Project Manual
Bio3055

Apoptosis:
Superoxide Dismutase I

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Apoptosis:  
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Introduction:
Apoptosis is another name for “programmed cell death”. It is a series of events in a cell that lead to its self-destruction. Apoptosis is a natural and important process that allows a cell to cleanly destroy itself when the cell senses an incorrectable problem. The types of cell problems that lead to apoptosis range from an increase in reactive oxygen species (for example, superoxide ion) to uncontrolled cellular growth. The process of apoptosis leads a cell to degrade itself and its parts to be engulfed and destroyed by surrounding cells. Apoptosis can provide a clean get-away for a damaged cell and prevent surrounding cells from being damaged. The signaling pathways and cellular conditions that lead to apoptosis are still being determined.

Superoxide dismutase is an enzyme that is important to cells for converting potentially damaging free radicals into hydrogen peroxide and oxygen. A form of this enzyme is found in mitochondria to scavenge any superoxide molecules that escape from Complex IV during oxidative phosphorylation. Mutations in the superoxide dismutase 1 gene cause the disease, familial amyotrophic lateral sclerosis (FALS), also called Lou Gehrig’s disease.

For this module, you will be working with a mutant superoxide dismutase I cDNA sequence that you can obtain at the course website under the following file name:

Saved in FASTA format in file “SOD1mutseq”

For your research project, you will analyze the mutant SOD1 protein using the bioinformatics tools presented in lab. You will investigate the structure of the SOD1 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 5).
This article will give you a summary of apoptosis and its role in human disease. Read the page in the Berg text as well as the pages in the paper below (which you can obtain under your project name at the course website). Answer the questions for reading 1 in your project packet and turn in your written answers at the second lab meeting.

Reading 1
Pages 80 – 83 of the following article:

Berg p. 521 (18.6.6)

Laboratory 3
Pre-lab assignment:
Complete the Structure Problem Set (page 7 –8)
Complete the questions for reading 2 (page 6). This reading will provide you with some background in working with the crystal structure of superoxide dismutase. The article listed below contains the crystal structure you will be studying. You need to read just an excerpt from this article which is available on the course website under your project name. The pages in Berg are assigned to provide an introduction to superoxide dismutase. 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. 506-7

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 apoptosis. The other group you will be meeting with has been researching a caspase I. 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
SOD1

Scientific American article p. 80 - 83:

1. Describe differences between necrotic cell death and apoptosis.

2. What types of events or signals can trigger apoptosis? Give two examples.

3. Describe the role of ICE-like proteases (caspases) in apoptosis.

4. Give an example for when an increase in apoptosis leads to disease and an example of when too little apoptosis leads to disease.

Berg (p.521)

5. What roles do mitochondria and cytochrome c play in apoptosis?
Questions for Reading 2
SOD1

Excerpt from:

1. Describe the three crystal structures that are reported in this article.

2. How is superoxide anion produced in organisms?

3. Describe the differences between Figure 1 and Figure 2.

Berg (p. 506 – 507)

4. What are reactive oxygen species (ROS)?

5. Write the reactions for SOD and catalase.

6. What can oxidative damage do to a cell?
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 SOD1

Translating the sequence

• Obtain the mutant cDNA sequence from the course website (SOD1mutseq)

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

NCBI – LocusLink

• Using LocusLink, find the entry for Homo sapiens (Hs) superoxide dismutase 1. Be sure to look for this exact protein name in the search results. Answer question 1.

Swiss-Prot Entry

• Use “SOD1” to search the SwissProt database and be sure to select the human protein from the search results. Check the EC number to make sure you have the right entry. Answer questions 2 – 7.

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 8 – 11.
Questions to accompany guide sheet 1
Superoxide Dismutase 1

Locus Link Entry

1. Fill in the following information from the LocusLink entry:
   a. Write the LocusLink entry number here ____________________.
   b. What is the gene name/symbol? (Hint: it is not “HGNC”, but it is short, 3 – 7 characters)
   c. What is the enzyme classification (EC) number(s)?
   d. Where on the human genome is this gene located?
   e. What is the RefSeq number for the mRNA sequence?
   f. 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 cofactors does this enzyme have?

4. Where in the cell is this protein found?

5. What is the chemical reaction catalyzed by superoxide dismutase 1?

6. What amino acids ligate copper (under “Features”)?

7. Which amino acid ligates both copper and zinc?
Multiple Sequence Alignment

8. 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.

9. What can you tell about the mutation based on your answer to question 6?

10. Is the secondary structure of the protein mostly helices, sheets, or a pretty even mixture of the two?

11. Is the mutation in a region of conservation?
Guide Sheet 2 Hints and Tips for SOD1

Searching for Structure Files: Since the crystal structure of human SOD1 has not yet been solved, you will analyze the crystal structure of the yeast SOD1. Since the sequences are very similar between the two species, it is thought that the protein structures are also very similar. 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 1B4L pdb file. The summary information page for 1B4L 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 “1B4L.pdb” file should now be on your desktop.

