A coordination chemist's view of the active sites of mononuclear molybdenum enzymes

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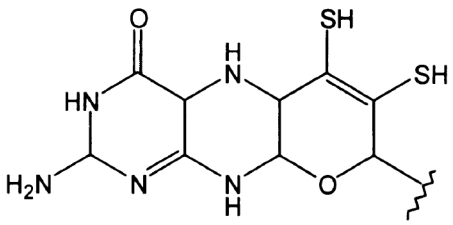
A comparison of the coordination environment of twelve structurally characterized mononuclear molybdenum-containing enzymes revealed many similarities that helped define specific features of the active sites. Using bond distances and angles, the types of bonds and terminal groups of significance were identified. For example, a distance between molybdenum and oxygen atoms ranging from 1.6 to 1.8 Å was considered as a Mo=O bond. The dithiolene sulphur to molybdenum single bond distances fluctuated around 2.40 Å. In most cases, the sulphur–sulphur interaction within the co-factors is weak, with distances above 3.00 Å, but both trimethylamine N-oxide and dissimilatory nitrate reductase (i.e. NapA) show strong sulphur–sulphur interactions with distances closer to 2.80 Å. In addition, the dithiolene bite angle (S Mo–S) was observed to be directly affected by the distance of the sulphurs from the metal centre. The bite angle is very small for co-factors that are further away from the molybdenum atom, as observed in TMAOR. A small bite angle is suggestive of ligand dissociation.

MOLYBDENUM is the only 4d transition metal required for all forms of life. With the exception of nitrogenase, molybdenum is generally found as mononuclear active centres, where the metal ion is coordinated by a specially modified pyranopterin co-factor (Structure 1)[1]. Mononuclear molybdenum-containing enzymes catalyse a wide variety of reactions such as the reduction of nitrate to nitrite, reduction of dimethyl sulphoxide to dimethyl sulphide, and oxidation of sulphite to sulphate. These reactions play critical roles in the global cycling of elements such as nitrogen, sulphur, carbon and arsenic. In recent years, significant progress has been made in isolating new and novel molybdenum-containing enzymes from a variety of organisms. In concert with the discovery of these new enzymes, important progress has been made in their structural characterization.



Structure 1.

According to Hille[2], the majority molybdoenzymes can be classified into three families represented by xanthine oxidase (XO), sulphite oxidase (SO) and DMSO reductase as the prototype members of each family. The crystal structures of members of all three families of mononuclear molybdoenzymes are now known, and essentially support the classification. The classification closely follows the composition of the metal centres, ca. the ratio of molybdenum to pyranopterin co-factor that varies from one to two. Similarly, the number of terminal groups attached to the molybdenum atom also varies from one to two. The number of terminal (oxo or sulphido) groups coordinated to molybdenum is related to the number of pyranopterin co-factors present in the molecule. Thus, when only one pyranopterin co-factor is present, in the fully oxidized state the metal centre can have two terminal linkages; in contrast where the metal centre is linked with two pyranopterin co-factors, only one terminal group is linked with the metal centre. In addition to these structural variations, the molybdenum centres are coordinated by endogenous ligands (e.g. serine, cysteine and selenocysteine). The ditholene moiety of the pyranopterin co-factor coordinates the molybdenum centres in a bidentate mode. In recent years, several excellent articles have discussed different structural aspects of the proteins[3,4]. Rees *et al.* have emphasized the crystallographically determined conformations of the tricyclic pyranopterin co-factor in relation to the redox chemistry[4]. In another article, Romao *et al.* have discussed organization of different domains and the structure-based catalytic mechanism of xanthine oxidase[5]. To this end, based on detailed sequence analysis, we have proposed that all nitrate reductases can be classified into three subfamilies[6]. This classification is also consistent with the phylogeny, the cellular localization of the active subunit, and the structural motifs. Here we will focus our attention on the catalytic site of representative structures and consider only the first coordination sphere of the molybdenum centre.

