Molybdenum is the only element in the second row of transition metals which has a defined role in biology. It exhibits redox states of (VI), (V) and (IV) within a biologically-relevant range of redox potentials and is capable of catalysing both oxygen atom transfer and proton/electron transfer. Apart from nitrogenase, all enzymes containing molybdenum have an active site composed of a molybdenum ion coordinated by one or two ene-dithiolate (dithiolene) groups that arise from an unusual organic moiety known as the pterin molybdenum cofactor or pyranopterin (1,2). The mononuclear molybdenum enzymes exhibit remarkable diversity of function and this is in part due to variations at the Mo active site that are additional to the common core structure. Prior to the appearance of X-ray crystal structures of molybdenum enzymes, EPR spectroscopy, X-ray absorption fine structure spectroscopy (EXAFS) and biochemical analysis had identified differences between the molybdenum hydroxylases, exemplified by xanthine dehydrogenase, and those enzymes that mostly functioned as ‘oxotransferases’ (3). The former possess a cyanolysable sulfido group at the Mo active site, while the latter are insensitive to cyanide and may possess oxo ligands (Fig. 1). Since 1995, X-ray crystal structures have raised the understanding of molybdenum enzymes to a much higher level and have led to a division of the oxotransferases into the sulfite dehydrogenase and the dimethylsulfoxide (DMSO) reductase families (Fig. 1) (4). The Mo hydroxylases and oxotransferases can act either as dehydrogenases or reductases in catalysis. This reaction can be summarised by the general scheme:

\[ X + H_2O \rightleftharpoons X=O + 2H^+ + 2e^- \]

During this process the Mo ion cycles between the (IV) and (VI) oxidation states with electrons being transferred to or from an electron transfer partner or substrate. Experiments with xanthine dehydrogenase using \(^{18}\text{O}\)-labelled water have confirmed that the oxygen is incorporated into the product during substrate oxidation and this distinguishes the mononuclear molybdoenzymes from monooxygenases where molecular oxygen rather than water acts as an oxygen atom donor (5).

The last decade has seen a resurgence of interest in Mo enzymes as a consequence of the new structural information and also because the remarkable bioenergetic diversity of microorganisms is underpinned to a large degree by Mo enzymes (6). Molybdenum hydroxylases and examples of the sulfite dehydrogenase family can be found in all three domains of life (4). In contrast, the DMSO reductase family appears to be restricted to bacteria and archaea (5).

Fig. 1. Structure of the molybdenum cofactors found in the 3 families of mononuclear molybdenum enzymes. Only the pyranopterin moieties of the organic component of the molybdenum cofactors are shown. For further details see http://metallo.scripps.edu/PROMISE/MOCOMAIN.html
Molybdenum Enzymes in Microbial Respiratory Processes

Two bioenergetic features which distinguish prokaryotes from eukaryotes are the ability to grow under anoxic conditions using oxidative phosphorylation (anaerobic respiration) and the ability to generate energy by respiration using inorganic compounds as electron donors (lithotrophy). Mo enzymes of the DMSO reductase family are pivotal in these two bioenergetic processes (Table 1). Examples of electron acceptors reduced by molybdenum enzymes in anaerobic respiration include nitrate, DMSO, TMAO (trimethylamine-N-oxide), selenate, while electron donors oxidised by molybdenum enzymes in anaerobic respiration include nitrate, of electron acceptors reduced by molybdenum enzymes during lithothrophic growth include nitrite, arsenite and dimethylsulfide.

Structure of Molybdenum Enzymes of the DMSO Reductase Family

In all enzymes of the DMSO reductase family it has been found that the Mo is ligated by four thiolates that arise from two pyranopterin molecules (Fig. 1). With the exception of arsenite oxidase, the Mo ion is also coordinated by an amino acid side chain (2). The presence of different amino acid side chains in enzymes correlates with their phylogenetic relationship and this leads to their classification as Type I (cysteine or selenocysteine ligand), Type II (aspartate ligand) or Type III (serine ligand) (Table 1) (9). In addition to the pyranopterin moiety, each enzyme has an oxygen ligand, either as an oxo-, hydroxo- or aqua-group. One function of the amino acid ligand is to help ‘tune’ the Mo centre to a redox potential which is appropriate for catalysis. It has been shown that in DMSO reductase, mutation of the serine ligand to a cysteine results in a lowered mid-point potential of the MoV/IV couple.

