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MECHANISMS FOR THE REACTIONS OF MOLYBDENUM IN ENZYMES

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CONTENTS

ABBREVIATIONS

acac	acetylacetonate ion
cys	cysteinate ion
dien	diethylenetriamine
dppe	1,2-bis(diphenylphosphino)ethane
EDTA	ethylenediaminetetraacetate ion
FAD	flavin adenine dinucleotide
hist	histidinate ion
IDA	iminodiacetate ion
MIDA	methyliminodiacetate ion
NTA	nitrilotriacetate ion
Q	8-oxoquinolate ion
Rcys	alkyl ester of cysteinate ion

A. INTRODUCTION

It might be expected that a clear relationship should exist between the chemistries of those proteins which contain molybdenum as an essential element for enzymatic activity and the chemistries of the rather simple molybdenum complexes which are studied by inorganic chemists. However, there is a wide range of structural variability of those simple complexes, particular-

ly with respect to the number and stereochemistry of oxo ligands which are bound invariably to the metal in its higher oxidation states in an aqueous environment. It is the intent of this review to examine both the role of molybdenum in enzymes and the aqueous chemistry of the higher oxidation states of that element. The results from the former will delineate the range of oxidation states which should be examined in the latter. Where data from aqueous solutions are sparse, it will be necessary on occasion to refer to results from nonaqueous systems. Further insight will be gained by comparing, when possible, molybdenum and tungsten systems under identical conditions. Finally, several mechanisms for enzymatic activity will be examined using the known chemistry of molybdenum as a guide.

Three earlier reviews [1,2] which undoubtedly inspired considerable research, are now out of date.

B. THE ROLE OF MOLYBDENUM IN ENZYMES

Molybdenum is found in a number of enzymes such as nitrogenases, nitrate reductases, and sulfite, xanthine, and aldehyde oxidases. A few of these and their sources are listed in Table 1. These enzymes catalyze the conversions of molecular nitrogen to ammonia, nitrate to nitrite ion, sulfite to sulfate ion, xanthine to uric acid, and aldehydes to carboxylic acids, respectively. The ex-

TABLE 1

Some enzymes containing molybdenum[a]

Enzyme	Source	Mo content in g-atom/mole	Ref.
Nitrogenase	Clostridium pasteurianum	2	b
	Klebsiella pneumoniae	2	c
	Azotobacter vinelandii	2	d
Nitrate reductase	Neurospora crassa	2	e
Sulfite oxidase	Bovine liver	2	f
Aldehyde oxidase	Rabbit liver	2	g
Xanthine oxidase	Cow's milk	2	h

[a] Taken in part from ref. 3.
[b] Ref. 10.
[c] Ref. 39.
[d] R.C. Burns, R.D. Holsten and R.W.F. Hardy, Biochem. Biophys. Res. Commun., 39 (1970) 90.
[e] Ref. 15.
[f] Ref. 12.
[g] K.V. Rajagopalan, I. Fridovitch and P. Handler, J. Biol. Chem., 237 (1962) 922.
[h] L.I. Hart, M.A. McGartoll, H.R. Chapman and R.C. Bray, Biochem. J., 116 (1970) 851; M.A. McGartoll, F.M. Pick, J.C. Swann and R.C. Bray, Biochem. Biophys. Acta, 212 (1970) 523.

act role of molybdenum in the catalytic reactions of these and other enzymes has been difficult to ascertain with certainty even with those enzymes which can be obtained in reasonable quantities and in a very pure state. The difficulty is due to the complexities of these enzymes and their low molybdenum contents (see Table 1). Unfortunately, data from X-ray studies, even at low resolution, are not available. It is reasonable to assume, nevertheless, that the metal atoms are bound to donor atoms from amino acid residues, and that each molybdenum atom has several oxidation states available to it. While it is clear that the highest available oxidation state is +6, the lowest oxidation state available should be dependent upon the nature of the reducing agent, the exact nature of the ligating atoms, and the pH. Thus, the resting state of the enzyme can involve any of several oxidation states of molybdenum. Furthermore, since lower or higher oxidation states are available, it can be assumed that oxidation or reduction of substrates involves the corresponding reduction or oxidation of molybdenum [3,4]. Experimental evidence will be discussed in a subsequent paragraph.

Additional prosthetic groups, such as flavin and Fe/S (a non-heme iron—sulfur center) in xanthine oxidase, are invariably present as well. The redox behavior of flavin is well known [5]. Structural studies of rubredoxin have shown that iron is found in a distorted $Fe(S—cys)_4$ tetrahedron (where S—cys indicates bonding to sulfur of a cysteinyl residue) [6]. Ferrodoxin, which has equal quantities of iron and acid-labile sulfur, contains two non-bonded Fe_4S_4 cubes [6]. Each iron atom is bonded additionally to the sulfur atom of a cysteinyl residue. There is also some evidence for the existence of a dinuclear $(cys—S)_2FeS_2Fe(S—cys)_2$ species in certain plant, mammalian, and bacterial proteins [7—9]. It seems probable that molybdoferrodoxin from Clostridium pasteurianum, which consists [10] of a tetramer (M.Wt. = 220,000) with 2 molybdenum atoms and approximately 20 iron atoms with an equal number of acid-labile sulfur atoms, contains a number of either Fe_4S_4 groups or Fe_2S_2 groups or both. Inorganic models for the Fe_4S_4 group have been discovered recently and their diverse redox behavior has been demonstrated [11]. In contrast, hepatic sulfite oxidases contain only a b-like cytochrome, rather than flavin and Fe/S [12—14], while the nitrate reductase from Neurospora crassa contains flavin and a cytochrome b [15]. At any rate it is clear that the additional prosthetic groups, as well as molybdenum, may participate in electron transfer reactions of the enzymes.

When a mutant variety of Neurospora crassa, which does not possess nitrate reductase activity, is incubated with acid-treated molybdenum enzymes, such as xanthine, sulfite, aldehyde oxidases and various nitrogenases, the in vitro assembly of an active nitrate reductase can be accomplished [16]. Since incubation with various Mo^{V} and Mo^{VI} complexes did not result in an active enzyme, these results suggest that all of the active enzymes contain a common, identical molybdenum cofactor, which can be transferred after treating an active enzyme with acid. Furthermore, it would appear that this component is a relatively small molecular species, since it is unlikely that a large molybdenum

polypeptide chain could be identical in organisms ranging from microbes to mammals. These important results imply that enzymes which contain molybdenum can be studied as a group rather than individually. Of course, it must be realized that substrate specificity, where it exists, must then be a result of a common molybdenum component binding to a unique polypeptide chain.

Electron spin resonance spectroscopy, which is ideally suited for following electron transfer reactions involving paramagnetic species, has been used to pinpoint the sequence of electron transfer within xanthine oxidase. The resting state of that enzyme gives no ESR signal, but treatment with xanthine in the presence of O_2 produces a sequence of signals [17]. The first has been assigned to a paramagnetic form of Mo^V, followed by a second due to flavin semiquinone radical, and followed finally by a signal due to Fe/S. Treatment with a reducing agent such as dithionite ion causes the Mo^V signals to appear, but on stronger reduction these signals disappear almost completely [18—21]. Furthermore, ESR signals arise only upon reduction and never upon treatment with an oxidizing agent. The natural conclusion [3] is that Mo^{VI} is involved in the resting rate of the enzyme and that the sequence of the reduction of molybdenum by substrates is

$Mo^{VI} \rightarrow$ paramagnetic $Mo^V \rightarrow$ diamagnetic Mo^{IV} .

