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PERSPECTIVE

# Chemical keys to molybdenum enzymes*

**Edward I. Stiefel**

*Exxon Research and Engineering Co., Clinton Township, Rt 22E, Annandale, New Jersey 08801, USA*

Nitrogenase, in which molybdenum is part of a unique multinuclear iron–molybdenum–sulfide cluster, has garnered much deserved attention. However, about 30 other distinct enzymes use molybdenum (and several more use tungsten) in a mononuclear site involving special pterin–ene-dithiolate [pterin = 2-amino-4(1*H*)-pteridinone, ene-dithiolate = dithiolene] ligation. These molybdenum enzymes have environmental, agronomic, and health relevance. Their 'molybdenum cofactor', Moco, is now viewed as a generic term for a family of prosthetic groups, although there are unresolved issues involving nomenclature. Oxygen-atom transfer and proton–electron transfer are convenient ways to stoichiometrically formulate the two-electron substrate and active-site regeneration reactions. Such processes are common in molybdenum chemistry and may have mechanistic relevance. Dithiolene ligands of the type present in Moco are redox active in free or complexed forms. Molybdenum–sulfur systems show facile internal electron–transfer chemistry and partial redox states that have potential structural and mechanistic implications for molybdenum enzymes. Chemical differences between molybdenum and tungsten may influence the respective presence or absence of these elements in enzymes from organisms that occupy diverse habitats.

The element molybdenum was known to the ancients in the form of its disulfide, $MoS_2$, molybdenite. The graphite-like properties of $MoS_2$ made it immediately useful for writing and as a lubricating material. The refinement of molybdenum from its ore (mostly $MoS_2$) and its technological use has grown since the start of the 20th century, as myriad metallurgical and chemical uses of molybdenum were discovered.[1]

In contrast to this knowledge of the technological importance of molybdenum, the use of molybdenum by biological systems was not recognized until the 1930s when Bortels[2] first reported that molybdenum (and vanadium) could have a stimulatory effect on the process of biological nitrogen fixation. The presence of molybdenum in the enzyme nitrogenase was fully appreciated by the early 1970s.[3] In the present decade, crystallographic elucidation of nitrogenase structures[4,5] confirmed the presence of molybdenum as part of FeMoco, the iron–molybdenum cofactor, the central part of the active site of nitrogenase.[6] Here, the molybdenum, bound by homocitrate and histidine ligands, is present as a *non-oxo* component of an unprecedented iron–molybdenum–sulfur cluster. The ability of this cluster to fix nitrogen is under intense study at the molecular mechanistic level.[7] However, there is a possibility that the molybdenum is not a crucial or direct participant in dinitrogen activation or reduction, especially in light of the existence of nitrogenases that use vanadium or iron in place of molybdenum.[8] In contrast to nitrogenase, the enzymes of the present Perspective have a mononuclear, pterin–ene-dithiolate [pterin = 2-amino-4(1*H*)-pteridinone, ene-dithiolate = dithiolene] ligated, (usually) oxo-bound molybdenum site, which demonstrably occupies the center of action in the substrate conversion process.

Beginning less than 50 years ago,[9] molybdenum was identified as the 'xanthine oxidase factor'. Since then, molybdenum has been discovered in a variety of enzymes, now roughly 30 in number.[10–13] Each enzyme contains a *mononuclear* Mo site with one or two unusual pterin–ene-dithiolate ligands and, usually, one or more oxo groups in the molybdenum co-ordination sphere. The molybdenum center in these enzymes, co-ordinated by the special ligand family, is designated Moco, 'the molybdenum cofactor'. The more recently discovered and (to date) less numerous tungsten enzymes share the same family of organic ligands.[14,15]

It is not the purpose of this contribution to review comprehensively the nature of the molybdenum enzymes or their 'cofactors'. Several recent reviews are available[10–13,16–20] and these are referred to heavily. Recent structural aspects of the Moco enzymes are summarized in the accompanying Perspective.[21] Nevertheless, for continuity of presentation, a brief survey and overview will be given of the mononuclear molybdenum enzymes. We highlight aspects of structure in relation to potential modes of activity. This Perspective largely deals with chemical systems, *i.e.* models in a strict or loose sense which can aid in our understanding of both structural and mechanistic features of the molybdenum enzymes.

## The Molybdenum Enzymes

The compositions and reactions of the molybdenum enzymes have been summarized in a number of recent publications.[10–13,18,19] Here we simply catalog the Mo enzymes in Table 1 according to their metabolic roles. The breadth of activity is wide and organisms that use these enzymes significantly impact several biogeochemical cycles.[10] In the nitrogen cycle, where both nitrogenase and nitrate reductase are key operatives, the preeminence of molybdenum in plant and microorganism metabolism is notable. In the metabolism of N-heterocycles, a large family of molybdenum enzymes encompasses a range of substrate specificities that allow hydroxylation of carbon centers in strategic regiospecificity. In the sulfur cycle, sulfite oxidation and dimethyl sulfoxide (dmso) reduction each play crucial roles. In carbon metabolism, both in the formation of methane and in the oxidation of formate, carbon monoxide, and various aldehydes, the molybdenum enzymes again have a prominent position. Clearly, the relatively rare second-row transition element, molybdenum, plays a disproportionately significant role in biogeochemistry on earth. Recently, several tungsten enzymes[14,15] have been isolated from thermophilic organisms in extreme environments. The tungsten enzymes (also listed in Table 1) are involved in carbon metabolism and usually have functions related to those of their molybdenum cousins.

In addition to these key environmental roles, knowledge of

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**Table 1** Molybdenum and tungsten enzymes

| Molybdenum enzymes | Tungsten enzymes |
|---|---|
| **Nitrogen cycle** | Aldehyde oxidoreductase (carboxylic acid reductase) |
| Nitrogenase (only molybdenum enzyme not containing the pterin dithiolene ligand) | Formate dehydrogenase |
| Nitrate reductase (assimilatory) | Formaldehyde ferredoxin oxidoreductase |
| Nitrate reductase (dissimilatory) | *N*-Formyl methanofuran dehydrogenase |
| Nitrate oxidase | Acetylene hydratase |
| Trimethylamine *N*-oxide reductase | |
| **N-Heterocyclic metabolism** | |
| Isonicotinic acid hydroxylase | |
| Nicotinic acid hydroxylase | |
| Nicotine hydroxylase | |
| Picolinic acid dehydrogenase | |
| Pyrimidine oxidase | |
| Isoquinoline oxidoreductase | |
| Quinaldic acid 4-oxidoreductase | |
| Quinoline oxidoreductase | |
| Xanthine dehydrogenase | |
| Xanthine oxidase | |
| **Acid and aldehyde reactions** | |
| Aldehyde oxidase (retinal oxidase) | |
| Aldehyde dehydrogenase | |
| Pyridoxal oxidase | |
| **Carbon metabolism** | |
| Formate dehydrogenase | |
| Carbon monoxide oxidoreductase | |
| *N*-Formyl methanofuran dehydrogenase | |
| 2-Furoyl dehydrogenase | |
| **Sulfur metabolism** | |
| Polysulfide reductase | |
| Sulfite oxidase | |
| Biotin sulfoxide reductase | |
| Dimethyl sulfoxide reductase | |
| Tetrathionate reductase | |
| **Miscellaneous** | |
| Pyrogallol transhydroxylase | |
| Arsenite oxidase | |
| Chlorate reductase | |

