Metabolic Disease:

Hypoxanthine-guanine phosphoribosyltransferase

Bednarski 2003
Funded by HHMI
Introduction:
Metabolic disease is a broad term that is generally used to describe diseases that result from enzyme deficiencies in either catabolic or biosynthesis 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.

Lesch-Nyhan disease is a metabolic disease caused by a deficiency in the enzyme, hypoxanthine-guanine phosphoribosyltransferase (HPRT). HPRT is an enzyme in the purine salvage pathway and when deficient, causes the metabolite, urate, to build up. This build-up can cause kidney stones and gout. Primary symptoms of Lesch-Nyhan disease include neurological problems such as mental deficiency and self-destructive behavior.

In this module, you will be analyzing a cDNA sequence from a patient suspected to have Lesch-Nyhan Syndrome. You can obtain this sequence by going to the course website and clicking on the file name shown below.

Saved in FASTA format in file “HPRT1mutseq”

Your research project will involve determining the HPRT1 mutation. Using bioinformatics tools, you will investigate the structure of the HPRT1 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 questions for reading 1 (page 4).
The first set of readings is designed to give you an overview of metabolic disease and Lesch-Nyhan syndrome (LNS). Go to the link below and read the
text. Answer the questions for reading 1 in your project packet and turn in your written answers at the second lab meeting.

Reading 1
NCBI online text, “Nutritional and Metabolic Diseases,” pages 1-2 and 10. (this link is available on the course webpage) under your project name.

Laboratory 3
Pre-lab assignment:
Complete the Structure Problem Set (p. 6, 7).
Complete the questions for reading 2 (page 5). This reading provides background of HPRT and purine metabolism. The readings were chosen to give you a background in the reaction catalyzed by HPRT and the role HPRT plays in purine metabolism. There is also an excerpt from the original article describing the crystal structure for HPRT which you can obtain on the course website. This information should help you with working with the HPRT crystal structure during Lab 4.

Reading 2
Berg p. 698 (section 25.2.1) and p. 711 (section 25.6.2)

On course website - excerpt from:

Laboratory 4
No pre-lab assignment
If you haven’t yet, you should begin working on 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 Diseases. The other group you will be meeting with has been researching phenylketonuria (PKU). 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
HPRT1

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

2. Do mutations in enzymes always result in metabolic disease? Please explain.

3. Describe the symptoms associated with Lesch-Nyhan Syndrome (LNS).

4. What metabolite builds up in Lesch-Nyhan Syndrome?
Questions for Reading 2
HPRT1

Berg p. 698 and p. 711

1. Describe what is meant by the “salvage” pathway for purine bases.

2. Write the reaction catalyzed by HGPT (including chemical structures of guanine, PRPP, and guanylate).

3. What is a hypothesis explaining how the reduction of HPRT could result in neurological problems?

Excerpt from *Nature Structure Biology*:

4. What is ImmGP supposed to resemble?

5. Describe the crystal structure that was solved for this article including the protein source and any molecules bound to the protein.

6. What is the proposed role for the loop from residues 100 – 117 in HPRT?

What role do the magnesium ions play in HPRT?
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]

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]

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 HPRT1

Translating the sequence

• Obtain your patient’s cDNA sequence from the course website (HPRT1mutseq)

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

NCBI – Gene

• Using Gene, find the entry for HPRT. Select the Homo sapiens entry (also may mention something about Lesch-Nyhan Syndrome). Answer question 1.

Swiss-Prot Entry

• Use the gene name/symbol to search the SwissProt database and be sure to select the human protein from the search results. Answer questions 2 - 8.

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 9 - 13.
Questions to Accompany Guide Sheet 1
HPRT1

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, trimer, or tetramer?

3. What metabolic pathway does this protein belong to?

4. Where in the cell is this protein found?

5. What is the chemical reaction catalyzed by HPRT1?

6. What metals are found in HPRT1?

7. What residue number binds magnesium 1 (under “Features”)?

8. What residue is modified by acetylation?
Multiple Sequence Alignment

9. 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.

10. Is the mutation in a region of conservation?

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

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

13. What secondary structure is predicted in this area of low conservation?
**Guide Sheet 2 Hints and Tips for HPRT1**

**Searching for Structure Files:** The crystal structure of HPRT1 bound to an inhibitor and magnesium ions 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 1BZY pdb file. The summary information page for 1BZY 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 “1BZY.pdb” file should now be on your desktop.

**Viewing the structure file:**

**IMPORTANT:**
As you know, the HPRT1 protein exists as a homotetramer. The crystal structure is of the homotetramer and each polypeptide chain is labeled A – D. There is an inhibitor bound in the active site of each polypeptide chain. This inhibitor is labeled “IMU300” in the control panel. There are also two magnesium ions bound in the crystal structure and these are labelled “MG900” and “MG901” in the control panel. These are listed in the control panel at the end of each chain.