One interpretation [3] of the reaction mechanism is that reducing substrates are bound at Mo^{VI} causing its reduction to Mo^V and Mo^{IV}. Reducing equivalents are then transferred from Fe/S and to flavin, either directly or through Fe/S. A more recent and considerably more ambitious approach [22] to the reaction mechanism supposes that no mutual interaction exists between the two active sites of the enzyme, each of which contains one molybdenum atom, one molecule of FAD, and two distinct Fe/S centers. Initial attack of xanthine on Mo^{VI} results in a two-electron reduction of the metal atom with the formation of Mo^{IV} and uric acid through a concerted mechanism involving a disulfide group which is presumed to be nearby. Reducing equivalents from Mo^{IV} are then distributed intramolecularly between molybdenum (yielding an equilibrium distribution of Mo^{IV}, Mo^V and Mo^{VI}), FAD (yielding an equilibrium distribution of FAD, FADH, and $FADH_2$), and Fe/S (yielding an equilibrium distribution of the oxidized and reduced forms of the two distinct sites). An empirical choice of relative reduction potentials for all oxidized species allowed a near-quantitative duplication of the equilibrium composition of the species which are observed during the titration of xanthine oxidase with dithionite ion. Still another choice of reduction potentials produced an adequate, but not exact, duplication of the time-dependent phenomena which are observed during the reaction of the enzyme with xanthine. The differences between reductive substrates were interpreted in terms of differential perturbations to reduction potentials caused by the binding of the substrate to the enzyme. It should be noted, however, that these obvious successes do not rule out a mechanism which includes mutual interaction between the two active sites of the enzyme. If mutual interaction were included, it would simply re-

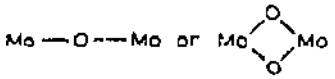
quire the introduction of one or more empirically chosen constants which describe the interaction. Although the available data may not allow the evaluation of these constants, this limitation is in no way indicative that mutual interaction does not exist.

Although it has been suggested that the enol form of flavin is bonded directly to molybdenum [23], there is sufficient evidence available to prove otherwise. The reductions of native and deflavoxanthine oxidases with xanthine proceed at similar rates and yield similar ESR signals due to Mo^{V} [24]. Consequently, the presence or absence of flavin does not influence the structure immediately adjacent to the molybdenum atoms to any significant degree. In addition to these important results, there is considerable ESR evidence, in the form of superhyperfine coupling, for the interaction of Mo^{V} with one or more exchangeable protons [25] while the relatively high g values and the relatively low Mo hyperfine couplings [26] suggest bonding to one or more sulfur atoms [27], probably from cysteinyl residues.

Sulfite and aldehyde oxidases have received less study but they exhibit similar behavior [28–30], although weak ESR signals due to Mo^{V} can also be found in the resting state of the latter. These become stronger upon reduction. It is noteworthy that, apart from subtle differences in substrate specificity, aldehyde and xanthine oxidases appear to have similar molecular weights, identical molybdenum contents, additional prosthetic groups which are identical and in the same proportion, and similar spectra in both their native and deflavo forms [31]. There is evidence that reducing equivalents are transferred directly from sulfite to molybdenum [12]. With nitrate reductase, reduction of the enzyme again causes the appearance of signals due to Mo^{V} [32]. Chemical evidence points to the direct transfer of reducing equivalents from molybdenum to NO_3^- [15,33]. ESR studies of nitrogenases have not revealed any evidence for paramagnetic Mo^{V} [34,35], although a redox function for molybdenum is clearly possible. In addition to N_2, the substrates which can be reduced by nitrogenases include N_3^-, N_2O, acetylene, nitriles, and CN^- [36].

All of the enzymes listed in Table 1 contain 2 molybdenum atoms. Enzymes from other sources, such as nitrate reductase from Escherichia coli, have been reported [37] to contain only a single molybdenum atom, but these results should be viewed with caution since contamination with demolybdo species has caused erroneous results with other systems, such as the nitrogenase from Klebsiella pneumoniae. The latter was first said to contain one molybdenum atom [38], but more recent results [39] have pushed the average molybdenum content up to 1.7. It is reasonable to assume that the true content is 2, as shown in Table 1.

There has been no ESR or chemical evidence for an interaction between these two atoms, yet their presence suggests that either in their formation, or in the resting state, or in a more reduced state the enzymes contain one of the following groups.



Both configurations are common to simple complexes obtained from aqueous solution with molybdenum in one of its higher oxidation states. Although the first configuration can be found with complexes of Mo^{VI}, which is the oxidation state of the metal in the resting state of the enzyme, there is evidence which suggests that this configuration is not present in the resting state. The competitive inhibitor, allopurinol, binds to molybdenum in a 1:1 ratio, apparently to produce two alloxanthine—Mo^{IV} complexes [40]. Apparently, each complex is formed with equal facility which certainly rules out any cooperativity between the two metal atoms and probably rules out an oxo-bridged species in the resting state.

It is of considerable importance to note that the oral administration of WO_4^{2-} to rats at levels of 1 to 100 p.p.m. in drinking water results [41] in proportionate decreases in the activities of xanthine and sulfite oxidases, decreases in the ESR signals due to molybdenum, and decreases in the total hepatic molybdenum content. However, the presence of as little as 1 p.p.m. of MoO_4^{2-} in the drinking water already containing 100 p.p.m. of WO_4^{2-} negated these effects. Restoration of the hepatic xanthine and sulfite oxidases activities could be accomplished by intraperitoneal injection under conditions in which new protein synthesis was inhibited. The implication is that reactivation is achieved by the incorporation of molybdenum into apoenzymes already in existence. However, in vitro reconstitution was not possible which points to the requirement for modification of either MoO_4^{2-}, the apoenzyme, or both as a prerequisite for binding.

In the case of sulfite oxidase, oral treatment with WO_4^{2-} was shown to produce 35% incorporation of tungsten into the enzymes, the remainder being metal-free [42]. While the native enzyme is reduced by sulfite ion in vitro within 1 minute, the tungsten protein showed 30—40% reduction in the course of 30 minutes, with full reduction only achieved by the addition of dithionite ion. Reduction produces an ESR signal at $g = 1.87$ which should be compared to the $g = 1.97$ signal due to Mo^{V} found in the reduced native enzyme. On the other hand, tungsten was not detected in xanthine oxidase [43]. Thus, in vivo incorporation of the metal (either molybdenum or tungsten) in the case of sulfite oxidase, must proceed by a mechanism which has substantially different requirements than the one for incorporation by xanthine oxidase.

Spinach plants have no nitrate reductase activity if they are grown in the absence of molybdenum but, if they are transferred to MoO_4^{2-} solutions, normal enzyme activity results within 24 h. If they are transferred instead to WO_4^{2-} solutions, tungsten uptake occurs, but no enzymatic activity is observed [44]. Tungsten also competitively inhibits molybdenum utilization by Azotobacter vinelandii [45], as well as nitrogen fixation [46].

These results suggest that the relative incorporation of molybdenum or tungsten into these proteins is not based solely on the relative binding constants of Mo^{VI} and W^{VI} in these enzymes. Indeed, evidence to be presented later suggests that Mo^{VI} and W^{VI} should have rather similar binding constants.

This evidence is in clear contrast to the restoration of full enzymatic activity at very low Mo/*N* ratios, as well as the inability of tungsten to populate fully the sites which would otherwise be available to it. Furthermore, these results raise another question of some interest. Although the gram-atom distribution of molybdenum and tungsten in crustal rocks is roughly comparable [47], while the distribution in sea water lies in favor of molybdenum by a factor of only 200 [47], it is clear that there must be strong reasons for the exclusive use of molybdenum, rather than its congener, in oxidases, reductases, and nitrogenases.

The evaluation of all these results makes the following conclusions readily apparent.

(1) A common, easily transferred molybdenum component of low molecular weight may be present in all of these enzymes.

(2) Reducing or oxidizing substrates appear to interact directly with molybdenum with reducing equivalents being passed from one to the other.

(3) Mo^{V} is intimately involved with the passage of reducing equivalents. However, ESR evidence suggests that an enzyme environment, at least with xanthine oxidase, can sustain Mo^{IV} for a finite lifetime.

(4) Ligating sulfur atoms, either from cysteinyl residues or possibly acid-labile sulfide ions, appear to be present.

(5) It is very possible that 2 molybdenum atoms are in close proximity joined by one or two oxo bridges in a reduced state of the enzyme.

(6) Tungsten, when it can be incorporated by an apoenzyme, produces a protein which is virtually inactive.

