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FEATURE ARTICLE

Metal chemistry relevant to the mononuclear molybdenum and tungsten pterin enzymes‡

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In the light of recent protein crystal structures, metal chemistry underpinning the action of oxygen-atom transfer enzymes which contain molybdenum and tungsten pterin centres is outlined.

Introduction

The group 6 metal molybdenum is an essential nutrient for all forms of life while its heavier congener tungsten is essential for certain anaerobic thermophilic bacteria.[1,2] The high solubility of Mo^{VI} and W^{VI} oxyanions in water ensures that the elements are available to biological systems (10^{-7} and 10^{-9} mol dm^{-3}, respectively, in sea water), despite their low terrestrial elemental abundances.[1]

Molybdenum enzymes fall into two general classes: the nitrogenases and the molybdopterin enzymes.[3,4] The former feature the iron–molybdenum cofactor (FeMoco), a cluster of stoichiometry Fe_7MoS_9. The latter possess mononuclear molybdenum cofactors (Moco) with metal bound by 1,2-dithiolate links to one or two pterin ring systems [Fig. 1(*a*)].[4–6] An oxidised derivative, urothione [Fig. 1(*b*)], is excreted during normal human metabolism. Known tungsten enzymes are related to the second class.[2]

Most pterin enzymes mediate formal oxygen atom exchange between substrate X and water [eqn. (1)].[7–9]

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

Specific half-reactions for some of the more extensively studied systems are given in Scheme 1. Molybdenum centres operate on C, N, S and As atoms of substrate X[8–10] while the known tungsten centres operate on carbon during its assimilation from various sources.[2,11] There is usually a second reaction centre (*e.g.* flavin, iron–sulfur, cytochrome) for the complementary half-reactions and the two active sites are connected by an internal electron-transfer chain. Two identified roles for the pterin ligand are to participate directly in this intramolecular electron transfer and to tune the redox potential of the site.[6,7,12]

Molecular features of the pterin enzymes have been illuminated by recent crystal structures (Fig. 2).[12–16] These provide a basis for further progress in defining the molecular mechanisms of action. This Article concentrates on the structural and reaction chemistry at the molybdenum and tungsten sites. The value of defining the fundamental chemistry was clear from the first significant modelling experiments. Electron paramagnetic resonance (EPR) signals characteristic of Mo^V appeared upon reaction of $[Mo^{VI}O_4]^{2-}$ and glycolic acids $HECH_2CO_2H$ (E = O, S).[17] Spectral parameters for the sulfur-based system were similar to the so-called 'very rapid' and 'rapid' signals of xanthine oxidase and established a major role for ligand sulfur.[18] The synthetic species generated have now been identified as square-pyramidal $[Mo^VO(SR)_4]^-$ and related species.[19–21]

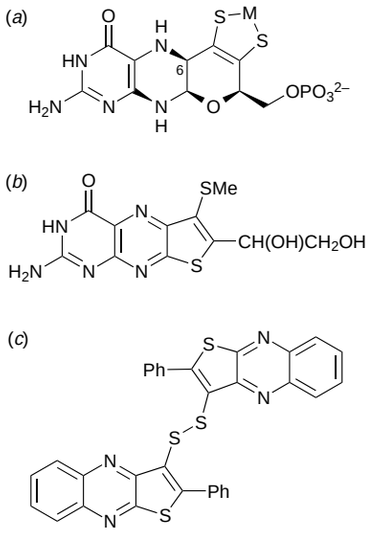
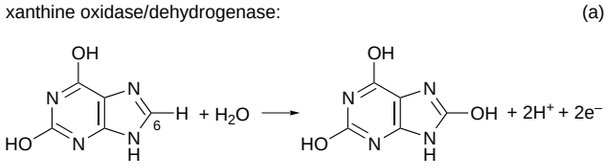


Fig. 1 (*a*) Pterin centre with pyran ring attached at positions 6 and 7. A 1,2-dithiolate unit capable of acting as a bidentate ligand to metal M occupies positions 1′ and 2′ of the pyran ring. It is represented as an ene-1,2-dithiolate, although this point is speculative as the redox level of the pterin–pyran system is undefined at present. The phosphate substituent is elaborated to a dinucleotide in enzymes from prokaryotic organisms. (*b*) Urothione and (*c*) 2-phenylthieno[2,3-*b*]-quinoxaline.

Molybdenum enzymes

xanthine oxidase/dehydrogenase: (a)



xanthine uric acid

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

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

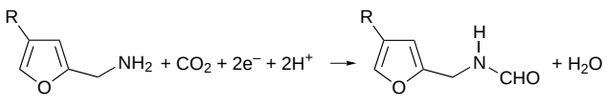
dimethyl sulfoxide reductase: $Me_2SO + 2H^+ + 2e^- \rightarrow Me_2S + H_2O$ (d)

Tungsten enzymes[a]

aldehyde oxido-reductase: $RCHO + H_2O \rightarrow RCO_2H + 2H^+ + 2e^-$ (e)

formate dehydrogenase: $HCO_2^- + H_2O \rightarrow HCO_3^- + 2H^+ + 2e^-$ (f)

formylmethanofuran dehydrogenase: (g)



Scheme 1 [a] Molybdenum versions of these enzymes are also known [*e.g.* compare Fig. 2(*a*) and (*c*)]. In certain bacteria, both molybdenum and tungsten versions can be isolated (see, *e.g.* refs. 4 and 33).

