General Introduction

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Aims and objective of this work

General Introduction

An outline about pterin-containing metalloenzymes is presented here, with the next section indicating the scope of research work on new pterin coordination compounds of molybdenum and a few first transition metals (Fe, Cu and Zn). The discussions include the current status of chemical approaches, the impact of x-ray structural data on enzymes, spectroscopic techniques and molecular modeling methods in giving clear descriptions of pterin coordination compounds in terms of molecular structures, electronic structures as well as correlation of structures with reactivity. Pterins (2-amino-4-oxidopteridines) are ubiquitous in nature including different classes of metalloenzymes containing molybdenum or tungsten or iron (non heme or heme type). For such enzymes the redox non-innocent nature of pterin is reciprocated by the ability of the associated transition metal ion in displaying multiple oxidation states. Their functional aspects need the tacit assumption that the redox processes at the metal center should be linked to the changes in the pterin / pyrazine ring oxidation level [34]

Rajagopalan, Johnson and co-workers found that molybdenum cofactor is extremely unstable and that the molybdenum atom is readily lost upon release of the cofactor from the protein. They proposed that the molybdenum cofactor contains a reduced pterin derivative that can bind molybdenum through the sulphur atoms of a dithiolene side chain [89,23c].

The synthetic analogue or model approach can provide insights into complex biomolecules through the design, synthesis and study of small molecules that mimic a component, typically an active site or prosthetic group, of the biomolecule. The approach is particularly valid for metal active sites where key chemical or spectroscopic information is required for the interpretation of the properties of the biomolecule. Valuable reviews and publications have enriched different aspects of our knowledge about Mo- or W- containing oxo-transferases, thereby providing guidelines for modeling strategy to be followed by synthetic bioinorganic [1a,1g,8a,9,13,27,33,34,72,89,77,78,87,91,97,103,107, 108].

An interest in the structure, function and applications of Mo and W enzymes serves as a platform for assimilation of ideas from different areas like bioinorganic chemistry, enzymology,

microbiology, biochemistry, crystallography and spectroscopy. Design and synthesis of small molecules as analogues (structural/functional/spectroscopic) of such enzymes is a challenging aspect of such studies; determining intrinsic geometries, electronic and reactivity properties is an integral part of such studies. The relevant biomolecules are essentially highly elaborated metal complexes equipped with the necessary protein structure for complementarity and all other aspects of function. Their structural and electronic properties are often modulated from those of small molecules (or analogues as above) containing the same metal ions (e.g., Mo/W). Understanding these differences is essential to understanding function and the optimal starting point for this endeavour is the crystal structures of the metallobiomolecules. From 1995 onwards many of the relevant enzymes have been defined x-ray crystallographically at atomic resolution.These structural results supplement the earlier EXAFS data effectively and affirm the reliability of such spectroscopic data. The enzymologists determine equilibrium and rate parameters and endeavors to relate function to structure as well as throwing light on the mechanistic aspect of the relevant catalytic cycles.

The above aspects converge to the central point of such bioinorganic research: **definition of function in terms of structures, centered around biological oxygen atom transfer involving Mo and W.** Such metals are wide spread in biology and participate in most of the reactions associated with environmental cycling of the fundamental elements of life: carbon, oxygen, sulfur and nitrogen.

A.Oxo-transferases containing molybdenum or tungsten

Natural selection of Mo/W: two aspects are to be considered.

i) Abundance

The biological importance of elements (either in microorganisms or in higher life forms) may be related largely to their natural abundance. The water solubility of Na_2MoO_4 helps to maintain an optimum concentration of Mo in oceans and its uptake for essential biological functions.

Some archaea (organisms which are believed to have evolved in pre-oxygenic times) include the hyperthermophiles that are able to survive at temperatures in excess of 100°C. These organisms are found in deep sea hydrothermal vents and terrestrial hot springs and are good sources of enzymes that contain W, the heaviest element known to be essential to life. The finding that W, Co and for the most part Ni are used only by more primitive life forms probably

reflects their special role in the early stages of evolution. For example, the use of cobalt by higher organisms is essentially restricted to cobalamin.

ii) Functional aspect

Apart from natural abundance, functional aspects also need attention for understanding the biological roles for Mo and W. The Frost diagram of the group VI elements (Cr, Mo and W) throws light on this aspects [Fig. (I-1)].

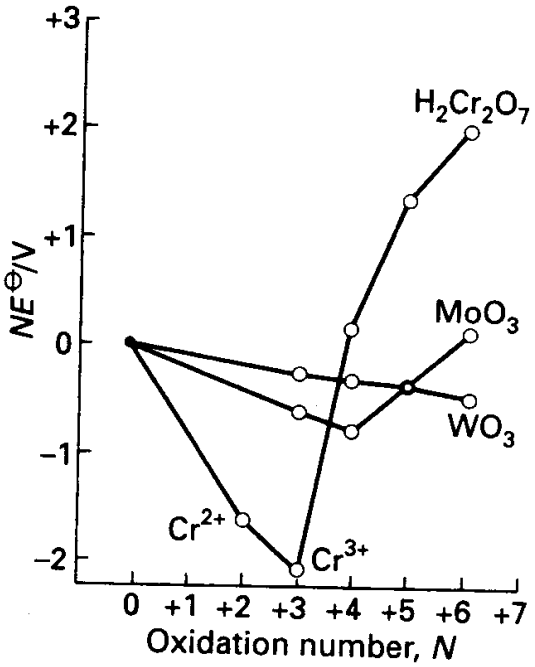


Fig. (I-1): A Frost diagram for the chromium group in the d-block (Group 6) in acidic solution (pH=0)

For the biologically relevant oxidation states (IV, V, VI) of Mo and W, the variation of redox potentials is restricted to a limited zone, close to those of biological electron carriers like NAD, FAD , etc. For Cr, a wider range of variation of redox potentials is observed, especially for the higher (V, VI) oxidation states. Organisms in most cases find chromium too difficult to oxidize from the trivalent (III) state and tungsten too hard to reduce from the hexavalent (VI) state.

The strong tendency of Mo to bind oxo groups (=O) is balanced by a capacity to lose a single oxygen atom easily, as needed by the proposed catalytic cycle of oxomolybdoenzymes. Apart from the formal oxygen atom transfer (to or from the substrate) process, the one- and two-electron transfer capability associated with Mo and its ability to couple ion (H^+ or O^{2-})transfer

with electron transfer may be crucial to the chemical role played by Mo in enzymes and its preferential selection for higher life forms as compared to W.

This class of enzymes catalyze hydroxylations and net oxygen atom transfer reactions (OAT) to and from a variety of biologically important substrates [1a,1g,8a,9, 13,27,33,34,72,89,77,78,87,91,97,103,107, 108]..

Hydroxylation : $RH + H_2O \rightleftharpoons ROH + 2H^{\oplus} + 2e^{\ominus}$

Net oxygen atom transfer : $X + H_2O \rightleftharpoons XO + 2H^{\oplus} + 2e^{\ominus}$

These redox active enzymes catalyse key reactions in the metabolism of carbon, nitrogen and sulphur; while molybdenum is essential for almost every life form, tungsten is proved to be essential for microorganisms, the hyperthermophilic archaea, which thrive near 100°C (hydrothermal vents on the ocean floor). So far more than three dozen oxomolybdoenzymes have been identified, in addition to over a dozen tungstopterin enzymes [1e,64].

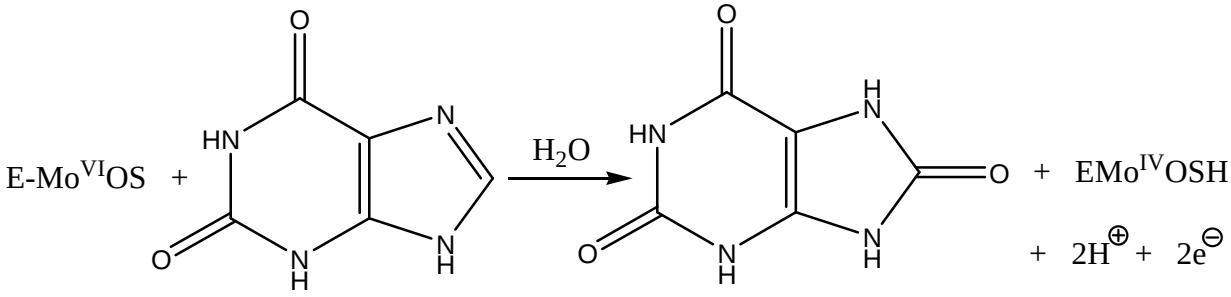
Nitrate reductase

$$E\text{-}Mo^{IV}O + NO_3^{\ominus} \longrightarrow E\text{-}Mo^{VI}O_2 + NO_2^{\ominus}$$

Sulphite oxidase

$$E\text{-}Mo^{VI}O_2 + SO_3^{2\ominus} \longrightarrow E\text{-}Mo^{IV}O + SO_4^{2\ominus}$$

Xanthine oxidase



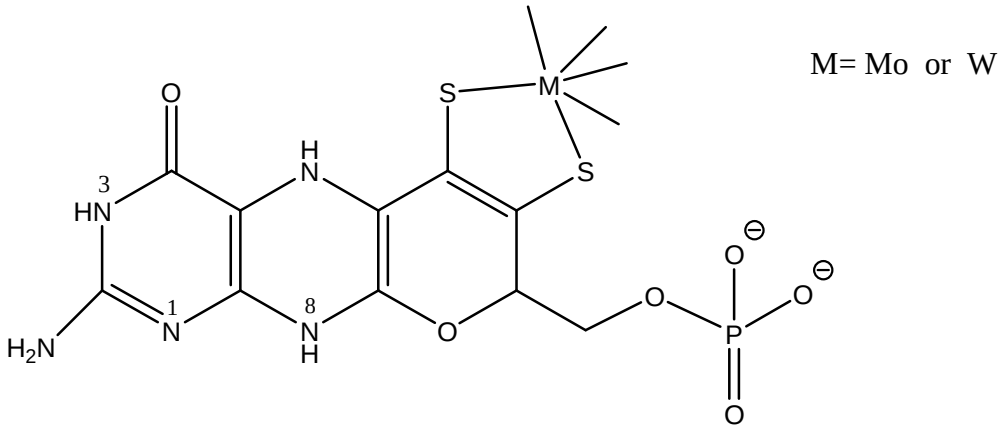
Xanthine Uric acid

E = enzyme without the metal centre

Scheme (I-1)

The above reactions mostly involve two-electron redox chemistry coupled to the transfer of an oxygen atom to or from water. During the catalytic cycle, the molybdenum / tungsten centre cycles between VI and IV oxidation states.

Pteridines are fused ring nitrogen heterocyclic compounds found as the core structures of folic acid and flavin adenine dinucleotide (FAD). The 2-amino-4-oxo substituted pteridines found in nature have the trivial class name pterin. The ene-dithiolate groups located on the pyran ring which is fused to the pterin have the name pyranopterin [scheme (I-2)]. Protein x-ray crystallography has revealed the structures of the active sites of many of these enzymes, in each of them the active site consists of either a molybdenum or tungsten atom coordinated by two sulfur atoms of one (or two) ene-dithiolate group.



Scheme (I-2): Structure of pyranopterin found by protein crystallography and its ene-thiolate mode of coordination to a metal atom (M= Mo or W]. In some enzymes the phosphate group is replaced by a dinucleotide.

