Project Manual
Bio3055

Metabolic Disease:
Phenylalanine Hydroxylase

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Metabolic Disease: Phenylalanine Hydroxylase

Introduction:

Metabolic disease is a broad term that is generally used to describe diseases that result from enzyme deficiencies in either catabolic or biosynthetic pathways. When these enzyme deficiencies occur, there is not only a decrease in the products of the pathway, but the intermediates in the pathway also build up. Symptoms of metabolic disease occur from one or both of these biochemical causes. Often, the build-up and storage of the metabolic intermediates cause the major symptoms associated with these diseases.

Phenylketonuria (PKU) is a metabolic disease that results from a deficiency in phenylalanine hydroxylase (PAH). This metabolic disease was one of the first to be well-characterized, and today is tested for in all newborns. In this module you will use web-based biotechnology tools to research how a genetic mutation in phenylalanine hydroxylase leads to the symptoms of PKU.

In this module, you will be analyzing a cDNA sequence for a female patient who may be a carrier for deficient phenylalanine hydroxylase. She does not have PKU, but is concerned for her future children based on her family history. Obtain the sequence for this patient’s cDNA from the course website under the file name shown below.

Saved in FASTA format in file “PAHmutseq”

For your research project, you will analyze the mutant PAH protein using the bioinformatics tools presented in lab. You will investigate the structure of the PAH protein, model the mutation, and find out what is known, if anything, about the biological impact of the mutation. Through your studies, you will form a hypothesis about what the structural and biological effects are of this mutation, and organize the results of your research into a report. At the last lab session you will present your report to a small group.

Laboratory 1
No Pre-lab assignment
Tutorial on web-based tools

Laboratory 2
Pre-lab assignment:
Complete the questions for reading 1 (page 4).

The first set of readings is designed to give you an overview of metabolic disease and phenylketonuria (PKU). Go to the website shown below and read
this short article. Answer the questions for reading 1 in your project packet and turn in your written answers at the second lab meeting.

Reading 1
http://www.meadjohnson.com/metabolics/intrometabolicmanage.html

Laboratory 3
Pre-lab assignment:
Complete the Structure Problem Set (page 7 – 8).
Complete the questions for reading 2 (page 5 - 6). This reading provides you with some background in working with the crystal structure of phenylalanine hydroxylase. This article contains the crystal structure you will be studying. Obtain the excerpt from the article from the course website. Read this article as well as the pages from the Berg text. Answer the questions for reading 2 in your project packet and turn in your written answers at the third lab meeting.

Reading 2
Excerpt from:
Berg p. 654 - 7

Laboratory 4
No Pre-lab assignment
If you haven’t yet, you should begin preparation for your final report.

Laboratory 5
Pre-lab assignment:
For this lab, you need to assemble all your research into a report format so you are ready to present your results to the other group working on Metabolic Disease. The other group you will be meeting with has been researching the gene responsible for Lesch-Nyhan Syndrome. Follow the format given in your lab manual for writing the report. At the last lab meeting, you will have 20 minutes to present your findings to the other group. Then they will present their findings. The rest of the lab will be spent working as a group to provide answers to a joint quiz. You will then hand in your reports to be graded.
Questions on Reading 1
PAH

Reading from website:
http://www.meadjohnson.com/metabolics/intrometabolicmanage.html

1. Briefly describe what is meant by the term, “metabolic disease.”

2. How can the effects of metabolic disease be prevented?

3. What are two reasons that the severity of a metabolic disease can differ in different individuals?

4. What type of inheritance pattern is often seen in metabolic diseases?

5. What enzyme is deficient or absent in PKU?

6. What amino acid levels are affected in PKU? Indicate which amino acid level is increased and which amino acid level is decreased.

7. Is PKU tested for in newborns in Missouri?
Questions for Reading 2
PAH

Berg p. 654 - 7

1. Write the reaction catalyzed by phenylalanine hydroxylase below. Be sure to draw the chemical structures of phenylalanine and tyrosine.

2. Normally, what fraction of phenylalanine is converted into tyrosine?

3. Without doing genetic testing, what is an indication that a person is a carrier of the PKU gene?

Excerpt from *J. Mol. Biol.* paper:

4. Describe the crystal structure that was solved for this paper including the protein source and any molecules bound to the protein.
5. Compare and contrast the structure of the substrate analog with phenylalanine.

