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Review

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Three classes of tetrahydrobiopterin-dependent enzymes

Abstract: Current knowledge distinguishes three classes of tetrahydrobiopterin-dependent enzymes as based on protein sequence similarity. These three protein sequence clusters hydroxylate three types of substrate atoms and use three different forms of iron for catalysis. The first class to be discovered was the aromatic amino acid hydroxylases, which, in mammals, include phenylalanine hydroxylase, tyrosine hydroxylase, and two isoforms of tryptophan hydroxylases. The protein sequences of these tetrahydrobiopterin-dependent aromatic amino acid hydroxylases are significantly similar, and all mammalian aromatic amino acid hydroxylases require a non-heme-bound iron atom in the active site of the enzyme for catalysis. The second classes of tetrahydrobiopterin-dependent enzymes to be characterized were the nitric oxide synthases, which in mammals occur as three isoforms. Nitric oxide synthase protein sequences form a separate cluster of homologous sequences with no similarity to aromatic amino acid hydroxylase protein sequences. In contrast to aromatic amino acid hydroxylases, nitric oxide synthases require a heme-bound iron for catalysis. The alkylglycerol monooxygenase protein sequence was the most recent to be characterized. This sequence shares no similarity with aromatic amino acid hydroxylases and nitric oxide synthases. Motifs contained in the alkylglycerol monooxygenase protein sequence suggest that this enzyme may use a di-iron center for catalysis.

Keywords: alkylglycerol monooxygenase; nitric oxide synthase; phenylalanine hydroxylase; tetrahydrobiopterin; tyrosine hydroxylase.

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Introduction

The present article is based on the Gowland Hopkins Award lecture given by the author on the 15th International Symposium on Pterins and Folates, May 9–13, 2012, in Antalya, Turkey. For a more detailed review on biochemistry and pathophysiology of tetrahydrobiopterin, the reader is referred to Ref. [1]. Here, a short historical overview of the discovery of tetrahydrobiopterin-dependent enzymes is presented, followed by a summary of the biochemical properties of these enzymes. The reactions catalyzed by the three classes of tetrahydrobiopterin-dependent enzymes are detailed in Figure 1.

Discovery of tetrahydrobiopterin-dependent enzymes

Aromatic amino acid hydroxylases

Phenylalanine hydroxylase was the first enzyme characterized to be dependent on a tetrahydropterin [2]. It then took five more years to identify the nature of the endogenous cofactor as tetrahydrobiopterin [3]. Soon after, the two other tetrahydrobiopterin-dependent aromatic amino acid hydroxylases, i.e., tyrosine hydroxylase (EC 1.14.16.2) [4] and tryptophan hydroxylase (EC 1.14.16.4) [5], were characterized to depend on tetrahydrobiopterin. These enzymes are required for the degradation of phenylalanine as well as for the biosynthesis of catecholamine and serotonin neurotransmitters. Studying the formation of serotonin in tryptophan hydroxylase (TPH1) knockout mice led to the discovery of the second isoform of tryptophan hydroxylase (TPH2) in 2003 [6]. The analysis of the protein sequences of the enzymes showed significant homologies between these tetrahydrobiopterin-dependent aromatic amino acid hydroxylases, and structural and mechanistic studies revealed a general similarity of these enzymes with only subtle differences [7, 8].

Alkylglycerol monooxygenase

In 1964, a tetrahydropteridine-dependent enzyme system for the cleavage of glyceryl ethers was first described [9]. The responsible enzyme, alkylglycerol monooxygenase

