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Molybdenum and Tungsten Oxidoreductase Models

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This microreview gives an overview about advances in bioinorganic model chemistry for molybdenum- and tungsten-dependent oxidoreductases with respect to specific aspects of the evolution, structure or function of distinct active-site properties. The approaches to evaluate catalytic properties fine-tuned by the ligand sphere, interpret and assign enzyme spectra and to understand why two different metals are used for the same task but in distinct habitats and respective evolutionary aspects are discussed on the basis of exemplary case studies. This short review provides a personal perspective and appreciation of the complex problems associated with the respective foremost synthetic and analytical model chemistry and their solutions partly accompanied and supported by theoretical results.

Introduction

In bioinorganic chemistry, in general, either metalloproteins of all kinds are investigated by chemical methods or, much more often, single-molecule inorganic compounds are designed to mimic metallosites of proteins, which are then investigated by the same methods and by methods that may be not applicable to large biomolecules. Both approaches serve the understanding of the chemistry of metalloproteins. Naturally, the frontiers between biology and chemistry are becoming increasingly blurred, but still the training of primarily synthetic inorganic chemists allows them, without necessarily applying biological methods but with carefully designed molecules, to shed light on nature's astounding efficiency, craftiness and potency with respect to problem solving and optimisation of processes.

The scope of this microreview is to illuminate the inorganic chemist's efforts to understand the evolution, detailed composition with respect to coordination geometry and electronic structure, catalytic efficiency and spectroscopic properties of the active sites of the molybdenum- and tungsten-dependent oxidoreductases. Enzymes of this kind catalyse the general reaction shown in Equation (1). An O^{2-} is transferred from water onto the substrate or vice versa in a two-electron redox reaction accompanied by a two-proton transport from or to the active site.[1]

$$R + H_2O \leftrightarrows RO + 2e^- + 2H^+ \quad (1)$$

These reactions are part of the carbon, nitrogen and/or sulfur metabolism of all organisms, and accordingly the respective enzymes are ubiquitous. In humans, three enzymes of this kind have been known to perform vital tasks (whose malfunctioning may even result in death[2]) for quite a while, and a fourth enzyme, whose exact role is still being investigated, has been found recently.[3]

The group of molybdenum- and tungsten-dependent enzymes (only Mo-dependent nitrogenase is an exception) has one common feature: the molybdopterin ligand (mpt; Figure 1). This name refers to the organic molecule only (excluding the active-site metal), which is structurally related to pterins that are found on butterfly wings, for instance, and is, in addition, remotely related to nucleosides (adenine, guanosine). The quite complicated biosynthesis of molybdopterin has been investigated in detail.[4] The role of this non-innocent ligand (potentially redox-active and interacting with the bound metal) has been and still is one of the focal points in the bioinorganic chemistry of molybdenum and tungsten. The ligand mpt can be present once or twice in the active site, and, on the basis of this and ad-

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ditional structural and, to a lesser extent, functional characteristics, the whole group of molybdenum- and tungsten-dependent enzymes (without nitrogenase) has been classified by Hille into different families (Figure 1).[1a,1b] It is evident that, despite some differences, molybdenum- and tungsten-dependent enzymes are very much alike with respect to structure and function and that they share the same evolutionary history. Because tungsten is found in organisms that belong to the first living beings that we know of, which still live in habitats that resemble the conditions on the early earth, it is agreed that the molybdenum-dependent enzymes evolved from the tungsten-dependent ones.[1c,1e] Why molybdenum actually could not be used early on is well understood today. But why nature deemed it necessary to change the well-functioning team of tungsten cofactor and protein at all is still being investigated.

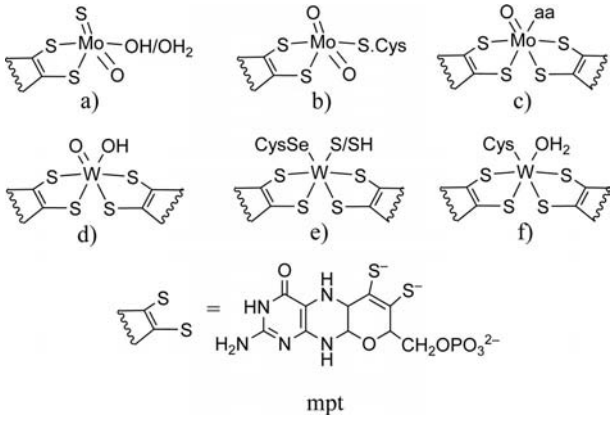

Figure 1. The typical active sites of the molybdenum- and tungsten-dependent oxidoreductases as derived from crystal structures of individual enzymes: (a) the xanthine oxidase family (fully oxidised), (b) the sulfite oxidase family (fully oxidised) (c) the DMSOR family (fully oxidised; aa = amino acid residue, that is, Asp, Ser, Cys or Se-Cys) (d) the aldehyde ferredoxin oxidoreductase family, (e) the formate dehydrogenase family, (f) acetylene hydratase.

In most cases, the exact compositions of the active sites have been verified with X-ray diffraction and absorption methods, but frequent fields of discussion are the small (one, two or three atom) ligands whose exact nature is often difficult to determine and which may be easily altered during the purification processes of the enzymes. Yet, another interesting field is the variety of amino acid residues found in the DMSO reductase (DMSOR) family and their possible role in the catalytic process. In one case, it was proposed on the basis of crystallographic data that selenocysteinate directly interacts with the substrate and that the selenium–metal bond is even broken in course of the catalytic cycle.[5] Further aspects are, for instance, of geometric (octahedral vs. trigonal prismatic), electronic (influence of spectator ligands, non-innocence etc.) or spectroscopic (for instance, differences in the EPR spectra dependent on isolation procedures) nature. The purpose of this microreview is to highlight examples of recent and/or particularly clever or ground-breaking approaches to answer open questions, which my group is particularly interested in, about the structure, function or the interplay of both and the evolution of molybdenum- and tungsten-dependent oxidoreductases.

Discussion

Dithiolene Ligands as Molybdopterin Mimics

Because the functional group of mpt with which it binds to molybdenum or tungsten is an ene-dithiol or dithiolene (dt), a moiety including the metal and one or two dithiolene ligands is one of the most common motifs in the chemical models for oxidoreductases.