The active sites of oxo-molybdenum and oxo-tungstenenzymes are further differentiated from one another by the number of terminal oxo and/or sulphide groups, OH and/or H_2O and by coordinated amino acid residues e,g., a cysteine sulphur in sulfite oxidase, serine oxygen in dimethyl sulfoxidereductase or selenocysteine selenium in formate dehydrogenase from the polypeptide backbone of the protein.

Few interesting aspects of the x-ray crystallographic results may be stated here:

(i) the 2:1 pterin : Mo ratio found in dimethylsulfoxidereductase [DMSO reductase];

(ii) the oxidized form of DMSO reductase possesses only one oxo-ligand in the Mo(VI) state;

(iii) the tricyclic form of the pterin, i.e., the pyranopterin structure is observed in every oxo-transferase structure;

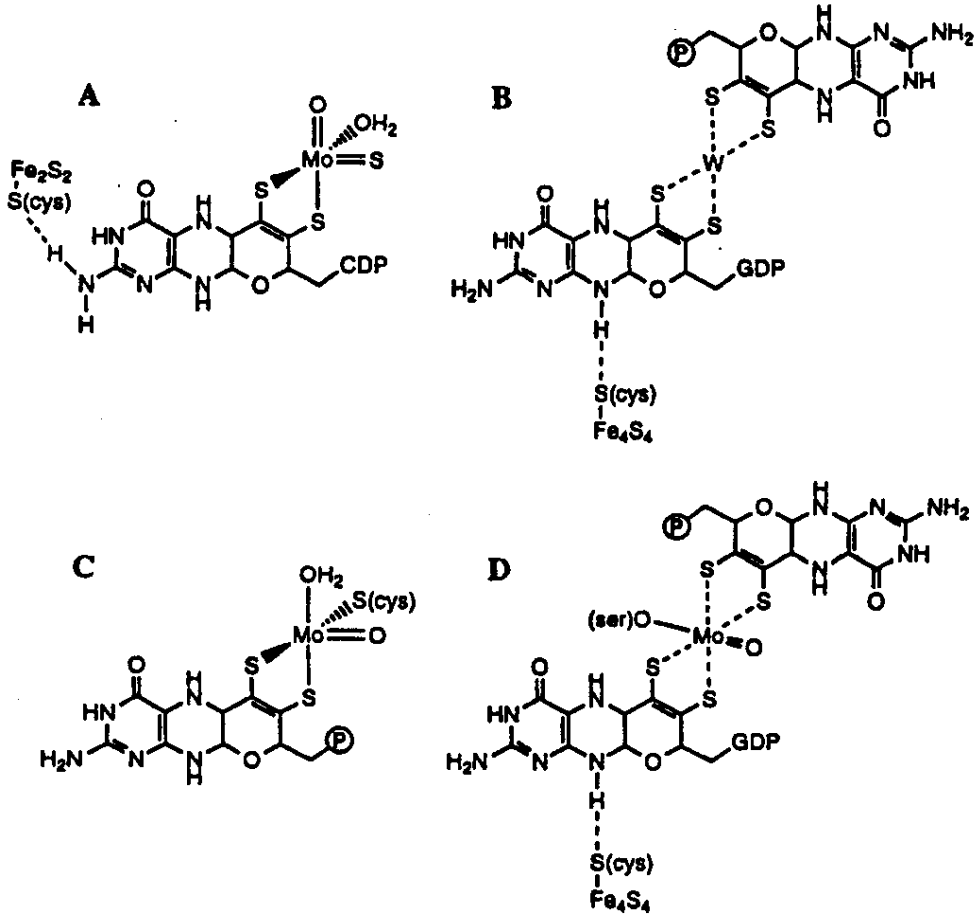


Fig.(I-2): The molybdenum site of several molybdoenzymes as determined by x-ray Crystallography: Structure **A** is the cofactor as seen in *Desulfovibriogigas* aldehyde oxidoreductase; structure **B** is the tungsten site from *Pyroccocusfurious* aldehyde oxidoreductase; structure **C** is the molybdenum site from chicken liver sulfite oxidase; structure **D** is the oxidized cofactor in *Rhodobactersphaeroides*dimethylsulfoxidereductase.

(iv) in some cases the pterin is observed to link the molybdenum (or tungsten) center to remote iron-sulphur clusters by hydrogen bonding through a pterinyl NH to a cysteinylsulphur coordinated to Fe (Fig. (I-2)]. The pterin effectively “hard-wires” the

metal center to other electron transfer prosthetic groups, facilitating electron flow out of the enzyme to the external acceptor. Finally, there are enzymes where the additional electron transfer group (e.g., heme) is far removed from both the pterin and the Mo atom, or, other enzymes without any direct linkage to additional prosthetic groups.

Functional aspects (catalytic / mechanistic) of oxomolybdoenzymes

The pterin- containing molybdenum enzymes possessing mononuclear active sites constitute a relatively large class of enzymes that can be divided into two categories on the basis of the reaction catalyzed.

1. Member of this large family of enzymes catalyze the oxidative hydroxylation of a diverse range of aldehydies and aromatic heterocycles in reactions that necessarily involve the cleavage of a C-H bond (e.g., xanthine oxidase). These enzymes are properly considered as hydroxylases, although product tautomerization in reactions involving heterocyclic substrate usually results in the keto rather than enol from predominating in aqueous solution.

2. The second category includes enzymes that typically catalyze proper oxygen atom transfer reactions to or from on available electron lone pair of substrate and can itself be subdivided into two fractions:

(a) the first includes well-known enzymes such as sulfite oxidase and the assimilatory nitrate reductases (i.e., those enzyme transfer whose physiological function is to reduce nitrate to nitrite in the first step of its reduction to ammonia for utilazition by the cell.)

(b) the second is a family made up of bacterial enzymes such as DMSO reductase and biotin-S oxidoreductase, as well as the bacterial dissimilatory (or respiratory) nitrate reductases: those membrane-associated enzymes that function as terminal respirator oxidases.

From a simple spectroscopic standpoint, the distinguishing features that sets the DMSO reductase family apart from that exemplified by sulfite oxidase, in the presence of a long-wavelength charge transfer band (λ_{max}> 700nm) in the electronic spectrum of the molybdenum centers of the former enzymes.

General active site structures of the three above-mentioned families of mononuclear molybdenum enzymes [e.g., xanthine oxidase (XO), sulfite oxidase(SO) and DMSO reductase) are shown below.

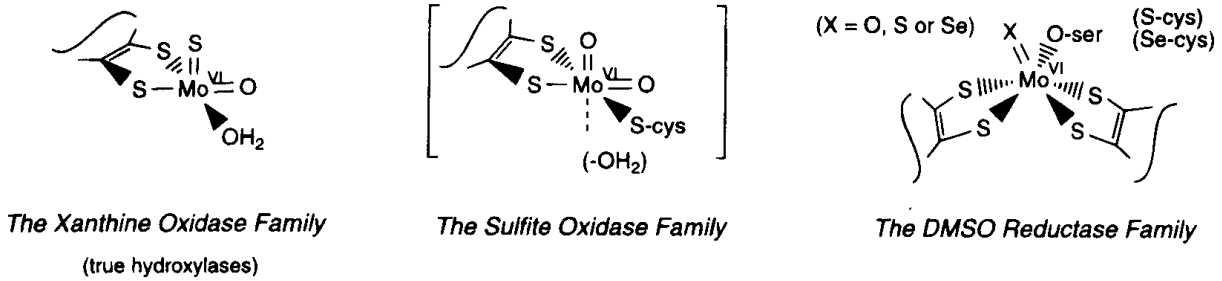


Fig. (I-3): The major families of mononuclear molybdenum enzymes. Members of the xanthine oxidase family (i.e., the true hydroxylases) have molybdenum centers consisting of a single cofactor dithiolene ligand coordinated to a *fac*MoOS(H_2O) unit. Members of the sulfite oxidase family are likely to possess a single cofactor dithiolene coordinated to a *cis* MoO_2 unit (additional coordination position may be taken up by water and/or a cysteine residue that is conserved within the family). Members of DMSO reductase family are distinguished by bis dithiolene coordination of the molybdenum, but this group of enzymes is likely to be more structurally diverse than the other two families. The Mo=X position may be taken up by oxygen, sulfur, or (possibly) selenium, while the sixth ligand coordination position may be occupied by serine, cysteine, or selenocysteine [13b].

Distinguishing core of different enzyme classes:

Apart from the ene-dithiolate pyranopterin coordination mode and the coordinated ancillary ligands (e.g., H_2O, cysteine, serine, etc), one or two atoms (O, S, or Se) multiply bonded to the Mo atom, characterize these active sites. A mixed oxosulfido core $(Mo^{VI}OS)^{2+}$, a dioxo core $(Mo^{VI}O_2)_2^{+}$ and monooxo /sulfide/ seleno core $(Mo^{VI}O/S/Se)^{4+}$ core embellish the mononuclear Mo active sites of the XO, SO and DMSO reductase families respectively. The last family of enzymes possesses an additional attribute of two pyranopterin-dithiolene coordination, instead of one for the other families.

Typical substrate reaction of these enzyme families are shown in Fig. (I-4).

Apart from the DMSO reductase family of enzymes which are of bacterial origin, most of the oxomolybdenumenzymes are quite complex with their full complement of iron-sulfur proteins, cytochromes, FAD, etc., in addition to the pyranopterin-molybdenum cofactor (Mo-co).

Hydroxylation: $RH + H_2O \rightleftharpoons ROH + 2H^+ + 2e^-$;

Net oxygen atom transfer: $X + H_2O \rightleftharpoons XO + 2H^+ + 2e^-$.