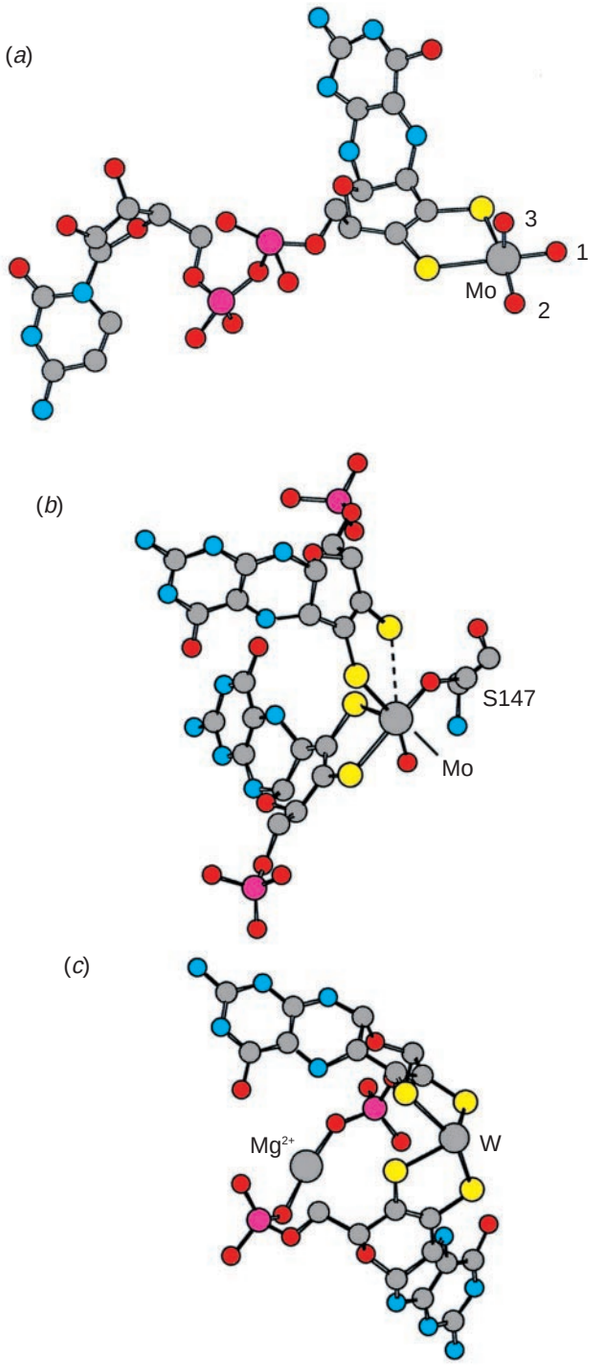


Fig. 2 Crystal structures: representations of metal centres. Colour code: oxygen, red; nitrogen, blue; sulfur, yellow; phosphorus, magenta; metal atoms (large grey circles) are identified on the figure. (*a*) Aldehyde oxidoreductase from *Desulfovibrio gigas* (resolution = 2.25 Å): a five-coordinate site interpreted as a $Mo^{VI}O_2(OH_2)$ fragment bound to a pterin 1,2-dithiolate.[12] The ligands assigned as oxo are labelled 1 and 3 while that assigned as aqua is labelled 2. The latter has direct access to an extended solvent channel. Data at 1.8 Å resolution is now available, including that for crystals soaked in buffer containing sulfide.[13] Ligand 3 appears to be S^{2-} in these latter forms. (*b*) Oxidised dimethyl sulfoxide reductase from *Rhodobacter sphaeroides* (resolution = 2.2 Å): a distorted trigonal prismatic site with a $Mo^{VI}O(OSer)$ fragment bound to two pterin 1,2-dithiolate ligands.[15] One of the latter bidentate ligands is bound asymmetrically with Mo–S distances of 2.4 and 3.1 Å. (*c*) Aldehyde oxidoreductase from *Pyrococcus furiosus* (resolution = 2.3 Å): the tungsten atom is coordinated to two pterin 1,2-dithiolate ligands whose sulfur atoms lie in an approximate square plane.[14] Two other non-protein ligands (oxo?) may be present to complete a distorted trigonal prismatic site. The two phosphate groups bind to a six-coordinate Mg^{2+} ion.

Background chemistry

High oxidation state metal-oxo chemistry is the key to catalysis of half-reaction (1) in these enzymes (for simplicity, coligands and overall charges are omitted) [eqns. (2) and (3)].

$$X + M^{VI}O \rightleftharpoons XO + M^{IV} \quad (2)$$

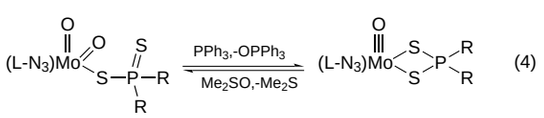
$$M^{IV} + H_2O \rightleftharpoons M^{VI}O + 2\,H^+ + 2\,e^- \quad (3)$$

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

Eqn. (2) represents an oxygen atom transfer (OAT) reaction.[7,8,22] Eqn. (3) is a coupled electron–proton transfer (CEPT) reaction in which the active site is regenerated.[8,9,23] The detailed molecular mechanism employed by an enzyme varies with the nature of its substrate X.

