BIO 451  
17th September 2001

EXAM I

This exam will be taken apart for grading. Please PRINT your name on each page.
If you do not have sufficient room for your answer in the space provided, please continue on the back of the page on which the question appears. It is in your best interest to read through the entire exam before starting to answer the questions.

*It typically requires 7 to 10 days to complete the process of grading and recording.*

<table>
<thead>
<tr>
<th>Question</th>
<th>Maximum Points</th>
<th>Earned Points</th>
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<td>I.</td>
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<td>VI.</td>
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<tr>
<td>XI.</td>
<td>16</td>
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</table>

Credit will be proportional to the accuracy, clarity, and legibility of your answers.
I. [8 points; one point for each correct response Only your first eight answers will be graded.]

Match the terms below with the appropriate accompanying statements. (More than one term may apply to a statement.) The point score corresponds to the number of correct responses expected.

A. Iodoacetate
B. Phenylisothiocyanate
C. Serine protease
D. 2-Mercaptoethanol
E. Cyanogen bromide
F. Dansyl chloride

_____ A reagent that carries out reductive cleavage of disulfide bonds.
_____ A reagent used to identify N-terminal residues.
_____ A reagent that introduces carboxymethyl group at C residues.
_____ A reagent that cleaves polypeptides into smaller fragments.
_____ A reagent that is used in sequencing polypeptides from the N-terminus.
_____ A class of enzymes that contains a catalytic triad
_____ A reagent that introduces an alkyl group at intact S-S bonds
II. [7 points]

The diagrams below represent two different conformations of a polypeptide.

A. The amide hydrogens of peptide bonds are not acidic; however, they will exchange with deuterium atoms if proteins are placed in deuterium oxide.

Would the amide hydrogens on these two conformers exchange at equal rates? Explain, being as thorough and specific as possible. (3 points)

B. Which of the above conformers best represents: (Explanation not needed)

_____ 1. poly-K at physiological pH?
_____ 2. poly-K at pH 11?
_____ 3. poly-E at physiological pH?
_____ 4. poly-E at pH 2?
III. [14 Points; *there are a total of 14 correct responses, 1 point for each correct selection*]

A. The specific activity of an enzyme increased from 3.6 units per mg homogenate protein to 435 units per mg protein after being bound to and eluted from a cation exchange column at pH 6.2. What is (are) the most valid conclusion(s) that you can draw from this information? [You do not need to know this in order to answer this question, but by international agreement, a unit of enzymatic activity is defined as that amount of enzyme that will convert 1 micromole of substrate to 1 micromole of product in 1 minute, under the conditions of assay.]

a. The yield of enzyme was greater than 80 percent.
b. The enzyme was positively charged at pH 6.2
c. The enzyme was purified over 100-fold.
d. The enzyme was globular in structure.
e. The enzyme was in an activated state.

B. The figure below shows the structure of immunoglobulin hydrolyzed by papain (a protease) to form two A fragments and one B fragment. The wavy line indicates where the polypeptides are cleaved by papain.

![Diagram](image)

Which of the following is true of the A fragments:

a. They will not cross-link to form a lattice of antigen and antibody
b. They contain only the heavy chain
c. They contain only the light chain
d. They will not functional as an antigen-binding site
e. They cannot be further dissociated by mercaptoethanol
Which of the following is true of the A fragments:

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b. They contain only the heavy chain
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d. They will not functional as an antigen - binding site
e. They cannot be further dissociated by mercaptoethanol

C. The side chains of all of the following amino acids are ionizable in proteins EXCEPT

a. leucine
b. histidine
c. terminal glycine
d. arginine
e. cysteine

D. All of the following amino acids are found in unusually high amounts in collagen as compared with other proteins EXCEPT

a. glycine
b. isoleucine
c. hydroxylysine
d. proline
e. hydroxyproline

E. All of the following assumptions apply to the Michaelis-Menton kinetic analysis of enzyme action EXCEPT

a. the total enzyme concentration studied at each substrate concentration is fixed in analysis of enzyme kinetics.
b. formation of enzyme-substrate complex does not appreciably decrease the concentration of substrate
c. $K_m$ decreases with competitive inhibition
d. maximal velocity is reached when the enzyme-substrate complex is equal to the total concentration of enzyme present
e. the initial reaction of velocity should be measured since most substrate has not been converted
to product

For each description below select the bond that is most appropriate match.

a. Ionic
b. Disulfide
c. Peptide
d. Hydrophobic
e. Hydrogen

F. A bond that contributes to forming the secondary structure of proteins but not the primary structure ______

G. A covalent bond that can only be involved in forming the tertiary structure of proteins ______

Match each parameter with the terms of measure used to describe it.

