Case 30
Phenylketonuria

Focus concept
The characteristics of phenylalanine hydroxylase, the enzyme missing in persons afflicted with the genetic disorder phenylketonuria (PKU), are examined.

Prerequisites
- Amino acid synthesis and degradation pathways.
- Integration of amino acid metabolic pathways with carbohydrate metabolic pathways.

Background
Phenylketonuria is an inherited disease which results from the lack of the enzyme phenylalanine hydroxylase (PAH). The PAH enzyme catalyzes the first step in the degradation of phenylalanine, as shown in Figure 30.1. In the phenylketonuric patient, phenylalanine accumulates which is eventually transaminated to phenylpyruvate, a phenylketone compound. Excess phenylpyruvate accumulates in the blood and urine and has the effect of causing mental retardation if untreated. Screening programs identify PKU babies at birth, and treatment consists of a low phenylalanine diet until maturation of the brain is completed. The structure and biochemical properties of the PAH enzyme have been well-studied.

The gene for PAH has been isolated and has been localized to chromosome 12. The PAH enzyme is a protein 451 amino acids in length with a molecular weight of 51,900 daltons. More than 60 different mutant genes giving rise to nonfunctional PAH proteins have been identified in PKU patients.

Questions
1. Is phenylalanine glucogenic, ketogenic, or both? Explain.

2. In order to learn more about the PAH enzyme, it was necessary to purify it. PAH has been isolated from both rats and humans. In the rat, three isozymes of PAH have been identified in the liver. Their molecular weights are identical, but their charges are different, as demonstrated by isoelectric focusing. The pI values are 5.2, 5.3 and 5.6. DEAE-cellulose (anion exchange) chromatography was one of the steps in the purification procedure of the enzymes. Predict the order of elution of these isozymes from the DEAE-cellulose column. What pH buffer would you choose in running the column?

3. Once the enzyme was purified, the investigators set out to determine its properties. They wanted to see if phenylalanine, in addition to serving as a substrate for the enzyme, had an additional role in the regulation of the enzyme. Polyacrylamide gels (under denaturing and non-denaturing conditions) were carried out with the rat liver PAH. The results are shown in Figure 30.2. How do you interpret these data?
**Figure 30.1.** Phenylalanine and tyrosine metabolism. BH₄ is tetrahydrobiopterin, an essential cofactor for phenylalanine hydroxylase.
Figure 30.2: PAGE, in the presence and absence of the denaturing agent SDS, and in the presence and absence of phenylalanine.

Figure 30.3: Reaction kinetics of PAH with and without preincubation with phenylalanine.

Figure 30.4: Percent activity (as compared to control) of PAH in the presence and absence of insulin, glucagon, and/or preincubation with phenylalanine.
4. Next, kinetic studies were carried out with the enzymes. A plot of velocity vs. phenylalanine concentration yields a sigmoidally shaped curve. What does this tell you about the enzyme?

5. Kinetic data in which PAH activity is compared with and without preincubation with phenylalanine is shown in Figure 30.3. Give a structural basis for the interpretation of these data.

6. The effect of the hormones glucagon and insulin on PAH activity were investigated. The results are shown in Figure 30.4. In addition, the amount of radioactively-labeled phosphate incorporated into PAH with glucagon treatment was found to be nearly seven-fold greater than in controls.
   a. How would you interpret these data?
   b. Draw a diagram demonstrating the mechanism for hormonal activation of PAH.
   c. Which hormone activates PAH, and why?

7. Tyrosine is not an essential amino acid in normal persons, but it is essential in persons with PKU. Explain why.

8. Patients with the disease PKU tend to have blue eyes, fair hair, and very light skin. Explain why.

9. More than 60 different mutant PAH genes have been identified in PKU patients. State, in general, what effect the following changes in the DNA would have on the resulting protein. Be specific.
   a. Nonsense mutations
   b. Splicing mutations
   c. Single base changes

10. The cause for the mental retardation associated with untreated PKU is not completely understood, but it is believed to arise from the high concentrations of phenylpyruvate, which is a product of a transamination reaction with phenylalanine and α-ketoglutarate. The phenylpyruvate is believed to be toxic to the developing brain. Write the balanced equation for the transamination of phenylalanine to phenylpyruvate, and include the structures of the reactants and products. Identify any cofactors needed to accomplish the reaction.

11. The mental retardation associated with phenylalanine can be avoided if the neonate is immediately placed on a low phenylalanine diet for the early years, and perhaps for life.
   a. Why is a PKU patient placed on a low phenylalanine diet instead of a phenylalanine free diet?
   b. The artificial sweetener Nutrasweet® contains the compound aspartame, which consists of a methylated Asp-Phe dipeptide. (The C-terminal carboxyl group is methylated.) Draw the structure of aspartame. If you were a physician, what advice would you give to a PKU patient regarding this product? If you were a manufacturer of aspartame, what would your responsibility to your customers be?
References

