CHAPTER I

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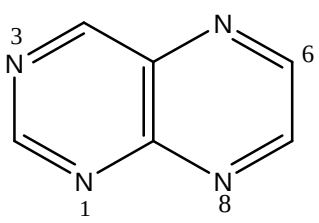
General Introduction
Aims and objectives of the work

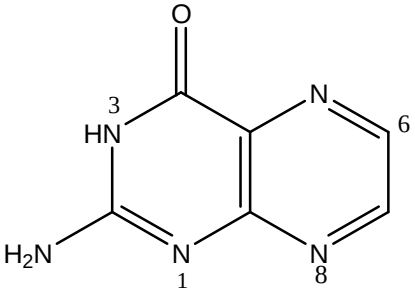
General Introduction

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 , nickel copper and zinc. 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.

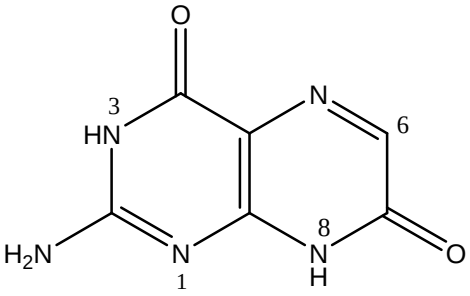
Pterins (2-amino-4-oxidopteridines) are ubiquitos in nature and their reduced forms act as essential components of different classes of metalloenzymes containing molybdenum or tungsten or iron (non heme or heme type).[5-35] Pterin is structurally related to guanine; isoalloxazine is another biomolecule possessing the pteridine core and this is present in flavin.[5]



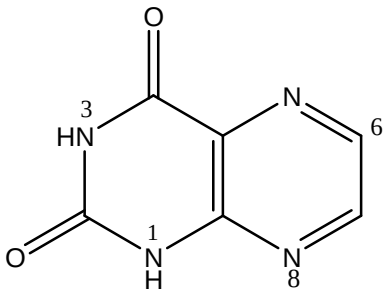
pteridine
(pyrazino [2,3-d] pyrimidine)



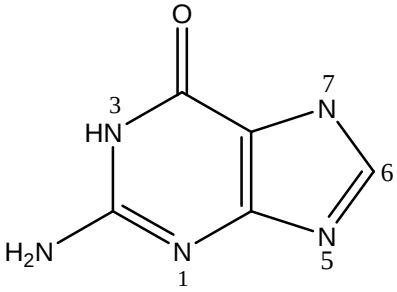
pterin
(2-amino- 4-oxidopteridine)



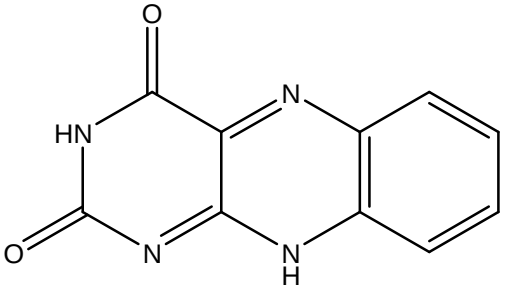
isoxanthopterin
(2-amino- 4,7-dioxidopteridine)



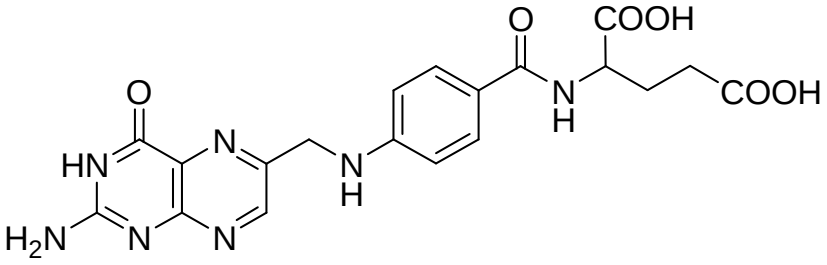
lumazine
(2,4- dioxidopteridine)



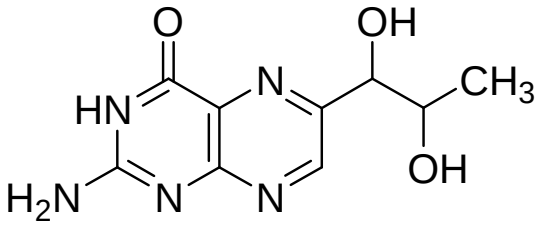
guanine



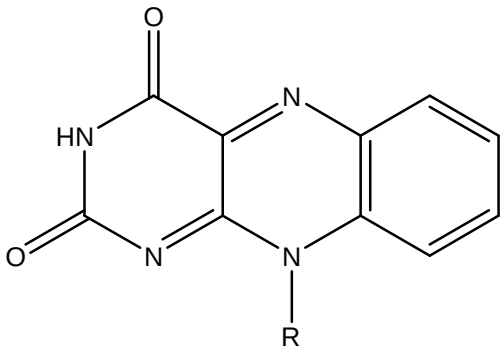
isoalloxazine



Folic acid



Biopterin



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

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

Biopterin (Scheme I -11) and folic acid are two important biomolecules with the pterin core.**6-substituted pterins dominate the biological forms of pterin**. The polar C=N bonds impart reactivity to pterin. The electron deficiency of the pteridine structure is compensated by the conjugation with the electron rich groups like amine, carbonyl, etc.[10]

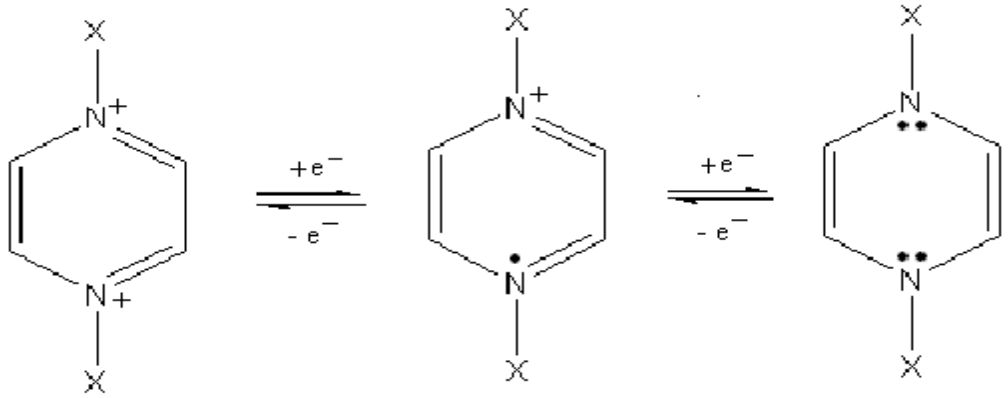
The above redox enzymes catalyze rich chemistries, ranging from oxygen atom transfer reactions using water as its source to and from a variety of biologically important substrates by oxomolybdoenzymes and tungstopterin enzymes, [1-6,10,99-104,106-109,133] activation of aromatic rings (of aromatic amino acids) towards hydroxylation by phenylalanine hydroxylases (PAH) containing non-heme iron [7-10,52-55,105,117-121] and finally heme iron- containing mammalian nitric oxide syntheses (NOSs) converting L-arginine to L-citrulline and nitric oxide, an important second – messenger molecule in neutral and cardiovascular systems; it is a overall five-electron oxidation process.[122-125]

Pterin has the unique ability of displaying multi-electron redox reactivity; this redox non-innocent nature of pterin is reciprocated by the ability of the associated transition metal ion in displaying multiple oxidation states, in the above enzymes. Their functional aspects require that

the redox processes at the metal centre should be linked to the changes in the pterin / pyrazine ring oxidation level, during each type of catalytic cycle.[9,10]

Redox non-innocent behavior of pterin

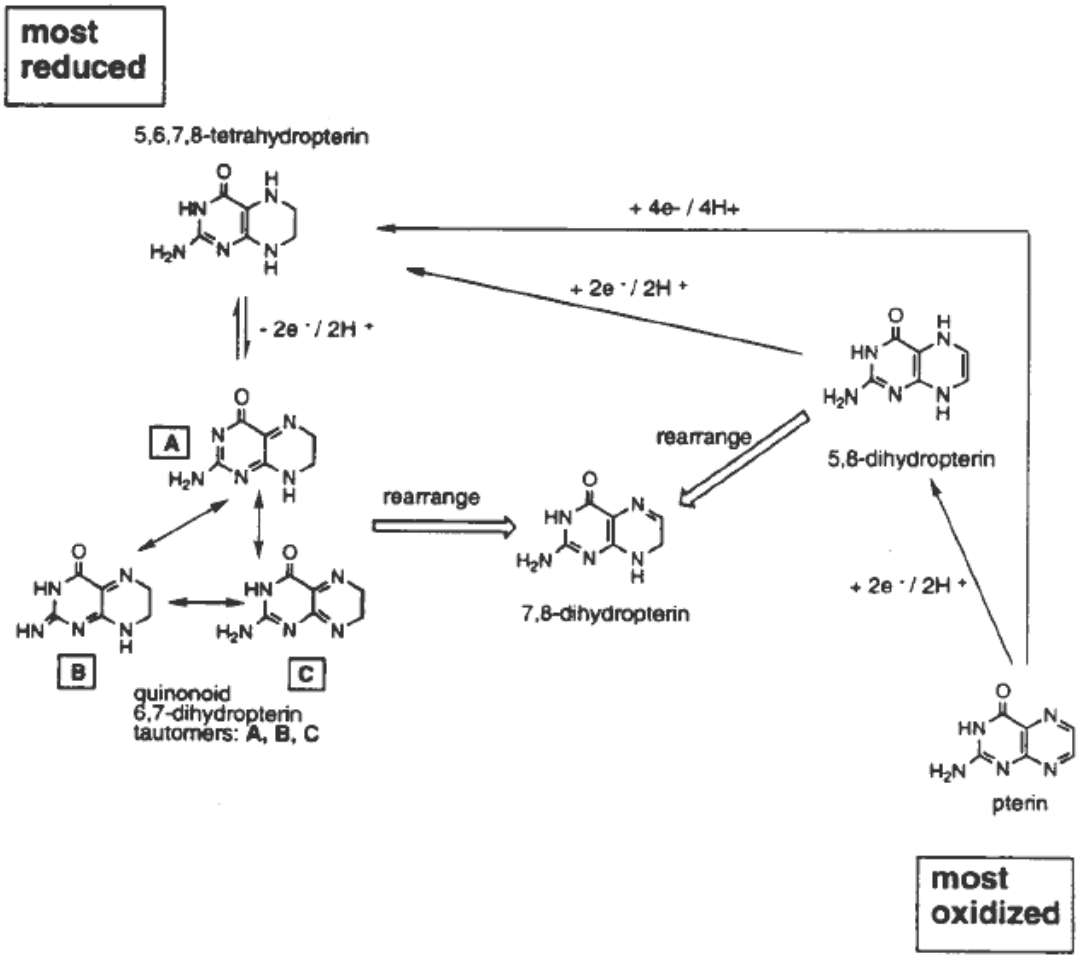
The pyrazine ring of ptein plays a decisive role in controlling the redox non-innocent behavior.[9] For example, N, N′-coordinated pyrazines undergo facile electron transfer between the 6π, 7π and 8π electron oxidation states.



6π 7π 8π

The two electrochemical potentials describing this two-step redox systems cover a broad range in the conveniently accessible potential region. For example, when the N, N′- substituents are changed as $CH_2Me \rightarrow SiMe_3 \rightarrow P(S)Me_2 \rightarrow C(O)Me$, the cyclic voltammetric data ($E^{0\prime}/E_{pc}/E_{pa}$ versus SCE) cover a range of 1.44 V(from -0.7 to +0.7V). This sequence reflects an increasing delocalization of electron density out of the pyrazine ring system with the maximum limit reaching at the acyl-substituted system.

As indicated in Scheme I-1 the most oxidized form (aromatic) of pterin can take up a total of 4-electrons/4-protons via proton-coupled electron transfer (PCET) or coupled electron-proton transfer (CEPT) to give the fully reduced form, tetrahydropterin.[9-11] Redox states between the fully

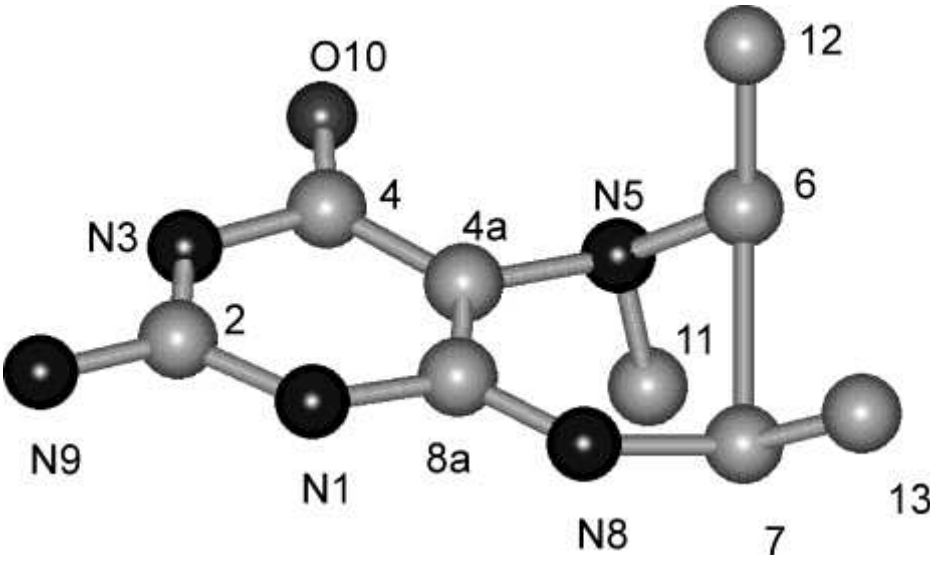


Scheme I-1

oxidized and fully reduced forms of pterin are also accessible. They mostly occur at the dihydro-level of reduction and are inter-related by tautomerism or proton rearrangement. The initial product of biopterin reduction is the 2-electron/2-proton product 5, 8- dihydropterin which is unstable and undergoes conversion to 7, 8- dihydropterin. The other dihydropterins such as the 2-electron/ 2-proton product of tetrahydropterin oxidation, exhibits tautomerism among different kinds of so-called quinonoid – dihydropterin (or 6, 7-dihydropterin), including one containing a C-N double bond at the 2-amino group. Like the 5, 8-dihydropterin, the 6,7-dihydropterin is also unstable and rearranges to 7,8-dihydropterin. Since this rearrangement involves H-transfer from

C6 position of the pterin ring, the quinonoid form can be isolated if C6 bears two methyl substituents to block this H6 loss.[124]

X-ray structural data on a few di-and tetrahydropterins throw light on their relative stabilities through π-electron delocalization. The tetrahydropyrazine ring of 5, 6, 7- trimethyl-5, 6, 7, 8-tetrahydropterin dihydrochloride-monohydrate, exists in a conformation in which the C6 (sp^3) atom deviates markedly from the mean plane of the other five atom; the pyrazine ring is planar with N8 and C7 coplanar to this ring.



On the other hand, for 6-methyl-7,8-dihydropterin- monohydrochloride-monohydrate, the 7, 8-dihydropyrazine ring is essentially planar with π-electron delocalisation into both pyrazine and pyrimidine rings. This aspect decides the grater stability of the 7,8-dihydro form among the three above-mentioned possibilities[124].