molybdenum continues to increase as to its physiological and pathological states in animals, including humans. The copper–molybdenum antagonism[22,23] provides a dramatic example, which involves the effect of thiomolybdate anions as ligands that bind copper and make the latter unavailable to fulfill its important role in metabolism. For molybdenum enzymes, sulfite oxidase deficiency[24,25] appears to induce fatal or devastating lesions. The recent suggestion[26] that retinal oxidase, an enzyme critical in development, is the molybdenum enzyme aldehyde oxidase points again to the essentiality of molybdenum. In the case of xanthine oxidase, which effects the terminal step in purine metabolism in primates, its metabolic product, uric acid [7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione], is implicated beneficially as an antioxidant[27–29] and, malevolently, in its precipitation, as the culprit in gout.[30] The need for viable molybdenum cofactor proteins for development, survival and success in many animal species is abundantly established.

## The Molybdenum Cofactor (Moco)

The molybdenum enzymes share a similar structural unit at their catalytic sites that overtly distinguishes them from other enzymes. This component is called the molybdenum cofactor (Moco) although it is not a cofactor in the classical sense of being dissociable from the enzyme during turnover. Moreover, we now know that there is no single universal molybdenum-containing unit in all these proteins. Rather, this class of enzymes is usefully designated as 'Moco enzymes' in the same sense that the designation 'hemoproteins' represents a large and diverse class of iron porphyrin-containing macromolecules. Thus, the molybdenum cofactor joins heme, coenzyme $B_{12}$ and iron–sulfur clusters as complex prosthetic groups in metalloproteins.

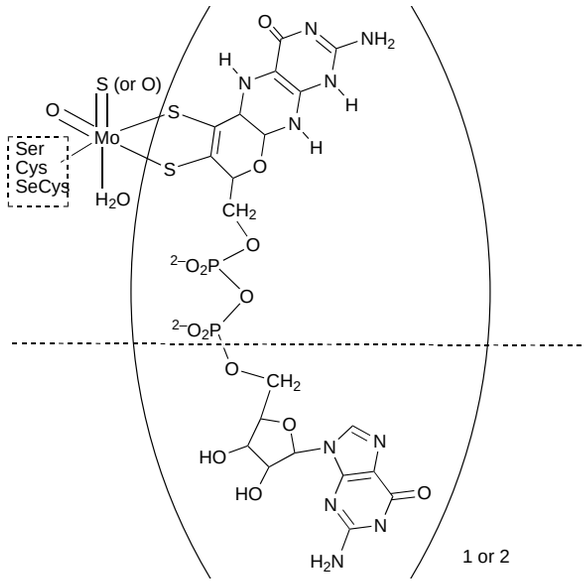


**Fig. 1** A general schematic representation of the extended structure of the molybdenum site in enzymes, generically designated as Moco. One or two pterin–ene-dithiolate ligands bind to molybdenum. The portion below the dotted line is only present in the dinucleoside version of Moco, present in some of the bacterial enzymes. The nucleoside is shown here as guanosine but can be adenosine, cytosine, or inosine. The molybdenum center is shown with terminal oxo, terminal sulfido, aqua, and side chain ligands from cysteine (Cys), selenocysteine (SeCys) or serine (Ser). The particular combination of ligands on the molybdenum center is characteristic of the specific enzyme and changes during turnover

The molybdenum cofactor is unique not only in containing molybdenum but also in its possession of a distinct organic ligand family that binds molybdenum (and tungsten). The dithiolate portion of this pterin–ene-dithiolate family binds the metal, modifying and, presumably, enhancing its properties. Both the co-ordination of molybdenum and the detailed nature of the organic ligand are subject to variation in the different molybdenum enzymes.[12,21] The essential features of the molybdenum cofactor family, with its pterin–ene-dithiolate and other non-protein ligand(s), are seen in Fig. 1. Either one or two pterin dithiolene ligands are co-ordinated to molybdenum.[12]

The last two years have witnessed the first crystal structure determinations of Moco enzymes.[21] In the crystallographic elucidation of the structures of ferredoxin aldehyde oxidoreductase,[31,32] dmso reductase,[33–35] formate dehydrogenase,[36] and, soon, sulfite oxidase,[37] many features of the Mo site were identified that were first assigned using chemical degradation, extended X-ray absorption fine structure (EXAFS), electron paramagnetic resonance (EPR) and other spectroscopic studies.[10–13,18,19] In particular, the dithiolene nature of the molybdopterin ligand has been unequivocally established. However, there is one significant difference between the ligand found crystallographically and that deduced by chemical and physical studies on derivatives of the isolated cofactor. The ligands in all the crystallographically elucidated centers contain three organic rings (*i.e.* a third ring on the pterin framework, not counting the fourth, metal–chelate ring).

The pterin–ene-dithiolate structure, masterfully deduced and supported by Rajagopalan and co-workers,[17,38] is fully

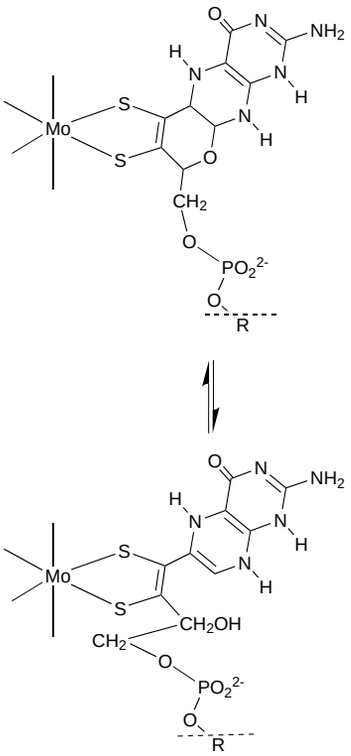

**Fig. 2** The interconversion of the open and closed forms of the pterin ligand. The open form is shown as a 5,8-dihydropterin but 7,8 or 5,6 forms are also possible. The reaction is viewed (bottom to top) as the addition of an alcohol functionality to the double bond. The resulting pyrazine ring of the pterin is formally in the tetrahydro state. The R group represents the nucleotide that may be appended to the pterin dithiolene structure