The molybdenum enzymes seem to fall into three families, exemplified by sulfite oxidase, dimethyl sulfoxide reductase and xanthine oxidase (Scheme 1).[4]

The OAT reaction is now well established for molybdenum.[7,8,22] In particular, interconversion of *cis*-$Mo^{VI}O_2$ and $Mo^{IV}O$ complexes is promoted by a range of oxygen atom donors and acceptors *via* associative mechanisms. The ability of a dimethyl sulfoxide reductase enzyme to mediate reaction (2) in either direction has been interpreted in these terms.[24] As illustration, a structurally well characterised catalytic system based upon the trispyrazolylborate ligand L-N_3 [see Fig. 3(*a*) for ligand structure] is presented in eqn. (4).[25] The forward and



(4)

reverse reactions are first order with respect to both complex and substrate, consistent with bimolecular reactions involving mononuclear species. The steric bulk of ligand L-N_3 and the ambidentate behaviour of the dithiophosphate ligand disfavour comproportionation reaction (5) which complicates many synthetic systems.

$$Mo^{VI}O_2 + Mo^{IV}O \rightarrow [Mo^{V}O]_2(\mu\text{-}O) \quad (5)$$

The CEPT reaction depends upon π bonding in M=O systems permitting dramatic modulation of the pK_a of the O atom upon change of oxidation state of metal M. Formal reduction of a *cis*-

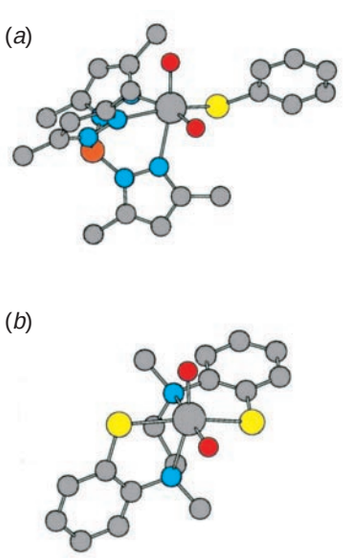


Fig. 3 Ligands. (*a*) L-N_3 = hydrotris(3,5-dimethylpyrazol-1-yl)borate monoanion as present in (L-N_3)MoO_2(SPh).[36] Colour code: boron, orange. (*b*) L-N_2S_2 = *N*,*N*′-dimethyl-*N*,*N*′-bis(2-sulfanylphenyl)ethylenediamine dianion as present in (L-N_2S_2)MoO_2: *cis*-oxo and *trans*-thiolate ligands.[26]

$Mo^{VI}O_2$ centre increases its basicity by population of π* orbitals with significant oxo character. Simple relevant examples are provided in eqn. (6) [L = L-N_2S_2 or (L-N_3)(SPh)]; Fig. 3.[26,27]

$$LMo^{VI}O_2 + e^- \rightarrow [LMo^{V}O_2]^- \xrightarrow{H^+} LMo^{V}O(OH) \quad (6)$$

The sulfite oxidase family: $Mo^{VI}O_2$ sites with one pterin 1,2-dithiolate ligand

A number of molybdenum pterin enzymes feature $Mo^{VI}O_2$ fragments bound to a single pterin 1,2-dithiolate [*e.g.* sulfite oxidase, assimilatory nitrate reductase; Scheme 1(b),(c)].[4] In addition, sulfite oxidase has a Mo–S–Cys linkage.[28] While active aldehyde oxidoreductase from *Desulfovibrio gigas* belongs to the [$Mo^{VI}OS$] class (*vide infra*), a crystallised form appears to contain a $Mo^{VI}O_2(OH_2)$ fragment.[12] A plausible model for the active site in sulfite oxidase would have a cysteinyl ligand substitute for OH_2 in position 2 of Fig. 2(*a*).

Unfortunately, catalytic intermediates are yet to be detected for enzymes in this family and so mechanism has been inferred from structural information obtained by X-ray absorption spectroscopy and EPR studies of resting forms [*e.g.* $Mo^{VI}O_2$, $Mo^{V}O(OH)$ and $Mo^{IV}O$ centres in sulfite oxidase[8]].

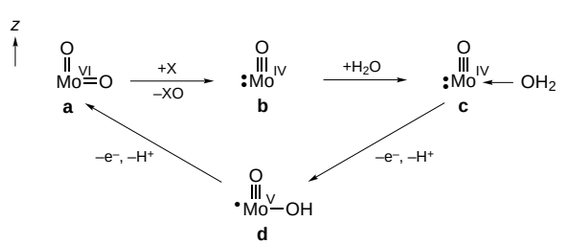
A consensus cycle has evolved in which a 'spectator' oxo ligand[29,30] controls the electronic structure allowing a second oxo ligand to participate in an OAT reaction with substrate X (Scheme 2 **a** → **b**). After coordination of ligand H_2O (**b** → **c**), the catalytic site is regenerated by two CEPT steps (**c** → **d** → **a**). Reductase enzymes would cycle in the opposite direction (*cf.* assimilatory nitrate reductase[31–33]). The OAT chemistry is represented as a concerted two-electron event, the CEPT chemistry as two consecutive (e^-, H^+) transfers (rather than hydrogen-atom transfers).[34]

A synthetic model based upon $LMo^{VI}O_2Y$ (L = trispyrazolylborate ligand; Y = anionic ligand) permits fine tuning of structural and redox properties *via* variation of Y and the substituents of L.[27,34–36] In particular, complex (L-N_3)$Mo^{VI}O_2$(SPh) [Fig. 3(*a*)] catalyses eqn. (1) (X = PPh_3) with O_2 acting as the formal oxidant.

$$PPh_3 + H_2{}^{18}O + 2O_2 \rightarrow {}^{18}OPPh_3 + 2HO_2 \quad (7)$$

Centres **a**, **b** and **d** of Scheme 2 were isolated or trapped while strong evidence for centre **c** was obtained by ^{18}O tracer experiments.[34] The energy profile of the cycle and its molecular mechanism have been examined theoretically.[37] Substrate PMe_3 is proposed to approach from a direction perpendicular to the *cis*-MoO_2 plane, then rotate to form intermediate $Mo^{IV}O(OPMe_3)$ with a dihedral angle OMoOP of 0°. This imposes a $(d_{xy})^2$ configuration permitting formation of a stabilising triple bond along the *z* direction to the spectator oxo ligand (Scheme 2). Substitution of product $OPMe_3$ by OH_2 proceeds by an associative mechanism leading to intermediate **c**. The direction of approach of the substrate could be under tight steric control at an enzyme site.

