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COMMENTARY

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The coordination chemistry and function of the molybdenum centres of the oxomolybdoenzymes

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Abstract The nature of the catalytic centres of the oxomolybdoenzymes is considered with particular reference to the results of recent protein crystallographic studies. The different nature of these centres, with one or two molecules of a special pyranopterin (molybdopterin) ligating the metal through a dithiolene group, the presence or absence of a nucleotide appended to the phosphate of the molybdopterin AND the variation in the coordination chemistry at the metal render the term "THE molybdenum cofactor" meaningless and confusing. Rather, there is a series of such cofactors, related by the common denominators of a single molybdenum atom bound to the dithiolene group of the molybdopterin and, at some stage in the catalytic cycle, at least one terminal oxo group. This Mo(O)(molybdopterin) moiety is considered to be the metal-centred functional unit (McFU) of the oxomolybdoenzymes. Variations in the coordination chemistry and, therefore, the properties of the metal centre occur with the binding of other ligands, which can include: a terminal oxo or sulfido group, OH^- and/or H_2O group(s), a second pterin, and/or a serine, a cysteine or selenocysteine group from the polypeptide backbone of the protein. The role of molybdopterin is considered with particular reference to its potential involvement in the various redox processes necessary for the operation of the catalytic cycles of these enzymes; special attention is given to the possible cooperativity between formally metal-based and pterin-based redox processes.

Key words Oxomolybdoenzymes · Molybdopterin · Metal-centred functional unit · Protein crystallography

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This is a very exciting time in the development of our understanding of the nature and mode of action of the active sites of the oxomolybdoenzymes and their tungsten counterparts. Many (ca. 30) fertile years of preparative and reaction chemistry were inspired by the EPR and XAS studies of the oxomolybdoenzymes [1] and led to much novel chemistry, including clear demonstrations of the suitability of oxomolybdenum centres ligated by S- or S/N-donor chelates for oxygen atom transfer [2]. The EPR and XAS investigations of the oxomolybdoenzymes not only served to demonstrate that molybdenum was clearly at the heart of the catalytic process, but also indicated that, typically, one or two oxo groups and three to four sulfur atoms were bound to the metal.

Following the results of several protein crystallographic investigations [3, 4], we now have the nature of the molybdenum and tungsten centres of these enzymes in much clearer focus. A striking feature of these crystallographic studies is the confirmation of the essence of the proposals by Rajagopalan et al. [5–7], made on the basis of spectroscopy and degradative chemistry, concerning the nature of the special pterin (molybdopterin) bound to the metal in these enzymes. The major structural difference between the structure of the pterin proposed by Rajagopalan et al. and those determined to date crystallographically (Fig. 1) is that the crystal structures each identify a pyran ring fused to the pterin, whereas the original proposal of Rajagopalan et al. [5–7] involved a four-carbon side chain attached to the pterin with an alcohol function on C3 of the side chain. The tricyclic pyranopterin structure (Fig. 1a) is actually *two hydrogen atoms more oxidised* than the bicyclic tetrahydropterin structure of Fig. 1b (vida infra). It has now become common usage to designate the structure of Fig. 1a as "molybdopterin", and that practice will be followed in this commentary.

Originally, the name "molybdopterin" was coined to indicate that the pterin ligates molybdenum. However, the pterin also ligates tungsten, a fact emphasised by

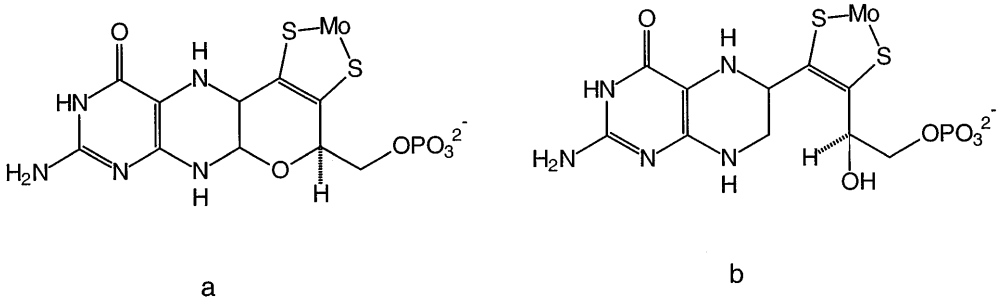

Fig. 1 **a** Structure of the pyranopterin found by protein crystallography [3, 4] and its mode of coordination to Mo (or W). **b** Tetrahydropterin structure and mode of coordination originally proposed for "molybdopterin" [7]. In some enzymes the phosphate group is replaced by a dinucleotide