Among the pioneers in bioinorganic model chemistry for molybdenum and tungsten oxidoreductases using dithiolene ligands are the groups of Richard H. Holm and David C. Garner. The former focussed in this respect on the immediate coordination sphere of the metal, including the additional ligands of the natural systems by using simple dithiolenes, whereas the latter addressed mainly the mpt ligand and its different aspects with more elaborated mpt models but with less effort on the remaining ligands. The Holm group was very often the first to synthesise and investigate (analytically and catalytically) a model complex, addressing the complete first coordination sphere of a specific enzymatic active site (Figure 2b–i).[6] The Garner group published the very first crystallographically characterised model for the DMSOR family with a rather simple dithiolene ligand (Figure 2a),[7] which is now known to be a quite good model of arsenite oxidase[8] but a less good model for the typical DMSOR active site. Their main focus, however, in cooperation with the organic chemistry group of Joule, was the development of a variety of ligand systems that mimicked different aspects of molybdopterin.[9] One important point here is the simple fact that most of their models are different from Holm's dithiolene ligands in that they are non-symmetrically substituted, as is mpt (Figure 2j). In combination with the proximity of nitrogen functions close to the dithiolene moiety, they developed many more and increasingly accurate model ligands of mpt that are still state of the art today. Two excellent model ligands that were developed closely resembled mpt but for the third ring, which was a benzene ring, instead of the nitrogen heterocycle, and coordinated to cobalt (Figure 2k).[10] The respective molybdenum or tungsten complex, however, could unfortunately not be isolated.

Very frequently, the mnt [maleonitrile = $H_2S_2C_2(CN)_2$] ligand has been used, which is certainly the easiest to synthesise, handle and react with the respective metal. Amazingly, the resulting comparably simple structural enzyme models were also shown to be very good functional models for quite a number of enzymatic reactions.[11] However, due to the presence of two strongly electron-withdrawing substituents on the ene function, the electronic properties of the metal site should be dramatically different from those in the enzymes in which the mpt ligand is considered to be electron-donating if non-innocent at all.

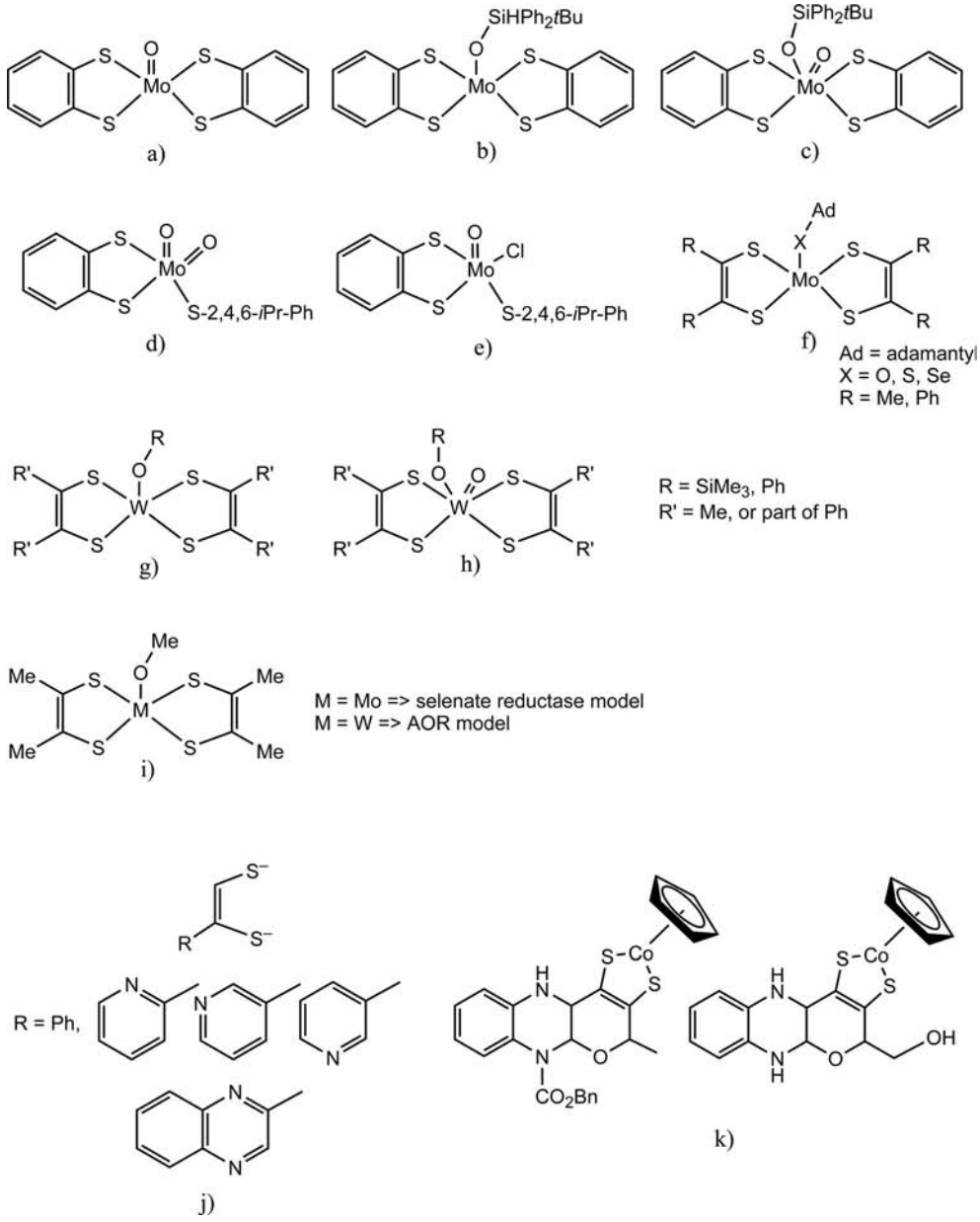

Figure 2. Typical models for the active sites developed by the Holm and Garner groups: (a) the first crystallographically characterised model for the DMSOR family, an excellent model for arsenite oxidase (AO) as revealed by its crystal structure; (b) higher developed and less accessible models for the DMSOR family (except AO), reduced and (c) oxidised; (d) model for oxidised sulfite oxidase (SO); (e) model for the high-pH form of SO (Cl^- replaces OH^-); (f) models for the reduced DMSOR active sites including distinct amino acid functional groups; (g) model for the aldehyde ferredoxin oxidoreductase family (AOR), reduced and (h) oxidised; (i) models for selenate reductase or AOR depending on the metal used; (j) unsymmetrically substituted ligands as models for mpt; (k) cobalt complexes of mpt models including the dithiolene, pyran and pyrazine functionalities.

