoO(L\text{-}NS_2)(DMF)$, as mononuclear **11**, did not possess a labile binding site such as is represented for the monomeric form **14**.



(9)

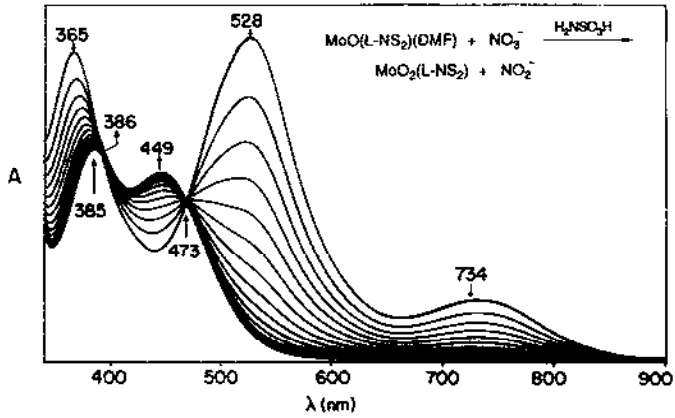
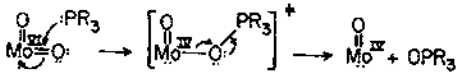


Fig. 6. Spectrophotometric time course of the reduction of nitrate by $MoO(L\text{-}NS_2)(DMF)$ in DMF solution. Sulfamic acid was added to discharge nitrite, which undergoes further reaction with the molybdenum complexes (reproduced with permission from ref. 60).

Transition states

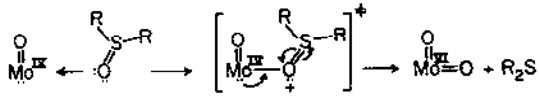
The activation parameters for four reactions of system (8) are collected in Table 3. A large negative activation entropy for the reaction of X ($X = (p\text{-}C_6H_4F)_3P$) is expected for a second-order reaction. Phosphine oxidation is represented by the simplified scheme (eqn. (10)), in which the phosphine interacts with the π^* orbital of an Mo=O group:



(10)

Coordination to the molybdenum atom as the initial event along the reaction coordinate cannot be excluded in this case, owing to the possibility of a labile binding site at five-coordinate molybdenum(VI). Other $Mo^{VI}O_2$ complexes with this property are also readily reduced with tertiary phosphines [15]. However, there are a number of other $Mo^{VI}O_2$ complexes that lack such a site, among them **10**, which are reduced by phosphines at comparable or faster rates [15,53].

Substrate reduction is illustrated with a sulfoxide in scheme (11):



(11)

The relatively small activation entropies for the reduction reactions imply transition states with structures similar to those of the ground state adducts of the molybdenum(IV) complex and substrate. Recently, thermochemical data have become available [15,75] that allow calculation of the enthalpy

change for reaction (12):

$$C_5H_5NO(g) + Me_2S(g) = C_5H_5N(g) + Me_2SO(g)$$

$$\Delta H = -14 \text{ kcal mol}^{-1} \quad (12)$$

If the substrates XO, where $XO = (p\text{-}C_6H_4F)_2SO$ and 3-fluoropyridine *N*-oxide, are likened to the reactants in reaction (12), the difference between their S–O and N–O bond energies is about 14 kcal mol^{-1}. The essential equality of activation enthalpies for the three oxidized substrates in Table 3 indicates no significant expression of these bond energies in achieving the transition states. Rupture of these bonds is certainly excluded in that process. It seems likely that the transition state represented in scheme (11) may overstate the degree of substrate bond-breaking. It is also conceivable that the transition state is different, perhaps involving a structural change of the adduct complex prior to Mo–O bond strengthening and N–O or S–O bond weakening. Any further interpretation of activation parameters requires the solution structure of $MoO(L\text{-}NS_2)(DMF)$ and its adducts, information that is currently unavailable.

Catalysis

With the demonstration that reaction system (8) can be operated in either direction, catalysis in the form of coupled reactions of oxo transfer to and from substrate becomes possible. One example of the generalized reaction (13) with $R = p\text{-}C_6H_4F$ and $XO = (p\text{-}C_6H_4F)_2SO$ is illustrated in Fig. 7 [59]:

$$R_3P + XO = R_3PO + X \quad (13)$$

There is no reaction between the phosphine and sulfoxide each at 1 M concentration in DMF solution for at least 5 days at 298 K. The reaction starts immediately upon the addition of the catalyst. The course of the reaction can be effectively monitored by ^{19}F NMR spectroscopy, owing to the complete resolution of the resonances of the four components of the reacting system. The system was allowed to proceed through 135 turnovers over 880 min, and it showed no signs of degradation at the end of this period. The catalytic velocity v is 3.6×10^{-5} s^{-1}. From $v/[Mo(VI)]$, $k_c = 7.2 \times 10^{-3}$ M^{-1} s^{-1}, close to the value of the rate constant for the reaction of the same phosphine with $MoO_2(L\text{-}NS_2)$ (Table 3). Consequently, the catalytic rate is limited by the rate of oxo transfer from $MoO_2(L\text{-}NS_2)$ to phosphine, behavior that also applies when $XO = Me_2SO$ and 3-fluoropyridine *N*-oxide [56,59].

The catalytic cycle is summarized in scheme (9) which depicts all the discrete steps. Although relatively efficient, the catalyzed reduction of *S*-oxides with tertiary phosphines is not likely to be a matter of practical

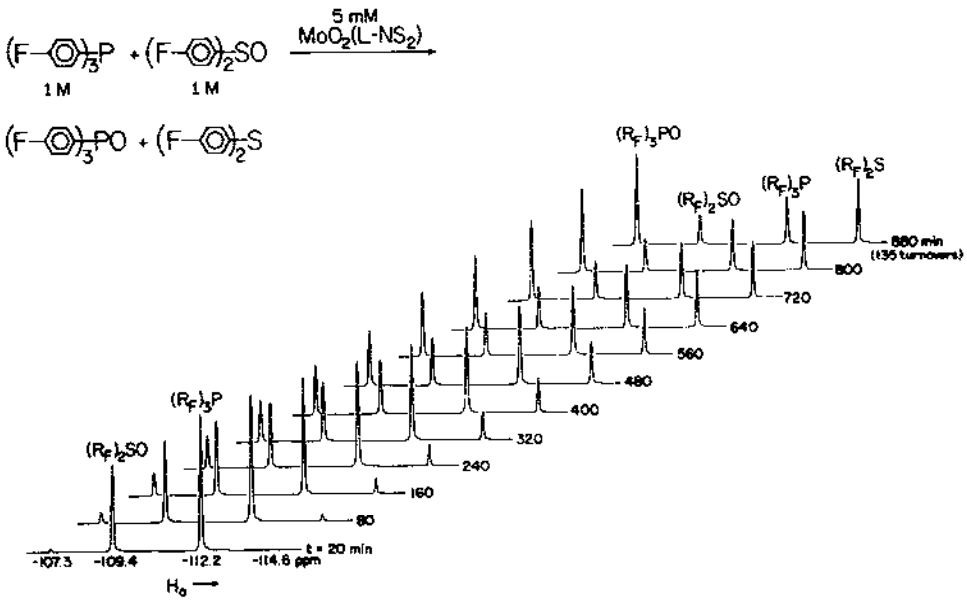


Fig. 7. Time course (to 880 min and 135 turnovers) of the molybdenum-catalyzed oxo transfer reaction between $(p\text{-}C_6H_4F)_3P$ and $(p\text{-}C_6H_4F)_2SO$ in DMF solution at 30 °C as observed by ^{19}F NMR spectroscopy (reproduced with permission from ref. 59).

importance. In the context of analogue reaction systems, the main point is that a catalytic system can be constructed. In enzymatic catalysis, the oxidized and reduced forms of the active site are connected by reactions of electron transfer and protonation (Figs. 4 and 5), processes that cannot be applied to reaction system (8) because of solubility and stability limitations. At the present stage of development of such catalytic systems, one must accept the simulation of these steps by the two-electron reduction of molybdenum(VI) with a tertiary phosphine when substrate reduction is desired. If the substrate of interest were to be oxidized in the catalytic system (8), the required two-electron oxidation of the reduced catalyst could be effected by a number of oxo donors such as Me_2SO. The system can be extended to other substrates. Similarly, the use of ^{19}F NMR as a means of following catalyzed reactions has broad applicability. Other catalytic oxo transfer reactions mediated by molybdenum complexes are summarized elsewhere [15].