the first structural characterisation of "molybdopterin" bound to tungsten in the aldehyde ferredoxin oxidoreductase from *Pyrococcus furiosus*, whose centre contains two pterins per tungsten [8]. An additional point of confusion is the difference between "molybdopterin", which contains no molybdenum, and the "molybdenum cofactor" (abbreviated Moco). These two terms are frequently used interchangeably, but the term "molybdenum cofactor" originally referred to the entity comprised of "molybdopterin", the Mo atom, and any other ligands (especially terminal oxo and sulfido groups) coordinated to the Mo atom. The recent protein structures of molybdenum and tungsten enzymes have now revealed the structures of their metal centres and the associated pterin(s). The different nature of these centres, with one or two molecules of molybdopterin ligating the metal (Fig. 1a), the presence or absence of a nucleotide appended to the phosphate of molybdopterin AND the variation in the coordination chemistry at the metal, renders the term "THE molybdenum cofactor" meaningless and confusing, and we suggest that this practice be abandoned. Rather, there is a series of molybdenum cofactors, related by the common denominators of a single molybdenum atom, bound to the dithiolene group of molybdopterin and, at some point in the catalytic cycle, at least one terminal oxo group. This Mo(O)(molybdopterin) moiety can, we believe, be regarded as the metal-centred functional unit (McFU, Table 1) of the oxomolybdoenzymes and should be regarded as analogous to the FeS_2Fe rhombohedra of the iron-sulfur clusters, the Fe-porphyrin core of hemes, and the $Cu(cys)(his)_2$ unit of Type 1 copper proteins [2]. Variations in the coordination chemistry and, therefore, the properties of the Mo centre occur with the binding of other ligands which can include: a terminal oxo or sulfido group, OH^- and/or H_2O group(s), a second pterin, and/or a serine, a cysteine or selenocysteine group from the polypeptide backbone of the protein. These considerations could also apply to the tungsten enzymes, but more structural studies will have to be accomplished to establish this perspective.

The protein crystallographic results [3, 4] clearly establish the pterin:metal ratio as one or two and confirm that, in the majority of the enzymes investigated, each molybdopterin binds to the metal by two sulfur atoms which are located on the pyran ring (Fig. 1a), a result consistent with the predictions of Rajagopalan et al. [5–7]. Although molybdopterin contains several other potential ligating groups, i.e. Lewis bases are present as part of the pyridone, pyrazine and pyran rings, the phosphate group and – for many cofactors – the dinucleotide, ligation of the metal by molybdopterin only involves the sulfur atoms of its dithiolene unit. The conformation of all of the molybdopterins identified crystallographically clearly shows that they are reduced, and it is now common to consider the pyrazine ring as being in its tetrahydro form (vide infra). Furthermore, all of the molybdopterins characterised by protein crystallography have the same absolute configuration, suggesting a common biological source [9]. The polar groups of the molybdopterin, the associated phosphate and, where present, the dinucleotide, are involved in an extensive series of bonding interactions with polar groups of the polypeptide. Collectively, these non-covalent bonding interactions serve to locate the metal cofactor within the protein matrix and, as is especially evident for the aldehyde oxido-reductase of *Desulfovibrio gigas* [4, 10], orientate the molybdopterin so that it could serve as a conduit for electron transfer to (and/or from) the catalytic centre.

Particularly interesting to biological inorganic chemists is the nature of the other ligands coordinated to the M(O)(molybdopterin) (M = Mo or W) centre and the overall coordination geometry at the metal. The crystal structure of the tungsten-containing aldehyde oxidoreductase from *P. furiosus* showed that the tungsten atom is coordinated by four sulfur atoms from two molyb-

