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Review

Tyrosine hydroxylase and regulation of dopamine synthesis

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ABSTRACT

Tyrosine hydroxylase is the rate-limiting enzyme of catecholamine biosynthesis; it uses tetrahydrobiopterin and molecular oxygen to convert tyrosine to DOPA. Its amino terminal 150 amino acids comprise a domain whose structure is involved in regulating the enzyme's activity. Modes of regulation include phosphorylation by multiple kinases at four different serine residues, and dephosphorylation by two phosphatases. The enzyme is inhibited in feedback fashion by the catecholamine neurotransmitters. Dopamine binds to TyrH competitively with tetrahydrobiopterin, and interacts with the R domain. TyrH activity is modulated by protein–protein interactions with enzymes in the same pathway or the tetrahydrobiopterin pathway, structural proteins considered to be chaperones that mediate the neuron's oxidative state, and the protein that transfers dopamine into secretory vesicles. TyrH is modified in the presence of NO, resulting in nitration of tyrosine residues and the glutathionylation of cysteine residues.

Introduction

Tyrosine hydroxylase (TyrH)[1] is the rate-limiting enzyme of catecholamine synthesis. It catalyzes the hydroxylation of tyrosine to L-DOPA [1]. The catecholamines dopamine, epinephrine and norepinephrine are the products of the pathway, important as hormones and neurotransmitters in both the central and peripheral nervous systems. In the latter, they are synthesized in the adrenal medulla [1,2]. The biosynthetic pathway is illustrated in Fig. 1. These catechol monoamines play roles in many brain functions, such as attention [3], memory [4], cognition [5], and emotion [6,7]. As the hormone of the fight-or-flight response, epinephrine produced in the adrenal gland affects many tissues throughout the body [8]. Therefore deficits and surfeits in the levels of the catecholamines have many repercussions, perhaps including high blood pressure, bipolar disorder, addiction, and dystonias [9–11]. Because of this, the activity of TyrH as the slowest enzyme in the pathway is of great interest in many fields of biomedical research.

Given the importance of the activity of TyrH, the complexity of its regulation is not surprising. Control of its expression by transcriptional mechanisms is a very active field of research, as is the relatively new field of its degradation in the proteosome after ubi-quitination [12]. This review, however, will focus on mechanisms of regulation that occur after the synthesis of TyrH and before its destruction. It will discuss the short-term reversible biochemical processes and conformational changes that TyrH undergoes while the enzyme is active and being modulated in response to the metabolic state of the neuron or the chromaffin cell.

When need for neurotransmitter increases at a catecholaminergic synapse, TyrH is activated to make more DOPA, which after decarboxylation to dopamine is transferred into the synaptic vesicle by the vesicular monoamine transporter (VMAT). Catecholamine synthesis then continues in vesicles *via* the actions of dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase. Influx of calcium causes the emptying of the vesicles into the synaptic cleft, and the nervous signal is passed on. Some catecholamine is taken up by the presynaptic neuron but more must be synthesized for the next transmission. TyrH activity must be sustained until the need is lessened, and its activity must be turned off when the need for neurotransmitters has passed. The post-translational mechanisms that accomplish all this include phosphorylation by kinases and dephosphorylation by phosphatases, feedback inhibition, oxidation by nitrites, and inclusion in protein complexes. Some of these complexes alter the activity of

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[1] *Abbreviations used:* AAAHs, aromatic amino acid hydroxylases; AADC, aromatic amino acid decarboxylase; BMHI, a 14-3-3 protein homolog from yeast; CaMKII, Ca^{++}/calmodulin-dependent protein kinase II; cAMP, cyclic AMP; C domain, catalytic domain; CdK5, cyclin-dependent kinase 5; DOPA, dihydroxyphenylalanine; ERK, extracellularly-regulated kinase; hTH1, human tyrosine hydroxylase isoform 1, etc.; MAPKAPKII, mitogen-activated protein kinase-activating protein kinase II; MSKI, mitogen- and stress-activated protein kinase; NO, nitric oxide; PheH, phenylalanine hydroxylase; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; PRAK, p38-regulated/activated protein kinase; R domain, regulatory domain; rTyrH, rat tyrosine hydroxylase; TrpH, tryptophan hydroxylase; TyrH, tyrosine hydroxylase; TyrHpser19, -pser31, and pser40, etc., TyrH phosphorylated at serine19, serine31, or serine40, etc.