In this context, the approach of my group was at the beginning focussed at an expansion from the immediate coordination sphere that was covered by Holm's work but without necessarily trying to mimic the whole N-heterocyclic part that Garner had addressed. We started out synthesising ligand systems that contained the dithiolene unit and the directly bound pyran ring (which, as such, had mostly been neglected previously), ignoring the pterin part of mpt. Molybdenum and tungsten oxo-bis(dithiolene) complexes with the flavanyl dithiolene ligand could be prepared (Figure 3a); they were the first complexes of the relevant metals with dt-pyran systems.[12] Unfortunately, no crystal structures were available, because of the presence of a mixture of *cis*- and *trans*- and *R*- and *S*-isomers compromising the crystallisation process. It was shown, however, that the electrochemical behaviour of these complexes was different from that of related dithiolene complexes without the pyran ring, suggesting that the respective model compounds carried particularly non-innocent ligands and consequently that the pyran ring had a substantial influence on the central metal. Later, the Sugimoto group was able to synthesise and structurally characterise a smaller pyrandi-

thiolene molybdenum model complex (Figure 3b) and could show that there were differences between this and related complexes, in which the oxygen was substituted by carbon or sulfur, with respect to spectroscopic and electrochemical properties, again supporting an electronic fine-tuning of the active-site metal by such a ligand system.[13]

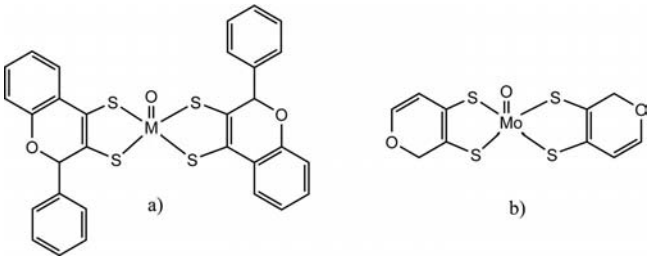

Figure 3. Model complexes with ligand systems mimicking the dithiolene and pyran part of mpt (M = Mo, W). (a) The first models of this kind employing the fdt ligand systems. (b) Ligand systems for which only the respective mpt functionalities have been characterised crystallographically.

General difficulties in modelling mpt-bound molybdenum and tungsten are firstly the complicated and so far not achieved synthesis of a ligand containing really all features of mpt and the instability of the free natural cofactor, indicating that this will also be a problem with a synthetic cofactor addressing every functional group of mpt.

Accordingly, our next step in this respect was primarily of a theoretical nature aimed at ascertaining what really needs to be mimicked in order to produce a mpt model that is the most economic to synthesise but the most accurate with respect to the electronic and geometric properties of the molecules. Commonly used model complexes with, for instance, bdt^{2-} (see Figure 1a) or $Me_2C_2S_2^{2-}$ ligands and increasingly accurate models for the mpt ligand were compared with oxo bis(molybdopterin) complexes of molybdenum and tungsten that were based on protein crystallographic data in which only the phosphate substituent of mpt was left aside.[14] Compared were NPA (natural population analysis) and NBO (natural bond orbital) data, energetic differences between the HOMO and the LUMO, bond lengths and angles, theoretical redox potentials and the folding angle. The latter describes an out-of-plane movement of the dithiolene ligand upon oxidation of complexes, which helps to buffer the electronic state and therefore the redox potential as a result of an enhanced overlap of sulfur p orbitals with metal d orbitals.[15] These studies revealed that the more of mpt was modelled the closer the results for molybdenum and tungsten were. Apparently, mpt is able to minimise differences based upon the character of both metals electronically and geometrically, which certainly helped the evolution of molybdenum-dependent from tungsten-dependent enzymes. With respect to the motivation for such a comparison, however, it was shown that, in order to mimic qualitatively and quantitatively all the investigated properties of mpt complexes the model has to include the dithiolene moiety, the pyran ring and the pyrazine ring whereas the third and furthest away ring could be neglected without compromising comparability to mpt. Our synthetic efforts are now focussed on developing new ligand systems that address these three structural and functional features of molybdopterin, similar to Garner and Joule's ligand (Figure 2k), and that are able to coordinate to molybdenum and tungsten. Recently, a promising precursor for such a model was published (Figure 4), containing all the needed non-hydrogen atoms but the pyrazine here is in its oxidised instead of reduced form and the dithiolene moiety would need to be deprotected in order to generate molybdenum or tungsten complexes. For a mpt model that truly mimics all its essential behaviour and properties and is able to bind molybdenum and tungsten, still a lot of dedication and synthetic persistence is required. But once achieved, such a model will certainly have been worth the effort, revealing nuances in analytic data and catalytic potential that will further our understanding of nature's choice of this rather complicated ligand system in the ubiquitous and indispensable oxidoreductases.

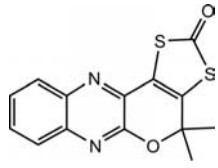

Figure 4. A promising precursor for a mpt model ligand, addressing the dithiolene, pyran and pyrazine functionalities of mpt.

Active-Site Geometry

An ongoing discussion in the field of molybdenum- and tungsten-dependent oxidoreductases is concerned with the importance of the coordination geometry around the active-site metal, that is, octahedral vs. trigonal prismatic geometry at hexacoordinate centres, in particular those with two molybdopterin ligands. The coordination of geometrically strained dithiolene ligands (planar moiety with a comparably small bite angle) to the enzyme's active-site metals and in tris(dithiolene) complexes supports the latter geometry. It was shown by theoretical studies on model compounds for the DMSOR family of enzymes that such a trigonal prismatic coordination geometry lowers the activation barrier of oxygen abstraction from substrate and reduces the exothermicity of the reaction, which subsequently makes it more reversible.[16] In the enzyme (DMSOR), this effect is even more pronounced because of further restraints put onto the active site through the protein environment and the substantially larger mpt. Synthetic bis(dithiolene) complexes, however, tend to be in a distorted octahedral geometry as are model compounds and enzyme active sites with only one mpt ligand. The challenge to investigate the influence of the geometry on the catalytic potential of a model compound therefore lies in the design of complexes that are forced to be in a trigonal prismatic geometry and (for comparison), in similar complexes, in an octahedral geometry. Naturally it is impossible to use the same ligand system for both geometries, and every comparison is neces-