Substrate analog: 3-(2-thienyl)-L-alanine (THA)
Structure Problem Set

Directions – Draw the chemical structures for the following amino acids. They are represented in cpk color mode (see Glossary for more information).

1.

2.

3.
4. Draw the chemical representation of the following tripeptide.

![Chemical representation of a tripeptide](image1)

5. Draw the chemical representation and represent H-bonds as dotted lines between the atoms where distances have been measured. You will need to add hydrogens that don’t appear in the picture below.

![Chemical representation with H-bonds indicated](image2)

6. What distance must two atoms be in order to be involved in hydrogen bonds and ionic bonds (use the Berg textbook, p. 9 – 10 if needed)?
Guide Sheet 1 Hints and Tips for PAH

Translating the sequence

• Obtain your patient’s cDNA sequence from the course website (PAHmutseq)

• Use “Reading Frame 2” when translating the sequence at the Sequence Manipulation Suite.

NCBI – Gene

• Using Gene, find the entry for phenylalanine hydroxylase. Be sure to select the Homo sapiens entry from the search results. Answer question 1.

Swiss-Prot Entry

• Use the full name “phenylalanine hydroxylase” to search the SwissProt database and be sure to select the human protein from the search results. Answer questions 2 - 6.

BLAST and ClustalW

• Be sure to choose a good variety of sequences from the BLAST search. The more varied the sequences, the more interesting the alignment will be to study.

• Be sure the wild type human (RefSeq) and mutant sequences only differ by one amino acid residue. If more differences are found, there may have been a mistake in the translation of the mutant sequence.

• Answer questions 7 – 11.
Questions to accompany guide sheet 1
Phenylalanine Hydroxylase

**Locus Link Entry**

1. Fill in the following information from the Gene entry:
   a. Write the GeneID number here ________________.
   b. What is the gene name?
   c. Where on the human genome is this gene located?
   d. What is the RefSeq number for the mRNA sequence?
   e. What is the RefSeq number for the protein sequence?

**Swiss-Prot Entry**

2. Does this protein exist as a monomer, dimer, or trimer?

3. What metabolic pathway does this protein belong to?

4. What is the chemical reaction catalyzed by phenylalanine hydroxylase?

5. Which three amino acid residue numbers bind the iron atom (under “Features”)?

6. Which residue is modified by phosphorylation (under “Features”)?

**Multiple Sequence Alignment**
7. What is the mutation? Write it in the following format “Res123Res” where the first Res is the three-letter code for the amino acid in the un-mutated (wild type) protein and the second Res is the amino acid in the mutated protein. In place of “123” put the amino acid residue number of the mutation.

8. Is the mutation in a region of conservation?

9. What is the secondary structure predicted for the region containing the mutation?

10. Based on the alignment, what span of amino acids is LEAST conserved?

11. What type of secondary structure(s) is the most common in the alignment? If it seems to be an even mix between alpha helices and beta sheets, state that.
Guide Sheet 2 Hints and Tips for PAH

Searching for Structure Files: The crystal structure of phenylalanine hydroxylase bound to its cofactor and a substrate analog has been solved. This is the structure you read about in the reading assignment due for this lab. To obtain the crystal structure data file (pdb file), follow these steps:

1. Go to the Protein Data Bank website [www.rcsb.pdb.org](http://www.rcsb.pdb.org) (see Glossary) which contains all of the macromolecule 3-D structure files (pdb files). Pdb files are named in 4 characters (numbers and letters).
   a. Search for the 1KW0 pdb file. The summary information page for 1KW0 contains a title for the entry, the compound crystallized, and the species of the source of the protein. Use this entry to answer questions 1 - 3.
   b. Click on “Download/Display” file at the left of the screen.
   c. On this page, choose to download the structure file in PDB format with no compression. It will be the “none” option in the second table. The “1KW0.pdb” file should now be on your desktop.

2. Swiss-Pdb Viewer/DeepView has been loaded on your desktop. To open 1KW0.pdb in this program, drag the file to the Swiss-Pdb Viewer/DeepView icon and drop it on the icon. In some cases, double-clicking on the file will also open the pdb file in DeepView.