Active Site Structure and Mechanism

The four thiolates of the two pyranopterin rings coordinate the Mo ion in a distorted square planar structure, but additional ligands to the Mo form a trigonal cap and these are in part responsible for the variation in the properties of Mo enzymes (Fig. 1). With the exception of arsenite oxidase, the Mo ion is also coordinated by an amino acid side chain (2). The presence of different amino acid side chains in enzymes correlates with their phylogenetic relationship and this leads to their classification as Type I (cysteine or selenocysteine ligand), Type II (aspartate ligand) or Type III (serine ligand) (Table 1) (9). In addition to the amino acid ligand, each enzyme has an oxygen ligand in the form of an oxo-, hydroxo- or aqua-group. One function of the amino acid ligand is to help ‘tune’ the Mo centre to a redox potential which is appropriate for catalysis. It has been shown that in DMSO reductase, mutation of the serine ligand to a cysteine results in a lowered mid-point potential of the MoV/IV couple.

Table 1. Enzymes of the DMSO reductase family for which a crystal structure has been determined.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Type</th>
<th>Reaction</th>
<th>Source</th>
<th>PDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO reductase</td>
<td>III</td>
<td>((\text{CH}_3)_2\text{SO} + 2\text{e}^- + 2\text{H}^+ \rightarrow (\text{CH}_3)_2\text{S} + \text{H}_2\text{O})</td>
<td><em>Rhodobacter sphaeroides</em></td>
<td>1EU1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Rhodobacter capsulatus</em></td>
<td>4DMR</td>
</tr>
<tr>
<td>Respiratory nitrate</td>
<td>II</td>
<td>(\text{NO}_3^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O})</td>
<td><em>Escherichia coli</em></td>
<td>1Q16</td>
</tr>
<tr>
<td>reductase (Nar)</td>
<td></td>
<td></td>
<td><em>Shewanella massiliana</em></td>
<td>1R27</td>
</tr>
<tr>
<td>Periplasmic nitrate</td>
<td>I</td>
<td>(\text{NO}_3^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O})</td>
<td><em>Desulfovibrio desulfiticans</em></td>
<td>2NAP</td>
</tr>
<tr>
<td>reductase (Nap)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMAO reductase</td>
<td>III</td>
<td>((\text{CH}_3)_3\text{NO} + 2\text{e}^- + 2\text{H}^+ \rightarrow (\text{CH}_3)_3\text{N} + \text{H}_2\text{O})</td>
<td><em>Shewanella massiliana</em></td>
<td>1TMO</td>
</tr>
<tr>
<td>Formate dehydrogenase-H</td>
<td>I</td>
<td>(\text{HCOOH} \rightarrow \text{CO}_2 + 2\text{e}^- + 2\text{H}^+)</td>
<td><em>Escherichia coli</em></td>
<td>1AA6</td>
</tr>
<tr>
<td>Formate dehydrogenase-N</td>
<td>I</td>
<td>(\text{HCOOH} \rightarrow \text{CO}_2 + 2\text{e}^- + 2\text{H}^+)</td>
<td><em>Escherichia coli</em></td>
<td>1KQF</td>
</tr>
<tr>
<td>Arsenite oxidase</td>
<td>I</td>
<td>(\text{AsO}_3^- + \text{H}_2\text{O} \rightarrow \text{AsO}_3^- + 2\text{e}^- + 2\text{H}^+)</td>
<td><em>Alcaligenes faecalis</em></td>
<td>1G8K</td>
</tr>
</tbody>
</table>


Crystal structures of seven molybdenum enzymes of the DMSO reductase family (Table 1) have revealed that they have a similar tertiary structure. The prototype is DMSO reductase from *Rhodobacter sphaeroides* which contains about 40% α-helix and 20% β-sheet and is composed of four domains that are folded around the Moco (8). Domains I, II and III define a ‘cup’ shape with the Mo ion located at the base. Domain IV forms the bottom of the cup and closes the cleft formed by the other domains. Domains II and III form a series of hydrogen bond interactions with the guanosine moiety of the GMP attached to the so-called P-pterin and Q-Pterin respectively, while Domain IV forms extensive interactions with the pyranopterin moieties (Fig. 2).
compared to wild-type enzyme, while the Mo$^{IV/IV}$ reox couple had a higher potential (10). This S147 C mutant form of DMSO reductase has almost no activity towards S-oxides, and reduced activity towards some N-oxides, but higher activity towards other N-oxides such as adenine N-oxide. Another important function of the amino acid side chains is to control the bond length of the oxygen ligand to the Mo centre since Mo-S bonds exhibit a greater degree of covalency which causes a weakening of Mo=O bonds. Thus, in DMSO reductase (Type III enzyme) an oxo group at the Mo active site has been confirmed using Raman spectroscopy (11,12) while in the Type II and Type I enzymes the Mo-oxygen atom bond is more consistent with the presence of a hydroxo or a water molecule. Amino acid side chains close to the Mo active site also have a key role in the reaction mechanism of Mo enzymes. In DMSO reductase critical roles in catalysis have been established for tyrosine (Y114) and tryptophan (W116) residues (5,13).