C. THE INORGANIC CHEMISTRY OF THE HIGHER OXIDATION STATES OF MOLYBDENUM

(i) Structural aspects

Aqueous solutions of molybdenum in its higher oxidation states (+6 and +5) are dominated exclusively by complexes containing the oxomolybdenum moiety. More than one oxo ligand may be bound with extremely short bonds to terminal sites in mononuclear or dinuclear complexes. Dinuclear complexes usually, but not always, have the oxo ligand in one or two bridging sites as well. Increasing evidence points to the importance of oxomolybdenum(IV) complexes, but the characterization of these complexes is far less complete than those of the higher oxidation states. Some structural results for Mo^{VI}, Mo^{V}, and Mo^{IV} are summarized below.

(a) Mo^{VI}

The simplest form of Mo^{VI} exists in basic aqueous solution as the tetrahedral MoO_4^{2-} anion [48]. Acidification of these solutions produces more or less complex behavior whose exact nature depends on the Mo^{VI} concentration as well as the pH. Nevertheless, the first steps involve protonation as well as expansion of the coordination number from 4 to 6 [49,50].

$$MoO_4^{2-} + 2H_2O + H^+ \rightleftharpoons MoO(OH)_5^-$$

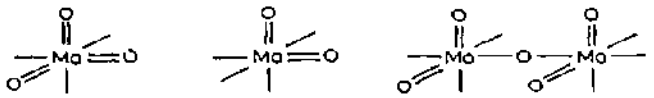
$$MoO(OH)_5^- + H^+ \rightleftharpoons Mo(OH)_6$$

$$Mo(OH)_6 + H^+ \rightleftharpoons Mo(OH)_6(H_2O)^+$$

These steps are in direct contrast to the protonation of CrO_4^{2-} where expansion of the coordination number does not occur. In the light of structural studies to be presented below, these products are perhaps better formulated with two or three oxo ligands. For example, $Mo(OH)_5(H_2O)^+$ might be written as $MoO_2(OH)(H_2O)_3^+$. More recent studies have shown further protonation of $Mo(OH)_5(H_2O)^+$ and an additional equilibration with dinuclear complexes which were written as $H_2Mo_2O_6^{2+}$ and $H_3Mo_2O_6^{3+}$ but whose precise forms are not known [100]. In more concentrated solutions, equilibrium ultracentrifugation studies [51] and temperature-jump measurements [50] point to polymerization of the mononuclear Mo^{VI} anions to give predominantly $Mo_7O_{24}^{6-}$ and $Mo_8O_{26}^{4-}$. Raman spectroscopy [51] has indicated that $Mo_7O_{24}^{6-}$ has the same structure in solution and in the solid state, while the same is probably true for $Mo_8O_{26}^{4-}$ as well.

When H_2S is passed through solutions containing MoO_4^{2-}, step-wise replacement of the oxo ligands occurs to yield [52] MoO_3S^{2-}, $MoO_2S_2^{2-}$, $MoOS_3^{2-}$, and MoS_4^{2-}.

When polydentate ligands are added to aqueous solutions of Mo^{VI}, the system appears to become somewhat simpler. There are three structural types which have been characterized by X-ray techniques. Metal–ligand bond distances are given in Table 2. I is found with MoO_3(dien) [53] and with each



I II III

half of the dinuclear, non-bridged $(MoO_3)_2EDTA^{4-}$ [54]. It is postulated with both MIDA and NTA complexes [55]. II is found with MoO_2Q_2 [56] and $MoO_2(S_2CNEt_2)_2$ [57]. A single authenticated example of III exists with $Mo_2O_5(C_2O_4)_2(H_2O)_2^{2-}$ [58], but infrared and pH studies have indicated [59] that the following reaction occurs at low pH.

$$2\ MoO_3(MIDA)^{2-} + 2H^+ \rightleftharpoons (MIDA)O_2Mo{-}O{-}MoO_2(MIDA)^{2-} + H_2O$$

The reaction of H_2S with $(MoO_3)_2EDTA^{4-}$ in aqueous solution leads [60] to $Mo_2O_4S(EDTA)^{2-}$ which is presumed to be a structural variant of III with the bridging oxo ligand replaced by sulfur.

The proton magnetic resonance spectra of $(MoO_3)_2EDTA^{4-}$ and MoO_3-$MIDA^{2-}$ contain AB quartets due to the non-equivalent methylene protons from the acetate portions of the ligands [55,61]. We have not observed collapse nor even broadening of these signals up to temperatures as high as 80°C

TABLE 2

Average bond distances (Å) for Mo^{VI} complexes

Compound	$Mo{=}O_T$[a]	$Mo{-}O_B$[b]	Mo—Y[c]	Ref.
(MoO_3)dien	1.74	—	N*:2.32	53
$Na_4[(MoO_3)_2EDTA]\cdot 8H_2O$	1.74	—	O*:2.20 N*:2.40	54
MoO_2Q_2	1.71	—	O:1.98 N*:2.32	56
$MoO_2(S_2CNEt_2)_2$	1.63	—	S:2.44 S*:2.63	57
$K_2[Mo_2O_5(C_2O_4)_2(H_2O)_2]$	1.69	1.88	ox O*:2.19 ox O**:2.09 H_2O*:2.33	58

[a] Terminal oxygen.
[b] Bridging oxygen.
[c] Atoms *trans* to a terminal oxygen are designated by a single asterisk while those which are *trans* to a bridging oxygen are given a double asterisk.

[62]. On the other hand, the spectrum of a solution containing either $(WO_3)_2$-EDTA^{4-} or WO_3MIDA^{2-} exhibits only a singlet, which is shifted from the corresponding absorption of the free ligand, due to the methylene protons [62]. Since equilibration of the two distinct sites demands at least partial dissociation along with inversion at the nitrogen atom [63], it is clear that these complexes of W^{VI} are considerably more labile than those of Mo^{VI}.

The stereochemical nonrigidity of $MoO_2(acac)_2$ has been established by proton magnetic resonance [64]. The limiting spectrum at low temperature for the methyl protons consists of two resonances, consistent with II, which coalesce at only 18°C in $CHCl_3$. The signal due to the ring protons remains sharp throughout the entire range of temperatures. These data, of course, are not sufficient to define the molecular process which causes coalescence of the signals. That problem is characteristic of virtually all work dealing with nonrigidity of complexes of the acetylacetonates.

Very little thermodynamic information pertaining to the formation of Mo^{VI} complexes appears to exist. Fortunately, a direct comparison of the formation constants [65] of Mo^{VI} and W^{VI} with several polydentate ligands is possible, as shown in Table 5. It can be seen that these constants are very similar, so that either metal can compete effectively with the other for binding to these ligands.

(b) Mo^V

There are again three structural types which have been found in X-ray studies. V is related to III by the loss of two oxo ligands. Two forms of V are actually known: in Va the terminal oxygen atoms are eclipsed while in Vb they

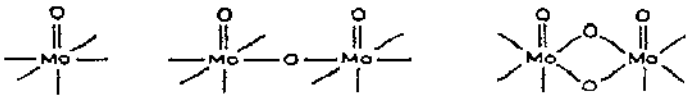
TABLE 3

Average bond distances (Å) for Mo^V complexes

Compound	$Mo{=}O_T$[a]	$Mo{-}X_B$[b]	Mo—Y[c]	Ref.
$(NH_4)_2[MoOBr_5]$	1.86	—	Br:2.55 Br*:2.83	66
$K_2[MoOF_5]\cdot H_2O$	1.66	—	Cl:1.88 Cl*:2.03	67
$Mo_2O_3(S_2COEt)_4$	1.65	1.86	S:2.49 S*:2.70 S**:2.54	72
$Mo_2O_3[S_2P(OEt)_2]_4$	1.65	1.86	S:2.47 S*:2.80 S**:2.55	73
$Na_2[Mo_2O_4(L\text{-cys})_2]\cdot 5H_2O$	1.71	1.93	O*:2.30 N**:2.23 S**:2.49	76
$Mo_2O_4(L\text{-hist})_2\cdot 3H_2O$	1.71	1.92	O*:2.21 N**:2.23	77
$Ba[Mo_2O_4(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$	1.70	1.90	ox O*:2.11 ox O**:2.14 H_2O**:2.22	78
$Mo_2O_4(L\text{-Etcys})_2$	1.71	1.93	N**:2.23 S**:2.49	79
$Mo_2O_2S_2(L\text{-Mecys})_2$	1.71	2.31	N**:2.23 S**:2.38	81
$Mo_2O_2S_2(L\text{-hist})_2\cdot H_2O$	1.71	2.32	O*:2.23 N**:2.24	82
$Cs_2[Mo_2O_2S_2EDTA]$	1.68	2.29	O**:2.11 N*:2.45	83

[a] Terminal oxygen.
[b] Bridging atom.
[c] Atoms which are *trans* to a terminal oxygen are shown with a single asterisk but those opposite a bridging atom are given a double asterisk.

are opposed. In VI the ligand atoms *trans* to the terminal oxo ligands are only weakly bound if they are present, i.e. VIa, but they can be completely absent, i.e. VIb. Bond lengths for various complexes are summarized in Table 3.