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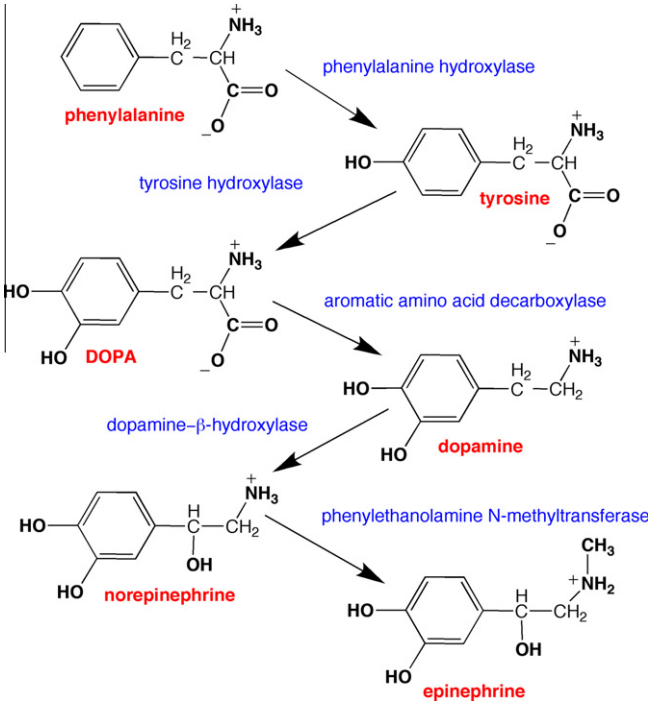

Fig. 1. The biosynthetic pathway for the catecholamine neurotransmitters. Phenylalanine hydroxylase converts phenylalanine to tyrosine, tyrosine hydroxylase hydroxylates tyrosine to L-DOPA. DOPA is converted to dopamine by aromatic amino acid decarboxylase. Dopamine-β-hydroxylase hydroxylates dopamine to norepinephrine, which is methylated to epinephrine by phenylethanolamine N-methyltransferase. Tyrosine hydroxylase is the rate-limiting enzyme of the pathway.

TyrH and some of them simply place the enzyme near other related proteins. The review will attempt to cover these steps using the following organization: First, structural changes in the regulatory domain, including phosphorylation and feedback inhibition, catecholamine binding, and R domain differences between rat and human TyrH; second, protein complexes with 14-3-3 proteins, Alpha-synuclein, AADC, GTPCH, VMAT, and DJ-1; and finally, nitrosylation/thiolation of TyrH.

Note: this review will focus heavily on experiments done *in vitro* with purified proteins whenever possible. For an excellent and comprehensive review of TyrH regulation by phosphorylation that encompasses *in vivo* and *in situ* studies the reader is referred to the 2004 article by Dunkley et al. [13].

Background: Description of the overall structure of the aromatic amino acid hydroxylases

TyrH is a member of a family of enzymes that also contains the aromatic amino acid hydroxylases (AAAHs) phenylalanine hydroxylase (PheH) and tryptophan hydroxylase (TrpH). PheH and TrpH will not be discussed in depth in this review. Nonetheless it is worth describing the entire family, because some of their structural similarities will be included in the discussion of TyrH regulation. All three enzymes perform hydroxylation of the aromatic ring of an amino acid. They all use diatomic oxygen and reduced biopterin in a reaction with a bound iron atom. The iron atom is held in place in the active site cleft by two histidine residues and a glutamate residue, and it must be in the ferrous state to carry out catalysis. Each of the three enzymes has a very similar active site. Similarities include the iron and its ligands, plus homologous residues for binding tetrahydrobiopterin and the aromatic amino acid