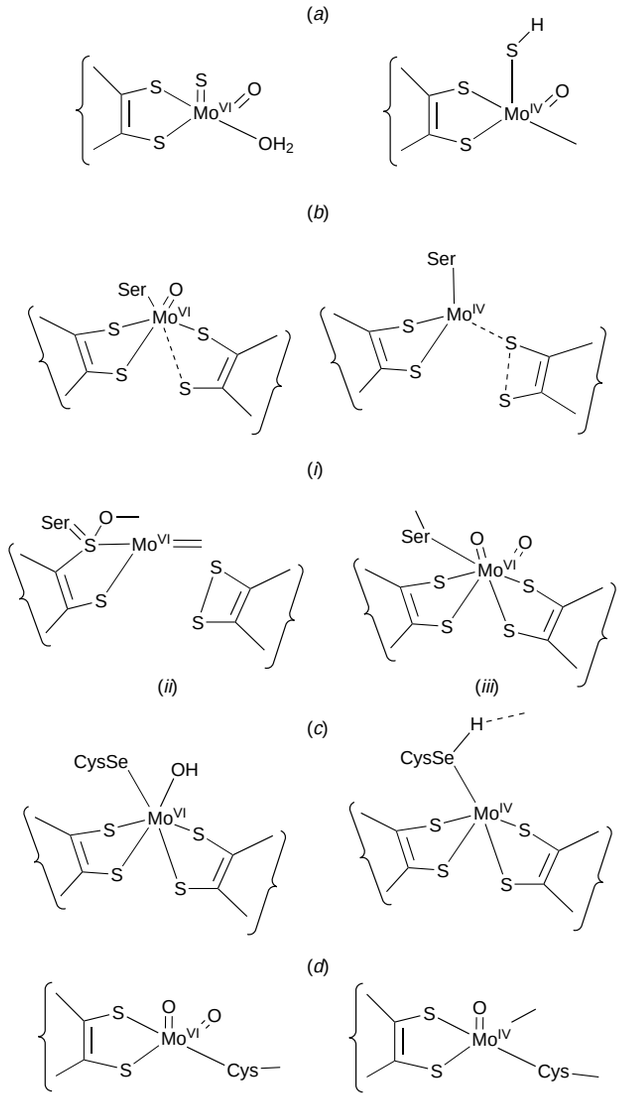

**Fig. 3** Schematic structures for molybdenum sites in enzymes based on X-ray crystallography. (*a*) Ferredoxin aldehyde oxidoreductase from *Desulfovibrio gigas*, a member of the xanthine oxidase family of proteins.[31,32] (*b*) Dimethyl sulfoxide reductase, three distinct structures have been determined: (*i*) from *Rhodobacter spheroides*;[33] (*ii*) from *Rhodobacter capsulatus*;[34] and (*iii*) from *Rhodobacter capsulatus*.[35] (*c*) Formate dehydrogenase from *Escherichia coli*.[36] No oxo groups and a selenocysteine have been identified in the molybdenum co-ordination sphere. (*d*) Sulfite oxidase, the structure shown based on EXAFS, spectroscopy, and genetics has recently received support from preliminary crystallographic studies[21,37]

confirmed by the X-ray analyses. However, the side chain on the pterin is not the linear four-carbon chain originally surmised from degradation experiments. Rather, a third ring, a 5,6-dihydropyran, is fused to the standard two-ring pterin framework at the 6 and 7 (pyrazine) positions of the pterin. Interestingly, earlier work had indicated a reduced dihydro, form of the pterin in sulfite oxidase and xanthine oxidase.[39] Significantly, dihydropterins form alkoxylated pterins in a facile reaction.[40] Therefore, it remains possible that, under some circumstances, the open form of the structure, or an equilibrium between an open and ring-closed form, is present under turnover conditions. Such an interconversion is shown in Fig. 2. The free hydroxyl group generated in the ring-opening reaction is more stereochemically flexible than the oxygen of the dihydropyran ring in the closed form.

## Nomenclature

The non-protein organic ligand in Moco was first characterized by Rajagopalan and co-workers,[17,18,38] The ligand was named molybdopterin (sometimes abbreviated MPT) in the hope that its unique role as a binder to molybdenum would provide an easily learned and flawlessly applied nomenclature. Unfortunately, three factors compromise this desired simplicity and transparency. Firstly, the name of the pterin contains the prefix molybdo- which has caused many uninitiated in the field to think that molybdopterin contains molybdenum, which it does not. Only Moco, the complete molybdenum cofactor, containing molybdenum, molybdopterin and other ligands, is the active entity. Secondly, the very same ligand family has been confirmed as being present in tungsten enzymes.[14,15,41,42] Hence we can have a 'molybdopterin-containing' cofactor that contains no molybdenum and yet is at the active site of a number of (tungsten) enzymes. Thirdly, in a number of the bacterial enzymes, the core pterin unit is part of a variety of extended dinucleoside structures. These dinucleotides involve adenosine, cytosine, guanosine, or other nucleotides, connected to the pterin unit by a pyrophosphate linkage (see Fig. 1). Nomenclature for these dinucleotide varieties of the pterin ligand has not been uniformly applied.

A further complication is presented by the additional (dihydropyran) ring appended on the pterin nucleus in all the extant crystal-structure determinations. The extra ring opens the door to renumbering of the entire ring system, but it is not obvious what the correct numbering scheme should be.

The need for systemization of nomenclature and numbering is apparent. It is hoped that researchers in the field will soon address the nomenclature issue and arrive at a recommendation that is workable and acceptable to (most) workers in the field and that does not cause unnecessary confusion in the uninitiated. Meanwhile, the various molybdopterin designations should be carefully defined and cautiously read.

**Table 2** Stoichiometric formulations for substrate reactions of molybdenum enzymes

| | |
|---|---|
| | Oxygen-atom transfer |
| Nitrate reductase: | $NO_3^- \longrightarrow NO_2^- + [O]$ |
| Dimethyl sulfoxide reductase: | $dmso \longrightarrow SMe_2 + [O]$ |
| Sulfite oxidase: | $SO_3^{2-} + [O] \longrightarrow SO_4^{2-}$ |
| Xanthine oxidase: | $xanthine + [O] \longrightarrow uric\ acid$ |
| Carbon monoxide oxidoreductase: | $CO + [O] \longrightarrow CO_2$ |
| | Proton–electron transfer |
| Nitrate reductase: | $NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O$ |
| Dimethyl sulfoxide reductase: | $dmso + 2e^- + 2H^+ \longrightarrow SMe_2 + H_2O$ |
| Sulfite oxidase: | $SO_3^{2-} + H_2O \longrightarrow SO_4^{2-} + 2e^- + 2H^+$ |
| Xanthine oxidase: | $xanthine + H_2O \longrightarrow uric\ acid + 2e^- + 2H^+$ |
| Carbon monoxide oxidoreductase: | $CO + H_2O \longrightarrow CO_2 + 2e^- + 2H^+$ |
| Polysulfide reductase: | $S\ (in\ polysulfide) + 2e^- + 2H^+ \longrightarrow H_2S$ |

## Crystallographic Molybdenum Sites

The structures of ferredoxin aldehyde oxidoreductase,[31,32] formate dehydrogenase,[36] sulfite oxidase[37] and three versions of dmso reductase[33–35] have been solved to various degrees of resolution. The proposed active sites are summarized in Fig. 3. Clearly, there is significant structural variety around the molybdenum and, surprisingly, in the way in which the dithiolene ligand binds (or does not bind) to the metal. Moreover, it is confirmed that the molybdenum site changes significantly during turnover, in agreement with the earlier spectroscopically derived conclusions.

