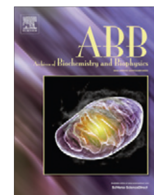
Archives of Biochemistry and Biophysics xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



A new model for allosteric regulation of phenylalanine hydroxylase: Implications for disease and therapeutics

Eileen K. Jaffe *, Linda Stith, Sarah H. Lawrence, Mark Andrake, Roland L. Dunbrack Jr.

Developmental Therapeutics, Institute for Cancer Research, Fox Chase Cancer Center, Temple Health, 333 Cottman Ave., Philadelphia, PA 19111, USA

ARTICLE INFO

Article history:
Received 7 November 2012
and in revised form 7 December 2012
Available online xxxx

Keywords:
Pharmacological chaperone
Protein homology modeling
ACT domain dimerization
Protein multimerization
Morpheein

ABSTRACT

The structural basis for allosteric regulation of phenylalanine hydroxylase (PAH), whose dysfunction causes phenylketonuria (PKU), is poorly understood. A new morpheein model for PAH allostery is proposed to consist of a dissociative equilibrium between two architecturally different tetramers whose interconversion requires a ~90° rotation between the PAH catalytic and regulatory domains, the latter of which contains an ACT domain. This unprecedented model is supported by *in vitro* data on purified full length rat and human PAH. The conformational change is both predicted to and shown to render the tetramers chromatographically separable using ion exchange methods. One novel aspect of the activated tetramer model is an allosteric phenylalanine binding site at the inter-subunit interface of ACT domains. Amino acid ligand-stabilized ACT domain dimerization follows the multimerization and ligand binding behavior of ACT domains present in other proteins in the PDB. Spectroscopic, chromatographic, and electrophoretic methods demonstrate a PAH equilibrium consisting of two architecturally distinct tetramers as well as dimers. We postulate that PKU-associated mutations may shift the PAH quaternary structure equilibrium in favor of the low activity assemblies. Pharmacological chaperones that stabilize the ACT:ACT interface can potentially provide PKU patients with a novel small molecule therapeutic.

© 2013 Published by Elsevier Inc.

Introduction

Diminished activity of human phenylalanine hydroxylase (PAH), which catalyzes the iron, dioxygen, and BH_4 dependent oxidation of phenylalanine (Phe) to tyrosine, is the most common inborn error of metabolism; the most severe cases result in phenylketonuria (PKU) [1]. Despite the success of dietary therapy in preventing severe PKU-associated mental deficits, patients continue to struggle with lifelong control of blood Phe levels. Poor control can compromise behavior, learning, and executive function [2,3]. Thus, alternative therapies are sought, including small molecule therapeutics [1,4]. Pharmacological chaperones that stabilize PAH structure are gaining support as a long term therapeutic approach [5–12]. The cofactor BH_4, which is an active site directed pharmacological chaperone, is in clinical use with some success [13]. Although biological and chemical chaperones are generally viewed as assisting in all aspects of protein folding, a focus on quaternary structure dynamics provides a different perspective that is applicable to PAH. Pharmacological chaperones can function allosterically (e.g. not at the enzyme active site) to assist a protein in achieving and/or maintaining its most active structure. PAH is known to be an allosteric enzyme where allostery involves major conformational changes and an equilibrium of multimers including dimers and tetramers. Although the structural basis for allosteric activation by Phe is poorly understood, PAH was recently identified as a putative morpheein, and the current work builds on this hypothesis [14,15]. Unlike previous views of PAH multimerization equilibria, the morpheein model contains two different PAH tetramers separated by a large kinetic barrier that requires multimer dissociation, conformational change in the dissociated state, and reassociation to an alternate tetramer. Herein we (1) detail the rationale behind developing a morpheein model for PAH allostery, (2) present protein structure models for components of a PAH quaternary structure equilibrium, and (3) provide experimental evidence on purified full length rat and human PAH consistent with the proposed equilibrium. The current work sets the stage for our long-term goal, which is to develop allosteric pharmacological chaperones to expand the PKU patient population responsive to small molecule therapy.

Abbreviations: DAH7PS, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; IEC, ion exchange chromatography; MBP, maltose binding protein; PS, phenyl sepharose; Phe, phenylalanine; PAH, phenylalanine hydroxylase; PKU, phenylketonuria; PAGE, polyacrylamide gel electrophoresis; PBGS, porphobilinogen synthase; PDB, protein data bank; SEC, size exclusion chromatography; SDS, sodium dodecyl sulfate; BH_4, tetrahydrobiopterin.

* Corresponding author.
E-mail address: Eileen.Jaffe@fccc.edu (E.K. Jaffe).

0003-9861/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.abb.2012.12.017

A quaternary structure equilibrium model for the dysfunction of PAH leading to PKU

A contemporary explanation for PAH dysfunction causing PKU identifies the disorder as one of PAH protein stability and/or folding [10,12,16–18]. For multimeric proteins, where the final step in the folding process is multimer assembly, perturbation of an equilibrium of functionally distinct alternative multimers provides both a mechanism for allostery as well as a mechanism for protein dysfunction [19,20]. Extensive literature describes mammalian PAH as an allosteric enzyme [21,22]. At low Phe levels the protein has basal activity which allows retention of sufficient Phe to support protein biosynthesis. At high Phe levels protein activation is accompanied by a large conformational change which dramatically changes various characteristics of the protein, including the fluorescence spectrum and the susceptibility to proteolytic digestion [23–25]. PAH allostery is associated with a quaternary structure equilibrium consisting of dimers and tetramers with some propensity to form larger aggregates [21,23,26–29]. Phe serves both as substrate and as allosteric activator; allosteric activation by Phe has been shown to draw an equilibrium of tetramers and dimers toward the tetramers [28,30]. A structural basis for allosteric Phe binding causing stabilization of a specific, fully active conformation of the tetramer is proposed herein.