D. A THERMODYNAMIC REACTIVITY SCALE FOR OXO TRANSFER

It is evident to anyone familiar with oxygen atom transfer reactions that high energy compounds such as iodosylbenzene, *m*-chloroperbenzoic acid,

dinitrogen oxide, percarbonate, and hydrogen peroxide are thermodynamically powerful, and for the most part, kinetically useful oxo donors. Similarly, tertiary phosphines and cyanide are easily recognized as strong oxo acceptors. What is considerably less evident are the relative reactivities of other real or potential oxo donors and acceptors, including complexes of a given metal in oxidation states that differ by one or two electrons. In an attempt to redress this situation, we have devised a simple thermodynamic scale of oxo transfer reactivity. Because the essential ideas and much of the thermodynamic data have been set out in some detail elsewhere [15,57], we provide a rather brief account, limiting considerations to analogue reaction systems of molybdenum.

(i) The scale of oxidation enthalpies

The enthalpy changes for selected reactions (couples) of the type $X + \frac{1}{2}O_2(g) = XO$ are presented in Table 4. A more extensive listing is available [15]. This table can be used for predicting reactions in a manner analogous to the use of a table of standard redox potentials. Under the criterion of exothermicity (many fewer ΔG data are available) and with favorable kinetics, the reduced member of a given couple can reduce the oxidized member of another couple with a larger ΔH, and conversely. For example, the enthalpic driving force for the reaction $ClO_3^- + SO_3^{2-} = ClO_2^- + SO_4^{2-}$ (couples *b*, *k*) is huge (-57 kcal mol^{-1}) and the reaction proceeds. The

TABLE 4

Selected thermodynamic data for the reaction $X + 1/2O_2(g) \rightarrow XO$

Couple	X [a]	XO [a]	$-\Delta H$ (kcal mol^{-1}) [b]
a	$S_2O_3^{2-}$(aq)	***$S_2O_4^{2-}$**(aq)	+12
b	ClO_2^-(aq)	***ClO_3^-**(aq)	−8
c	C_5H_4N(g)	**C_5H_4NO**(g)	−13 [c]
d	C_2H_4(g)	C_2H_4O(g)	−25
e	NO_2^-(aq)	**NO_3^-**(aq)	−25
f	Me_2S(g)	**Me_2SO**(g)	−27
g	$MoO(S_2CNEt_2)_2$	$MoO_2(S_2CNEt_2)_2$	−35 [d]
h	MoO(L-NS_2)(DMF)	MoO_2(L-NS_2)	< −35 to > −54 [e]
i	**Me_2SO**(g)	Me_2SO_2(g)	−52
j	***MeCHO**(g)	$MeCO_2H$(g)	−64
k	***SO_3^{2-}**(aq)	SO_4^{2-}(aq)	−65
l	Ph_3P(g)	Ph_3PO(g)	−67
m	***CO**(g)	CO_2(g)	−68

[a] Enzyme substrates in bold face. [b] Data from ref. 15 unless otherwise indicated. [c] Ref. 75. [d] Ref. 67; 1,2-dichloroethane solution. [e] See text.

driving force for the reduction of Me_2SO with Ph_3P (couples *f*, *l*) is also very large (-40 kcal mol^{-1}) but this reaction, which is of the type shown in eqn. (13), requires a catalyst. The enthalpy of couple *g* results from a direct calorimetric measurement which is part of the thermochemical work of Watt et al. [67] on the reactions, including oxo transfer, of complexes **10–12** in 1,2-dichloroethane solution. This pioneering research has aided materially in the evolution of a reactivity scale.

There are no direct enthalpy data for couple *h*. However, because of the occurrence of the intermetal oxo transfer reaction (14) and the reduction reaction (15) [57]

$$MoO(L\text{-}NS_2)(DMF) + MoO_2(S_2CNEt_2)_2 \rightarrow MoO_2(L\text{-}NS_2) + MoO(S_2CNEt_2)_2 \quad (14)$$

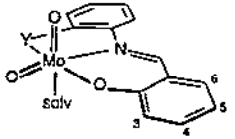
$$MoO_2(L\text{-}NS_2) + 2PhSH \rightarrow MoO(L\text{-}NS_2)(DMF) + PhSSPh + H_2O \quad (15)$$

the ΔH value of this couple can be bracketed. This result, together with the enthalpy of couple *g*, leads to an extremely interesting observation which we have emphasized before [14,15,57]. The Mo(IV,VI) couples *g*, *h* are positioned so as to oxidize or reduce all enzymatic substrates for which thermodynamic data are available. Thus $MoO_2(L\text{-}NS_2)$ and $MoO(L\text{-}NS_2)(DMF)$ are thermodynamically competent as site analogues, a property demonstrated by the transformations of the unmarked enzymatic substrates in Table 4. This necessary attribute could not have been securely predicted, but as we have argued [14,57], it originates in large part because of the design aspect of the $L\text{-}NS_2$ ligand which, as the enzymatic sites, contains thiolate ligands.

(ii) Thermodynamic fitness of molybdenum complexes

The effect of thiolate ligands on oxo transfer reactivity is illustrated by the following observations. (i) The bis(alkoxide) variant of **13** is not reduced with Ph_3P, in contrast to the ready reduction of **13** itself with this phosphine. (ii) The Schiff base complex $MoO_2(ssp)(solv)$ (**16**) is reduced with Ph_2EtP at a moderate rate ($k = 1.0 \times 10^{-3}$ M^{-1} s^{-1} at 30 °C) whereas $MoO_2(sap)(solv)$ (**17**) does not react at any appreciable rate under the same conditions [76,77]. (iii) Complex **17** is reducible with the more basic Ph_2RP (R = Me, Et) but elevated temperatures (above 70 °C) are required for convenient reaction times [78,79]. The available evidence suggests that the couples based on **16** and **17** are below couple *h* in Table 4. In this event, the corresponding $Mo^{IV}O$ complexes, which have not been isolated (vide infra), will be relatively strong oxo acceptors and be capable of reducing all oxidized enzymic substrates. In terms of thermodynamic fitness, a (hypo-

thetical) $Mo^{IV}O$ site without anionic sulfur ligation will presumably be competent to reduce oxidized substrates, but not necessarily catalytically. Its oxidized ($Mo^{VI}O_2$) form may be too stable and thus not reducible by the electron transfer and protonation pathway of Fig. 4 at the potentials of physiological reductants. Similarly, a tungsten-substituted enzyme, even with sulfur ligation to the metal, may not be catalytic owing to the relative stabilization of tungsten(VI) vs. molybdenum(VI) in equivalent environments. Assuming analogous oxo species, the $W^{IV}O$ state, if accessible at all, should be a powerful oxo acceptor, but the $W^{VI}O_2$ state is likely to be too stable to be reduced back to $W^{IV}O$ under physiological conditions. This appears to be the case with rat liver tungsten sulfite oxidase [80,81], which is inactive and can be reduced to tungsten(V) but presumably not lower. Recent results in this laboratory bear out the greater difficulty of reducing $W^{VI}O_2$ species by atom transfer [82]. For example, $WO_2(S_2CN(CH_2)_5)_2$ is inert to Ph_3P and reacts only very slowly with Me_3P and Et_3P at 50 °C in 1,2-dichloroethane. It is reduced with the very strong oxo acceptor $(MeO)_3P$ ($\Delta H \approx -90$ kcal mol^{-1}), the product isolated being $W_2O_3(S_2CN(CH_2)_5)_4$.



16 (Y = S, ssp)
17 (Y = O, sap)

To proceed further with an assessment of the factors that influence oxo transfer reactivity, potentials for the half-reaction $MO_2L_n + 2H^+ + 2e^- = MOL_n + H_2O$ or calorimetrically measured enthalpies of reactions such as $MOL_n + XO = MO_2L_n + X$, determined as a function of M and L, would be ideal. Unfortunately, no such information is available. If one-electron reduction potentials are accepted as a measure of intrinsic reducibility, the foregoing conclusions are upheld. Thus for complexes whose only significant difference is in the ligand groups RS^- for RO^- (the only comparison available), the sulfur-ligated complexes are more easily reduced than their oxygen-ligated counterparts [57]. Potential differences do, however, depend strongly on the nature of the remaining ligands.

Somewhat more striking is the effect of Mo/W substitution. While it is axiomatic that, at parity of environment, molybdenum is less difficult to reduce than tungsten, there has been little quantitative data to support this until the last few years. The majority of available redox potentials have been collected [57], and without exception they bear out the expected order of reducibilities. In a particularly significant contribution, Heath et al. [83] have determined the potentials for the successive reversible one-electron reductions of the hexachlorometalates of 4*d* and 5*d* elements in dichloromethane. The results for molybdenum and tungsten are summarized as

$E_W - E_{Mo}$ (V) in series (16):

$$[MCl_6]^0 \overset{-0.61}{\longleftrightarrow} [MCl_6]^{1-} \overset{-0.65}{\longleftrightarrow} [MCl_6]^{2-} \overset{-0.87}{\longleftrightarrow} [MCl_6]^{3-} \quad (16)$$

The potentials are especially interesting because chloride, but not NH_3 in this regard, is nonetheless an innocent hard ligand that will not strongly attenuate intrinsic differences between isoelectronic metal ions in their complexes. In comparison, thiolate, phosphine, and carbonyl ligands do markedly reduce potential differences [57]. While this same effect probably operates in enzymes, because in cofactor structure **5** there are two anionic sulfur ligands, it seems unlikely that a functional tungsten-substituted version of most oxotransferases can be produced.