Viewing the structure file:

IMPORTANT: Remember you are analyzing the YEAST SOD1 structure! You may notice some differences in the amino acid numbering between the human and yeast sequences. Review the sequence around the residue of interest to help orient you.

2. Swiss-Pdb Viewer/DeepView has been loaded on your desktop. To open 1B4L.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.
d. Put check-marks in the “ribbon” column for all the A chain residues. (Stop when you see the B chain.) For now, it is easier to just view one monomer. The Cu and Zn atoms are listed last in the A chain “residues”. When you locate the Cu and Zn in the list, put a check in the “show” column for these atoms. In the box in the same row as the amino acid in the control panel, click to obtain a color wheel. When the color wheel appears, choose pink for the Cu atom. Repeat this process for the Zn atom, but choose orange instead of pink.
e. 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.
f. 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”.
g. 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.
h. 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 something short with .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 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 residues that ligate the copper atom (which includes the residue you are studying) in the control panel. Remember that the residue numbers may not be exactly the same as they were for the human SOD1. Show the side chains 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 copper and the side chains. The copper should be colored pink since you made it that color in step 3d. The side chains will be colored based on the atom type in the CPK mode shown below:

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

9. Now measure the distances between the copper atom and each nitrogen of the side chains that ligate the copper. 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.
10. Confirm the identity the amino acid side chains by using the identity tool on the toolbar. Include this information in your figure in question 6.

11. Follow steps 4 – 6 to print the current view of your protein.

**Modelling the Mutation**

12. To change your selected side chain to a different side chain, use the mutate tool on the toolbar. Select the amino acid asparagine to mimic the mutation. Use the distance tool to measure the distance between the closest terminal arginine nitrogen and the copper atom. Use steps 4 – 6 to print the view of the mutated side chain. Answer questions 8 and 9.

**To put in report:**
For this lab, you will need the three figures printed in steps 6, 11, 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
SOD1

1. What organism is the superoxide dismutase 1 from in the 1B4L crystal structure?

2. Write the first author and title for the primary citation for this crystal 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 copper and the 4 side chains of the amino acids that ligate the copper. Include the distance of each ligation to the copper in angstroms as well as the identity of each side chain. Be sure to draw a correct chemical representation of the side chains including lone pair electrons and correct placement of hydrogens.

7. Calculate the average distance in angstroms of the copper ligation using your distances in question 4.

8. Draw a correct chemical representation of the arginine side chain, including the proper placement of lone pair electrons and hydrogens? Is the arginine side chain in your patient’s SOD1 still able to ligate copper?

9. What is your hypothesis for how the arginine mutation affects the activity of SOD1? Do you hypothesize the mutant SOD1 to be more active, less active, or no change from wild type SOD1? Please explain.
Guide Sheet 3 Hints and Tips for SOD1

OMIM search

• First, go back to your first guide sheet to obtain the gene name for your protein. Go to the NCBI homepage and search the “OMIM” database for your gene name. Double click on the search result that contains the correct name for your protein.

• Scroll down until you see the paragraph that describes the two forms of superoxide dismutase and answer questions 1 and 2. (Hint: paragraph starts with “The 2 distinct forms…”)

• Scroll down until you see the paragraph about the H46R mutation (Hint: look for “Liu et al.” at the start of the paragraph.) Answer question 3.

• Click on the link immediately following “H46R”. It should be the number “147450.0013”. This will take you to the section that discusses this mutation in detail. Answer questions 4 and 5.

• Now look up the OMIM entry for ALS-1. Read under “Text” and answer question 6.

• Go to the NCBI homepage. Under “Hot Spots” on the right of the page, find the link to “Genes and Diseases” and click. This will take you to an online book on genetic diseases. Select “Muscle and Bone” and then “amyotrophic lateral sclerosis”. Answer question 7 – 9.

KEGG pathway

• Go to the LocusLink entry for human SOD1. Scroll down to the “Additional Links” and select the “KEGG database: Neurodegenerative Disorders” link. Answer question 10.

• Go to the LocusLink entry again. This time select the “KEGG database: ALS” link. Answer question 11.
Questions to Accompany Guide Sheet 3
Superoxide Dismutase 1

1. What metal does SOD2 contain?

2. Where is SOD2 found in cells?

3. What did Liu et al. (2000) find out about the copper binding site in the H46R mutant?

4. How does the mean survival after disease onset differ for patients with ALS who have the H46R SOD1 mutations from patients with other types of mutations?

5. What did the Aoiki et al. 1993 article report about why the H46R SOD1 enzyme is less active?

6. What distinguishes Type 1, Type 2, and Type 3 forms of ALS?
7. Describe the symptoms and disease progression of ALS.

8. When did they discover SOD1 mutations are linked to ALS?

9. What famous athlete had ALS?

10. What gene has been linked to both ALS and Huntington’s disease?

10. What metabolite is formed when SOD1 misfolds?

11. How is apoptosis linked to SOD1?