

The Role of Phenylalanine Hydroxylase in the Metabolism of Phenylalanine:

A Review

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ABSTRACT

Phenylalanine hydroxylase (PAH) is essential for metabolism of phenylalanine in the body. It has an α/β structure organized in the form of $\beta\alpha\beta\beta\alpha\beta$ fold and consists of two domains, regulatory domain and catalytic domain. In the case of increased blood phenylalanine concentration, PAH convert it into tyrosine. Sometimes mutations occurs in the phenylalanine hydroxylase gene and PAH level decreases in the body. Different diagnostic tests including mutation scanning, duplication analysis and some other are used to detect the phenylalanine hydroxylase deficiency in the body. PAH deficiency can be treated by a number of ways including dietary inhibition, phenylalanine ammonia lyase injection and intake of glycomacropeptide. Phenylalanine restricted diet is used to control blood phenylalanine concentration. Large neutral amino acids can also be used to control phenylalanine concentration in blood and brain. In the case of pregnant women, blood phenylalanine concentration is very crucial.

Keywords - phenylalanine, phenylalanine hydroxylase, phenylketonuria

I. INTRODUCTION

Phenylalanine hydroxylase is an aromatic amino acid containing enzyme that catalyzes the conversion of phenylalanine (an aromatic amino acid) to tyrosine (Zhang et al., 2015). The enzyme is produced by liver. Defects in PAH enzyme lead to the increase plasma level of phenylalanine and its related neurotoxic defects. This enzymatic defect can lead to the different diseases like hyper phenylalaninemia and phenylketonuria. In severe conditions it can lead to mental retardation. It only happens when a lot of neurotoxic metabolites of phenylalanine are accumulated in body, therefore, their accumulation should be prevented. Different diagnostic tests are used to find the causes of PAH defects and then

these defects are treated with different methods (Carluccio et al., 2013).

II. STRUCTURE OF PHENYLALANINE HYDROXYLASE

Phenylalanine hydroxylase occurs in tetrameric form. It consists of two dimers. Each monomeric unit is composed of 452 amino acids having a molecular weight of 52 kDa. The enzyme exists as α/β structure. It consists of domains. An N-terminal regulatory domain (1-117 amino acid), a catalytic domain (118-410 amino acids) where substrate, iron, cofactor bind and a tetramerization domain (411-452 amino acids). N-terminal contains ACT domain (33-111 amino acids) which is composed of 4 β and 2 α sheets in the form of $\beta\alpha\beta\beta\alpha\beta$ fold. It has been observed that regulatory domain of human PAH enzyme has 84% sequence similarity with that of rat PAH enzyme (Carluccio et al., 2013).

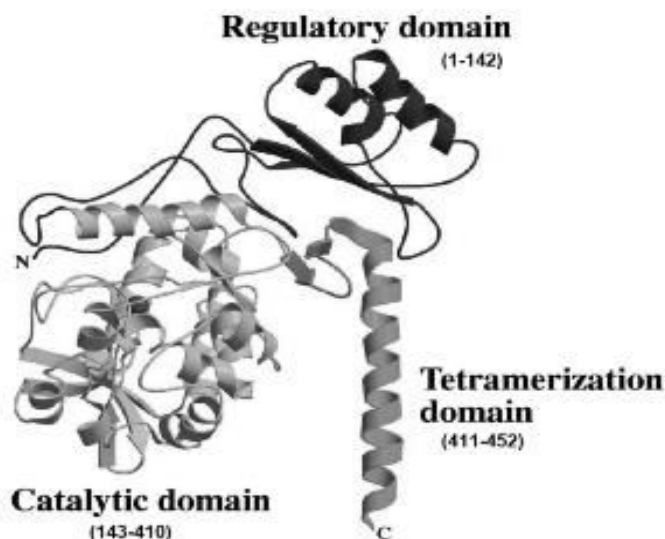


Fig 1: Structure of Phenylalanine Hydroxylase

III. METABOLIC PATHWAY OF PHENYLALANINE BY PHENYLALANINE HYDROXYLASE

Phenylalanine hydroxylase requires a phenylalanine (substrate), tetrahydropterin (BH_4) as cofactor, iron and molecular oxygen for its proper functioning. When BH_4 binds to the enzyme in the absence of phenylalanine then it forms a phenylalanine hydroxylase- BH_4 (PAH- BH_4) complex which is inactive. But in the presence of phenylalanine, BH_4 binds to the phenylalanine hydroxylase and enhance the catalytic activity of enzyme (Carluccio *et al.*, 2013). Conversion of phenylalanine to tyrosine takes place in the liver and it is an irreversible process (Shiman *et al.*, 1990). Phenylalanine hydroxylase is also controlled by cyclic AMP dependent protein kinase and calmodulin dependent phosphorylation and dephosphorylation. They also regulate the interaction of BH_4 with phenylalanine hydroxylase (Williams *et al.*, 2008).