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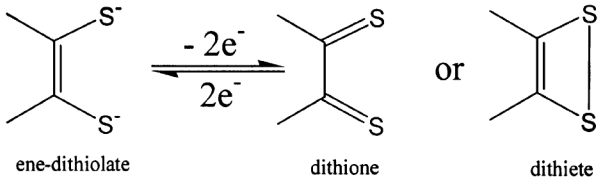
Functionally, mononuclear molybdenum enzymes catalyse a net oxygen atom transfer reaction represented in equation (1).

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

This net reaction is generally considered to be a two-electron process. Mechanistically, these reactions take the form of true oxygen atom transfer reactions, hydroxylation reactions, hydride transfer reactions, or hydration reactions. Regardless of the nature of the reaction catalysed, the metal centre functions as an electron-transferring unit. Because the co-factors themselves are redox non-innocent, they may play an active role as a conduit in the electron transfer process. For example, the ene-dithiolate (or dithiolene) moieties and their metal complexes are well known for their non-innocent redox behaviour, which has previously been investigated in detail[7], and there has been a resurgence of interest in their fundamental aspects[8], in part as models for mononuclear molybdenum and tungsten enzymes[9]. The ene-dithiolate can exist in the reduced (i.e., dithiolate), oxidized dithione or dithiete forms (Scheme 1). Accurate evaluation of bond distances and angles can often differentiate these forms.



Scheme 1.

In addition to the two forms mentioned above, dithiolenes can also exist in one electron oxidized form and in such cases, differences in the bond distances and angles are less distinct.

Experimental approach

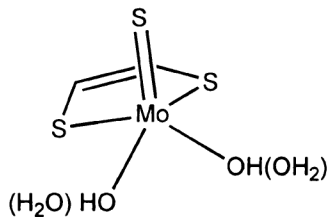
We have used small molecule crystal structures (where the structural resolution is higher than the protein structures) deposited in the Cambridge Crystallographic Structural Database (CSD) as a benchmark for the metal ligand bond distances. A search of the database in February of 2002 returned 3544 hits for Mo=O bonds. The mean Mo=O distance calculated from these data is 1.705 (± 0.073) Å at a 95% confidence level. Similarly, a search for Mo=S gave 154 hits and the mean distance in this case was calculated to be 2.145 (± 0.060) Å at a 95% confidence limit. Also searching the metal ligand single bond distances, we only considered mononuclear molecules, and we excluded units with four-membered chelate rings such as those found in dithiocarbamate ligands. These restrictions gave 1637 and 1640 hits for Mo–O and Mo–S linkages, respectively, for which the mean (at a 95% confidence limit) Mo–O and Mo–S single bond distances were found to be 2.056 (± 0.159) Å and 2.452 (± 0.092) Å, respectively. These metric parameters provide a benchmark for comparing the bond distance obtained from protein crystallography. The coordinates for the protein structures were downloaded from the protein data bank and investigated using the WebLab Viewer software. Platon[10] software was used to determine the least square planes. The statistical parameters were calculated using Quatrro Pro software.

Results and discussion

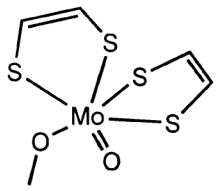
The coordination features of the active sites in each family are discussed and the bond distances are compared with those obtained from crystallographically characterized inorganic complexes. Representative active site structures of all three families are shown in Figure 1.

The xanthine oxidase family

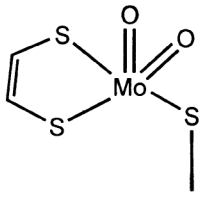
Past studies have defined the XO family as the enzymes that catalyse the hydroxylation of substrates in the pre-



from Bt-XDH (pdb code:1FO4)



from Rc-DMSOR (pdb code:1DMR)



from Gg-SO (pdb code:1SOX)

Figure 1. Schematic representation of active sites of all three families. The structures are drawn in light of the protein structure mentioned.

sence of an electron acceptor and include XO, AOR and CODH[2]. The crystal structures of one AOR[11], two XDH[12,13] and two CODH[14,15] are discussed. The details are provided in Chart I and the specific structural parameters are tabulated in Table 1.

