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Biomimetics of some active sites for non-heme iron enzyme: future approach

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ABSTRACT

Pterin-dependent oxygenases, represented by aryl amino acid hydroxylases, are a small family of closely related enzymes essential for physiological processes that use tetrahydrobiopterin (BH4) as a cofactor.

INTRODUCTION

This class of enzymes includes phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH) that perform aromatic hydroxylations of the corresponding amino acid, leading to the biosynthesis of several biogenic neurotransmitters such as serotonin, which are known to be involved in several physiological processes are [1-12]. Questions of enzymology have been the subject of several detailed articles. [13-17] Three-dimensional structures by X-ray diffraction are available for characterization of each of these three enzymes. [18-20], These three enzymes are homotetrameric, contain mononuclear iron, and use dioxygen and tetrahydrobiopterin as substrates in the hydroxylation reaction. Their metallic active site is shown in Figure 1:

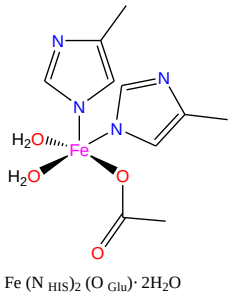


Figure1: Active site of pterino-dependent oxygenases

The PAH enzyme resides primarily in the liver and is involved in the main pathway of phenylalanine catabolism. In contrast, the other two enzymes act mainly in the central and peripheral nervous systems and catalyze rate-limiting steps in the biosynthetic pathways of

neurotransmitters such as dopamine, norepinephrine, and adrenaline for TH and serotonin and melatonin for TPH (Figure 2). Dysfunction of these essential enzymatic activities is implicated in several serious neurological and psychological diseases.

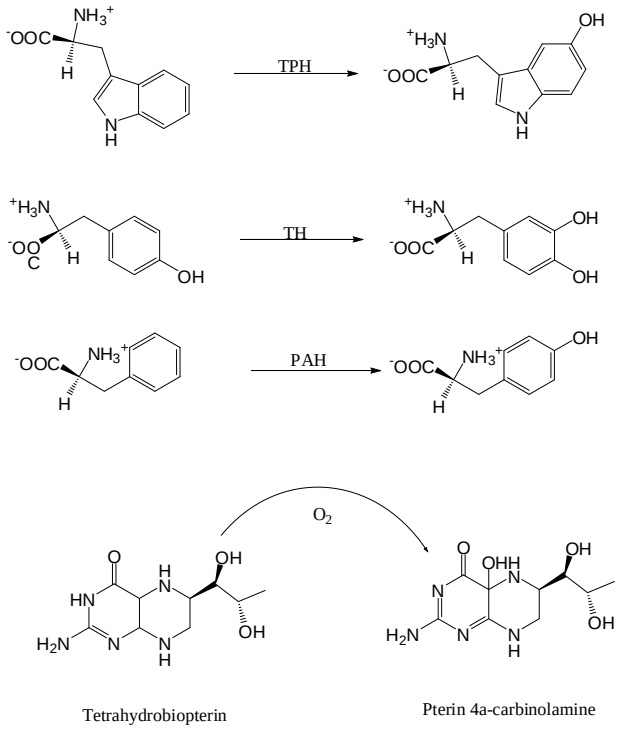


Figure 2 Coupled hydroxylations of the BH4 cofactor and the side chain of aromatic amino acids catalyzed by PAH, TH and TPH hydroxylases.

The extensive sequence and structural homology between PAH, TH, and TPH suggests that these three hydroxylases share a similar catalytic mechanism. This mechanism should further explain the experimental observation that the oxygen atom of the hydroxy cofactor and the oxygen atom of the hydroxy amino acid derive from molecular oxygen. Figure 3 shows two proposed mechanisms for oxygen activation of these enzymes. The two pathways differ in the initial role of iron in this type of catalysis.

