

DIRECTIONS: Please make sure that you have all 13 pages of this exam. There are **SIX** questions. Answer all parts of all questions. Read through the entire question before you answer. Note that each question indicates an approximate time you should allot to it--do not overextend your welcome at any particular question! The point values of each question are also noted.

Answer all questions thoroughly with appropriate detail, however, please be succinct. Most require no more than 3-6 sentences for an answer.

PLEASE WRITE CLEARLY.

Note that question 6 will take the majority of your time, so plan accordingly

***** GOOD LUCK *****

Question 1. (5 minutes, 5 points) A new protein of unknown structure has been purified. Gel filtration chromatography reveals that the native protein has a molecular weight of 240,000 daltons (240 kDa). Chromatography in the presence of 6M guanidine hydrochloride yields only a peak for a protein of 60 kDa. Chromatography in the presence of 6M guanidine hydrochloride and 10mM β -mercaptoethanol yields peaks for proteins of 34 kDa and 26 kDa. Explain what can be determined about the structure of this protein from these data and why.

Guanidine hydrochloride is a powerful denaturing agent that disrupts tertiary and quaternary structure by disrupting hydrogen bonds. In the presence of 6 M guanidine-HCl, only a 60 kDa species is observed, indicating that the native protein may in fact be a tetramer of four 60 kD subunits. Upon denaturation in the presence of β -mercaptoethanol (a disulfide reducing agent), two protein peaks are observed, one at 34 kDa and one at 26 kDa. This indicates that the 60 kDa subunit is a heterodimer of two chains, 34 kDa and 26 kDa, held together by a disulfide bond.

Question 2. (5 minutes, 5 points) A major difficulty in investigating the properties of proteases is that these enzymes, being proteins themselves, are self-digesting. This problem is less severe, however, for solutions of chymotrypsin than it is for solutions of trypsin. Explain. (Recall that chymotrypsin cleaves on the carboxy-terminal side of residues such as phenylalanine.)

Chymotrypsin cleaves on the carboxy-terminal side of large hydrophobic residues like phenylalanine. Such residues are likely to be buried in the interior of globular proteins (such as chymotrypsin) and thus are inaccessible to a protease. On the other hand, trypsin cleaves on the carboxy-terminal side of lysine and arginine, which are charged at physiological pH. These two residues are thus typically found on the surface of globular proteins (such as trypsin), exposed to proteases (such as trypsin).

Question 3. (10 minutes, 10 points) There are two parts to this question.

Carboxypeptidase, which sequentially removes carboxyl-terminal amino acid residues from its peptide substrates, is a single polypeptide of 307 amino acids. The two essential catalytic groups in the active site are Arg¹⁴⁵ and Glu²⁷⁰.

(3a) If the carboxypeptidase chain were a perfect α -helix, how far apart (in Angstroms - Å) would Arg¹⁴⁵ and Glu²⁷⁰ be? Please show your work.

Arg¹⁴⁵ and Glu²⁷⁰ are $270-145=125$ amino acids apart

in an α -helix there are 3.6 amino acids per turn:

$$125 / 3.6 = 4.16 \text{ turns}$$

in an α -helix there is a rise of 5.4 Å per turn:

$$5.4 \times 4.16 = 22.46 \text{ Å}$$

The two essential catalytic groups in the active site would be 22.46 Å apart in a perfect α -helix.

Question 3 continues on the next page.

(3b) Explain how two amino acids separated by this distance can catalyze a reaction occurring in the space of a few Angstroms.

The two amino acids are separated by a long distance (125 amino acids) in the primary amino acid sequence. However, proteins fold into distinct secondary and tertiary structures that allow amino acids that are far apart in the primary sequence to be near each other in the native protein.

Question 4. (15 minutes, 25 points) There are TWO parts to this question - use the space below and the attached graph paper for your answers.

Prostaglandins are a class of fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. Prostaglandins are responsible for producing fever and inflammation and its associated pain. They are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme uses oxygen to convert arachidonic acid to PGG₂, the immediate precursor of many different prostaglandins.

(4a) The kinetic data given below are for the reaction catalyzed by prostaglandin endoperoxide synthase. Focusing here on the first two columns, determine the V_{\max} and K_M of the enzyme. Graph paper is on the next page. Please show all of your work and your *labeled* graph.

(4b) Ibuprofen is an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. Using the data in the table above, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase. Again, please show all of your work.

We need to do a Lineweaver-Burk Plot here. The first thing to do is translate the information given into $1/[S]$ and $1/V$.