We have examined the coordination environments of these five structurally characterized enzymes. In each case, the overall geometry of the metal centre is square pyramidal with a terminal oxo-group at the apical position, while the two sulphur atoms from the ene-dithiolate ligand occupy the equatorial positions. The remaining equatorial position is occupied by either an oxygen donor from water, hydroxo, oxo group or a sulphur donor from a sulphido group. Typically, the molybdenum atom is above the equatorial plane by 0.6 Å. The terminal group is tilted 104–123° away from the centroid of the two sulphur atoms of the dithiolene moiety.

In oxo-molybdenum complexes, the terminal oxo-group dominates the ligand field making the metal d_{xy} orbital as the energetically isolated redox orbital. Although in the fully oxidized state there could be two terminal groups, the presence of the oxo-group in the apical position is significant. This geometry orients the redox orbital on the equatorial plane with the ditholene sulphur atoms, whose energy can be changed by modulating the interaction with the dithiolene unit. Recently, using discrete analog molecules, distortion at the dithiolene moiety with respect to the terminal group has been implicated in modulating the reduction potential of the metal centre[16].

Among the five structures discussed here, only the structure of Dg-AOR was solved as a monomeric protein where a single polypeptide chain held the molybdenum centre, while the other structures have additional subunits[11]. Both CODHs are dimers of heterotrimers; the Bt-XDH is a homodimer[12] while the Rc-XDH is a hetero-tetramer[13]. In the case of multimeric structures, we discuss the average metric parameters unless the parameters of individual monomeric units differ significantly, such as in the case of Bt-XDH. For Bt-XDH, we limit the discussion to the subunit that has metric parameters closer to discrete inorganic molecules.

The crystal structure of Dg-AOR indicates a dioxo-molybdenum centre and not the oxo-sulphido-molybdenum form that is required for catalytic activity. The presence of the thio-molybdenum centre in the Rc-XDH and Bt-XDH suggest that these proteins were active, although the distances were not within the expected range especially in the case of the Bt-XDH structure. The low resolution of the structure may contribute to the deviation. The dithiolene bite angles are consistent with each other and the C–C bond distances along with the C–S distances support the ene-dithiolate formulation of the co-factor.

Chart I. Crystallographically characterized members of the XO family

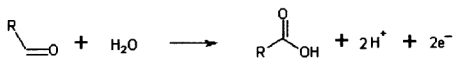
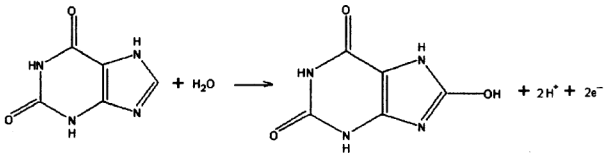
Enzyme	*Reaction catalysed*	*General description*
Dg-AOR		One co-factor per Mo, no amino acid coordination and no sulphide coordination.
Bt-XDH		Oxidized form; oxo-sulphido Mo-centre. One co-factor per Mo.
Rc-XDH	Same as above	Oxidized and alloxanthine inhibited forms; oxo-sulphido Mo-centre. One co-factor per Mo.
Pc-CODH	$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$	Oxidized dioxo-Mo-centre with a Se-linked cysteine residue in in the second coordination sphere and one co-factor per Mo.
Hp-CODH	$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$	Oxidized form; the dioxo-Mo centre is coordinated by one ene-dithiolate moiety.