The two CEPT steps are mediated normally by internal electron transfer to another redox enzyme site where the formal oxidant binds. In the present model system, the first CEPT step involves comproportionation between **a** and **c** (Scheme 2) to produce 2 equivalents of **d** which are then oxidised by O_2. The ligand steric barriers are balanced to allow hydrogen atom transfer between **a** and **c** but to disfavour formation of binuclear species [*cf.* eqn. (5)].



Scheme 2

The other system for which good evidence for Scheme 2 is available mimics enzymic reduction of aromatic nitro compounds, a multi-electron process [eqn. (8)].

$$ArNO_2 + 6H^+ + 6e^- \rightarrow ArNH_2 + 2\,H_2O \quad (8)$$

When intercalated into a layered double hydroxide host lattice, the $[Mo^{VI}O_2\{O_2CC(S)Ph_2\}_2]^{2-}$ ion catalyses reaction (8) cleanly.[21] An interpretation consistent with experiment has reductant PhSH inducing the initial CEPT steps (Scheme 2: **a** → **d** → **c**), followed by substitution of substrate $ArNO_2$ for ligand H_2O. The OAT step (**c** → **a**) then produces intermediate ArNO which is reduced directly by PhSH.

The OAT step of Scheme 2 (**a** ↔ **b**) has been demonstrated for many enzymic substrates.[7,24,38–40] For example, reduction of $[NO_3]^-$, Me_2SO and Me_3NO *via* electrophilic attack on $Mo^{IV}O$ has been demonstrated in systems with bi- and tridentate ligands.[7,24,38] Oxidation of $[HSO_3]^-$ is effected cleanly by $[Mo^{VI}O_2(mnt)_2]^{2-}$ (Fig. 4) in MeCN–H_2O.[40]

The dimethyl sulfoxide reductase family: $Mo^{VI}O_x$ (x = 1,2) sites with two pterin 1,2-dithiolate ligands

Dimethyl sulfoxide reductases had been assumed to be related to the sulfite oxidase family until recent X-ray crystallographic and absorption data for the enzyme from *Rhodobacter sphaeroides* indicated [Fig. 2(*b*)]:[15,41] (*i*) the presence of two inequivalent 1,2-dithiolate ligands. One is a symmetric bidentate ligand. The other is also bidentate but asymmetrically bound in the oxidised form and monodentate in the reduced form; (*ii*) the presence of a serine sidechain providing a Mo–alkoxo link; (*iii*) that a single oxo ligand (rather than two) is present in the oxidised form and (*iv*) that no oxo ligand (rather than one) is present in the dithionite-reduced form, and that one of the ligands is monodentate.

The situation is somewhat uncertain at the moment as two separate crystal structures of the dimethyl sulfoxide reductase from a closely related organism *R. capsulatus* suggest the presence of two oxo ligands (rather than one) in the oxidised form.[16,42] In addition, there are differences in the detail of the 1,2-dithiolate coordination modes. In one structure, both dithiolate ligands are bidentate and symmetrical,[42] while in the other, one is free and not bound to the molybdenum centre.[16] It is possible that different crystal forms have been examined.

The detailed nature of the individual sites remains to be clarified. Certainly, both $Mo^{VI}O_2$-based models and the dime-

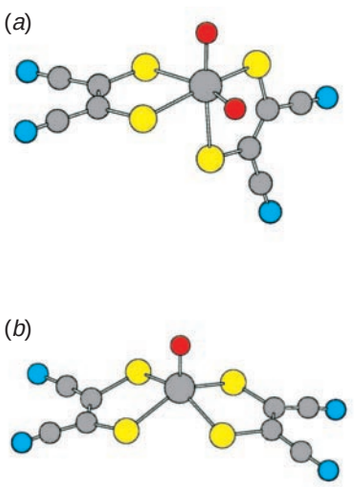


Fig. 4 Molecular structures (M = Mo, W; mnt = dianion of 1,2-dicyano-1,2-dithioethene)[40,71,72] of (*a*) $[M^{VI}O_2(mnt)_2]^{2-}$ and (*b*) $[M^{IV}O(mnt)_2]^{2-}$

thyl sulfoxide reductase from *R. sphaeroides* can catalyse the OAT reaction (9).[7,22]

$$PR_3 + OSMe_2 \rightarrow OPR_3 + SMe_2 \quad (9)$$

This enzyme is unusual in that the molybdenum site is its sole redox site. This apparently allows efficient cycling of PR_3 oxidation by Mo^{VI} and $OSMe_2$ reduction by Mo^{IV}. Equivalent processes are expected to be more difficult to achieve in other enzymes if rapid electron transfer to or from another redox site occurs after each step.

