CHAPTER I

General Introduction

Aims and objectives of the 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 cobalt and tungsten. 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 centre should be linked to the changes in the pterin / pyrazine ring oxidation level [7].

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 [62].

A cofactor is a non-protein chemical compound that is bound to a protein and is required for the protein's biological activity. These proteins are commonly enzymes. Cofactors can be divided into two broad groups: organic cofactors and inorganic cofactors. Organic cofactors are further divided into coenzymes and prosthetic groups.

Coenzyme refers to the functional properties of a protein; prosthetic group refers to the nature of the binding of a cofactor to a protein.

In humans, inorganic cofactor are metal ions like iron, magnesium, manganese, cobalt, copper, zinc, selenium, molybdenum. Other organisms like bacteria, thermophilic archaea, marine diatom require vanadium, tungsten, cadmium.

Metabolism involves the transfer of functional groups in which cells use a small set of metabolic intermediates to carry chemical groups between different reactions. These group-transfer intermediates are the loosely-bound organic cofactors, often called coenzymes. Each class of group transfer reaction is carried out by a particular cofactor, which is the substrate for a set of enzymes that produce it, and a set of enzymes that consume it. These cofactors are continuously recycled as part of metabolism.

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.

A. Oxo-transferases containing molybdenum or tungsten

This class of enzymes catalyse hydroxylations and net oxygen atom transfer reactions (OAT) to and from a variety of biologically important substrates [2,7,22,62,68-73,95-97,118,124,127-135].

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

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

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 [22].

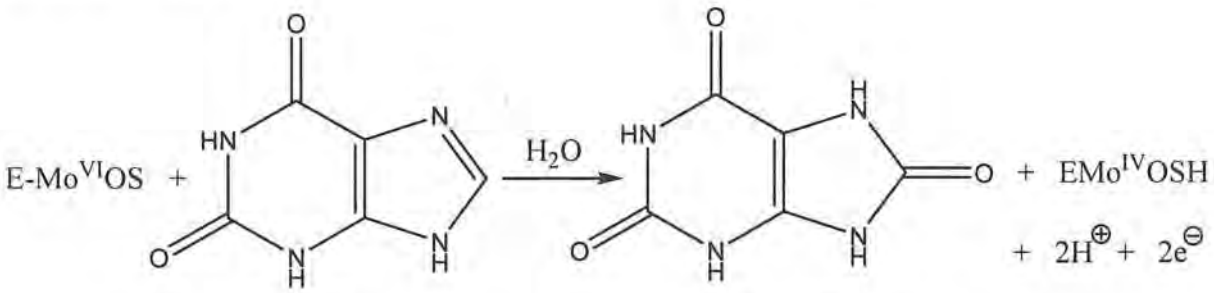
Nitrate reductase

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

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

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. Molybdenum is available in natural waters as molybdate, exceeding in concentration such essential trace elements as Mn, Fe, Co, Cu and Zn. This availability and a remarkable chemical versatility make

Mo a crucial component of enzymatic systems.

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 fused to the pterin have the name pyranopterin [Figure (I-1)]. 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 sulphur atoms of one (or two) ene-dithiolate group.

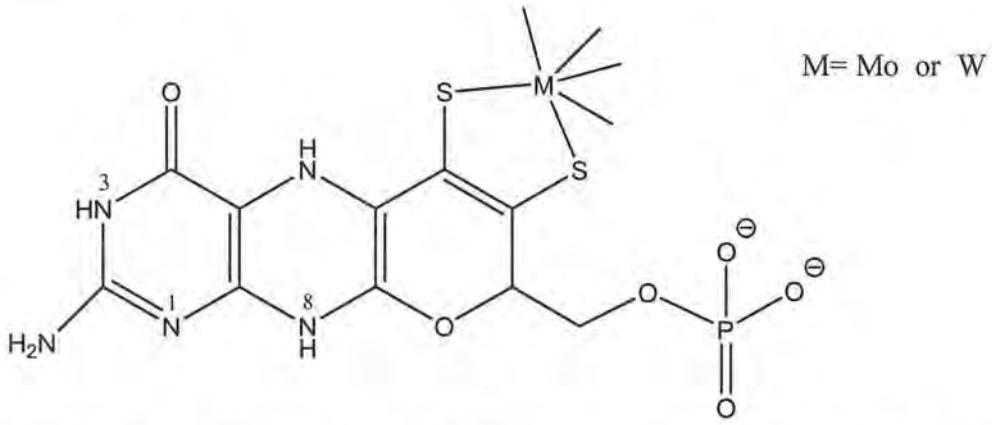


Fig.(I-1): Structure of pyranopterin found by protein crystallography and its ene-thiolate mode of coordination to a metal atom (M= Mo or W]. In some enzymes the phosphate group is replaced by a dinucleotide.

The active sites of oxo-molybdenum and oxo-tungsten enzymes 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 sulfoxide reductase 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 dimethylsulfoxide reductase [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;

(iv) in some cases the pterin is observed to link the molybdenum (or tungsten) centre to remote iron-sulphur clusters by hydrogen bonding through a pterinyl NH to a cysteinyl sulphur coordinated to Fe (Figure (I-2)]. The pterin effectively "hard-wires" the metal centre to other electron transfer prosthetic groups, presumably

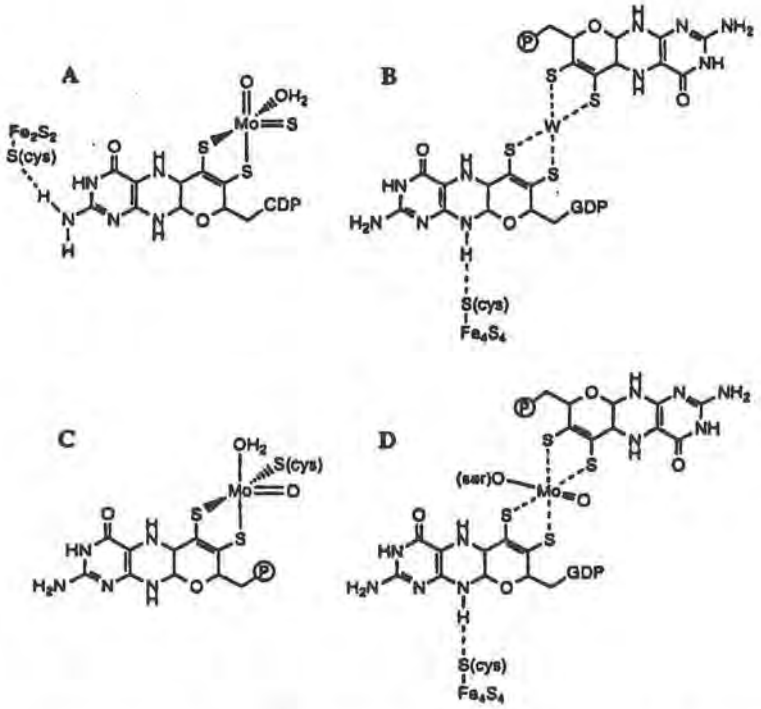
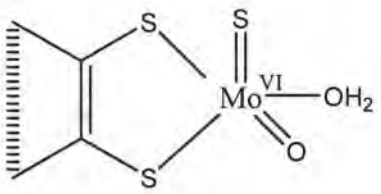


