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The regulatory domain of human tryptophan hydroxylase 1 forms a stable dimer

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ABSTRACT

The three eukaryotic aromatic amino acid hydroxylases phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase have essentially identical catalytic domains and discrete regulatory domains. The regulatory domains of phenylalanine hydroxylase form ACT domain dimers when phenylalanine is bound to an allosteric site. In contrast the regulatory domains of tyrosine hydroxylase form a stable ACT dimer that does not bind the amino acid substrate. The regulatory domain of isoform 1 of human tryptophan hydroxylase was expressed and purified; mutagenesis of Cys64 was required to prevent formation of disulfide-linked dimers. The resulting protein behaved as a dimer upon gel filtration and in analytical ultracentrifugation. The s_w value of the protein was unchanged from 2.7 to 35 μM, a concentration range over which the regulatory domain of phenylalanine hydroxylase forms both monomers and dimers, consistent with the regulatory domain of tryptophan hydroxylase 1 forming a stable dimer stable that does not undergo a monomer-dimer equilibrium. Addition of phenylalanine, a good substrate for the enzyme, had no effect on the s_w value, consistent with there being no allosteric site for the amino acid substrate.

Tryptophan hydroxylase (TPH) belongs to the family of the aromatic amino acid hydroxylases, together with tyrosine hydroxylase (TyrH) and phenylalanine hydroxylase (PheH) [1]. They each catalyze the hydroxylation of the aromatic ring of their respective aromatic amino acid substrate, using oxygen and tetrahydrobiopterin as the other substrates. TPH converts tryptophan to 5-hydroxytryptophan in the biosynthesis of the neurotransmitter serotonin. TPH is also the first enzyme in the synthesis of melatonin in the pineal gland [2]. Thus, dysfunction of TPH can result in decreases in serotonin and melatonin, leading to depression and other disorders [3,4]. Two distinct isoforms of TPH (TPH1 and TPH2) have been identified in humans and other mammals, with an amino acid sequence identity of ~71% [5]. TPH1 is expressed mainly in the gut and pineal gland, while TPH2 is expressed almost exclusively in the brain [6].

All three hydroxylases are homotetramers, with three functional domains: a divergent N-terminal regulatory domain, a homologous catalytic domain, and a C-terminal tetramerization domain [1]. The catalytic domains have three-dimensional structures that are essentially identical [7–9]. The regulatory domains show a much lower sequence identity and vary in length (Fig. 1), suggesting that these enzymes have distinct regulatory mechanisms. Indeed, the regulatory mechanisms of TyrH and PheH have been established to be different [10]; however, the regulatory mechanism of TPH is poorly understood due largely to difficulties in obtaining stable recombinant enzyme for study.

PheH is activated by its substrate phenylalanine and by phosphorylation at Ser16 and inhibited by tetrahydrobiopterin [11]. The resting form of PheH is inactive, with the N-terminus of the regulatory domain partially occluding the active site [12]. Phenylalanine binding to an allosteric site in the regulatory domain [13,14] causes a significant conformational change that opens up the active site [15,16]. Key to this conformational change is formation of an ACT domain dimer by regulatory domains from two subunits [17,18]. This model is supported by recent studies of the isolated regulatory domain [14,19,20] and of the intact protein [13,18]. TyrH is activated by phosphorylation of Ser40 in its N-terminal regulatory domain and inhibited by catecholamines [10,21]. While there is no available structure of TyrH with both the regulatory and catalytic domains, several studies support a model

Abbreviations: TPH, tryptophan hydroxylase; PheH, phenylalanine hydroxylase; TyrH, tyrosine hydroxylase; RDTPH, regulatory domain of tryptophan hydroxylase; AUC, analytical ultracentrifugation; s_w, weight average sedimentation coefficient.

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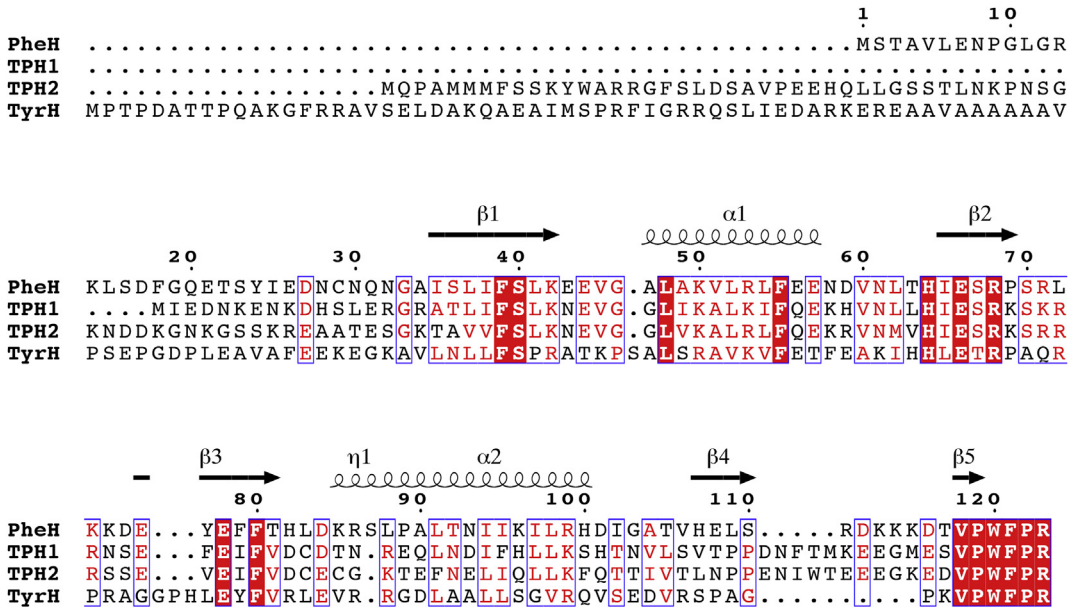