The presence of a $Mo^{VI}O$ site in the enzymes would mean that a controlling spectator oxo ligand is not necessary (*cf.* Scheme 2): the two 1,2-dithiolate ligands and their apparent ambidentate nature may impart an equivalent electronic environment. While the couple $[Mo^{VI}O_2(mnt)_2]^{2-}$–$[Mo^{IV}O(mnt)_2]^{2-}$ (Fig. 4) features two 1,2-dithiolate ligands and is active in OAT reactions, an equivalent $Mo^{VI}O$–Mo^{IV} couple is not known. The $[Mo^{IV}(mnt)_3]^{2-}$ ion is known.[40,43]

Sequence homologies suggest that the serinyl ligand in dimethyl sulfoxide reductase is replaced by a cysteinyl ligand in dissimilatory nitrate reductase and in trimethylamine *N*-oxide reductase (*cf.* sulfite oxidase) and by a selenocysteinyl ligand in formate dehydrogenase.[15,44] This variation is a straightforward method of tuning the active site.

The xanthine oxidase family: $Mo^{VI}OS$ sites with one pterin 1,2-dithiolate ligand

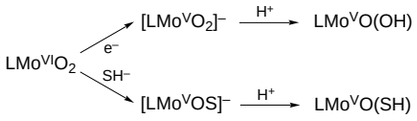
This class includes the xanthine oxidase/dehydrogenase systems [Scheme 1(*a*)] and the molybdenum aldehyde oxidoreductases which, in contrast to the other enzymes, are able to activate C–H bonds.[4,8,9] A crystal structure of the aldehyde oxidoreductase from *D. gigas* with the active site present in its hydrolysed 'desulfo' form indicates the presence of a five-coordinate site with a $Mo^{VI}O_2(OH_2)$ fragment bound to a pterin 1,2-dithiolate [Fig. 2(*a*)].[12] The aqua ligand has direct access to an extended solvent channel where substrate would be expected to bind. Structures are now available for oxidised and reduced forms of the enzyme and forms in which the thio ligand S^{2-} appears to occupy the apical position in the coordination sphere.[13] X-Ray absorption spectroscopy data for the Mo^{VI} and Mo^{IV} levels of xanthine oxidase/dehydrogenase are consistent with $Mo^{VI}OS$ and $Mo^{IV}O(SH)$ centres. Both $Mo^{V}OS$ ('very rapid') and $Mo^{V}O(SH)$ ('rapid') centres are observed by EPR during catalytic turnover.

The complex $(L\text{-}N_3)MoOS(S_2PPr^i_2)$, synthesised with the sulfur atom donor propylene sulfide [eqn. (10)], provides a structurally characterised example of a relevant *cis*-$Mo^{VI}OS$ centre [Fig. 5(*a*)].[9,45]

$$(L\text{–}N_3)Mo^{IV}O(S_2PPr^i_2) + C_3H_6S \rightarrow (L\text{–}N_3)MoOS(S_2PPr^i_2) + MeCH{=}CH_2 \quad (10)$$

The Mo=S distance is 2.227(2) Å (compared to 2.15–2.25 Å estimated for xanthine oxidase/dehydrogenase by EXAFS) and the structure features a significant stabilising S···S interaction [2.396(3) Å] between the Mo=S and S=P functions. The apical Mo=S link observed in aldehyde oxidoreductase from *D. gigas* appears to be hydrogen bonded to a nearby histidine sidechain which may induce an equivalent stabilisation.[13]

Molybdenum(v) centres have been generated synthetically *via* Scheme 3 [L = $L\text{-}N_2S_2$ or $(L\text{-}N_3)Y$; Fig. 3) and characterised in the solid state or in solution *via* EPR hyperfine interactions in species substituted with ^2H, ^{17}O, ^{33}S, ^{95}Mo and ^{98}Mo.[26,27,34–36,46–48] Combination of the synthetic results with those from enzyme EPR[49,50] and single catalytic turnover experiments[51–53] led to the minimal catalytic scheme shown in Scheme 4 for xanthine oxidase/dehydrogenase under substrate limiting conditions.[9,48]



Scheme 3

The resting form was formulated as a *fac*-$Mo^{VI}OS(OH)$ centre.[48] This structure is supported by density functional calculations[54] and is compatible with the structural results for aldehyde oxidoreductase [Fig. 2(*a*)].[13] In this latter case, a nearby glutamic acid residue is available to act as a base for effective conversion of ligand OH_2 to OH during turnover. Product uric acid [Scheme 1(*a*)] bound as an anionic ligand RO^- appears in both the Mo^{IV} and Mo^{V} (very rapid) centres of xanthine oxidase. Reduction of the resting form leads to occupation of a highly delocalised MoS π_{xy}* orbital allowing the thio ligand to become a strongly basic centre facilitating activation of the C–H bond of xanthine.[9,48]

Classification of individual steps as OAT or CEPT chemistry becomes blurred somewhat in this cycle. Generation of bound product may be classified as an OAT step while regeneration of the Mo^{VI} site with H_2O is a CEPT step. The state of protonation of the thio ligand and its role as a base upon reduction is also CEPT chemistry. Further complications are observed when substrate is in excess and can interact with a Mo^{V} form of the enzyme generating the rapid centres [*fac*-Mo^{V}-O(SH)(OH)?].[4,48] A detailed molecular mechanism is still evolving.[4,13,55]

Cyanide ion inactivates xanthine oxidase/dehydrogenase under oxidising conditions to produce a 'desulfo' $Mo^{VI}O_2$ centre and SCN^-.[8] Complex $(L\text{-}N_3)Mo^{VI}OS(S_2PPr^i_2)$ reacts with cyanide to produce SCN^- quantitatively *via* a formal sulfur atom transfer, forming $(L\text{-}N_3)Mo^{IV}O(S_2PPr^i_2)$ under dry anaerobic conditions and $(L\text{–}N_3)Mo^{VI}O_2(S_2PPr^i_2)$ in the presence of H_2O and O_2 [eqn. (11)].[45]