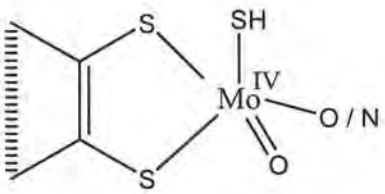
Fig.(I-2): The molybdenum site of several molybdoenzymes as determined by X-ray Crystallography: Structure **A** is the cofactor as seen in *Desulfovibrio gigas* aldehyde oxidoreductase; structure **B** is the tungsten site from *Pyroccocus furious* aldehyde oxidoreductase; structure **C** is the molybdenum site from chicken liver sulfite oxidase; structure **D** is the oxidized cofactor in *Rhodobacter sphaeroides* dimethylsulfoxide reductase .

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.

Hille has classified oxomolybdoenzymes into three families based upon their protein sequences and the structures of their oxidized active sites; each family is named as par its most prominent member, e.g., xanthine oxidase, sulphite oxidase and DMSO reductase families. Figures (I-3) to (I-5) show the schematic structures of their active sites in the oxidized [Mo(VI)] and reduced [Mo(IV)] forms. For the DMSO reductase family of enzymes, the Mo(IV) centre in the reduced form is devoid of any terminal oxo (=O) group [22(b)].

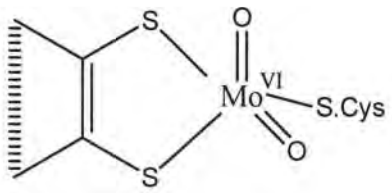


(oxidised form)

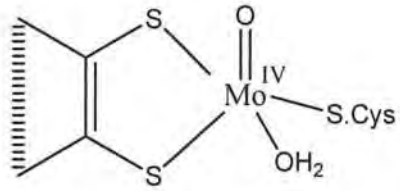


(reduced form)

Fig.(I-3): The xanthine oxidase family (true hydroxylase)

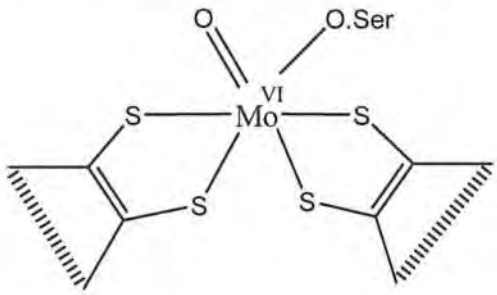


(oxidised form)

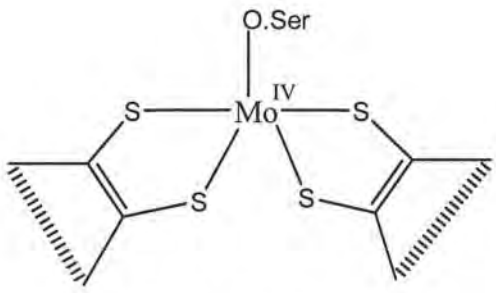


(reduced form)

Fig.(I-4): The sulphite oxidase family



(oxidized form)



(reduced form)

Fig.(I-5): The DMSO reductase family

The tungstopterin enzymes are classified into three families according to their active site, determined by X-ray crystallography [22(c),128].

1. the aldehyde-ferredoxin oxidoreductase family (AOR)
2. the formate dehydrogenase family (FDH)
3. the acetylene hydratase family (AH)

The X-ray crystal structure of aldehyde ferredoxin oxidoreductase (AOR) from Pyrococcus furiosus (thermophiles) shows a homodimeric protein with two subunits bridged by a monomeric Fe site. Each subunit contains a Fe_4S_4 cluster and a W active site in a distorted square pyramidal geometry. Each aldehyde

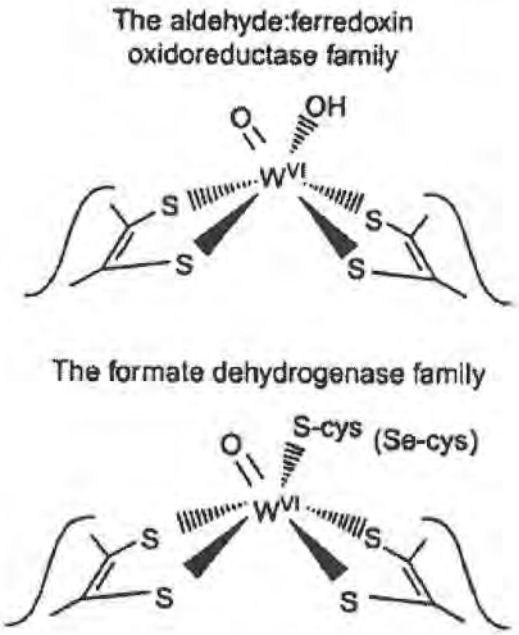


Fig.(I-6): The tungsten enzyme families

ferredoxin oxidoreductase subunit contained two molybdopterin molecule that coordinate a tungsten by a total of four sulphur ligands and the pterin system was modified by an intramolecular cyclisation in which the two pyranopterins are linked through the coordination of their phosphate group to a Mg^{2+} cation that generated a three-ringed

structure. The coordination sphere about the tungsten contains a glycerol and/or oxo ligands. Presence of a relatively large number of both ion-pairs and buried atoms may contribute to the extreme thermostability of this enzyme (~grows at 100° C).

Formate dehydrogenase (FDH) family contains two pyranopterins, -OH, selenocysteine.