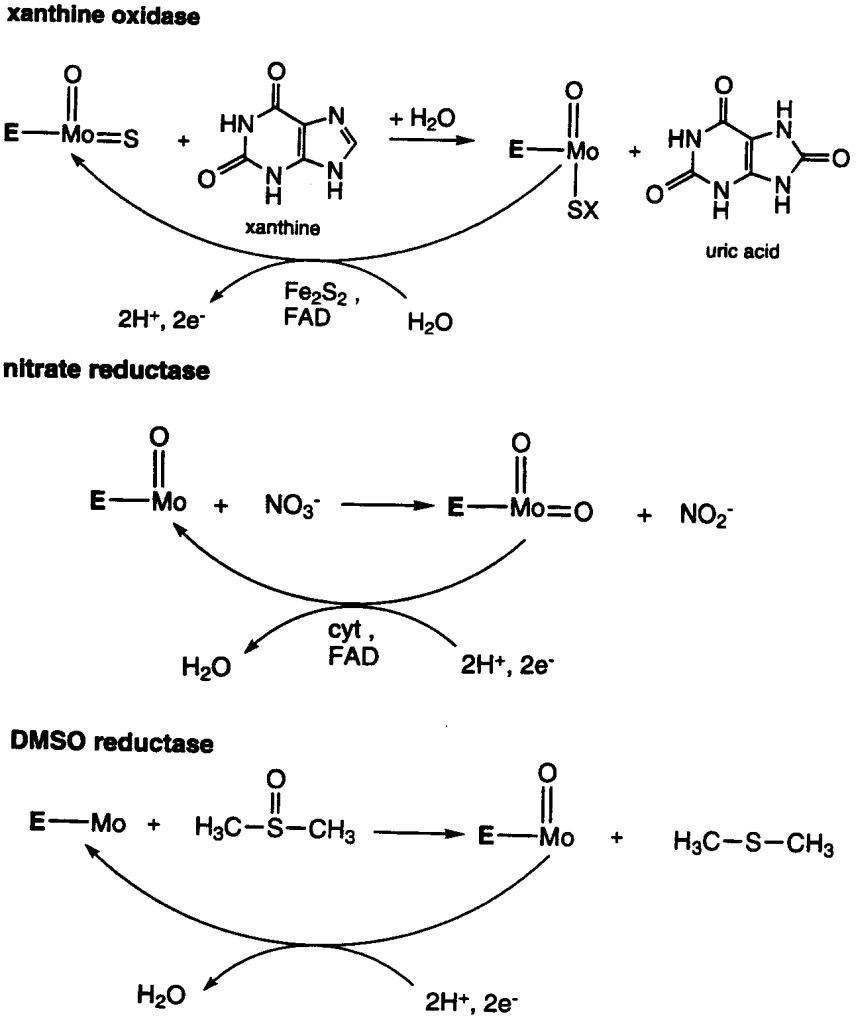
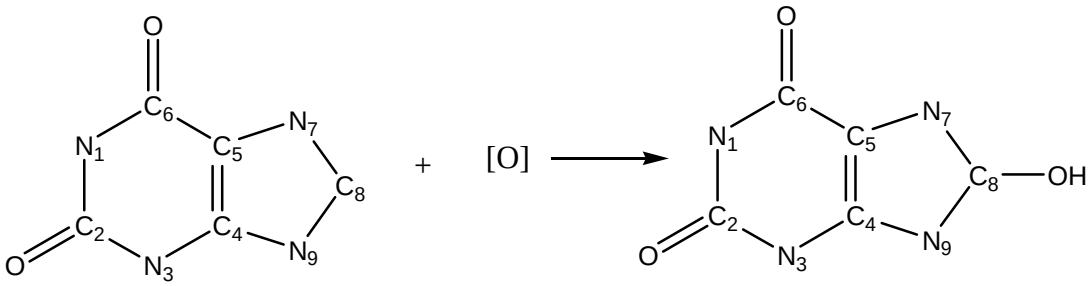


Fig. (I-4)

The intimate catalytic mechanisms proposed for either hydroxylation or oxygen atom transfer (vide later) involve the coordination of water to metal atom (M) to give M-OH_2, M-OH or M=O species which cycle between the M(VI) and M(IV) oxidation states. That is, protonation stage of the aqua ligand is related to the M(VI), M(V) and M(IV) oxidation states. Single turnover experiments on DMSO reductase and XO in $H_2{}^{18}O$ provide support for this hypothesis. The resting state of the enzyme is regenerated by two consecutive one-electron processes involving the prosthetic groups (FAD, haems and Fe-S centers) that usually accompany the Mo or W cofactor in these enzymes.

The different steps involved in the catalytic reaction of xanthine oxidase are summarized below. XO has come to represent the prototypical molybdenum hydroxylase because of its ease of

isolation in gram quantities from cow's milk and its high degree of stability under a variety of conditions. XO is a complex metalloflavoprotein containing in each of its two independent subunits a molybdenum center (containing 1 g atom of molybdenum), FAD and two 2Fe-2S centers, that is, a total of four redox centers. Xanthine is converted to uric acid at the molybdenum center, a reaction that formally involves abstraction of hydride from position 8 of the substrate followed by its replacements with hydroxide. Overall conversion is shown by Scheme (I-3).



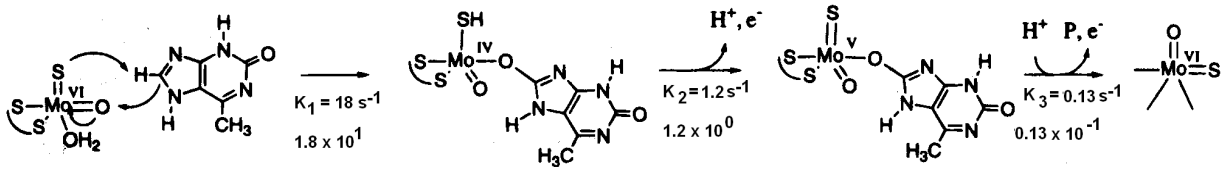
(2,6-dioxopurine) (8-hydroxy-2,6-dioxopurine)

Scheme (I-3)

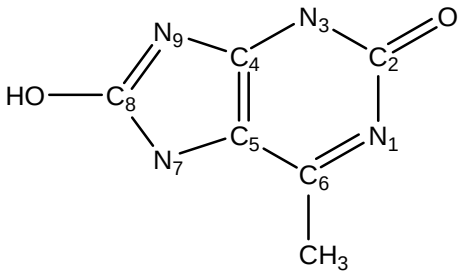
Oxidative hydroxylation of a variety of aromatic heterocycles and simple aldehydes is catalysed by XO; molecular oxygen is the accepter of reducing equivalents thus obtained by the enzyme(from the substrate which is being oxidized). Under normal catalytic conditions (with xanthine as reducing substrate and molecular oxygen as oxidizing substrate) the ultimate source of the oxygen atom incorporated into the substrate is solvent water.

Mechanistic studies have shown that substrate hydroxylation occurs at the molybdenum center (the reductive half-reaction), whereas O_2 reacts at the flavin site (the oxidative half-reaction) and intermolecular electron transfer between the molybdenum center and flavin (mediated by the iron-sulfur center)is an integral aspect of catalysis. XO (a prototypical molybdenum hydroxylase) is a small but important class of monooxygenases that utilizes water rather than O_2 as the source of the oxygen atom incorporated into the product. The mechanism of action of these enzymes is of intrinsic chemical interest, since it appears to be unique in the highly energetic oxygenating species corresponding to those seen in other hydroxylating system (flavin or pterin 4a peroxidase and ferry oxides, i.e., the Fe^{IV}=O entity, for example) are formed in the course of the catalytic cycle.

The **kinetics of the reductive half-reaction** have been followed by UV-VIS spectroscopy, e.g., the reaction of XO with the physiological substrate xanthine or a substrate analogue like 2-oxo-6-methyl purine [13b]. Reaction of enzyme (XO) with substoichometric concentrations of 2-oxo-6-methyl purine in aerobic condition (pH 10.0) when followed UV-VIS spectroscopically reveal two reaction intermediates. The rate constants for formation of the first intermediate, conversion of the first to the second and the decay of the second to give oxidized enzyme are 18, 1,2 and 0.13 sec^{-1} respectively. Such studies supported by EPR data indicate that these spectral intermediates arise from the molybdenum center of the enzyme in the Mo^{IV} and Mo^{V}valance states; these results can be represented schematically as follows.



P = product, 2-oxo-6-methyl-8-hydroxy purine



Scheme (I-4)

During the above process, the rate constants change by a factor of 10^2 (1.8×10^1 s^{-1} to 1.3×10^{-1} s^{-1}). The associated structural changes as followed using XAS result, are shown below.

The following scheme summarizes above steps incorporating the oxygen atom into the substrate from H_2O.

XO catalytic cycle

Overall reaction:

Xanthine + $H_2O \rightarrow$ uric acid + $2H^+$ + $2e^-$

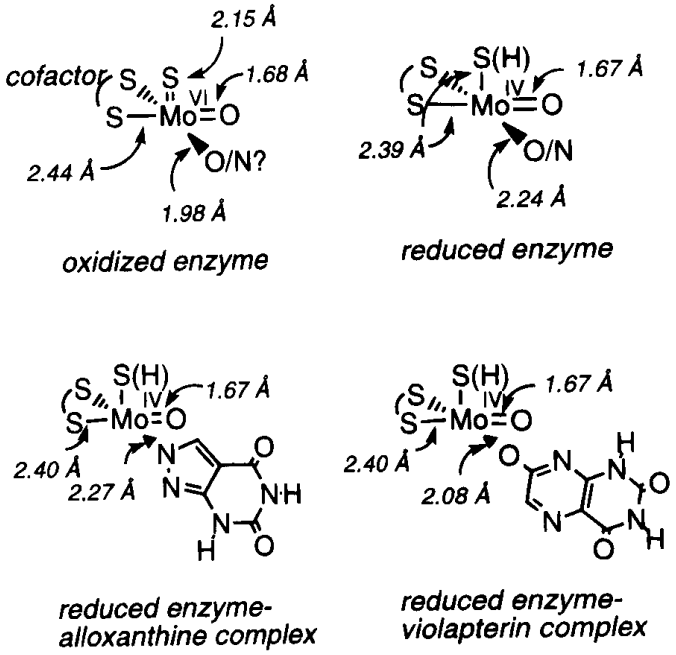
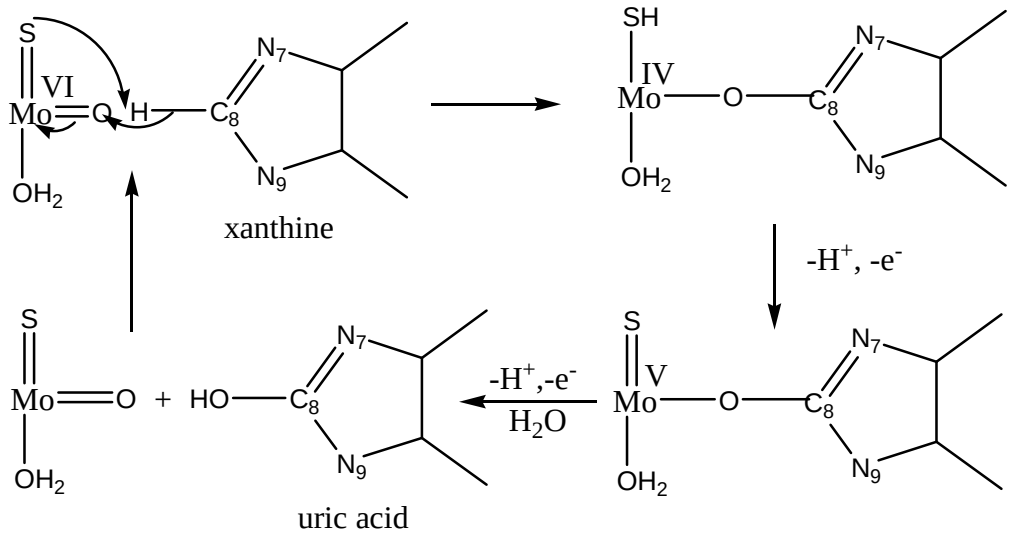


Fig. (I-5): A summary of XAS results obtained with the several forms of xanthine oxidase shown. The coordination geometry of the molybdenum center is inferred froem that for the *D. gigas* aldehyde oxidoredtase.