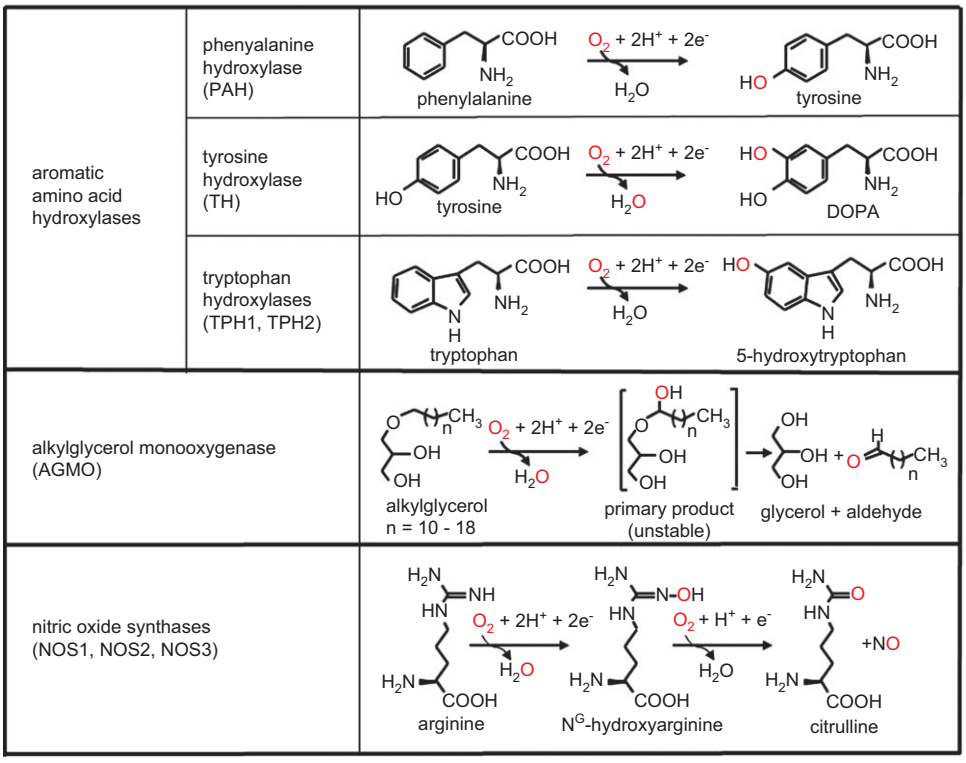

Figure 1 Chemical reactions catalyzed by the three classes of tetrahydrobiopterin-dependent enzymes.
The respective gene symbols in humans are in parentheses. The intermediate semiacetal as initial reaction product of the alkylglycerol monooxygenase is assumed to occur by plausibility, an experimental proof of it as well as knowledge on whether or not its rearrangement is assisted by an additional enzyme is currently missing. The figure was modified from Ref. [1].

(EC 1.14.16.5.), is a very labile integral membrane protein that could not be purified up to now. Sequence assignment was achieved in 2010 by bioinformatic selection of candidate genes and recombinant expression in Chinese hamster ovary cells [10]. This sequence turned out to be unrelated to the other groups of tetrahydrobiopterin-dependent enzymes, the aromatic amino acid hydroxylases and the nitric oxide synthases (EC 1.14.19.39). Alkylglycerol monooxygenase is the only enzyme known to cleave the ether bond of alkylglycerols, which are major membrane constituents, e.g., in the brain. The platelet-activating factor, a very potent inflammatory mediator, is also an alkylglycerol derivative. The precise physiological role of alkylglycerol monooxygenase remains to be elucidated. Current knowledge on alkylglycerol monooxygenase is summarized in Ref. [11].

Nitric oxide synthases

In the late 1980s, a new class of tetrahydrobiopterin-dependent enzymes, the nitric oxide synthases, was discovered [12–14], which, from a biochemical point of view, behaves quite differently from the currently known tetrahydrobiopterin-dependent enzymes (see below). Nitric oxide synthases occur in mammals in three isoforms encoded by three different genes. They serve a variety of important functions, including neurotransmission, vasorelaxation, and host defense to pathogens and tumors [15–17]. Their protein sequences share significant sequence homology but are unrelated to the two other sequence clusters of tetrahydrobiopterin-dependent enzymes, the aromatic amino acid hydroxylases and the alkylglycerol monooxygenase. The role of tetrahydrobiopterin in the three isoforms is very similar, with only subtle differences [18, 19].

Biochemical characteristics of the three classes of tetrahydrobiopterin-dependent enzymes

A summary of the current knowledge on the biochemistry of the three classes of tetrahydrobiopterin-dependent enzymes is presented in Table 1; for a more

Table 1 Some characteristics of the three classes of tetrahydrobiopterin-dependent enzymes.

	Aromatic amino acid hydroxylases	Alkylglycerol monooxygenase	Nitric oxide synthases (NOS)
Members in humans (gene symbols)	Phenylalanine hydroxylase (PAH) Tyrosine hydroxylase (TH) Tryptophan hydroxylases (TPH1, TPH2)	Alkylglycerol monooxygenase (AGMO)	Neuronal NOS (NOS1) Inducible NOS (NOS2) Endothelial NOS (NOS3)
Substrate atom hydroxylated (cf. Figure 1)	Aromatic carbon atom	Aliphatic carbon atom	Guanidino nitrogen atom
Affinity of tetrahydrobiopterin (K_m)	~30 µM	~3 µM	~0.3 µM
Recycling of tetrahydrobiopterin	External (PCDB1+QDPR)	External [PCBD1 (?)+QDPR]	Internal
Chemistry of cofactor assisted catalysis	Two-electron	Two-electron	One-electron
Selectivity for the side chain in position 6 of the pterin	Medium	Medium	High
Form of the catalytically active iron	Non-heme	Di-iron center	Heme
Oxygen activation by tetrahydrobiopterin	Yes	Yes	No
Soluble – membrane association	Soluble	Integral membrane protein	Soluble (NOS3 myristoylated and palmitoylated)
Distinct sequence cluster	Aromatic amino acid hydroxylases	Alkylglycerol monooxygenases	Nitric oxide synthases
Structure	Crystal structures available	Only a predicted structure of a part of the protein available	Crystal structures of oxygenase domains available