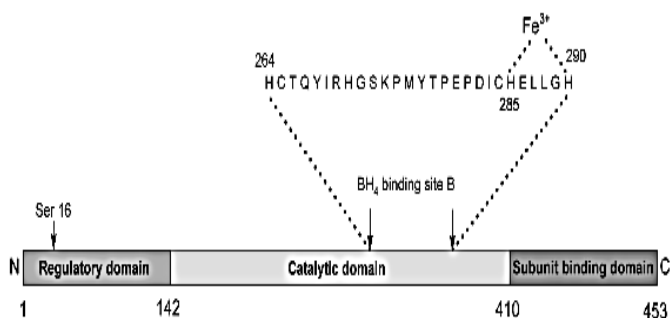


Fig 2: Binding Site of Tetrahydropterin

Phenylalanine hydroxylase activation by phenylalanine involves a feed-forward effect because phenylalanine itself is responsible for PAH enzyme activation and is degraded by activated PAH enzyme (Shiman *et al.*, 1990). Basically phenylalanine hydroxylase causes the hydroxylation of phenylalanine which result in the formation of tyrosine (Wettstein *et al.*, 2015).

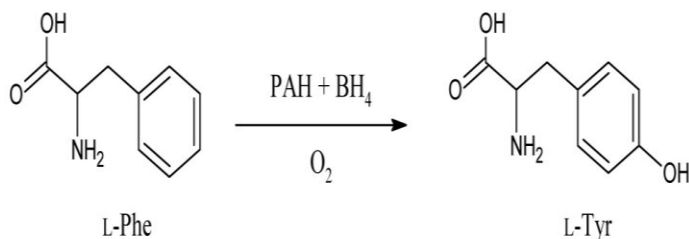


Fig 3: Conversion of Phenylalanine to Tyrosine

The sources of phenylalanine in the body include dietary protein and recycling of endogenous amino acids. The amount of the phenylalanine which is desired by the body is used and the rest of the phenylalanine is converted into tyrosine which is catalyzed by phenylalanine hydroxylase. Other metabolic products are also produced due to the metabolism of phenylalanine as is shown in the figure below (Zhang *et al.*, 2015).

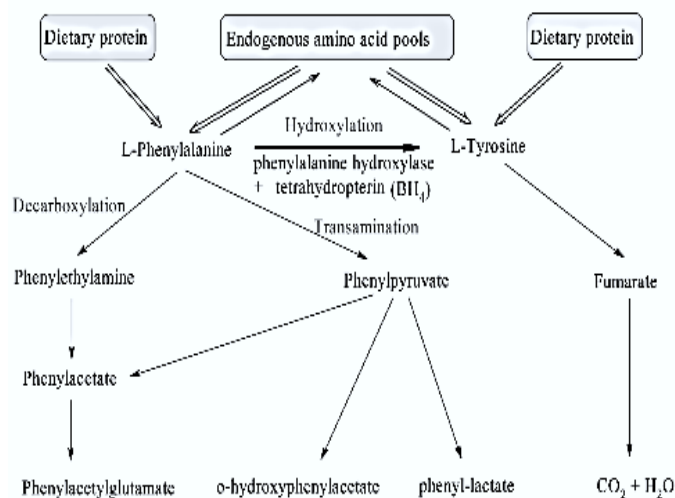


Fig 4: Metabolism of Phenylalanine in the Body

IV. GENETICS OF PHENYLALANINE HYDROXYLASE

Phenylalanine hydroxylase coding gene is located on chromosome number 12. Its size is 171 kb. It consists of 13 exons. Its complementary DNA is of 2.4 kb and codes for a monomer of 452 amino acids (Williams *et al.*, 2008).

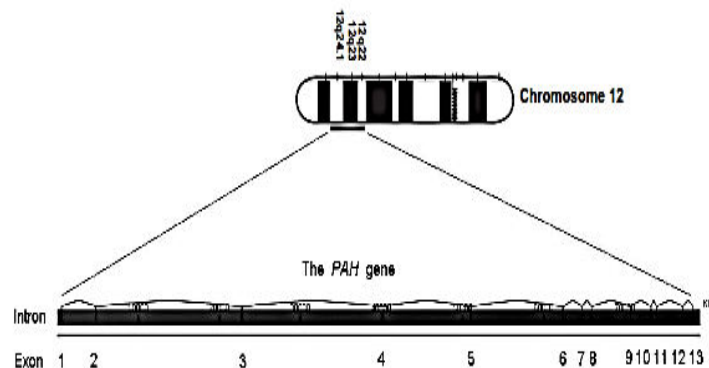


Fig 5: The Location of Phenylalanine Hydroxylase Coding Gene on Chromosome 12

Mutations occur in the PAH gene coding for phenylalanine hydroxylase. Sometimes these mutations have a slight impact on enzyme but in some cases these mutations can lead to the major changes and can cause diseases. About 500 different mutations of PAH gene have been identified which can cause disease. These mutations can be missense, deletions, splice, silent, nonsense and insertions and they appear in the different percentages (Mitchell *et al.*, 2011). Mostly the mutations results in improper folding of PAH enzyme protein, loss in the activity of enzyme and change in turn over number of enzymes (Vockley *et al.*, 2014).