A third family, exemplified by acetylene hydratase, might be similar to that of aldehyde-ferredoxin oxidoreductase.

AOR family

$$CH_3CHO + H_2O \rightleftharpoons CH_3COO + 3H + 2e^{\ominus}$$

In addition to their structural similarities, a unifying feature among the three types of tungstopterin enzyme in the hyperthermophiles is that they catalyse the oxidation of aldehydes and use the redox protein ferredoxin (Fd) as the physiological electron acceptor. Aldehyde oxidation is a two-electron process but Fd contain a single [4Fe-4S] cluster and undergo only a one-electron redox reaction; for maintaining electron stoichiometry one catalytic turnover requires the reduction of two molecules of Fd.

FDH family

They utilize CO_2 as the substrate.

$$CO_2 + H^{\oplus} + 2e^{\ominus} \rightleftharpoons HCOO^{\ominus}$$

NADPH acts as the physiological electron donor.

Acetylene hydratase

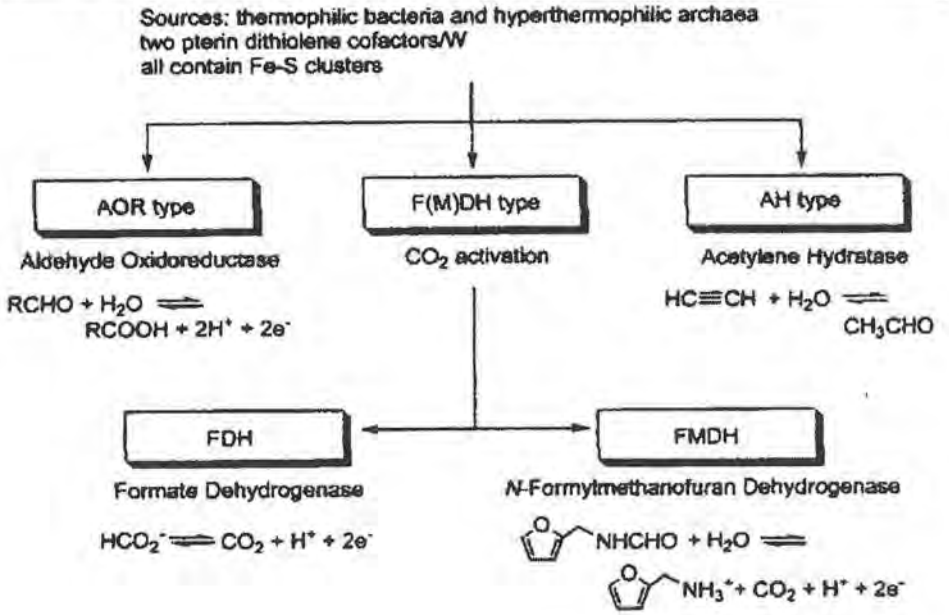
The third class of tungstoenzyme has just one member and it is termed acetylene

hydratase (AH) and was purified from the acetylene-utilizing anaerobe Pelobacter acetylenicus (Pa). The enzyme catalyzes the hydration of acetylene to acetaldehyde.

$$CH{\equiv}CH + H_2O \rightleftharpoons CH_3CHO$$

The functional aspects of tungstoenzymes are summarized below.

Types of Tungstoenzymes and Reactions Catalyzed



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 centres 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 $Fe^{IV}_2=O$ (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 hyperthermophilic archaea, 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.

Given the hot, anaerobic conditions under which life probably arose, tungsten might have been the first of these elements to be acquired by living organisms. For example, the tungsten-sulphur bonds, such as those found in tungsten-containing enzymes, are more stable than their molybdenum counterparts. Low-valent tungsten sulphides are also more soluble in aqueous solutions and thus would be more available in the anaerobic, highly reducing environments of the early earth. Lastly, the reduction potentials of tungsten-containing complexes are usually lower than those of molybdenum and therefore more useful to early life forms, which probably had a low intracellular redox poise. However, as the earth's crust cooled and photosynthetic organisms made its atmosphere aerobic, the O_2-sensitivity of tungsten compounds lowered their effectiveness and the water-solubility of high-valent molybdenum oxides became useful; the elevated

intracellular redox poise of aerobic organisms would have also made molybdenum more suitable. Because tungsten and molybdenum have similar chemistry, it is possible that, initially, as the transition to an oxygen-rich environment occurred, the latter substituted the former in enzyme active sites. Indeed, molybdenum- and tungsten- containing enzymes often catalyze the same kind of chemistry- such as the oxidation of aldehydes to carboxylic acids.

In addition to the metal, an unusual pterin cofactor is an integral part of the active site in both molybdenum- and tungsten- containing enzymes; it is coordinated to the metal via a dithiolate side chain [Figure (I-1)]. The pathway by which the pyranopterin core of the cofactor is synthesized involves >12 proteins and appears to be universally conserved in biology. Although organisms lacking hemes are known (e.g., lactobacilli and some enterococci), all known organisms contain the biosynthetic pathway for this pterin cofactor. This attests both to the early appearance of the pathway in the course of evolution and to the importance of the processes in which these centres are involved.

X-ray structural data on several such enzymes containing Mo or W, supported by spectroscopic (e.g., EXAFS, EPR), kinetic methods as well as computational approaches, are helpful in suggesting the following scheme [Figure (I-7)] of enzyme reaction; it represents a simple cycle of reactions that describes the oxidation (or in reverse, reduction) of a substrate (X) at an oxo-molybdenum centre, such that present in sulphite oxidase [7,22,24,27,28,49,50,68-73,96,97,130]. The intimate catalytic mechanism involves coupled electron-proton transfer (CEPT) and the oxygen atom transferred to the substrate is derived from the solvent (e.g., H_2O). The metal oxidation states (VI / V/ VI) control the level of protonation of the water-based ligand (H_2O / OH^{-1} / O^{2-})

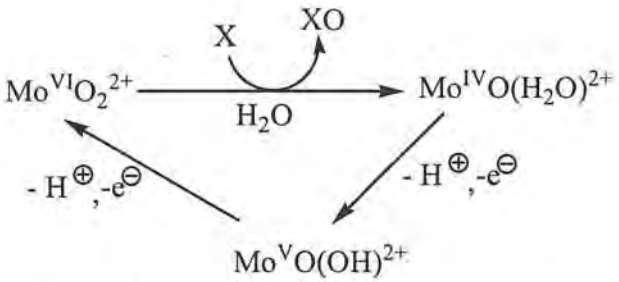


Fig.(I-7)

Exceptions do exist. For example, Holm and coworkers have demonstrated that DMSO-reductase from R. sphaeroids is an oxo-transferase and the oxygen atom (^{18}O) transferred did not arise from the solvent [136].