E. BIOLOGICAL OXO TRANSFER

The purpose of functional models and analogue reaction systems is to provide, for biological reactions, feasible reaction pathways based on demonstrated chemistry. One advantage of such a system is that a given reaction can be inspected separately rather than as a transformation coupled to others in a catalytic system. This reaction is then a counterpart of a single turnover enzymatic reaction, the accomplishment and study of which is usually a very demanding endeavor. The results of system (8) are considered to provide the type of information sought from analogue reaction systems. Consequently, direct atom transfer to the catalytic site is not only a conceivable but also a feasible reaction pathway for the reduction of *S*-oxide and *N*-oxide substrates. In the case of nitrate, this pathway had been conceived earlier [84–86] but it lacked experimental support. Nearly all the early work on the reduction of nitrate with molybdenum complexes involved molybdenum(V), and in a number of these systems the initial reaction product is not nitrite but nitrogen dioxide [15]. Abiological reduction of nitrate has been accomplished with mononuclear [87] and binuclear molybdenum(III) [88], and for the latter the oxo transfer pathway has been proven by isotope labeling. However, there is no clear indication at present that a binuclear site or molybdenum(III) is involved in nitrate reductase, but the matter remains open.

(i) Initial experiments

The first evidence that an atom transfer mechanism might apply to any of the enzymes in Table 1 was developed in experiments reported in 1966 by Murray et al. [29]. They showed that liver xanthine oxidase when supplied with xanthine and ^{18}O-labeled nicotinamide *N*-oxide incorporated 0.67 atom

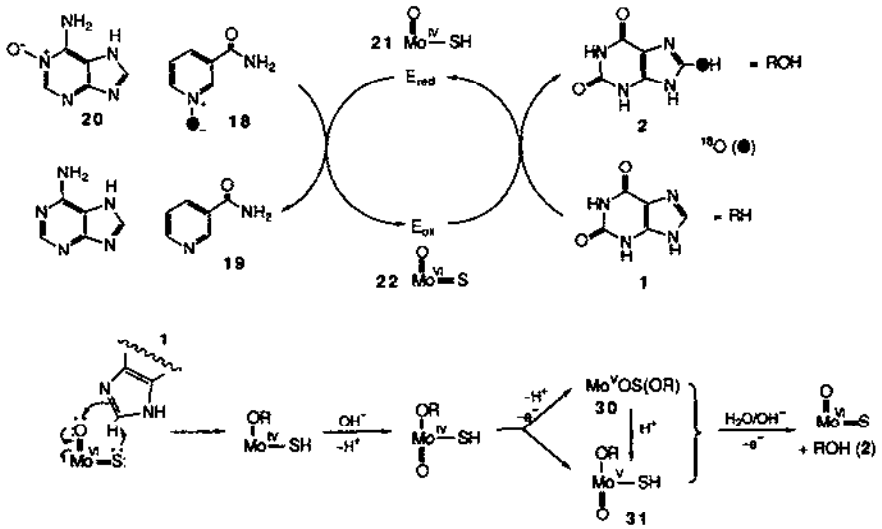


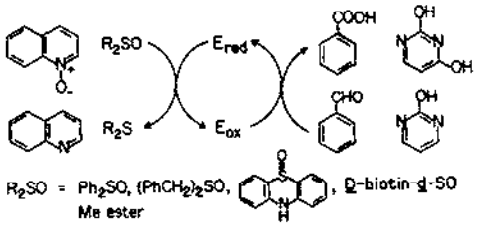
Fig. 8. Upper scheme: an interpretation of the ^{18}O isotope transfer from nicotinamide *N*-oxide (**18**) to xanthine (**1**) and the reduction of adenine 1-oxide (**20**) in the course of uric acid (**2**) formation catalyzed by xanthine oxidase [29,72]. The scheme is based on the oxo transfer hypothesis, but it is not necessarily the only catalytic pathway in the systems. Lower reaction scheme: minimal mechanism for the oxidation of xanthine to uric acid.

fraction of ^{18}O into xanthine in the course of forming uric acid. We have interpreted that result under the oxo transfer hypothesis in terms of the cycle in Fig. 8 [14,15,57]. The *N*-oxide (**18**) acts as an oxo donor to the reduced enzyme, converting it to its oxidized form which reacts with the natural substrate xanthine (**1**) to form uric acid (**2**). With milk xanthine oxidase, the extent of incorporation was 0.54. It was further proven with $^{18}O_2$ that the oxygen introduced did not come from dioxygen or from water, a point confirmed by the use of $[^{18}O]H_2O$.

In the interpretive cycle in Fig. 8, **18** functions as an electron acceptor by being an oxygen atom donor to molybdenum(IV), the atom transferred being reduced to oxide and the donor converted to nicotinamide (**19**). Adenine 1-oxide (**20**) was found to fulfill the same function [89]. In this picture the oxo donor interacts directly with the molybdenum site, and is a device for converting molybdenum(IV) to molybdenum(VI) in order that the next turnover can occur. Thus xanthine oxidase is also an *N*-oxide reductase. In the physiological mode of enzyme action, however, electrons flow from the molybdenum site to a flavin, through the two Fe_2S_2 clusters present in the enzyme, to dioxygen, which is the terminal electron acceptor. That water is the source of oxygen atoms transferred may be appreciated from the minimal reaction scheme in Fig. 8, which is a summary of the

considerations of several investigators but does not include all views [13,14,18,90–93]. We will return to this scheme, but for now it is seen that hydrolysis of reduced intermediates restores the oxygen atom in the Mo=O bond found in oxidized site **8** by EXAFS.

The appearance of ^{18}O in **2** unambiguously requires that the isotope label be transferred to the catalytic site and then to **1** when it is transformed to product. Thus in xanthine oxidase both the molybdenum(IV) and molybdenum(VI) states are capable of oxo transfer, in a manner that may be likened to the behavior of the components of reaction system (8). The lack of higher extents of incorporation of the isotope label in the product may arise from oxygen exchange of **21** and/or **22** (Fig. 8) with bulk water. Current evidence indicates that the reduced form of the enzyme exchanges more rapidly [92,94]. Possibly some of the label was also lost in the reaction $ArNO + 2H^+ + 2e^- = ArN + H_2O$ wherein the *N*-oxide is reduced and molybdenum(IV) is oxidized by electron transfer. Oxo transfer capability is probably not confined to xanthine oxidase, although no isotope tracer work necessary to prove this statement has yet been carried out. As one example, guinea pig liver aldehyde oxidase under anaerobic conditions will reduce *S*-oxides such as diphenyl and dibenzyl sulfoxide in the presence of aldehydes or 2-hydroxypyrimidine as electron donors [28]. These results are summarized in terms of the oxo transfer hypothesis in scheme (17) [57]:



(17)

Because of the absence of dioxygen, a non-atom transfer pathway would appear to require reduction of the *N*-oxide or *S*-oxide by proton-coupled electron transfer.

(ii) The Hille–Sprecher experiments

These highly significant experiments, which probed the occurrence of oxo transfer in the mechanism of action of milk xanthine oxidase [92], are schematically summarized in Fig. 9. Two separate experiments were conducted. The uric acid product from each was silylated with *N,O*-bis(trimethylsilyl)acetamide to provide volatility for mass spectrometric analysis. One experiment was conducted with an excess of enzyme such that it was

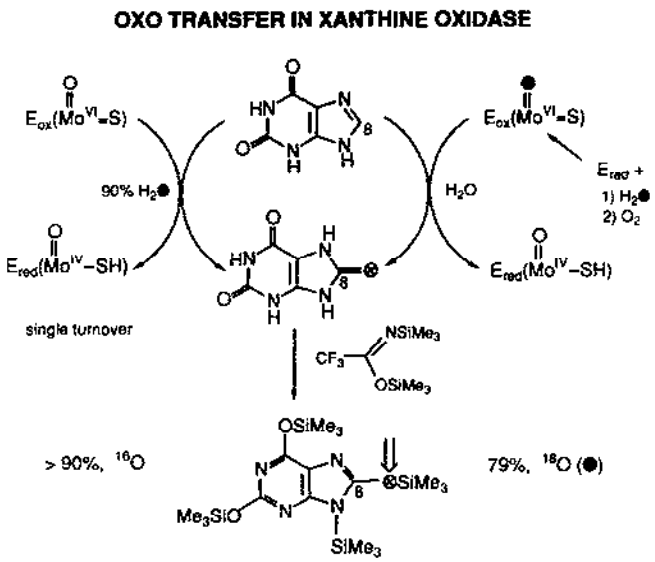


Fig. 9. Schematic representation of the Hille–Sprecher experiments which demonstrated an oxo transfer pathway for milk xanthine oxidase [92]; ⊗ is the oxygen atom introduced in oxidation and whose indicated isotopic contents were determined by mass spectrometry.