2. Swiss-Pdb Viewer/DeepView has been loaded on your desktop. To open 1BZY.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 sidechain) column by clicking on them. 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 in Chain A (stop when you see Chain B). It is easier to view just one monomer.

e. Go to IMU300, POP400, MG900, and MG901 in the A chain and put checks in the “show” column to add the inhibitor and magnesium and phosphate ions 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.

g. 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”.

h. Click in the center window to make sure that window is the active one. The tool bar for this window is located at the top and is described in your lab manual. Select the “rotate” tool. To rotate the protein, click and hold on the picture while moving the mouse. The other two buttons are “zoom” and “transverse” for zooming in on the protein and for moving the protein from side to side across the screen.

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.tiff”. 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 Asp193 (the residue that is mutated in the 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.

8. Zoom in on this amino acid. Rotate the structure until you can get a good view of the Asp side chain. The Asp side chain will appear in CPK color mode which is colored by atom type:

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

9. Confirm the identity the Asp side chain by using the identity tool on the toolbar.

10. It should appear that the Asp side chain is ligated to one of the magnesium ions. Measure the distance of the magnesium ligands using the distance tool. The magnesium ion is located in the center of the gray bonds. 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. Print this view by repeating steps 4 - 6, naming the picture “Asp.tiff”. Answer question 6.
Modelling the Mutation

11. To change your selected side chain to a different side chain, use the mutate tool on the toolbar. Select the amino acid asparagine (Asn) to mimic your patient’s mutation. The Asn 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 deselect “Show H-bonds”.

12. Use the distance tool to measure the distance between the Asn side chain and the magnesium ion. 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, “Asn.tif”. Answer questions 7 – 8.

To put in report:
For this lab, you will need the three figures printed in steps 6, 10, and 12. 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
HPRT

1. What organism was HPRT1 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 Asp193 side chain and the magnesium and phosphate ions from the crystal structure. Include the magnesium ligands based on the CPK code and the structure file. Draw the correct chemical structures of the Asp and phosphate ions including atom types, correct placement of lone pair electrons, double bonds, hydrogens, etc. Draw the distance of the ligands to magnesium as a dotted line with the distance in angstroms labelled.

7. Draw the Asn side chain. How does the Asn side chain affect the protein structure? (Refer to your drawing above and your view of the Asn side chain relative to the magnesium ion.)

8. What is your hypothesis for the effect of the Asn mutation on HPRT1 function? Will the mutation affect Mg binding? Is the Mg important to HPRT1 function? Please explain.
Guide Sheet 3 Hints and Tips for HPRT1

OMIM search:

• Go to the NCBI homepage and search the OMIM data base for “Lesch-Nyhan syndrome”. Double click on the search result that states this exact disease name. Answer questions 1 - 3.

• In the first paragraph of the OMIM entry there is a link to the OMIM entry for HPRT1. Double click on this link. Answer questions 4 and 5.

• Read the text under “Allelic Variants” and find the entry for your patient’s mutation. Answer questions 6 and 7.

KEGG pathway

• Go to the Gene entry for human HPRT1. Scroll down to the “General gene information” section and select the “Purine metabolism” link to the KEGG database.

• 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 HPRT1 (2.4.2.8). Answer questions 8 and 9.

• Click on a box with the EC number for HPRT1. Answer question 10.

• Go back to the pathway and double click on the circle next to guanosine. Answer question 11.
Questions to Accompany Guide Sheet 3
HPRT1

1. What syndrome (other than LSC) is also associated with reduced activity of HPRT1?

2. Besides having high levels of urate, what observations are important for diagnosing Lesch-Nyhan Syndrome (LNS)?

3. Changes in the levels of what neurotransmitter is thought to lead to the neurological problems of Lesch-Nyhan Syndrome (read under “Pathogenesis”)?

4. What type of inheritance pattern is seen with HPRT1?

5. Are males or females more likely to get Lesch-Nyhan disease? Please explain your answer.
6. Why are there two different ways of numbering Asp 193(4)?

7. Write the first authors, publication date, and PubMed ID numbers (PMID’s) for the two articles that describe patients with the D193(4)N mutation.

8. HPRT1 is listed 4 times in this pathway scheme. Briefly write the four reactions HPRT1 is shown to catalyze in this scheme.

9. What product of HPRT1 is linked to glyoxylate and dicarboxylate metabolism?

10. List 4 types of information listed in this entry. (for HPRT1)

11. List 2 types of information that is listed in this entry. (for guanine)