3. A black screen should appear with the protein shown in wire form. This is a difficult form to view the protein, so we are going to change it to the ribbon form mode. To do this, follow these steps:
   a. First make sure the control panel is open. If you don’t see it, select “control panel” under “Wind”
   b. Click on the control panel window. You can see that all the amino acid residues in the protein are listed in the first column by 3-letter code and residue number. The next columns allow you to change what is displayed. In order to clean up the display of the enzyme, follow these steps:
c. Erase all the check marks in the “show” column and the “side” (meaning side chain) column by clicking on them. For now, we are only going to view the protein backbone in a ribbon diagram.

d. Put check marks in the “ribbon” column for all the residues including the substrate analog, the iron, and the tetrahydrobiopterin.

e. Locate the substrate analog, the iron, and the tetrahydrobiopterin in the control panel and put checks in the “show” column to these molecules to the display.

f. Go to the main window and click on the “Display” menu and select “Render in Solid 3-D”. You should now be viewing a ribbon diagram of your protein.

h. You can change the ribbon colors to any color you think looks best by selecting “ribbon” under “Prefs”. In this window, make sure the “render as solid ribbon” option (near the top) is selected. You can select different colors for the top, side, and bottom of the ribbons. This allows you to choose a darker version of the same color for the bottom of the ribbon to enhance the 3-D viewing. Take a minute to play around with this option and to color your protein the way you want. You can also change the background to any color by choosing “Colors” under “Prefs”, then “background”.

i. Once you have a view that you like of your protein, save it by going to “File” then “Save”. Then select “Layer”. Name your file hprt1.pdb and save to desktop. When you open this file, all your colors and the orientation should be saved, but you will have to select “Render in Solid 3D” again under “Display” to see it. Answer questions 4 – 5.

**Printing the 3D Figure of Your Protein**

4. To save the pdb file as a photo file, we will use the program Grab. You can open Grab by clicking on the scissors icon in the toolbar of your desktop.

5. Make sure the figure is visible exactly the way you want it in Swiss-Pdb Viewer. Then, in Grab, go to “Capture” then “Selection”. You can now draw a box around the part of the view in Swiss-Pdb Viewer that you want to save. Save the file as “pah.tif”. Save it to your desktop. You can use
this same method to create a protein structure figure for a PowerPoint presentation.

6. Open the “.tiff” file in Preview. Choose Page Setup under “File” and change the scale to 70% to make sure the figure prints on one page. Print a copy of your “.tiff” file.

**Viewing an amino acid side chain**

7. Locate the Glu280 (the residue that is mutated in your patient’s protein) in the structure. Show the side chain by clicking on the “show” and “side” columns in the control panel for that amino acid. The side chain will appear in the CPK coloring mode which is based on atom type:

- red = oxygen
- blue = nitrogen
- orange = phosphorous
- yellow = sulfur and phosphorous
- gray = carbon
- light blue = hydrogen

At this point, you can erase the check marks in the “show” column for all the other residues in order to focus on the Glu280, the cofactor (BH4426), the substrate (TIH427), and the Fe^{2+} (Fe2425).

8. Zoom in on the Glu280. Rotate the structure until you can get a good view of the glutamate side chain.

9. Confirm the identity the Glu side chain by using the identity tool on the toolbar and clicking on the side chain in the display window.

**Investigating the environment of the side chain**

In order to investigate the non-covalent interactions of the side chain, use the distance tool on the toolbar to find atoms that are close enough to atoms in the side chain to be involved in H-bonds, ionic bonds, or Van der Waal’s interactions. Keep in mind the atom type when determining what type of interactions may be occurring.

10. Use the radius tool to identify all the residues within 4 angstroms of the glutamate side chain. To do this, select the radius tool. The command line will then instruct you to click on an atom of the glutamate side chain. When you do this, a window should open where you can enter in the
radius. Enter 4 angstroms. All the side chains within 4 angstroms of the glutamate should now appear.

11. Use the distance tool to measure the distances between the glutamate side chain and the closest residue. You may need to measure several distances until you find the one that is closest. Measure the distances between the atoms that appear closest to the glutamate side chain. You will click on the two atoms that you want to measure the distance between, then the distance, in angstroms, should appear. This step may take several attempts. If you need to erase distances or labels, you can go under “Display” to “labels”, then select “erase user labels.”

Keep in mind the resolution of the crystal structure provides the error in the distances that you are measuring. For example, if the distance is 5 angstroms and the resolution is 2 angstroms, the distance between the atoms is estimated to be 5 angstroms ± 2 angstroms.