Table 1. The structural parameters of representative members of the XO family

	AOR from Dg (at 1.28 Å)	Av XDH from Rc (at 2.7 Å)	XDH from Bt (at 2.1 Å)	Av CODH from Hp (at 2.25 Å)	Av CODH from Pc (at 2.20 Å)
d(Mo=O)	1.74, 1.79	1.71		1.69	1.68, 1.71
d(Mo=S)		2.04	1.82		
d(Mo-O)	1.99	2.02	2.25, 2.05	2.31	2.28
d(Mo-S)	2.41, 2.49	2.26, 2.39	2.42, 2.29	2.31, 2.55	2.45, 2.33
d(C-S)	1.80, 1.76	1.66, 1.73	1.71, 1.59	1.74, 1.75	1.77, 1.79
d(S. . .S)	3.14	2.94	3.22	3.14	3.08
dithiolene d(C=C)	1.34	1.44	1.50	1.33	1.37
< S-Mo-S	79.5	77.9	86.3	80.3	80.1

The crystal structure of Bt-XDH exhibits a longer C–C distance (1.5 Å) and shorter C–S distances, suggesting a considerable single bond character in C–C distance and double bond character in C–S bonds. This would be consistent with a partial thio-ketone formulation as opposed to pure ene-dithiolate formulation. The S . . . S distance in Rc-XDH is less than 3 Å, suggesting a partial sulphur–sulphur bond character, which is also consistent with the lower bite angle. Functionally this may imply that the co-factor may take part in the electron transfer process during the regeneration steps of the catalytic cycle.

It is interesting to note that unlike XO, CODH may not require an oxo-sulphido-molybdenum centre for catalytic competency. In CODH, the active form is proposed to be a dioxo-molybdenum centre that can be inactivated with cyanide treatment leading to the formation of selenocyanate. The inactivation by cyanide is well-known for XO, where cyanide removes the terminal sulphide by forming thiocyanate. However, in CODH, the cyanizable selenium of the modified cysteine residue is not in the first coordination sphere of molybdenum. The role of this distal selenium has not been demonstrated but Dobbek *et al.*[14] have suggested that it may be involved in proton shuttling and binding to CO. Despite the structural differences, CODH exhibits a high degree of sequence homology with other members of XO family.

The sulphite oxidase family

Members of the sulphite oxidase family contain an O–Mo–O unit in contrast to the S–Mo–O in the XO family. Presumably these enzymes function via an oxygen atom transfer process, although direct verification of such reactivity has not been demonstrated in the enzymatic systems. Only one crystal structure of this family is known, the chicken liver sulphite oxidase[17]. The description of this enzyme is shown in Chart II and the metric parameters are listed in Table 2.

The molybdenum centre is found to be in a square pyramidal arrangement with the two sulphurs from the dithiolene units occupying two equatorial positions, with another oxygen and the cystenato sulphur occupying the other equatorial positions. The terminal oxygen attached to molybdenum in sulphite oxidase has a bond distance of 1.74 Å, while longer equatorial Mo–O distance indicates a water or hydroxo group. This is unexpected as the crystals were grown from a fully oxidized sample. A detailed EXAFS investigation suggested that under the intense X-ray beam (where the crystallography was conducted) the metal centre might have been reduced[18]. Such X-ray induced redox reactions in metalloproteins are not uncommon. Recently Berglund *et al.* have used X-ray radiation to drive the reduction of dioxygen in the crystal of horseradish peroxidase[19]. The C–C bond distance is 1.46 Å, which again indicates a partial double bond character similar to that of XDH. The C–S distances are little shorter than the others, perhaps suggesting a partial double bond character. The molybdenum to cysteinato sulphur bond distance (2.47 Å) is close to the expected value while the other two Mo–S bonds are slightly shorter. The 84.4° bite angle is as predicted for a coordinated dithiolene. The S . . . S contact of 3.21 Å is above the S . . . S partial disulphide distance.

