Crystallization of a Stable form of Human Phenylalanine Hydroxylase: Towards the 3D Structure Determination

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1. Human Phenylalanine Hydroxylase (hPAH)

Background

Phenylalanine hydroxylase (PAH; EC 1.14.16.1) constitutes together with tyrosine hydroxylase (TYH; E.C.1.14.16.2) and tryptophan hydroxylase (TPH; E.C. 1.14.16.4) a family of aromatic amino acid hydroxylases (AAOHs), structurally and functionally related, which catalyse the hydroxylation of an aromatic amino acid in the presence of the pterin cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4) (Fig.1).

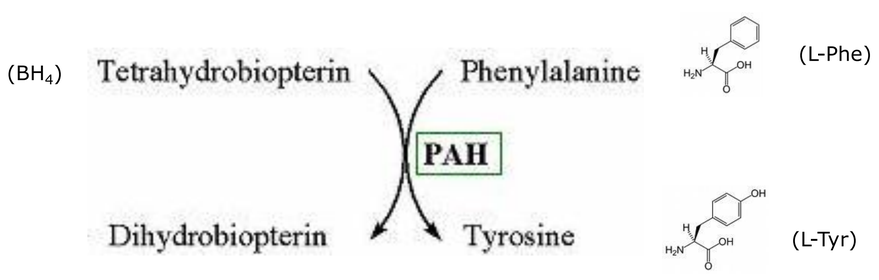
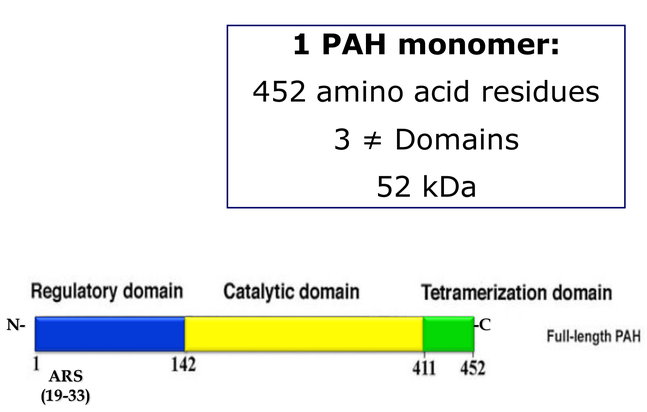


Fig.1 General representation of the Phenylalanine Hydroxylase catalytic reaction

PAH is a cytosolic enzyme, expressed mainly in the liver and in the kidney that supplies the systemic circulation with additional L-Tyr for protein synthesis, and prevents the accumulation of L-Phe in body fluids above normal levels, specially after dietary intake. The enzyme is distributed in a dimer – tetramer equilibrium. Each subunit is constituted by 452 residues and organized in three different domains: a N-terminal domain (12 -19 kDa) with regulatory properties; a catalytic domain (38 kDa); and a small C-terminal domain (5 kDa) involved in protein tetramerization (Fig.2).

1 PAH monomer:
452 amino acid residues
3 ≠ Domains
52 kDa



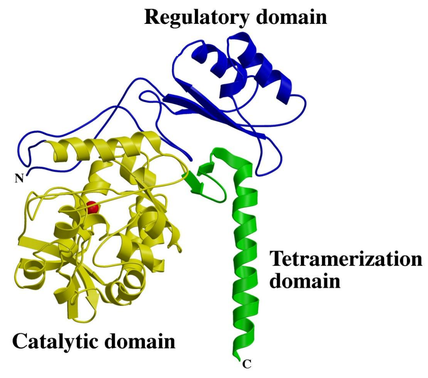


Fig.2 Representation of Phenylalanine hydroxylase domains per monomer.
(Adapted from Erlandsen & Stevens, 1999)

2. PAH and Phenylketonuria Disease

New Treatment approach

Phenylalanine Hydroxylase assumes particular importance in Human health, since a reduced or complete loss of its catalytic activity causes phenylketonuria (PKU; OMIM 261600). This is the most frequent disorder of amino acid metabolism with an average incidence in Caucasians of ≈1:10000 [1]. When untreated, this conformational disease is characterized by a severe psychomotor delay which can be prevented with a strict dietetic treatment for life. However, dietary compliance is very difficult and the results are severe neurophysiological dysfunction with a severe social-economical impact.

New Treatment Approach

⇩

Enzymatic Reposition Teraphy ⇨ Production of hPAH Chimerical forms with higher activity / stability

⇩

TARGETS:

C29S = Balk Cys Oxidation

E360K =Protein external charge modification

3. hPAH Chimeric Forms

C29S and E360K

The two chimeric hPAH proteins produced (C29S and E360K), showed not only enhanced enzymatic activity but also higher protein stability (unpublished results), when comparing with the wild-type form. Also the quantity of expressed protein (mg/L) proved to be higher in the chimeric forms (Fig.3).