The differences seen in the independently determined structures of dmso reductase[33–35] could reflect artifacts of the crystallographic structure determinations, but may be revealing the subtle structural and electronic fluidity of which the active site is capable.

## Substrate Reactions and Catalytic Cycles

A few of the substrate reactions of molybdenum enzymes are shown in Table 2.[12,18] The substrate clearly differs from the product by a two-electron redox process. In a stoichiometric sense, there are two simple ways in which many of the substrate conversions can be formulated. In one, the substrates differ from their products by some combination of two electrons, protons and, usually, water.[43] Alternatively, many of the conversions can be formulated as oxo-transfer reactions.[44] Both the proton/electron and oxo-transfer formulations are valid ways of formally accounting for the substrate transformations. However, neither formulation can be translated into mechanistic promulgation without the marshalling of appropriate evidence (which, fortunately, is accumulating inexorably in the case of some of the better studied enzymes). A change in molybdenum oxidation state between VI and IV fully accommodates the required substrate conversion.

Once the substrate conversion has occurred, the molybdenum site must be regenerated for the next round of reaction. This occurs invariably through the electron, proton, water type reaction.[12] For example, in the regeneration of the $Mo^{VI}$ site in sulfite oxidase, equation (1) is a representation of the reactivation step. In general, electrons go to (or come from) the biological redox partner and protons go to (or come from) the aqueous phase. The reactivation process removes oxo and generates water or regenerates oxo from water in the molybdenum co-ordination sphere. Interestingly, although the overall reactivation is a two-electron process, abundant evidence in all enzymes studied indicates tandem one-electron steps, each often coupled to the transfer of a single proton. The molybdenum site is able to link the required two-electron reaction of substrate to the one-electron transfer processes typical of its biological redox partners (hemes, Fe–S centers, flavins).

$$Mo^{IV}O^{2+} + H_2O \longrightarrow Mo^{VI}O_2^{2+} + 2e^- + 2H^+ \quad (1)$$

## Insights from Co-ordination Chemistry

### Proton and electron transfer

The coupling of proton and electron transfer is well established in co-ordination chemistry, including that of molybdenum.[43] Such coupling highlights the relationship between redox and acid–base properties of metal centers. The state of protonation of a complex depends upon its state of oxidation. This is particularly true for families of ligands such as $H_2O$, $OH^-$, $O^{2-}$ and $H_2S$, $SH^-$, $S^{2-}$. In the higher metal oxidation states, these ligands tend to be fully deprotonated, *i.e.* strongly acidic with dissociated protons. Witness such species as $MoO_4^{2-}$, $MoS_4^{2-}$ and $MoO_2S_2^{2-}$ and the prevalence of $MoO_2^{2+}$ units in the chemistry of molybdenum VI.[45,46] In contrast, reduced compounds have fewer oxo groups and/or possess protonated ligands.

The effect of oxidation state on p$K_a$ of the co-ordinated ligands is profound with differences of 6–10 p$K_a$ units not uncommon. Some of the more dramatic and quantitative work in this area comes from studies of ruthenium oxo bipyridyl compounds. For example, the ruthenium(II) ion, $[Ru(bipy)_2(py)(H_2O)]^{2+}$ (bipy = 2,2′-bipyridyl, py = pyridine), has a p$K_a$ of 10.8 whereas the ruthenium(III) ion, $[Ru(bipy)_2(py)(H_2O)]^{3+}$ has a p$K_a$ of 0.8; a change of ten orders of magnitude in acidity for a unit change in oxidation state.[47]

The large effect of oxidation state on p$K_a$ provides the driving force for the coupling of proton and electron transfer. The electron resides predominantly on the metal center but the ligand donor atom bears the proton. The transfer of one electron and one proton is equivalent to a hydrogen-atom transfer, while a transfer of two electrons and one proton is the equivalent of a hydride transfer. However, the placement of the electrons and protons on metal and ligand sites, respectively, indicates that hydride transfer, *per se*, is usually not the mechanistically most accurate description of these reactions, at least in high oxidation state metal ions,[13,18,19] such as those found in the molybdenum and tungsten enzymes.

### Oxygen-atom transfer

The ability of molybdenum and tungsten sites in their higher oxidation states to undergo oxygen-atom transfer is well established.[44] Mechanistically, this process can be viewed as the transfer of two electrons and an oxide ion in opposite directions. Oxygen-atom transfer is the two-electron counterpart of classical one-electron inner-sphere electron transfer involving, for example, a chloride bridge. The oxide ligand bridges the redox partners, allowing the two-electron transfer to occur, and ends up bound to the oxidized reaction partner. Clearly, in chemical systems, the reactions of small molecules such as phosphines, sulfides and tertiary amine oxides appear to proceed by simple 'oxo' transfer.[44]

## Enzyme Mechanisms

The electron-transfer processes occurring in mononuclear molybdenum enzymes have been extensively discussed.[11,12] The reactivation step invariably involves coupled proton–electron transfers, but the substrate reaction pathways are far less clear. It is tempting, in light of the stoichiometries, to invoke simple oxo transfer for many of the molybdenum enzymes, in particular those in the class that resembles dmso reductase and nitrate reductase. While this reaction type clearly occurs in 'model' systems,[44] and may occur in some of the enzymes,[12] it is not

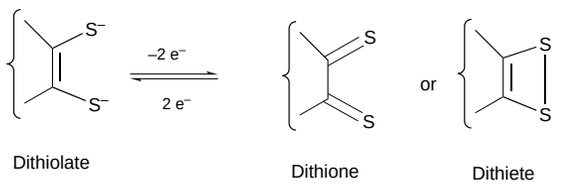


**Fig. 4** The redox activity of a generic ene-dithiolate ligand. The two-electron oxidation of the dianionic dithiolate form of the ligand yields a neutral dithione or dithiete form

established mechanistically in most of the enzymes. An oxygen-atom transfer *stoichiometry* doesn't necessarily implicate an oxygen-atom transfer *mechanism* for the substrate reaction.