$1/[S]$	$1/V$	$1/V$ (+ibuprofen)
2.0	0.042	0.059
1.0	0.031	0.039
0.67	0.027	0.032
0.40	0.023	0.026
0.28	0.022	0.025

Now we can plot it - see next page - I am aware that your numbers may be slightly different, so don't worry too much about that. That is why I asked for you to show your work and your labeled graph.

From the y-intercept we can calculate V_{max} .

$$1/V_{max}=0.018$$

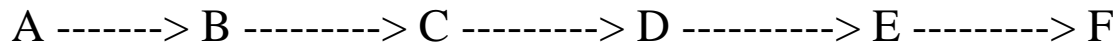
$$V_{max} = 55 \text{ mM/min}$$

From the x-intercept we can calculate K_m :

$$-1/K_m = -1.6 \quad K_m = 0.625 \text{ mM}$$

4b - plotting the points for when the inhibitor ibuprofen is present we see the same y-intercept, that is, the V_{max} doesn't change if the inhibitor is there. This is indicative of a competitive inhibitor. The inhibition can be overcome by a sufficiently high concentration of substrate. (see Stryer pg 197)

Question 5. (10 minutes, 10 points) This question is made up of two parts. Below is a hypothetical pathway of five reactions. The G° for each step is given in the table.



Reaction	Enzyme	G°
A \rightarrow B	E1	+0.5
B \rightarrow C	E2	-3.4
C \rightarrow D	E3	+1.2
D \rightarrow E	E4	+0.4
E \rightarrow F	E5	-5.0

(5a) Is the overall reaction A \rightarrow F energetically favorable? Explain.

Yes, because G° 's are additive in a reaction pathway - the reactions are energy coupled. If all the G° are added, we get -6.3. A negative G° indicates that the overall reaction of A \rightarrow F is spontaneous.

(5b) Given the information in the table, and knowing that B is a substrate for other pathways, suggest a mechanism by which E2 could be regulated. Feel free to draw diagrams to clarify your points. The next page is empty if you need extra space. Please limit your text to less than 8 sentences.

There are several mechanisms by which E2 could be regulated. Feedback inhibition by downstream products (most likely F), feed-forward stimulation by A or an analog of A. Such interactions would most likely be allosteric. Other molecules (maybe ATP) could stimulate or inhibit the activity of E2. Or there may be covalent modification (i.e. addition or removal of a phosphoryl group) that could stimulate or inhibit the activity of E2.

Question 6. (25 minutes, 45 points) There are two main parts to this question. Each part asks *several* questions - be sure to answer ALL of them.

You have discovered a new protein in macrophages (a type of white blood cell) and have made some progress into its characterization. In first trying to purify the protein, you found that it could only be extracted after treating the macrophages with a strong detergent such as SDS. After purifying the protein you sent it away for Automated Edman Sequencing. The report from the company showed that your protein is composed of 340 amino acids. A partial amino terminal sequence is shown below:

Asn-Asp-Ser-Ala-Val-Asn-Asp-Ser-His-Gly-Ser-Pro-Ser-Gly-Ala-Asn-Asp-Ser-

A partial carboxy terminal sequence is shown here:

- Arg-Arg-Gly-Ser-Phe-Gly-Leu-Ile-Leu-Met-Leu-Ala-Lys-Leu-Ala-Gly-Ala-Leu

An ultracentrifugation experiment yields a molecular weight for the protein of 42000 daltons (Da). Recall that the average mass of an amino acid is 110 Da.

You plug the entire sequence into a computer program that produces a hydrophathy plot for your protein. It is shown below.

Question 6 continues on the next page

(6a) What can you tell about the protein from the information you've gained so far?

The first thing we learned is that this is an INTEGRAL MEMBRANE PROTEIN because SDS was required to purify it from the cells. We know that the protein is 340 amino acids long, but if an amino acid averages about 110 Da in mass then:

$$340 \times 110 = 37400 \text{ daltons}$$

this is 4600 daltons LESS than the ultracentrifugation experiment yielded (total mass of the protein is 42000 Da). So, where are those extra 4600 daltons coming from? Carbohydrate modification. There must be carbohydrates on our protein. Looking at the sequences given, notice that the amino-terminal sequence is loaded with Ser and Asn residues, both of which can be modified by O-linked and N-linked carbohydrates. Moreover, the sequence Asn-Asp-Ser is repeated 3 times, this is the consensus sequence for N-linked carbohydrates. Alternatively, the carboxy-terminal sequence has only one Ser and no Asn.