Formation of a pterin radical in the reaction of the heme domain of inducible nitric oxide synthase with oxygen, could be detected by EPR spectroscopy. Here the bound cofactor tetrahydrobiopterin (BH_4) undergoes one-electron oxidation to the radical ($BH_3^{\bullet}$) where the unpaired electron localizes mainly at the N5 position of the pterin ring[115,122]

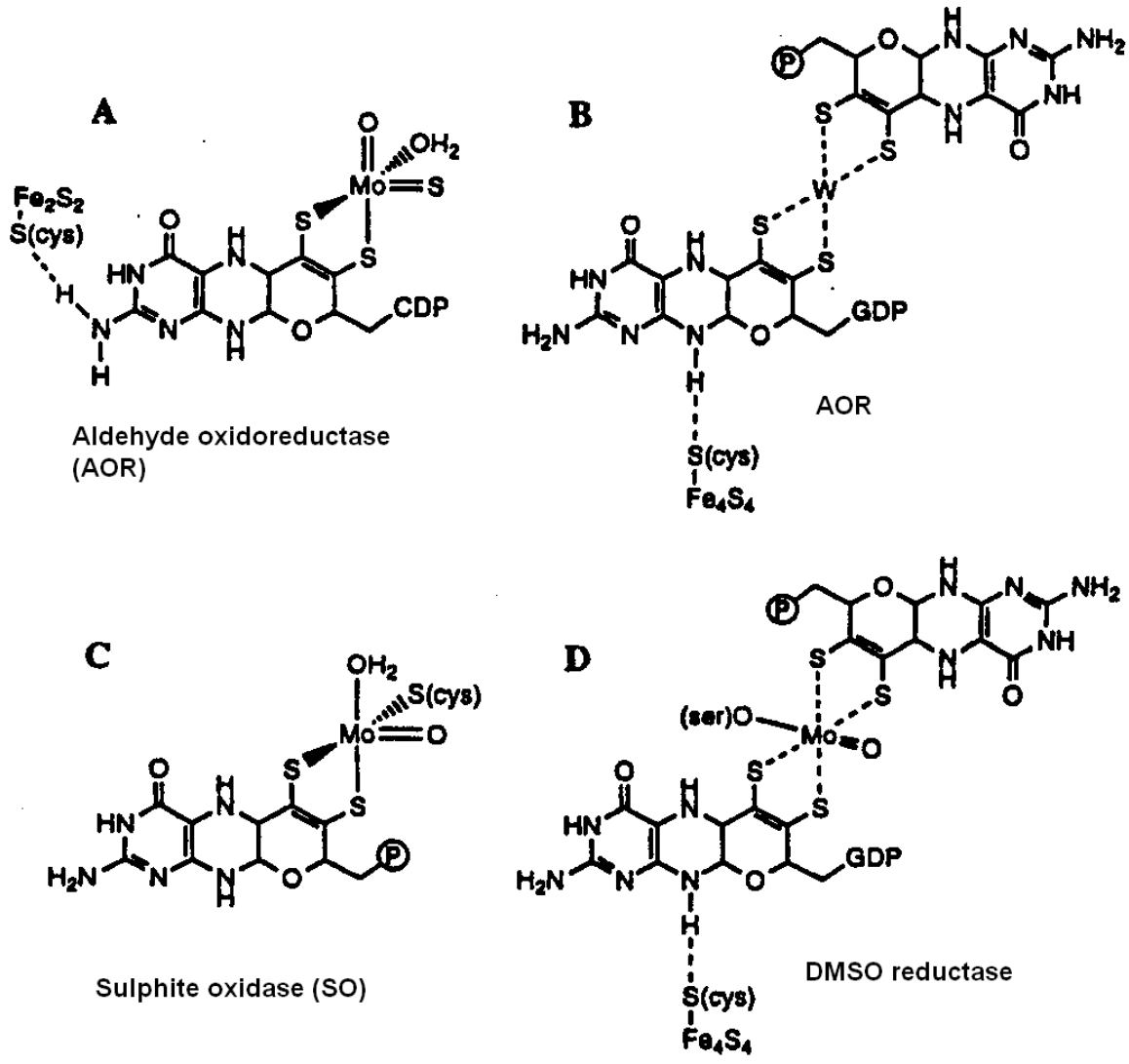
In connection with the intimate mechanism for phenylalanine hydroxylase (PAH) catalysis, formation of a 4a-hydroperoxydihydrobiopterin has been identified involving the addition of O_2 at the C4a bridgehead site of pterin (Scheme I-2). This reaction has precedence

Scheme I-2

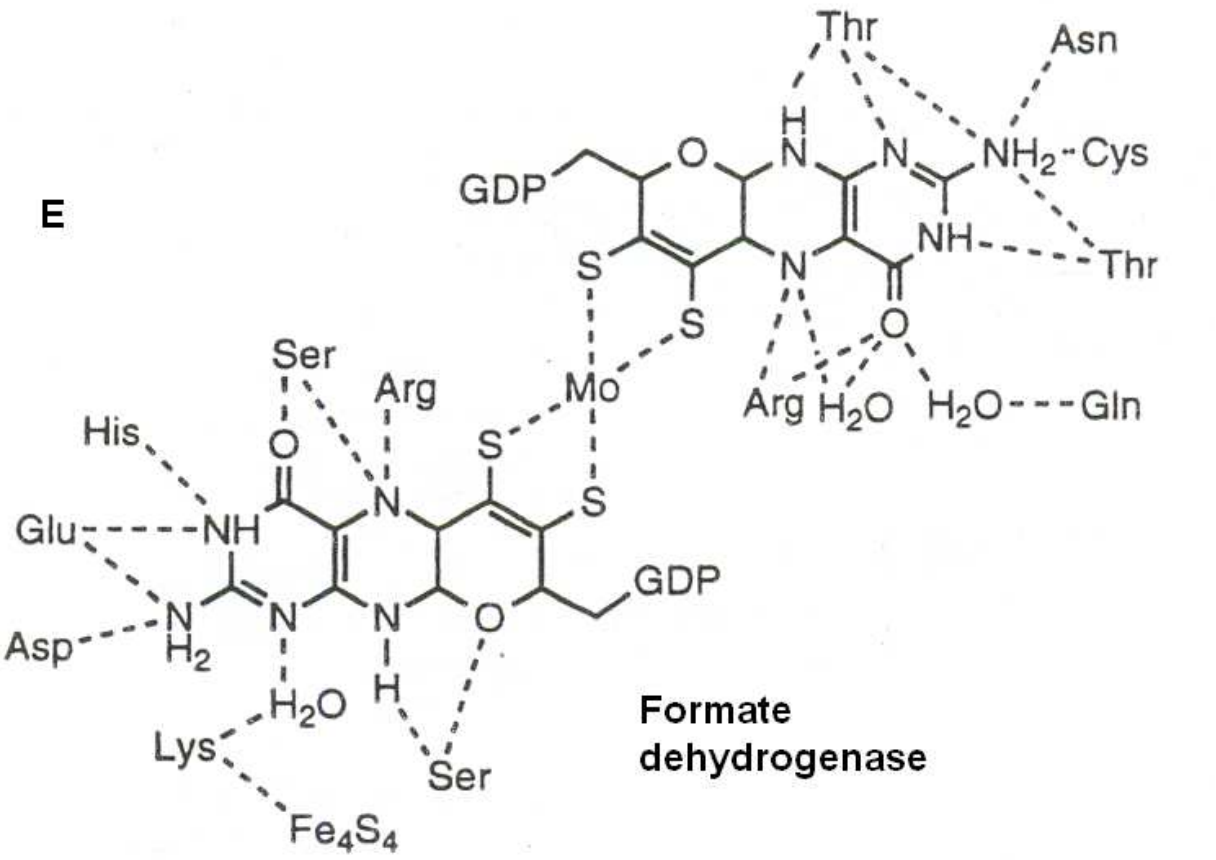
in flavin chemistry. Most likely O_2 activated in this manner, generates a reactive species capable of hydroxylating the unactivated aromatic rings.[10] Theoretical calculations in vacuo predicted that C4a of tetrahydropterin has the maximum electron density and that the adjacent nitrogen (N5) enhanced the reactivity of C4a. The low energy π^* orbital of biopterin and large orbital coefficent of C4a, are suggested to be responsible for the above observations[124].

The various reactivities of pterin-containing enzymes originate from the regulation of redox potentials of pterin cofactors by noncovalent interactions between pterins and functional groups of protein backbones. The noncovalent interactions include multiple hydrogen bonding towards pterin cofactors at the active sites and π-π interactions in the vicinity of the metal centres.[126,127]

The extensive hydrogen bonding interactions as revealed by x-ray crystallography, arround the active sites of Mo or W-containing oxotranferases are shown below schematically (Scheme I-3 & I-4).

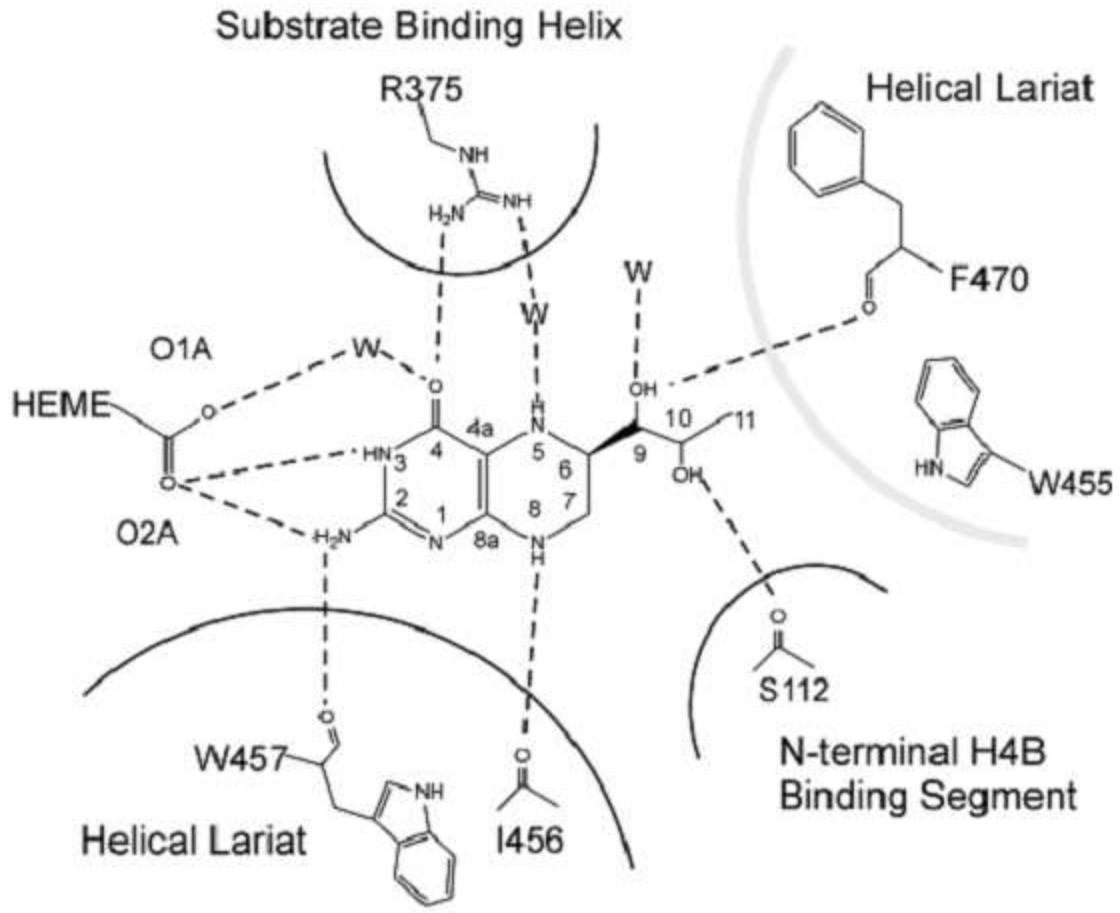

A
Fe2S2
S(cys)
Mo
OH2
S
CDP
Aldehyde oxidoreductase
(AOR)
B
W
GDP
S(cys)
Fe4S4
AOR
C
OH2
S(cys)
Mo
O
Sulphite oxidase (SO)
D
(ser)O
Mo
O
GDP
S(cys)
Fe4S4
DMSO reductase

Scheme I-3



Scheme I-4

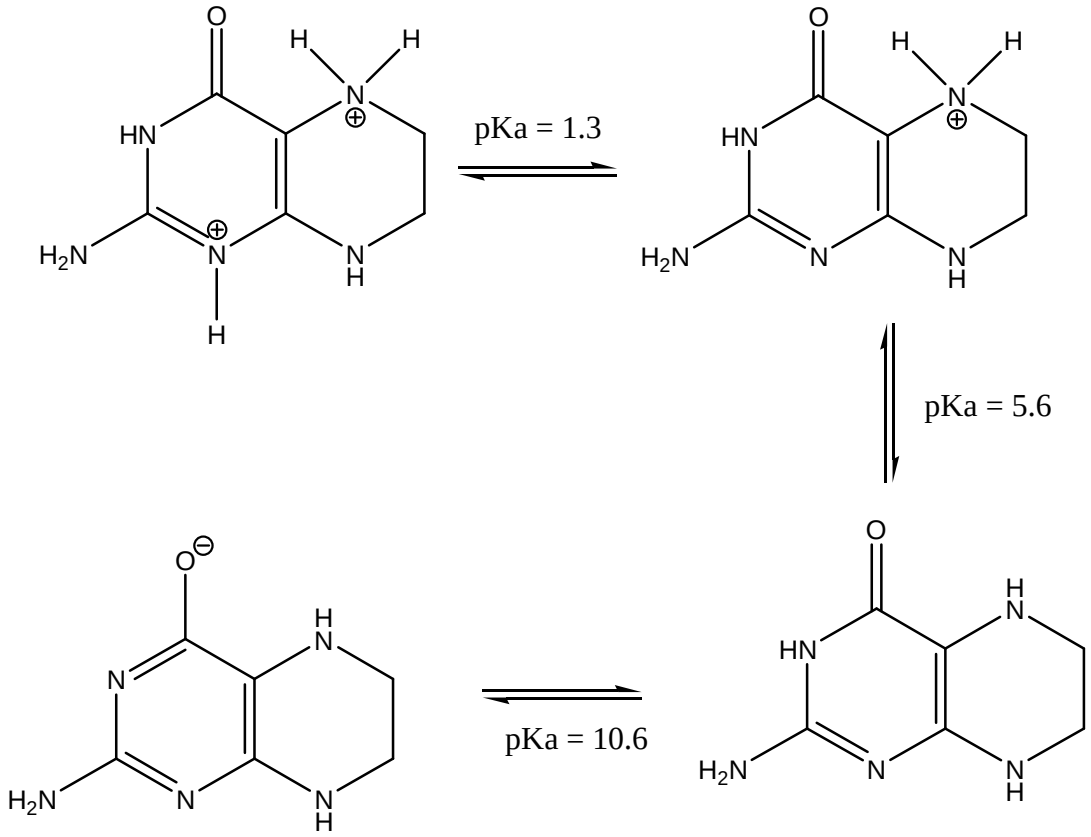
The 2-amino group and the 8-NH group are the points of the pterin structure involved in the majority of the hydrogen bonding interactions that include other electron-transfer groups such as iron-sulphur clusters as observed in oxotranferases. For nitric oxide synthases (NOSs) the tetrahydrobiopterin cofactor (BH_4) uses three additional positions of the pterin ring (e.g., 3-NH, 4-O and 5-NH) for hydrogen bonding interactions. Scheme I-5 highlights this aspect.