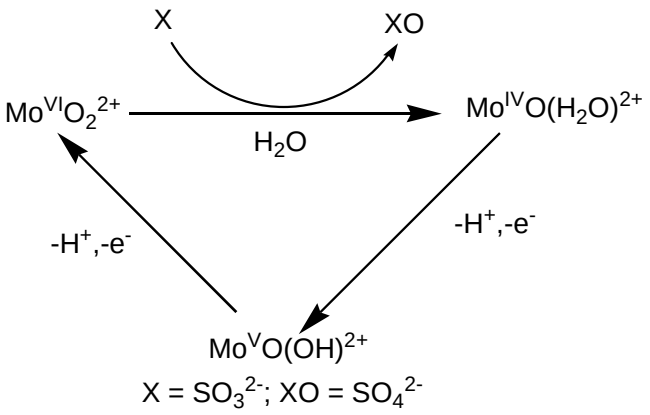
The initial step involves a concerned attack with proton transfer from the C8 position of the purine ring and attachment of the terminal oxo group of the $(Mo^{VI}OS)^{2+}$ core at the same position, associated with two electron reduction of the metal center [Mo(VI) → Mo(IV)] (the redution half-reaction). Two coupled electron-proton transfer steps restores the original catalytic $[Mo^{VI}OS(H_2O)^{2+}]$ core through the transient paramagnetic Mo(V)species (the oxidation half-reaction) along with the release of the product (uric acid). FAD assists this process through transfer of reducing equivalent from the Mo(IV) center to the ultimate acceptor O_2.The oxidation state of the metal center has a profound effect on the pKa of the coordinated ligands e.g., the magnitude of this dependence may be as great as 8 pKa units per unit oxidation state change, Mo(VI)/Mo(IV) redox cycle could vary ligand acidity by 16 pKa units (or roughly the difference between HNO_3 and H_2O). This effects help to deprotonate the coordinated ligands(-SH/H_2O) of the initial Mo(IV) center, thereby sustaining the catalytic cycle, underlying the need of different components of such enzymes, as indicated in Scheme (I-5).



Scheme (I-5)

A comment is needed at this stage about the substrate concentration used in the kinetic analysis. Under pseudo-first order condition using excess of the substrate (e.g., xanthine) over the enzyme (XO), six reducing equivalents are required to fully reduce the enzyme under anaerobic condition (two at the molybdenum centers, one at each of the two iron-sulfur centers, and two at the FAD). As a consequence, under condition of excess substrate, each enzyme molecule reacts sequentially with three substrate molecules (each of which donates two reducing equivalents to the enzyme), resulting in a triplication of the reductive half-reaction sequence. However, using substoichiometric concentration of the substrate, the above multiple turnover could be avoided and the kinetic analysis simplified. Under this condition the proposed catalytic cycle [involving (Mo(IV) and Mo(V) intermediates] for XO appears to be valid.

Sulfite oxidase (SO) catalytic cycle:



Scheme (I-6)

This scheme is also able to interpret the formation of the EPR active Mo(V) intermediate as well as the utilization of H_2O as the source of the oxygen atom transferred. Additional prosthetic group assist the coupled electron proton transfer steps. For nitrate reductase (NR) a similar catalytic cycle is valid, with Mo(IV) state of the enzyme being the starting point of the reaction with the substrate (NO_3^-), leading to the abstraction of oxygen atom {$NO_3^- \rightarrow NO_2^- + [O]$} .

Reduction potentials of the different redox centers associated with the oxomolybdo-enzymes: electron flow pathways

In organisms, electrons are abstracted from food (fuel) and flow to an oxidant, down the potential gradient formed by the sequence of acceptors and donors known as a **respiratory chain**[Fig. (I-6)]. Apart from flavins and quinones, which are redox-active organic cofactors, these acceptors and donors are metal-containing electron transfer(ET) centers, which fall into three main classes, namely FeS clusters, cytochromes, and Cu sites. These enzymes are generally bound in a membrane, across which the energy from ET is used to sustain a transmembrane proton gradient: this is the basis of the **chemiosmotic theory**. The counter flow of H^+, through a rotating enzyme known as ATP synthase, drives the phosphorylation of ADP to ATP. Many membrane-bound redox enzyme are **electrogenic proton pumps**, which means they directly couple long-range ET to proton transfer through specific internal channel.

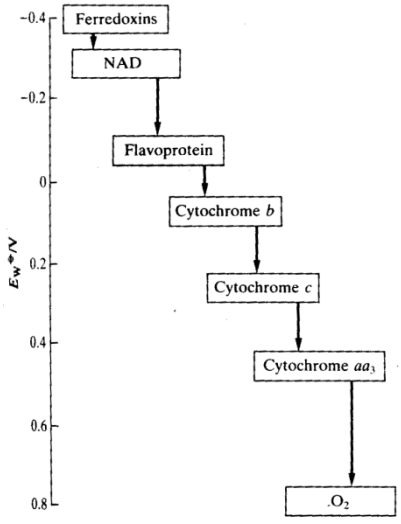


Fig. (I-6): Reduction potentials of some important electron transfer mediators in biological cells at pH = 7

Energy can be acquired as a flow of electron from fuel to oxidant. Important fuels include fats, sugars, and H_2, are important biological oxidants include O_2, nitrate and even H^+. As estimated from Fig. (I-7), oxidation of sugar by O_2 provide a lot of energy (over 4eV per O_2 molecule) and is the reason for the success of the aerobic organisms over anaerobic ones that once dominated the earth.

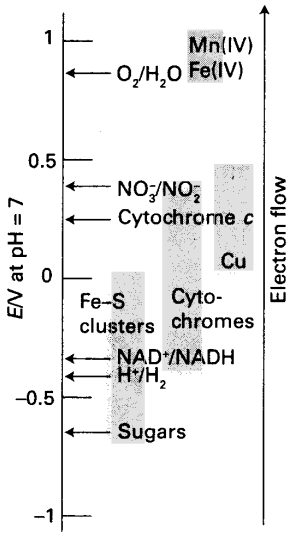


Fig. (I-7): The 'redox spectrum' of life.

Electron flow along electron-transport chain is coupled to chemical processes such as ion transfer (particularly H^+); the simplest electron-transfer centers have evolved to optimize fast electron transfer.

These general ideas can be used for analyzing the redox chemistry associated with the oxomolybdoenzymes.

For example, the molybdenum center of xanthine oxidase (XO) participates directly only in the reductive half-reaction of the catalytic cycle, that is the oxidative hydroxylation of xanthine that introduces reducing equivalents into the enzyme. Reoxidation of the molybdenum center takes place via simple electron transfer to the other redox active center of the enzyme, ultimately, to the FAD where electrons are removed from the enzyme by reaction with O_2.Intramolecular electron transfer within XO and related enzymes is thus an integral aspects of catalysis and has been extensively studied from both a thermodynamic and kinetic standpoint.

Table (I-1and 2) represent reduction potential data of the different redox center associated with some typical oxomolybdoenzymes.

The active sites of Mo and W enzymes are unique in their ability to perform mild, coupled two electron/OAT process between water and substrate near 0.0V versus the standard hydrogen electrode (SHE) and to undergo successive one-electron transfers. This is achieved by utilizing the M(VI)/M(IV), M(V)/M(IV) and M(VI)/M(V) couples of the [(ene-dithiolate)MOS], [(ene-dithiolate)MO_2(SCys)] or [(ene-dithiolate)$_2$MO(OSer)] units that span a potential range Table (I-1) that fits into the biological potential window of -400 to +800 mV versus SHE [Fig. (I-7)]. The bonding of at least one oxygen atom and three sulfur atoms to the metal in the M(VI) state appears to be a prerequisite for OAT catalytic activity. The oxidations of more resistant substrates, such as alkyl chain and aromatics, do not appear to be catalyzed Mo and W enzymes. Such reaction are catalysed by cytochrome P450 and methane monoxygenese, the iron-containing active sites of which possess an aggressive Fe^{IV}=O (cytochrome P450) or Fe_2^{IV}=O (methane monoxygenese) group that attacks the substrate, This intermediate is derived from dioxygen rather than from water as in Mo and W enzymes.

Table (I-1): Mid-point potential (E° versus SHE/mV) for representative members of the four classes of molybdenum and tungsten-enzymes.

Protein	E° vs SHE/mV
Xanthine oxidase from milk pH = 7.7 [43]	
Mo(VI)/Mo(V)	349
Mo(V)/Mo(IV)	-315
Sulfite oxidase from chicken liver, pH =7.0, 0.1 KCl [44]	
Mo(VI)/Mo(V)	+70
Mo(V)/Mo(IV)	-90
DMSO reductase from *R. sphaeroides*, pH = 70 [45]	
Mo(VI)/Mo(V)	+144
Mo(V)/Mo(IV)	+160
Aldehyde ferrodoxinereductase from P. furiosus, pH = 7.8	
W(VI)/W(V)	-365
W(V)/W(IV)	-436

Table (I-2): Reduction potentials of xanthine oxidase and related families.

	Mo^{VI}/Mo^{V}	Mo^{V}/Mo^{IV}	Fe-S_I	Fe-S_{II}	FAD/FADH$^{\bullet}$	FADH$^{\bullet}$/$FADH_2$
xanthine oxidase[a]	−345	−315	−310	−217	−301	−237
xanthine oxidase[b]	−373	−377	−310	−255	−332	−234
chicken liver xanthine dehydrogenase[c]	−357	−337	−280	−275	−294	−330
milk xanthine dehydrogenase[d]	ND	ND	−310	−235	−270	−410
rabbit liver aldehyde oxidase[e]	−359	−351	−207	−310	−258	−212
	Mo^{VI}/Mo^{V}	Mo^{V}/Mo^{IV}	heme		FAD/FAD$^{-\bullet}$	FAD$^{-\bullet}$/$FADH_2$
chicken liver sulfite oxidase[f]	38	−239	68		NA	NA
spinach nitrate reductase[g]	2	−6	−123		−380	−180
Chlorella vulgaris nitrate reductase[h]	15	−25	−164		−372	−172

The milder reduction potential range of the Mo or W centers [Table (I-2)] allow them to use the oxygen atom derived from H_2O for the catalytic purpose [Scheme (I-6)]; here control of pKa values of the aqua ligands (H_2O/OH/=O) or by the sulfide ligand (=S/-SH) by the oxidation state of the associated metal center is of paramount importance. The biological electron transfer agents [e.g., FAD; Table (I-1 and 2), Fig. (I-7)] with their E° values matching with those of metal center (VI/V/IV) work in tandem to sustain the catalytic cycle. The pH dependence of the reduction potentials of xanthine oxidase have been extensively studied [13b].

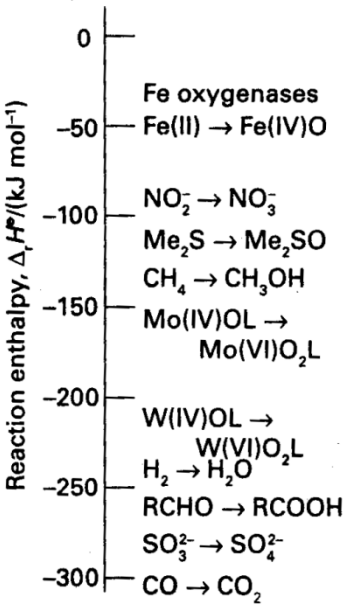


Fig. (I-8): Scale shows relative enthalpies for O-atom transfer. Fe(IV) oxido species are powerful O-atom donors, whereas Mo(IV) and W(IV) are good O-atom acceptors.

This kind of oxygenation reaction can be distinguished from that of the Fe and Cu enzymes (described previously), because with Mo enzymes the oxido group that is transferred is not derived from molecular O_2 but from water. The Mo(VI)=O unit can transfer an O atom, either directly (inner sphere) or indirectly to reducing (oxophilic) substrate, such as SO_3^{2-} or AsO_3^{2-}, but cannot oxygenate C–H bonds. Fig. (I-8) shows the reaction enthalpies for O-atom transfer: we see that the highly oxidizing Fe species formed by the reaction with O_2 are able to oxygenate all substrate, whereas Mo(VI) oxo species are limited to more reducing substrates and Mo(IV) able to extract an O atom from nitrate.

