The first X-ray crystal structure of full-length mammalian phenylalanine hydroxylase

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Abstract

Mammalian phenylalnine hydroxylase (PAH) is a non-heme iron-dependent mono-oxygenase that uses tetrahydrobiopterin (BH₄) to catalyze the rate-limiting step of phenylalanine catabolism, synthesizing tyrosine from phenylalanine (Phe, Phe). At elevated concentrations, Phe allosterically activates PAH, improper allosteric regulation and/or reduced enzymatic activity permit accumulation of neurotoxic levels of phenylalanine in the blood; this is characteristic of one of the most common inborn errors of metabolism, phenylketonuria (PKU). We previously proposed that allosteric regulation of PAH involves major inter-domain motions that interconvert multiple architecturally distinct tetramers that have low- or high-activity; however, no full-length structure has yet been reported. This previously underappreciated fact—that PAH exists in a dynamic equilibrium of tetrameric assemblies in solution—has likely been a hurdle in obtaining diffraction-quality crystals of the full-length protein. By including an additional ion-exchange purification step that separates tetramers, crystallization was successful and we now report the first X-ray crystal structure of full-length rat PAH at 2.9 Å resolution. This structure supersedes a long-accepted consensus homology model. Furthermore, using small-angle X-ray scattering (SAXS), we confirm that this crystal structure is in a conformation consistent with the structure of a low-activity tetramer in solution. Comparison of this full-length structure with all other X-ray crystal structures of PAH—at least the two Phe receptors—reveals features that likely play a role in tetramer interconversion, and in the regulation of active-site access. From the full-length structure we can now appreciate inter-domain interactions not previously reported, some of which may give new insight regarding the role of disease-associated single-residue substitutions. Ultimately, the goal is to discern the structures of the multimer tetramers available to PAH such that drugs can be rationally designed to bind to an allosteric ligand-binding site that stabilizes a high-activity form, or destabilizes a low-activity form. Such a drug could increase the activity of disease-associated variants whose dimeric equilibrium is perturbed towards low-activity forms.

PAH is a multimeric multi-domain non-heme iron-dependent mono-oxygenase that uses BH₄ to catalyze the hydroxylation of Phe to tyrosine. All figures herein are colored according to this domain map. Our model of allosteric regulation of mammalian PAH includes at least two architecturally distinct tetrameric assemblies in an dynamic equilibrium. The high-activity tetramer shown is a homology model based in part on the ACT-ACT interface found in the related, constitutively active aromatic amino acid hydroxylase, tyrosine hydroxylase. The mechanism of interconversion is an active research pursuit.

The full-length structure reveals intra-(LEFT) and inter-(RIGHT)-chain interactions (present in all four chains of the ASU) that are potentially important to the interconversion of tetrameric assemblies.

The four monomers in the asymmetric unit (ASU) can be arranged as a tetramer that represents the low-activity tetrameric assembly of mammalian PAH

SAXS confirms that the crystal structure of the full-length protein is consistent with the structure of a low-activity tetramer in solution.

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