Table 1 Some metal-centered functional units (McFUs) in bioinorganic chemistry

McFU	Occurrence	Function
Mo(O)(molybdopterin)	Oxomolybdoenzymes	(Net) oxygen atom transfer
$Cu(his)_2(cys)$	Blue copper proteins	Electron transfer
FeS_2Fe	Iron-sulfur clusters	Electron transfer
Co(corrin)	Coenzyme B_{12}	Diverse
Fe(porphyrin)	Heme proteins	Diverse

dopterins in what appears to be square-pyramidal geometry [8]. The structure also clearly showed that there are no coordinated amino acid residues from the protein. However, the large electron density from the tungsten atom precluded the identification of light atoms from other likely ligands in the coordination sphere, such as oxo or hydroxo groups or water molecules [3]. The X-ray absorption spectroscopic studies of George et al. clearly identified the presence of at least one terminal oxo group bound to the tungsten of this enzyme [11]. The crystallographic studies of the molybdenum-containing enzymes have identified other groups bound to the molybdenum in addition to the molybdopterin(s) [3]. However, by themselves the crystallographic results do not establish unequivocally the number and the nature of the other ligands in the coordination sphere of the metal, the oxidation level of the molybdopterin cofactor(s), and whether the metal is ligated by an ene-1,2-dithiolate (=dithiolene) or a 1,2-dithiolate group. Hence, it is vital that the protein crystallographic results are complemented by further detailed spectroscopic studies of the metal centres of these enzymes. XAS/EXAFS [1], EPR/ENDOR/ESEEM [12], UV/VIS/NIR absorption, CD and MCD and resonance Raman [13] and other spectroscopies are capable of providing important information concerning the nature of the catalytic centre for various states of the oxomolybdoenzymes and their tungsten counterparts. Furthermore, the development of improved chemical analogues for the catalytic centres for these metalloenzymes is crucial, not only to calibrate the information obtained from each of the spectroscopic techniques, but also to enable chemical and electrochemical studies to be accomplished which are relevant and complementary to studies of the function of the catalytic centres of the enzymes.

The structure of each of the molybdenum and tungsten centres identified by protein crystallography [3, 4] represents a challenge to the coordination chemist. In part, this challenge derives from the limitations imposed by the resolution of the site achieved in some of the crystallographic studies. This is an important perspective which means that not all of the coordination sphere of the Mo (or W) can be regarded as having been unambiguously characterised, and the metal-ligand bond lengths have an uncertainty of $>\pm 0.1$ Å [3]. Many of the ambiguities in the coordination about the Mo (or W) centres will be resolved by further protein crystallographic studies and complementary spectroscopic investigations, especially EXAFS measurements, which can generally provide very accurate bond lengths (± 0.02 Å). However, such high accuracy may not be possible for the M-S distances because the presence of multiple (2–4) M–S distances in these enzymes restricts the resolution of individual distances. The smallest resolvable difference by EXAFS for metal-ligand distances involving similar ligands is usually >0.10 Å [1]. Nevertheless, the coordination environments that have been deduced to date from the protein crystal structures have no precedent in presently known coordination chemistry of molybdenum and tungsten. This situation will not change as the structures of these metal sites are refined and, therefore, significant challenges remain for coordination chemists to reproduce the essence of these sites in their syntheses.

As an indication of the sophistication of the coordination chemistry at the catalytic centres of the oxomolybdenum enzymes, it is instructive to consider the present situation concerning the DMSO reductases. The structure of the molybdenum centre of "native" (presumed to be the oxidised, Mo(VI), form) DMSO reductase from *Rhodobacter sphaeroides* has been reported by Rees et al. [14] and that from *R. capsulatus* by both Huber et al. [15] and Bailey et al. [16, 17]. Although the structures of the proteins and the locations of the two pterins (P and Q) are essentially the same in each case, the coordination at the molybdenum is significantly different in the three structures. Of these three structures, the structure of the molybdenum centre proposed by Bailey et al. (Fig. 2), complemented by the Mo K-edge EXAFS studies of Baugh et al. [18], appears to be the most straightforward, because all four S atoms of the two molybdopterins are clearly bonded to the molybdenum. The metal's coordination environment is comprised of two essentially symmetrically bidentate dithiolene ligands with Mo-S 2.32–2.47 Å, an oxo group with Mo=O 1.71 Å, an oxo or hydroxo group with Mo-O 1.92 Å, and a serine oxygen with Mo-O 2.27 Å; the geometry of the seven-coordinate site can be approximated to that of a capped trigonal prism in which the oxo group (at 1.71 Å from Mo) caps a rectangular face. Figure 3 presents an idealised depiction of this environment. However, such a centre has no precedent in molybdenum coordination chemistry, especially because there are no examples of seven-coordinate bis-dithiolene complexes. Furthermore, the donor atom distribution of this site is notable, with all four S atoms approaching the metal from the same side, a situation directly comparable to that for tungsten in the aldehyde oxido-reductase of *P. furiosus* [8].