In the case of enzymes in the well studied xanthine oxidase family, our knowledge has been enriched by the recent crystal structure determination of an enzyme in that class.[32] In xanthine oxidase and related enzymes, there is a terminal oxo and a terminal sulfido ligand in the oxidized $Mo^{VI}$ state. The molybdenum site is transformed during substrate oxidation to a reduced $Mo^{IV}$ state that maintains the oxo but not the *terminal* sulfido ligand. It has been suggested that the sulfur becomes a co-ordinated sulfhydryl ligand and thus serves as the proton acceptor in the substrate oxidation process.[12,18,32]

Labelling experiments[48] clearly show that, in a single turnover, an $^{18}O$ label on the enzyme is transferred to xanthine (3,7-dihydro-1*H*-purine-2,6-dione) in the production of uric acid. But, is it the oxo that gets transferred or some other labelled oxygen atom at or near the active site? The simpler explanation, based on crystallographic[32] and chemical[18] arguments, holds the oxo as a 'spectator' ligand[49] and invokes a different oxygen atom, probably co-ordinated $OH^-$ or $H_2O$, as the ligand that is transferred to the substrate.[32] This process then involves a coupling of proton and electron transfer (two electrons and one proton or a hydride equivalent), in conjunction with the nucleophilic attack at the susceptible C–H bond by the labelled (non-oxo) oxygen. While this reaction stoichiometrically looks like a simple oxygen-atom transfer, mechanistically, based on the structure and labelling experiments[32,48] it combines a coupled proton–electron transfer with a nucleophilic attack on the substrate in the oxidation process.[43]

## Dithiolene Ligands

The chemistry of dithiolene ligands (1,2-ene-dithiolate or 1,2-benzene dithiolate ligands) had been under study long before[50–52] it was suspected that such ligands are present in molybdenum (much less tungsten) enzymes. The impetus for the study of dithiolenes came from their formation of stable, highly colored, redox active complexes for virtually all transition elements. The late transition metals form redox series of bis(dithiolene) complexes while the early transition metals form tris complexes with co-ordination geometries ranging from trigonal prismatic to octahedral.

The structure, bonding and redox interconversions of dithiolene complexes attracted considerable attention[50–52] but there was little inkling that this ligand could join porphyrins and corrins as specific organic ligands in a biological context. One of the consequences of this lack of knowledge was the concentration of early efforts on homoleptic dithiolene complexes, *i.e.* on complexes where non-dithiolene ligands are absent. As we now know, the mono- and bis-dithiolene systems are the biologically 'chosen' stoichiometries. Such a dithiolene grouping leaves room in the molybdenum (or tungsten) co-ordination sphere for other ligands, *e.g.* oxo, aqua, sulfido or protein residues, to play key functional roles in the enzyme reactions. Indeed, recent studies on bis(dithiolene) complexes of molybdenum and tungsten have revealed features of structure and reactivity which resemble those of the molybdenum and/or tungsten enzymes.[53–58] What properties of dithiolene ligation

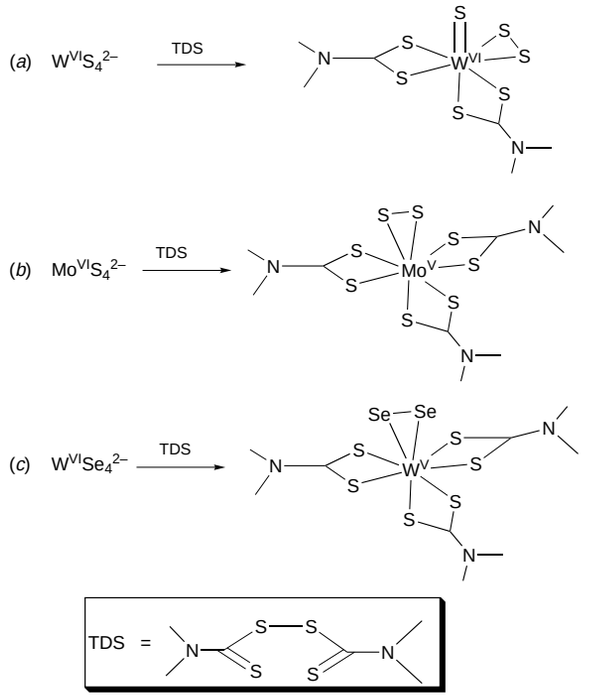


**Fig. 5** Redox reactions between tetrachalcogenidometalates and thiuram disulfide. (*a*) The reaction[59] involves ligand redox; the oxidation state of tungsten does not change. (*b*) The reaction involves internal electron transfer[59] in which sulfide on the tetrathiomolybdate reduces the disulfide of the thiuram disulfide as well as the molybdenum center. (*c*) The reaction shows that tetraselenotungstate reacts in a manner identical to tetrathiomolybdate[60]

are important with respect to the evolutionary choice of this ligand type?

Dithiolene ligands form stable conjugated five-membered chelate rings. The dithiolene ligand delocalizes the 'metal' electron density in the non $d^0$ systems that are often present. Moreover, dithiolenes are themselves redox active and a great deal of early work went into sorting out the metal or ligand character of the highest occupied (HO) and lowest unoccupied (LU) molecular orbitals (MOs).[50–52] A vivid illustration of the redox participation of the dithiolene ligand is given by the well characterized complex $[V\{S_2C_2(C_6H_5)_2\}_3]$.[51] Here, if the dithiolene ligands are formulated in their dianionic (dithiolate) form, this complex contains the Group 5 element vanadium in the oxidation state VI; clearly an impossible situation! The enigma is resolved by invoking the redox activity of the dithiolene ligands. Clearly, contributions from forms of the ligand that are more oxidized, see Fig. 4, must be important in the vanadium complex. Indeed, the oxidized free ligand form of one of the dithiolenes, namely the dithiete of the bis(trifluoromethyl) 1,2-dithiolene is uncharged and contains an internal disulfide linkage. Complete dithiolene oxidation may be a part of the action of molybdenum or tungsten enzymes. Moreover, the ability of dithiolene ligands to undergo oxidation by one or two electrons at potentials comparable to those of metal centers is consistent with an electronic structure of the metal–dithiolene system that involves extensive delocalization and at least partial ligand participation in redox processes.

## Ligand and Internal Redox Reactions (W and Se Comparison)

The closeness of the redox manifolds for sulfur redox systems and early transition metals manifests itself in unusual redox behavior that is not commonly encountered in transition-metal chemistry of non-chalcogenide ligands. First, there is the possibility of purely ligand-based redox processes. An example is shown in Fig. 5(*a*) wherein the tetrahedral tungsten(VI)

tetrathiotungstate ion is transformed into a new complex with different ligands (dithiocarbamato and disulfido) by addition of the disulfide of the dithiocarbamate (the thiuram disulfide).[59] The tungsten atom does not change its state of oxidation. The green diamagnetic tungsten(VI) complex has formed *via* redox processes that involve only the ligands.