The new model for allosteric regulation of PAH, its relationship to PKU, and the promise of developing pharmacological chaperones that function allosterically follows our recently reviewed work with the prototype morpheein porphobilinogen synthase (PBGS) whose dysfunction causes the inborn error of metabolism known as ALAD porphyria [31,32]. Four important corollaries exist between the behavior of PBGS and the model proposed for PAH.

(1) On the level of protein structure dynamics, the nature of the conformational change that dictates subunit assembly into either a high activity multimer or a low activity multimer is a rotation or hinge motion between two domains of each subunit. For human PAH, which is a three-domain protein detailed in Fig. 1a, the proposed rotation is around the hinge at position 117, which lies between the regulatory and catalytic domains. The rationale for this proposal is based on an analysis of ACT domains in the Protein Data Bank (PDB) [33–36], described in more detail below. In support of the proposed PAH conformational change, hydrogen/deuterium exchange studies indicate that allosteric activation by Phe involves a dramatic reorientation of the regulatory and catalytic domains [37].

(2) The structural commonality that imparts low activity on an assembly derives from protein–protein interactions that modulate molecular motions governing active site access. Fig. 1b includes one orientation of the PAH domains, as seen in a rat PAH crystal structure (PDB id 1PHZ), where the N-terminal portion of the regulatory domain partially blocks active site access and is thus predicted to be the low activity conformation [38]. Reorientation of the entire regulatory domain would disallow this inhibitory interaction, placing the N-terminal portion out of reach of the enzyme active site.

(3) The link between protein structure dynamics and disease is the effect of disease-associated mutations on the protein quaternary structure equilibrium. In the case of ALAD porphyria, which is a very rare disease, all eight disease-associated point mutations, which are dispersed throughout the protein structure, cause the quaternary structure equilibrium to be shifted toward the low activity assembly relative to the wild type protein [32]. With regard to PAH and PKU, there are hundreds of disease-associated point mutations throughout the protein structure [39], most of which are not at the enzyme active site, and some of which have already been demonstrated to affect an equilibrium between alternate multimers, though a model consisting of alternate tetramers has not previously been considered [16,17,23,24,27,40–42]. We posit that disease-associated PAH mutations that shift a quaternary structure equilibrium will respond to pharmacological chaperones designed to act allosterically to stabilize the high activity multimer(s).

(4) A strong precedent for the feasibility of discovering effective allosteric pharmacological chaperones is set by PBGS, where both *in silico* and *in vitro* screening methods have successfully identified small molecules that can perturb an equilibrium between low activity hexamers and high activity octamers and modulate enzyme activity [43–48]. The search for these allosteric regulators was based on oligomer-specific small-molecule binding sites. For PAH, the proposed structure for the high activity multimer has a protein–protein interface not present in the low activity multimer; portions of this interface are proposed as the oligomer-specific binding site for allosteric Phe. Other portions of this interface can be targeted for the development of pharmacologic chaperones.

The goals of this study are to prepare and support a model for the quaternary structure equilibrium that governs PAH activity and to develop methods that can evaluate the position of the PAH quaternary structure equilibrium in a variety of physiologically relevant environments. The developed methods will allow future investigation of the effect of PKU-associated variants on the position of the PAH quaternary structure equilibrium. The developed protein structure models will assist in pursuit of allosteric pharmacologic chaperones that promote PAH to achieve and maintain its active multimeric assembly.

Materials and methods

Building the models

Both human PAH tetramer models are based on the human PAH structure 2PAH, which is a tetramer of a truncated form of PAH that contains only the catalytic and multimerization domains. A human regulatory domain homology model (residues 19–117) was prepared from the rat PAH crystal structure 1PHZ, which is of a truncated rat PAH containing only the regulatory and catalytic domains. Modeling was performed using our program MolIDE, which includes side chain rotamer optimization [49,50]. The low activity human PAH tetramer model was prepared using the program CHIMERA [51,52] to align the catalytic domains of the rat structure 1PHZ with the human structure 2PAH and the human regulatory domain model. Loop modeling (amino acids 113–121) was used to attach a regulatory domain to each of the human PAH subunits to yield our structure of 4mer*, using the program YASARA [53]. Following loop modeling, loop optimization included energy minimization by steepest descent methods. A final optimization of side chain rotamers was done in the context of the full multimer using SCWRL4 [54]. Preparation of the high activity human 4mer model started with the preparation of a dimer of the human PAH regulatory domain homology model, prepared by aligning two regulatory domains with the ACT domain dimer seen in crystal structure 1PSD. Following energy minimization and size chain optimization, the human PAH regulatory domain dimers were manually positioned to optimize the connection to the catalytic domains in the region of amino acid 118 of two catalytic domains with amino acid 117 of two halves of the regulatory domain

Download English Version:

https://daneshyari.com/en/article/8290909

Download Persian Version:

https://daneshyari.com/article/8290909

Daneshyari.com