Project Manual
Bio3055

Cholesterol Homeostasis:

HMG-CoA Reductase

Cholesterol Homeostasis: HMG-CoA Reductase

Introduction:

HMG-CoA Reductase is an enzyme in the cholesterol biosynthesis pathway. It is the target of the family of statin drugs, which have become very important in the treatment of high cholesterol and reducing the risk for cardiovascular disease in many people. In this module, you will learn about the gene for HMG-CoA reductase, how statins inhibit this enzyme, and how this enzyme is involved in cholesterol homeostasis.

Many individuals who are currently taking statins have been diagnosed with "familial hypercholesterolemia". In this module, you will also be learning about this condition, its genetic basis, and why inhibition of HMG-CoA reductase is important to the treatment of this condition.

For this module, you will be working with a patient's cDNA who is completely unresponsive to statin therapy. This is very unusual. Normally serum cholesterol levels drop in response to statin therapy. This patient's cDNA sequence is available from the course website under the file name:

"HMGmutseq" (Saved in FASTA format)

Your job for the following 4 labs is to determine why this patient is not responding to statin therapy. You will use the above sequence and web-based bioinformatics tools to solve this problem. You will investigate the structure of HMG-CoA reductase and model 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 the enzyme, statins, and their role in the prevention of coronary artery disease. Read the following paper as well as the pages in your Berg textbook. The paper can be downloaded from your project page on the lab website.

Reading 1
Nabel, E.G., "Cardiovascular Disease", *N. Eng. J. Med.,* vol. 349, pp. 60-62. (Read until heading for "Hypertension" on page 62.)

Berg – p.723 (Section 26.2.1), p. 731 (Section 26.3.6)

Laboratory 3

Pre-lab assignment:

Complete reading questions 2 (page 5).

Complete the structure problem set (page 6-7). This set of readings provides you with some background in working with the crystal structure of HMG-CoA Reductase and its bound statin. This article contains the crystal structure you will be studying.

Reading 2
Excerpt from:
Istvan, E.S., and Deisenhofer, J., "Structural Mechanism for Statin Inhibition of HMG-CoA Reductase", *Science*, vol. 292, pp. 1160 – 1164 (2001).

Berg - p. 726-7 (Section 26.3) and p. 730 – 733 (Sections 26.3.5 – 26.4.1)

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 Cholesterol Biosynthesis. The research focus of the other group you will be meeting with has been the LDL receptor. 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 for Reading 1
HMG-CoA Reductase

Berg p. 723:

1. What are the substrates for HMG-CoA reductase? (Note: mevalonate is considered a product, not a substrate.)

Berg p. 731 (Section 26.3.6)

2. Briefly describe how statins work to lower **BLOOD** cholesterol levels. (Note: this is a two-step process.)

NEJM article:

3. How are LDLs cleared from the blood?

4. You're a cardiologist who is treating a patient that has high serum cholesterol levels.   Your patient's serum cholesterol level is 300 mg/dl. You suspect your patient has familial hypercholesterolemia which affects 1 in 500 people. Mutations in what gene is the cause of familial hypercholesterolemia?

5. Mutations in what other genes have been found to result in elevated cholesterol?

Questions on Reading 2
HMG-CoA Reductase

Berg p. 726 – 7

1. What are the four ways that HMG-CoA reductase is controlled?

Berg p. 730-733

2. What is the difference in treatments for homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia?

3. Name three types of molecules that are derived from cholesterol and a biological function for each.

Excerpt from *Science* article:

4. Where on the protein does the statin molecule bind in relative to the substrate HMG?

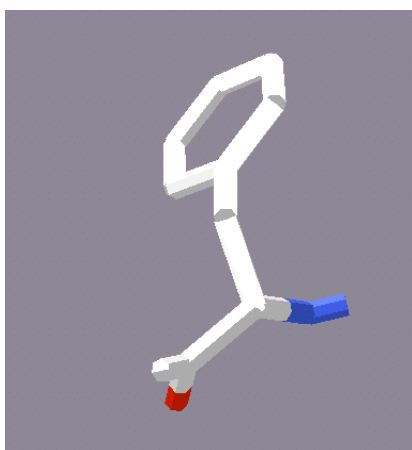5. In Figure 4, which of the structures (a – f) shows a hydrogen bond between Asp690 and the statin?