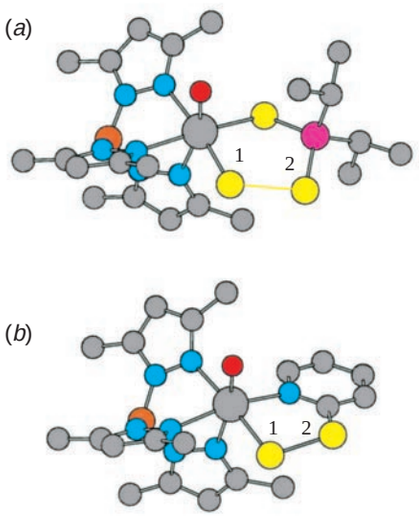
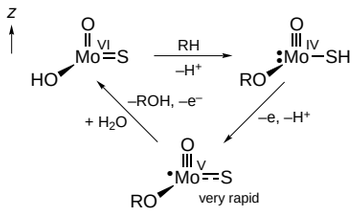


Fig. 5 Molecular structures of (*a*) $(L\text{-}N_3)Mo^{VI}OS(S_2PPr^i_2)$[45] and (*b*) $(L\text{-}N_3)Mo^{IV}O(pyS_2)$[56]



Scheme 4 For the very rapid centre, the Mo=S notation implies occupation of a MoS π_{xy}* orbital by the unpaired electron leading to a formal MoS bond order of 1.5[47,48]

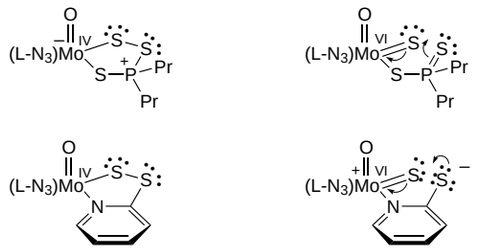
$$Mo^{VI}OS + CN^- + H_2O + O_2 \rightarrow Mo^{VI}O_2 + SCN^- + H_2O_2 \quad (11)$$

The structure of (L-N_3)MoO(pyS_2), produced from (L-N_3)MoO(pyS) *via* a sulfur atom transfer reaction entirely similar to that of eqn. (10), is shown in Fig. 5(*b*).[56] The Mo–S and the S–S distances of 2.323(2) and 2.108(3) Å, respectively, approach those typical of a single bond (*ca.* 2.4 and 2.05 Å). The compound is best formulated as an oxodithiomolybdenum(IV) species. The alternative formulations for (L-N_3)$Mo^{IV}O(pyS_2)$ as a Mo^{VI} species or for (L-N_3)$Mo^{VI}OS(S_2PPr^i_2)$ as a Mo^{IV} species are less likely due to the unfavourable charge separations illustrated in Scheme 5. The redox properties of these complexes are fascinating: EPR-active Mo^V species are formed upon reduction or oxidation.[56] Both (L-N_3)$Mo^{VI}OS(S_2PPr^i_2)$ and (L-N_3)$Mo^{IV}O(pyS_2)$ produce oxothiomolybdenum(V) anions upon reduction and oxodithiomolybdenum(V) cations upon oxidation (Scheme 6).

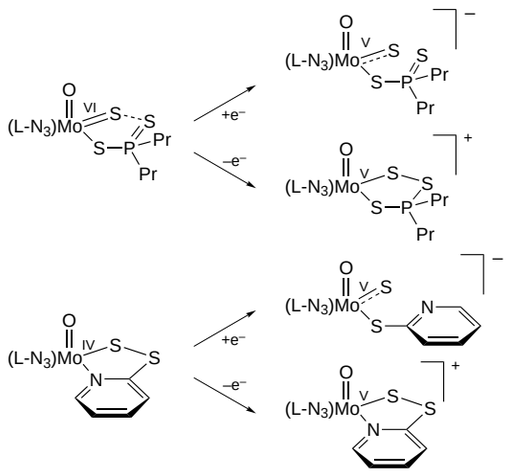
These examples highlight the facile redox interplay of molybdenum and its sulfur ligands in these systems.[57] It is apparent that the fine details of both the acid–base and redox interactions of the MoOS centre with its co-ligands (including the 1,2-dithiolate function of the pterin, in particular) and with nearby amino acid sidechains[12–16] will be crucial to a molecular understanding of each stage of catalysis in this class of enzymes.

Tungsten enzymes

The rapid development of this field is due largely to the work of Ljungdahl, Adams, Simon and Thauer.[2,11] Tungsten enzymes are largely restricted to thermophilic organisms, which thrive in niche environments such as hot marine sediments and hydrothermal vents. In these extreme environments, the thermodynamic and kinetic attributes of tungsten appear to enhance the stability and performance of tungsten enzymes over molybdenum enzymes. The unusual biochemistry of the thermophiles is highlighted by their use of sulfur rather than dioxygen as terminal electron acceptor. However, it is in the assimilation of carbon from sources such as carbon dioxide, complex carbohydrates or proteins that the tungsten enzymes play key roles. A sample of half reactions is given in Scheme 1(e)–(g). The specific couples have very low potentials (≤400 mV *vs.* the standard hydrogen electrode[11]).