Oxygen atom transfer using H_2O as its source versus oxygen atom transfer using O_2 as it is source as well as O_2 evolution capacity of different metalloenzymes. Molybdenum is the only second-row transition metal that is required by most living organisms, and the few species that do not require molybdenum use tungsten, which lies immediately below molybdenum in the periodic table. Because of their unique chemical versatility and unusually high bioavailability, these two transition metals have been incorporated into the active sites of enzymes over the course of evolution. Both are redox-active under physiological conditions (ranging between oxidation states VI and IV); because the V valence state is also accessible, they can act as transducers between obligatory two-electron and one-electron oxidation-reduction systems; they can catalyze reactions such as the hydroxylation of carbon centers under more moderate conditions than are required by other systems. The oxidations of more resistant substrates, such as alkyl chains and aromatics, do not appear to be catalysed by Mo and W enzymes. Such reactions are catalysed by cytochrome P-450 and methane monooxygenase, the iron-containing active sites of which present an aggressive Fe^{IV}=O (Cyt P-450) or Fe_2^{IV}=O (methane monooxygenase) group that attacks the substrate. This intermediate is derived from dioxygen rather than from water as in the Mo and W enzymes and the former needs higher operating redox potential. While Mo is needed by both microorganisms and higher life forms, W is utilized by microorganisms, the hyperthermophilicarchaea, which thrive near 100°C. Clearly, the chemical properties of W and Mo are sufficiently different that biology can distinguish between them, either at the levels of their uptake and / or incorporation into enzymes or in the properties of the enzymes themselves, which

function with Mo but not with W. Several factors control the above crucial biological process where the reduction potentials of the pertinent system play a crucial role. Few such data are presented here along with the Forst diagrams of the relevant systems.

$FAD + 2H^+ + 2e^- \rightleftharpoons FADH_2$ -0.18V

$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$ (pH = 7) 0.816V

$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$ (pH = 0) +1.23V

Cell constration for O_2 evolution:

$E_{cell} = E_+ - E_- = E_t - (1.23)$ = +ve values for spontaneous O_2 evolution using the following half-cells:

(i) oxidizing agent absorbing electrons, +ve electrode (E_+);

(ii) $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$;

reducing agent, generating electron, -ve electrode (E_-).

Thus for a +ve E_{cell} value where

$E_{cell} = E_+ - (1.23)$,

E_+ should be greater than +1.23 for making the O_2 evolution process feasible. As evident from the relevant half-cell reaction, the origin of the barrier to this reaction is the need to transfer four electrons and to form an oxygen-oxygen double bond.

As per the Forst diagrams, Fe and Mn in their high oxidation states can achieve such high E_+ values make O_2 evolution possible [Fig. (I-9)].

Example:

The Mn-protein in photosynthesis (with Mn_4O_6/Mn_4O_4 clusters and Mn (IV/III) oxidation states) is able to catalyze water splitting reaction, involving the net transfer of 4 electrons: $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$

The reducing equivalents released ($4H^+ + 4e^-$ or [4H]) reduce CO_2 to glucose.

The same is true for catalase catalyzing the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$, where an energetic species like $Fe^{IV}=O$ is involved.

The same is true for an O_2 utilizing oxygenase like Cyt P450 where an $Fe^{IV}=O$ species is involved.

The Frost diagram for Mo/W indicate that their electrode potential range of operation, matches with that of the FAD system [Fig. (I-1)].

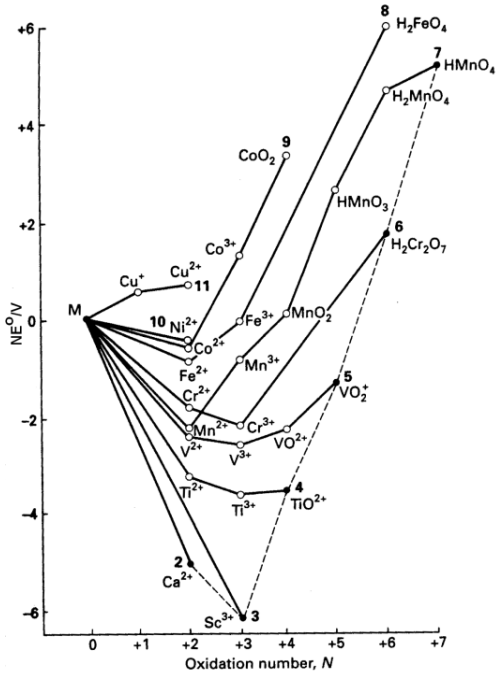
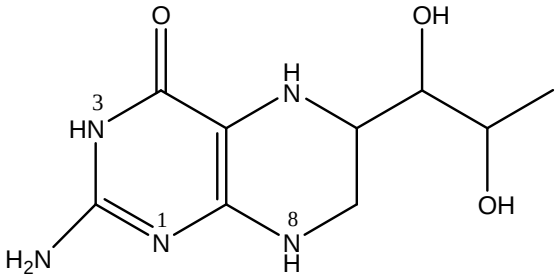


Fig. (I-9): A Forst diagram for the first series of d-block elements in acidic solution (pH = 0). The bold numbers designate the group numbers and the broken line connects species in their group oxidation states.

B.Aromatic aminoacid hydroxylases

This class of pterin-containing metalloenzymes utilize a different pterin cofactor, a tetrahydropterin called biopterin [Scheme (I-7)]; just like the molybdopterin cofactor or the pyranopterin without the metal center as shown in Scheme (I-2), it is also a 6-substituted pterin derivative.



Scheme (I-7)

Such enzymes catalysehydroxylations of the aromatic amino acids phenylalanine, tyrosine and tryptophan [Fig. (I-10)]. For example, phenylalanine is converted into tyrosine in presence of the cofactor tetrahydrobiopterin (BH_4). One molecule of O_2 is utilized in

the reaction; one oxygen atom is inserted into the substrate as an hydroxyl group, while BH_4 supplies the two electrons needed for reducing the other oxygen atom to the level of water.

The four-electron reduction of oxygen thermodynamically drives the hydroxylation of phenylalanine (a formal two electron oxidation) in tandem with the two electron oxidation of tetrahydrobiopterin (BH_4) to its quinonoiddihydropterin (BH_2). NADH most likely reconverts BH_2 to BH_4.

$$RH + O_2 \xrightarrow{2H\ ,\ 2e^{\ominus}} ROH + H_2O$$

or

$$RH + O_2 + BH_4 \longrightarrow ROH + H_2O + BH_2$$

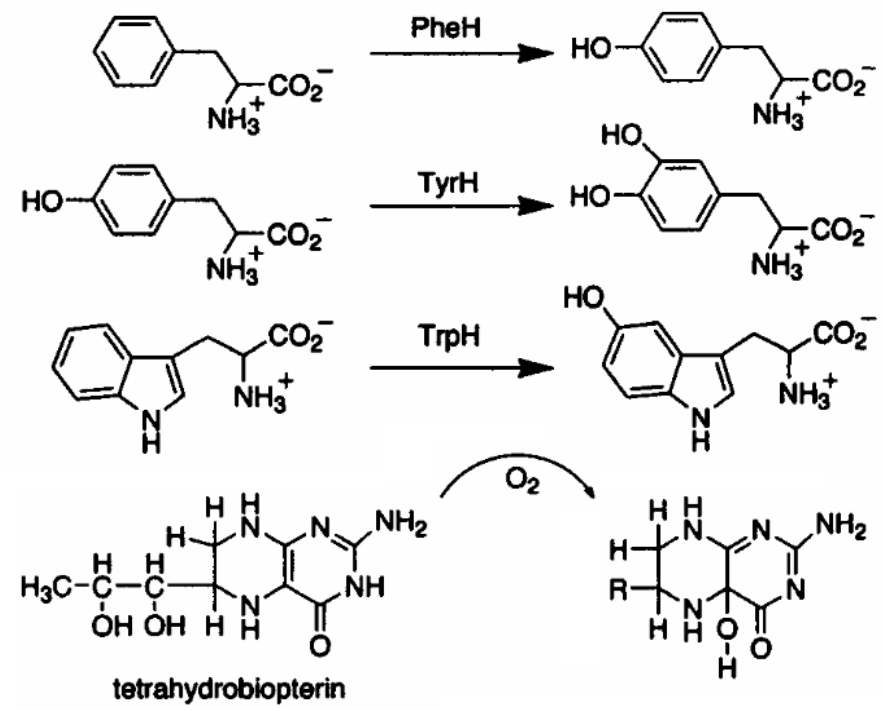


Fig. (I-10)

The mixed function oxygenase nature of the enzyme was demonstrated by ^{18}O incorporation into [^{18}O] tyrosine and H_2O^{18}. A non-heme iron atom is essential for the functioning of phenylalanine hydroxylase (PAH). PAH is a liver enzyme that catalyzes

the catabolism of excess phenylalanine in the diet to tyrosine; thevast majority of cases of phenylketonuria are due to deficiencies in this enzyme [27,35,59-61].

A few of such hydroxylases have been characterized X-ray structurally [35].

For tyrosine hydroxylase (TyrH) the iron atom lies at the bottom of 10 Å deep cleft in the enzyme surface [35] It is bound to three amino acid residues, e.g., two histidine (His) residues and one glutamic acid (Glu) residue. This arrangement of ligand, that is, two histidines and one acidic residue (e.g., Glu), has been seen in a number of metalloproteins with divergent functions [98]**In contrast to the other proteins with this metal-binding motif**, such as α-ketoglutarate dependent enzymes and the intra- and extradioldioxygenases, **there is no evidence that a substrate becomes a metal ligand during the reaction**. In the absence of substrates, up to three water molecules make up the remaining ligands to the metal in all three eukaryotic enzymes, resulting in a distorted octahedral arrangement of ligands [Fig. (I-11)A][61a,99,100]The actual number of water molecules varies with the structure[101,102]

The kineticmechanism has been determined for some of these hydroxylases (e.g., PAH and TyrH); all three substrates must be bound before catalysis occurs (e.g., O_2, tetrahydropterin and the amino acid), but the order of binding is somewhat random for all these enzymes.

It is clear that an enzyme – tetrahydropterin complex can form since structures are available for all three enzymes with a pterin bound. Fig. (I-11)A shows the interactions between tetrahydrobiopterin and PAH in the binary complex of ratPAH[101] Most of the interactions involve the pyrimidine ring of the pterin; the carboxylate of Glu286 provides the only electrostatic interaction between the protein and the pterin.

No structure is yet available with only an amino acid bound. However, a structure is available for the catalytic domain of human PAH with both tetrahydrobiopterin and β-thienylalanine [Fig. (I-11)B]. It has not been established directly whether β-thienylalanine is hydroxylated. Fig. (I-12) shows the interactions between the amino acid substrate and the protein in the ternary complex; the carboxylate ofβ-thienylalanine is bound to the side chain of Arg270.