The MoOS(molybdopterin) centre of the oxidized form of the aldehyde oxido-reductase from *D. gigas* [4,

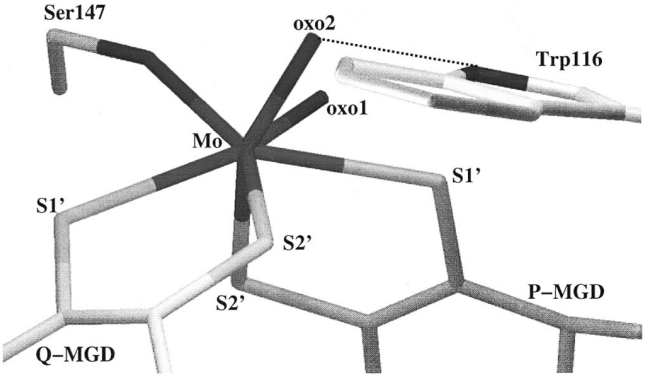

Fig. 2 The structure of the molybdenum centre of native DMSO reductase from *Rhodobacter capsulatus* [17]

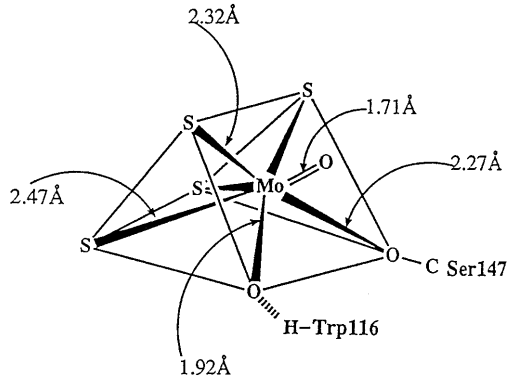

Fig. 3 A representation of the coordination environment of the molybdenum centre of native DMSO reductase from *Rhodobacter capsulatus* [18]

10], a representative member of the xanthine oxidase family [19], poses a synthetic challenge because of the tendency for high-valent molybdenum sulfido compounds to undergo internal redox reactions [20].

Beyond the difficulties of defining the structure at the metal centres for particular states of the oxomolybdoenzymes and their tungsten counterparts lies the considerable challenge to achieve a clear description of how the nature of each of these metal centres changes during the operation of the enzyme's catalytic cycle [21]. At the centre of these considerations is the relationship between the atomic arrangements at these novel molybdenum (and tungsten) centres, their electronic structures, and how changes in both of these relate to the catalytic reactivity. The most detailed information about the electronic structures of the Mo centres can be obtained from studies of the paramagnetic Mo(V) state by EPR/ENDOR/ESEEM [12] and/or MCD spectrocopy; however, the Mo(VI) and Mo(IV) states are most easily generated for protein crystallography studies. If the geometry of the Mo(V) states is nearly congruent with the Mo(VI) and/or Mo(IV) forms, then the protein crystal structures will provide a firm foundation for: (a) interpreting the ligand field at the metal, (b) elucidating the nature of the HOMO and LUMO of the metal centre, (c) interpreting the location and orientation of substrate binding, (d) interpreting pathway(s) for electron transfer to or from the metal centre.