statistically improbable that a given enzyme molecule would encounter more than one molecule of substrate. The reaction was carried out in 90% $[O^{18}]H_2O$ for a single turnover. More than 90% of the uric acid contained ^{16}O at position 8. In the other experiment, the enzyme was reduced with an excess of xanthine sufficient to maintain reduction while the oxygen atom was exchanged with $[^{18}O]H_2O$. It was then oxidized and separated from the reaction mixture. The enriched enzyme was incubated with xanthine in $[^{16}O]H_2O$. The product contained 79% ^{18}O at position 8. The 21% isotope dilution doubtless arises because of slow exchange of the oxidized enzyme over the time course of the experiment. Controls established that the oxygen atoms of uric acid do not exchange with water. The results prove that the oxygen atom incorporated into uric acid in the event of oxidation arises from the enzyme and not from the solvent. The most reasonable interpretation is that the reaction proceeds by oxo transfer from the molybdenum(VI) atom, given the preceding body of demonstrated chemistry of this type. It is improbable that the $Mo^{VI}OS$ group **22** (Fig. 8) would not have a similar reactivity to the $Mo^{VI}O_2$ group. However, it is the case that no site analogue containing this group has been synthesized. Indeed, the preparation of such a complex remains one of the significant challenges in oxotransferase analogue chemistry.

F. SYNTHETIC MOLYBDENUM(IV, V) COMPLEXES AND THEIR RELATIONSHIP TO ENZYME STATES

Current interpretations of the mode of action of oxotransferases implicate three oxidation states, molybdenum(VI, V, IV). The relationship of synthetic oxomolybdenum complexes in these oxidation states to states of the enzymes, primarily in terms of structures and electronic features, has been examined by Garner and Bristow [17]. We add here other considerations based on recent developments in molybdenum(IV, V) chemistry primarily due to the experiments of Enemark, Spence, and their coworkers in the United States, and Hanson, Pilbrow, Wedd, and their coworkers in Australia [95–106].

(i) Oxo transfer

Oxidation of substrate in reaction system (8) is unexceptional in the sense that the reactions are first order in the $Mo^{VI}O_2$ complex and XO (Table 3). All other reaction systems containing analogous components also exhibit bimolecular kinetics [15,53,103]. However, reduction of substrate in other systems does not necessarily follow the scheme of system (8) wherein saturation kinetics are observed. Reaction (6) with $XO = Me_2SO$ is a rather slow second-order process ($k_2 = 1.6 \times 10^{-4}$ M^{-1} s^{-1}, 298 K) at the ratio $[Me_2SO] : [Mo(IV)] \approx 500 : 1$ [53]; activation parameters were not determined. Reaction (18) proceeds with second-order rate constant $k_2 = 1.5 \times 10^{-5}$ M^{-1} s^{-1} in toluene at 298 K ($\Delta H^{\ddagger} = 15.3(2)$ kcal mol^{-1}, $\Delta S^{\ddagger} = -29(2)$ e.u.) [103]:

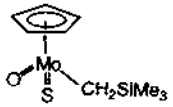
$$\{HB(Me_2pz)_3\}MoO\{S_2P(OEt)_2\} + Me_2SO \rightarrow \{HB(Me_2pz)_3\}MoO_2\{S_2P(OEt)_2\} + Me_2S \qquad (18)$$

Even at very large $[Me_2SO] : [Mo^{IV}O]$ ratios, the reaction remains bimolecular. The absence of saturation kinetics behavior is probably due to the lack of a labile binding site on the molybdenum(IV) complex, whose X-ray structure reveals the presence of bidentate dithiophosphate and tridentate tris(pyrazolyl)borate ligands. While the number of systems studied is rather limited, the present results indicate that a labile binding site (as in **11**) does not necessarily lead to saturation behavior and a first-order pathway. Further, such a site, at least when recognized by being vacant or having solvent or some other weak ligand present, is obviously not a general requirement for substrate reduction. The large negative activation entropy of reaction **18** implies an associative transition state with Me_2SO bound. It is considered that, because of the $HB(Me_2pz)_3$ ligand, the molybdenum(IV) complex is too hindered to bind substrate without dissociation of one sulfur

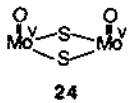
atom of the dithiophosphate ligand to give a six-coordinate transition state [103]. The analogous complex containing the $Et_2NCS_2^-$ ligand does not react with neat Me_2SO for at least 48 h, suggesting that an alteration in the binding of the bidentate ligand is required for reaction. It is not known whether the product $Mo^{VI}O_2$ complex is six- or seven-coordinate.

(ii) Oxo / sulfido substitution

The presence of a terminal sulfido or hydrosulfido ligand in active sites of xanthine oxidase/dehydrogenase, aldehyde oxidase, and possibly carbon monoxide dehydrogenase places an imperative on an understanding of Mo=S/SH interactions in those oxidation states where they occur in enzymes. The $Mo^{VI}OS$ fragment is present in species such as $[MoO_2S_2]^{2-}$ [107] and $MoOS(R_2NO)_2$ [108,109], which are derivatives of $[MoS_4]^{2-}$ and cannot be construed as site analogues. Very recently, Faller and Ma [110] have prepared $Cp^*MoOS(CH_2SiMe_3)$ (**23**) as red crystals from the reaction of the dioxo compound with H_2S in CS_2 solution. The $Mo^{VI}OS$ group is thus far unknown in any molecule in which the remaining binding sites are occupied by $2e^-$ ligands. The most likely mode of decomposition of the group, once formed, is by autoreduction owing to an overly reducing ligand environment or by reduction with the sulfiding reagent with likely formation of the bridge unit **24**. This group is known [111,112]; considerably more prevalent is the analogous group $Mo_2^VO_4$ which is accessible by reduction of $Mo^{VI}O_2$ complexes [15]. One possible means of stabilizing the $Mo^{VI}OS$ group is by its generation in a complex, through O/S ligand substitution of an $Mo^{VI}O_2$ group or oxidative addition from an $Mo^{IV}O$ or $Mo^{IV}S$ species, whose ligand steric properties and coordination saturation block the formation of bridge **24**. In this event the $Mo^{VI}OS$ complex should be more resistant to reduction unless, of course, there is some other feasible means of stabilizing molybdenum(V). Complex **23** is not an acceptable site model. Given the existence of compounds such as $Cp_2^*MoO_2(\mu_2\text{-}S)_2$ and $Cp_2^*MoS_2(\mu_2\text{-}S)_2$ [113], both of which have a transoid conformation, it seems unlikely that **23** owes its stability to steric impedance of dimerization.



23

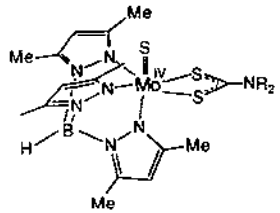


24

Progress has been made in the introduction of sulfido ligands into molybdenum(IV, V) complexes. Recent work by Young et al. [100] in achieving the Mo^{IV}=S group is summarized in reactions (19):

$$MoO(S_2CNR_2)_2 + K[HB(Me_2pz)_3] \rightarrow \{HB(Me_2pz)_3\}MoO(S_2CNR_2) + KS_2CNR_2 \quad (19)$$

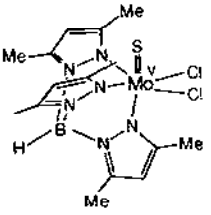
$\downarrow B_2S_3\text{–}CH_2Cl_2$



25

Here complex **11** serves as a precursor for the mixed-ligand oxo complex which affords the gold-yellow product **25** in good yield. The complex with R = Et has a distorted octahedral stereochemistry with an Mo=S bond distance of 2.129(2) Å. The complexes do not sustain a reversible oxidation step. In a similar manner, the Mo^{V}=S complex **26** was obtained by reaction (20) as an orange-brown solid [101]:

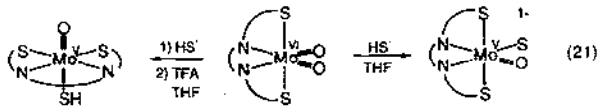
$$\{HB(Me_2pz)_3\}MoOCl_2 \xrightarrow[CH_2Cl_2]{B_2S_3} \quad (20)$$



26

Its structure has not been determined by X-ray analysis but is not in question. An analysis of the EPR spectrum of **26** has been presented [101]. Both **25** and **26** exhibit weak near-IR absorption bands at 1000–1100 nm which are likely to arise from $d \rightarrow d\pi^*$ transitions. These may be of some general utility in establishing the presence of $Mo^{IV,V}$=S groups.