In contrast to the behavior of the tungsten complex, the nominally analogous reaction with the $Mo^{VI}$ tetrathiomolybdate ion is shown in Fig. 5(*b*). This reaction clearly involves reduction of the molybdenum center to its $Mo^{V}$ state.[59] The $4d^1$ $Mo^{V}$ nature of the molybdenum is unequivocally indicated by the EPR spectrum, which displays the expected value for molybdenum(V) hyperfine splitting. The only reductant in this system is the sulfide ($S^{2-}$) ligand in the molybdenum co-ordination sphere of the starting material. The internal redox reaction, in which the sulfide ligand reduces the molybdenum from VI to V, has been induced by the addition of an oxidant in the form of the thiuram disulfide. This oxidant is reduced by the co-ordinated sulfide to the dithiocarbamate ligand. The non-organic sulfur remaining in the molybdenum co-ordination sphere is the (oxidized) disulfido ligand ($S_2^{2-}$).

Recently, we have looked at the internal redox reaction involving other transition-metal chalcogenide systems. For example, tetrathioperrhenate[61,62] and tetrathiovanadate[63] each undergo facile internal electron-transfer processes. Indeed, for the rhenium system the process can involve reduction of the rhenium from the VII to the III oxidation state.[61,62] All the reducing equivalents come from co-ordinated sulfide, which is oxidized to elemental sulfur in the process. Why are some compounds more susceptible to internal redox than others?

To address the proclivity toward internal redox we examined the position of the lowest energy ligand to metal charge transfer (LMCT) transition. This electronic transition resembles the chemical internal electron-transfer process insofar as an electron is moved from a sulfur lone pair level to a largely metal centered d orbital. We examine the spectra of the tetrathiometalate ions which are relatively simple and, at least for the lowest energy transitions, assigned without controversy to LMCT.[64] The energy of the lowest energy LMCT increases in the order $[VS_4]^{3-}$ (18 600) < $[ReS_4]^{-}$ (19 800) < $[MoS_4]^{2-}$ (21 400) < $[WS_4]^{2-}$ (25 500 $cm^{-1}$).[65] In a qualitative sense, this is the observed order for the ease and extent of internal redox reactivity. The rhenium complex, with the low LMCT energy undergoes facile internal redox. The tungsten complex does not undergo internal redox under many of the same conditions where the corresponding molybdenum complex does. Does this correlation have predictive value?

To investigate the usefulness of the correlation we studied the reactions of the tetraselenotungstate ion, $[WSe_4]^{2-}$.[60] The lowest LMCT for this ion occurs at 21 600 $cm^{-1}$, a value almost identical to that of the similarly red-orange $[MoS_4]^{2-}$ ion. The increase in the energy of the LMCT in going from Mo to W (W is more difficult to reduce) is almost exactly offset by the decrease in the LMCT energy in going from sulfur to selenium (where selenium is easier to oxidize). If the controlling factor in reactions such as those in Fig. 5(*a*) and 5(*b*) is the nature of the metal, then we might expect the reaction of $[WSe_4]^{2-}$ to be similar to that of $[WS_4]^{2-}$ [reaction 5(*a*)]. However, if the reactivity trend corresponds to the spectroscopic LMCT process, we would predict reactivity analogous to $[MoS_4]^{2-}$. Indeed, the reaction between $[WSe_4]^{2-}$ and the thiuram disulfide [Fig. 5(*c*)] gives, as the sole product, $[W(Se_2)(S_2CNR_2)_3]$ (R = alkyl), which is isostructural with $[Mo(S_2)(S_2CNR_2)_3]$, that is, the internal redox product. Clearly, the lowest energy LMCT is an effective indicator of the proclivity of chalcogenide metal complexes to undergo internal redox reactions.

The implications of internal redox proclivity are discussed further below, but there is one possibly direct connection to the enzyme systems. In recent years, tungsten has joined molybdenum as a heavy transition metal present at the active center of metalloenzymes.[14,15] In light of the observed similarity in reactivity of molybdenum–sulfur and tungsten–selenium systems, it is fascinating to note that the first identified tungsten enzyme[66,67] is also found to contain selenium. It is possible that, in this formate dehydrogenase, for which there are molybdenum–sulfur analogs, the combination of tungsten and selenium is similar to the molybdenum sulfur unit in its reaction or redox ability. While this is an interesting conjecture, its generality may not be great as most of the tungsten enzymes do not contain selenium while some molybdenum enzymes do. Therefore, it seems more likely that nature chooses a particular combination of metal, chalcogenide, and protein to facilitate the specific redox or substrate reaction process that the enzyme has evolved to carry out.

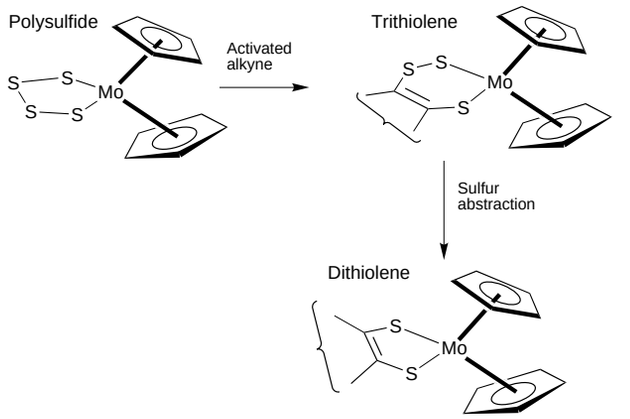


**Fig. 6** Reaction of a polysulfide molybdenum complex with an activated alkyne initially yielding a trithiolene bound to molybdenum.[70,71] The excess sulfur atom in this sulfur rich molecule is removed with sulfur extracting agents such as triphenylphosphine to yield the molybdenum dithiolene complex

## Trithiolene Ligation

The major emphases in the creation of chemical analogs of the molybdenum sites in enzymes have involved structural/spectroscopic and reactivity studies. These have been reviewed extensively and will not be emphasized here.[18–20,68] A related approach has targeted chemical synthesis[69,70] of the pterin ligand and its metal complexes, ultimately aiming to achieve the full complexity of Moco itself.

Here we summarize aspects of the synthesis of the pterin and related quinoxaline ene-dithiolate chemistry on molybdenum.[70] One potential step in a chemical strategy to synthesize Moco involves reaction of a pterin or quinoxaline substituted acetylene with a molybdenum polysulfide linkage to directly form a molybdenum ene-dithiolate linkage.[70,71] As shown in Fig. 6, this reaction was ultimately realized, but, only in a two-step reaction. Instead of the desired five-membered dithiolene ring, the reaction first generated an unprecedented six-membered 'trithiolene' ring where the additional sulfur is part of a persulfido linkage directly co-ordinated to the molybdenum center. The extra sulfur is, however, simply removed by treatment with sulfur-extracting agents such as triphenylphosphine. The chemistry in Fig. 6 occurs on the $Mo^{IV}$ oxidation state [albeit, on the admittedly non-biological bis(cyclopentadienyl) molybdenum framework].