IV V VI

IV is found with $MoOBr_5^{2-}$ [66] and $MoOF_5^{2-}$ [67], while $MoOCl_5^{2-}$ is known to exist in concentrated HCl solutions [68], and weakly acidic or neutral solutions probably contain [69] diamagnetic $Mo_2O_4(H_2O)_6^{2+}$ (type VIa). At intermediate acidities, however, V is believed to exist [70]. The reaction of

TABLE 4

Average bond distances (Å) for Mo^{IV} complexes

Compound	$Mo{=}O_T$[a]	Mo—Y[b]	Ref.
$NaK_3[MoO_2(CN)_4]\cdot 6H_2O$	1.83	C:2.20	91
$[MoOCl(dppe)_2][ZnCl_3]\cdot 2(CH_3)_2CO$	1.69	Cl*:2.46 P:2.57	92
$MoOCl_2(PMe_2Ph)_3$	1.67	Cl:2.46 Cl*:2.55 P:2.53	94
$MoOCl_2(PEt_2Ph)_3$	1.80	Cl:2.48 Cl*:2.43 P:2.55	94

[a] Terminal oxygen.
[b] Atoms which are *trans* to the terminal oxygen atom are designated by a single asterisk.

$Mo_2O_4(H_2O)_6^{2+}$ with NCS^- leads to $Mo_2O_4(NCS)_6^{4-}$ [71]. Va has been shown to exist with $Mo_2O_3(S_2COEt)_4$ [72] and Vb is found with $Mo_2O_3[S_2P(OEt)_2]_4$ [73]. Analytical and spectroscopic data are in accord with the existence of $Mo_2O_3Q_4$ [74]. Reactions of type V complexes with H_2S led to oxygen-free products which appear to be polymers and are poorly characterized [75]. VIa exists with $Mo_2O_4(L\text{—cys})_2^{2-}$ [76], $Mo_2O_4(L\text{—hist})_2$ [77], and $Mo_2O_4(C_2O_4)_2\cdot(H_2O)_2^{2-}$ [78] while VIb has been shown to occur upon esterification of the carboxyl group in cysteine as in $Mo_2O_4(L\text{—Etcys})_2$ [79]. Results from proton magnetic resonance studies [80] are consistent with type VIa for $Mo_2O_4\cdot(EDTA)^{2-}$. Reactions with H_2S have led to the authenticated structures of $Mo_2O_2S_2(L\text{—Mecys})_2$ [81], $Mo_2O_2S_2(L\text{—hist})_2$ [82], and $Mo_2O_2S_2(EDTA)^{2-}$ [83] where the bridging oxo ligands have been replaced by sulfur but the terminal oxo ligands remain unchanged.

An interesting new type of dinuclear complex of Mo^V is the result of the reaction of the pyridine adduct of $Mo_2O_4Q_2$ with 2-mercaptoethanol [84]. The resulting complex, $Mo_2O_3Q_2(SCH_2CH_2O)$, has three bridging groups. Two of these are the donor groups from the 2-mercaptoethoxide dianion while the third is the normal oxo ligand.

It has been possible in a few instances [85,86] to demonstrate by means of synthetic reactions the interconversion, V ⇌ VI. It is unfortunate that this important reaction has received so little attention. Interconversion has not been accomplished when bridging sulfide ligands are present. No thermodynamic data are available.

Since Mo^V has a d^1 electronic configuration, the mononuclear complexes of type IV are expected to be paramagnetic. Electron coupling usually occurs in dinuclear complexes (V and VI) to cause diamagnetism. Although $Na_2[Mo_2O_4\cdot(L\text{—cys})_2]\cdot 5H_2O$ is essentially diamagnetic in the solid state [87], aqueous solutions of the compound, enriched in ^{95}Mo ($I = 5/2$), exhibit [27] a 6-line

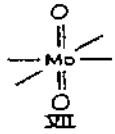
ESR spectrum whose intensity only accounts for about 2% of the molybdenum which is present. Small equilibrium quantities of a mononuclear complex are presumed to be responsible for the ESR signal. Similar results have been obtained [27] for the Mo^{V} complexes of β-mercaptopropionic acid, 1,2-ethanedithiol, and 2-aminoethanethiol, but no ESR signal was observed with *L*-alanine, *L*-histidine, *L*-cystine, *L*-propanethiol, or EDTA. Thus, ESR activity in an aqueous environment, however small, depends upon the presence of a sulfhydryl group in a chelating ligand. In a polar, nonaqueous solvent, such as DMF, the 1:1 complexes of oxomolybdenum(V) with 8-mercaptoquinoline, 8-aminoquinoline, and 3,4-dimercaptotoluene are apparently mononuclear since they are essentially 100% ESR active [88]. It is noteworthy that the ligands with sulfur donors again caused higher *g* values and lower hyperfine coupling constants.

Thermodynamic data for the formation of Mo^{V} complexes are known in far less detail than those for Mo^{VI}. The formation of $Mo_2O_4(H_2O)_5NCS^+$ from $Mo_2O_4(H_2O)_6^{2+}$ has an equilibrium constant of 200 ± 40 l $mole^{-1}$ with $[H^+]$ = 0.5 M [89], while the formation of $Mo_2O_4EDTA^{2-}$ according to the following reaction [90] has an equilibrium constant of 2.5×10^{27} l $mole^{-1}$.

$$Mo_2O_4(H_2O)_6^{2+} + EDTA^{4-} \rightleftharpoons Mo_2O_4EDTA^{4-} + 6H_2O$$

(c) Mo^{IV}

Two structural types have been identified by X-ray methods. These are IV, described above, and VII.



VII

The only known example of VII occurs with $MoO_2(CN)_4^{4-}$ [91]. IV occurs with $MoOCl(dppe)_2^+$ [92], and $MoOCl_2(PR_2Ph)_3$ (R = CH_3 [93] or C_2H_5 [94]). Bond lengths are summarized in Table 4. Reduction [95] of $MoO_2(S_2CNR_2)_2$ or $MoO_2[S_2P(OR)_2]_2$ leads to diamagnetic $MoO(S_2CNR_2)_2$ and $MoO[S_2P(OR)_2]_2$. The molecular structure of $MoO(S_2CNPr^n_2)_2$, in a paper [96] which unfortunately is unavailable to the author thus far, is said [97] to be a square pyramid, but the small bite angle due to the bidentate ligand causes the virtual symmetry to be close to C_{2v}. However, the proton magnetic resonance spectrum of $MoO(S_2CNPr^i_2)_2$ indicates that structural differences occur in $CDCl_3$ solutions [98]. At ambient temperature, the 100 MHz spectrum consists of a single doublet from the methyl groups and a single septet from the methine protons due to one or more rapid averaging processes. The spectrum at low temperature, however, consists of two 1:2:1 triplets of equal intensity for the methyl protons and two 1:4:6:4:1 quintets of equal intensity for the methine protons. Although fortuitous overlap has occurred, this spectrum is consistent only with a structure possessing either a plane of symmetry or a two-fold axis, as in an idealized trigonal bipyramid with the oxo ligand in the equator to achieve

TABLE 5

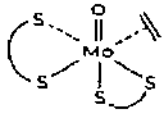
Formation constants for some complexes of Mo^{VI} and W^{VI}[a]

Reaction	log K_{Mo}	log K_W
$MO_4^{2-} + IDA^{2-} + 2H^+ \rightleftharpoons MO_3IDA^{2-} + H_2O$	18.3 ± 0.1	18.5 ± 0.2
$MO_4^{2-} + MIDA^{2-} + 2H^+ \rightleftharpoons MO_3MIDA^{2-} + H_2O$	18.73 ± 0.04	18.70 ± 0.01
$MO_4^{2-} + NTA^{3-} + 2H^+ \rightleftharpoons MO_3NTA^{3-} + H_2O$	18.94 ± 0.03	18.86 ± 0.05
$MO_4^{2-} + EDTA^{4-} + 2H^+ \rightleftharpoons MO_3EDTA^{4-} + H_2O$	18.6 ± 0.4	18.9 ± 0.4
$MO_4^{2-} + MO_3EDTA^{4-} + 2H^+ \rightleftharpoons (MO_3)_2EDTA^{4-} + H_2O$	17.5 ± 0.3	16.9 ± 0.2

[a] All data were taken from ref. 65.

maximum bonding. Of course, distortion of this idealized structure toward a square pyramid is possible as long as these elements of symmetry are preserved on the NMR time scale. Five-coordinate complexes of the vanadyl ion (VO^{2+}), with one less *d* electron, generally adopt either the geometry of a square pyramid or one which is intermediate between a square pyramid and a trigonal bipyramid. There is only a single authenticated example of a trigonal bipyramid [99], and in that case the oxo ligand is found in the equatorial plane.