We also know from the hydropathy plot that this integral membrane protein has 7 membrane-spanning regions.

(6b). Draw a diagram of what this protein might look like. Label the N-terminus and C-terminus and all other salient features of your diagram. (You do not need to write out the sequences in your diagram - just draw a spacial representation of what is going on with appropriate labels.)

Question 6 (continued)

Your curiosity is peaked. You decide to do an experiment involving radioactive phosphate (^{32}P). In a miracle protocol, you can incorporate radiolabeled trinucleotides (i.e. ATP, CTP, GTP - all containing ^{32}P) into the cytosol of the macrophages. You run three different experiments. All of these experiments are done in cell media that contains various nutrients, hormones, and growth factors (but no trinucleotides).

(Experiment 1) You add radioactive ATP to the cells along with non-radioactive CTP and GTP. You find that, after this treatment, your protein becomes radioactive.

(Experiment 2) You add radioactive CTP and GTP along with non-radioactive ATP and find that your protein does not incorporate any radioactivity.

(Experiment 3) You add radioactive ATP but, beforehand, you completely deplete the cells of both GTP and CTP. You find that your protein does not become radioactive.

You think you may have an idea of what is happening in the system. You decide to do one more experiment.

(Experiment 4) You deplete the cells of all CTP, GTP and ATP. You then add back radioactive ATP, non-radioactive GTP, and a potent, specific inhibitor of adenylate cyclase. Once again, you find no incorporated radioactivity in your protein. But, when you also add nonradioactive dibutyryl cAMP (a derivative of cAMP that hydrolyzes to cAMP once inside the macrophage) you do recover radioactive protein.

(6c) What is the function of your protein? (1-2 sentences)

The protein is a hormone receptor. It has 7 membrane-spanning regions which is a hallmark of β -adrenergic receptors such as epinephrine (refer to Stryer pg 341). This fits with the experimental data that show that a cyclic AMP cascade is occurring in these cells.

Question 6 continues on the next page

Question 6 continued

(6d) How is your protein becoming radioactive (i.e. how is it incorporating ^{32}P ? Draw a diagram of what might be going on in the cell that would be consistent with all the information that you have gained so far. Be sure to label all important features/reactions in the diagram.

The experimental data show that the radioactive moiety is being transferred from ATP to our protein. They also show that GTP is required for this process to occur. cAMP is also needed, because when adenylate cyclase is inhibited, no radioactivity is transferred. Adenylate cyclase catalyzes the formation of cAMP (see Stryer pg 340). But, if cAMP is added directly (in the form of dibutyryl cAMP), the beginning of the signal transduction pathway can be circumvented (i.e. the requirement for GTP and adenylate cyclase activity).

So, the hormones in the media are stimulating our protein. This causes a G-protein to be activated - GTP replaces GDP in the G-protein (this is why GTP is necessary). The G-protein then activated adenylate cyclase, which then catalyzes the formation of cAMP. cAMP stimulates protein kinase A (PKA). PKA either targets our protein directly, or activates other proteins (kinases) that then phosphorylate our target protein. Kinases use ATP as the donor in the phosphorylation reaction. Thus, when radioactive ATP is incorporated into the cells, the kinases use it to transfer the radioactive phosphoryl group to the cytosolic side of our protein.

Question 6 continues on the next page.

Question 6 continued.

(6e) Referring to the partial sequences of the protein given earlier (and copied below for you convenience), determine where the radioactive moiety may be bound. Simply indicate where it may be binding (circle or underline).

A partial amino terminal sequence is shown below:

Asn-Asp-Ser-Ala-Val-Asn-Asp-Ser-His-Gly-Ser-Pro-Thr-Gly-Ala-Asn-Asp-Ser-

A partial carboxy terminal sequence is shown here:

- Arg-Arg-Gly-Ser-Phe-Gly-Leu-Ile-Leu-Met-Leu-Ala-Lys-Leu-Ala-Gly-Ala-Leu

From earlier, we know that the carboxy terminus of the protein is in the cytosol. Reviewing the sequence we see that there is only one Ser which must be where the phosphoryl group from the radioactive ATP is linked. Moreover, the sequence Arg-Arg-X-Ser-Z (where X is a small aa like Gly and Z is a large hydrophobic aa like Phe) is the consensus sequence for phosphorylation by PKA.

CONGRATULATIONS!

END OF EXAM

**PLEASE TURN IT IN AND STAPLE YOUR GREEN SHEET
TO THE EXAM**