The existence of a trithiolene–$Mo^{IV}$ linkage raises the interesting admittedly speculative possibilities outlined in Fig. 7. Focusing on the sulfur ligation, one recognizes that a $Mo^{IV}$ trithiolene unit is an internal redox isomer of a sulfido dithiolene $Mo^{VI}$ center. This latter combination of sulfur ligands and molybdenum oxidation state is exactly that present in the oxidized form of the xanthine oxidase family of Moco enzymes.[12,32] While we stress that there is at present no evidence for a trithiolene unit in any enzyme, the potential existence of

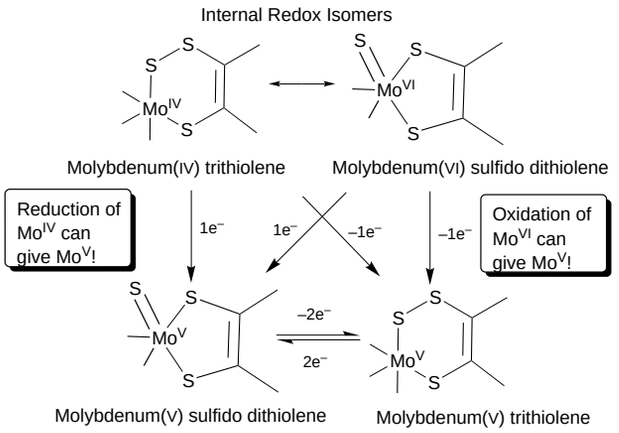


**Fig. 7** The hypothetical redox interconversions that are possible in molybdenum (or tungsten) enzymes that contain a sulfido (selenido) ligand. Seemingly paradoxical behavior occurs for example in oxidation of $Mo^{VI}$ compound to give a $Mo^{V}$ compound. The paradox disappears when one takes account of sulfur oxidation that accompanies the reduction of the molybdenum center

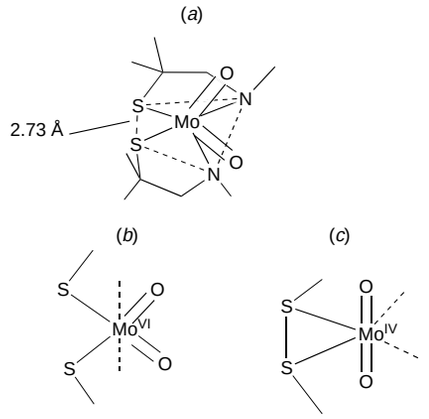


**Fig. 8** (*a*) Schematic structure of the bis(substituted cysteamine) dioxo molybdenum complex[74,75] with an unusual geometry and unusually short sulfur–sulfur contact in the molybdenum co-ordination sphere. (*b*) and (*c*) represent the two extreme cases, a *cis*-dioxo $Mo^{VI}$ site and a *trans*-dioxo $Mo^{IV}$ site, respectively

this redox isomer offers some interesting possibilities. The upper portion of Fig. 7 shows internal redox in which the two electrons released by generation of the persulfide bond reduce $Mo^{VI}$ to $Mo^{IV}$. This simple internal redox reaction transforms the sulfido dithiolene $Mo^{VI}$ to the trithiolene $Mo^{IV}$. The process reduces the co-ordination number on molybdenum by one, potentially generating an open co-ordination site. Such a site could be exploited by the enzymes for binding of substrate or other ligands during turnover.

The left half (and diagonal) of Fig. 7 shows that paradoxical (or conventional) redox reactions may lead to the formation of the molybdenum(V) state. Thus, one-electron reduction of the sulfido dithiolate $Mo^{VI}$ state can lead conventionally to a $Mo^{V}$ state (diagonal). However, one-electron *reduction* of the trithiolene $Mo^{IV}$ (left) can also lead to the same $Mo^{V}$ state, if it is coupled to the internal redox reaction. The disulfide bond is reduced by two electrons: one electron from the $Mo^{IV}$ and one from the external reductant. This induced internal electron transfer appears contradictory since addition of reductant leads to an oxidized metal site. Similarly, as shown on the right side of Fig. 7, a one-electron *oxidation* of the sulfido dithiolate $Mo^{VI}$ grouping is possible if the persulfido linkage is formed concomitantly. The $Mo^{V}$ state is produced with one of the electrons released from the formation of the persulfido linkage, the other going to the external oxidant; again, an induced internal redox reaction. The two $Mo^{V}$ products on the bottom of the diagram are related by a two-electron redox process involving only the ligands. The redox richness of this chemistry is apparent.

While there is no evidence for processes such as those in Fig. 7 in molybdenum enzymes, the reactions are chemically possible and researchers seeking to sort out the myriad states that have been identified by EXAFS, EPR and crystallography, especially for the large xanthine oxidase subclass of molybdenum enzymes, should keep in mind the possibility of ligand or internal redox. Indeed, a process such as this could explain the behaviour in the tungsten enzyme aldehyde oxidoreductase, where unexpected redox behaviour is encountered.[72]

## Partial redox

The complete transfer of electrons between metal and ligand unambiguously changes the oxidation state of both. However, it is possible that the redox may not be complete. There are several examples in molybdenum–sulfur chemistry. It has been suggested that the somewhat short (*i.e.* less than van der Waals) interligand sulfur–sulfur contacts in dithiolene complexes are an indication of weak sulfur–sulfur bonding, which may help stabilize the dithiolene complexes in planar and trigonal prismatic co-ordination geometries.[73] While there is no strong experimental or hard theoretical evidence for such bonding, it can be recognized as one extreme, with formation of a strong sulfur–sulfur bond combined with two-electron reduction of the metal as the other.