sarily compromised. But, if the ligand systems chosen are not too distinct with respect to the donor atoms used and the respective discussion is taken with caution, some interesting experimental trends may be found. In order to force a sixfold coordinated molybdenum centre into a trigonal prismatic coordination geometry, Most et al. used nitrogen-donor pyrazolate (pz) ligands instead of sulfur-donor dithiolene ligands, the former having significantly smaller bite angles (Figure 5a).[17] The resulting $[MoO_2(pz)_2]$ complexes were indeed of trigonal prismatic geometry, as evidenced by X-ray crystallography. For a comparison, octahedral Schiff base (SB) complexes, $[MoO_2(SB)_2]$ (Figure 5b), which coordinated to molybdenum via nitrogen and oxygen donors, were synthesised.[18] Although replacing one nitrogen by one oxygen donor in the used ligand systems certainly diminishes the comparability of the two types of complexes, both were actually carefully designed. In the two different ligands, formally one of the donor atoms is involved in a double bond to the three-carbon backbone of the respective ligand, whereas the other is connected to it via a single bond, and the double bonds that are present are mobile, allowing different mesomeric species of the respective ligand to form.

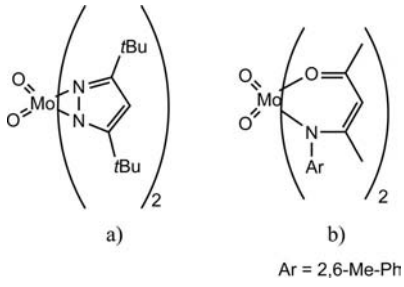

Figure 5. Models with distinct coordination geometry. (a) Trigonal prismatic geometry enforced by the small bite of the pyrazolate ligands. (b) Octahedral geometry with the more flexible Schiff base ligands.

Both types of complexes were tested with respect to their catalytic oxygen-atom-transfer potential, revealing that, at least with certain substituents on the ligands, they were indeed active. The typically investigated oxo-transfer model reaction is the oxidation of PR_3 with DMSO, involving two oxo transfers, from DMSO onto the metal and from the metal onto the phosphane. This reaction can be neatly followed by ^{31}P NMR spectroscopy and does not occur in the absence of a catalyst [Equations (2) and (3)].

$$LMoO + PR_3 \leftrightarrows LMo + OPR_3 \quad (2)$$

$$LMo + DMSO \leftrightarrows LMoO + DMS \quad (3)$$

In agreement with the theoretical results mentioned above, it was indeed shown that the trigonal prismatic complex was far more active with respect to the catalytic oxidation of specific PR_3 species by DMSO than the octahedral one. The latter could only process less bulky PR_3 molecules and had lower rate constants even at higher temperatures relative to the pyrazolate complex. Nevertheless, at this point it is impossible to distinguish the impact of the coordination geometry from the steric effect that the large substituent attached to one of the donor atoms of the octahedral complex may have.

Another issue regarding the geometry of DMSOR enzymes was investigated by Kirk, Basu and their co-workers. They were particularly interested in the geometric control of the redox potential by the serinate ligand present in some members of the family. Complexes *cis*-$[(L1O)Mo^{V}OCl_2]$ and *trans*-$[(L1O)Mo^{V}OCl_2]$ [L1OH = (3-*tert*-butyl-2-hydroxy-5-methylphenyl)bis(3,5-dimethylpyrazolyl)methane] were synthesised and isolated, the *cis* and *trans* notation describing the position of the phenolate oxygen relative to that of the terminal oxo ligand (Figure 6).[19] Both compounds have been studied electrochemically and show well-defined one-electron reduction signals ($Mo^{V} \rightarrow Mo^{IV}$ at –940 and at –1160 mV vs. Fc/Fc^{+}, respectively). This result reveals that the *trans* isomer is by about 200 mV easier to reduce than the *cis* isomer in an otherwise identical coordination sphere. This is a strong indication of a critical role the serinate oxygen-donor atom plays in gating the electron transfer process of active-site regeneration as part of the catalytic cycle by its position relative to the terminal oxo ligand at the molybdenum centre of DMSOR. These findings also show that an exchange of the donor atom of the peptide should have an influence on the catalytic performance of the active site, since Mo–O, Mo–S and Mo–Se bond lengths and bond strengths are necessarily distinct.

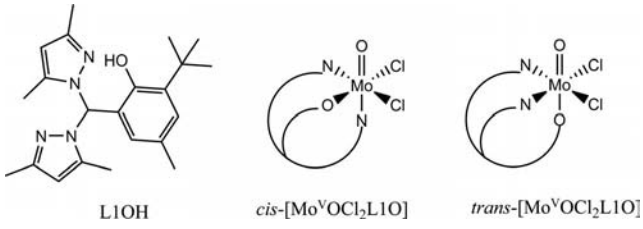

Figure 6. Mo^{V} complexes (*cis* and *trans*) with the $L1O^{-}$ ligand, used to investigate fine-tuning of the redox potential of the active site by serinate coordination.[19]

Though much has been achieved in this respect, the complicated issue of geometric control of the molybdenum- and tungsten-dependent enzymes is not resolved, and further effort and inventiveness by synthetic chemists is required in order to understand and appreciate the importance of the active-site coordination geometry in full detail.

A combination of synthetically controlled geometry with the use of dithiolene or at least sulfur-donor ligands in carefully designed ligand systems is certainly the most desirable and challenging task lying ahead.

Amino Acid Coordination

Staying with the specific enzymes of the DMSOR family, whose active site is directly bound to the peptide through an amino acid residue (only AO is an exception), this specific