Scheme I-5

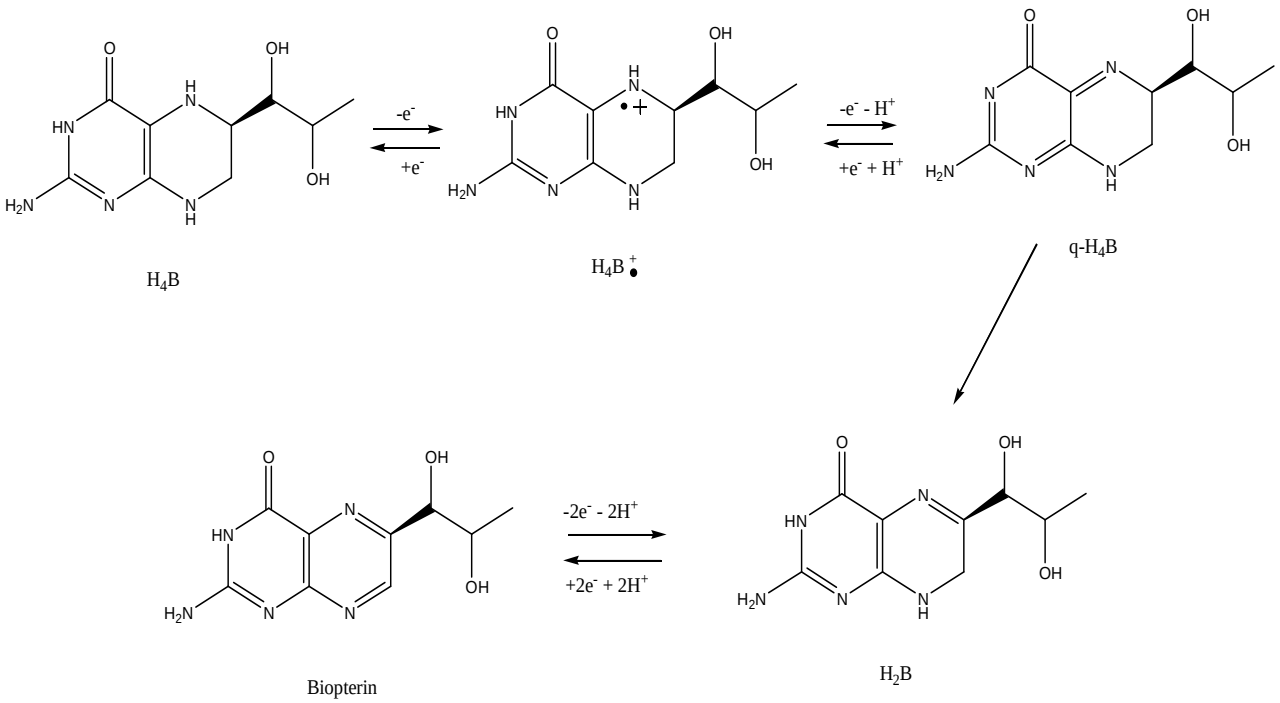
The pterin site is well designed to facilitate electronic interaction between heme and BH_4 by virtue of direct hydrogen bonds between a heme carboxylate and pterin N2 and N3. The same heme carboxylate group integrates BH_4 binding with substrate binding by also forming hydrogen bonds to the substrate amino group.[115,122-125] Furthermore, π-stacking interactions, an overall negative potential at the pterin site and interaction of an Arg residue with pterin O4 may all facilitate BH_4 radical formation in NOS.

Scheme I-6 shows protonation of the pterin nitrogen (amide group) and phenolate groups as a function of pH. The pKa of N1 and N5 are 1.3 and 5.6 respectively, such that at physiological pH, biopterin exists essentially as an uncharged species.



Scheme I-6 Protonation states of pterin

Biopterin can pass through different oxidation states including a free radical one where the electron density is localized mainly on the N5 atom. As indicated in Scheme I-7, heteroatom



Scheme I-7 Redox states of H_4B

protonation follows the same order in both the dihydro and tetrahydro forms of pterin. Here BH_2 stands for the most stable dihydro form, i.e., 7, 8- dihydropterin due to essentially planarity of the partly reduced pyrazine ring, as elucidated earlier[124]. The pterin ring protonation state is a vital parameter that regulates biopterin binding and function in enzymes.

Tetrahydropterins like BH_4 are labile in solution and can react with O_2, superoxide, H_2O_2 etc. (Scheme I-2.). Oxidations under anaerobic conditions have been studied using chemical oxidants like ferricyanide or by electrochemical means.

Evolutionary process has endowed nature with sufficient maturity for utilizing the intricate properties of pterin deravatives as above, to the optimum limit in different classes of redox metalloenzymes. Folic acid, an essential nutrient for mammalian organism, is a vital pterin derivative. Riboflavin and flavin enzymes are based on a common structural motif called lumazine; the latter possesses a pteridine core. Flavoenzyme oxidases are unique in the sence that they are the only class of such oxidases in which a metal ion is not essential for the activation of O_2.[124]

Essential aspects of the relevant metalloenzymes are presented below.

1. **Oxo-transferases containing molybdenum or tungsten**

This class of enzymes catalyse hydroxylations and net oxygen atom transfer reaction (OAT) to and from a variety of biologically important substrates.[1-6,10,99-104,106-109,133]

Hydroxylation:

$$RH + H_2O \rightleftharpoons ROH + 2H^+ + 2e'$$

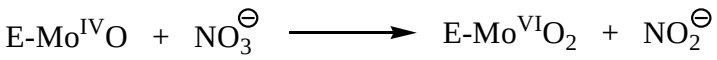
Net oxygen atom transfer:

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

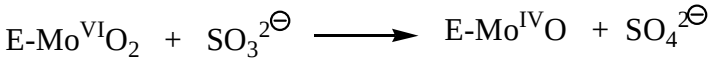
The above reactions mostly involve two-electron redox chemistry coupled to the transfer of an oxygen atom to or from water. During the catalytic cycle the Mo/W centre cycles between VI and IV oxidation states. These redox enzymes catalyse key reactions in the metabolism of carbon, nitrogen and sulphur; while molybdenum is essential for almost every life form, tungsten is proved to be essential for microorganisms, the hyperthermophilic archaea, which thrive near 100^0C (hydrothermal vents on the ocean floor). From 1995 onwards many of such enzymes have been characterized x-ray structurally. Molybdenum is available in natural waters as molybdate, exceeding in concentrations such essential trace elements as Mn, Fe, Co, Cu and Zn. This

availability and a remarkable chemical versatility make Mo a crucial component of enzymes. Few typical examples are shown below.

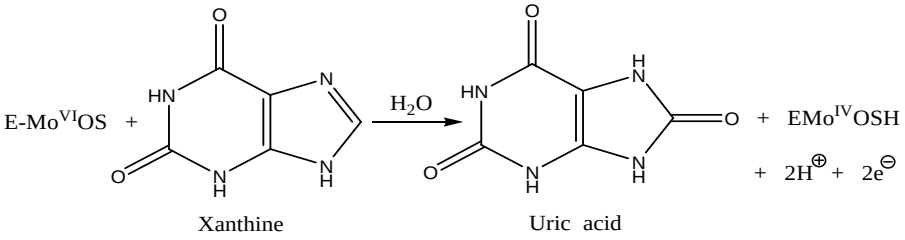
Nitrate reductase



Sulphite oxidase

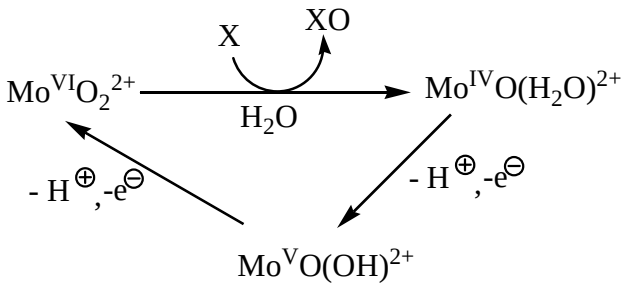


Xanthine oxidase



E = enzyme without the metal centre

A typical enzyme cycle for net oxygen atom transfer is shown below.

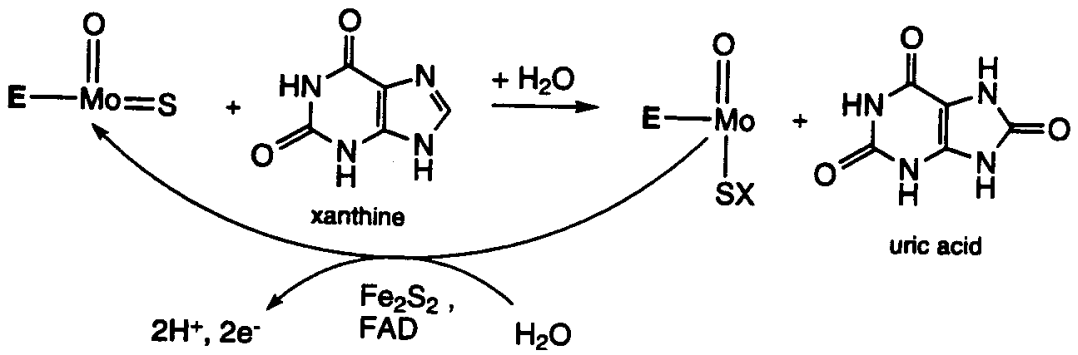


This cycle can operate in either direction. For sulphite oxidase (X = SO_3'') it is valid as shown above; in case of nitrate reductase (XO = NO_3') the cycle operates in the reverse direction. The above scheme is based on x-ray structural data of Mo- or W- containing enzymes, supported by spectroscopic (e.g., EXAFS,EPR) kinetic methods as well as computational approaches.[1,2,108,109,133] 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

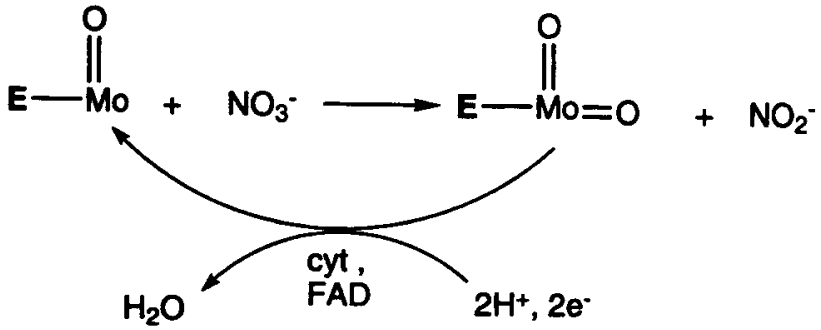
metal oxidation states (VI/V/IV) control the level of protonation of the water-based ligand ($H_2O/OH'/O^{2-}$).

As indicated above, both Mo and W are redox active under physiological conditions (ranging between oxidation states VI and IV); since the V oxidation state is also accessible, they can act as transducers between obligatory two-electron and one-electron redox systems; they can catalyse reactions such as hydroxylation of carbon centers (e.g., xanthine → uric acid conversion) under more moderate conditions than are required by other systems, using H_2O as the source of the oxygen atom transferred. The oxidations of more resistant substrates, such as alkyl chains and aromatics, do not appear to be catalysed by Mo or W enzymes. Such reactions are catalysed by cytochrome P-450 and methane monooxygenase with the iron – containing aggressive active sites like Fe^{IV}= O (cyt P-450) or $Fe_2{}^{IV}$ = O (methane momooxygenase) group, attacking the substrates. Such intermediates are derived from dioxygen rather than water and higher operating redox potentials are needed here.

xanthine oxidase



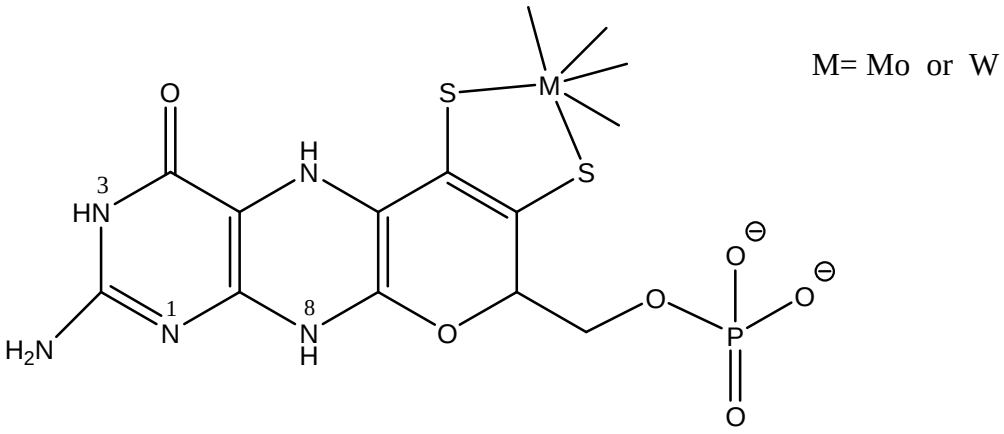
nitrate reductase



Presence of prosthetic groups (FAD, cyt, Fe-S centres) are essential for mediating the reducing equivalents [$2H^+$, 2e′] or [2H] involved in each enzyme turnover cycle.`

The stoichiometry of the reaction $RH + H_2O \rightarrow ROH + 2H^+ + 2e'$ associated with the xanthine oxidase family is unique among biological systems catalyzing hydroxylation reactions in that reducing equivalent are generated rather than consumed in the course of the reaction and water rather than dioxygen is utilized as the ultimate source of the oxygen atom incorporated into the substrate.[1] As outlined above, the overall reaction mechanism of all such enzymes can be broken down into reductive and oxidative half- reactions of the catalytic cycle. For xanthine oxidase the reductive half-reaction is associated with the hydroxylation process, coupled with the reduction Mo(VI)→Mo(IV).

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 (Scheme I-8)

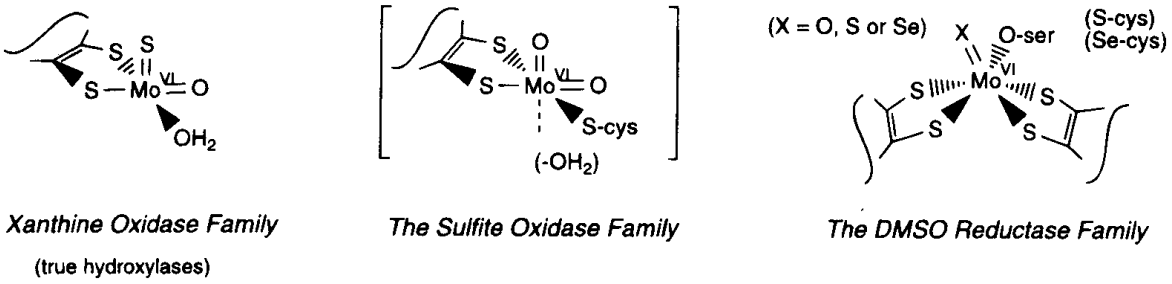


Scheme I-8

The ene-dithiolate group located on the pyran ring which is fused to the pterin, has the name pyranopterin. Schemes I-3& I-4 reveal few additional aspects of x-ray data.