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.
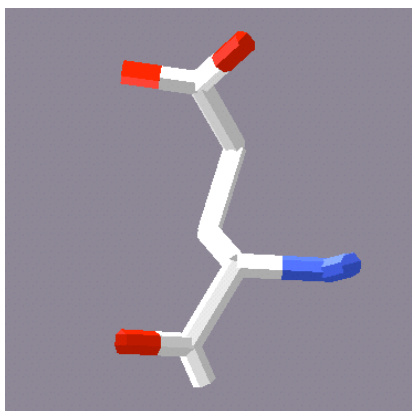
2.

3.

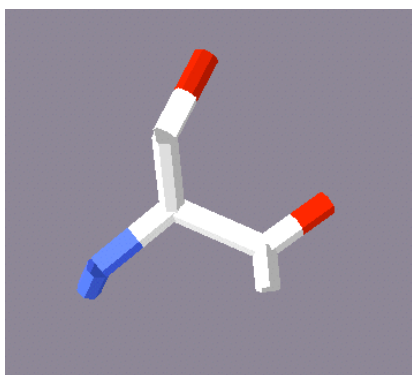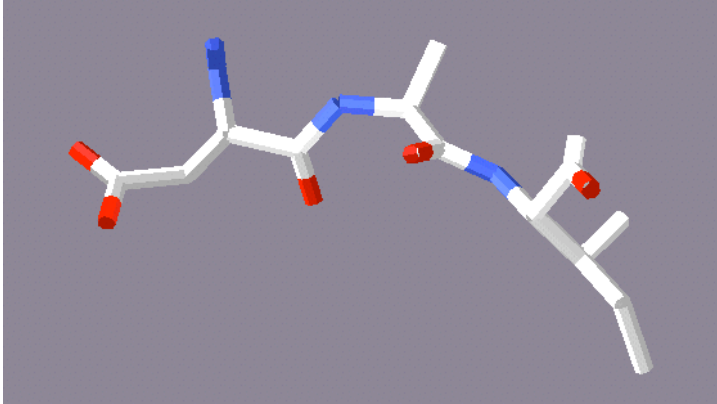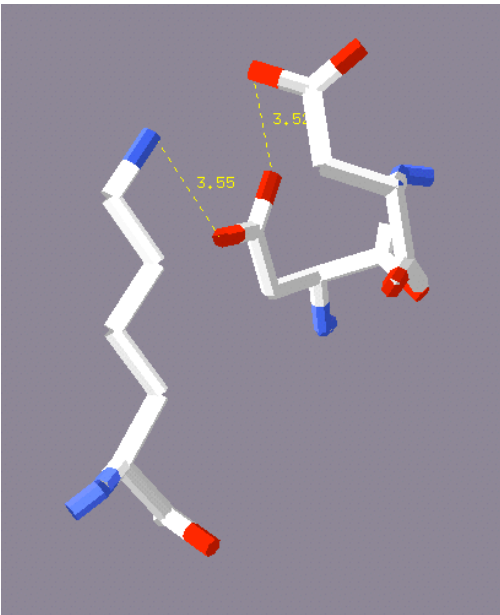4. Draw the chemical representation of the following tripeptide.

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.

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 HMG-CoA Reductase

Translating the sequence

- Obtain your patient's cDNA sequence from the course website (HMGmutseq). When copying and pasting the sequence into the box on the Sequence Manipulation Suite, be sure to select the ENTIRE sequence. It may be on more than one page of the pdf document.

- Use "Reading Frame 1" when translating the sequence at the Sequence Manipulation Suite.

NCBI – Gene

- Using Gene, find the entry for HMG-CoA reductase. Scroll down until you see the entry for the *Homo sapiens* gene, then select that link. Answer question 1.

Swiss-Prot Entry

- Use "HMGCR" to search the SwissProt database and select the human entry from the search results.

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.

- Be sure to download both the MEMSTAT and PSIPRED predictions from your project page on the course website and map both predictions onto your alignment.

Questions to accompany Guide Sheet 1
HMG-CoA Reductase

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. Where in the cell is this protein found?

5. What is the chemical reaction catalyzed by HMG-CoA reductase?

6. What span of amino acids make up the catalytic domain (under "Features")?

7. What span of amino acids make up the transmembrane domain?
8. What 3 residues potentially form the active site?

Multiple Sequence Alignment

9. What is your patient's 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 your patient's mutation in a region of conservation?