Various unsaturated molecules readily bond to $MoO(S_2CNR_2)$. The authenticated structure of the adduct with $(NC)_2C{=}C(CN)_2$, which has become available recently [101], is shown below.



A true square-pyramidal structure undoubtedly exists with the diamagnetic phthalocyanine complex of oxomolybdenum(IV) because of the rigidity of the ligand [102].

The paucity of compounds of oxomolybdenum(IV) is due probably to difficulties surrounding the synthetic methods. Indeed aqueous solutions of Mo^{IV} have been obtained only recently [103]. Ion-exchange experiments [103] have led to the conclusion that the species present in solution is $[MoO(H_2O)_4]_2^{4+}$. Polarographic reduction of Mo^{VI} in either aqueous HCl [104], citric acid [134], or gluconic acid [136] solutions gives rise to an unstable Mo^{IV} species. There are other indications that ligands containing oxygen as the donor atom provide an unstable environment for Mo^{IV}. Thus, while $MoO_2(S_2CNR_2)$ is readily reduced to the Mo^{IV} compound (see above), no reduction of $MoO_2(acac)_2$ was observed under comparable conditions [95].

In dimethylsulfoxide, it would appear that some stability can be attained. The two-electron reduction of the dinuclear complex of Mo^{V} and the riboflavin radical anion (VI), which has no ESR spectrum because of spin coupling between the electrons localized on the ligand and metal, produces a dinuclear Mo^{IV} species having an ESR spectrum which is very similar to the uncomplexed radical anion [105].

(ii) Reactivity

The substitution reactions of Mo^{VI} appear to be very rapid, but reduction is fairly slow. Thus the rate constants for the formation of the 1:1 octahedral complexes with catechol and EDTA are 3×10^2 and 2×10^5 M^{-1} s^{-1} respectively [106,107]. The reduction of Mo^{VI} with $N_2H_5^+$ in a phosphate buffer at pH 1.6 and 60°C to yield Mo^{V} and N_2 is slow, with the rate constant being about 0.5 M^{-1} s^{-1} [108]. The detection of N_2H_2 led to the conclusion that the rate-determining step of the mechanism was the two-electron redox process

$$N_2H_5^+ + Mo^{VI} \rightarrow N_2H_2 + Mo^{IV} + 3H^+$$

followed by more rapid steps

$$2N_2H_2 + H^+ \rightarrow N_2 + N_2H_5^+$$

$$Mo^{IV} + Mo^{VI} \rightarrow 2Mo^{V}$$

$$2Mo^{V} \rightarrow [Mo^{V}]_2$$

where $[Mo^{V}]_2$ is probably similar to $Mo_2O_4(H_2O)_6^{2+}$ discussed above, but probably with one or more buffer anions coordinated to each metal atom. Cysteine will also reduce Mo^{VI} to yield cystine and a new complex which was formulated as $Mo_2O_3(L\text{-cys})_4^{4-}$ (V) [109,110]. It is unfortunate that the latter is converted to $Mo_2O_4(L\text{-cys})_2^{2-}$, i.e. VI, during attempts at isolation. The rate constant for the reaction (pH 7.5, 60°C, phosphate buffer), which is first order in Mo^{VI} and second order in cysteine, is approximately 1.0×10^{-2} M^{-2} s^{-1}.

Recent unpublished results from this laboratory [62] have shown that $(MoO_3)_2EDTA^{4-}$ will reduce hydrazine at a rate which is approximately 10^2 more rapid than MoO_4^{2-} under identical conditions. Neither WO_4^{2-} nor $(WO_3)_2$-$EDTA^{4-}$, however, is reduced under the same conditions. Similarly, WO_4^{2-} is not reduced by cysteine at pH 7.5. Polarography has shown that, while $(MoO_3)_2$-$EDTA^{4-}$ in an acetate buffer is reduced at -0.63 V vs. SCE, the corresponding W^{VI} complex is inactive [133]. Similarly, only Mo^{VI} is reduced in citrate [134] or tartrate [135] buffer solutions containing both Mo^{VI} and W^{VI}.

The oxidation of aldehydes by $MoO_2(L\text{-Etcys})_2$ to yield carboxylic acids has been claimed to proceed according to the following equation in DMSO or DMF [111].

$$RCHO + 2MoO_2(L\text{-Etcys})_2 \rightarrow RCOOH + Mo_2O_3(L\text{-Etcys})_4$$

This reaction, whose kinetics have not been studied, is of considerable interest because of its apparent relationship to functioning of an aldehyde oxidase.

The kinetics of the reactions of $MoOCl_5^{2-}$ with Sn^{II} [112] and $[Mo^V]_2$ with O_2 [113], I_3^- [113], NH_2OH [114], NO_2^- [115], and NO_3^- [116], have been studied in various buffers. All of these reactions are fairly slow. Qualitative results from this laboratory have indicated that the reaction of Mo_2O_4-$(EDTA)^{2-}$ with NO_3^- to yield NO (and a trace of N_2O) is also very slow. The reaction of that complex with O_2 is said to be nonexistent [117]. It is noteworthy that one explanation [113] of the mechanism of reactions of O_2 and I_3^- is the slow disproportionation of $[Mo^V]_2$ to yield Mo^{IV} and Mo^{VI}, with the former being the species which causes reduction of O_2 and I_3^-. However, an alternative explanation [113] is that mononuclear Mo^V, resulting from dissociation of the dinuclear $[Mo^V]_2$, is the reactive species. This dissociation, which was not actually observed in the kinetic experiments, can be measured under favorable conditions [118]. Either explanation will also explain the half-order dependence on the concentration of $[Mo^V]_2$ in the reduction of NO_3^- by reduced flavin (reduced by Ti^{III} or dithionite ion) which is catalyzed by Mo^V [119]. However, it should also be noted that it has been demonstrated [120,121] by polarography that Mo^{IV} is involved as an active participant in the molybdenum-catalyzed reduction of NO_3^- and ClO_4^- by Sn^{II}.

A clear-cut case for disproportionation of type V complexes has recently been demonstrated [86] with $Mo_2O_3(S_2CNR_2)_4$. Studies of the absorption spectrum, which showed that Beer's Law is not obeyed at ~510 nm, have suggested an equilibrium

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

The equilibrium constant for this reaction is 4×10^{-3} M at 41°C in chlorobenzene with $R = n\text{—}C_3H_7$ [122]. However, the Mo^{IV} complex, which is coordinatively unsaturated, can be trapped and isolated as the 1:1 complex with diethylazodicarboxylate. Hydrolysis of this complex leads to $MoO_2(S_2CNR_2)_2$ and the corresponding hydrazide. The Mo^{IV} complex will also form adducts with $CH_3O_2CC{\equiv}CCO_2CH_3$ and $(NC)_2C{=}C(CN)_2$ [123]. The structure of the latter has been determined by X-ray studies [101].