The active sites of such 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 sulphite oxidase, serine oxygen in DMSO reductase or selenocysteine selenium in formate dehydrogenase from the polypeptide backbone of the protein.[104]

Hille has classified the oxo-molybdenum enzymes into three families based on their protein sequences and the structures of their oxidized active sites; each family is named in terms of its most prominent member (Scheme I-9). [1,103,133]



The Xanthine Oxidase Family
(true hydroxylases)

The Sulfite Oxidase Family

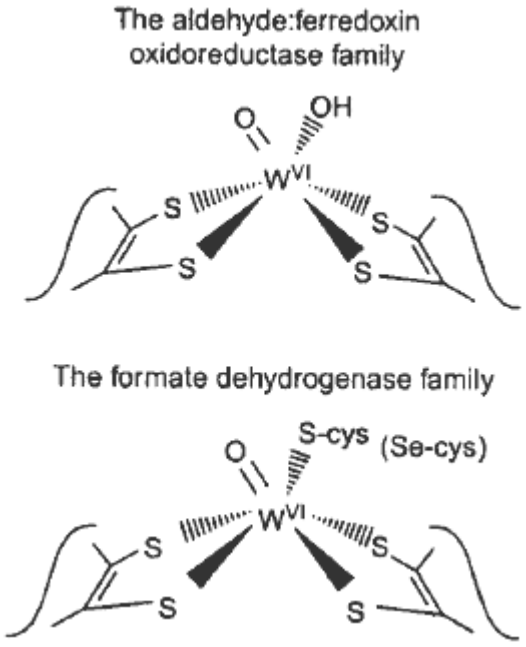
The DMSO Reductase Family

Scheme I-9

The tungstopterin enzymes are classified into three families according to their active sites, determined by x-ray crystallography.[2]

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

The active site structures are shown for two cases (Scheme I-10).



Scheme I-10

Presence of a relatively large number of both ion-pairs and buried atoms may contribute to the extreme thermostability of these enzymes (approx. 100^0C).

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

Their functional aspects are stated below briefly.

AOR family

$CH_3CHO + H_2O \rightarrow CH_3COO' + 3H^+ + 2e'$

Aldehyde oxidation is a two-electron process but ferredoxin (Fd) contains a single [4Fe-4S] cluster and undergoes only a one-electron redox reaction; for maintaining electron stoichiometry one catalytic turnover requires the reduction of two molecules of Fd.

FDH family

They utilise CO_2 as the substrate.

$$CO_2 + H^+ + 2e' \rightleftharpoons HCOO'$$

Here NADPH acts as the physiological electron donor.

Acetylene hydratase (AH)

The enzyme catalyses the hydration of acetylene to acetaldehyde:

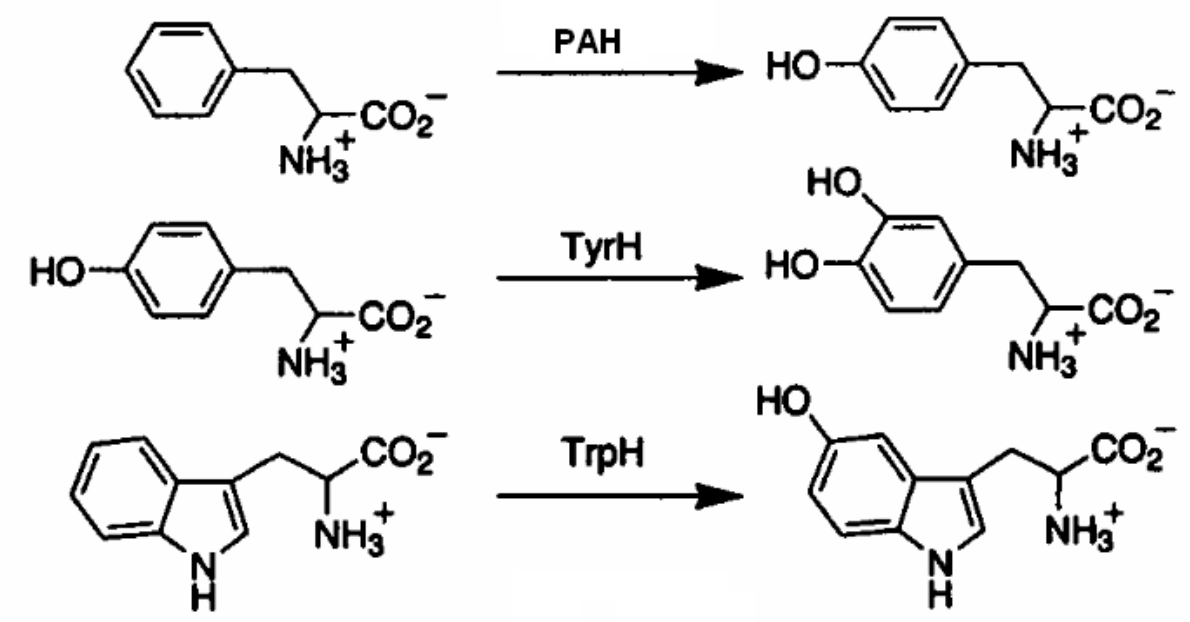
$$HC{\equiv}CH + H_2O \rightleftharpoons CH_3CHO$$

2. Aromatic amino acid hydroxylases

The aromatic amino acid hydroxylases (AAHs) constitute a family of mononuclear non-heme iron(II)-containing enzymes – phenylalanine hydroxylases (PAH), tyrosine hydroxylases (TryH) and the tryptophan hydroxylase (TrpH) – which all catalyse the hydroxylation of aromatic amino acids like phenylalanine, tyrosine and tryptophan respectively (Scheme I-11).

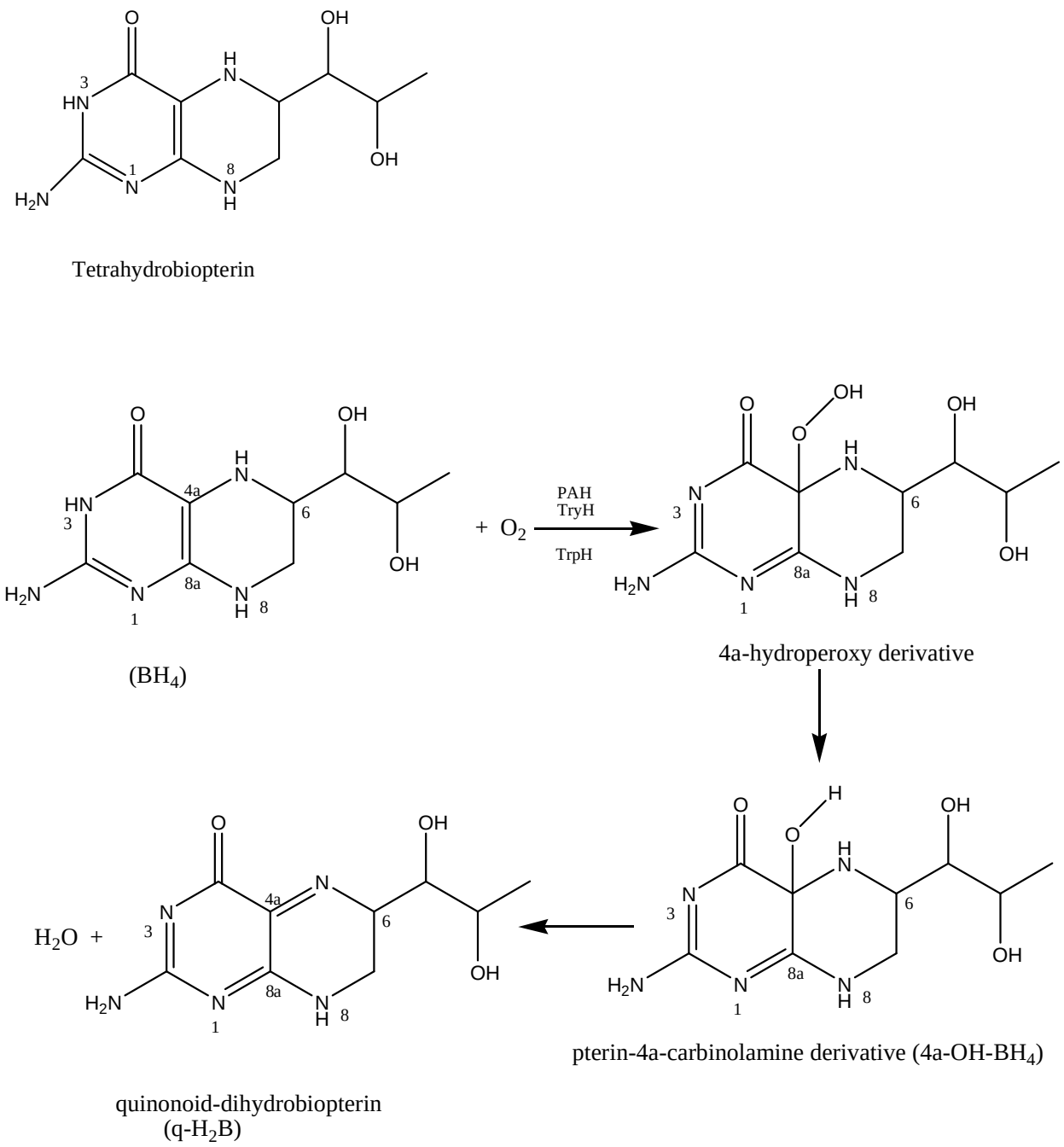
The conversion of phenylalanine to tyrosine by phenylalanine hydroxylase is belived to play an obligatory role in the catabolism of phenylalanine for energy production. One of the products of tyrosine breakdown, fumarate, can be oxidized to CO_2 and water or it can lead to the formation

of glucose. Since gluconeogenesis takes place mainly in the liver, it seems appropriate that phenylalanine hydroxylase is present in highest concentration in that tissue. The only other organ capable of significant glyconeogenesis, the kidney, has also been shown to contain significant phenylalanine hydroxylase activity.[139,146,147]



Scheme I-11

The common features within the AAH family are the dependence on the tetrahydrobiopterin cofactor, BH_4 which is oxidized to pterin-4a-carbinolamine (4a-OH-BH_4) during the catalytic cycle and the use of molecular oxygen, the atoms of which are incorporated into the amino acid and the cofactor (Scheme I-12).[53,55,105,117,119-121]



Scheme I-12 Role of BH_4 cofactor in activation/splitting of the dioxygen molecule.

One molecule of O_2 is consumed in the reaction; one oxygen atom is inserted as an hydroxyl group into the substrate (Scheme I-11), while the second oxygen atom is reduced to water (Scheme I-12).

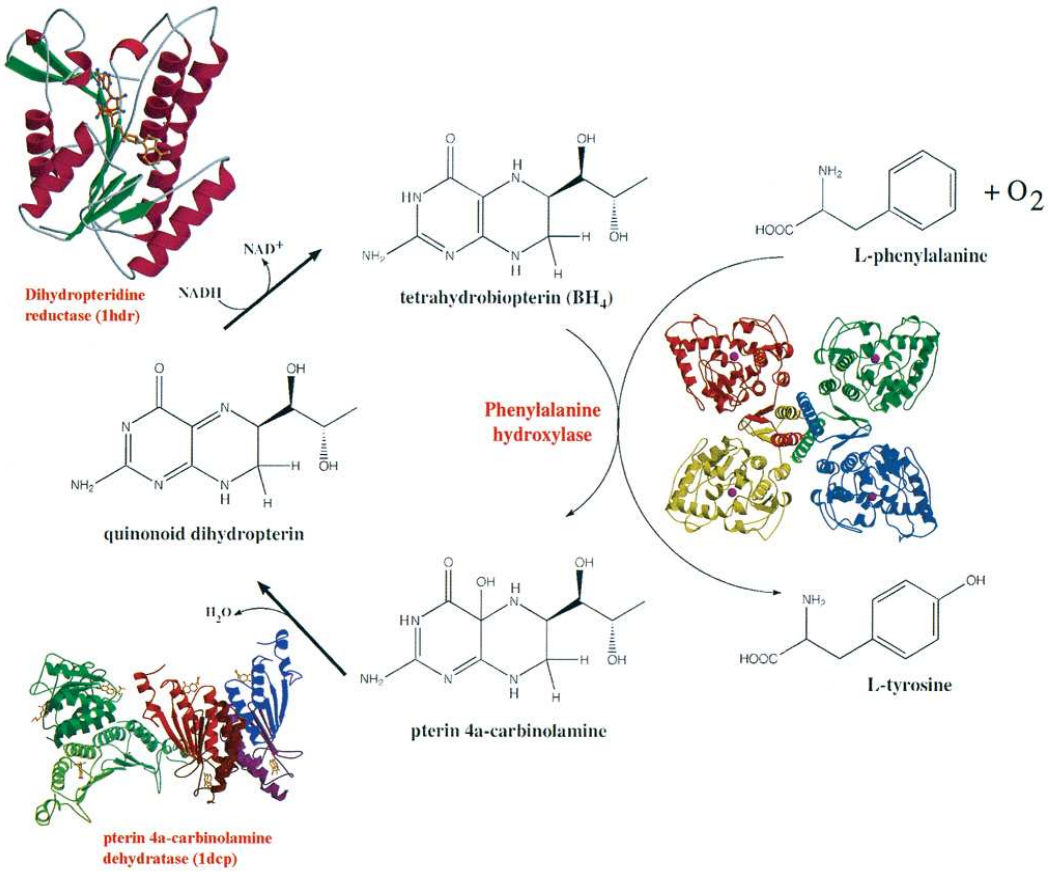
$$\text{Phenylalanine} + O_2 + BH_4 \longrightarrow \text{Tyrosine} + H_2O + BH_4$$

or

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

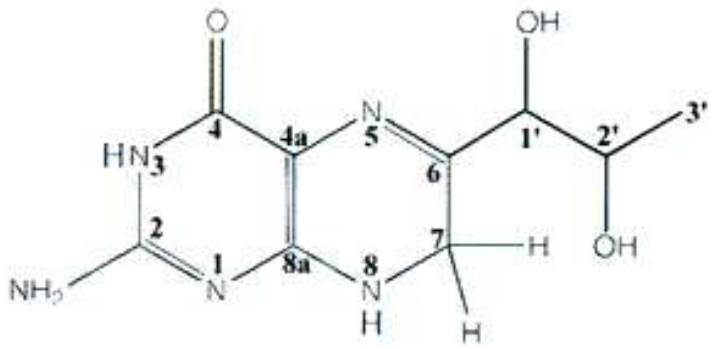
The four-electron reduction of oxygen thermodynamically drives the hydroxylation of the substrate, namely, phenylalanine (a formal two electron oxidation) in tandem with the two electron oxidation of tetrahydrobiopterin (BH_4) to its quinonoide dihydropterin state (q-BH_2). NADH most likely reconverts BH_2 to BH_4 (Scheme I-13). The mixed function oxygenase nature of the enzyme (PAH) was demonostrated by ^{18}O incorporation into [^{18}O] tyrosine and H_2O^{18}.