11. Based on the transmembrane predictions, can you explain why there are large gap regions at the beginning of some of the sequences in your multiple sequence alignment? (Hint: look at the protein domain information on the Swiss-Prot entry)

Guide Sheet 2 Hints and Tips for HMG-CoA Reductase

Searching for Structure Files: The crystal structure of HMG-CoA reductase complexed with a statin molecule 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 (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 1HW9 pdb file. The summary information page for 1HW9 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.
   a. Click on "Download/Display" file at the left of the screen.
   b. 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 "1HW9.pdb" file should now be on your desktop.

Viewing the structure file:

IMPORTANT:
   The 1HW9 crystal structure was solved with simvastatin bound in the active site. The simvastatin is called "SIM3" in the control panel. The crystal structure is a homotetramer, meaning it contains 4 identical polypeptide chains labelled A – D.

2. Swiss-Pdb Viewer/DeepView has been loaded on your desktop. To open 1HW9.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. When the file opens, 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, you will only view the protein backbone in a ribbon diagram.
d. Put check marks in the "ribbon" column for all the A and B chain residues. (Stop when you see the C chain.) It will be easier to view just one homodimer.
e. Locate the SIM3 in the control panel and put a check in the "show" column to show the molecule in the display. Simvastatin should appear in ball and stick form in 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 prefer 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 to get the colors the way you would like them. You can also change the background to any color by choosing "Colors" under "Prefs", then "background".
h. Click in the display 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 "hmg.pdb" and save to desktop. When you open this file, all your colors and the orientation should by saved, but you will have to select "Render in Solid 3D" again under "Display" to see it. Answer questions 4 and 5.

Printing the 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 something ending with ".tiff". Save it to your desktop. Now you can now open this file in PowerPoint to use it in a presentation, or Preview to print the figure.

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 Asp690 (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.

8. Click on the structure to re-select the center window. Zoom out until you can see the Asp690 side chain. Zoom in on the side chain.

9. The Asp690 and SIM3 molecules are colored in CPK mode which is:

> 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 Asp690 and SIM3.

10. Zoom in on the SIM3 molecule. Find the carbonyl of the SIM3 molecule that H-bonds to Asp690 using the figure from your reading for today for help.

Investigating the environment of the side chain

In order to investigate the non-covalent interactions between Asp 690 and SIM3, use the distance tool on the toolbar to find atoms that are close enough 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.

11. Now measure the distance between the nearest Asp oxygen and the carbonyl on SIM3 by using the distance-measuring tool in the toolbar and selecting the two atoms. You will click on the distance tool, then the two

atoms that you want to measure the distance between.   The distance, in angstroms, should then 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 Asp 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 aspartate side chain to a different side chain, use the "mutate" tool on the toolbar. Select the amino acid alanine (Ala) to model the mutation. The Ala side chain will appear in the lowest energy conformation (most stable). Some green lines may appear which represent potential H-bonds. You can make these disappear under "Display", then deselect "Show H-bonds".

14. Use the "distance" tool to measure the distance between the Ala side chain and the SIM3 as you did in step 11 with aspartate. 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, "Ala.tiff". 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
HMG-CoA Reductase

1. What molecule is HMG-CoA crystallized with in this structure file?

2. Write the author and title for the primary citation for this structure.

3. What is the resolution for this crystal structure? Explain what "resolution" means 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 Asp690 side chain and the carbonyl of simvastatin involved in the H-bond. Draw the H-bond as a dotted line and include the distance in angstroms. This should include the correct chemical structure of the aspartate side chain including atom types, double bonds, etc.

7. Analyze the model of the Ala mutation. Can the same non-covalent interaction(s) occur with Asp and Ala? Draw a chemical representation of the Ala residue and the simvastatin. Show any changed non-covalent interactions and distances measured between atoms.

8. What is your hypothesis for how the alanine mutation affects the binding of simvastatin?

Guide Sheet 3 Hints and Tips for HMG-CoA Reductase

OMIM search:

- Search the "OMIM" database using the gene name. Double click on the search result that contains the correct name for your protein.

- Read the "Gene Function" and "Biochemical Features" sections. Answer questions 1 – 4.