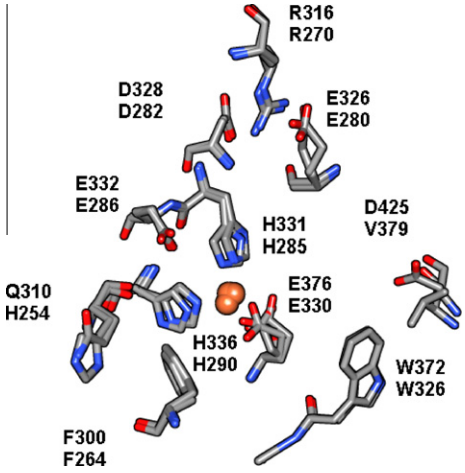

Fig. 2. The active sites of PheH (1DMW) and TyrH (1TOH) are overlaid on each other and the residues that play identifiable roles in catalysis are highlighted. The top # in each pair of residues is the residue in TyrH and the bottom # is the residue in PheH. In particular, histidines 331 and 336 (285 and 290 in PheH) and glutamate 376 (330) are the ligands to the iron atom. Residues on the left in this view, gln310/his254, phe300/264, and glu332/286 form the binding site for tetrahydrobiopterin, and on the right, arg316/270, asp328/282 and asp425/val379 form the binding site for the aromatic amino acid.

[14,15]. The active sites of TyrH and PheH from crystallographic data, overlaid, are shown in Fig. 2.

In addition to these similarities in the active site, the family shares other features of three-dimensional structure. Each enzyme has a multi-domain structure, with an amino-terminal regulatory domain (R) of varying size from 100–160 amino acid residues, followed by a catalytic domain (C) of about 330 residues, and a coiled-coil domain at the carboxyl terminus of about 40 amino acids. All three enzymes form tetramers. The C domains display 50% identity in primary structure, while the R domains are as little as 15% similar [16]; rat PheH and TyrH are ~75% similar in their C domains. A scheme illustrating the domains of the AAAHs is shown in Fig. 3. Also shown are the domains of TyrH and PheH *via* coloration of their crystal structures [17,18]. An alignment of the amino acid sequences of TyrH and PheH by CLUSTALW is shown in Fig. 4.

Despite little similarity in R domains, all three hydroxylases are phosphorylated at serine residues that lie in the R domains. They are all phosphorylated by cAMP-dependent protein kinase (PKA) [19]. When TyrH is phosphorylated by PKA, it is less susceptible to feedback inhibition by catecholamines [20], and when PheH is phosphorylated by PKA, lower levels of phenylalanine are required for substrate activation [21]. While there is a fine crystal structure of the C domain of TyrH [17], the R domain of TyrH has never been crystallized, presumably due to its flexibility. Attempts to crystallize it with ligands have been unsuccessful. Therefore some study of the R domain of TyrH relies on the crystals of PheH [18], because the structure of PheH has been solved, though it is missing its amino terminal 18 residues. These are the structures shown in Fig. 3. PheH is activated by phosphorylation at ser16 (missing from the crystal structure). Although the serine of interest is missing the structure is still very informative. The R domain of PheH, a lobe consisting of two α-helices and four strands of β-sheet, lies mainly above and to the side of the opening to the active site. However, a short portion of the R domain (from residues 19 to 33) spans the distance from that somewhat removed position to the opening of the active site, lying across it and possibly restricting access to the active site. Although no crystal structures prove it, it is logical to hypothesize that phosphorylation moves the R domain out of the opening of the active site, and dephosphorylation by a phosphatase returns it to its obstructive position. A simple graphical representation of this model, applied to TyrH, appears in Fig. 5.