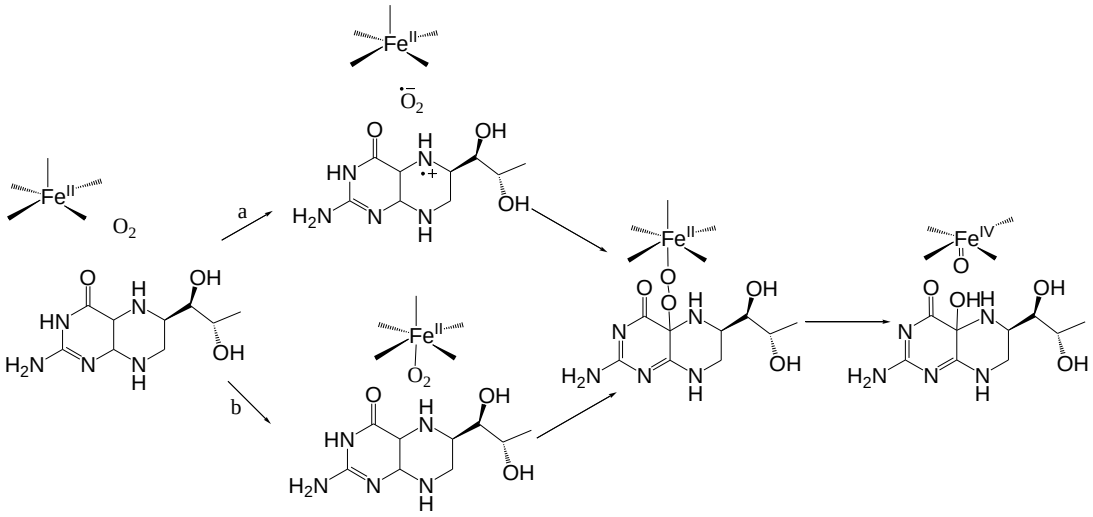


Figure 3: The two pathways suggested for oxygen activation in aromatic amino acid hydroxylases: a) the metal-free pathway; b) the metal-catalyzed route. Both mechanisms lead to an Fe(II)-peroxy-pterin intermediate.

In one case (pathway a), termed the metal-free mechanism, oxygen activation is initiated by the transfer of an electron from the BH4 cofactor, and this transformation does not directly involve the metal. In the other proposed mechanism (pathway b), termed the metal-active pathway, reduction of molecular oxygen by the cofactor occurs after coordination of oxygen to iron. The two pathways shown in Figure 3 lead to the formation of an Fe(II) peroxypterin intermediate, which may itself be responsible for the aromatic hydroxylation of the substrate. But alternatively, there could be heterolysis of the O-O bond, generating the hydroxylated cofactor and a Fe(IV)-oxo intermediate. The latter would then perform the hydroxylation of the substrate.

It can also be seen that the pterins in solution are oxidized by the action of oxygen to produce superoxide and a pterin cationic radical. The association of the two species results in a pterin peroxide whose reaction with iron could produce the oxidizing species. No direct experimental evidence is currently available to support either pathway shown in Figure 3 [21]. For example, experiments with apoenzyme show that pterin is oxidized by oxygen even in the absence of iron, albeit at a slower rate. It has therefore been suggested that this activity might be due to the presence of other metals such as nickel or copper [22,23]. In addition, PAH mutants, which are characterized by a low affinity for iron, do not show any enzymatic activity [24]. As mentioned above, in the case of activated rings, the peroxypterin species resulting from the reduction of the two dioxygen electrons by the cofactor can affect certain aromatic hydroxylations. But it seems that the observed reactivity reflects the presence of a more efficient oxidizing agent, e.g., B. a high-valent Fe-Oxo species required.

We will detail in this part an example of these pterino-dependent hydroxylases, it is the enzyme PAH.

All the starting materials described in this section were synthesized in our laboratory from inexpensive commercial products. The synthetic methods used are well known and easily accessible, and we favored methods based on methylene halides (and not those involving Schiff bases from aldehydes).

Originally, bromination of the TPA ligand was considered in the laboratory to answer two questions:

1) Is it possible to use a brominated TPA as a synthon in order to prepare more sophisticated molecules by implementing coupling reactions?

2) Is it possible, thanks to bromination, to obtain electrode-deficient ligands likely to improve the performances of complexes in oxidation, as had been described in porphyrin chemistry?