The absorption spectra of the xanthate complexes, $Mo_2O_3(S_2COR)_4$, in benzene or $CHCl_3$ deviate only slightly from Beer's Law at ~510 nm [124]. Although it is clear that some reaction is occurring, it was not impossible to distinguish between dissociation of the xanthate ligands and disproportionation. The former seems unlikely in these solvents. Finally, time-dependent changes [125] in the absorption spectrum of $Mo_2O_3(L\text{-Rcys})_4$ (R = CH_3 or C_2H_5) may well indicate the slow disproportionation of that compound.

The importance of this disproportionation is illustrated further with the catalysis of the oxygenation of Ph_3P by $Mo_2O_3(S_2CNR_2)_4$ [122]. The results show convincing evidence that Ph_3P abstracts an oxo ligand from MoO_2-$(S_2CNR_2)_4$ which is present due to the disproportionation described above.

The resulting compound, $MoO(S_2CNR_2)_2$, is then oxidized by O_2 to give back the catalytically active Mo^{VI} compound.

The oxidation of aldehydes by $MoO_2(L\text{-Etcys})_2$ [111] has already been discussed. Although the observed product is $Mo_2O_3(L\text{-Etcys})_4$, oxo ligand abstraction by the aldehyde is a real possibility, providing the Mo^{IV} complex then suffers a rapid reaction with $MoO_2(L\text{-Etcys})_2$.

$$RCHO + MoO_2(L\text{-Etcyl})_2 \rightarrow RCOOH + MoO(L\text{-Etcys})_2$$

$$MoO(L\text{-Etcys})_2 + MoO_2(L\text{-Etcys})_2 \rightarrow Mo_2O_3(L\text{-Etcys})_4$$

Since disproportionation of $Mo_2O_3(L\text{-Etcys})_4$ is either slow (see above) or nonexistent, complete conversion to the Mo^{IV} complex may not be possible.

Emulation of nitrogenase activity has been accomplished [126] by the reduction of $Mo_2O_4(L\text{-cys})_2^{2-}$ in the presence of a substrate and ATP. The substrates include N_2, C_2H_2, CN^-, N_3^-, and N_2O but turnover numbers are, under the best conditions, at least 10^3 times less than those obtained from the nitrogenase from Clostridium pasteurianum. The reduction of N_2 by this system produces diimide and hydrazine prior to the formation of NH_3 [36]. However, it is of interest to note that there exists a linear dependence between the total product formation and $[Mo_2O_4(L\text{-cys})_2^{2-}]^{\frac{1}{2}}$ where the substrate is CN^-. Thus, the molybdenum species, whatever its actual composition, must be mononuclear. Furthermore the requirement for a reducing agent makes it clear that an oxidation state lower than +5 is involved. Cyclic voltammetry has indicated [127] that $Mo_2O_4(L\text{-cys})_2^{2-}$ in aqueous solution is reduced at -1.29 V vs. SCE. Unfortunately, the number of electrons involved in the reduction was not determined. Recently, it has been claimed that all experimental evidence points to Mo^{IV} species as the catalytic reagent [36]. Furthermore the reduction of Mo^{VI} or Mo^{V} is catalyzed by the cluster, $Fe_4S_4(SR)_4^{2-}$ [36].

Some aspects of nitrogenase activity can also be found in the tungsten–cysteine system [126]. Relative rates (W vs. Mo) for the reactions with N_2, CN^-, N_3^-, and N_2O are 0, 4.3, 0.75, and 0.52, respectively.

However, small amounts of ammonia, formed by the reduction of N_2, have been claimed [128] in far simpler systems containing either molybdenum or tungsten. Acidic solutions of Mo^{VI} or W^{VI}, through which N_2 is bubbled, will catalyze the formation of ammonia either upon electrolytic reduction or in the presence of the reducing agents, Zn or Sn^{II}. The formation of ammonia ceased when all the Mo^{VI} was converted to Mo^{III} after about 24 h, or in the presence of catechol. However, when the reduction of N_2 by Sn^{II} was catalyzed by W^{VI}, the formation of ammonia continued beyond two weeks. This result was attributed to the greater stability of the highest oxidation state of tungsten.

(iii) Exclusion of Mo^{III}

A discussion of the aqueous chemistry of Mo^{III} has not been included for two reasons. First, complexes of Mo^{III} are expected to be inert to substitu-

tion. Indeed, recent work has indicated that the order of increasing lability of octahedral complexes is $Mo^{III} < Mo^{V} < Mo^{VI}$ [89]. Inertness to substitution reactions would not be an attractive feature for a catalytic substance. Second, there is absolutely no evidence that Mo^{III} results after strong reduction of an enzyme. If it did, it would be expected to be observed either directly through its ESR spectrum or indirectly through a determination of the number of reducing equivalents which are available to the reduced enzyme.

(iv) Evaluation

Several conclusions can be obtained from this account.

(1) Oxo complexes are the invariable result with the higher oxidation states of molybdenum in an aqueous environment.

(2) Dinuclear, oxo-bridged compounds can sometimes be found with Mo^{VI} but they prevail with Mo^{V} in either of two types, V or VI. Under favorable conditions, V can equilibrate with VI. However, if bridging sulfide ligands are present, no equilibration occurs.

(3) Dissociation of dinuclear Mo^{V} complexes is never extensive in aqueous solutions. With ligands which contain sulfur as donor atoms, dissociation is sufficient to detect small quantities of ESR-active, mononuclear complexes of unknown structures. This dissociation can be enhanced in a polar, nonaqueous environment.

(4) There is a recurring theme in the literature that Mo^{IV} is involved in many oxidation—reduction reactions. It is often postulated that Mo^{IV} is responsible for further reductive reactions. This theme has found partial substantiation in the observations that certain Mo^{VI} compounds are readily reduced by oxo ligand transfer reactions to the corresponding Mo^{IV} complexes. Furthermore, disproportionation of certain type V complexes provides a facile route to Mo^{IV} complexes. Mo^{IV} appears to be stabilized by ligands which contain sulfur as a donor atom.

(5) Electron transfer reactions of both Mo^{VI} and Mo^{V} are fairly slow but catalysis by certain iron compounds, such as $Fe_4S_4(SR)_4^{2-}$, can produce significant increases in the rates of these reactions.

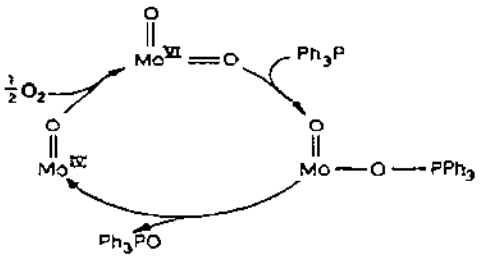
(6) Although not much data exist, equilibrium studies for a few cases indicate that W^{VI} should compete effectively with Mo^{VI} for a given binding site. However, it is possible to show that the resulting W^{VI} complexes are not reduced as easily as those of Mo^{VI}.

D. CORRELATIONS AND REQUIREMENTS

A comparison of the conclusions from Sections B and C of this review indicate some strong similarities. It is clear that both biochemists and inorganic chemists are convinced that Mo^{IV} plays an important and perhaps essential role in the oxidation—reduction reactions of molybdenum. It would also appear that ligands which contain sulfur as a donor atom, but apparently not

sulfide ligands, are important for both ESR activity of Mo^{V} complexes in approximately neutral solutions and finite lifetimes for Mo^{IV} species. It is also apparent that molybdenum-bound oxo ligands are to be expected in the enzymes, but whether or not these are useless appendages needs further consideration. When the obvious need for Mo^{VI} is included, these correlations should serve to outline roughly the active site of oxidases, reductases, and nitrogenases which contain molybdenum.