coordination is worth exploring further, as was shown in the previous paragraph. In contrast to the sulfite oxidase family, in which the respective cysteine is conserved among the family members, in the DMSOR family a variety of amino acids has been found. These are serinate (O-functional), aspartate (O-functional), cysteinate (S-functional) or selenocysteinate (Se-functional). Interestingly, there is no strict correlation between the coordinated amino acid and the catalytically processed atom (C, N, S).[20] For each kind of substrate, at least two examples of different amino acid coordination are known. It is not known, however, whether the different amino acids were changed in a targeted way during evolution in order to optimise enzymatic catalysis or this variety is simply the result of random mutation. Our synthetic and analytical approach in this respect was aimed at evaluating a potential influence of the coordinated ligand atom on catalytic performance. The corresponding studies were focussed on a comparison of sulfur coordination with selenium coordination. When trying to synthesise structural and functional models for the two kinds of active sites, two main obstacles are involved: firstly, the metal centres should be in a high oxidation state (IV or VI) to be relevant for the oxidoreductases. The reaction of such a metal centre with a thiolate or selenolate usually leads to a reduction of the metal, oxidation of the ligand and subsequently to the generation of disulfides and diselenides, respectively, which are not necessarily coordinated to the metal any more. Secondly, the investigated compounds should be active and stable catalysts. The latter is often compromised by a comproportionation of reduced (M^{IV}) and oxidised (M^{VI}) form to a M^{V}-O-M^{V} core, which is no longer catalytically active. It was therefore decided to turn to model compounds that are not really structural models for the active sites, though all donor atoms are from the chalcogenides that are already binuclear (to avoid generation of the notorious M-O-M core), that are not too reactive (so that they are stable enough to survive several catalytic turnovers and last a reasonable time) and that contain the donor atoms of interest as -ether (-C-X-C-) or -one (-C=X) type function (to avoid redox reactions between ligand and metal; X = S, Se).[20–23] All used compounds were rather slow catalysts, but they were absolutely stable at catalytic conditions and processed 100% of the lower concentrated substrate (PPh_3; DMSO was added in excess, see the catalytic procedure described above). For all used pairs of complexes, the studies showed that, despite nearly identical structural and electrochemical

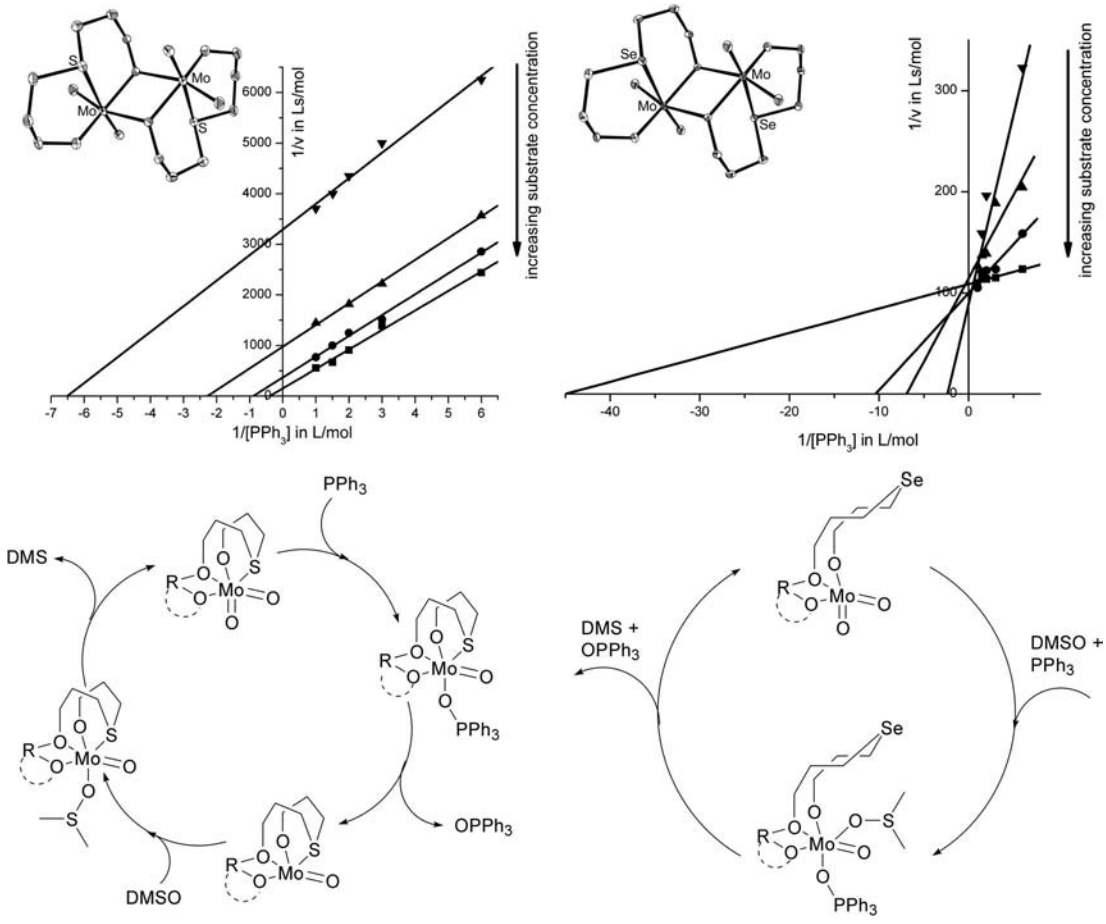

Figure 7. Almost identical molecular structures of $[Mo_2O_4(OC_3H_6SC_3H_6O)_2]$ (left) and $[Mo_2O_4(OC_3H_6SeC_3H_6O)_2]$ (right), Lineweaver–Burk diagrams for both catalysts, obtained by varying the concentrations of both substrates {on the linear graphs for PPh_3 and different linear graphs for DMSO [DMSO concentrations increase from 1.75 M (triangles) over 3.5 M and 7 M to 14 M (squares)]} and the proposed catalytic reaction mechanisms that have been confirmed by the Lineweaver–Burk plots.[20,22] Reproduced with permission from ref.[20]

properties the catalytic activity was always different for sulfur- and selenium-based ligands, though for some complexes the Se compounds were better catalysts and for others it was vice versa. In one particularly interesting case, it could be shown that, though the molecular X-ray structures were nearly identical (isomorphous actually; Figure 7), the structure in solution was different. For the sulfur compound, the S–Mo bond stayed intact in solution (proved by extended X-ray absorption fine structure spectroscopy), whereas the Se–Mo bond was disconnected in solution (revealed by ^{77}Se NMR spectroscopy). This led to the proposal of two distinct catalytic reaction mechanisms, the typical consecutive or ping pong mechanism for the S complex and a concerted mechanism for the Se complex. And indeed, by using a Michaelis–Menten type kinetic approach and Lineweaver–Burk diagrams, the proposed mechanisms for the two complexes, which are dramatically different from each other (Figure 7), were strongly supported. Interestingly the observation of a disconnected Se–Mo-bond supports the proposed enzymatic reaction mechanism for periplasmic nitrate reductases, which also involves removal of the selenium coordination from the active-site metal.[5]

At the present state of our studies, we can certainly say that the change from sulfur to selenium coordination has an influence on catalytic properties, which could be enhanced or diminished, and that it is therefore certainly possible that the distinct amino acid residues in the different members of the DSMSOR family may be there to really optimise the respective catalytic reaction. Further efforts in this respect are going to turn to ligand systems having relevant functions of an -ate nature (-C–X$^-$) while still maintaining stability and catalytic activity of the resulting complexes. Currently we are exploring the always reliable Schiff base and newly developed macrocyclic ligands. In order to really establish a significant influence of the coordinated amino acid on enzymatic catalysis, it will, however, be necessary to not only vary the donor atoms of the models but also the processed substrates and to investigate a whole series of catalysts and catalytic reactions.