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 chemists [2,7,22,24,62,72,73,96,97, 121, 125-129,137].

B. Aromatic amino acid hydroxylases

This class of pterin-containing metalloenzymes utilize a different pterin cofactor, a tetrahydropterin called biopterin [Figure (I-8)]; just like the molybdopterin cofactor or the pyranopterin without the metal centre as shown in Figure (I-1), it is also a 6-substituted pterin derivative.

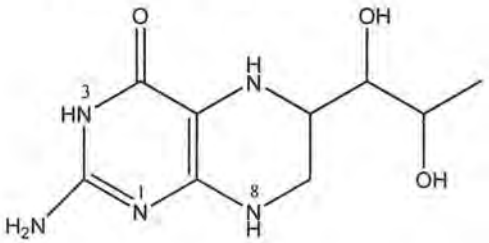


Fig.(I-8)

Such enzymes catalyse hydroxylations of the aromatic amino acids phenylalanine, tyrosine and tryptophan. 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.

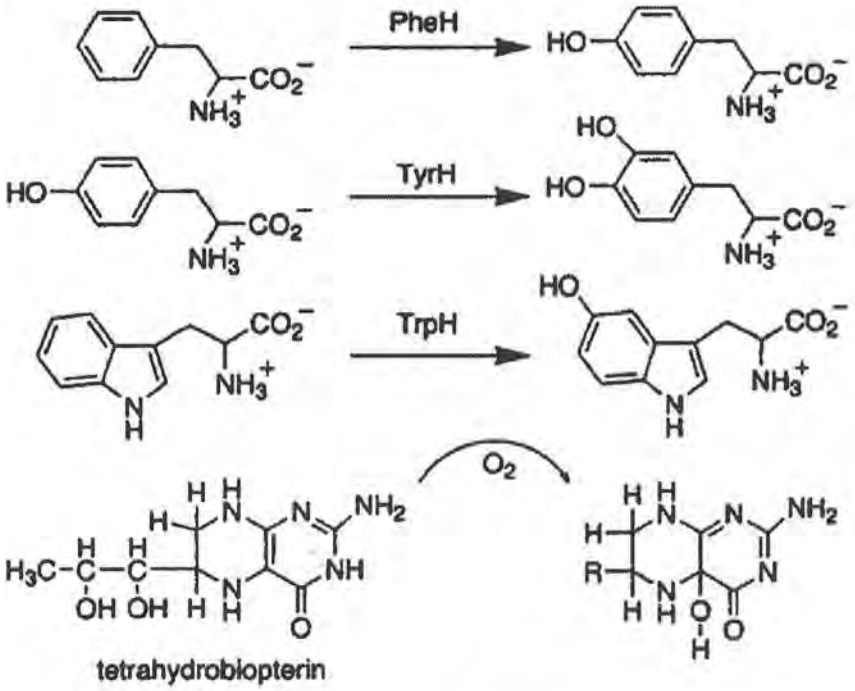


Fig.(I-9)

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 quinonoid dihydropterin (BH_2). NADH most likely reconverts BH_2 to BH_4.

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

or

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

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; the vast majority of cases of phenylketonuria are due to deficiencies in this enzyme [9,19-21]. Few of such hydroxylases have been characterized X-ray structurally [9].

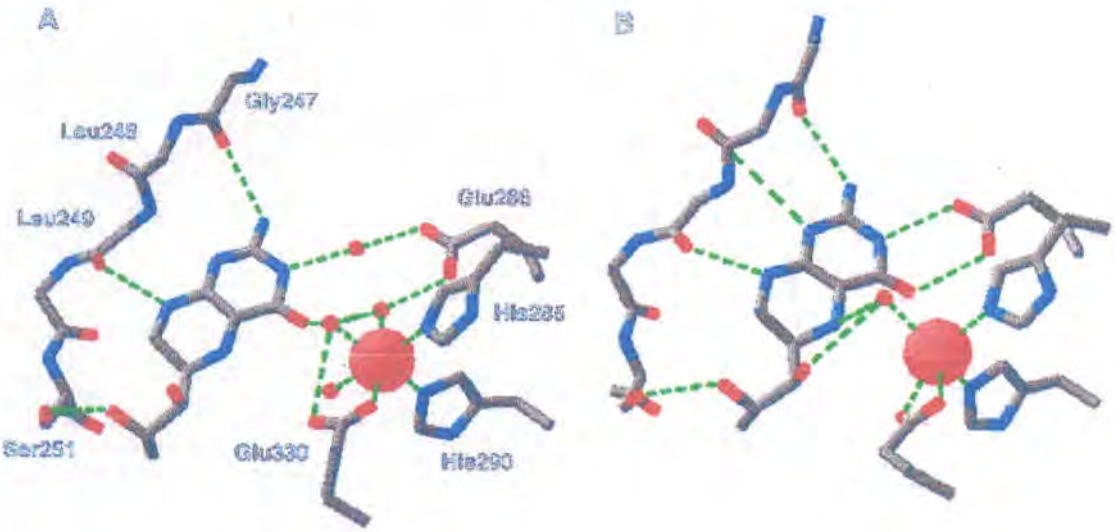


Fig.(I-10): Comparison of the iron and tetrahydrobiopterin binding sites of PAH in the absence (A) and presence (B) of *β*-thienylalanine. The structures are from the PDB files 1J8U and 1KWO. Reference 9 gives further details.

For tyrosine hydroxylase (TyrH) the iron atom lies at the bottom of 10 Å deep cleft in the enzyme surface [9]. 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 [138]. **In contrast to the other proteins with this metal-binding motif**, such as α-ketoglutarate dependent enzymes and the intra- and extradiol dioxygenases, **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 [Figure (I-10)A] [139-141]. The actual number of water molecules varies with the structure [142,143].