We proposed above that the MoO(molybdopterin) entity is the metal-centered functional unit (McFU) for oxomolybdenum enzymes. This McFU (Table 1) must carry out two key functions: (a) (net) oxygen atom transfer (inner sphere electron transfer) to (or from) the substrate, and (b) outer-sphere electron transfer to regenerate the active catalytic centre. Function (a), the (net) O-atom transfer reaction, is somewhat analogous to reactions of cytochrome P450. For oxomolybdoenzymes, the additional groups that are coordinated to the McFU [MoO(molybdopterin)] will depend, at least in part, on the thermodynamics and kinetics of the reaction being catalysed. Function (b), on the other hand, requires another redox partner such as an iron-sulfur centre, a flavin, or a heme, which often occurs within the same protein. The W- and Mo-containing aldehyde oxido-reductase structures both have iron-sulfur centres positioned to undergo electron transfer reactions with the molybdopterin fragment of the McFU [4, 8, 10]. For enzymes such as DMSO reductase, which possess no other prosthetic groups but which have two molybdopterins per metal, the second molybdopterin may act as a "cofactor" or "second prosthetic group" in the electron transfer pathway of the McFU, the Mo(O)(molybdopterin) centre. The structural variations in the binding of molybdopterin Q between oxidised and reduced DMSO reductase found by Rees et al. [14] could be indicative of such behaviour. A direct view of oxygen atom transfer at the molybdenum centre of DMSO reductase has been obtained by Bailey et al. [16, 17] and Baugh et al. [18]. These workers investigated native DMSO reductase reduced – to the Mo(IV) level – by the addition of dimethylsulfide (DMS) and have achieved the first structural characterisation of a substrate-bound form of an oxomolybdoenzyme. With reference to their structural characterisation of the molybdenum centre of the oxidised form of this enzyme given above, addition of DMS forms a DMSO molecule which is bound to the metal at the site of the oxo (or hydroxo) group with an Mo-O distance of 1.91 Å. This oxygen-atom transfer reaction takes place with virtually no other structural change occurring at the molybdenum centre. Thus, it would appear that, in this enzyme, the unusually long "terminal" Mo-O bond (at ca. 1.9 Å) is poised for atom transfer (either to or from the substrate) in a manner which may be compared directly with the oxygen-atom transfer chemistry accomplished by Holm et al. [2]. However, the protein crystallographic results of Bailey et al. [16, 17] show that H-bonding, notably to an adjacent tryptophan group (Fig. 2), is responsible for the lengthening and presumed weakening of the "reactive" Mo-O bond; such activation of an oxo-group for atom transfer is not readily realised in small-molecule chemistry.

Catalysis achieved by simple oxygen-atom transfer at an oxomolybdenum (or tungsten) centre, although appealing, would appear to ignore the chemical versatility present within the ubiquitous ligand of these centres, the molybdopterin. Although the oxidation state of the molybdenum (or tungsten) in an enzyme can, in principle, be determined spectroscopically, especially from the position of the Mo K-absorption edge [1] or the manifestation of an EPR signal characteristic of Mo(V), such measurements do not address the oxidation level of the ligands bound to the metal centre. As Young has clearly documented [20], metal-assisted sulfur-redox chemistry can be facilitated at molybdenum and tungsten centres, and such behaviour augments any metal-based redox processes. In addition to these considerations, it is important to note that the molybdopterin bound to molybdenum or tungsten (in their re-

spective families) also has the potential to become involved in the redox processes which take place at these catalytic centres.

Inorganic chemists have long recognised the "non-innocent" nature of dithiolene ligands, since the SC=CS group can adopt different oxidation levels, with consequent changes in the intraligand C-S and S···S interactions [22]. However, the oxidation state of a metal in a dithiolene complex is generally assigned on the basis that each dithiolene ligand has a charge of 2–. This conclusion has received support from ^{95}Mo and ^{183}W chemical shifts of neutral tris(dithiolene) complexes, i.e. the metals have been effectively stripped of their valence electrons which are placed on the ligands [23]. Nevertheless, protein crystallographic results have shown that the S···S distances of the molybdopterins vary as the oxidation level of the protein changes [3, 4]; hence the redox process of the oxomolybdoenzymes must not be regarded as exclusively metal based. In addition, the planarity of the metal-dithiolene MS_2C_2 ring produces an "aromaticity" [24] which could allow the dithiolene ring to be a conduit for the electronic coupling of the metal centre to the pterin nucleus. Such coupling will be especially favoured if the adjacent carbon atom can assume sp^2 hybridisation, since this would allow conjugation of the metal-dithiolene ring to the pyrazine ring [25].