Catalytic Oxygen-Atom Transfer, Proton-Coupled Electron Transfer and Intermediates

To advance one's insight into the actual catalytic mechanism of the various molybdenum- and tungsten-dependent oxidoreductases, the knowledge not only of substrate, product and catalyst is required but also of intermediates of the catalytic reaction. This is particularly difficult to investigate directly with the enzymes, since intermediates in the proteins are naturally short-lived, and spectroscopic data are rare if available at all. So, in this respect the use of model compounds is nearly indispensable if one does not want to rely on theory alone.

The typical catalytic cycle involves the transfer of O^{2-} from the metal site to the substrate or vice versa accompanied by a two-electron reduction or oxidation of the active site and for its regeneration two-proton-coupled electron transfer (PCET) steps, the latter being the more challenging part to model.

The very first model complex with which it was possible to include two distinct PCETs for the regeneration of the active catalyst was [Tp*Mo^{VI}O$_2$(SPh)] {Tp*: hydrotris(3,5-dimethyl-1-pyrazolyl)borate}.[24] In this complex with a sterically highly demanding ligand system, the unwanted comproportionation is successfully impeded through steric hindrance though it has not become entirely impossible (Figure 8).

Reaction of [Tp*MoO$_2$(SPh)] with PPh$_3$ in DMF or acetonitrile gives [Tp*Mo^{IV}O(SPh)] with or without weakly bonded solvent. Depending on the used solvent, this species

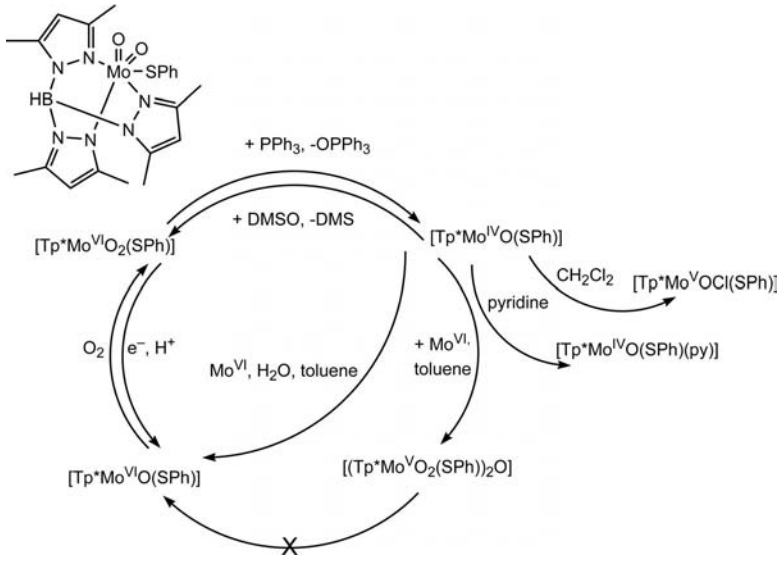

Figure 8. Reaction scheme covering all reactions that have been observed with the [Tp*Mo^{VI}O$_2$(SPh)]/[Tp*Mo^{VI}O(SPh)] couple and its chemical structure (left top corner).

shows distinct behaviour. In pyridine or in CH_2Cl_2 it can be trapped as [Tp*$Mo^{IV}O$(SPh)(py)] or [Tp*$Mo^{V}O$Cl(SPh)], respectively. In wet THF or toluene the water present leads to the formation of [Tp*$Mo^{V}O$(OH)(SPh)] including one-electron oxidation of molybdenum and removal of a proton from water. This species can now quantitatively be oxidised to Tp*$Mo^{VI}O_2$(SPh) again, removing one electron and one proton. All observed reactions based on this system are summarised in Figure 8.

By using H_2O labelled with oxygen isotope ^{18}O, the authors could show that the oxygen in the product $OPPh_3$ was provided by water. This synthetic system was the first that ran through the full catalytic cycle, featuring the $[Mo^{VI}O_2]^{2+}$ resting state and closely modelling sulfite oxidase catalysis.[25]

The more elusive $Mo^{IV}/Mo^{VI}O$ couple, again with the Tp* ligand, modelling the reduced and oxidised form of the DMSOR family active sites (excluding AO), was first employed as catalyst in both OAT and PCET reactions by Kirk, Basu and their co-workers.[26] Using $(NH_4)_2Ce(NO_3)_6$ as oxidant providing one electron to [Tp*$Mo^{V}O$(p-OC_6H_4-OC_2H_5)$_2$] gives the corresponding monooxo Mo^{VI} species [Tp*$Mo^{VI}O$(p-OC_6H_4-OC_2H_5)$_2$]$^+$. Dissolved in acetonitrile together with PPh_3, this complex undergoes OAT (oxygen-atom transfer), forming the desoxo species [Tp*Mo^{IV}(p-OC_6H_4-OC_2H_5)$_2$]$^+$ and $OPPh_3$. Combined with 2,3-dicyano-5,6-dichloro-1,4-benzoquinone (DDQ) as oxidant in the presence of water, the Mo^{IV} desoxo species is transformed back to [Tp*$Mo^{V}O$(p-OC_6H_4-OC_2H_5)$_2$], completing the catalytic cycle (Figure 9). Again, the formation of [Tp*Mo^{V}(^{18}O)(p-OC_6H_4-OC_2H_5)$_2$] when $H_2{}^{18}O$ was used in the final step proved water to be the source of oxygen in the catalytic process.

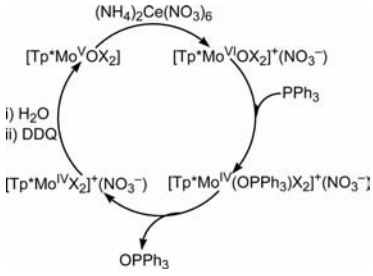

Figure 9. The combination of PCET and OAT reactions of the catalytic desoxo Mo^{IV} and monooxo $Mo^{VI}O$ couple; X = p-O-$C_6H_4OC_2H_5$.