The kinetic mechanism 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. Figure (I-10)A shows the interactions between tetrahydrobiopterin and PAH in the binary complex of rat PAH[142]. 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 [Figure (I-10)B]. It has not been established directly whether β-thienylalanine is hydroxylated. Figure (I-11) shows the interactions between the amino acid substrate and the protein in the ternary complex; the carboxylate

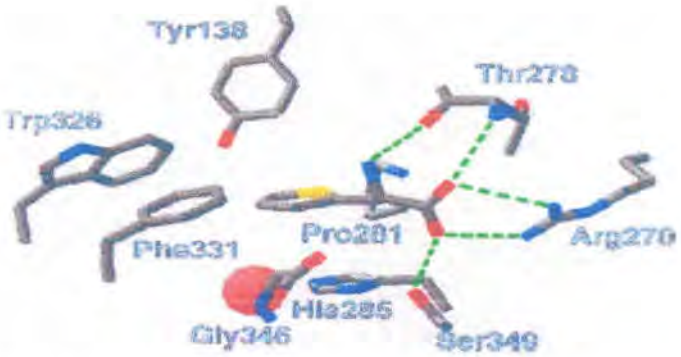


Fig.(I-11): Amino acid substrate binding site ofPAH. The structure is from the PDB file 1KWO.

of β-thienylalanine is bound to the side chain of Arg270.

As far as the mechanism of oxygen activation is concerned, the results of different studies are summarized in Figure (I-12). 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.

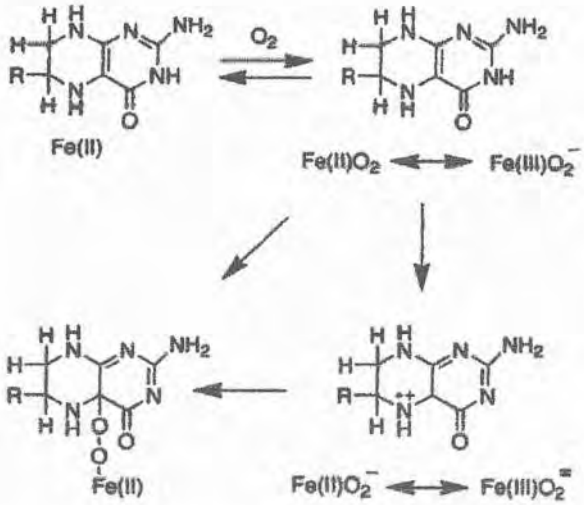


Fig.(I-12): Details regarding the formation of the 4a-peroxy-pterin intermediate are given in reference 9.

Deeper understanding about the mechanism of aromatic amino acid hydroxylation can only be obtained through synthetic modelling 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 [144]. 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. Eukaryotic NOS isozymes are catalytically self-sufficient. The electron flow in the NO synthase reaction is :

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

Tetrahydrobiopterin provides an additional electron during the catalytic cycle which is replaced during turnover. NOS is the only known enzyme that binds flavin adenine

dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH_4) and calmodulin.

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 relevant

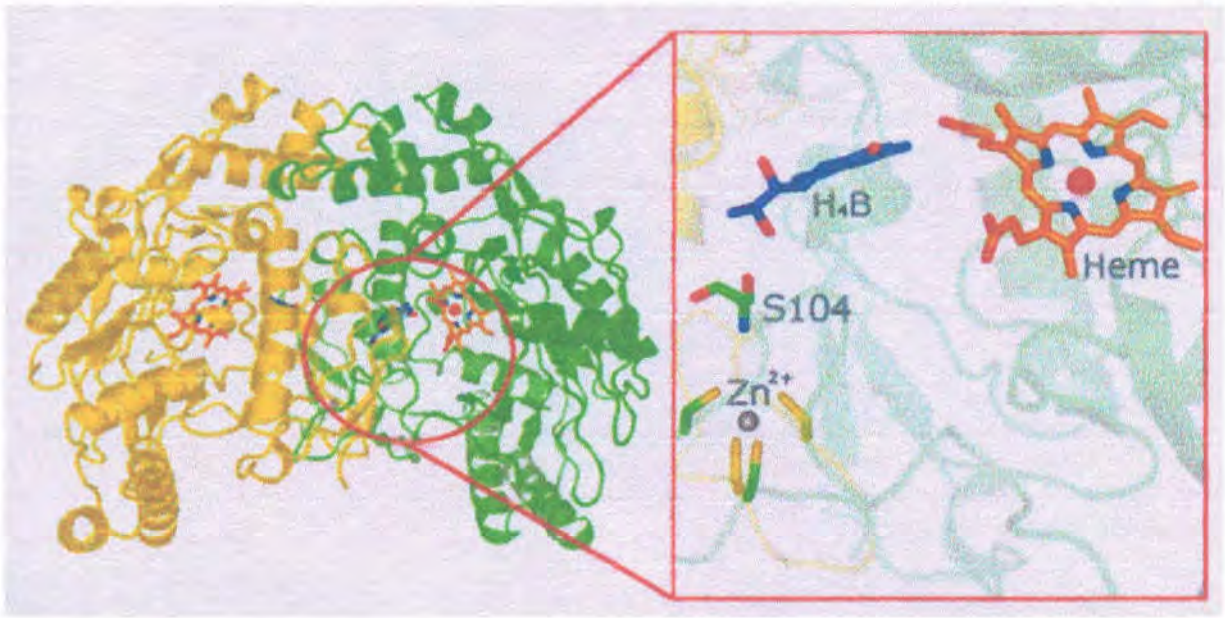


Fig.(I-13): Overall structure of the bovine eNOS dimer in complex with H_4B (Proton Data Bank entry 9NSE). The Zn^{2+} binding site is located at the dimer interface and ~15 Å from the center of the pterin binding pocket in both molecules A and B of the dimer. Chain A is colored green, chain B yellow, pterin blue, and heme orange. All structural figures were prepared with PyMol (http://www.pymol.org). Reference 144 gives further details.

X-ray structure is shown in Figure (I-13) 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 af a Zn^{2+} ion ligated to two cysteine thiols from

each subunit (ZnS_4) [Figure (I-13)]. 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;

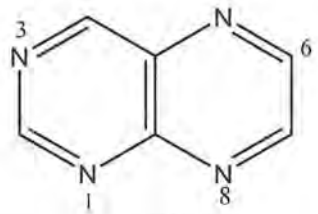
(iv) achievement of stability of the homodimer through Zn^{2+} coordination;

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

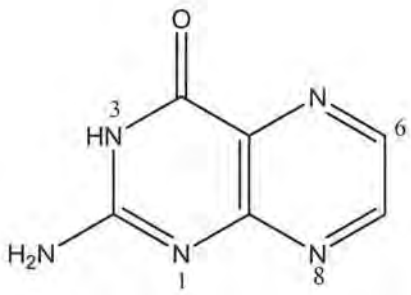
A scope is opened up for studying synthetic model compounds for elucidating the catalytic reaction mechanism and devising suitable functional models.