12. Once you have identified possible non-covalent interactions involving the Glu side chain, print this view with the distances using the same method as in steps 4 - 6. Save this view as something “.tiff” You will need the distance measured here for question 6 of your guide sheet 2 questions.

Modelling the Mutation

13. To change the glutamate side chain to a different side chain, use the mutate tool on the toolbar. Select the amino acid lysine (Lys) to mimic your patient’s mutation. The Lys side chain will appear in the lowest energy conformation (most stable). Some strange green lines may appear which represent potential H-bonds. You can make these disappear under “Display”, then deselected “Show H-bonds”.

14. Use the “distance” tool to measure the distance between the Lys side chain and the closest residue(s) as you did in step 11 with glutamate. You will click on the two atoms that you want to measure the distance between, then the distance, in angstroms, should appear. Save and print this view following steps 4 - 6 and naming the file, “Lys.tif”. Answer questions 7-8.

To put in report:
For this lab, you will need the three figures printed in steps 6, 12, and 14. Make sure the residue numbers and distances are labelled. The distances and labels can be added by hand to the figure if they are difficult to see in the print-out.
Questions to Accompany Guide Sheet 2

PAH

1. What organism was PAH obtained from for the crystal structure?

2. Write the first author and journal name for the primary citation for this crystal structure.

3. What is the resolution for this crystal structure and what does “resolution” mean for a crystal structure?

4. Carefully examine the secondary structure in the crystal structure and record any positions where the PSIPRED predictions were incorrect.

5. PSIPRED states its predictions are ~80% correct. Do you agree this is a good estimate of the accuracy?
6. Draw the Glu280 side chain and the non-covalent interactions of Glu280 as determined from the crystal structure. Include the distances between the atoms you measured. This should include the correct chemical structure of the glutamate side chain, including atom types, correctly placement of hydrogens, charges, etc.

7. Draw the lysine side chain including the non-covalent interactions predicted by modelling this mutation. Draw the correct chemical structure for Lys including charges, hydrogens, etc. How are the non-covalent interactions the same of different from Glu280?

8. What is your hypothesis for the role of the glutamate side chain and how the protein is affected when this side chain is substituted with a lysine side chain?
Guide Sheet 3 Hints and Tips for PAH

OMIM search:

• Go to the NCBI homepage and search the OMIM database for “Phenylketonuria”. Select the search result that states this exact disease name.

• Read the text under “Allelic Variants” and find the entry for the mutation you are studying. Answer questions 1 - 3.

• Go to the NCBI home page. Select “PubMed” from the pull-down menu and search for PMID 12655545 (just enter the number in the search box).

• Click on the author line to view the abstract for this article. Read this abstract and answer question 4.

• Go to the website that is listed in the abstract. At this website, click on “Mutation Search” from the menu on the left. Then click on the “Human Data Listing” link under “In Vitro Expression”. Scroll until you find E280K (this entry will be more than halfway down the page). Answer questions 5 and 6.

KEGG pathway

• Go back to the Gene entry for human PAH. Scroll down to the “General gene information” section and select the KEGG pathway link for “Phenylalanine, tyrosine and tryptophan biosynthesis.” You should see a nice graphic for the enzyme complexes in the pathway. The diagram also contains boxes with the EC numbers for enzymes in each of the reactions. Each box is a link to a data base entry similar to GenBank. Find the EC number for PAH (1.14.16.1). Answer question 7.

• Click on “Tyrosine Metabolism.” This will take you to a new pathway figure. In this figure, locate “dopamine” and click on the circle next to dopamine. Answer questions 8 - 9.
Questions to Accompany Guide Sheet 3
PAH

1. What is mentioned about the enzyme activity of E280K PAH?

2. How common is the E280K mutation in PAH?

3. Write the first author and PubMed ID number (PMID) for two articles that describe patients with the E280K mutation.

4. Write the website mentioned in this abstract and what is mentioned to be found at this website.

5. What is the highest residual activity seen in the enzyme with the E280K mutation?

6. What additional observations are noted for E280K mutant protein?
7. What pathways are most directly affected when PAH is deficient?

8. Dopamine is a neurotransmitter in our brain and could be a possible link between PKU and learning disabilities. From these pathways, how could a deficiency in PAH lead to a deficiency in dopamine biosynthesis?

9. Draw the structure of dopamine and compare it to a metabolite that is deficient in PKU patients (tyrosine).