Employing a slightly modified tris(pyrazolyl)borate (tpb) with one isopropyl instead of two methyl substituents on the pyrazole rings, it was even possible to isolate catalyst–substrate adducts [Tp$^{iPr}Mo^{IV}$OX(OPPh$_3$)] (X = Cl$^-$, OR$^-$, SR$^-$), which are key intermediates in the catalytic oxidation of PPh_3.[27] The O=Mo–O–P torsion angle could, by comparison with previous theoretical results,[28] be identified as the likely suspect responsible for retention or disruption of the Mo–O bond by fine-tuning the Mo–OP π bonding.

More recently, the tris(pyrazolyl)borate ligand system was used as complex backbone to access the usually very labile $M^{VI}OS$ core, which is prone to undergo a metal–ligand redox reaction and dimerise to a $Mo^{V}O$(S-S)$Mo^{V}O$ species.[29]

All this shows the usefulness of the tpb ligands for a variety of issues that need to be addressed when trying to understand properties and catalytic reaction mechanisms of the molybdenum- and tungsten-dependent oxidoreductases. The disadvantage of this ligand, however, certainly is the fact that it provides a very different coordination sphere compared to the dithiolene coordination of mpt. So it is always possible that systems with a closer resemblance to the natural active site behave at least slightly differently, with consequences for our understanding of the enzymes.

The Heinze group, in addition to the typical single molecule approach,[30] developed an entirely different strategy in order to investigate OAT and PCET in systems with relevance for molybdenum-dependent oxidoreductases. They immobilised mononuclear dioxomolybdenum(VI) cores by covalently linking the bidentate nitrogen-donor ligands [phenyl(pyrrolato-2-ylmethylene)amine] to a polymer support.[31] This has several advantages: firstly, deactivation through dimerisation becomes impossible; secondly, the reactivity can be controlled to a much higher extent than is possible with dissolved and diffuse systems, allowing that, thirdly, even water, just as in nature, could be used as oxygen source for the OAT to molybdenum(IV). In this way, a truly functional model involving all biologically relevant molybdenum species (Mo^{IV}, Mo^{V} and Mo^{VI}) in a rather stable form has been made available.

Our attempts to detect and characterise catalytic intermediates and to include PCET in the investigated reactions with our model systems as catalysts containing dithiolene ligands have been so far unexpectedly unsuccessful. Processing of the substrates occurs too fast to allow the detection of reaction intermediates, and the use of proton and electron donors led to decomposition. This shows that, without some kind of control and protection, that for instance a protein shell could provide, these catalysts are too reactive to allow investigation of key intermediates. Though probably rather difficult, further attempts in this field will consider modification of the dithiolene ligands with respect to steric hindrance (i.e. slowing down) of the catalytic reaction or interaction with the substrate (for instance, hydrogen bonding) to bind it more strongly to allow isolation of the sought-after intermediates.

Interpreting Spectra

Since the molybdenum- or tungsten-dependent enzymes are typically mononuclear (i.e., containing the respective metal only once, in contrast to, for instance, iron, which is quite often present more than once in a metalloprotein) metal-specific spectroscopy can be used to gain detailed insight into the geometric and electronic structure of the active site. This requires, however, the spectroscopic data to be interpreted correctly on the basis of some understanding of the active-site structure that has already been developed. Model compounds in this respect are extremely valuable

tools for the interpretation of complex enzyme spectra, in particular when it comes to subtle differences.

One example of an early and remarkably successful comparative study of model compounds and milk xanthine oxidase allowed the interpretation and consequent assignment of different enzyme EPR spectra to specific active-site species.[32] The observed EPR spectra of milk xanthine oxidase varied depending on the isolation and subsequent reduction methods to obtain the enzyme in its d^1 Mo^V oxidation state. The spectra comprised characteristic signals, and the respective species were named after these typical signals: Very Rapid, Rapid Type 1, Rapid Type 2 and Slow. By employing a ligand system that has only limited similarity with mpt but which is reliably attached and flexible enough [LH_2 = *N*,*N*′-dimethyl-*N*,*N*′-bis(2-mercaptopheny1)-1,2-diaminoethane (Figure 10)], the metal core could be effectively modified with respect to the one- and two-atom ligands that were believed to be involved in milk xanthine oxidase catalysis (O, S, OH, SH). A careful examination of the respective EPR spectra including the definition of ^{95}Mo and ^{33}S hyperfine matrices from multifrequency EPR experiments finally allowed the unambiguous assignment of [$Mo^VO(SH)$], [$Mo^VO(SH)(OH)$] and [$Mo^VO(OH)$] cores as being responsible for the Rapid Type I, Rapid Type 2 and Slow EPR signals, and thus confirmed previous tentative assignments. Compelling evidence that [Mo^VOS] caused the Very Rapid signal was obtained in addition. Since this latter and the Rapid Type 1 signal were implicated in the enzyme's turnover, this investigation not only allowed the definite assignment and interpretation of the enzyme's spectra but also further insight and understanding of the catalytic mechanism. The success of this study shows that, even with a comparably simple ligand system that does not necessarily mimic the natural coordination environment of the active site, it is certainly possible to acquire important and relevant information. One just needs to tailor the properties of the compounds to the requirements of the issue that is to be addressed. With today's available theoretical and spectroscopic methods and the already gathered knowledge about the active-site chemistry, this kind of study may have become less important recently, but this could easily change with the discovery of an as of yet unknown enzyme of this type.

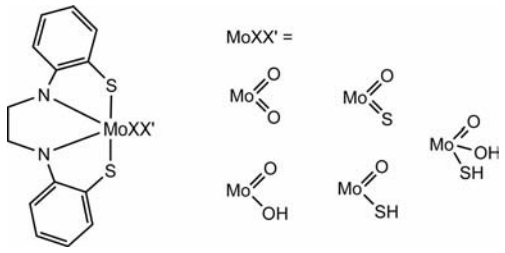

Figure 10. Chemical structures of the complexes that were used for identifying the xanthine oxidase species responsible for distinct ERP spectra.