In a somewhat different approach starting from the $Mo^{VI}O_2$ oxidation level, sulfido and hydrosulfido groups have been introduced into complexes containing the N_2S_2 ligand L^1. Following solution experiments that served to demonstrate the insertion of these ligands [98,102], the preparative reactions (21) starting from the fully characterized dioxo complex **27** [102] were carried out [105]:



28 27 29

(21)

Complex **28** was prepared by reaction with excess hydrosulfide followed by treatment with excess trifluoroacetic acid to afford a deep purple product. It is the only known synthetic compound containing the $Mo^{V}O(SH)$ grouping. Treatment of **27** with excess hydrosulfide but no acid gives a monoanion formulated as **29**, which was isolated as a Ph_4P^+ salt after the addition of Ph_4PCl in methanol and then water. Protonation of **29** is reported to give a transient blue color, attributed to the *cis* isomer of **28**, followed by rearrangement to the *trans* form. The structures of **28** and **29** have not been confirmed by X-ray crystallography but they have been examined by molybdenum and sulphur K-edge XANES and EXAFS [105].

The structure of **28**, with one Mo=O bond at 1.66 Å and 3–4 Mo–S bonds at 2.39 Å, is not exceptional. The near-edge spectrum of **29** provides no evidence for a short (ca. 2.15 Å) Mo=S bond whereas it is clearly detected in **26** by such means. Further, the EXAFS transform indicates the presence of one Mo=O bond at 1.68 Å, and 2–3 Mo–S distances at or near 2.36 Å with some heterogeneity. Elemental analysis of the Ph_4P^+ salt is accurate for an S : P atom ratio of 3 : 1 and the sulfur content is such that a difference of ± 1 atom would be detectable. Provided the solid is not a mixture, this rules out certain alternatives such as binuclear μ_2-S and $(\mu_2\text{-}S_2)_2$ and mononuclear $\eta^2\text{-}S_2$ structures involving sulfido or persulfido ligands. In addition to the proton-linked conversion **29** → **28**, the two compounds have different EPR spectra and cyclic voltammetric behavior, showing that they are indeed different compounds. One conclusion is that **29** contains a terminal Mo–S single bond of length ca. 2.36 Å [105], although an Mo–Cl bond would be indistinguishable by molybdenum EXAFS. Terminal M–S single bonds are not unknown but are extremely rare, and are expected to be strongly polarized with the sulfide atom highly nucleophilic. Examples are found in the compounds Na_5FeS_4 [114] and Na_3FeS_3 [115], both prepared by high temperature synthesis. In these compounds the sulfido atoms are surrounded by sodium ions, providing some stabilization by charge neutralization. A similar effect is absent in the isolated salt of **29**, which, moreover, was separated from its reaction mixture by the addition of water. The elemental composition and the Mo–S distance are compatible with bridge unit **24**, which would be expected to have an Mo–Mo separation near 2.8 Å [111,112]. No evidence for this feature in the EXAFS was reported [105]. At the time of writing, the exact nature of this compound remains open. It may be noted that this complex, being a six-coordinate d^1 species, is a potential candidate for bond stretch isomerism [116]. Further, because of the heterogeneity in Mo–S bond distances, the difference between the actual distance in **29** and a normal Mo=S bond may be less than ca. 0.2 Å and thus in the range of known cases of bond stretch isomerism. As yet, no metal–sulfido bond has been implicated in this type of isomerism.

(iii) Relation to enzyme sites

The potential importance of model structural work on molybdenum(V) complexes is the deduction of at least some of the ligands present in enzymes of the same oxidation state. Enzymes, particularly xanthine oxidase, have been the subjects of extensive EPR studies [9–13,48,91,117–122]. Bray and coworkers have provided highly significant information through detailed spectroscopic analysis, including determination of hyperfine interactions in different states of the enzymes by introduction of appropriate isotopes of molybdenum, sulfur, oxygen, carbon and hydrogen. While a detailed consideration of these results in relation to model compounds is beyond the purview of this report, some pertinent conclusions are summarized.

The proton-coupled redox series (22) has been established in aprotic solvents with complexes derived from tetradentate N_2S_2 ligands L = L^1 and L^2 [97,102,104]. The reduction of $Mo^{VI}O_2$ complexes is usually not reversible on the cyclic voltammetry time scale but these complexes are exceptions; potentials in THF vs. the standard calomel electrode (SCE) are given [102,123]:

$$\left[LMo^{VI}O_2\right] \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} \left[LMo^{V}O_2\right]^{1-} \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} \left[LMo^{V}O(OH)\right] \qquad (22)$$

-1.0 V (L^1), -1.3 V (L^2)

Upon reduction, the intensely basic $Mo^{V}O_2$ group is protonated by added water. Neither molybdenum(V) species has been isolated. However, $L^2MoO(OSiMe_3)$ has been prepared and its X-ray structure shows a *cis* arrangement of oxo and trimethylsiloxo ligands [104]. The close agreement between its EPR spectrum and that of the generated oxo–hydroxo complex supports a *cis* stereochemistry for the latter. These observations enhance the viability of the $Mo^{V}O(OH)$ unit in the overall catalytic cycle of those enzymes which do not contain sulfido or hydrosulfido ligands (Figs. 4 and 5).

The recently developed four-membered series (23) [98,102,105] is relevant to xanthine oxidase and other enzymes that contain unit **22** in their oxidized forms:

$$\left[L^1Mo^{VI}OS\right] \underset{-0.62\ \text{V}}{\overset{+e^-}{\rightleftharpoons}} \left[L^1Mo^{V}OS\right]^{1-} \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} \left[L^1Mo^{V}O(SH)\right] \xrightarrow[\text{ca. } -0.7\ \text{V}]{+e^-} \left[L^1Mo^{IV}O(SH)\right] \qquad (23)$$

Here the entry point is complex **29**, which may be oxidized to molybdenum(VI) or protonated to a *cis* form that rapidly rearranges to the

trans isomer. Reduction of the latter to the molybdenum(IV) level occurs in a step that is not strictly reversible. Potentials were determined in DMF solutions vs. SCE [105]. The existence of members of this series adds some credibility to the minimal mechanism of action of xanthine oxidase in Fig. 8. The well-known Very Rapid and Rapid EPR signals during turnover are attributed to intermediates **30** and **31** respectively. While the scheme is indeed minimal, it is nonetheless contentious in certain respects except direct oxo transfer from the catalytic site to substrate. If structure **29** and the non-protonated intermediate **30** were to be accepted, one of the Mo–O or Mo–S bonds could be abnormally long. In one example of bond stretch isomerism, both isomers of $[(Me_3\text{-tacn})WOCl_2]^{1+}$ were prepared and isolated by separate routes under thermal conditions [124,125].

(iv) A biologically irrelevant but otherwise pervasive reaction

As far as is currently known, the catalytic sites of oxotransferases are mononuclear. At first glance, this might appear to be an uneventful matter, but not so in the context of the synthetic analogue approach. The reason is the widespread occurrence of the μ-oxo molybdenum(V) dimerization reaction (4), of which the well-studied reaction (7) [67,70] is a particular case. Indeed, this is one of the few such reactions that is a reversible equilibrium; the others also involve complexes of bidentate sulfur ligands [70,126,127]. In synthetic systems, this reaction proceeds unless impeded and is frequently irreversible. Such a reaction is absent in the enzymes owing to the dilution of sites by protein structure. Depending on the relative rates of reactions (24) and (25)

$$Mo^{VI}O_2L_n + X \rightarrow Mo^{IV}OL_n + XO \tag{24}$$

$$Mo^{VI}O_2L_n + Mo^{IV}OL_n \rightleftharpoons L_nMo^{V}\overset{\overset{O}{\|}}{-}O\overset{\overset{O}{\|}}{-}Mo^{V}L_n \tag{25}$$

even stoichiometric product formation might not be possible. These considerations led to the design of reaction system (8) with its sterically obstructing ligands.

Reaction (25) is in effect an inner-sphere electron transfer reaction in which the bridged intermediate persists as product, or, alternatively, it is an incomplete intermetal oxo transfer reaction [15]. The $Mo^{V}_2O_3$ group in the product has been structurally confirmed by X-ray analysis in over 20 compounds.