Scheme 5



Scheme 6

The best characterised enzyme is the aldehyde oxidoreductase of *Pyrococcus furiosus* [Scheme 1(e)].[58] A crystal structure has revealed a square pyramidal tungsten centre bound to two 1,2-dithiolate bidentate ligands [Fig. 2(*c*)].[14] It was not possible to identify positively the other ligands in the crystallised form. Chemical and EXAFS studies of active forms are consistent with the presence of W=O, W=S and/or W–SH functions.[58–60] These forms lose activity upon treatment with the thiophiles cyanide and arsenite. Redox titrations of active enzyme monitored by EPR suggests that some aspects of the redox chemistry may be based on the pterin ring.[61]

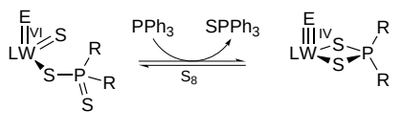
A formate dehydrogenase from *Clostridium thermoaceticum* [Scheme 1(f)] is proposed to feature selenocysteine and 1,2-dithiolate ligands [Fig. 6(*a*)].[62] The tungsten formate dehydrogenases from two *Methanobacterium* strains are resistant to cyanide inactivation.[4] Intriguingly, a molybdenum isoenzyme is not [*cf.* eqn. (11)].

Despite remarkable insights provided by EXAFS and X-ray crystallography, the detailed nature of the active sites in the tungsten enzymes remains uncertain. The presence of oxo, thio, mercapto, thiolato, selenolato and O- and N-donor ligands are all possible. The chemistry is expected to involve tungsten in oxidation states IV–VI as these dominate established compounds bearing the above ligands.

Thiotungsten chemistry

The $W^{VI}O_2$ group is relatively unreactive and nature may exploit sulfur ligands to enhance the oxidative capacity of tungsten centres. Series of complexes featuring $W^{VI}OS$ and $W^{VI}S_2$ centres bound to ligands L-N_3 and L-N_2S_2 have been isolated using both high-valent $W^{VI}O_2$ and low-valent $W^0(CO)_x$ complexes as starting points (*e.g.* see Fig. 7).[63–66]

Under similar conditions, the reduction potentials of the $W^{VI}OS$ and $W^{VI}S_2$ complexes are 0.5–0.8 V more positive than those of the $W^{VI}O_2$ analogues and fall into the range observed for related $Mo^{VI}O_2$ species. Terminal thio ligation at W^{VI} clearly facilitates reduction. The oxothio- and dithio-tungsten(VI) complexes are reducible at biologically accessible potentials whereas most of the dioxotungsten(VI) complexes are not.



(12)

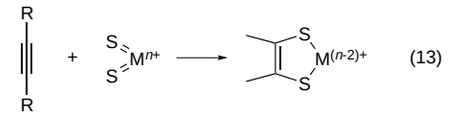
The $W^{VI}OS$ and $W^{VI}S_2$ complexes interconvert with $W^{IV}O$ and $W^{IV}S$ species by sulfur atom transfer reactions [*e.g.* eqn. (12); E = O, S; L = L-N_3)].

The reaction of cyanide with the $Mo^{VI}OS$ centres of xanthine oxidase/dehydrogenase and (L-N_3)$MoOS(S_2PR_2)$ results in the abstraction of thio ligand [eqn. (11)]. In contrast, several tungsten enzymes are not deactivated by cyanide,[4,67] and this may be interpreted to indicate the absence of thiotungsten centres in the enzymes. However, there is no reaction between (L-N_3)WS_2Cl and NEt_4CN in refluxing benzene over 24 h. The stability of this thiotungsten complex toward cyanolysis indicates that it is dangerous to dismiss the possible presence of thiotungsten centres in enzymes on the basis of their failure to react with cyanide.

Tungsten ene-1,2-dithiolate complexes

The formate dehydrogenase enzyme from *C. thermoaceticum* is proposed to feature selenocysteine and 1,2-dithiolate ligands

[Fig. 6(*a*)]. Solutions of (L-N_3)$W^{VI}S_2Y$ react with alkynes to form ene-1,2-dithiolate tungsten(IV) complexes [eqn. (13)].



(13)

These remarkably facile reactions are very rapid at room temperature, even with unactivated alkynes and including acetylene. The structure of (L-N_3)W(OPh){$S_2C_2(CO_2Me)_2$} confirms the formation of the ene-1,2-dithiolate ligand [Fig. 6(*b*)]. The W–S bond lengths of 2.267(4) and 2.279(4) Å are considerably shorter (by 0.07 Å) than those reported previously for tungsten enedithiolates. The average W–S distance for such compounds is 2.40 Å and the W–S_{av} distance for the above formate dehydrogenase is close to this average at 2.39 Å. The short W–S distances in (L-N_3)W(OPh){$S_2C_2(CO_2Me)_2$} are indicative of considerable d_π–p_π bonding between the W and S atoms; the ene-1,2-dithiolate S_2C_4-framework is planar. Similar chemistry was recently reported for the complex [(η-C_5Me_5)WS_3]$^-$ which reacts with alkynes to give [(η-C_5Me_5)$WS(S_2C_2R_2)$]$^-$.[68] The complex (L-N_3)W(SePh){S_2C_2(Ph)(2-quinoxalinyl)} provides a structural and spectroscopic benchmark for the proposed site in the formate dehydrogenase [Fig. 6(*a*)]. Interestingly, it decomposes rapidly in methanolic solution in air to give (L-N_3)WO_2(OMe), diphenyldiselenide and the disulfide shown in Fig. 1(*c*). This latter species is also formed in the oxidative decomposition of [Mo{S_2C_2(Ph)(2-quinoxalinyl)}$_3$]$^{2-}$ in a reaction reminiscent of the formation of urothione upon degradation of the molybdenum cofactor [Fig. 1(*a*),(*b*)].[69] It is possible that degradation of pterin tungsten enzymes might produce urothione.