Although the crystal structure did not reveal the expected dioxo-Mo(VI) form, the equatorial oxygen is thought to be the reactor oxygen while the apical oxo-group is the spectator one. In the crystal structure, the equatorial oxygen is found to be in close proximity to a sulphate ion (the physiological product of SO) while the apical oxygen is sterically constraint. This description is further corroborated by detailed spectroscopic and kinetic investigations[20,21]. As mentioned, the crystal structure of SO did not show two terminal oxo groups. Therefore, the difference in their bond distances are not known. However, recent crystallographically characterized model complexes of SO demonstrated that the $Mo{=}O_{eq}$ (equatorial terminal oxygen) distance is longer than the $Mo{=}O_{ap}$ (apical terminal oxygen)[22]. The enzymatic centre is also expected to have a very similar feature. The shorter Mo=O bond is expected to dictate that the redox orbital be placed on the equatorial plane with the two sulphur atoms from the dithiolene, similar to XO. The crystal structure shows the O_t–Mo–S_{cys}–C torsion angle to be 90°. This angular restriction precludes any direct bonding interaction between the sulphur *p*-orbitals and the molybdenum d_{xy} orbital. Thus, the primary role of the cysteine has been proposed to poise the reduction potential[23].

Table 2. Representative metric parameters of Gg–SO from the crystal structure solved at 1.90 Å resolution

d(Mo=O)	1.75	d(C-S)	1.64, 1.60
d(Mo-O)	2.21	d(S. . .S)	3.21
d(Mo-S-cyst)	2.49	dithiolene d(C=C)	1.42
d(Mo-S)	2.37, 2.41	< S-Mo-S	84.4

Chart II. General description of SO

Enzyme	*Reaction catalysed*	*General description*
Gg–SO	$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e^-$	One co-factor per molybdenum; the metal atom is coordinated by oxo, hydroxo/water and a cysteinato sulphur.

The DMSO reductase (DMSOR) family

Members of this family are all prokaryotic in origin. Like sulphite oxidase, the members of this family presumably function via an oxygen atom transfer reaction. In the case of DMSO reductase, direct atom transfer reaction has been demonstrated by isotope labelling experiments[24]. Several structures of this family are known, including dissimilatory nitrate reductase (i.e. NapA)[25], formate dehydrogenases[26,27], trimethylamine-*N*-oxide reductase[28], and dimethylsulphoxide reductases[29–31]. Chart III outlines the nature of these enzymes, while Table 3 lists the metric parameters.

In the members of the DMSO reductase family of enzymes, the molybdenum centre is associated with two pyranopterin co-factors. With the exception of arsenite oxidase[32], an amino acid residue also coordinates the metal centre. The co-factor may dissociate from the molybdenum due to the inactivation by molecular oxygen[33] and especially long Mo–S distances in some structures lend support to this hypothesis. For example, the average Mo-S distance in the oxidized Rs-DMSOR is

Chart III. General description of the members of DMSO reductase family of enzymes

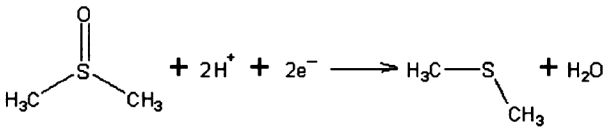
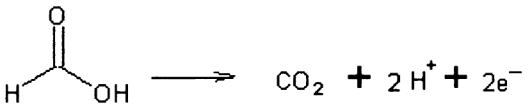
Enzyme	*Reaction catalysed*	*General description*
Af-ArO	$As^{III}O_2^- + 2H_2O \rightarrow As^{V}O_4^{3-} + 4H^+ + 2e^-$	Reduced Mo-centre with a terminal oxo group; two co-factors per Mo-atom.
Rs-DMSOR		Oxidized Mo-centre is associated with two dithiolene ligands; one coordinated and the other one is dissociated. In addition, a serinato oxygen coordinates the metal centre. The metal centre is disordered between penta and hexa-coordinated forms.
Rc-DMSOR	Same as above	The oxidized Mo-centre is associated with four sulphurs from the two dithiolene ligands, a serinato oxygen and two terminal oxo groups; upon reduction one of the terminal oxo group is lost.
Ec-FDH-H		Oxidized, reduced and nitrite inhibited. Two coordinated dithiolene ligands per molybdenum atom and a seleno-cysteine coordinated to Mo.
Ec-FDH-N	Same as above	Two coordinated dithiolene ligands per molybdenum atom and a selenocysteine coordinated to Mo.
Sm-TMAOR	$Me_3NO + 2H^+ + 2e^- \rightarrow Me_3N + H_2O$	Dioxo-Mo centre in the oxidized form, coordinated by two dithiolene moieties and a serinato oxygen.
Dd-NapA	$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	Oxidized form, two coordinated dithiolene ligands per Mo atom and a cysteinato sulphur coordination to Mo.