The answer to the first question is obviously positive, and a wide range of mono and disubstituted ligands is easily accessible by Suzuki-type coupling reaction.

The answer to the second question has never really been given. It has indeed appeared that with halogenated ligands, ferrous complexes become sensitive to oxygen. Was it due to the steric effect for which the halogens impose a bipyramidal trigonal geometry?

The use of peroxides, currently under study in the laboratory, should make it possible to answer this second question, but this remains to be done because as far as we are concerned, it is on the

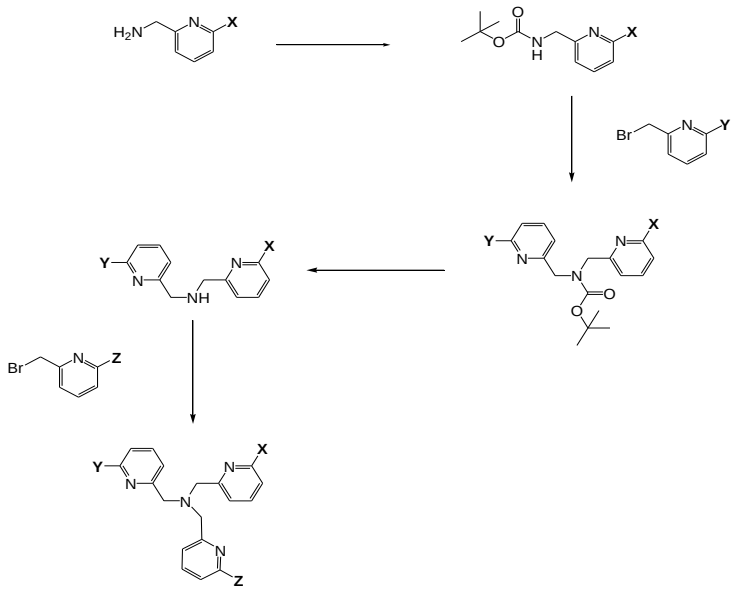
dioxygen-metal interaction that we have focused our attention, and not on the use of already reduced forms of oxygen.

Following a previous article [25-29], the idea that the coordination of dioxygen to iron in the chemistry of TPAs could be governed not only by steric factors but also by the Lewis acidity at the metal center led us to prepare ligands substituted in the α position by non-bulky groups or atoms. It was as much as possible to compare the activity of complexes with fixed geometry, of the deformed Oh type. This is how the ideas for preparing the fluorinated and cyanic series were born.

It was then a question of estimating the basic character of the ligands obtained. It should be noted that in mono- and bisubstituted series, we are in the presence of asymmetric ligands, whose pyridines must have very different pKa values from each other. As a first approximation, we have chosen to examine an easily observable oxidation potential in the ranges used in cyclic voltammetry, that of the central amine. This has the additional advantage of being able to measure a single potential.

In fact, we observe very clear variations, with for the α-substitution by nitriles, the highest potentials: the oxidation at Ea = 1.450 V/ECS of the derivative [(CN)3TPA] highlights a very strong pyridine electrodeficiency. The study of reactivity, carried out on the complexes, should make it possible to determine the influence of the electronic factor. In the future, one can imagine a finer modulation of the redox potentials by the preparation of α-hetero substituted ligands. A possible synthetic scheme is given below (Scheme 1):