Aims and objectives of the work

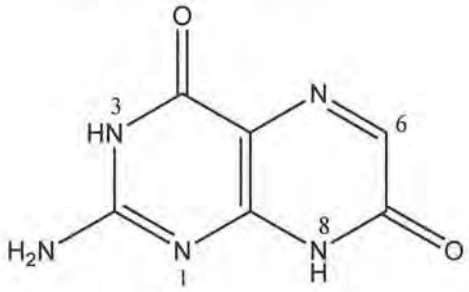
The primary motivation for pursuing the coordination chemistry of pterins (and also related heterocycles possessing the pteridine ring system, like isoxanthopterin, lumazine, etc.,) is the presence of this heterocyclic system in a substantial number of metalloenzymes, as outlined above. Important bioinorganic chemistry has grown up, centred around this structural motif. The general features of the active sites of pterin-containing metalloenzymes, are unprecedented in coordination chemistry. Apart from the intellectual attractiveness of this subject, a considerable experimental challenge is posed by the redox non-innocent nature of pterin, coupled with its poor solubility.



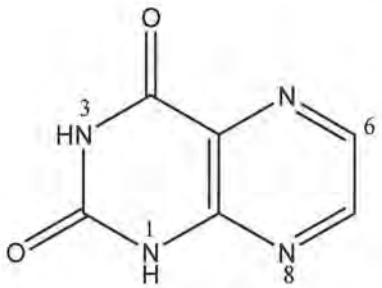
pteridine
{pyrazino [2,3-d] pyrimidine}



pterin
(2-amino- 4-oxidopteridine)

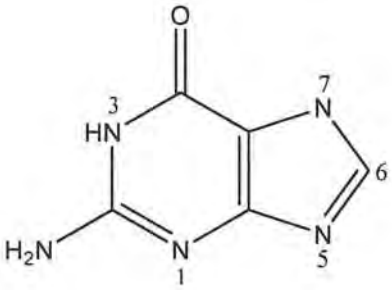


isoxanthopterin
(2-amino- 4,7-dioxidopteridine)

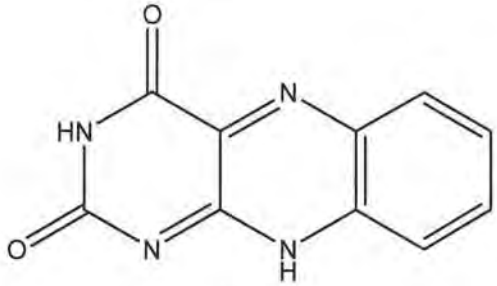


lumazine
(2,4- dioxidopteridine)

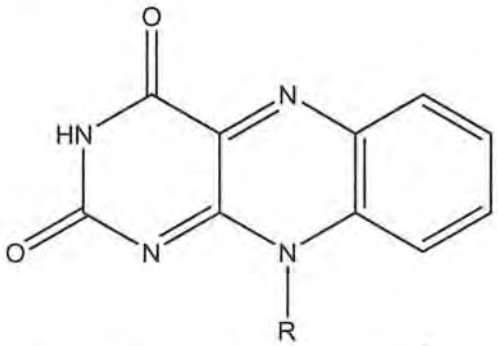
Pterin is structurally related to guanine. Isoalloxazine is another ubiquitous biomloecule, possessing the pteridine core. It is present in flavin.



guanine



isoalloxazine



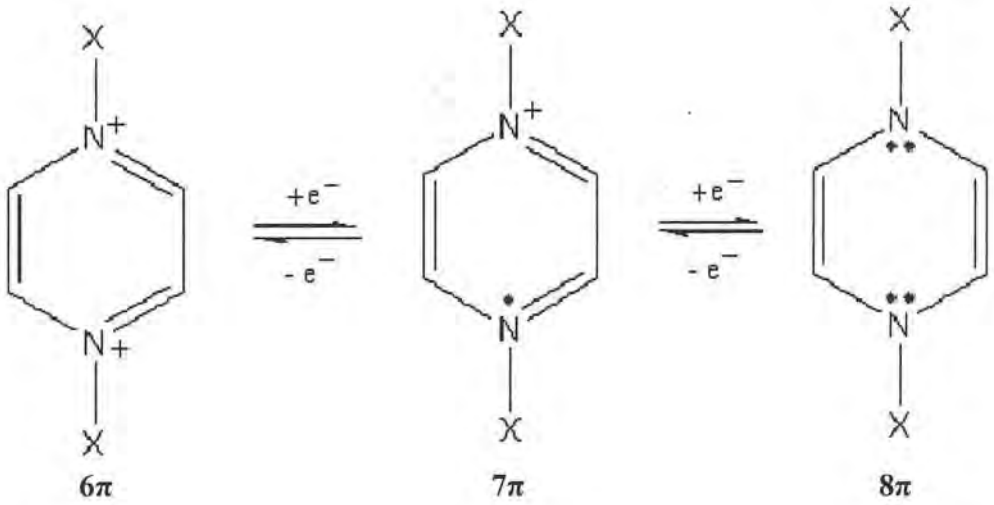
R = $-CH_2(CHOH)_3CH_2OPO_3^{2\ominus}$ in FMN

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

The polar -C=N- bonds make the pteridine structure inherently reactive. Electron-rich functional groups like amine, carbonyl, etc., stabilize the electron deficient rings of pteridine.

However, the redox non-innocent nature of pteridines, as discussed below, adds a new dimension to their chemistry, among the important biological ligand systems.

The well-known ability of pterins to act as redox partners in biological redox systems is intimately connected to the ability of the pyrazine moiety (of the pterin ring) to exist in different oxidation states (in terms of π electrons), thereby exhibiting multiple redox activity[23].