- Now look up the OMIM entry for hypercholesterolemia. Choose the "autosomal dominant" link, but NOT the "TYPE B" entry. Read the Description, Pathogenesis, Clinical Management, and Gene Therapy sections. Answer questions 5 – 9.

KEGG pathway

- Go back to the Gene entry for human HMGCR. Scroll down to the "General gene information" section and select the link for the KEGG pathway for "Biosynthesis of steroids."

- Use the EC number (1.1.1.34) to find HMGCR on the metabolic map. Answer question 10.

- **Questions to accompany guide sheet 3**
  HMG-CoA Reductase

1. What other role(s), if any, does HMG-CoA Reductase play besides cholesterol biosynthesis?

2. If an inhibitor was found that irreversibly inhibited HMG-CoA Reductase, so that no cholesterol could be formed, would this be desirable? Why or why not?

3. Describe how statin molecules bind HMG-CoA Reductase. Are statins competitive or non-competitive inhibitors?
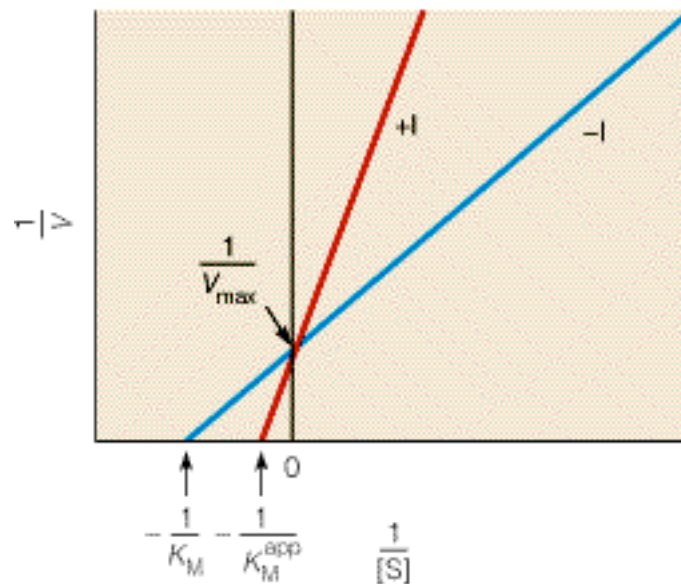
4. Before the crystal structure was solved of statins bound to HMG-CoA Reductase, researchers already knew what type of inhibitor statins were by performing enzyme assays in the presence of statins and plotting the data in a Lineweaver-Burke plot. Based on your answer in question 4, which of the following plots describes what the researchers found?

Plot A:
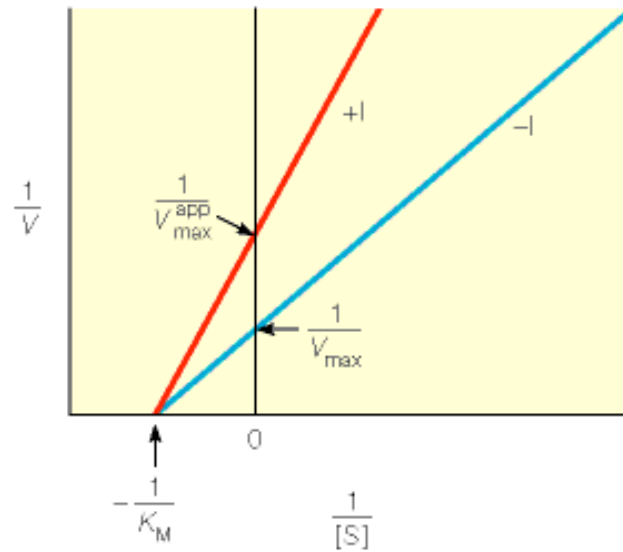
Plot B:

5. What is the primary genetic defect in the disease?

6. How is HMG-CoA Reductase related to the disease, familial hypercholesterolemia?

7. Is your patient the only known case of someone with familial hypercholesterolemia not responding to statin therapy? Explain your answer.

8. What types of gene therapy have been tried?

9. Who are the authors for the article describing these experiments?

10. The concentration of what metabolite is directly lowered by inhibiting HMG-CoA reductase? What other pathways (besides steroid biosynthesis) are affected by the lowering of this metabolite?