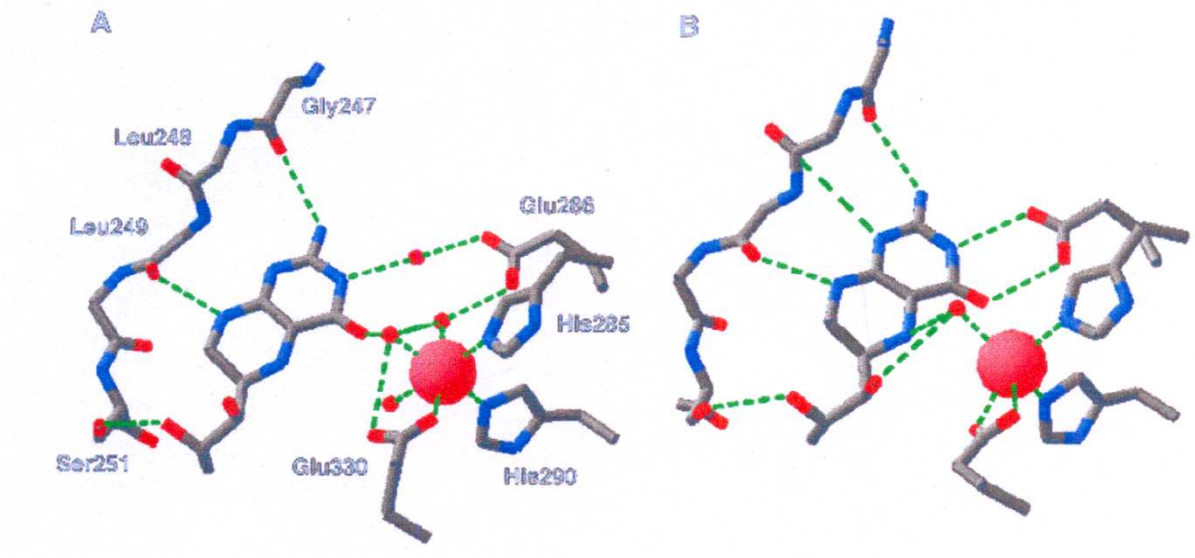


Fig.(I-11): Comparison of the iron and tetrahydrobiopterin binding sites of PAH in the absence (A) and presence (B) of *β*-thienylalanine. The structures are from the PDB files 1J8U and 1KWO. Reference 9 gives further details.

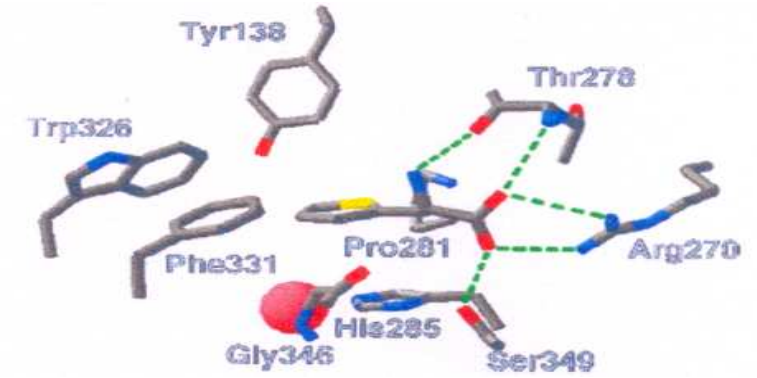


Fig.(I-12): Amino acid substrate binding site ofPAH. The structure is from the PDB file 1KWO.

As far as the mechanism of oxygen activation is concerned, the results of different studies are summarized in Fig. (I-13). It involves the formation of a 4a-peroxy-pterin intermediate. Such a mechanism would avoid the spin-forbidden direct reaction of triplet oxygen with the tetrahydopterin, through the binding of oxygen to the Fe(II) atom and generating a complex equivalent to $Fe(III)O_2^-$ which could attack the C(4a) position of the tetrahydopterin.

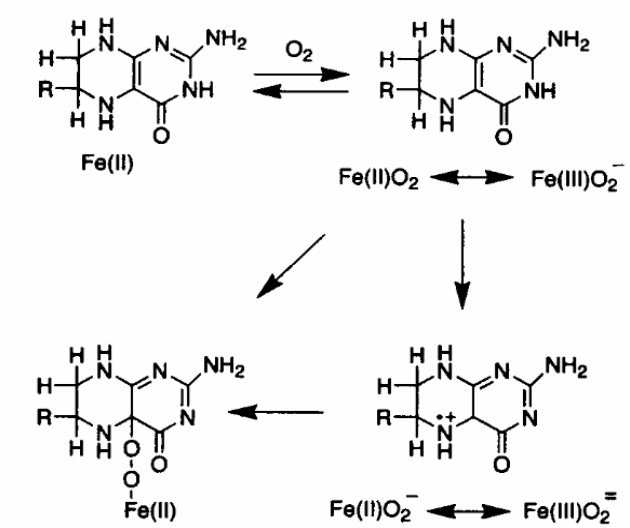


Fig.(I-13): Details regarding the formation of the 4a-peroxy-pterin intermediate are given in reference [35].

Deeper understanding about the mechanism of aromatic amino acid hydroxylation can only be obtained through synthetic modeling studies, where the well-designed experiments will help to elicit the unambiguous inference about the process.

C. Nitric oxide syntheses (NOSs)

Mammalian nitric oxide syntheses (NOSs) require the cofactor (6R) – 5,6,7,8-tetrahydro-biopterin (H4B) to convert L-arginine to L-citrulline and nitric oxide, an important second-messenger molecule in neutral and cardiovascular systems [37] NOS catalyzes the reaction :

$$\text{L-arginine} + 3/2\ \text{NADPH} + H^{\oplus} + 2O_2 \rightleftharpoons \text{Citrulline} + \text{nitric oxide} + 3/2\ \text{NADP}$$

NOS isoforms catalyze other leak and side reactions, such as superoxide production at the expense of NADPH. As such, this stoichiometry is not generally observed, and reflects the three electrons supplied per NO by NADPH.

NOSs are unusual in that they require five cofactors. EukaryoticNOSisozymes are catalytically self-sufficient. The electron flow in the NO synthase reaction is:

$$\text{Tetrahydro} \quad \text{NADPH} \longrightarrow \text{FAD} \longrightarrow \text{FMN} \longrightarrow \text{heme} \longrightarrow O_2$$

biopterin provides an additional electron during the catalytic cycle which is replaced during turnover. NOS is the only known enzyme that binds flavindeninedinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH_4) and calmodulin.

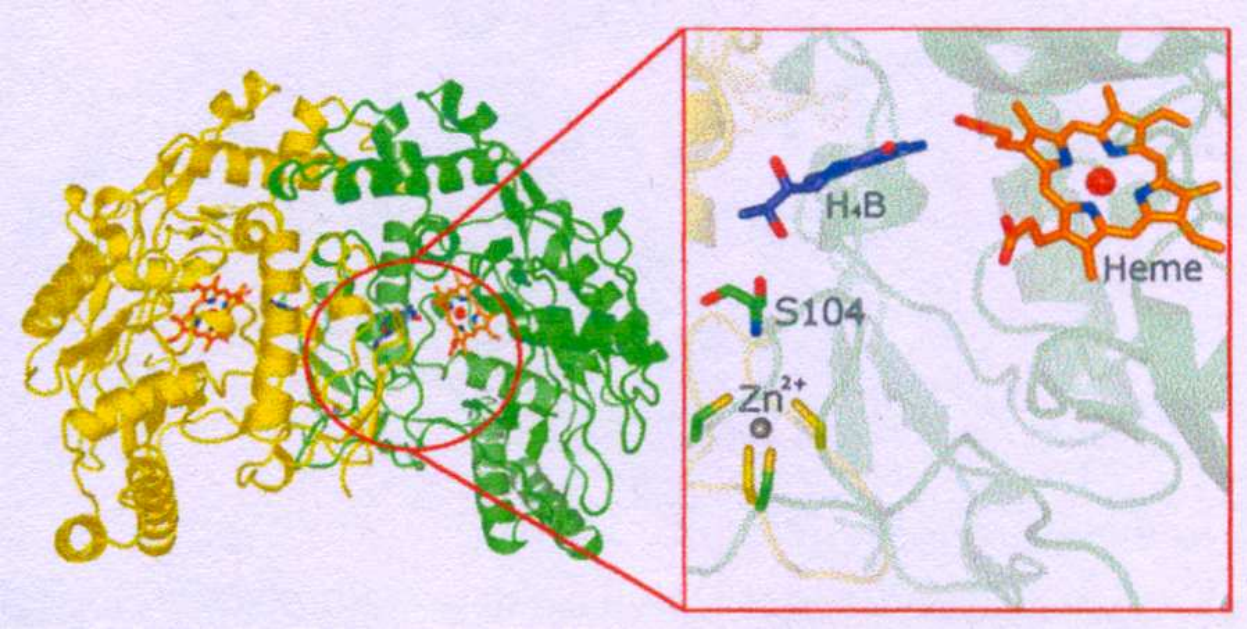


Fig.(I-14): Overall structure of the bovine eNOS dimer in complex with H_4B (Proton Data Bank entry 9NSE). The Zn^{2+} binding site is located at the dimer interface and ~15 Å from the center of the pterin binding pocket in both molecules A and B of the dimer. Chain A is colored green, chain B yellow, pterin blue, and heme orange. All structural figures were prepared with PyMol (http://www.pymol.org). Reference [37] gives further details.

The mammalian NOS enzyme family consists of three isoforms, neuronal NOS (n NOS), inducible NOS (i NOS) and endothelial NOS (e NOS). Each isoform is active only as a homodimer because the pterin binding site is located right at the dimer interface and monomeric NOS does not bind H_4B or the substrate. A relevantx-ray structure is shown in Fig. (I-14) indicating the heme and pterin (H_4B) sites, along with other sites of the bovine e NOS dimer in complex with H_4B. The dimer interface is formed between two N-terminal heme binding oxygenase domains that is further stabilized by the coordination of a Zn^{2+} ion ligated to two cysteine thiols fromeach subunit (ZnS_4) [Fig. (I-14)]. H_4B plays the role of a redox active one-electron donor that activates the heme-bound O_2, resulting in the formation of an H_4B radical.

With L-Arg as the substrate, this radical is then re-reduced by obtaining an electron from the ferrous NO complex generated at the end of the catalytic reaction, thus

allowing the release of NO from the ferric heme[145-151]. All NOS isoforms share a strikingly similar pterin binding pocket with comparable H_4B binding affinities, and cofactor and substrate binding events have been shown to synergistically stabilize the NOS dimer[152].

The above characterization data about NOSs highlight several aspects having bioinorganic relevance :

(i) the catalytic reaction itself with unique stoichiometry;

(ii) the electron transfer pathway from NADPH to the electron sink O_2;

(iii) the need of a class of heme-thiolate proteins for oxygenase type activity;

(iv) achievement of stability of the homodimer through Zn^{2+} coordination;

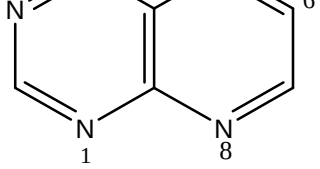
(v) the role of H_4B in the catalytic reaction.

A scope is opened up for studying synthetic model compounds for elucidating the catalytic reaction mechanism and devising suitable functional models.

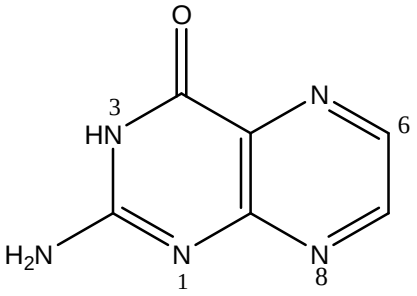
Aims and objectives of this work

The presence of pterin [Scheme (I-8)] (more precisely, molybdopterin, a heterocyclic system) in a substantial number of metalloenzymes, as outlined above, is the primary impetus of this work. Besides this, the importance of bioinorganic chemistry grown up, centered around this structural motif cannot be ignored. The general features of the active sites of pterin-containing metalloenzymes, are unprecedented in coordination chemistry. The poor solubility, moisture as well as air sensitivity along with non-innocent nature of the pterin ligand throw challenge to synthesis as well as preservation aspects of these complexes.