In view of the foregoing information, the reported reduction of Schiff base complexes **16** and **17** in reaction (24), where $X = Ph_2EtP$, to afford the

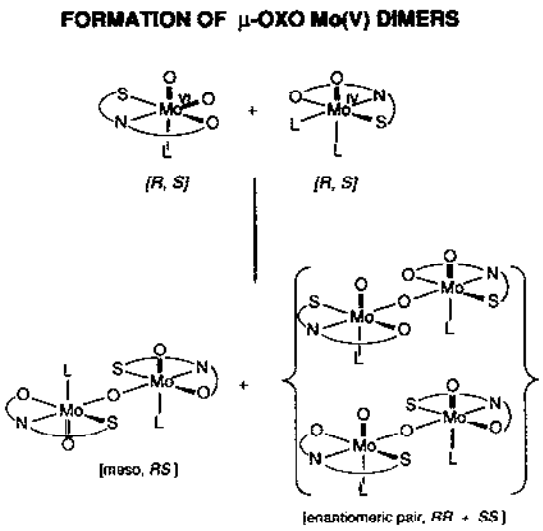


Fig. 10. Schematic depiction of the formation of μ-oxo molybdenum(V) dimers by reaction (25) involving chiral molybdenum(VI, IV) complexes (L = solv). The molybdenum(IV) complex has the stereochemistry of MoO(3-^{t}Bussp)(bipy) [79] where 2L = bipy (reproduced with permission from ref. 79).

molybdenum(IV) products MoO(ssp)(solv) and MoO(sap)(solv) [78] is anomalous. Product formulations were supported by analytical data and electrochemical behavior. Subsequently, Topich and Lyon [76,77], in their valuable work on the kinetics of reaction (24) with this same phosphine, adopted the molybdenum(IV) product formulation. In these studies, very clean isosbestic points in the visible region were observed. The lack of an evident steric and/or electronic barrier to the occurrence of reaction (25) caused us to re-examine reaction (24) using several substituted Schiff base ligands to improve solubility and crystallinity. The results of that investigation are schematically summarized in Fig. 10 for ssp complexes; the behavior of sap complexes is the same.

We have verified the reduction of complexes **16** and **17** with Ph_2RP (R = Me, Et) in reactions that afford sharp isosbestic points corresponding to those previously observed. The products were shown to be of the $Mo_2O_3L_2$ type, ultimately by X-ray diffraction. However, this study served to develop several criteria for the detection of μ-oxo complex formation without the necessity of X-ray structural analysis: (i) parent ion peaks in mass spectra; (ii) reaction stoichiometry; (iii) absorption spectral properties; (iv) product stereochemistry. No single criterion is necessarily adequate but the set is persuasive. We consider briefly the results for complexes where L = ssp [79].

The FAB mass spectrum of $Mo_2O_3(ssp)_2$ contained the proper parent ion peaks with the correct relative intensities arising from isotope distributions. Determination of the mole ratio $[R_3PO]$: $[Mo(VI)]_0 = 0.46$ at the completion of the reaction of $MoO_2(ssp)(DMF)$ with Ph_2MeP is consistent with reaction (26), the sum of reactions (24) and (25), rather than reaction (24) occurring separately:

$$2Mo^{VI}O_2L_n + X \rightleftharpoons Mo_2^{V}O_3L_{2n} + XO \qquad (26)$$

However, a distinction between these two reactions can be made only if the forward reaction (25) is fast and irreversible. This has proven to be the case for all complexes **16** and **17** examined as well as for $MoO_2(acac)_2$, which affords $Mo_2O_3(acac)_4$ upon reduction with phosphine. Reduced compounds of ssp and sap exhibit an intense absorption band at 440–490 nm and a band or shoulder at 300–340 nm which appear to be diagnostic for molecules containing the $Mo_2^{V}O_3$ group with combinations of nitrogen and anionic oxygen and sulphur ligands. As an example, the spectrum of $Mo_2O_3(ssp)_2$ in DMF has $\lambda_{max} = 319$ nm, $\epsilon_M = 24\,700\ M^{-1}\ cm^{-1}$ and $\lambda_{max} = 464$ nm, $\epsilon_M = 20\,200\ M^{-1}\ cm^{-1}$. For complexes of bidentate anionic sulphur ligands such as **12**, which are usually purple, the principal bands are at 330–380 and 500–520 nm. In the formation of μ-oxo dimers depicted in Fig. 10, each half-dimer is chiral. In this case, the products can be formed as *meso* and racemic diastereoisomers, which are not interconvertible by rotation about an Mo–O bridge bond. Most are detectable by small differences in chemical shifts in ^1H NMR spectra. For example, in THF solution the two azomethine proton signals of $Mo_2O_3(ssp)_2$ appear at 9.31 and 9.39 ppm. The spectrum of $Mo_2O_3(acac)_2$ is particularly impressive in this regard, containing two sets of six signals each, in an intensity ratio of ca. 2 : 1. There are, of course, numerous complexes which upon μ-oxo bridge formation do not generate diastereoisomers. Final confirmation of the stuctures of the bridged complexes was obtained by X-ray analysis of $Mo_2O_3(3\text{-}Rssp)_2(DMF)_2$ (R = EtO, ^{t}Bu) [79].

It is unlikely that any mononuclear molybdenum(IV) complex of the type MoOL(solv) (L = sap, ssp) has ever been isolated. However, it is entirely probable that the $Mo^{IV}OL$ entity is the instantaneous product of oxo transfer to substrate. The isosbestic points in the spectrophotometry of the reaction arise because the forward reaction (25) is much faster than reaction (24). To address the point of $Mo^{IV}OL$ formation in these systems, the product of reaction (27) (L = 3-^{t}Bussp)

$$MoO_2L(solv) + Ph_2MeP + bipy \xrightarrow{THF} MoOL(bipy) + Ph_2MePO + solv \qquad (27)$$

with excess bipyridyl was examined crystallographically and found to have the indicated formulation with the bipyridyl ligand occupying positions *cis* and *trans* to the oxo atom [79]. An earlier description of the unsubstituted ssp compound is correct [78]. This trapping experiment supports the proposition that molybdenum(IV) is the initial product of reaction with phosphine. It offers justification for the molybdenum(IV) formulations given elsewhere by ourselves and others for the instantaneous reaction product of oxo transfer from $Mo^{VI}O_2$ species [14,15,57,76,77].

The structures of molybdenum(VI, V, IV)–ssp complexes provide a clear indication of the facility of the dimerization reaction (25) for tridentate Schiff base complexes. With reference to Fig. 10, the ssp ligand has the same *mer* disposition in the two reactants and in the product. If L = solv in the molybdenum(IV) complex, dimer formation requires only dissociation or displacement of the solv ligand *cis* to oxo followed by the lengthening of one Mo–O bond originating in the molybdenum(VI) complex and the formation of a bridge Mo–O bond in concert with electron transfer. Clearly, reaction (25) is generally favorable. There are no oxo transfer systems in which the μ-oxo dimerization reaction does not occur, either irreversibly or as an equilibrium, unless suppressed by steric factors or added ligands [79]. In addition to those in reaction system (8), sterically embellished $Mo^{VI}O_2$ complexes designed to suppress reaction (25) have been synthesized [95,103,128]. The spatial properties of the $HB(Me_2pz)_3$ ligand appear to be largely responsible for the occurrence of oxo transfer reaction (18) without μ-oxo dimer formation.

Lastly, while $Mo_2^VO_3$ complexes are not usually desirable products in oxo transfer systems, they are not necessarily devoid of oxo transfer reactivity. The reverse of reaction (26) has been observed with L = ssp and sap complexes and XO = R_2SO and NO_3^- and its stoichiometry established [79]. Certainly any $Mo^{IV}O$ complex present would be expected to reduce these substrates. However, it was not possible to detect the known ^1H NMR resonances of the corresponding $Mo^{VI}O_2$ species in any solution prepared from a number of $Mo_2^VO_3$ complexes in solvents such as DMF, methanol, THF, and pyridine [79]. If, as seems likely, the substrate reacts directly with the molybdenum(V) complex, the following sequence of reactions (28) and (29) is probable:

$$Mo_2O_3L_2(solv)_2 + XO \rightleftharpoons Mo_2O_3L_2(solv)(XO) + solv \quad (28)$$

$$Mo_2O_3L_2(solv)(XO) + solv \rightarrow 2MoO_2L(solv) + X \quad (29)$$

CONCLUSION

It is hoped that this brief chronicle of events in this laboratory, together with selected recent developments in the laboratories of others, has conveyed

some of the progress and excitement in oxo transfer chemistry related to the mode of action of a broad class of molybdoenzymes. It is gratifying that our endeavors and those described in Sections F(i)–F(iii) are at once complementary in providing information that may ultimately lead to a satisfactory definition of all enzyme states over a catalytic reaction cycle. Certainly, the synthetic analogue approach to the reactivities, stereochemistry, and electronic properties of oxomolybdenum(VI, V, IV) units in enzymes has been a beneficial contributor. However, it must be remembered that, for analogue reaction systems in particular, this approach can only reveal what is possible —founded on demonstrated chemistry and the correctness of the model. What is true must be elicited from the natural system, but with less tribute to the conjectural than to the possible.