The pyrazine ring of a pterin can adopt three different oxidation levels: tetrahydro, dihydro, and fully oxidised. These levels correspond to four, two, or no hydrogen atoms bound to the pyrazine ring or tautomers of these forms. The protein crystallographic results imply that the pyrazine ring of the molybdopterin is fully reduced and electronically equivalent to a tetrahydropterin (Fig. 1). However, there is a subtle aspect of the pyranopterin system (Fig. 1a) that requires special mention. Opening the pyran ring (Fig. 4) yields a dihydropterin, and, formally, represents a two-hydrogen-atom oxidation of the pterin nucleus; the reverse reaction represents a two-hydrogen-atom reduction of the pterin nucleus. Thus, in Fig. 1, the tricyclic pyranopterin structure (Fig. 1a), which has a saturated central ring, is actually *two hydrogen atoms more oxidized* than the bicyclic tetrahydropterin structure with a side-chain alcohol (Fig. 1b) that was originally proposed for "molybdopterin" by Rajagopalan et al. [7]. However, subsequent oxidative studies of xanthine oxidase and sulfite oxidase by Gardlik and Rajagopalan indicated a dihydro oxidation level for the pterin [26]. This result is consistent with both the pyranopterin structure found from protein structures [3, 4] and with ring-opened dihydropterin structures (Fig. 4), because they all have two H atoms bound to carbon atoms of the pyrazine ring. The organic component of the molybdopterin could be involved in the handling of the protons which are obligatorily involved in the (net) oxygen atom transfer process of the overall general catalytic reaction:

$$X + H_2O \rightleftharpoons XO + 2H^+ + 2e^-$$

We note that all of the basic sites of the pterin are involved in the extensive network of hydrogen bonding interactions in the protein structures (vida supra) [8, 10, 14, 15, 17], and this network could provide a route for facile proton diffusion to(or from) the pterin moieties. The chemical properties of pyranopterins have been little studied to date, but there is evidence for lability of the acetal bond [27, 28]. In principle, several isomers are possible for the ring-opened dihydropterins [29]; those which have sp^2 hybridisation at the carbon atom attached to the dithiolene will have the pyrazine ring conjugated to the metal centre. Figure 4 shows how the opening of the pyran ring can produce this conjugation. Hence, it is fascinating to speculate about the role of the pyranopterin structure in these enzymes. Rajagopalan has proposed that the formation of the pyran adduct "stabilizes the pterin ring against oxidative modifications that could disrupt the structure of the cofactor" [30]. Another possibility is that scission and condensation reactions of the pyran ring (Fig. 4) are employed by the oxomolybdoenzymes and their tungs-

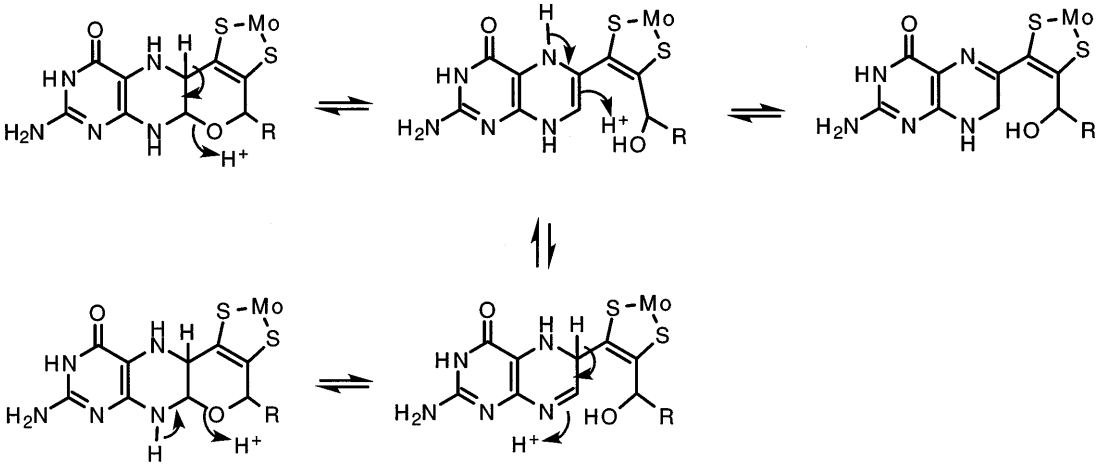

Fig. 4 A scheme illustrating scission/condensation of the pyran ring of the pyranopterin to generate a dihydropterin

ten counterparts during the operation of their catalytic cycles. Such scission/condensation reactions could conceivably provide a way of controlling the redox state of the entire McFU. If so, this could lead to important new perspectives of the mode of action of these enzymes.

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