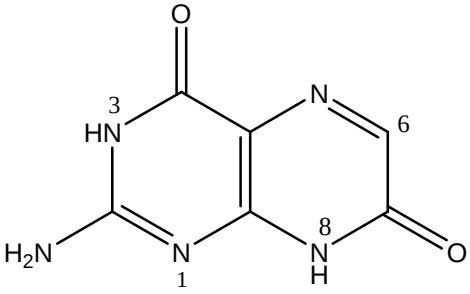
Pterin is structurally related to guanine [Scheme (I-9)] and another biomoleule, possessing pteridine core named isoalloxazine, found to be present in flavin.



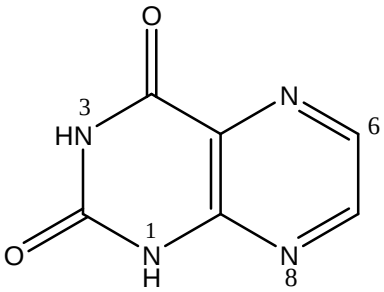
pteridine
(pyrazino [2,3-d] pyrimidine)



pterin
(2-amino- 4-oxidopteridine)



isoxanthopterin
(2-amino- 4,7-dioxidopteridine)

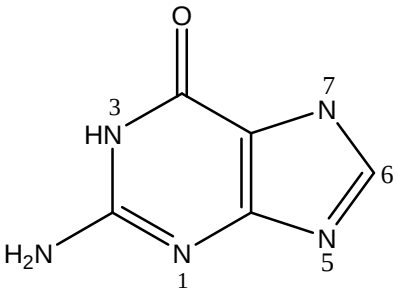


lumazine
(2,4- dioxidopteridine)

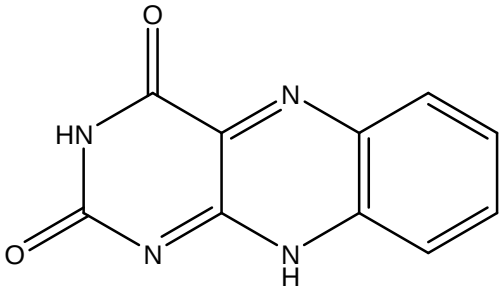
Scheme (I-8)

The reactivity of the pterin is due to the presence of polar -C=N- bond in the molecule and may act as an electron reservoir. The electron deficiency of the pterin is compensated by the conjugation of the electron rich groups like amine, carbonyl etc.

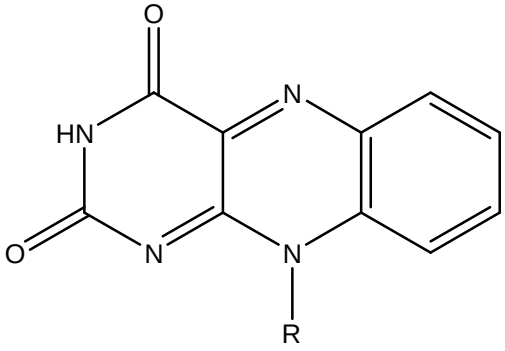
The presence of this pterin ring in metalloenzyme is due the ability of existence of the pyrazine ring of pterin molecule in different oxidation state (in terms of π – electron) as well as the matching of the redox potential with the metal partners [70]. However, the redox non-innocent nature of pteridines[Scheme (I-10)], as discussed below, adds a new dimension to their chemistry, among the important biological ligand systems.



guanine



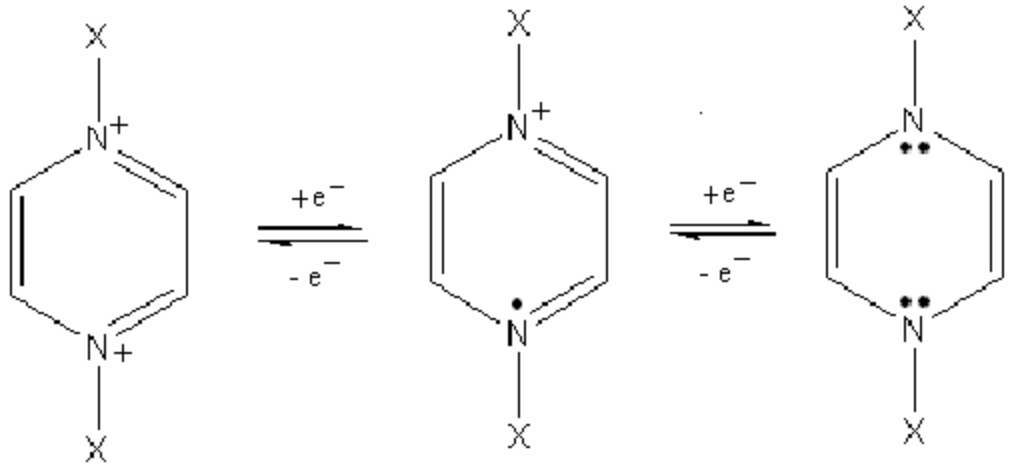
isoalloxazine



R = $-CH_2(CHOH)_3CH_2OPO_3^{2\ominus}$ in FMN

R = $-CH_2(CHOH)_3CH_2-$ ADP in FAD

Scheme (I-9)



6π **7π** **8π**

Scheme (I-10)

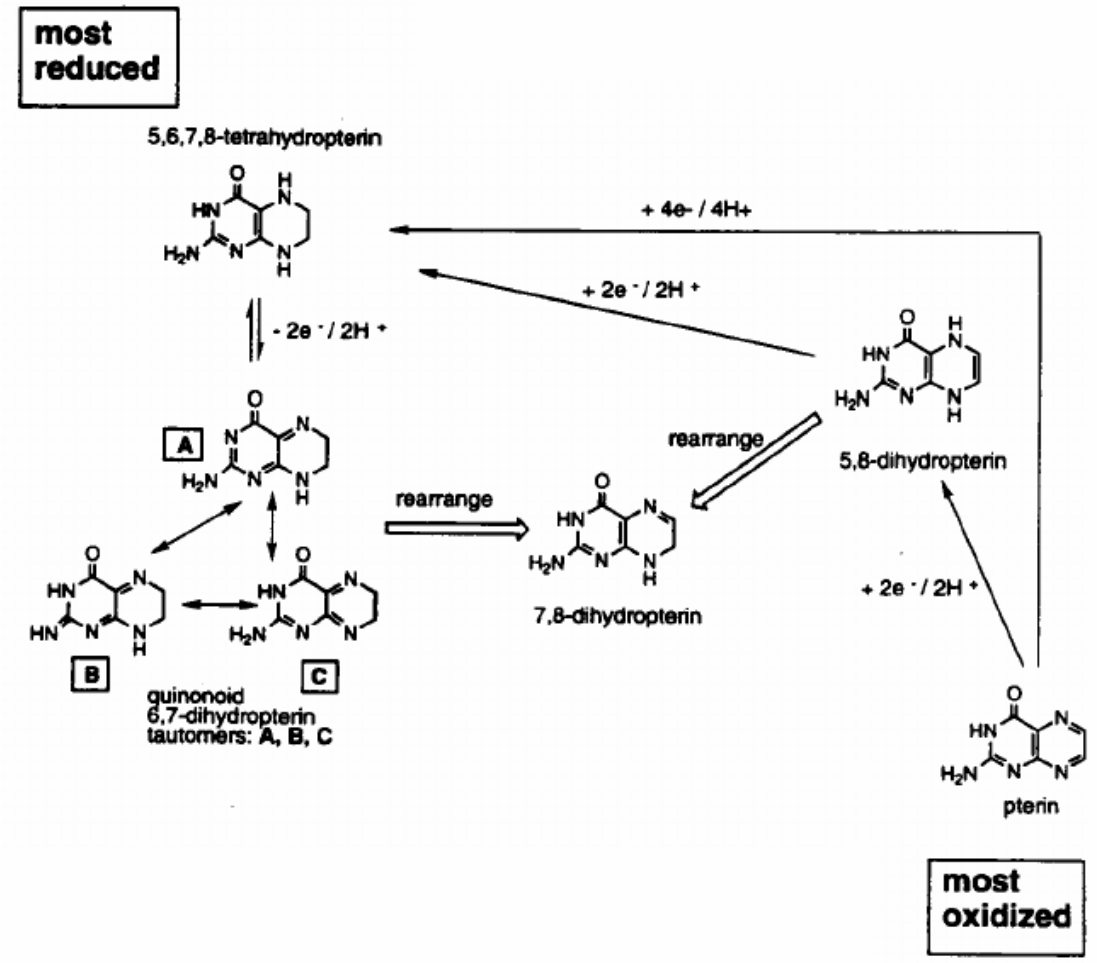


Fig.(I-15): Redox reactions that inter convert tetrahydro-, dihydro- and oxidizedpterins.

Burgmayer et. al proposed that the transformation of the bicyclic pterin molecule from the most oxidized state to the most reduced state (tetrahydropteridine) is occurred by acceptance of four electrons and four protons as in Fig. (I-15). In the intermediate redox state, i.e., in dihydro level, the tautomerism as well as proton rearrangement is possible. A closer look at Fig. (I-15) reveals that the 7,8-dihydropterin is most stable and both 5,8-dihydropterin and the 6,7-dihydropterin isomers are transformed into the former one in dihydro level. Even a radical trihydropterin species can be produced from reactions of radical initiators [34].

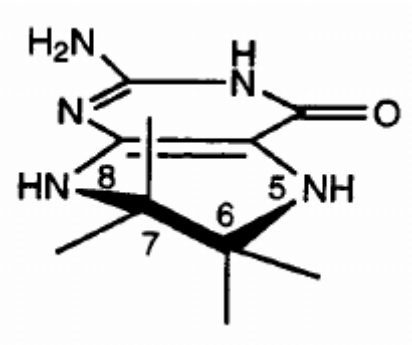


Fig. (I-16): Half-chair conformation within pyrazine ring of tetrahydropterin.

The planar structure of oxidized pterin becomes reffuled when the saturated region in the pyrazine ring spanning atoms N5, C6, C7 and N8 adopts a half-chair conformation [Fig. (I-16)][34].

The various biological redox reactions, catalyzed by metalloenzymes, take place by multi-electron redox activity as well as by redox capability of the transition metals.The fine tuning of the changes of oxidation states of pterin and transition metals (e.g., Mo, W ,non-heme and heme Fe) and the substrates like DMSO, $Me_3N \rightarrow O$, PPh_3, phenylalanine etc, is utmost required.

The above idea inspired the synthesis of new metal – pterin complexes along with suitable ancillary ligands as well as the study of reactivity aspects of these new complexes. References [1, 4, 12,33, 34, 45, 49, 63, 64, 72,77, 92, 93, 94, 96, 97] give a good overview of the available literature on the coordination chemistry of such ligands. They cover complex compounds of mainly molybdenum, first transition metals and a few later transition metals like ruthenium, rhenium, silver and cadmium. The relevant data are concerned with a significant number of x-ray structurally characterized compounds, throwing light on the metal-pterin / pteridine ligation aspects.

In the following areas this thesis may throw some light and may act as benchmark data:

1.Molybdenum - pterin coordination chemistry : It should be explored in every possible aspect, e.g., synthesis, characterization, spectroscopic, reactivity, etc., for drawing inferences which will be biochemically relevant to molybdopterin enzymes.