While the elucidation of the structure and mechanism of action of nitrogenase remains one of the great scientific challenges of this and the next decade, the accelerating evolvement of the fundamental chemistry and biology of the oxotransferases, or hydroxylases, is an achievement of some magnitude. At the center of this is the "universal" cofactor and its structure and reactivity. Surely we will see before long the total synthesis of molybdopterin [129] in quantities sufficient to allow synthesis of the cofactor and interrogation of all its properties. It is perhaps an irony of nature that in the pursuit of understanding of the two classes of large complex molybdoenzymes, one of their smaller integral components, their cofactors, present major obstacles at the same time. Finally, this account may have dispensed some modicum of evidence in favor of the answer proffered to the question posed at the outset of this contribution to the Centennial Volume of *Coordination Chemistry Reviews*.

ACKNOWLEDGEMENTS

Research described herein and performed at Harvard has been supported by National Science Foundation Grants CHE 81-06017 and 85-21365. Jeremy Berg initiated research on the bioinorganic chemistry of molybdenum oxo transfer reactions in this laboratory and developed reaction system (8). Martha Reynolds made important contributions to our initial kinetics studies. Research on this subject has been continued, expanded, and refined by John Caradonna, Julia Craig Loutens, Ed Harlan, and P.R. Reddy. I am grateful to all for participating in a research adventure that continues. I thank Professor Jack Spence for preprints of refs. 105 and 106, Professor Jack Faller for a preprint of ref. 110, and Sonny Lee and Jack Spence for useful discussions.

REFERENCES

1 G. Wilkinson, R.D. Gillard and J. McCleverty (Eds.), Comprehensive Coordination Chemistry, Vol. 3, Pergamon, Oxford, 1987, Part 36.

2 E.I. Stiefel, Prog. Inorg. Chem., 22 (1977) 1.
3 F.A. Cotton and R.A. Walton, Multiple Bonds Between Metal Atoms, Wiley-Interscience, New York, 1982.
4 M.H. Chisholm, Angew. Chem., Int. Ed. Engl., 25 (1986) 21.
5 F.E. Massoth, Adv. Catal., 27 (1978) 164.
6 R.R. Chianelli, Catal. Rev.-Sci. Eng., 26 (1984) 361.
7 R.J. Angelici, Acc. Chem. Res., 21 (1988) 387.
8 W.H. Orme-Johnson, Ann. Rev. Biophys. Biophys. Chem., 14 (1985) 419.
9 T.G. Spiro (Ed.), Molybdenum Enzymes, Wiley-Interscience, New York, 1985.
10 M. Couglan (Ed.), Molybdenum and Molybdenun-Containing Enzymes, Pergamon, Oxford, 1980.
11 R.C. Bray, Adv. Enzymol., 51 (1980) 107.
12 K.V. Rajagopalan, Biochem. Elem., 3 (1984) 149.
13 R.C. Bray, Q. Rev. Biophys., 21 (1988) 299.
14 R.H. Holm and J.M. Berg, Acc. Chem. Res., 19 (1986) 363.
15 R.H. Holm, Chem. Rev., 87 (1987) 1401.
16 R. Barral, C. Bocard, I. Serée de Roch and L. Sajus, Tetrahedron Lett., (1972) 16.
17 C.D. Garner and S. Bristow, in ref. 9, Chapter 7.
18 R. Hille and V. Massey, in ref. 9, Chapter 9.
19 O. Meyer, J. Biol. Chem., 257 (1982) 1333.
20 O. Meyer and K.V. Rajagopalan, J. Bacteriol., 157 (1984) 643.
21 J.M. Bell, J. Colby and E. Williams, Biochem. J., 250 (1988) 605.
22 K.V. Rajagopalan, in ref. 10, Chapter 7.
23 M.W.W. Adams and L.E. Mortenson, in ref. 9, Chapter 10.
24 E.J. Hewitt and B.A. Notton, in ref. 10, Chapter 8.
25 A. Del Campillo-Campbell and A. Campbell, J. Bacteriol., 149 (1982) 469.
26 J.H. Weiner, D.P. MacIsaac, R.E. Bishop and P.T. Bilous, J. Bacteriol., 170 (1988) 1505.
27 T. Satoh and F.N. Kurihara, J. Biochem. (Tokyo), 102 (1987) 191.
28 S. Yoshihara and K. Tatsumi, Arch. Biochem. Biophys., 242 (1985) 213; 249 (1986) 8.
29 K.N. Murray, J.G. Watson and S. Chaykin, J. Biol. Chem., 241 (1966) 4798.
30 G. Stöhrer and G.B. Brown, J. Biol. Chem., 244 (1969) 2498.
31 S. Kitamura and K. Tatsumi, Biochem. Biophys. Res. Commun., 120 (1984) 602.
32 I. Yamamoto, N. Okubo and M. Ishimoto, J. Biochem. (Tokyo), 99 (1986) 1773.
33 K.V. Rajagopalan, in W.B. Jakoby (Ed.), Enzymatic Basis of Detoxication, Vol. I, Academic Press, New York, 1980, Chapter 14.
34 S.P. Cramer and E.I. Stiefel, in ref. 9, Chapter 8.
35 S.P. Kramer, J.L. Johnson, A.A. Ribeiro, D.S. Millington and K.V. Rajagopalan, J. Biol. Chem., 262 (1987) 16357.
36 K.V. Rajagopalan, Biochem. Soc. Trans., 13 (1985) 401.
37 S. Kramer, R.V. Hageman and K.V. Rajagopalan, Arch. Biochem. Biophys., 233 (1984) 821.
38 T.R. Hawkes and R.C. Bray, Biochem. J., 219 (1984) 481.
39 H.D. May, N.L. Schauer and J.G. Ferry, J. Bacteriol., 166 (1986) 500.
40 B. Krüger and O. Meyer, Eur. J. Biochem., 157 (1986) 121.
41 T.R. Hawkes and R.C. Bray, Biochem. J., 222 (1984) 587.
42 G.N. George, T.R. Hawkes, G.D. Jones and R.C. Bray, Polyhedron, 5 (1986) 587.
43 J. Bordas, R.C. Bray, C.D. Garner, S. Gutteridge and S.S. Hasnain, Biochem. J., 191 (1980) 499.
44 S.P. Cramer, R. Wahl and K.V. Rajagopalan, J. Am. Chem. Soc., 103 (1981) 7721.