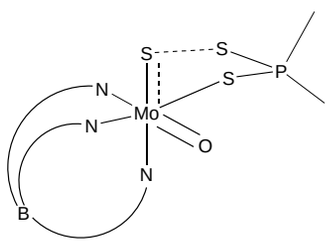

**Fig. 9** Schematic structure of a dithiophosphinato sulfido complex with a short 'interligand' sulfur–sulfur contact that is intermediate between the sulfido dithiolate and trithiolate structures described in the text and shown in Fig. 7. The tridentate nitrogen-donor structure on the left is a tris(pyrazolyl)borate ligand[76]

An example of partial redox involves dioxo bis(thiolate) molybdenum complexes. In a series of hindered bis(cysteamine) dioxo $Mo^{VI}$ complexes, unusual six-co-ordinate structures were found of the type illustrated in Fig. 8.[74,75] Here, the sulfur–sulfur distance, at ≈2.73 Å, is much shorter than the expected *cis* sulfur–sulfur contact and the S–Mo–S angle of ≈70° is unusually small. Moreover, the molybdenum oxo bonds, at ≈1.72 Å, are somewhat longer and the O–Mo–O angle, at 122°, larger than those typically found in the very common *cis*-dioxo octahedral molybdenum complexes.[45,46] However, the co-ordination geometry is not octahedral (or trigonal prismatic) but rather, closely fits the arrangement of a skew trapezoidal bipyramid.[75] The structure can be viewed as intermediate between a *cis*-dioxo molybdenum(VI) complex, with two *cis*-thiolate ligands [Fig. 8(*b*)], and a *trans*-dioxo molybdenum(IV) complex, with one organic disulfido ligand [Fig. 8(*c*)] albeit closer to the former.

A more recent example of partial internal redox involves an oxo sulfido dithiophosphinate complex (Fig. 9) in which the sulfur–sulfur distance is, at 2.396 Å, even closer to single bond distances.[19,76] The ability of a nominally terminal sulfido group to enter into such an interaction is particularly relevant to the xanthine oxidase family of enzymes, where this type of linkage is present.

## The Need for Three Sulfur Donors

Along with two dithiolene sulfur atoms, there is always at least one additional sulfur (or selenium) donor in all enzymic molybdenum co-ordination spheres. This ligand may be a ter-

minal sulfide (and possibly a selenide), a cysteine thiolate, a selenocysteine selenolate or a second dithiolene. Any of these combinations is a candidate for complete internal redox involving S–S (or S–Se) bond formation. However, partial redox involving far weaker and longer interchalcogenide distances are also possible. In short, all molybdenum (and tungsten) enzymes contain at least three oxidizable chalcogenide donors in their metal co-ordination spheres. That choice of co-ordination sphere may allow facile complete or partial internal redox. Such capability may make the site more fluid, both electronically and structurally, allowing the enzyme great flexibility in its interaction with substrate and/or its redox (re)activation system during turnover.

When two dithiolenes are present the additional possibility is open that the two ligands serve different functions. One dithiolene may be primarily responsible for the activation of oxo or sulfido ligands. Since oxo and sulfido are invariably *cis* to one another in molybdenum(VI) compounds, in an octahedral structure, one of these ligands is necessarily *trans* to one dithiolene sulfur. Alternatively, distortion of the molybdenum co-ordination sphere away from the conventional octahedral (or even from the less common trigonal prismatic geometry), or reduction (or possibly incrementation) in co-ordination number may occur.

If the role of the first dithiolene involves preparation of the active site for substrate activation, the second dithiole may then be involved in other functions, such as local or long-distance electron transfer. The presence of a putative dithiete in the representation of one of the two dithiolenes in one[34] of the crystal structure determinations of dmso reductase (Fig. 3) shows that local redox is viable in the vicinity of the enzyme active site. The extended nature of the pterin dithiolene structures, especially for the dinucleotide varieties, make the outer fringes of the ligands interesting candidates for long-distance electron-transfer sites where the protein may contact its redox partner(s). The intricate and specialized pterin dithiolene ligand may have evolved to play multiple roles in the turnover cycle of the mononuclear molybdenum enzymes.

## Tungsten *vs.* Molybdenum in Enzymes

Table 1 shows that there is now a number of well established tungsten enzymes. These enzymes are mostly from thermophilic or hyperthermophilic bacteria found, for example, at deep-sea hydrothermal vents.[14,15] The tungsten enzymes catalyze reactions that are related to those catalyzed by molybdenum enzymes. Moreover, the tungsten active sites contain the same type of pterin–ene-dithiolate ligand that the molybdenum enzymes employ.[14,15,41,42] One is forced to ask: why do both tungsten and molybdenum enzymes exist?

The *raison d'etre* of tungsten enzymes may involve chemical differences between molybdenum and tungsten that are selected by the special conditions under which the tungsten enzymes function. For example, tungsten is more difficult to reduce than molybdenum. Hence, if tungsten enzymes are required to work at lower redox potentials than their molybdenum analogs, as they appear to be, then tungsten could be evolutionarily favored over the more common molybdenum system. Moreover, the greater inertness of tungsten toward substitution reactions could be advantageous in hyperthermophilic environments (≈100 °C) where hydrolytic reactions could compromise the integrity of the active site. Therefore, the fitness of tungsten over molybdenum in certain cases may involve tungsten's ability to better function or survive in the chemical or physical environment characteristic of the organism in which the enzyme is found.

On the other hand, the chemical differences between molybdenum and tungsten may manifest themselves, not in different reactivity within the formed enzymes, but, in the geochemical environment in which the organisms find themselves. Molybdenum deposits are invariably found in nature in the form of $MoS_2$, while the more oxophilic tungsten is found as $CaWO_4$ and $PbWO_4$. Clearly, their chemical difference is sufficient to effect geochemical differentiation of these elements. Chemically, it is much more difficult to convert tungstate to tungsten sulfide compounds than it is to convert molybdenum to molybdenum sulfide compounds. Therefore, in the sulfur-rich hydrothermal environments in which many of the organisms that contain tungsten enzymes have been found, it is possible that molybdenum is not available, having precipitated along with the massive sulfide deposits found at such sites. The microorganisms therefore use tungsten because molybdenum is not available. Further studies on the enzymes, the organisms, the biogeochemistry of the habitats, and the coevolution of these entities should help sort out the teleology of the tungsten *versus* molybdenum.

## Conclusion

The molybdenum (and tungsten) enzymes are a broad class of catalysts in which a mononuclear metal site transforms a variety of substrates by net two-electron redox processes. The molybdenum is complexed by a pterin–ene-dithiolate ligand and the full non-protein co-ordination of molybdenum is called the molybdenum cofactor, Moco. The ability of molybdenum and tungsten to engage in one- or two-electron redox processes facilitates both substrate redox processes and reactivation of the active site. Oxygen-atom transfer and proton–electron transfer are convenient ways to stoichiometrically designate reactions and may have mechanistic relevance in particular cases. The chemistry of early-transition-metal chalcogenide systems reveals facile internal redox chemistry. Moreover, especially for the molybdenum–sulfur systems, evidence for partial redox states is adduced from short sulfur–sulfur distances within the molybdenum co-ordination sphere. The minimal presence of three potentially oxidizable chalcogenide donor atoms in all molybdenum and tungsten enzymes may facilitate internal (full or partial) redox reactions that are crucial for the activity of the enzymes. The differences in the chemistry of molybdenum and tungsten may be responsible for the occurrence of these two Group 6 metals in different organismic or enzymic systems.

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