Alkylglycerol monooxygenase could not be purified so far, and the enzymatic characteristics are therefore based on measurements in rat liver microsomes or in homogenates of transfected cells. The two recycling enzymes of tetrahydrobiopterin are shown by their gene symbols in the table.

detailed description, the reader is referred to Ref. [1]. The entries for the alkylglycerol monooxygenase are based on current knowledge, but they should be treated with caution because no pure enzyme has been prepared so far to study the biochemical parameters. These were observed mainly in rat liver microsomal preparations or in homogenates of transiently transfected Chinese hamster ovary cells.

The three classes of tetrahydrobiopterin-dependent enzymes all catalyze a mixed-function oxygenase reaction, i.e., one oxygen atom of the oxygen molecule is incorporated into the substrate, whereas the other one is converted to water (Figure 1). However, the nature of the substrate atom hydroxylated is different for each class of tetrahydrobiopterin-dependent enzymes. It is an aromatic carbon atom in the aromatic amino acid hydroxylases, a guanidino nitrogen in nitric oxide synthases, and an aliphatic carbon atom in alkylglycerol monooxygenase. The affinity for the tetrahydrobiopterin cofactor varies over two orders of magnitude, being highest in the nitric oxide synthases and lowest in the aromatic amino acid hydroxylases. In addition to the highest affinity for tetrahydrobiopterin, nitric oxide synthases are also the class of enzymes with the highest selectivity for the 6*R*-1′,2′-dihydroxypropyl side chain of tetrahydrobiopterin. Although nitric oxide synthases are almost exclusively stimulated by tetrahydrobiopterin, with the action of other tetrahydropterins only at orders-of-magnitude-higher concentrations, aromatic amino acid hydroxylases and alkylglycerol monooxygenase can be stimulated with some other tetrahydropterins to a more comparable extent. Aromatic amino acid hydroxylases and presumably also alkylglycerol monooxygenase use tetrahydrobiopterin for oxygen activation. This is not the case for nitric oxide synthases [20]. The tetrahydrobiopterin cofactor leaves the enzymatic reaction of aromatic amino acid hydroxylases as 4a-hydroxy derivative in aromatic amino acid hydroxylases (and presumably also in alkylglycerol monooxygenase). This compound is recycled by pterin 4a-carbinolamine dehydratase (PCBD1) and quinoid dihydropteridine reductase (QDPR) back to the active tetrahydrobiopterin cofactor (reviewed in Ref. [1]). In contrast, in nitric oxide synthases, the cofactor is thought to undergo only one-electron transformations to a tetrahydrobiopterin radical, which is regenerated at the enzyme without the aid of additional external enzymes. All three classes of tetrahydrobiopterin-dependent enzymes contain an iron, which is essential for catalysis, but in a different form. This is a non-heme iron bound to two histidines and a glutamate in aromatic amino acid hydroxylases and a heme-bound iron in nitric oxide

synthases. The occurrence of the fatty acid hydroxylase motif in the protein sequence of alkylglycerol monooxygenase suggests that this enzyme may contain a di-iron center in its active site [10]. This feature may be linked to the exceptional instability of this enzyme because no protein containing the fatty acid hydroxylase motif has ever been purified in its active form so far [21]. Another feature related to experimental difficulties with handling alkylglycerol monooxygenase is that it is an integral membrane protein occurring in the endoplasmic reticulum [10], which has nine transmembrane regions [22], whereas the other tetrahydrobiopterin-dependent enzymes are soluble proteins. One of these, endothelial nitric oxide synthase, is myristoylated and palmitoylated post-translationally, targeting it to the plasmalemmal caveolae [23]. The crystal structures of the aromatic amino acid hydroxylases (reviewed in Refs. [7, 8]) and of the oxygenase domains of the three nitric oxide synthases (reviewed in Ref. [24]) are available. For alkylglycerol monooxygenase, only a predicted structure of a part of the protein has been suggested [22].

Conclusion

The three classes of tetrahydrobiopterin-dependent enzymes are encoded by proteins belonging to three different sequence clusters; they use three different forms of iron for catalysis; and they hydroxylate three different kinds of substrate atoms, all in monooxygenase-type reactions. Although the biochemistry of tetrahydrobiopterin in alkylglycerol monooxygenase and in aromatic amino hydroxylases shares many features, nitric oxide synthases use tetrahydrobiopterin in a considerably different manner.

Conflict of interest statement

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