6π **7π** **8π**

Fig. (I-14)

Bicyclic pteridines accept a total of four electrons and four protons during the transformation to their most reduced state, tetrahydropteridine and Figure (I-15) illustrates this reaction for the unsubstituted pterin. Redox states between the fully oxidized pteridine and the fully reduced tetrahydropteridine are also accessible, e.g., the dihydro-level of reduction where different possibilities can arise by tautomerism or proton rearrangement. Both the 5,8-dihydropterin and the 6,7-dihydropterin isomers are unstable and rearrange to 7,8-dihydropterin. This rearrangement involves H-transfer from C6 and the quinonoid isomer can be stabilized and isolated if C6 bears two methyl substituents to block H6 loss. Even a radical trihydropterin species can be produced from reactions of radical initiators [7].

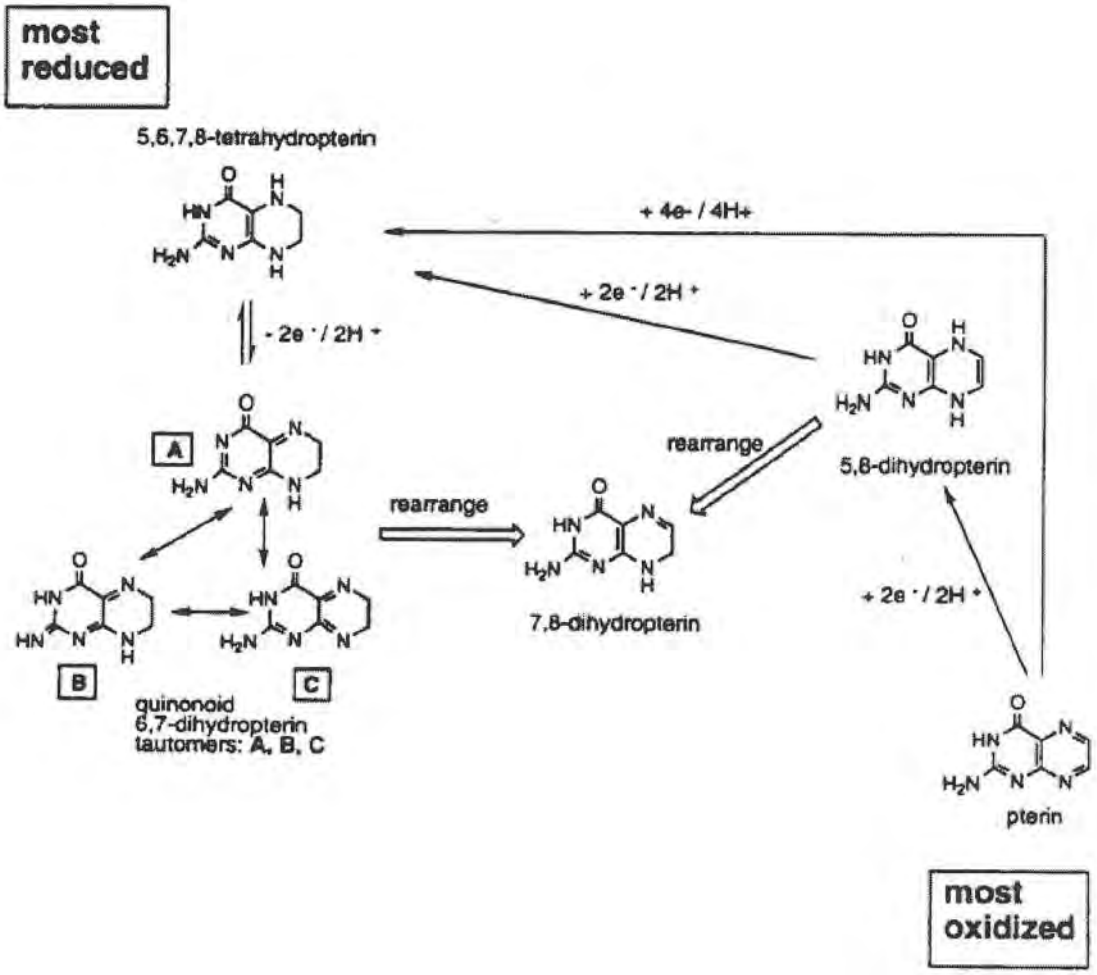
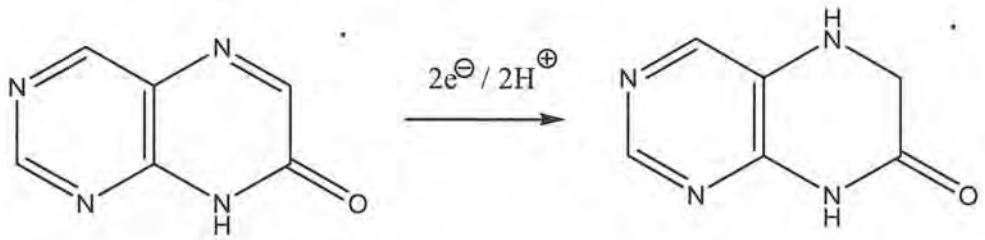


Fig.(I-15): Redox reactions that inter convert tetrahydro-, dihydro- and oxidized pterins

Pteridines and pterins having keto, carboxyl or amino substituents at C7 that participate in ring conjugation, are observed to yield 5,6-dihydropteridines on reduction.



5,6-dihydro-7-oxido-pteridine

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 [Figure (I-16)] [7].

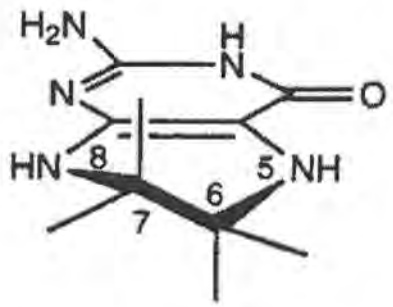


Fig.(I-16): Half-chair conformation within pyrazine ring of tetrahydropterin

The multi-electron redox reactivity of pterin is reciprocated by the redox capability of the transition metals in the relevant metalloenzymes, catalyzing a variety of redox reactions. A synchronization of the changes in the oxidation levels of the pterin ring, the transition metal ion (e.g., Mo, W, non heme and heme Fe) and the substrate (DMSO, $Me_3N \rightarrow O$, PPh_3, phenylalanine, etc.,) is needed for the successful completion of the catalytic reaction (or cycle) for each class of pterin-containing metalloenzymes.

The above ideas have catalysed symbiotic developments of coordination chemistry of pterin ligands in particular and pteridine ligands in general. References 2, 7, 12, 13, 24, 25, 27-30, 98-100, 106, 110, 117, 124, 125 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.