Although the primary product of PAH activity is the quinonoid isomer of dihydrobiopterin (q-H_2B, Scheme I-12) the cofactor isolated from rat liver, however, was 7,8-dihydrobiopterin (Scheme I-1). In the presence of the 7, 8-dihydro isomer, another enzyme (e.g., dihydrofolate reductase) was shown to be an essential component of the hydroxylation system.[139,146,147]



Scheme I-13. Reaction of phenylalanine hydroxylase (PAH) and regeneration of tetrahydropterin cofactor (BH_4).[53]

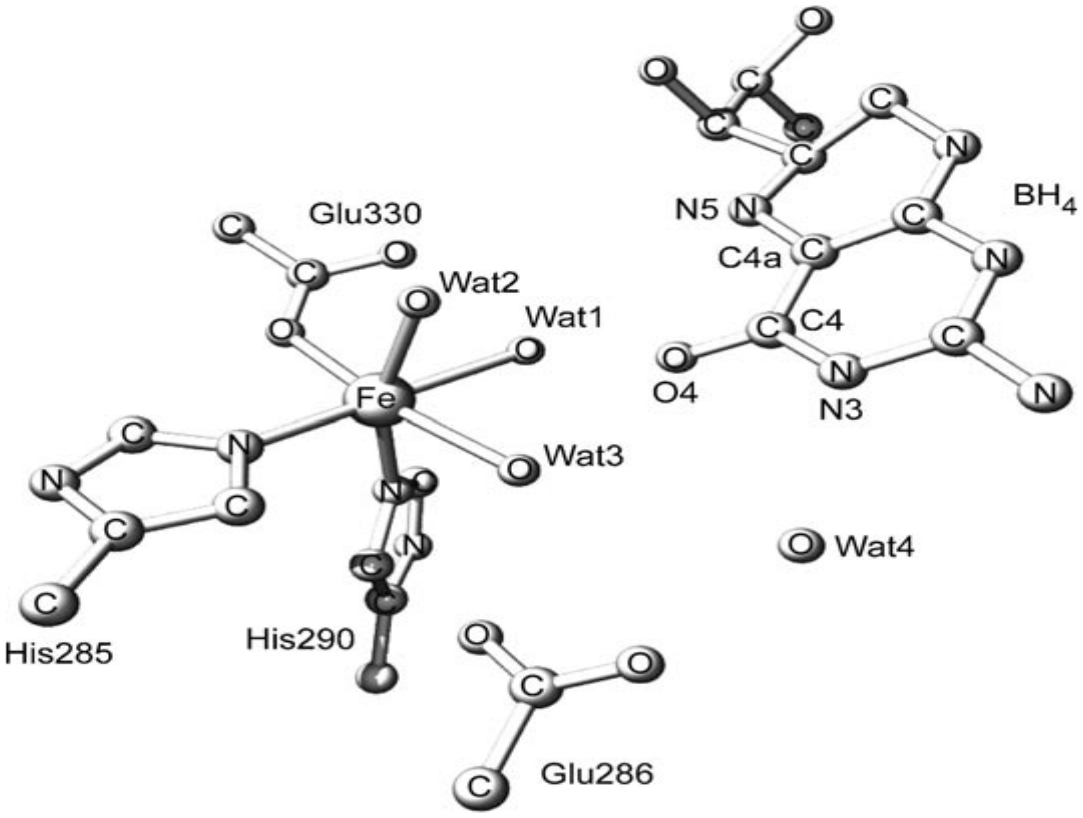
The quinonoid dihydropterin (q-BH_2) can isomarise to 7, 8-dihydro-L-biopterin (BH_2) (Scheme I-14), stressing the stability of the 7, 8-dihydro form among different possible dihydropterins.[9,10]



7,8-dihydro-L-biopterin

Scheme I-14

As an example, the x-ray crystal structure of the active iron centre in the catalytic domain of the human (h) PAH in a binary complex with the cofactor BH_4, e.g.,(hPAH-Fe^{II}-BH_4) is shown in Scheme I-15. In the binary complex, two His, one Glu and three water molecules



Scheme I-15 The x-ray crystal structure of the active iron centre in the catalytic domain of the hPAH – Fe^{II} – BH_4 binary complex.[120]

coordinate to the metal, which results in a hexacoordinate complex with essentially octahedral geometry. The cofactor (BH_4) is located in the second coordination sphere of iron[120].

3. Nitric oxide syntheses (NOSs)

Mammalian nitric oxide syntheses (NOSs) require the tetrahydrobiopterin (BH_4 Scheme I-7) to convert L-arginine to L-citrulline and nitic oxide, an important second-messenger molecule in neutral and cardiovascular systems.[122-125] NOS catalyses the reaction:

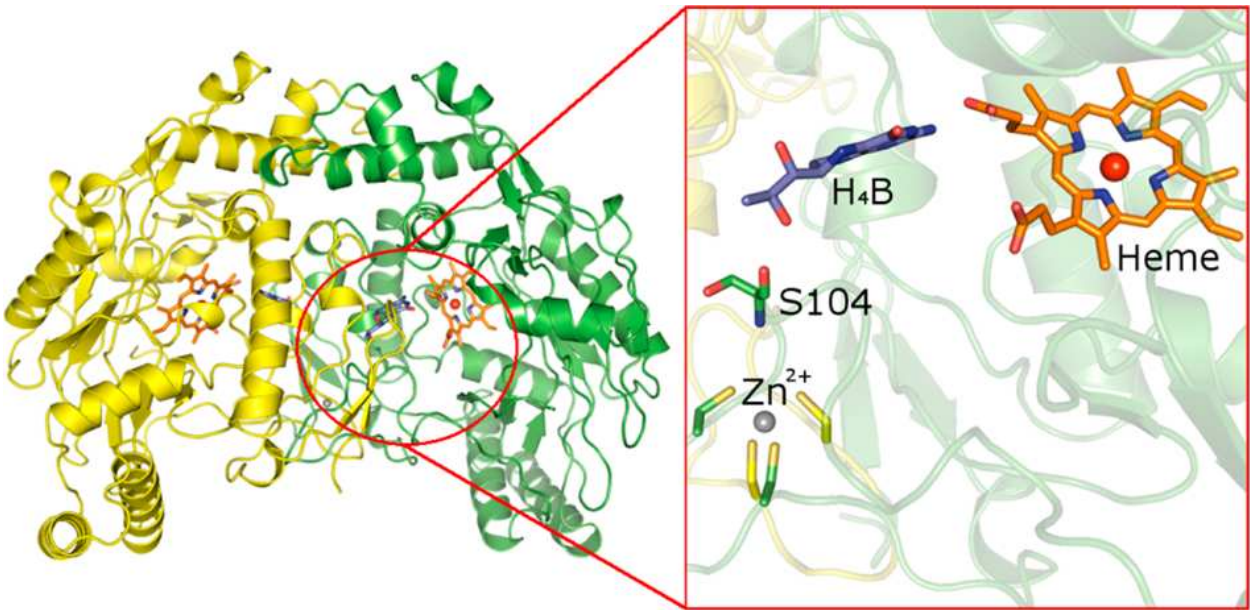
L-arginine + 3/2 NADPH + H^+ + 2 O_2 $\rightleftharpoons$ Citrulline + nitric oxide + 3/2 NADP.

The electron flow in the NOS reaction is :

NADPH → FAD → FMN → heme → O_2 .

Tetrahydrobiopterin (BH_4) provides an additional electron during the catalytic cycle which is replaced during turnover.

A relevant x-ray structure is shown in Scheme I-16 indicating the heme and pterin (BH_4)



Scheme I-16 Overall structure of the bovine eNOS dimer in complex with H_4B. The Zn^{2+} binding site is located at the dimer interface and ~ 15Å from the centre of the pterin binding pocket in both molecules A and B of the dimer.[125]

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sites, along with other sites of the bovine e NOS dimer in complex with BH_4. The dimer surface 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 from each subunit (ZnS_4)[125].

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 class of heme-thiolate proteins for oxygenase type activity;

iv. Achivement of stability of the homodimer through Zn^{2+} coordination;

v. The role of BH_4 in the catalytic reaction.

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 substaintial number of metalloenzymes, as outlined above. Important bioinorganic chemistry has grown up, centred around this structural motif. The general features of the active site 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.

The aforesaid information about the structural and functional aspect of pterin-containing redox metalloenzymes have catalysed symbiotic developments of coordination chemistry of pterin ligands in particular and pteridine ligands in general. References 10-18, 25-28, 76, 83, 84, 101, 110-116, 118, 129, 143 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 cover a significant number of x-ray structurally characterized compounds, throwing light on the metal-pterin/pteridine bonding interactions.

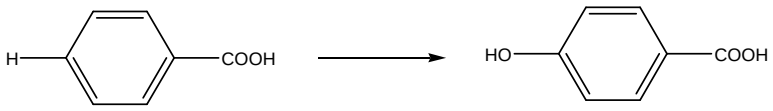
However, continued efforts are needed on the synthetic and reactivity aspects of pterin coordination chemistry, for improving our understanding about the above redox metalloenzymes, especially their functional aspects and their mechanism of action. The associated modeling studies may throw up new pathway/ideas for studying the redox non-innocent pterin ligands their coordinaton compound and correlating their properties with molecular and electronic structures. Few basic guidelines may be stated here.

1. The synthetic chemist is to assemble minimal active site representations and to determine intrinsic geometric, electronic and reactivity properties of the resulting new compounds.

2. To define function in terms of structure (both geometric and electronic)- a basic goal of bioinorganic research.

3. For the above-mentioned pterin-containing redox metalloenzymes, the electron transfer pathway to or from the metal centre is to be understood along with the possible cooperativity between formally metal-based and pterin-based redox processes as well as the maintenance of

stoichiometric compatibility of the electron transfer activity of the metal site with the redox process/oxidation state change at the substrate active site (e.g., $S^{IV}O_3^{2-} \rightarrow S^{VI}O_4^{2-}$, $N^{V}O_3' \rightarrow N^{III}O_2'$, etc;



etc.;).

4. The above aspect immediately raises the question about the formal oxidation states of both the metal-and pterin-ligand centres in the new synthetic models.

5. Bench-mark data (spectroscopic, electrochemical/cyclic voltammetric and kinetic data on group/electron transfer reactions, etc.) are to be gathered on such synthetic model compounds where the oxidation states of both the metal and pterin ligand centres are to be carefully controlled, together with the donor atoms from the ancillary ligands; development of such coordination chemistry will also enable chemical and electrochemical studies to be accomplished which are relevant and complementary for studying the functional aspects of the enzyme catalytic centres, including the exact role of the metal and pterin ligand centres in oxygen atom transfer or O_2 activation, etc.

6. Even the source of the oxygen atom transferred to or from the substrate by different pterin-containing enzymes, need attention. For example, water is the ultimate source of the oxygen atom transferred to the substrate by the Mo/W-containing oxotransferases. On the other hand, dioxygen is the source of the oxygen atom involved in the phenylalanine → tyrosine conversion, with the other oxygen atom being reduced to the level of water. For a couple of other classess of monooxygenases, the iron-containing aggressive active sites attack the substrates, e.g., Fe^{IV}=O (cytochrom P450) or Fe_2^{IV}=O (methane monooxygenase) and such intermediates are derived from dioxygen rather then from water as in case of the Mo and W enzymes.

Answers to such fundamental problems are to be found from the stand point of basic parameters of inorganic chemistry, e.g., the operating reduction potentials of the enzyme active sites, O_2/H_2O systems and the associated free energy relationships in terms of the Frost diagrams. **Such interpretations are to be carefully tested on the synthetic model compounds.** These studies will also bring to the focus a few other fundamental aspects like kinetics and thermodynamics of long-range electron transfer, multielectron reduction and ultimately the pathways adopted by nature to harness the redox non-innocent nature of pterin for fruitful purposes in diversified metalloenzymes.

7. **Information about the frontier orbitals of the synthetic models** are to be obtained using theoretical methods (e.g., DFT calculations) and their basic properties (e.g., energy in eV, percentage compositions, energy gaps, etc.) are to be **correlated with the reactivities and different physico-chemical properties.** Ligand fields at the metal centres are to be interpreted carefully for a correct description of the associated chemical bonding and their role in controlling reactivity/functional aspects.

For a better conceptual frame work of this proposed synthetic study aimed apart from anything else, at modeling the PAH type activity, a closer look is needed into the $NADH/NAD^+$ as well as the dioxygen systems, as they are intimately connected with the PAH catalytic cycle (Scheme I-13).[53] There are four major classes of redox enzymes based on the nature of the associated electron mediators.[94,145] (1) the pyridine-linked dehydrogenases catalyzed reversible transfer of electrons from substrates to the loosely bound coenzymes $NAD^+/NADP^+$ to/ from NADH/NADPH, respectively; the reduction of the pyridine ring is stereospecific and is accompanied by a spectral change. (2) The flavin-linked dehydrogenase contain tightly bound FMN or FAD as prosthetic groups and often a metal; their intensely colored oxidized froms are

bleached on reduction. (3) The iron-sulfur protein containing enzymes. (4) The redox systems utilizing the cytochromes, acting in series, transferring electrons from flavoproteins to oxygen. Their reversible Fe(II) – Fe(III) transition can be followed spectrophotometrically.[94]

Few relevant potentials are indicated here:

Couple	$E^{0'}$(V)
$1/2O_2 + 2H^+ + 2e' \rightleftharpoons H_2O$	+ 0.816
$[Fe(CN)_6]^{3-} + e' \rightleftharpoons [Fe(CN)_6]^{4-}$	+0.36
$NAD^+ + 2H^+ + 2e' \rightleftharpoons NADH + H^+$	-0.320
$NADP^+ + 2H^+ + 2e' \rightleftharpoons NADPH + H^+$	-0.324
Ferredoxin ox/red (algal)	-0.41
Flavodoxin ox/red (clostridial)	-0.31
$2Cyt\ b_k(ox) + 2e' \rightleftharpoons 2Cyt\ bk(red)$	+0.030
$2Cyt\ C_{ox} + 2e' \rightleftharpoons 2Cyt\ Cred$	+0.254
$2Cyt\ a_3(ox) + 2e' \rightleftharpoons 2Cyt\ a_3(red)$	+0.385

It may be added that $NAD^+/NADP^+$ can be reduced nonenzymatically by sodium dithionite or $NaBH_4$. NADH/NADPH can in turn be nonenzymatically reoxidized with $K_3[Fe(CN)_6]$, but they are not oxidized directly by dioxygen at pH 7.0[94]

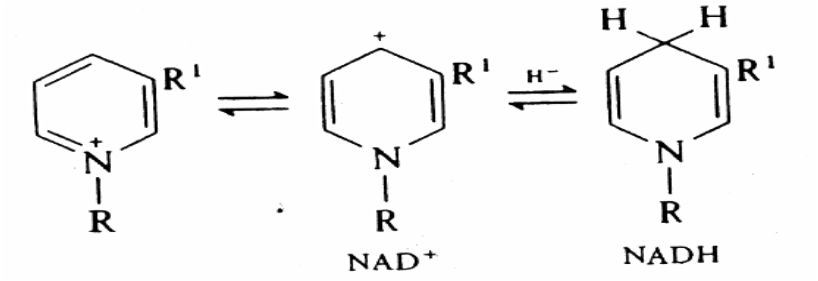
Coenzyme of electron transport: NAD^+

The coenzyme most frequently employed as acceptor of electrons from the substrate is nicotinamide adinine dinucleotide (NAD^+). When bound to the appropriate site on a dehydrogenase protein, a hydride ion is liberated from the substrate to the nicotinamide moiety and a proton is liberated into the medium.

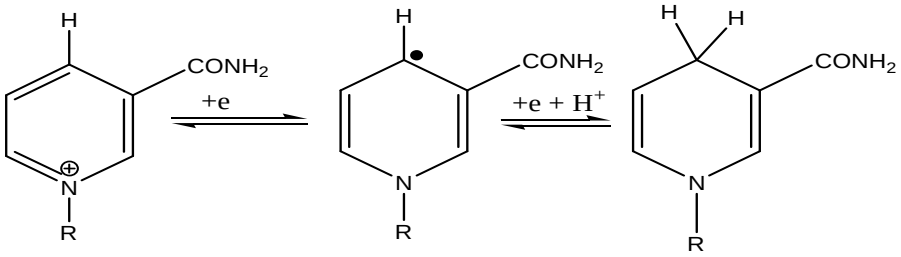
$MH_2 + NAD^+ \rightarrow M^+ + NADH + H^+$

Nicotinamide adenine dinucleotide phosphate ($NADP^+$) also functions in the same manner. The reduced coenzyme is not metabolized further on the same enzyme surface, but after dissociation from the original location, it transfers its electrons to an acceptor on the surface of a second enzyme.