Evolution

The distribution of molybdenum- and tungsten-dependent enzymes throughout distinct habitats appears to be based on the evolution of the respective environments. Tungsten is found in archaea, which were among the first organisms to populate the earth, and with only very rare exceptions, these organisms still live in confined surroundings that resemble the conditions on the early earth, that is, high to very high temperatures with high sulfur or sulfide concentrations. Molybdenum-dependent enzymes, in contrast, are used by organisms that live in habitats with (from our point of view) normal temperatures or moderately high temperatures and with, in general, typical (for today) sulfur concentrations. Consequently, there are two issues involved when discussing the abundance and distribution of molybdenum- and tungsten-dependent enzymes. Firstly, tungsten-dependent enzymes are obviously older than those utilising molybdenum. Secondly, molybdenum seems to be used preferentially, as the majority of these enzymes have been switched to it. As indicated above, the conditions of thermophilic habitats and of the early earth involve high temperatures and high sulfur/sulfide concentrations. Such an environment promotes the precipitation of the molybdenum present as hardly soluble molybdenum disulfide. Soluble molybdenum is therefore scarce in these surroundings. Tungsten, in contrast, keeps its oxidation state VI and is available as soluble $WO_xS_{4-x}^{2-}$ species.[33] Subsequently, molybdenum was not mobilisable when these enzymes first evolved, while tungsten was. This explains why tungsten was used first and why it is still used in habitats where molybdenum is significantly less abundant than tungsten. This abundance-based explanation of the distribution of molybdenum and tungsten cofactors settles the first issue and is commonly accepted today. However, it is not possible to take one enzyme, remove tungsten and replace it with molybdenum without compromising activity.[34] So, from nature's point of view, the change from one metal to the other required costly adjustments of the protein to accommodate and complement the new metal. This brings us to the second issue: what is it that makes molybdenum preferential, prompting the change to it wherever it is available, though both metals are very similar and perform basically the same tasks? To answer this, we hypothesised that temperature, in combination with redox potential, may play a role here. Consequently, an extensive, comparative, temperature-dependent electrochemical study was undertaken on twelve pairs of molybdenum and tungsten compounds. Starting with actual models for the enzymes (AO from the DMSOR family, mainly) utilising dithiolene ligands, the set of compounds was soon expanded to include also very simple, some strictly inorganic as well as organometallic complexes.[12,35] The results quite surprisingly showed that tungsten's redox potential is generally more temperature-sensitive than molybdenum's (Figure 11). Not one exception to this rule was observed. This in itself is already an advantage for molybdenum-dependent enzymes, since they are able to provide more stable catalytic conditions than their tungsten

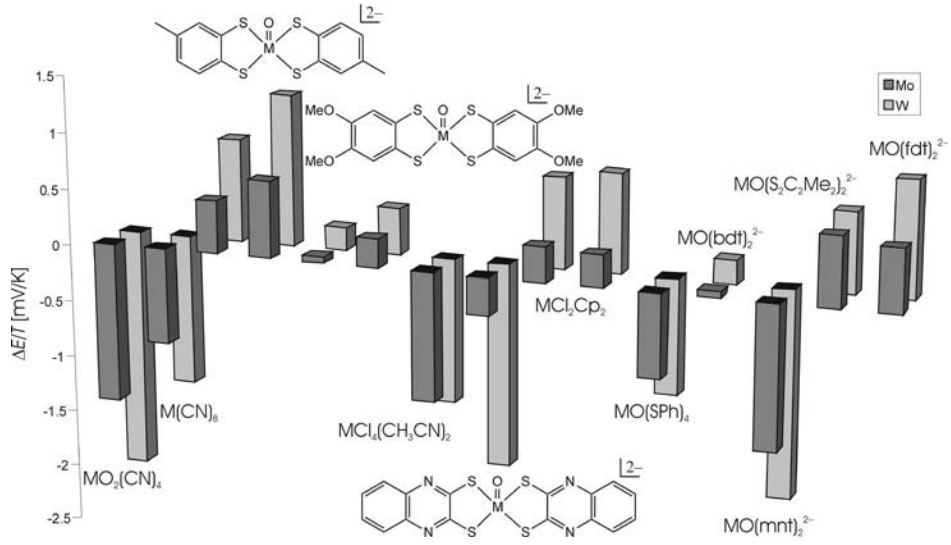

Figure 11. Measured gradients for temperature-dependent redox potential changes (ΔE/T in mV/K) for twelve investigated pairs of molybdenum and tungsten compounds. M = Mo, W; Ph = phenyl; Me = methyl; Cp = cyclopentadienyl; for bdt see Figure 2a, for fdt Figure 3a. The tdt and vdt complexes have been investigated for both oxidation processes ($M^{IV} \leftrightarrow M^{V}$ and $M^{V} \leftrightarrow M^{VI}$): columns three and four and five and six, respectively. The MCl_2Cp_2 pair has been measured in two different solvents: columns nine and ten.

counterparts in environments that are subject to temperature changes. But there is even more to it. The change of redox potential is directly related to the entropy change of the reduction. A larger temperature dependence of the redox potential also means a larger entropy change, which is, in other words, a larger change in the degrees of freedom. This again is related to a larger change in geometry around the active-site metal, which certainly disadvantageously increases the energies required for changing from one state to the other in the active-site pocket of an enzyme. This is because a change of the inner shell geometry is necessarily accompanied by a reorganisation of the outer shell geometry in particular for, but not limited to, active sites in which the metal is directly coordinated by an amino acid residue of the protein. The reasons for this distinction between molybdenum and tungsten is thought to be related to the higher relativity of the tungsten atom allowing its less tightly bound valence d orbitals to be influenced to a larger degree by external stimulation. Though still a lot of work from different disciplines is required to understand this phenomenon in more detail, at least it provides a reasonable explanation why an already well working team of protein and tungsten was changed, particularly considering that this required quite some protein modification effort to accommodate and complement the new metal well enough to restore activity. The slightly higher abundance of molybdenum at ambient conditions would, in our opinion, not justify a complete turn to it wherever it is available but an energetic optimisation of the redox processes involving geometric change should indeed be a significant enough driving force.

Conclusion

To conclude this microreview, it can certainly be said that much has been achieved regarding the understanding of nature's reason to generate the active sites of the molybdenum- and tungsten-dependent oxidoreductases with all their common and distinct functional groups and properties. But there is also still so much to do for a bioinorganic chemist interested in this field (and the so far not created models for specific sites in specific oxidation states have not even been discussed here) who is willing to accept the challenge that much more elaborate models than those currently available pose. This exciting field will without doubt keep all of us involved quite busy for some time to come.

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