However, lacuna exists in our knowledge on several important areas of this subject, as outlined below.

1, Tungsten-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 tungstopterin enzymes.

2. Cobalt-pterin coordination chemistry :

This aspect is covered by only a few X-ray structurally characterized compounds, with little reactivity data [1,3,8,11]. Couple of factors are responsible for the choice of cobalt for this work, e.g., the avidity of cobalt(II) complexes for molecular oxygen as well as the ability of this d^7 system 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 :

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

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 :

This step is the elucidation of electrochemical and group transfer reactivity data of the new pterin coordination compounds in terms of their molecular and electronic structures. Different spectroscopic data and molecular modelling calculations using a suitable computational program, will stand in good stead for this purpose. 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) corresponds to the pyran ring oxygen atom of "molybdopterin", as revealed through X-ray structural work on the relevant enzymes [Figure (I-1)]. The redox non-innocent nature of pterin as par Figures (I-14) and (I-15), may be further accentuated through electronic redistribution involving this oxo group [72].

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 ($SO_3^{2-} \rightarrow SO_4^{2-}$; $CH_3CHO \rightarrow CH_3COO^-$, etc.,) or away from it ($NO^{3-} \rightarrow NO^{2-}$; $Me_2SO \rightarrow Me_2S$; $Me_3NO \rightarrow Me_3N$, etc.,), the metal centre (Mo or W) shuttles among the oxidation states VI, V and IV [Figure (I-7)] involving dioxo / oxosulphido / monooxo / non oxo sites like :

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

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

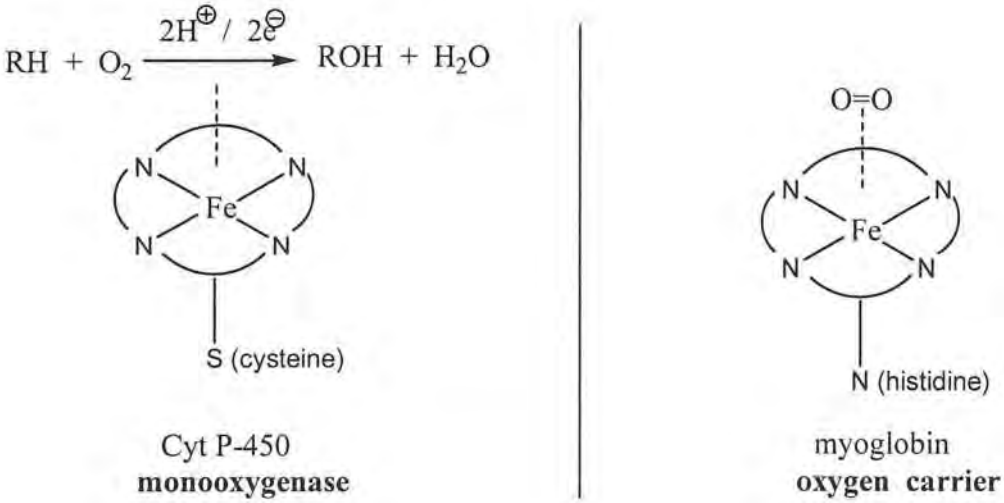
$$Mo^{VI}OS^{2\oplus} + \text{xanthine} \longrightarrow Mo^{IV}OSH^{2\oplus} + \text{uric acid}$$

► All Mo (or W) cofactors share a common non-protein organic component, called molybdopterin, that acts as a ligand to the metal centre [Figure (I-1)]; besides this, the coordination sphere around the metal centre is completed by oxo / sulphido

groups and coordinated protein ligands or even a second pterin ene-dithiolate ligand.

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. 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

Fig.(I-17)

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

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

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 [Figure (I-2)] [7]. Their possible role in facile electron flow into or out of the enzyme reaction centre, working in tandem with the redox non-innocent pterin ring, will be facinating.

2. For the heme enzyme cytochrome P-450, the oxygen atom needed for monooxygenase activity is derived from the dioxygen molecule; but for most of the oxotransferases containing Mo (or W), water supplies the oxygen atom for their catalytic function. As evident from Figure (I-7) 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 flavin centres in such large and complex enzymes [22]. Here the metal (Mo or W) oxidation state controls the level of protonation of the water-based ligand (H_2O / OH^- / O^{2-}) [62(a),127]. The increase in oxidation state of the metal centre (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 centres 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 centre,, redox reactions at the metal centres, role of the redox non-innocent pterin ligands in the associated electron transfer process, etc.

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 cobalt(II)-pterin complex of the ligand [H_2L^1] with 1,10-phenanthroline (phen) as the ancillary ligand and its X-ray structural characterization. New light is shed on the redox non-innocent property of this pterin ligand. Besides this, its X-ray structurally determined geometric parameters have been compared with those of its CHEM3D model (obtained through molecular mechanics calculations, MM2). A reasonable tally between the above two sets of data, justifies the extensive use of CHEM3D models for correlating molecular structures with the spectroscopic and kinetic data in this thesis.

In Chapter III, a new dinuclear cobalt(II)-pterin complex of [H_2L^2] is reported along with its reactivity studies towards phenylalanine and bromobenzene. Formation

of tyrosine and 4-bromophenol in the respective reaction sequences, indicates activation of aromatic rings towards hydroxylation by this new cobalt(II) complex. Modelling the phenylalanine hydroxylase (PAH) type activity even on a limited scale could be achieved.

Chapter IV reports the studies on a new binuclear tungsten(IV)-pterin complex exhibiting reactivity towards an enzyme substrate like trimethylamine N-oxide (Me_3NO) [22]. A substrate saturation type kinetics is observed. This W(IV) complex is a low-spin, diamagnetic d^2 system. It permits effective ^1H NMR spectral studies throwing light on the electron transfer ability of the pterin ligand towards the substrate Me_3NO.

Synthesis of new mixed ligand tungsten(IV) complexes of $[H_2L^2]$ and $[H_2L^3]$ are presented in chapters V and VI respectively, along with their characterization and reactivity data. Carefully chosen ancillary ligands permit a variety of studies, including redox non-innocent behaviour of a few of these new complexes. Optimum interpretation of reliable 2D-NMR data and correlation of reactivity with electronic structures, are some of the highlights of these chapters.

References are grouped together at the end of this thesis.