It is known in organic chemistry that pyridinium compounds react as if the para carbon were positively changed; thus they can add hydride ions. NAD^+ carries out this reaction in biological system.



Alterenatively, the above transformation can be represented as a two electron transfer step along with a proton ($H' \equiv e' + e' + H^+$), since the one electron reduced intermediate radical is not stable.



In the mitochondrial system, no enzyme catalyzes a reaction in which the metabolite (MH_2) reacts directly with O_2.

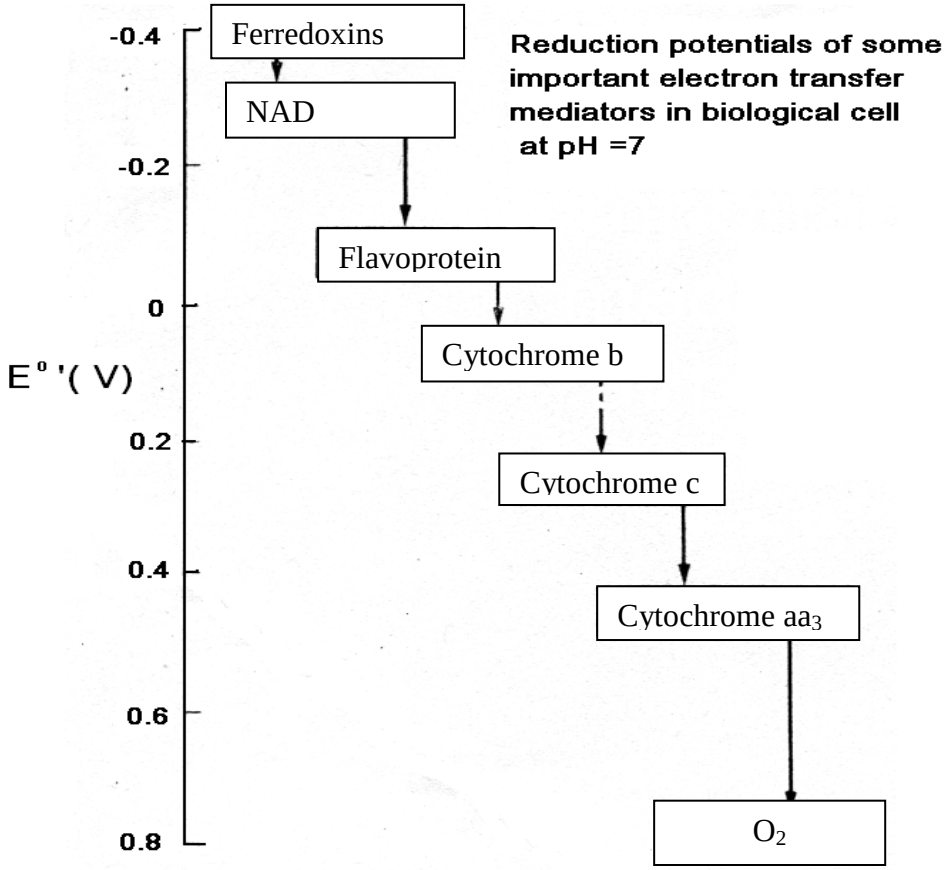
$$MH_2 + 1/_2\, O_2 \longrightarrow M + H_2O.$$

Rather, a transfer of electrons occurs from substrate to coenzyme (coenz),

$$MH_2 + Coenz \longrightarrow M + CoenH_2$$

with subsequent oxidation of the reduced coenzyme in an independent process.

The arrays of electron carriers that convey electrons from the dehydrogenated substrates to O_2, are shown below schematically.



These series of mediators, which act like a series of locks on a canal, allowing oxidation to occur in stages. Here control is of paramount importance, for uncontrolled oxidation by oxygen is combustion. Such mediators include the heme-containing cytochromes and flavoproteins, which also mediate the reaction that invest oxygen into the organic molecules.

The energietics of electron transport from NADH ($E^{0'}$ = -0.32V) to dioxygen ($E^{0'}$ = +0.82V), that is , along the entire length of the respiratory chain, can be calculated using the relation:

$\Delta G^{0'} = -nF\Delta E^{0'}$

where n is the number of electrons transferred, F is the faraday (23,062 cal), and $\Delta E^{0'}$ is the $E^{0'}$ of the electron-accepting couple minus the $E^{0'}$ of the electron donating couple.

$\Delta G^{0'} = -2 \times 23062 \times [0.82 - (-0.32)]$

$= -52{,}700 \text{ cal mol}^{-1}$

$= -52.7 \text{ Kcal mol}^{-1}$.

This value may be compared with the standard free energy of formation of ATP at pH 7.0 from ADP and phosphate:

$$Pi + ADP \rightleftharpoons ATP + H_2O, \quad \Delta G^{o'} = +7.3 \text{ Kcal mol}^{-1}.$$

Thus the formation of many ATP molecules, is needed to absorb the energy released during the passage of a pair of electrons down the respiratory from NADH to oxygen as above .[59,94] Two striking characteristics of the electron-transport process are relivent to the mechanism of energy conservation during electron transport: (i) the fact that a large number of sequential electron – transferring steps is involved, which suggests stepwise release of energy, and (ii) the fact that H^+ ion are absorbed and released at some of these steps, suggesting that proton exchanges are involved in energy conservation.[94]

Alcohol dehydrogenase

This enzyme catalyze the NAD^+- dependent conversion of primary alcohols to aldehydes:

$$RCH_2OH + NAD^+ \rightleftharpoons RCHO + NADH + H^+$$

Although the reaction involves redox chemistry, the enzyme contains zinc, which serves to bind and activate the substrate molecule prior to a hydride-transfer step; it does so much as it does in carboxypeptidase and carbonic anhydrase. Conclusive evidence indicates that zinc promotes hydride transfer to NAD^+ via the formation of a Zn(II)-alcoholate complex.[137,144]

The nitrogenase system

Here the overall reaction is :

$$N_2 + 6H^+ + 6e' + 12ATP + 12H_2O \longrightarrow 2NH_3 + 12ADP + 12Pi\ .$$

Reduced ferredoxin serves as the immediate electron donor. Regeneration of reduced ferredoxin occurs through the enzyme NADH-ferredoxin reductase:

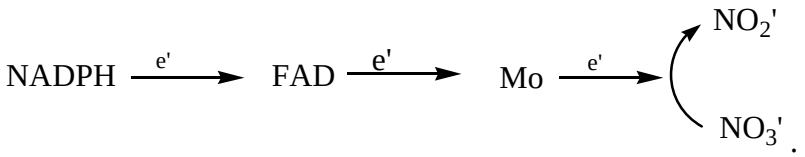
$$NADH + Fdox \longrightarrow NAD^+ + Fd\ red + H^+\ ,$$

or by the pyruvate dehydrogenase system.[94] The active site of nitrogenase containing a MoFeS cluster has been characterized x-ray structurally.

Nitrate reductase

Nitrate is the pricipal form of nitrogen available to higher plants from the soil. This metabolic assimilation of nitrate into the form of ammonia proceeds in two major steps: (i) reduction of nitrates to nitrite and (ii) reduction of nitrite to ammonia.

The first reaction in nitrate utilization is catalyzed by nitrate reductase, which is widely distributed in plants and fungi.[94] This enzyme is a flavoprotein containing both a molybdenum cofactor and cytochrome b; it employs NADPH as electron donor. The overall process of electron flow to nitrate can be shown schematically:



At the active site, the molybdenum centre shuttles between the Mo(VI) and Mo(IV) oxidation states through Mo(V).

NADPH or NADH dependent monooxygenases

The monooxygenases or hydroxylases, catalyze insertion of one oxygen atom of molecular oxygen into the organic substrate; the other oxygen atom is reduced to water:

$$AH + XH_2 + O_2 \longrightarrow AOH + H_2O + X$$

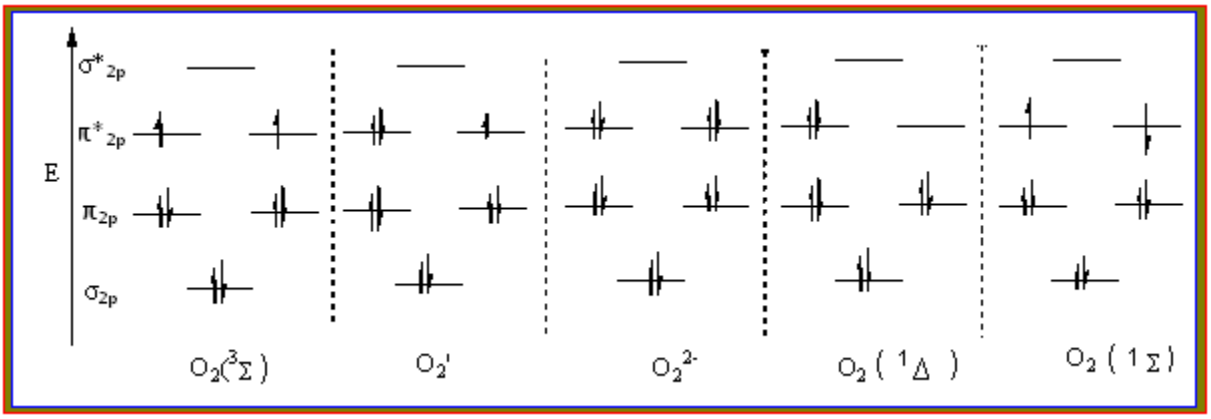
Monooxygenases require a second substrate to donate electrons for the reduction of the second oxygen atom in the oxygen molecule, the one reduced to water. Hence the monooxygenases are also called mixed-function monooxygenases; this was established by using ^{18}O as tracer.

In most monooxygenases reactions the second substrate that furnishes electrons to reduce one atom of oxygen to water is ultimately NADH or NADPH; however, different electron carriess are employed to transfer electron from NADPH or NADH to the oxygen.

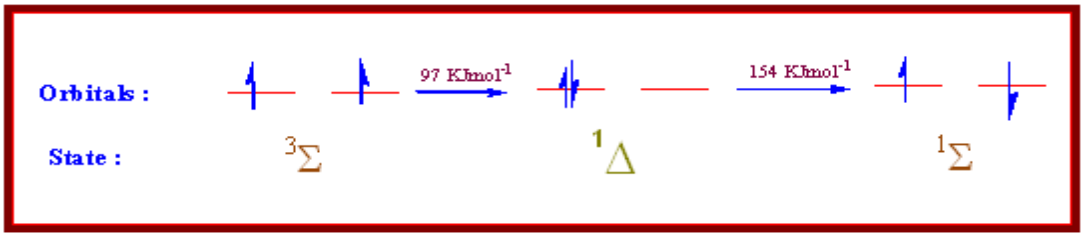
In the reaction catalyzed by the liver enzyme **phenylalanine hydroxylase, the reducing equivalents are transferred via the tetrahydrobiopterin cofactor.**

Dioxygen: thermodynamic and kinetic aspects of its behavior as an oxidant

Dioxygen is a powerful oxidant, but it is kinetically inert. This paradox has allowed life, in evolution to come to terms with dioxygen in the atmosphere and to make its controlled use in biosynthesis, biodegradation and respiration. Thus the two problems of survival are to activate dioxygen sufficiently for reaction, while ensuring that dangerous by products are not allowed to damage the organism[137,138]. Flavin is the only organic cofactor known to interact enzymatically with dioxygen. Otherwise in the biological systems metal-containing biomolecules (almost exclusively Fe and Cu) are used to deal with dioxygen , superoxide and peroxide.[139] Their electronic structure are shown below; here x axis is the internuclear axis and the π_{2p}/π^*_{2p} levels have two components along with y and z axis respectively.



The paramagnetism (with two unpaired electrons) of the dioxygen molecule is easily explained in terms of MOT with its degenerate π^*_{2p} orbitals. The MO designations of the superoxide ($O_2^{'}$) and peroxide (O_2^{2-}) are shown above along with the exited-state configurations of O_2, that is, $O_2(^1\Delta)$ and $O_2(^1\Sigma)$ which are 97 and 154.5 KJmol^{-1} above the ground state ($^3\Sigma$) respectively.



The characterization data of O_2, $O_2^{'}$ and O_2^{2-} are shown below, indicating the extent of electron transfer (e.g. from metal) to dioxygen (or specifically its antibonding MOs).

	O_2	O_2^-	O_2^{2-}
Bond order	2	1.5	1
Bond length(Å)	1.21	1.28	1.49

$\nu_{O-O}(cm^{-1})$	1560	1150-1100	850-740

The overall geometry of the $M(O_2)$ or $M(O_2)M$ group is also a useful guide on this matter, e.g. peroxo complexes $M(O_2)M$ are usually non-planer.

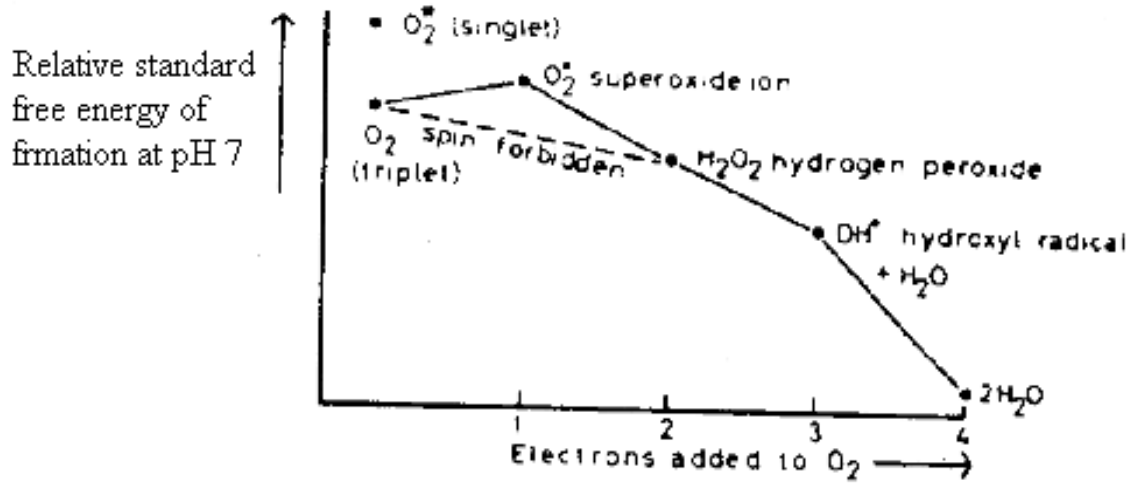
The kinetic inertness of O_2 and its activation

The kinetic inertness of oxygen is emphasized by the fact that autooxidation of organic compounds (that is, reaction of O_2) at room temperature usually takes place through metal-ion catalysis, by photochemical activation (for example, of butadienes) or by chain reactions promoted by an extraneous radical (for example, of ethers). Simple bimolecular reactions between O_2 and organic compounds are unusual. Two factors are responsible for the above observations:

(i) the high energy of formation of superoxide;

(ii) the stability of triplet O_2 ground state.