Table 3. Representative metric parameters from crystallographically characterized members of the DMSO reductase family

	d(Mo=O), Å	d(Mo-O) Å	d(Mo-S) Å	d(Mo-Se) Å	d(C-S) Å	d(C=C) Å	d(S. . .S) Å	< S-Mo-S
Av Af-ArO (1.64 Å)	1.61		2.40, 2.35; 2.37, 2.32		1.77, 1.72; 1.75, 1.73	1.41, 1.43	3.17, 3.09	82.5, 83.8
Rc-DMSOR (1.82 Å), oxidized	1.55, 1.81	1.90	2.51, 2.48, 2.55, 2.42		1.80, 1.79, 1.82,1.79	1.35, 1.35	3.12, 3.00	77.4, 74.3
Rc-DMSOR (1.9 Å), reduced	1.60	2.02	2.36, 2.45; 2.51, 2.35		1.80, 1.79, 1.82, 1.79	1.35, 1.35	3.04, 3.10	80, 77.3
Rs-DMSOR (1.3 Å), oxidized	1.71, 1.75	1.92	2.45, 2.5, 4.53, 3.62		1.78, 1.73, 1.81, 1.77	1.35,1.36	3.20,3.13	80.6, 43.3
Ec-FDH-H (2.9 Å) NO_2^- inhibited		2.58 (NO_2^-)	2.48, 2.48, 2.50, 2.21	2.71	1.77, 1.76; 1.77, 1.77	1.39, 1.42	3.06,3.23	80.8, 81.4
Ec-FDH-H (2.3 Å) Reduced			2.34, 2.47, 2.28, 2.09	2.54	1.76, 1.77; 1.76, 1.77	1.40, 1.40	3.20,3.15	83.4, 92.1
Ec-FDH-H (2.8 Å) oxidized		2.14	2.37, 2.44; 2.35, 2.49	2.6	1.77, 1.77; 1.77, 1.76	1.42, 1.39	3.23, 3.04	84.2, 77.8
Ec-FDH-N (1.6 Å) oxidized		2.16	2.53, 2.44; 2.41, 2.57	2.6	1.79, 1.79; 1.77, 1.77	1.35,1.38	3.24,3.27	81.3, 82.1
Sm-TMAOR (2.5 Å) oxidized	1.57, 1.64	1.73	2.69, 2.80; 2.54, 2.59		1.77, 1.73; 1.76, 1.73	1.40,1.34	2.80, 2.79	61.2, 65.8
Dd-NapA (1.9 Å), oxidized		2.06	2.39, 2.4, 2.19, 2.36, 2.59 (cyst)		1.76, 1.81; 1.77, 1.79	1.43, 1.39	3.03,2.82	78.5, 76.4

Resolutions of the crystal structures are shown in the parentheses.

3.28 Å, where one co-factor seems to be moved away from the Mo-centre[29]. In fact, such movement is manifested in the small bite angle (43°) of one of the co-factors. Small ligand bite angles are also observed in the oxidized form of TMAOR, although the Mo–S distances were in the normal range[28]. In this case, the metal ion is placed significantly above the equatorial plane. Interestingly, the S . . . S contacts are found to be less than 3 Å, strongly suggesting a partial disulphide bond formation, which is also consistent with the C=C and the C–S bond distances. In the case of DMSOR and TMAOR, the terminal oxo-molybdenum bond distances are smaller than the expected value. In the case of the nitrite bound form of FDH–H, the nitrite is found to bind in a monodented mode. For both FDH–H[26] and FDH–N[27], the metal ion is also coordinated by oxygen, either from a water molecule or a hydroxo group. The average Mo–O (aquo/hydroxo) distance is larger (by 0.2 Å) than the average Mo–O (ser) distance of 1.93 Å found in the DMSOR and TMAOR.