**Electron Transport in Molybdenum Enzymes**

The mechanism of electron transfer to and from the Mo ion in enzymes of the DMSO reductase family is not well defined. It is tempting to suggest that one of the pyranopterins has a role but there is no strong evidence to support such a role at present. Crystal structures of Type I and Type II enzymes which contain an additional prosthetic group within the Mo-containing catalytic subunit have helped define an electron transfer pathway (9,14). For these enzymes the distance from the Fe-S cluster associated with Domain I of the Mo-containing subunit to the edge of a pyranopterin is close enough (7 Å in the case of respiratory nitrate reductase) for electron transfer at a rate sufficient for enzyme catalysis as defined by Dutton’s Ruler (15). For Type III enzymes (Table 1) that lack this Fe-S cluster, the electron transfer pathway to the Mo ion is not known. The majority of enzymes of the DMSO reductase family are connected to respiratory or photosynthetic electron transfer chains and so they are often oligomeric enzymes with additional subunits with redox-active prosthetic groups. For example, respiratory nitrate reductase is composed of a Mo-containing catalytic subunit (NarG), a subunit containing four [Fe-S] clusters (NarH), and a di-haem subunit (NarI) (7). These additional electron transfer components connect the Mo catalytic subunits to cytochromes or the quinone pool in the cytoplasmic membrane. Many Mo enzymes are water-soluble and are located in the periplasmic space of Gram negative bacteria (6). Thus, they do not participate directly in energy conserving processes since electron transfer to these enzymes via cytochromes or quinol is not linked to charge separation across the cytoplasmic membrane. However, since these enzymes often terminate a respiratory chain that involves proton-translocating NADH dehydrogenase (e.g. DMSO reductase) or NADH dehydrogenase and the cytochrome $b_{c_1}$ complex (selenate reductase) the cells are able to generate a proton motive force.
Formate Dehydrogenase-Nitrate Reductase Respiratory System: Two Classical Protonmotive Redox Loops

In contrast to periplasmic enzymes which can only catalyse scalar reactions, formate dehydrogenase (FdH-N) and respiratory nitrate reductase (NarGHI) are organised so that they participate in vectorial movement of charge across the membrane (16). In formate dehydrogenase, electrons from the oxidation of formate are separated from protons on the periplasmic face of the membrane and translocated across the membrane dielectric by the dihaem subunit of formate dehydrogenase to the site of quinone reduction (14). Generation of quinol consumes two protons from the cytoplasm and results in a net charge movement across the cytoplasmic membrane and leads to the generation of a membrane potential and ΔpH. The quinol generated by formate dehydrogenase diffuses to the periplasmic face of the cytoplasmic membrane where it is oxidised by the dihaem NarI subunit of nitrate reductase. Protons are released into the periplasm and electrons are conducted across the cytoplasmic membrane to its cytoplasmic face where the Mo-containing subunit (NarG) catalyses the reduction of nitrate in a reaction that consumes two electrons. Again, the quinol-nitrate oxidoreductase activity is linked to vectorial charge movements. The solution of the crystal structure of formate dehydrogenase and nitrate reductase (see Table 1 for PDB references) provides a beautiful illustration of a proton-translocating redox loop which was first postulated by Peter Mitchell as a mechanism for energy conservation. The $\text{H}^+/\text{e}^-$ ratio in such systems is 1 and their ability to conserve energy is entirely dependent upon the topological organisation of the enzyme’s prosthetic groups and does not involve proton pumping as seen in cytochrome oxidase (17).

Concluding Remarks

The DMSO reductase family of enzymes illustrates the remarkable way in which enzymes can use a metal ion to catalyse highly specific reactions. However, in spite of the strong similarities in structure, the subtle differences in Mo active site mean that there exists a substantial degree of diversity of reaction mechanism within the DMSO reductase family: formate dehydrogenase catalyses a dehydrogenation but no oxygen atom transfer, while enzymes such as polysulfide reductase catalysing S-atom transfer rather than O-atom transfer (18), and even those enzymes which catalyse the classical oxygen atom transfer may be mechanistically different. It should also be noted that tungsten, a third row transition metal immediately below molybdenum in the periodic table, is also found in enzymes of the DMSO reductase and xanthine dehydrogenase family in thermophilic bacteria and archaea that grow under anaerobic reducing conditions (5,19). Tungsten operates at a lower redox potential compared to molybdenum and this property, coupled to the greater stability of W-sulfur bonds, means that tungsten enzymes are ideal for a thermophilic lifestyle. It is likely that tungsten enzymes are the most ancient enzymes within the superfamily of molybdenum and tungsten enzymes.

Acknowledgements

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References