2. First transition metal-pterin coordination chemistry: This aspect is covered by only a few x-ray structurally characterized compounds, with little reactivity data. Couple of

factors are responsible for the choice of Fe(II), Cu(II) and Zn(II) for this work, e.g., the avidity of some of these complexes for molecular oxygen as well as the ability of these d^n systems to balance the extent of M→L π bonding (with molecular oxygen) with the transmission of reducing property of the pterin ligand to the following reaction site as discussed here :

$$\text{phenylalanine} + O_2 \xrightarrow{\text{M(II)-pterin complex}} \text{tyrosine} + H_2O$$

where, M = Fe / Cu

The results will be helpful for understanding the functional aspects of phenylalanine hydroxylase (PAH), which is able to activate the aromatic ring towards hydroxylation.

3. Correlation of reactivity with molecular and electronic structures: The electronic structure as well as redistributions during complex formation process can be followed by some spectroscopic data such as ^{1}H-NMR spectra, fluorescence spectra as well as cyclic voltammetric data. Some computational calculations (e.g., energy gap calculations of frontier orbitals obtained through Huckel surface calculations of these complexes) support the above mentioned spectra. The results will be biochemically significant for understanding the functional aspects of pterin-containing metalloenzymes.

The schematic structures of the pterin ligands used for the present study, have already been shown in the Preface. The 7-oxo group of some of these ligands (H_2L^2, $H_2L^3.H_2O$) corresponds to the pyran ring oxygen atom of "molybdopterin", as revealed through x-ray structural work on the relevant enzymes [Fig. (I-2)]. The redox non-innocent nature of pterin as par Scheme (I-10) and Fig. (I-15), may be further accentuated through electronic redistribution involving this oxo group [77].The present investigations as outlined above, have been pursued with the following **broader research goals** in mind.

1. Any synthetic modeling strategy aimed at elucidating the functional aspects of pterin-containing oxo-transferases should consider two basic factors :

► During the oxygen atom transfer step to the substrate (SO_3^{2-}→SO_4^{2-} ; CH_3CHO→CH_3COO^-, etc.,) or away from it (NO^{3-}→NO^{2-} ; Me_2SO→Me_2S ; Me_3NO→Me_3N, etc.,), the metal center (Mo or W) shuttles among the oxidation states VI, V and IV [Scheme (I-6)] involving dioxo / oxosulphido / monooxo / non oxo sites like :

$Mo^{VI}O_2^{2\oplus} + SO_3^{2\ominus} \longrightarrow Mo^{IV}O + SO_4^{2\ominus}$

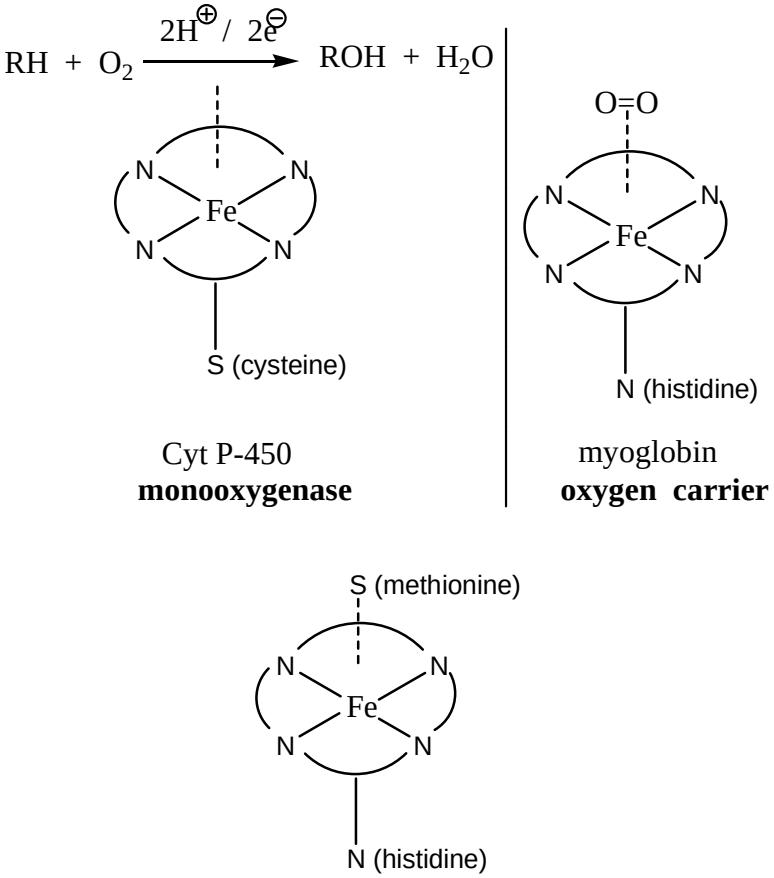
$Mo^{IV} + DMSO \longrightarrow Mo^{VI}{=}O + DMS,$ and

$Mo^{VI}OS^{2\oplus} + \text{xanthine} \longrightarrow Mo^{IV}OSH^{2\oplus} + \text{uric acid}$

► All Mo (or W) cofactors share a common non-protein organic component, called molybdopterin, that acts as a ligand to the metal center [Scheme (I-2)]; besides this, the coordination sphere around the metal center is completed by oxo / sulphido groups and coordinated protein ligands or even a second pterinene-dithiolate ligand [Fig. (I-2)].

The reaction locant of the substrate (carbon, nitrogen or sulphur, i.e., the atom to or from which the oxygen atom transfer takes place) varies from one enzyme to another. This fine tuning of catalytic property (with respect to substrate selection) of the same common cofactor [consisting of the above-mentioned Mo (or W)-molybdopterin complex] by other donor ligands in the coordination sphere is to be understood through future research work. Synthesis, characterization and reactivity studies on new mixed ligand complexes where the pterin ligand is supplemented by carefully chosen ancillary ligands, will be helpful in such an endeavour. Apart from throwing light on the functional aspects of Mo- or W- containing oxo-transferases, these studies may open up new pathways of pterin coordination chemistry.

A parallel case involving a well-characterized bioinorganic system may be cited as an example; here the hemoproteins use the extra ligand trans to the O_2 binding site to adjust / tune the reactivity of heme towards dioxygen [Scheme (I-12)]. For example, cytochrome P-450 has a cysteine thiolate in this position trans to where O_2 binds, which facilitates dioxygen activation; in myoglobin, a histidine nitrogen in this position facilitates the reversible binding of O_2. When the sixth coordination position of the heme iron is blocked by a methionine thioether, an electron carrier activity becomes possible involving the Fe(III) / Fe(II) states (i.e., cytochrome c).



Cyt P-450
monooxygenase

myoglobin
oxygen carrier

Cyt C
electron carrier

Scheme (I-12)

Mechanistically speaking, this fine tuning of property of the heme cofactor by the sixth ligand involves both thermodynamic and kinetic control as summarized below:

$$L_nM(S) + O_2 \underset{}{\overset{K}{\rightleftharpoons}} L_n(S)M(O_2) \xrightarrow{k} LnM\text{-}(S)_{ox} + (O_2)_{red}$$

The oxygen carrying property is favoured when K is large and k is small, while oxidase (e.g., monooxygenase) activity will occur when k is also large. From the angle of coordination chemistry, the cysteine thiolate coordination promotes O_2 activation, while the histidine nitrogen ligand permits O_2 carrier activity.

As far as pterin coordination chemistry is concerned, the elucidation of reactivity at a level of clarity as above, is yet to be achieved. Additional queries are added to this problem by the presence of electron transfer prosthetic groups (e.g., iron-

sulphur proteins) hydrogen-bonded to the pterin rings, as established x-ray structurally [Fig. (I-2)] [34] Their possible role in facile electron flow into or out of the enzyme reaction center, working in tandem with the redox non-innocent pterin ring, will be fascinating.

2. For the heme enzyme cytochrome P-450, the oxygen atom needed for monooxygenase activity is derived from the dioxygen molecule [Scheme (I-12)]; but for most of the oxotransferases containing Mo (or W), water supplies the oxygen atom for their catalytic function. As evident from Scheme (I-6) representing the latter system, coordination of water to the metal (Mo) atom gives Mo-OH_2, Mo-OH or Mo=O species which cycle between the Mo(VI) and Mo(IV) oxidation states, through the transient intermediate Mo(V) state. Two electrons and two protons are released in this process, thereby justifying the presence of heme, Fe-S and / or flavincenters in such large and complex enzymes [13,27]. Here the metal (Mo or W) oxidation state controls the level of protonation of the water-based ligand (H_2O / OH^- / O^{2-})[9,89a] The increase in oxidation state of the metal center (Mo / W) is accompanied by a considerable lowering of pK_a value (or increase in acidity) of the protonated ligand. Since the magnitude of this dependence may be as great as 8 pK_a units per unit oxidation state change, a Mo(VI) / Mo(IV) redox cycle could vary ligand acidity by 16 pK_a units or roughly the difference between nitric acid and water.

3. Details about the frontier orbitals (HOMO, LUMO, etc.,) are needed for the synthetic model compounds for interpreting the ligand fields at the metal centers and correlating their reactivities with the electronic structures.

4. Careful experimental designs are needed while applying the different physico-chemical and spectroscopic methods for the synthetic model systems, so that the correct inferences could be elicited regarding characterization aspects, ascertaining the oxidation states of the metal center, redox reactions at the metal centers, role of the redox non-innocent pterin ligands in the associated electron transfer process, etc.

5. To explore the role of the sulfur donor atom, coordinated to the Mo(IV/V/VI) center of the suitable ancillary ligand, to facilate the electron migration to pterin ligand via metal center.

The above results will serve as bench-mark data for understanding the properties of pterin-containing metalloenzymes; besides these, pathways will be paved for effective designing of synthetic model systems with closer approach to reality.

Preface of this thesis indicates the pterin ligands (Schemes 1, 2 and 4) used for the present synthetic studies. Chapter II is concerned with the synthesis of a new mixed ligand zinc(II)-pterin complex of the pterin ligand [H_2L^1] with 1,10-phenanthroline (phen) as the ancillary ligand and its x-ray structural characterization. In this chapter geometric parameters obtained from x-ray structural characterization are compared with those obtained from CHEM3D model of this complex. A fare agreement finds the applicability of these models to other complexes of the rest of this thesis.

In Chapter III and IV, two new series of mononuclear as well as binuclear complexes of Mo(IV/V) with the pterin ligand (H_2L^2) as the primary one and suitable ancillary ligands with sulfur, nitrogen and oxygen donor atoms are reported. The reactivities of these complexes with enzyme like substrates like $Me_3N\rightarrow O$, PPh_3 along with physico-chemical characterization are discussed in these two chapters.

Chapter V reports the synthetic, spectroscopic and reactivity aspects of seven new mononuclear as well as binuclear complexes of Mo(IV/V/VI) of the pterin ligand ($H_2L^3.H_2O$) as the primary one and carefully chosen secondary ligands with sulfur, nitrogen donor atoms.

In these chapters1D and 2D ^{1}H-NMR data play a vital role to follow the electron movement in these complexes. Elemental analysis, ESIMS data as well as IR data play a decisive role to acertain the structures of binuclear complexes.

In Chapter VI, three new complexes of the pterin ligand (H_2L^2) and first transition metals [e.g., Fe(II), Cu(II) and Zn(II)] are reported. The spectroscopic data as well as model monooxygenese activity with phenylalanine will be relevant with respect to phenylalanine hydroxylase activity.

References are grouped together at the end of this thesis.