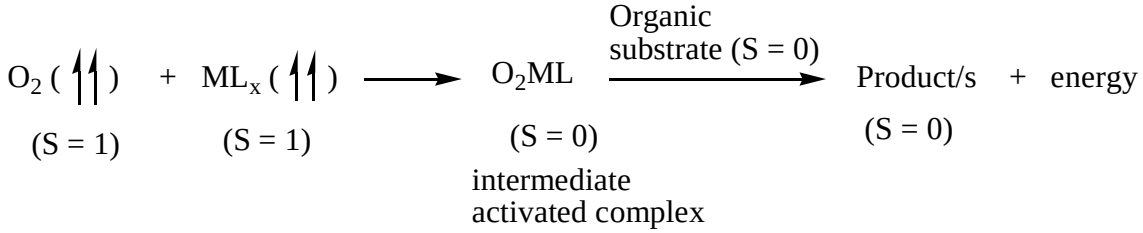
The thermodynamic aspect dioxygen reduction is shown below schematically.[138]



Thermodynamics of dioxigen reduction. E^0 for a couple is represented by the slope of the line joining the two species. An intermediate can only disproportionate to the two species if it lies above such line.

One-electron reduction of O_2 to superoxide ion (O_2^-) is thermodynamically unfavorable, requiring a reducing couple of -0.33 V. Reuction to any other species is exothermic, but now we encounter a spin restriction. O_2 has a triplet ground state (S=1), with two unpaired electrons. Quantum mechanical laws require that if it is to be rapidly reduced to peroxide, a diamagnetic (singlet) molecule, then the reductant supplying the two electrons must be a free radical or must change from a singlet to a triplet state, or O_2 itself must be exited to its singlet state($^1\Delta$, S=0). This rules out most stable organic substrates(S=0), since free radicals are generally unstable. Two-electron reduction of O_2 (S=1) by singlet molecules (S=0), being spin forbidden, will not take place in the lifetime of a simple collision complex (ca. 10^{-13}s). This is due to the fact that spin is weakly coupled with molecular vibration. Molecular vibration is about 10^4 times faster than spin inversion.[137,138]

The activation energy of dioxygen (97 KJ mol^{-1}) is too high for biological systems, but nature has desgined suitable pathways requiring low activation energy for achieving fast reactions with O_2. One typical example is the quenching of O_2 spin ($^3\Sigma$) by the unquenched spin (e.g., a triplet) of a transition metal complex-a process involved in the action of O_2 as the terminal oxidant in the respiratory chain.



Transition metal reacts with O_2 in various ways by virtue of their labile d-electron configurations. Favin, by contrast, probably activates O_2 by adopting a semiquinone radical state. If Cu or Fe salts are added to tissue slices or cultures, they promote O_2-toxicity, while in vivo they are pressed into service as cofactors to control its reactions, and destroy its dangerous by-products.[138]

For the above spin quenching interaction, the geometry of the π-antibonding MOs of the O_2 molecule will impose limitations on the feasibility of different redox reactions occurring. Reduction will involved the transfer of electrons from the valence shell of the metal ion to the oxygen molecule and therefore there will be a symmetry requirement on the orbital of the metal ion. The implications of the Franck-Condon principle will also hold here. For example, metal t_{2g} orbitals can overlap with ligand orbitals having π symmetry, whereas metal e_g orbitals are suitable for overlap with ligand σ orbitals.[137]

Oxygen donor ligands will particularly affect the rate of electron transfer as these bases have π-donor properties and hence will enhance the transfer of electron density from the appropriate metal d orbital into the oxygen molecule. In contrast σ donors, such as nitrogen ligands, trans to the oxygen molecule will have little influence.

Molecular oxygen is a strong oxidizing agent and the full reduction potential will be realized only when the oxidation process involves an essentially synchronaous foure-electron transfer reaction.

$$O_2 + 4H^+ + 4e' \longrightarrow 2H_2O\ (l)\ ;\ E = 1.23 - (0.059v)pH \quad \text{.....(i)}$$

To derive the pH dependence from the Nernst equation, it is assumed that the partial pressure of oxygen is 1 bar and setting $\nu = 4$.[63] from the above equation we get

in 1N acidic medium, $E^{0'} = 1.23V$ ……..(ii) and

in neutral medium (pH =7), $E^{0'} = 0.817V$ …..(iii).

Few other reduction reactions of oxygen in the neutral medium (pH =7, 25^0C) have been suggested for biological oxidations, involving one-or two- electron steps.

	$E^{0'}(v)$
$O_2 + e' \longrightarrow O_2'$	- 0.33 .....(iv)
$O_2 + 2H^+ + 2e' \longrightarrow H_2O_2$	+0.68 .....(v)
$O_2 + H^+ + e' \longrightarrow HO_2$	- 0.1 .....(vi)

Evidently, the atmospheric oxygen is innocent to us, but not the singlet oxygen. However, the ordinary oxygen can react through hydrogen atom abstraction which generates free radicals to initiates chain reaction.

Finally, the ligating property of singlet-O_2 may be compared with that of triplet O_2. Singlet-O_2 with the distinguishing electronic configuration $(\pi^*_{2p})^2(\pi^*_{2p})^0$ can use the π^*-pair for σ- donation and the vacant π^*-MO for π-acceptance. Thus, singlet-O_2 ($^1O_2$) can act as a better π-acid ligand and consequently with higher crystal field splitting property.

Activation of dioxygen through complex formation with transition metal ion

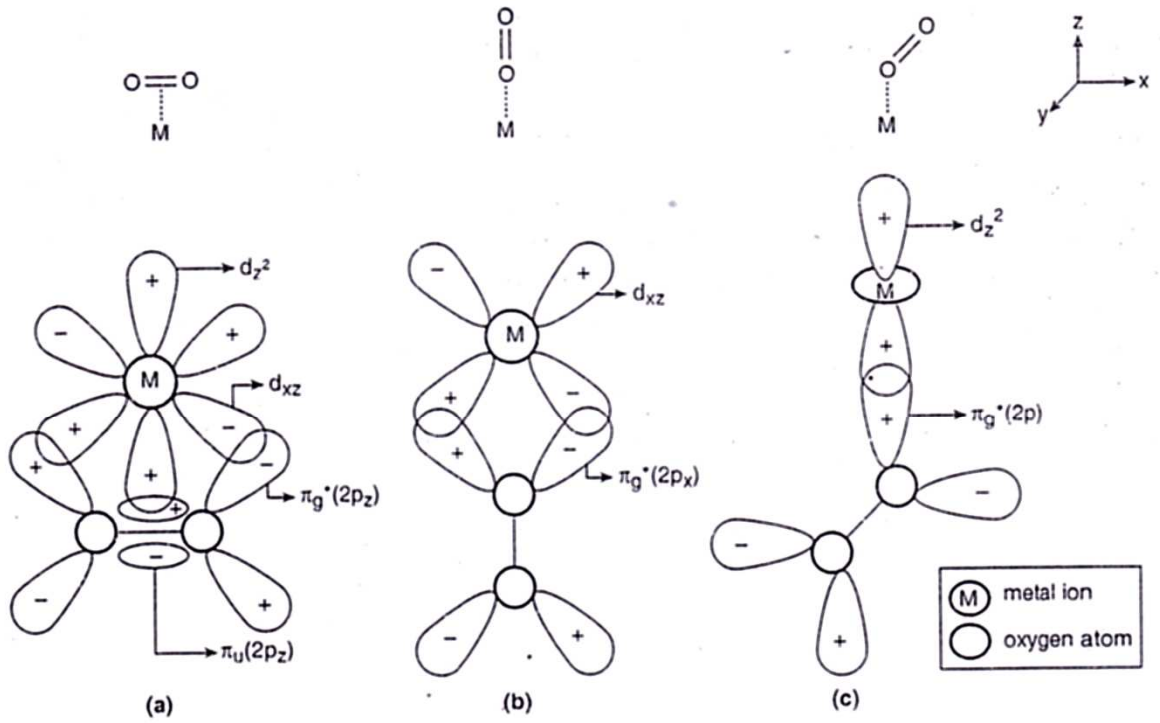
Nature has developed different energetically favorable pathways to convert the triplet oxygen to singlet oxygen which is utilized in biochemical redox reactions. Complex formation of triplet oxygen ($^3O_2$, S = 1) with transition metal ion (possessing d orbitals with unpaired electrons) produces singlet oxygen ($^1O_2$, S = 0) in the resulting complex. The common modes of binding of O_2 with a metal centre are:

(a) perpendicular (i.e., edge-on-overlap)

(b) linear (i.e., end-on-overlap) and

(c) angular (i.e., bent end-on-overlap).

These possibilities are shown below schematically. They are based on the Dewar-Chatt-Duncanson bonding model of alkene complexes as well as the bonding considerations about the dioxygen-carrying Co(II)-Schiff base complexes.[134-141]



Different modes of bonding in M-O_2 complexes. (a) Perpendicular mode (edge - on overlap); (b) Linear mode (end - on overlap) (c) Bent end - on overlap

As delineated below the perpendicular and angular modes of O_2 binding to a suitable transition metal centre can remove the degeneracy of the π^*_{2p} orbitals of O_2, but the linear mode of binding cannot remove the said degeneracy.[140]

In the linear mode (b), both the π^*_{2p} orbitals of O_2 overlap (π-interaction) equally with d_{xz} and d_{yz} orbitals of the metal centre and consequently the said degeneracy of the π^*_{2p}

orbitals is retained even after complex formation [the π-interaction are (π^*_{2px} + d_{xz}) and (π^*_{2py} + d_{yz}) respectively]. Here the M – O bond direction is assumed to lie along the z-axis.

The following overlapping interactions can be considered for the perpendicular mode of bonding (a). The filled π_{2pz} orbital of O_2 forms a σ-bond with the d_z^2 orbital of the metal centre. The π^*_{2pz} orbital of O_2 can overlap with the metal d_{xz} orbital; but only a weak overlap is possible between the π^*_{2py} (perpendicular to the plane of the paper) orbital of O_2 with the metal d_{yz} orbital. After the above complex formation process π^*_{2pz} will have lower energy, while π^*_{2py} will be of relatively higher energy. Since their degeneracy is now lost, the original two unpaired electrons of the π^*_{2p} level of O_2 ($^3O_2$), will now be accommodated in the lower energy orbital (that is, π^*_{2pz}). Here the metal d_{xy} and $d_{x^2-y^2}$ orbitals remain nonbonding.

For the bent manner of bonding (c), one π^*_{2p} orbital of O_2 is oriented to overlap with the metal d_z^2 orbital to from a σ-bond, while the other π^*_{2p} orbital combines with the metal d_{yz} orbital to from a π-bond. This mode of bonding removes the degeneracy of the π^*_{2p} orbitals of O_2 through complex formation. In this mode of coordination, the metal d_z^2 orbital is stabilized; the $d_{xy,}$ $d_{x^2-y^2}$ and d_{xz} orbitals remain nonbonding.

Actually for the ML_5O_2 complexes of Co(II)(d^7) and Fe(II)(d^6), O_2 binds strongly in a bent manner and the metal d_z^2 orbital electrons are located in the resulting MO where the π^*_{2p} orbital character of O_2 is significant. The net result is the flow of electron density from the metal to the π^*_{2p} orbital of O_2, implying oxidation of the metal centre as well as reduction of O_2. In other wards, $^3O_2$ is activated through an intermediate complex, O_2ML_5 (S = 0) for reaction with the substrate X (S = 0), leading to the product as pointed out earlier. The basic requirements of the above bonding interaction are:

(i) the transition metal complex must have an active site to accommodate O_2, e.g. the formation of a 5-coordinate ML_5 intermediate through the loss of a ligand from the z-axis;

(ii) for the bent bond formation by O_2 in the adduct, the HOMO (acting as the donor orbital) of the metal centre should be d_z^2, assuming M – O bond formation in the z-direction;

(iii) the metal centre should be able to display multiple oxidation states;

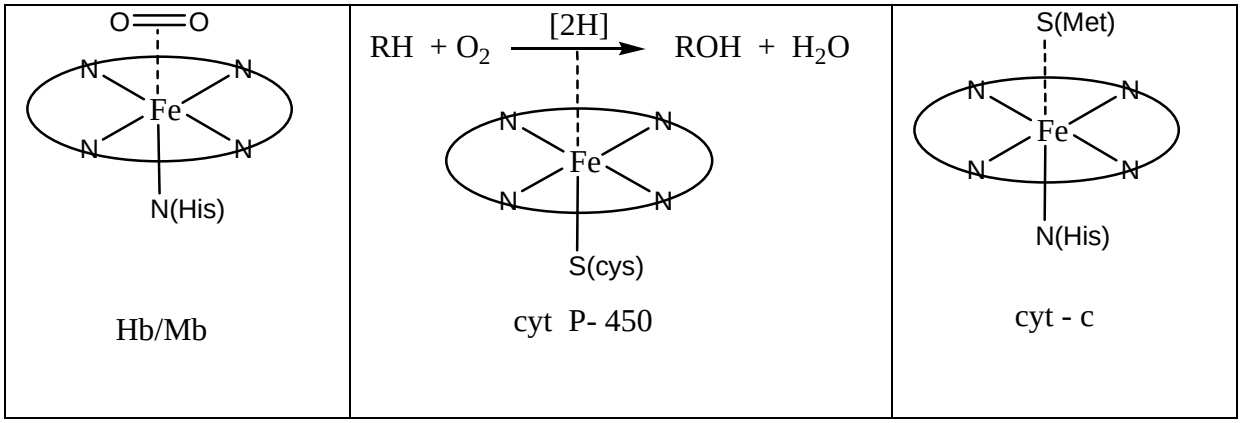
(iv) the deoxygenated and oxygenated forms should have the comparable stability.[137,140]

The above discussions throw lights on the attributes of both an oxidase enzyme (e.g., cytochrome O-450 enzyme) and an oxygen carrying protein (Mb/Hb); the thermodynamic stability constant (K) and the electron transfer rate constant (k) of the intermediate complex $L_nM(S)O_2$ (where S is an oxidizable substrate) decide the issue .

$$L_nM(S) + O_2 \overset{K}{\rightleftharpoons} L_nM(S)(O_2) \xrightarrow{k} L_nM + (S)_{ox} + (O_2)_{red}$$

The oxygen carrying property is favoured when K is large and k is small; a large value of k favours the oxidase activity.

The above situations may be represented schematically, using the iron (II)-prophyrin system as an example. When the sixth coordination position is blocked (e.g., by a

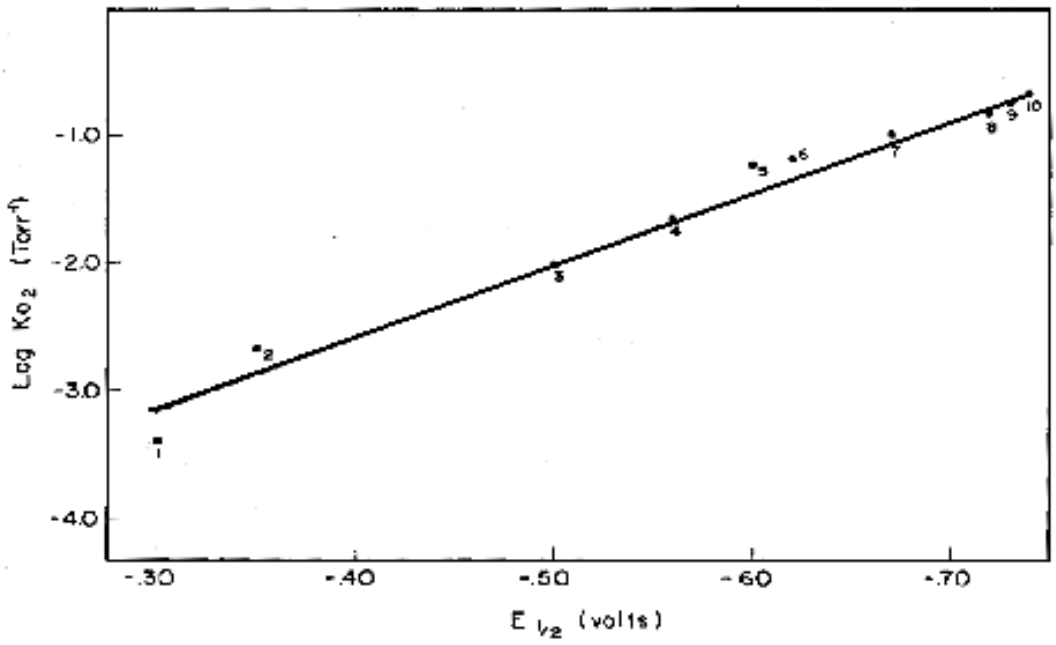


methiononine sulphur ligand), an electron carrier (cyt-c) property is manifested.