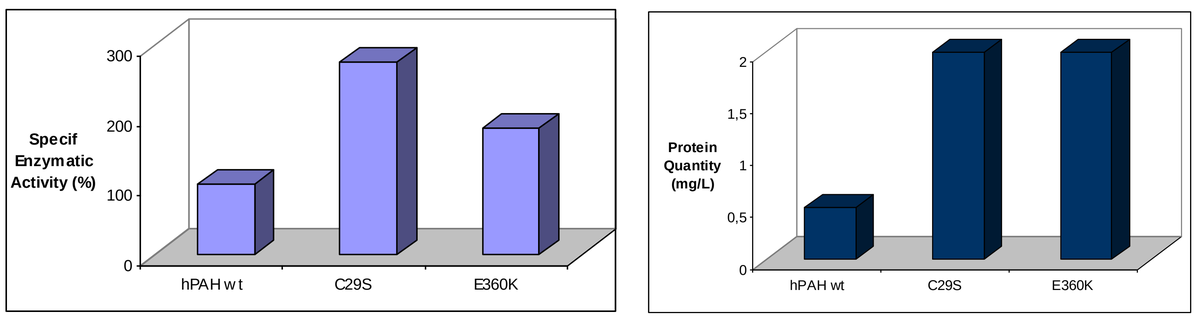


Fig. 3 Comparasion of percentual specific enzymatic activity (left) and amount of protein expression (right) , for wild-type PAH and C29S, E360K mutants

4. C29S 3D Structure Determination

Preliminary Results

To date, there is no crystal structure of the full length hPAH protein, and the only structures reported in the PDB database correspond to truncated forms of the enzyme [2]. The determination of the 3D structure of the full length enzyme would provide new and valuable information namely in the protein active-site, the identification of amino acid residues involved in enzyme catalysis and regulation, the binding of substrate and inhibitors, as well as on the molecular basis of PAH disease-causing mutations.

Based on the enhanced properties of the mutant forms C29s and E360K, when compared to the native hPAH, they can be considered as good candidates for trying to elucidate the full protein crystal sructure. We succeeded in obtaining protein crystals (four different crystal forms) of the C29S mutant, grown at 20ºC and using polyethylene glycol 3350 and 8000, and sodium tartrate as precipitant (Fig.4 and Fig.5).

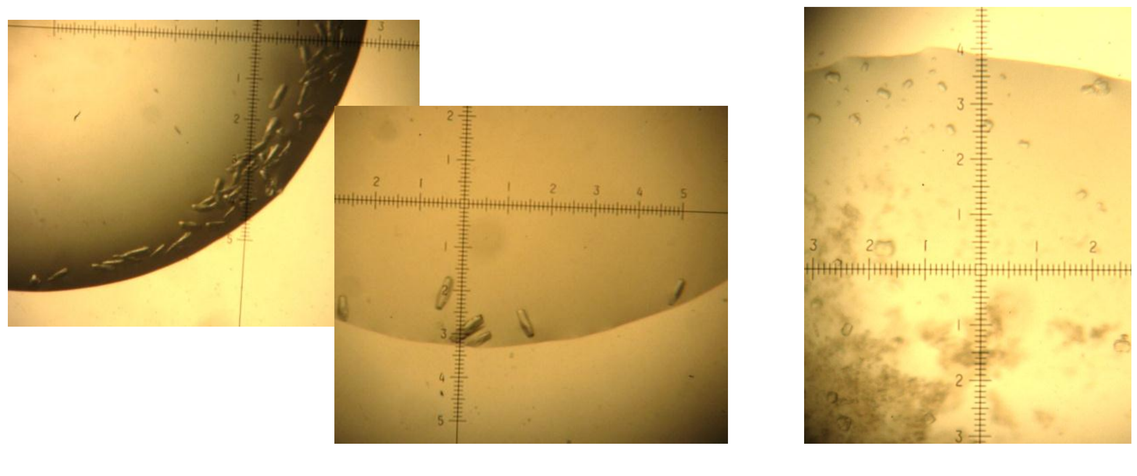


Fig.4 Crystals of PAH C29S obtained using sodium tartrate as precipitant, in two different crystallization conditions. Approximate crystal dimensions 0.2 mm x 0.2 mm.

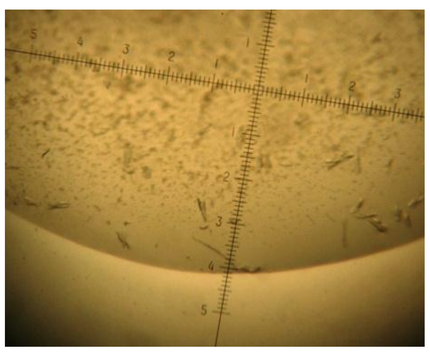


Fig.5 Crystals of PAH C29S obtained using polyethylene glycol as precipitant. Elongated crystals that need to be improved (dimensions 0.1 mm x 0.05 mm)

These crystals were tested for x-ray diffraction, and they produced diffraction data but to only limited resolution. The crystallization conditions are still being improved and optimized. These results are very promising, and will contribute to the understanding of the structure and molecular dynamics of phenylalanine hydroxylase enzyme, and to the development of new therapeutic approaches to Phenylketonuria disease.

References: [1] Lichter-Konecki U., Hipke CM, Konecki DS. Mol Genetics and Metabolism 1999, 67:308-316.
[2] Erlandsen H, Martinez A, Knappskog PM, Haavik J, Hough E, Flatmark T. Febs Lett, 1997, 406:171-4.

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