However, other factors must also be considered. Two obvious and essential requirements for enzymatic activity are rapid substitution or addition of the substrate at the active site and rapid electron transfer at that site. Considering each requirement in turn; if the substrate attacks a site containing Mo^{VI}, which should occur with the oxidases, then rapid formation of a molybdenum-substrate complex is to be expected since fast reactions are the rule with Mo^{VI}; if the substrate attacks a site containing Mo^{IV}, which should be the case with the reductases, then a somewhat slower reaction might ensue if the coordination geometry of the metal prior to attack is octahedral. This conclusion is at least in line with a recent statement [89] that the lability of octahedral Mo^{IV} lies somewhere between those of Mo^{III} and Mo^{V}. However, if coordinative unsaturation is also attributed to this site, then rapid addition or oxidative-addition of the substrate would be expected. Fast addition reactions certainly appear to be the case with $MoO(S_2CNR_2)_2$. Rapid electron transfer reactions, the second requirement for these enzymes, are not the general case for Mo^{VI} or Mo^{V} in the absence of catalysis. However, it would appear that Fe/S groups can provide the required catalysis. Oxo bridges, either between molybdenum atoms or between molybdenum and a substrate, may also provide a feasible and efficient pathway for electron transfer. Thus, the disproportionation of $Mo_2O_3(S_2CNR_2)_4$ is established instantly. Furthermore, the catalytic oxygenation of Ph_3P by $MoO_2(S_2CNR_2)_2$ probably proceeds through an oxo bridge according to the following fast sequences.

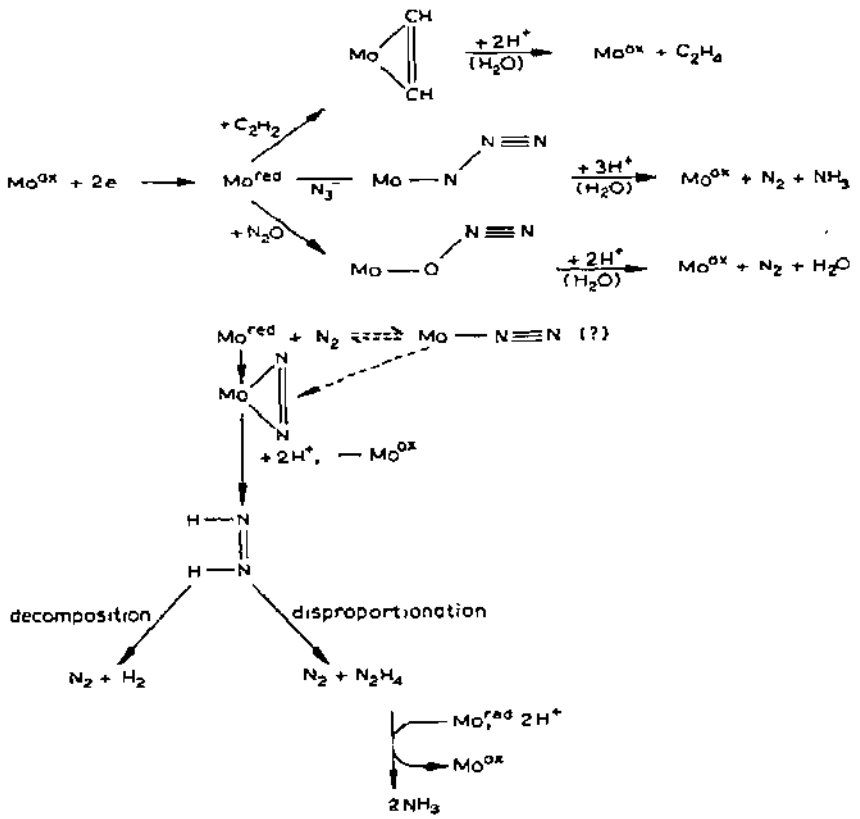


Although tungsten appears to mimic molybdenum reasonably well in model systems in the presence of rather strong reducing agents, it does not do so under biological conditions. Since compounds of W^{VI} should be more stable to reduction than their molybdenum counterparts, biological reducing agents are probably not capable of reducing W^{VI} at physiological pH values. This statement applies not only to the actual functioning of the metalloenzyme

with various substrates, but also to the formation of these proteins. If their formation (or the formation of a cofactor which contains the metal [16]) requires a change in oxidation state, then synthesis of the metalloenzyme (or cofactor) may not be possible. Even relatively small changes in the local environment (such as the pH) may suffice to inhibit a required oxidation—reduction reaction which might occur under different conditions. This reasoning may explain the partial incorporation of tungsten into sulfite oxidase [42] and its exclusion from the apoenzyme corresponding to xanthine oxidase [43].

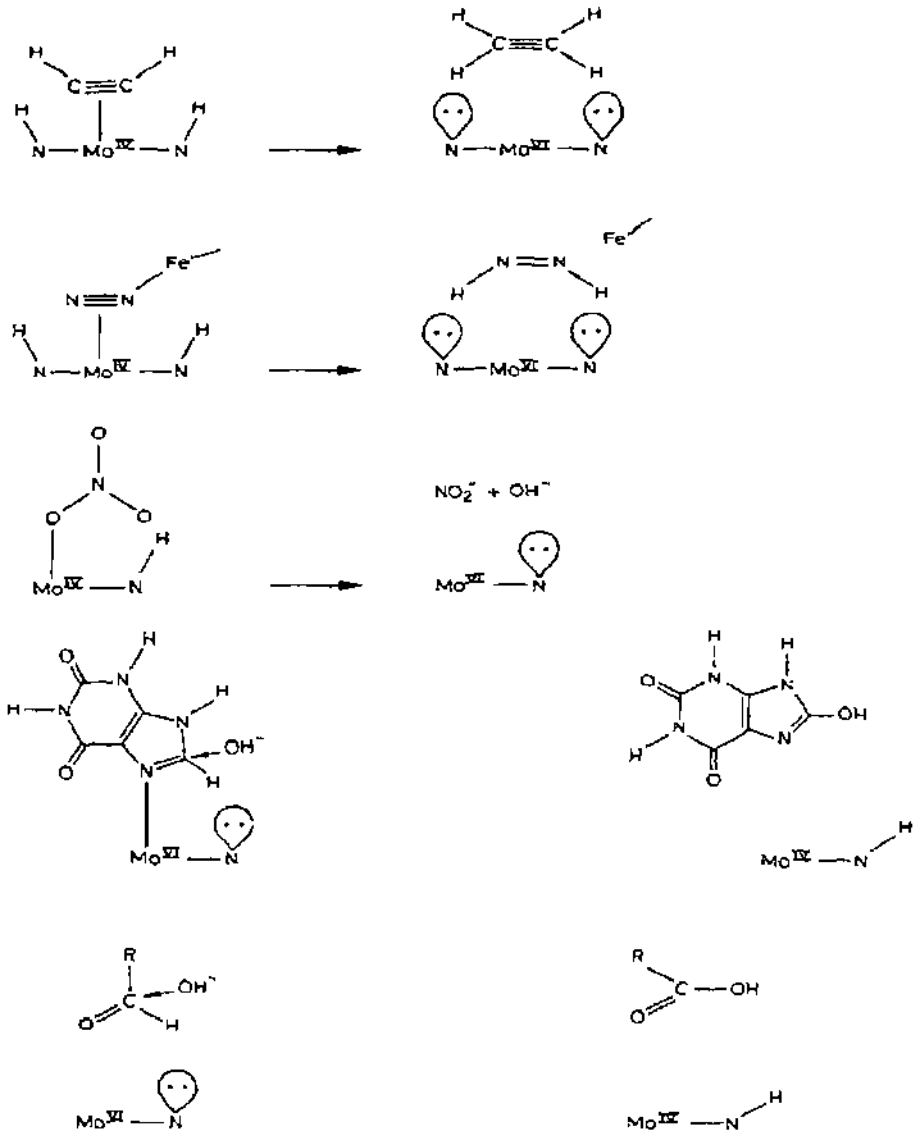
E. MECHANISMS FOR REACTIONS

Current theories for the mechanism of the interaction of a substrate with one of these enzymes center around the probability that Mo^{IV} is an active species, unlike older theories which focused attention on Mo^{V}. However, the mode of interaction between the substrate and the active site readily distinguishes the current opinions.



Scheme 1. Essential steps in the reduction of C_2H_2, N_3^-, N_2O, and N_2 by a nitrogenase according to Schrauzer.

Schrauzer [36,126] has considered the action of a nitrogenase with its various substrates to be either simple substitution or addition at Mo^{IV}, followed by oxidation to either Mo^{V} or Mo^{VI} and release of the reduced substrate. A few of these reactions are shown in Scheme 1, wherein Mo^{red} is equivalent to Mo^{IV} while Mo^{ox} represents either Mo^{V} or Mo^{VI}. The reaction with acetylene



Scheme 2. Reduction of C_2H_2, N_2, and NO_3^- and oxidation of xanthine and aldehydes according to Stiefel.