Fig. 1. Sequence alignment of the regulatory domains of the human aromatic amino acid hydroxylases. The secondary structure locations are from the crystal structure of rat PheH (2PHM). Residues that are identical in all four proteins are highlighted in red. The alignment was generated using CLUSTALW [35]. The figure was generated using ESPript [36]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in which the protein exists in two conformations, one stabilized by catecholamine binding in which the N-terminus extends across the active site and prevents substrate access in a similar fashion to PheH, and one stabilized by phosphorylation in which the active site is accessible [22–24]. Studies of the isolated regulatory domain of TyrH have established that it forms a stable ACT domain dimer in solution even in the absence of tyrosine [25]. This supports a model in which TyrH has a quaternary structure similar to the active form of PheH, with two regulatory domains forming a side-by-side ACT domain dimer, but the N-terminus of the regulatory domain still extending across the active site [10]. TyrH contains a flexible N-terminal tail of ~70 residues [25], much longer than that in PheH, allowing it to accommodate the increased distance to the active site when the regulatory domain dimerizes. Thus, while regulation of TyrH and PheH both involve an N-terminal extension from an ACT domain regulatory domain hindering access to the active site, they differ in whether the regulatory domain forms a dimer reversibly.

PheH and TyrH provide discrete models for the arrangement of regulatory domains in TPH, with clear implications for regulation of that enzyme. To better understand the regulation of TPH, we have expressed the isolated regulatory domain of human TPH1 (RDTPH1) and analyzed the oligomerization state of the protein. The results suggest that the regulatory domain of TPH1 forms a dimer in the resting form of the enzyme similar to that in TyrH despite the greater sequence similarity to PheH.

1. Experimental procedures

1.1. Materials

Dithiothreitol was from Inalco, S.p.A. (Milan, Italy). Leupeptin and pepstatin A were from Peptide Institute, Inc (Osaka, Japan). Restriction and DNA modification enzymes were purchased from New England Biolabs (Ipswich, MA). All the other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO). The plasmid pGro7 for co-expression of GroEL and GroES was from TaKaRa Bio Inc.

1.2. Protein expression and purification

A synthetic gene for human TPH1 optimized for expression in *Escherichia coli* was obtained from DNA2.0 (Menlo Park, CA). The gene, which contains a histidine tag at the N-terminus, was extracted from the commercial vector and cloned into pET23d using the restriction enzymes NcoI and BamHI. The plasmid for expressing only the regulatory domain of TPH1 (RDTPH1, residues 1–101) was constructed by introducing a stop codon into the gene for TPH1 in pET23d. Cys64 in RDTPH1 was mutated to serine using the QuikChange Mutagenesis protocol (Agilent Technologies). Sequences of the resulting plasmids were verified by DNA sequencing (Genscript, NJ). Both plasmids were used to transform BL21 (DE3) cells containing pGro7.

For protein expression and purification, a single colony from an agar plate was used to inoculate LB media containing 150 μg/L ampicillin, 50 μg/L chloramphenicol, and 0.5 mg/ml arabinose. After overnight growth 14 ml were transferred to 1 L of the same media. When the absorbance at 600 nm reached 0.3, the temperature was reduced to 18 °C. Expression was induced with 0.25 mM isopropyl ß-D-1-thiogalactopyranoside when the absorbance at 600 nm reached 0.6; cells were harvested after 20 h. The cell pellets were suspended in lysis buffer (300 mM NaCl, 10 mM imidazole, 1 μM pepstatin A, 1 μM leupeptin, 50 mM phosphate, pH 8.0) plus 100 μg/ml phenylmethylsufonyl fluoride, and lysed by sonication. The resulting cell suspension was centrifuged at 30,000 g for 30 min at 4 °C. The supernatant was loaded onto a 5 mL HisTrap HP column (GE Healthcare) equilibrated with lysis buffer. The column was washed with 50 ml lysis buffer; the protein was then eluted with a 100 ml gradient of lysis buffer containing 0–0.5 M imidazole. The fractions showing a band with an apparent molecular weight of ~13 kDa in a SDS-polyacrylamide gel were pooled and concentrated using an Amicon Ultra-15 10 K centrifugal filter (10 K molecular weight cutoff, Millipore). The concentrated sample was then loaded to a HiPrep 16/60 Superdex 200 prep grade (GE Healthcare Life Science, Piscataway, NJ) gel filtration column equilibrated with 50 mM phosphate, 100 mM NaCl, pH 8.0. Fractions containing pure protein were pooled and stored at −80 °C. The protein