Table 1: Different Mutations of PAH Gene

Percent of Mutation	Genetic Mechanism
62	Missense
13	Deletion
11	Splice
6	Silent
5	Nonsense
2	Insertion
<1	Deletion or duplication of exon or whole gene

Different PAH genes determine different phenotypes. The phenotype is not only determined by PAH genes but it is also determined by environment. According to the scientists, genotype of PAH gene only determines biochemical phenotype but clinical phenotype is determined by the environment. It has been observed that some individuals has same genotypes but different biochemical and clinical phenotypes. This is due to different kinetics of Phenylalanine absorption and uptake across blood-brain barrier. In some cases the individuals having same PAH genotype show different plasma concentrations of phenylalanine.

V. DISEASES CAUSED BY PHENYLALANINE HYDROXYLASE GENE MUTATION

Mutation in the PAH gene can lead to the intolerance of dietary phenylalanine due to which phenylalanine concentration increases in blood and it leads to two kinds of diseases Hyperphenylalaninemia (HPA) and phenylketonuria(PKU). In **Hyperphenylalaninemia (HPA)**, plasma concentration of phenylalanine is more than the normal concentration i.e. greater than 120 $\mu\text{mol/L}$. This condition is not dangerous. Therefore if the patient is left untreated then there is no problem of impaired cognitive development in patient. In **phenylketonuria (PKU)** condition can be fatal because in this case the phenylalanine concentration in blood is more than 1000 $\mu\text{mol/L}$ which is the abnormal concentration. In this case patient suffer from impaired cognitive development, if they are left untreated.

VI. CLINICAL DESCRIPTION OF PHENYLKETONURIA (PKU)

If phenylketonuria (PKU) is left untreated then it can lead to different mental illnesses including epilepsy, intellectual disability, microcephaly and some problems related to behavior. In the secretions of the patients musty odor appears. Untreated phenylketonuria affect the pigments of skin and hairs. Cognitive development and cerebral metabolism is also disturbed due to the elevated phenylalanine concentration in blood because it interferes with transport of neutral amino acids on neurotransmitter from blood to brain and synthesis of protein. It also causes depletion of dopamine, norepinephrine, serotonin and pathology of white matter. Other problems like tendon reflexes, tremor and paraplegia occurs later in life. If the problem left untreated for a long interval of time, then it can lead to psychological problems including depression, phobias and anxiety. In some cases the persons with elevated phenylalanine concentration show normal intelligence and this elevated phenylalanine level is indicated only when some psychological problems appears.

As the disease of phenylketonuria is concerned, it is caused due to the increase in phenylalanine concentration in the blood. If the diet of the patient is maintained in such a way that it contains a less amount of phenylalanine amino acid then phenylalanine concentration in the blood can be maintained. This treatment can reduce the disease conditions to some

extent. Despite this treatment, diseased individuals show less ability of planning, problem solving and cognitive development.

VII. DIAGNOSIS OF PHENYLKETONURIA

Phenylketonuria is diagnosed at molecular level in order to find out the change which is present in the gene. If a person has a mutant phenylalanine hydroxylase gene then there is an elevated concentration of phenylalanine in plasma. The reason is that the mutant PAH gene will not be able to code for phenylalanine hydroxylase enzyme which is responsible for the conversion of excess amount of phenylalanine into tyrosine. PCR techniques are used for molecular analysis of mutations in phenylalanine hydroxylase gene (Mitchell *et al.*, 2011).

VIII. NEWBORN SCREENING

The normal level of phenylalanine in the humans ranges from 360 $\mu\text{mol/l}$ to 600 $\mu\text{mol/l}$. If Phenylalanine level exceeds from this level then it requires testing and treatment. The new born children are screened for the PAH gene. Guthrie card blood spot test is done for the screening of PAH gene. The blood spot is taken from the heel of the new born. In America this PAH screening has been included in the routine test of neonates because this early diagnosis help to prevent the long term consequences. The new born children having mutant PAH gene show small size of head and birth weight is lower than normal weight of a neonate. Tandem mass spectrometry is used to detect the mutation in PAH gene of new born. The blood spot taken from the new born is tested for detecting the amount of phenylalanine. The amount of tyrosine is also detected in order to determine phenylalanine to tyrosine ratio in the blood. The amount of the two amino acids is determined using plasma amino acid analysis. But this method does not tells clearly whether the elevated plasma phenylalanine concentration is due to genetic defect or due to some other reason, therefore, there remains a need for further testing which includes testing about BH_4 and some other aspects of enzyme. This method has been found to be cost effective.