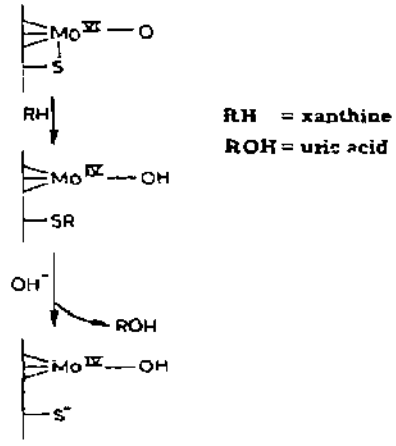
is in accord with the known ability of $MoO(S_2CNR_2)_2$ to form a 1:1 complex with activated acetylenes. Furthermore, the hydrolysis products of the Mo—N_2 complex are in accord with the detection of diimide in the reaction of reduced $Mo_2O_4(cys)_2^{2-}$ with N_2. Diimide then disproportionates by a well known reaction to provide hydrazine and N_2. There is, however, no current evidence that a simple Mo^{IV}—hydrazine complex can be found, although it would seem to be a logical possibility, More complicated modes of interaction, resulting in fragmentation of substituted hydrazines, are known [129]. One other point is worthy of note. Since both MoO_4^{2-} and $(MoO_3)_2EDTA^{4-}$ actually oxidize hydrazine, it is clearly true that cysteine in Schrauzer's model system and perhaps cysteinyl residues in the enzyme, probably by virtue of sulfur donor atoms, substantially alter the free energy of the reduced complex with respect to its oxidized form.

Stiefel [130] has provided a somewhat more subtle view based on mechanisms which employ coupled proton and electron transfer between the substrate and the active site. These mechanisms, shown in Scheme 2, depend upon the availability of bound ligands which can serve as proton donors or acceptors. These are shown as primary or secondary amine groups in the Scheme but oxo ligands might function equivalently. The logic behind this mechanism is the well known increase in the acidity of a bound ligand which accompanies an increase in the oxidation state of the metal. Clearly, these mechanisms make a more substantial use of the immediate environment around each molybdenum atom. Stiefel accounts for the observed ESR activity in terms of Mo^V by sequential electron transfer steps

$$Mo^{VI} \rightarrow Mo^V \rightarrow Mo^{IV}$$

or by a redistribution process which is shown below.

$$Mo^{VI} + Mo^{IV} \rightarrow 2Mo^V$$



Scheme 3. Oxidation of xanthine according to Bray.

Proton couplings are presumably due to acidic hydrogen atoms of the bound amine groups which are exchanging slowly with those from the aqueous environment.

Another mechanism which appears to use the entire active site is the one favored by Bray [131] for xanthine oxidase. As shown in Scheme 8, an oxo ligand accepts a hydride ion from xanthine, which causes the two-electron reduction of Mo^{VI}, while the oxidized xanthine residue is bound to a nearby sulfur atom. Hydrolysis then provides uric acid and a Mo^{IV} species. Loss of hydride ion from the latter returns the sequence to the original active site. This mechanism appears to be the first to make specific use of an oxo ligand, althougb Stiefel has admitted the possibility [130].

None of these mechanisms draws heavily from the known inorganic chemistry of molybdenum, although Stiefel's mechanism does use certain postulated aspects of it. The results which were previously discussed indicate that Mo^{VI} is invariably bound to two or three oxo ligands, as in I or II. Reduction to mononuclear, paramagnetic Mo^{V} complexes results only at high acidities or in a polar, nonaqueous environment. At physiological pH values, one-electron reduction will result in a dinuclear Mo^{V} complex, however.

$$I + 2H^+ + e^- \rightleftharpoons \tfrac{1}{2}VI + H_2O$$

$$II + 2H^+ + e^- \rightleftharpoons \tfrac{1}{2}V + H_2O$$

Paramagnetism can then result from hydrolytic dissociation of the bridged complexes [20,127,132]. Rather than supposing that Mo^{IV} results from the addition of two electrons to V or VI, a less demanding route would be disproportionation [86], as in the following reaction.

$$V \rightleftharpoons II + VIII$$

One of the products, VIII, is an oxo Mo^{IV} species of unspecified geometry. Earlier arguments concerning reaction rates suggest that VIII could be coordinatively unsaturated.

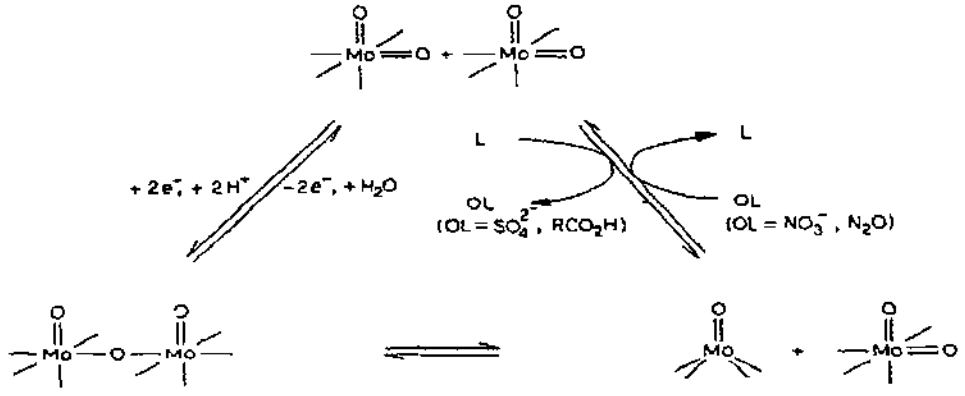
If a direct pathway between Mo^{VI} and Mo^{IV} is included, the result is a fully reversible series of sequences which should be equally applicable to both oxidases and reductases. It is evident that this pathway is the one that must account for the interaction of the substrate with the active site. Oxo ligand transfer between molybdenum and the substrate will provide the necessary link between the two oxidation states [4], as shown below.

$$VIII + OL \rightleftharpoons VIII{-}OL \rightleftharpoons II + L$$

$$(L = SO_3^{2-},\ NO_2^-,\ RCHO,\ \text{xanthine},\ N_2O)$$

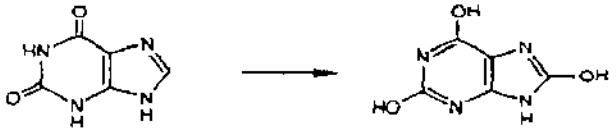
The combination of these sequences is shown in Scheme 4, wherein the resting state of the enzyme is presumed to contain II. An equally acceptable series of reactions can be devised for I.

This scheme requires a certain cooperativity between the two molybdenum



Scheme 4. A new proposal for oxidases and reductases.

sites, but only in reduced oxidation states and never in the resting state of the enzyme. The manner in which allopurinol inhibits the action of xanthine oxidase [40] is a legitimate question requiring an explanation. Two possibilities are immediately obvious. First, reduction of the enzyme by allopurinol results in a Mo^{IV}—alloxanthine complex of considerable stability. If VIII—OL will not release the oxidized substrate, then it is clear that inhibition will occur. Second, a comparison of allopurinol and xanthine or their oxidation products, alloxanthine and uric acid, indicates that the hydroxyl group has been introduced at an entirely different site (see below). As a consequence, different binding modes at the active site, each with differing steric requirements, must result, Subsequent reactions of the Mo^{IV}—alloxanthine complex could then be inhibited because of steric hindrance.



xanthine uric acid

allopurinol alloxanthine

If cooperativity exists only in the reduced oxidation states of molybdenum and not in the highest oxidation state, then movement of the protein chain,

possibly restricted, would be required. The trigger for this movement could be the changes in the molybdenum—donor atom bond lengths which must occur inevitably upon reduction of molybdenum [4]. As shown in Tables 2, 3 and 4, these bond lengths are largest when *trans* to a terminal oxygen atom. Thus, considerable changes in the conformation of the protein would accompany the reduction of II to V or VIII since a decrease in the number of terminal oxygen atoms occurs. This trigger may also cause other electron transfer centers to move into the proximity of molybdenum which would then allow the passage of reducing equivalents between the centers [4].

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