Thus, the oxidized molybdenum centres in this class of enzymes are coordinated by at least four sulphur atoms from the dithiolene moieties. Either an amino acid or a water molecule, and a terminal oxo group provide additional coordination. The overall geometry of these sites are closer to trigonal prismatic. It has been postulated that this geometric arrangement leads to a high-energy 'entatic state' at least in the case of DMSO reductases[34]. We note that the near-trigonal prismatic geometry places the terminal groups in close proximity with the donor atom from the endogeneous ligand such as serinato oxygen or cystenato sulphur. In nitrate reductases, for example, the O–Mo–S (cys) angle is only 55° and the through space contact between the terminal oxygen and cysteinato sulphur is 2.2 Å[25]. Once the substrate is reduced, the oxidized metal centre is reduced first to a hydroxo and then to an aquo species. We propose that the small angle suggests that the cysteinato sulphur may be involved in coupled electron–proton–transfer reactions during the regeneration process. In fact, the cysteinato sulphur is 1.7 Å away from the hydrogen of the terminal hydroxo-group, which is within the hydrogen bonding distance. Defining the role of coordinated amino acids in the DMSO reductase family of enzymes is an important target for understanding the structure–function relation in these enzymes. Boyington *et al.*[26] have suggested that different amino acids set the substrate specificity that has been confirmed experimentally by site-directed mutagenesis studies[35]. Our suggestion of coordinated amino acids playing a key role in the proton dislocation process is consistent with Boyington's proposal. We anticipate that future research with suitably designed model studies will test the suggestion made here.

Summary and outlook

Clearly, significant progress has been made in understanding the structural aspects of mononuclear molybdoenzymes and protein crystallography has significantly helped in this endeavour. Here we have discussed selected examples of the first coordination sphere of structurally characterized mononuclear molybdoenzymes. Comparison of the structural parameters with those of small inorganic molecules, where the resolution is higher, has provided a point of reference for distances obtained by protein crystallography. We emphasize that the bite angle analysis can be a good indicator of the complete or partial dissociation of the ene-dithiolate ligand from the metal. Also, shortening of the S . . . S distances is suggestive of a redox role for the ene-dithiolate ligands under catalytic turnover conditions. Within a family, the structures are comparable, although small differences exist, which can influence the reactivity of the molybdenum centres. For example, in DMSOR the Ot–Mo–Oser angle found to vary from 77°–146°, such angular modulation may serve as a gate to electron transfer process in DMSO reductases[36]. Here, we also suggest that in the case of the members of the DMSO reductase family, the coordinated amino acid residues may be involved in the proton shuttling process. We anticipate that small molecule model chemistry such as those reported recently[37,38], will continue to provide a detailed understanding of these exciting chemistries.

Abbreviations. XO, xanthine oxidase; AOR, aldehyde oxidoreductase; XDH, xanthine dehydrogenase; CODH, carbon monoxide dehydrogenase; SO, sulphite oxidase; ArO, arsenite oxidase; DMSOR, dimethylsulphoxide reductase; FDH-H, formate dehydrogenase-H; FDH-N, formate dehydrogenase-N; NapA, periplasmic nitrate reductase; TMAOR, trimethylamine oxidoreductase; Rs, *Rhodobacter sphaeroides*; Rc, *Rhodobacter capsulatus*; Ec, *Escherichia coli*; Sm, *Shewanella massilia*; Dd, *Desulfovibrio desulfuricans*; Af, *Alcaligenes faecalis*; Hp, *Hydrogenophaga pseudoflava*; Pc, *Pseudomonas carboxydovorans*; Dg, *Desulfovibrio gigas*; Gg, *Gallus gallus*.

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