The above well-characterized metalloproteins/metalloenzymes illustrate how the same cofactor (iron-prophyrin) unit can be made to perform drastically different functions by the donor atoms from the protein chain.

In oxyhemoglobin, the stretching frequency for the O – O bond is 1106 cm^{-1} which is closer to the value of 1150 – 1100 cm^{-1} for O_2^-. X-ray structural studies indicate that oxy-Hb is a bent superoxo complex of Fe(III) with a Fe – O – O angle of about 120^0.

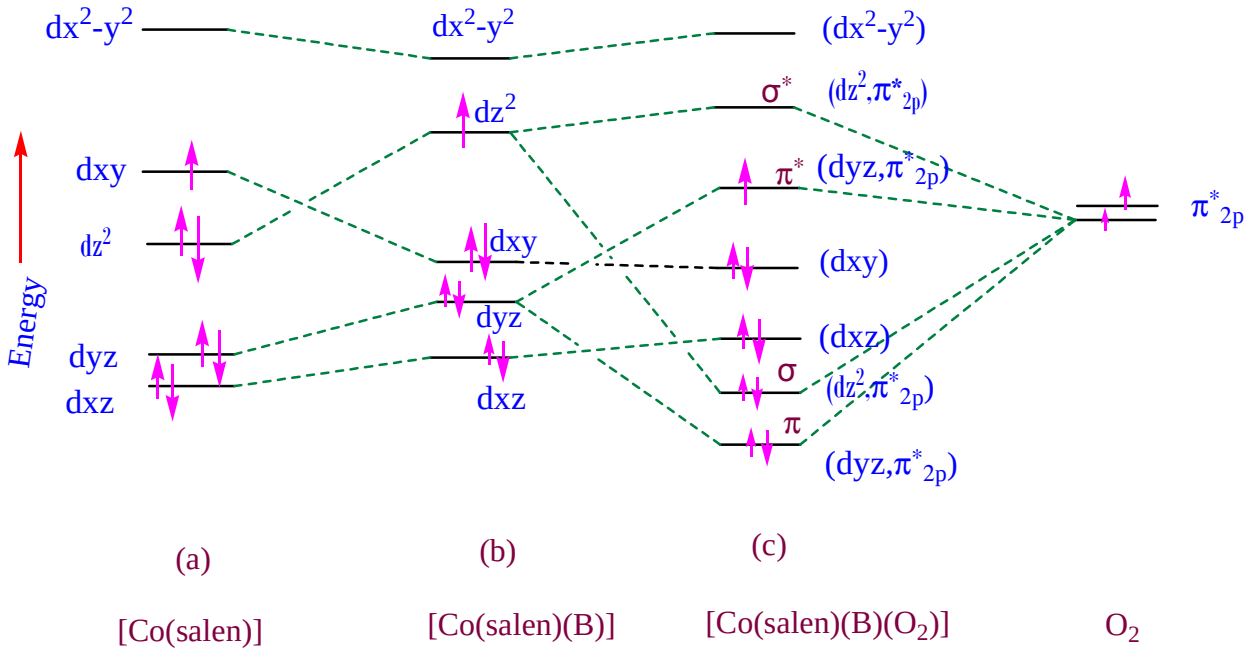
Model (synthetic) oxygen carriers have received considerable attention including the Co(II)-Schiff base complex like salcomine, [Co(salen)] which reversible binds O_2 in solution containing a base, e.g., pyridine (py) to give the superoxo complex [Co(salen)(B)(O_2)]. It supports the O_2 binding model leading to oxidation of the metal centre, ie., $M^{n+} + O_2 \rightarrow M^{(n+1)+} - O_2^-$. Studies indicate that logK (K is the O_2 – binding constant) increases linearly with the reduction potential ($E_{1/2}$) for the



Comparison of oxygen uptake ($\log K_{O2}$, - 21 ^{0}C) with $E_{1/2}$ values for Co(II)→Co(III) of Co(benacen)(B): (1)PPh_3; (2) 4-CNpy; (3) py; (4) 3,4-Me_2py; (5) pip; (6) sec-$BuNH_2$; (10) n-$BuNH_2$.

Co(III)/Co(II) complexs. It is reasonable to infer that electron transfer from cobalt(II) (and reduction of O_2 e.g., $O_2 \rightarrow O_2^-$) is involved in O_2 binding. However, an increase of 0.4v (40 KJmol^{-1} in terms of $\Delta G^0 = -nFE^0$) in E^0 leads to an increase in log K of only about 2.1(12KJmol^{-1} in terms of $\Delta G^0 = -RT\ln K$). The difference suggests that electron transfer to O_2 is incomplete and that Co(II) – O_2 and Co(III) – O_2^- are idealized extremes [134-136]. The above data also indicate that greater the σ-donor property of B, higher is the O_2 binding constant, favouring the step Co(II) – O_2 → Co(III) –$O_2^{-\cdot}$ If the base (B) is a good π-acid ligand, then formation of the O_2 – adduct will not be favored (where O_2 acts as a π-acid ligand at the trans-position of B). The importance of the trans-axial ligand B in imparting the O_2 carrying properties of [Co(II)-(salen)] compound can

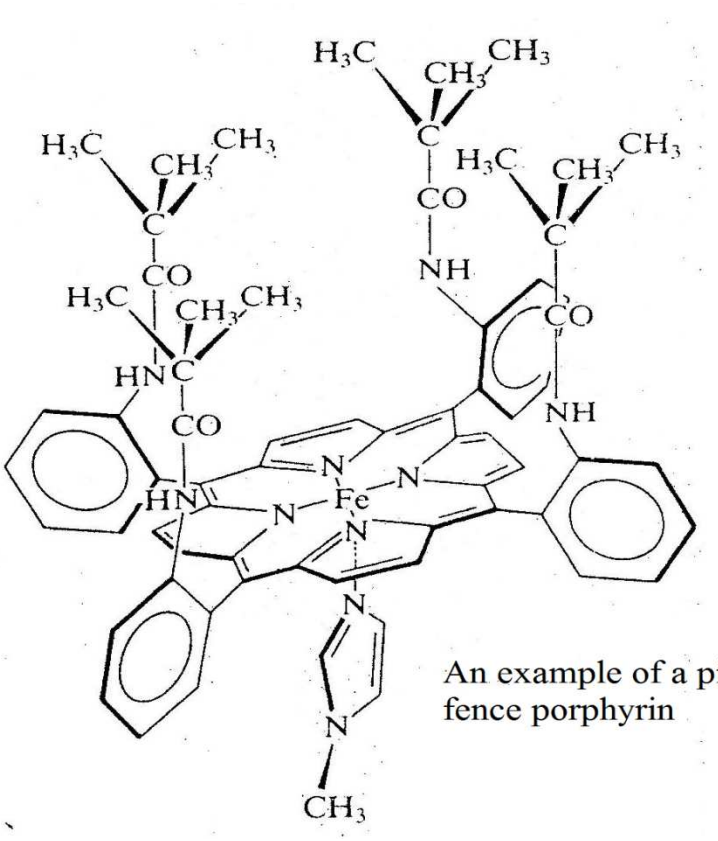
be explained as follows in terms of bent – on bonding (c) stated earlier; here the d_z^2 orbital is used for π-bonding, with the $dx^2_{-y}{}^2$, d_{xy} and d_{xz} orbitals remain nonbonding.[135,140,141]



Energy level diagram (qualitative) for the d orbitals of Co(II) in (a) square -planar and (b) square-pyramidal fields in CoL_4 and CoL_4B; (c) MO diagram (qualitative) for Co - O_2 bond in $CoL_4B(O_2)$ involving the d orbitals of Co(II) in CoL_4B with the antibonding π MO-s of O_2.[135]

The net result of the above bonding interaction between [Co(salen)(B)] and O_2 is that the unpaired electron of the metal is located in a π* MO which is energetically close to the $\pi^*_{2p}(O_2)$ orbital. This is equivalent to the electron transfer Co(II) – O_2 → Co(III) – O_2^-, forming the superoxide ion. EPR spectral data indicate that about 80% of the odd electron resides in the superoxide ion, while x-ray structural data provide with an 'O – O' bond distance of 1.26Å. This can be compared with the corresponding distance of 1.28Å (in the O_2^-) in KO_2 and 1.21Å in O_2,

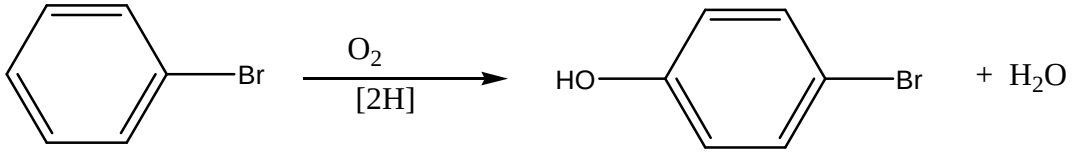
respectively. It is comparable to the formulation Fe(III) – O_2^- frequently used for oxy-Hb, with bent Fe – O - O bond.



In this connection it is worthwhile to refer to the picket fence model of the oxygen carrying protein myoglobin (Mb).[136] Here imidazole (Im) is an effective σ-base that favors coordination of a π-acid lying trans to itself. The blocking substituents create a pocket for O_2 coordination and prevent the formation of a μ-O_2 species through reaction with a second Fe centre. It reacts with O_2 giving a structure with an Fe – O – O angle of 136^0 and O – O distance of 1.25Å. The O – O stretching frequency lies at 1107 cm^{-1}, which is closer to the O_2^{2-} value of 1145 cm^{-1} than the O_2 value of 1550 cm^{-1}. In the light of the above MO model, this diamagnetic (low spin) compound may be considered to be a low - spin d^6 Fe(II) complex of singlet O_2; this would mean that Fe(II) → O_2 reduction is less important than in the above Co(II) complex.[136]

Alternatively, the above low – spin character could arise as the result of spin pairing of Fe(III) and O_2^-, in which case the complex would be similar to the cobalt model compound. That such a possibility is quite real is underlined by the observation that a d^3 Cr(III) porphyrin complex of oxygen has been synthesized which has only two unpaired electrons. Since d^3 Cr(III) must be high-spin, the only explanation for the spin observation is spin-pairing with an electron from O_2^-.

The above bonding consideration are **pertinent in the present context** because dioxygen (O_2) has been used here extensively for modeling the phenylalanine hydroxylase (PAH) activity using bromobenzene as a model substrate and a reduced metal-pterin complex as the source of reducing equivalents:[7,52-55]



Scheme I-13 represents the phenylalanine hydroxylase (PAH) catalytic cycle including the regeneration of tetrahydrobiopterin cofactor (BH_4) through the action of NADH on quinonoid dihydropterin.[53] As detailed out in this treatise **$NaBH_4$ been used here as the source of reducing equivalents (instead of NADH)** towards the oxidized from of a suitable metal-pterin complex; the reduced from of the corresponding complex is able to drive the above reaction, that is , hydroxylation of the aromatic ring (of bromobenzene). Experimental data indicate **that direct transfer of reducing equivalent from $NaBH_4$ to the above reaction system is not possible** . But the presence of a suitable mediator is essential for this purpose, e.g., a metal-pterin complex as above. As per the above MO diagram, the reduced metal centre initially activates O_2 through electron transfer (involving the π^*_{2p} orbital) and the reduced pterin ring completes the process of reducing equivalent transfer to the above reaction system.[139]

Scheme I-15 shows the x-ray structural data of the active iron centre in the catalytic domain of the hPAH-Fe-BH_4 bianary complex.[120]

Developing functional models of PAH type activity

To achive the above objectives using the later members of the 3d transition series, is a major research goal of this study. The synthetic and characterization aspects of the proposed new compounds pose considerable challenge due to the presence of redox non-innocent pterin ligand.[9,10] Possible understanding of the mechanism of action of the PAH type enzymes, will be a gratifying result of such an endeavour.[55,120]

Preface of this thesis indicates the pterin ligand (H_2L) and the ancillary ligands (Schemes 1 to 6) used for the present research work. H_2L plays a pivotal role for entire study, including highlighting the role of its redox non-innocent nature in imparting unique redox properties to its new coordination compounds.

Chapter II presents the synthetic, characterization and reactivity aspects of two new copper (I, II) complexes of H_2L, with 1, 10-phenanthroline (phen) as the ancillary ligand. Reactivity studies of the copper(II) complex towards $NaBH_4$ and that of the copper (I) complex towards a mixture of bromobenzene – O_2, elicit unique redox properties which can be rationalized in the light of the electronic structures (DFT). The diamagnetic copper(I) complex presents itself here as an excellent candidate for 2D NMR study.

Chapter III is concerned with the synthesis, characterization and reactivity studies of a new cobalt(II) complex with 1, 10-phenanthroline as the ancillary ligand. The corresponding cobalt(I) complex can be accessed on the time scale of cycic voltammetry as well as isolated through $NaBH_4$ reduction and characterized (ESIMS, μ_{eff}, etc., data). Reactivity of the latter

compound with a mixture of bromobenzene and O_2, affords 4-bromophenol. Significance of such reactivity data has been analyzed on the basis of frontier orbitals/electronic structures (DFT).

Chapter IV reports the synthesis of a new mixed ligand nickel(II) complex with 1,2-diaminoethane (en) as the ancillary ligand. The corresponding quatarnary complex, obtained through substitution of its aquo group with imidazole (Im), could also be synthesized. Both of them have been characterized x-ray structurally. Their group transfer and redox reactivity studies elicit an important aspect, e.g. a considerable change over in property is associated with the substitution of the aquo group with imidazole. The corresponding nickel(I) complex could be isolated using $NaBH_4$ as the reducing agent and characterized (ESIMS data). Properties of their frontier orbitals (DFT) throw light on their redox properties.

In chapter V two new chiral mixed nickel(II) complexes are reported, using the ancillary ligand like R-(+)-propylene diamine and S-(-)-propylene diamine. Both of them have been characterized x-ray structurally and different physico-chemical data including CD spectroscopy. X-ray structural data point towards δ-conformation of the ancillary ligands in both these cases. These two chemically identical compounds show unique difference among their reactivity properties; their electronic structures (DFT) throw light on such reactivity differences.

Synthesis of several new copper(II), cobalt(II) and nickel(II) mixed ligand complexes are reported in chapter VI; the ancillary ligands include 2, 2′ – bipyridyl (bipy) and 1, 10-phenanthroline (phen) . Their chemical compositions have been established on the basis of elemental analysis (C-H-N data), ESIMS data as well as different physico-chemical studies; such data include those of a new zinc(II) complex whose x-ray data have been presented elsewhere. In all these cases, optimized molecular structures have been obtained through DFT calculations; the computed bond length/bond angle data tally with those reported in chapters II-V. The

diamagnetic zinc(II) complex is helpful in recording the 2D NMR data. Their reactivities and considerations about electronic structures complete this chapter.

References are grouped together at the end of this thesis.

Chapter II