45 S.P. Cramer, L.P. Solomonson, M.W.W. Adams and L.E. Mortenson, J. Am. Chem. Soc., 106 (1984) 1467.
46 S.P. Cramer and R. Hille, J. Am. Chem. Soc., 107 (1985) 8164.
47 G.N. George, C.A. Kipke, R.C. Prince, R.A. Sunde, J.H. Enemark and S.P. Cramer, Biochemistry, 28 (1989) 5075.
48 S.P. Cramer, Adv. Inorg. Bioinorg. Mech., 2 (1983) 259.
49 R.H. Holm and J.A. Ibers, in W. Lovenberg (Ed.), Iron–Sulfur Proteins, Academic Press, New York, 1977, Chapter 7.
50 J.A. Ibers and R.H. Holm, Science, 209 (1980) 223.
51 W.H. Armstrong, in L. Que, Jr., (Ed.), Metal Clusters in Proteins, ACS Symposium Series 372, 1988, Chapter 1.
52 J.M. Berg and R.H. Holm, J. Am. Chem. Soc., 106 (1984) 3035.
53 M.S. Reynolds, J.M. Berg and R.H. Holm, Inorg. Chem., 23 (1984) 3057.
54 R.H. Holm and J.M. Berg, Pure Appl. Chem., 56 (1984) 1645.
55 J.M. Berg and R.H. Holm, J. Am. Chem. Soc., 107 (1985) 917.
56 J.M. Berg and R.H. Holm, J. Am. Chem. Soc., 107 (1985) 925.
57 E.W. Harlan, J.M. Berg and R.H. Holm, J. Am. Chem. Soc., 108 (1986) 6992.
58 J.P. Caradonna, E.W. Harlan and R.H. Holm, J. Am. Chem. Soc., 108 (1986) 7856.
59 J.P. Caradonna, P.R. Reddy and R.H. Holm, J. Am. Chem. Soc., 110 (1988) 2139.
60 J.A. Craig and R.H. Holm, J. Am. Chem. Soc., 111 (1989) 2111.
61 J. Halperin and H. Taube, J. Am. Chem. Soc., 72 (1950) 3320; 74 (1952) 375.
62 L. Ricard, J. Estienne, P. Karagiannidis, P. Toledano, J. Fischer, A. Mitschler and R. Weiss, J. Coord. Chem., 3 (1974) 277.
63 J.M. Berg and K.O. Hodgson, Inorg. Chem., 19 (1980) 2180.
64 P.C.H. Mitchell and R.D. Scarle, J. Chem. Soc., Dalton Trans., (1975) 2552.
65 X. Lu, J. Sun and X. Tao, Synthesis, (1982) 185.
66 X. Lu and J. Sun, Synth. React. Inorg. Met.-Org. Chem., 12 (1982) 427.
67 G.D. Watt, J.W. McDonald and W.E. Newton, J. Less-Common Met., 54 (1977) 415.
68 K. Tanaka, M. Honjo and T. Tanaka, Inorg. Chem., 24 (1985) 2662.
69 K.G. Moloy, Inorg. Chem., 27 (1988) 677.
70 T. Matsuda, K. Tanaka and T. Tanaka, Inorg. Chem., 18 (1979) 454.
71 B. Kaptein, L. Wang-Griffin, G. Barf and R.M. Kellogg, J. Chem. Soc., Chem. Commun., (1987) 1457.
72 K.N. Murray and S. Chaykin, J. Biol. Chem., 241 (1966) 2029.
73 N. Brot, L. Weissbach, J. Werth and H. Weissbach, Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 2155.
74 N. Brot and L. Weissbach, Trends Biochem. Sci., 7 (1982) 137.
75 L. Shaofeng and G. Pilcher, J. Chem. Thermodyn., 20 (1988) 463.
76 J. Topich and J.T. Lyon III, Polyhedron, 3 (1984) 61.
77 J. Topich and J.T. Lyon III, Inorg. Chem., 23 (1984) 3202.
78 I.W. Boyd and J.T. Spence, Inorg. Chem., 21 (1982) 1602.
79 J.A. Craig, E.W. Harlan, B.S. Snyder, M.A. Whitener and R.H. Holm, Inorg. Chem., 28 (1989) 2082.
80 J.L. Johnson, H. Cohen and K.V. Rajagopalan, J. Biol. Chem., 249 (1974) 5046.
81 J.L. Johnson and K.V. Rajagopalan, J. Biol. Chem., 251 (1976) 5505.
82 S.-B. Yu and R.H. Holm, Inorg. Chem., 28 (1989), in press.
83 G.A. Heath, K.A. Moock, D.W.A. Sharpe and L.J. Yellowlees, J. Chem. Soc., Chem. Commun., (1985) 1503.
84 R.J.P. Williams, Biochem. Soc. Trans., 1 (1973) 1.

85 R.A.D. Wentworth, Coord. Chem. Rev., 18 (1976) 1.
86 E.J. Hewitt, B.A. Notton and C.D. Garner, Biochem. Soc. Trans., 7 (1979) 629.
87 P.A. Ketchum, R.C. Taylor and D.C. Young, Nature (London), 259 (1976) 202.
88 K. Wieghardt, M. Woeste, P.S. Roy and P. Chaudhuri, J. Am. Chem. Soc., 107 (1985) 8276.
89 K.N. Murray and S. Chaykin, J. Biol. Chem., 241 (1966) 3468.
90 R.C. Bray, S. Gutteridge, D.A. Stotter and S.J. Tanner, Biochem. J., 177 (1979) 357.
91 G.N. George and R.C. Bray, Biochemistry, 27 (1988) 3603.
92 R. Hille and H. Sprecher, J. Biol. Chem., 262 (1987) 10914.
93 R.C. Bray and G.N. George, Biochem. Soc. Trans., 13 (1985) 560.
94 S. Gutteridge and R.C. Bray, Biochem. J., 189 (1980) 615.
95 P. Subramanian, J.T. Spence, R. Ortega and J.H. Enemark, Inorg. Chem., 23 (1984) 2564.
96 B.B. Kaul, J.H. Enemark, S.L. Merbs and J.T. Spence, J. Am. Chem. Soc., 107 (1985) 2885.
97 F. Farchione, G.R. Hanson, C.G. Rodrigues, T.D. Bailey, R.N. Bagchi, A.M. Bond, J.R. Pilbrow and A.G. Wedd, J. Am. Chem. Soc., 108 (1986) 831.
98 C.J. Hinshaw and J.T. Spence, Inorg. Chim. Acta, 125 (1986) L17.
99 G.R. Hanson, G.L. Wilson, T.D. Bailey, J.R. Pilbrow and A.G. Wedd, J. Am. Chem. Soc., 109 (1987) 2609.
100 C.G. Young, S.A. Roberts, R.B. Ortega and J.H. Enemark, J. Am. Chem. Soc., 109 (1987) 2938.
101 C.G. Young, J.H. Enemark, D. Collison and F.E. Mabbs, Inorg. Chem., 26 (1987) 2927.
102 D. Dowerah, J.T. Spence, R. Singh, A.G. Wedd, G.L. Wilson, F. Farchione, J.H. Enemark, J. Kristofzki and M. Bruck, J. Am. Chem. Soc., 109 (1987) 5655.
103 S.A. Roberts, C.G. Young, W.E. Cleland, Jr., R.B. Ortega and J.H. Enemark, Inorg. Chem., 27 (1988) 3044.
104 G.L. Wilson, M. Kony, E.R.T. Tiekink, J.R. Pilbrow, J.T. Spence and A.G. Wedd, J. Am. Chem. Soc., 110 (1988) 6923.
105 R. Singh, J.T. Spence, G.N. George and S.P. Cramer, Inorg. Chem., 28 (1989) 8.
106 C.J. Hinshaw, G. Peng, R. Singh, J.T. Spence, J.H. Enemark, M. Bruck, J. Kristofzki, S.L. Merbs, R.B. Ortega and P.A. Wexler, Inorg. Chem., 28 (1989), in press.
107 A. Müller, E. Diemann, R. Jostes and H. Bögge, Angew. Chem., Int. Ed. Engl., 20 (1981) 934.
108 K. Wieghardt, M. Hahn, J. Weiss and W. Swiridoff, Z. Anorg. Allg. Chem., 492 (1981) 164.
109 S.F. Gheller, T.W. Hambley, P.R. Traill, R.T.C. Brownlee, M.J. O'Connor, M.R. Snow and A.G. Wedd, Aust. J. Chem., 35 (1982) 2183.
110 J.W. Faller and Y. Ma, Organometallics, 8 (1989) 609.
111 N.C. Howlader, G.P. Haight, Jr., T.W. Hambley, M.R. Snow and G.A. Lawrance, Inorg. Chem., 23 (1984) 1811.
112 T. Shibihara, H. Kuroya, K. Matsumoto and S. Ooi, Bull. Chem. Soc. Jpn., 56 (1983) 2945.
113 M. Rakowski DuBois, D.L. DuBois, M.C. VanDerveer and R.C. Haltiwanger, Inorg. Chem., 20 (1981) 3064.
114 K.O. Klepp and W. Bronger, Z. Anorg. Allg. Chem., 532 (1986) 23.
115 P. Müller and W. Bronger, Z. Naturforsch., Teil B, 34 (1979) 1264.
116 Y. Jean, A. Lledos, J.K. Burdett and R. Hoffmann, J. Chem. Soc., Chem. Commun., (1988) 140; J. Am. Chem. Soc., 110 (1988) 4506.

117 R.C. Bray, Biol. Magn. Reson., 2 (1980) 45.
118 S. Gutteridge, M.T. Lamy and R.C. Bray, Biochem. J., 181 (1980) 285.
119 S. Gutteridge and R.C. Bray, Biochem. J., 189 (1980) 615.
120 R.C. Bray and S. Gutteridge, Biochemistry, 21 (1982) 5992.
121 F.F. Morpeth, G.N. George and R.C. Bray, Biochem. J., 220 (1984) 235.
122 J.P.G. Malthouse, J.W. Williams and R.C. Bray, Biochem. J., 197 (1981) 421.
123 C. Pickett, S. Kumar, P.A. Vella and J. Zubieta, Inorg. Chem., 21 (1982) 908.
124 G. Backes-Dahmann and K. Wieghardt, Inorg. Chem., 24 (1985) 4049.
125 K. Wieghardt, G. Backes-Dahmann, B. Nuber and J. Weiss, Angew. Chem., Int. Ed. Engl., 24 (1985) 777.
126 S. Miyake, K. Tanaka and T. Tanaka, J. Chem. Soc., Dalton Trans., (1981) 292.
127 T. Tanaka, K. Tanaka, T. Matsuda and K. Hashi, in W.E. Newton and S. Otsuka (Eds.), Molybdenum Chemistry of Biological Significance, Plenum, New York, 1980, pp. 361–367.
128 C.J. Cook and J. Topich, Inorg. Chim. Acta, 144 (1988) 81.
129 E.C. Taylor and L.A. Reiter, J. Am. Chem. Soc., 111 (1989) 285.