In new born child it is necessary to test the BH_4 level in blood in order to confirm that the increased level of phenylalanine in blood is due to the cofactor deficiency or due to some other reason. The neonate blood sample is taken on the filter paper and is checked

for cofactor deficiency. The results are then compared with given control. If the abnormal results are found, they lead to the further enzymatic analysis for the enzymes involved in the cofactor generation or degeneration pathway including GTP cyclohydrolase, dihydropteridine reductase, 1,6-pyruvoyl tetrahydrobiopterin synthetase and pterin carbinolamine.

IX. TREATMENT OF PHENYLKETONURIA

Phenylketonuria can be treated by using a number of strategies.

A) CONTROLLED INTAKE OF PHENYLALANINE THROUGH DIET

Basically tolerance level for phenylalanine vary from individual to individual. Normally phenylalanine concentration ranges from 120-360 $\mu\text{mol/L}$ or 40-240 $\mu\text{mol/L}$ is regarded as safe. When phenylalanine concentration increases from this normal range then it becomes necessary to control the dietary intake of phenylalanine. Depending upon the tolerance level of a particular person, dietary intake of phenylalanine is controlled. It should not be decreased to such an extent that causes deficiency of phenylalanine. A moderate level of phenylalanine should be maintained in body for the sake of appropriate growth.

The patients of phenylketonuria are prescribed to take phenylalanine restricted diet but it can lead to certain problems in the body. When phenylalanine restricted diet is given to the patient for a long time, then it can lead to its deficiency, therefore, important functions of body which are performed in the presence of phenylalanine are effected. It can lead to bone diseases and deficiency of long chain polyunsaturated fatty acids (LCPUFA) which is required for mental growth. So the diet should be controlled accordingly. Aspartame is an artificial sweetener which contains phenylalanine, therefore, the diets which contains aspartame should be avoided by the patients of phenylketonuria.

Special foods are prepared which contains an adequate amount of all amino acids but phenylalanine is maintained at low level so that the patients with phenylalanine hydroxylase deficiency can obtain a protein diet. One such food is Glycomacropeptide. Cheese whey is the main source of **Glycomacropeptide**. It is a protein which contains different essential amino acids in adequate amounts but phenylalanine is present

in relatively low amount. If the patients are given Glycomacropeptide then it can maintain an adequate amount of proteins along with phenylalanine.

B) TRANSPORTERS OF LARGE NEUTRAL AMINO ACIDS (LNAA)

Phenylalanine shared LNAA transporters in order to control its concentration in brain. Basically these transporters maintain a level of phenylalanine at blood-brain barrier. They help to maintain high phenylalanine concentration in blood as compared to brain because high phenylalanine concentration in brain can prove to be toxic. LNAA can also be used for the treatment of high phenylalanine concentration but its use is limited to the older patients. Its use is restricted in pregnant women because the effect of LNAA has not been completely understood in pregnant women and the foetus yet (Vockley *et al.*, 2014).

C) PHENYLALANINE AMMONIA-LYASE (PAL) SUBSTITUTION

One method to decrease the blood phenylalanine concentration is to introduce the PAL enzyme in body of patient. This enzyme converts phenylalanine into trans-cinnamic acid and ammonia. In this way it helps to reduce blood phenylalanine concentration. When this enzyme is introduced in body through oral route, it is subjected to proteolytic digestion. If the enzyme is injected in the body then immunity response is produced against that enzyme. In order to prevent this immunogenicity, the enzyme is PEGylated (conjugation of enzyme with polyethylene glycol). In this way the enzyme efficiency is increased.

X. PHENYLALANINE HYDROXYLASE DEFICIENCY IN PREGNANT WOMEN

If a women suffer from PAH deficiency then there will be high phenylalanine concentration in her body. If such women becomes pregnant then this high phenylalanine concentration will prove to be toxic for the foetus. The maternal blood having high amount of phenylalanine can cause different diseases in fetus including microencephaly, impaired cognitive development and heart diseases. Therefore the pregnant women should have a regular checkup for her plasma phenylalanine concentration. The recommended range of blood

phenylalanine concentration is 120-360 $\mu\text{mol/L}$. If a women has PAH deficiency then the phenylalanine concentration will increase from this normal range and foetus is affected. In order to control blood phenylalanine level, phenylalanine restricted diet is recommended to the pregnant women.

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