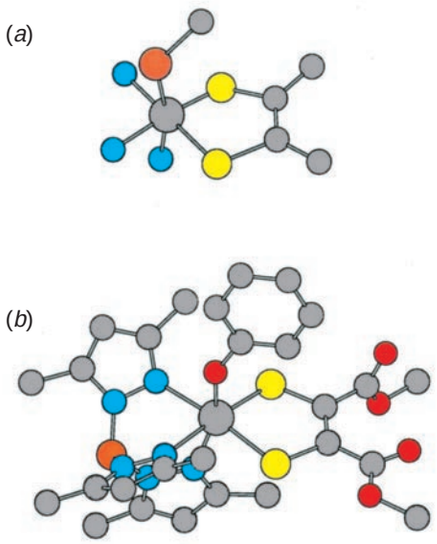


Fig. 6 (*a*) Proposed structure at the active site of formate dehydrogenase from *Clostridium thermoaceticum*.[62] Colour code: selenium, orange. (*b*) (L-N_3)W(OPh){$S_2C_2(CO_2Me)_2$}.[64] Colour code: boron, orange.

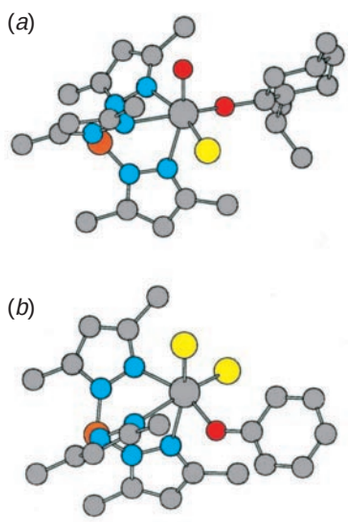


Fig. 7 Molecular structures[64] of (*a*) (L-N_3)W^{VI}OS{(−)-mentholate} and (*b*) (L-N_3)$W^{VI}S_2$(OPh)

Structural and functional models for the aldehyde oxidoreductase of *P. furiosus* incorporating ene-1,2-dithiolate ligands at high-valent oxotungsten centres have been developed by Nakamura and Sarkar.[70,71] They represent exciting developments in a challenging area of coordination chemistry. The benzene-1,2-dithiolate complex $[W^{VI}O_2(bdt)_2]^{2-}$ converts benzoin to benzil [eqn. (14)].[70]

$$[W^{VI}O_2(bdt)_2]^{2-} + PhCH(OH)COPh \rightarrow [W^{IV}O(bdt)_2]^{2-} + PhCOCOPh + H_2O \quad (14)$$

The ease of this reaction contrasts with the very slow reaction of $[W^{VI}O_2(bdt)_2]^{2-}$ with PPh_3 and suggests that reduction of $W^{VI}O_2$ centres may be facilitated by CEPT rather than OAT reactions. Recently, the interconversion of anions $[W^{VI}O_2(mnt)_2]^{2-}$, $[W^{IV}O(mnt)_2]^{2-}$ (Fig. 4) and $[W^{VI}O(\eta^2\text{-}S_2)(mnt)_2]^{2-}$ in enzymatically relevant reactions, such as the oxidation of aldehydes to carboxylic acids, has been demonstrated.[71] The stoichiometries of these bis(ene-1,2-dithiolate)tungsten(VI) complexes are similar to the centre revealed in the aldehyde oxidoreductase [Fig. 2(*c*)] although their coordination geometries deviate from the proposed trigonal-prismatic structure at the enzyme centre. The effects of such variations on the chemistry of the tungsten centres remains to be assessed.

Conclusions

The recent enzyme crystal structures emphasise the structural diversity available to a cofactor ligand such as the pterin 1,2-dithiolate centre. Further refinement of these structures and the anticipation of new ones in the near future (formate dehydrogenase; xanthine oxidase and dehydrogenase) will further illuminate the fundamental chemistry.

This article has but touched the surface of the chemical diversity exhibited by the molybdenum and tungsten enzymes. This diversity follows from variation of metal, ligand (oxo → thio; serine → cysteine → selenocysteine), pterin redox level and interaction with protein sidechains. Investigations will move to a new sophistication *via* synergy between the enzyme structural work, active site modification by site-directed mutagenesis and continuing development of the fundamental chemistry.

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Footnotes

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‡ Abbreviations: bdt = dianion of benzene-1,2-dithiol, *C.* = *Clostridium*, CEPT = coulpled electron proton transfer, *D.* = *Desulfovibrio*, EXAFS = extended X-ray absorption fine structure, FeMoco = iron-molybdenum cofactor of nitrogenase, L-N_2S_2 = dianion of *N*,*N*′-dimethyl-*N*,*N*′-bis(2-sulfanylphenyl)ethylenediamine, L-N_3 = hydrotris(3,5-dimethylpyrazol-1-yl)borate anion, mnt = dianion of 1,2-dicyano-1,2-dithioethene, Moco = molybdenum pterin cofactor; OAT = oxygen atom transfer, *P.* = *Pyrococcus*, *R.* = *Rhodobacter*, pyS = anion of

pyridyl-2-thiol, pyS_2 = anion of pyridyl-2-dithiol, X = reduced substrate, Y = monoanionic ligand.

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