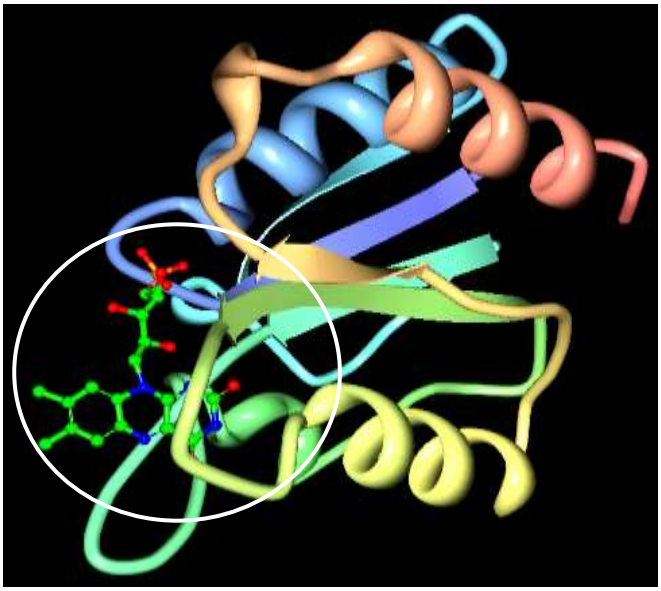
We have synthesized many hexa- and penta-chelated ligands and studied their complexes with iron (II). We were able to determine the oxidation and reduction potentials and control the strength of Lewis acid in the mineral center by modifying the structure. Through this control, the reaction speed with molecular oxygen was controlled. These associations were later linked to riboflavin [30-40]



Schema1: Possible synthetic scheme for preparing tris α-hetero substituted derivatives.

We have also described the preparation of a compound in which a DPA "clamp" is connected to a redox-active spacer such as riboflavin.

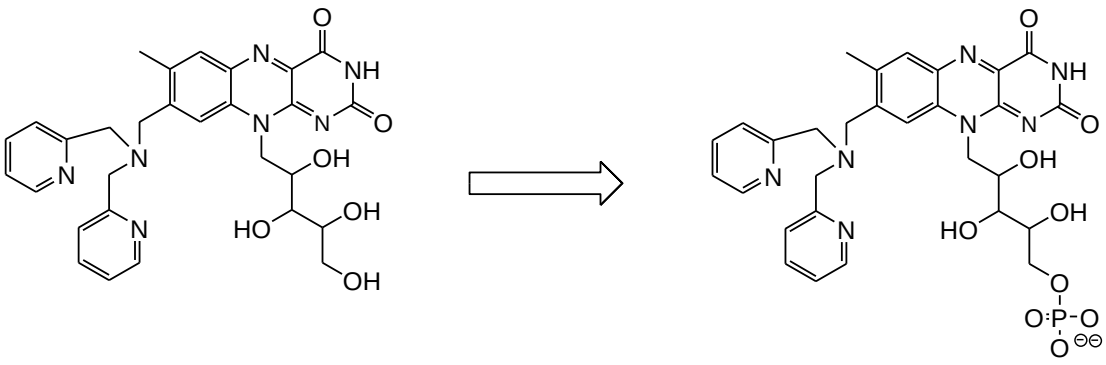
One could imagine directly complexing the compound 8α-[bis(2-pyridylmethyl) amine] N-riboflavin on flavoproteins which contain a receptor [35-44]. But this operation is only well documented on one particular flavoprotein, that from Desulfuvibrio Vulgaris [40-49]. Furthermore, the stability constants of FMNs are greater than those of simple flavins [26] (Schema 2).



Schema 2: Overview of the FMN coordination site at a flavodoxin from *Desulfuvibrio Vulgaris present tow free methyl group given possible attachment site for the ligands*

It therefore seems important to us to transform the riboflavin part into FMN, so as to be able to complex this fragment on a wider range of flavoproteins, a well-documented operation.

Different protection/deprotection sequences should make it possible to obtain the derivative drawn below in Schema 3:



Schema 3: DPA-Riboflavin can be bound with phosphate.

Subsequently, complexation with flavodoxins can be considered by the methods described [47-57] and suggests the preparation of new molecular objects associating proteins and synthetic metal sites.

We also see in Schema 2that the flavoproteins are small molecules at the biological level, and that the complexed FMN part is protected by the protein, but that, located rather at the periphery, it remains perfectly accessible. The presence of a DPA substituent should not have major structural consequences.

Very simple modifications make it possible to modulate the electronic properties of the ligands, and consequently to hope to modulate the reactivity of future complexes.

But the chemist studies the object that he himself has made, and he is always attracted by new syntheses. A nitrile can be seen as a protected aminomethyl; a methoxyl such as a phenol; and independently of the passage through a spacer, we could consider using these groups to directly link our ligands to proteins with accessible amino acids. This would be a direct method of accessing artificial proteins.

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