a. Millimoles/litre
b. Units/milligram protein
c. Micromoles/minute
d. Units/minute
e. $t^{1.1}$

H. $K_m$ (Michaelis constant) ______

I. Specific activity of enzyme ______

J. All the following correctly describe the active site of an enzyme EXCEPT

a. it is small relative to the entire enzyme
b. specificity is defined by the arrangement of certain atoms
c. it is two-dimensional
d. it is usually a crevice or cleft
e. it initially binds substrates by weak attractions
IV. [10 points]
A. At what substrate concentration will an enzyme with a $k_{cat}$ of 30s$^{-1}$ and a $K_m$ of 0.005M show one-quarter of its maximum rate? (5 points)

B. Estimate the $V_{max}$ and $K_m$ of the enzyme-catalyzed reaction for which the following data were obtained. Explain your rationale. (5 points)

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<th>[S] (M)</th>
<th>$V_0$ (µM/min)</th>
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<tr>
<td>2.5 x 10$^{-6}$</td>
<td>28</td>
</tr>
<tr>
<td>4.0 x 10$^{-6}$</td>
<td>40</td>
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<td>1 x 10$^{-5}$</td>
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<td>1 x 10$^{-4}$</td>
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<tr>
<td>2 x 10$^{-3}$</td>
<td>139</td>
</tr>
<tr>
<td>1 x 10$^{-2}$</td>
<td>140</td>
</tr>
</tbody>
</table>

Please do not waste your time trying to plot these data.
V. [5 points] Answer ONE of the following; only your first answer will be graded.

A. You have isolated two samples of collagen, one from fibroblasts of a normal individual, and one from fibroblasts of an individual who is thought to produce a mutant form of collagen in which several G residues have been replaced by A. Unfortunately the labels have come off during rearrangement of the ! 70°C freezer, before you have had time to perform the relevant analyses. A bright Bio500 student suggests that you can identify the two samples by performing a thermal transition analysis (‘melting point’) of aliquots of the two collagen samples. Is this a good suggestion? Explain. (5 Points)

B. Explain the following observations:
Mature collagen fibers contain a very significant number of hydroxyproline residues, but if radiolabeled hydroxyproline is added to a culture system that is actively engaged in collagen synthesis, none of the added hydroxyproline is incorporated into the newly-synthesized collagen fibers. (5 points)

C. How is it that Class I collagen (a water insoluble, extracellular protein) is synthesized in the endoplasmic reticulum without falling out of solution? Be as specific as you are able. Full credit will be given for the most concise, but informative answers. (5 points)
VI. [12 points]
Native lactate dehydrogenase is a tetramer (~140-kDa) in all animal tissues. It is found in several forms in various tissues; while each form catalyzes the same reaction, they differ in electrophoretic mobility in the native state. In heart muscle it is predominately represented by a molecule comprised of four identical polypeptide chains (subunits); this form is designated H$_4$. The predominate species in white skeletal muscle is a tetramer designated as M$_4$. While highly homologous, their primary structures are not identical.

Figure 1 (see next page) illustrates the net charge on H and M as a function of pH.

A. What is the approximate isoelectric point of M$_4$ and H$_4$, respectively? Explain your rational. Be specific.(3 points)

B. If LDH M$_4$ and H$_4$ were subjected to NATIVE electrophoresis at pH 7 and the gel was stained for enzymatic activity, what would the pattern look like. Use the rectangle below to indicate your answer. Assume that the samples are applied in the middle of the rectangle and that the positive pole is to the right and the negative pole is to the left. Explain your rationale. (3 points)
C. Assume that the subunits of LDH have virtually identical conformations, and that a mixture of M₄ and H₄ was dissociated into monomers at pH 2.3 and then allowed to refold at neutral pH. What would you expect the pattern to look like if this reactivated, NATIVE, sample was applied to a similar gel and electrophoresed as described above. Explain your rationale. (3 points)

D. What would an SDS-PAGE gel look like if M₄ and H₄ were run under reducing conditions? Please use the rectangle below to show the pattern. Assume that samples were applied at the top, that the positive pole is at the bottom of the gel and the negative pole at the top. (3 points)

Continued on Page 10
VII. (6 points)

1  KETAAKFERQHMDSTSAA SSSNYCNQMM KSRNLTKDRC KPVTFFHES
51  LADVQAVCSQ KNVACKNGQT NCYQSITMS ITDCRETGSS KYPNCAVKTT
101 QANKHIIVAC EGNPYVPVHF DASV

The figure above shows the primary structure of bovine ribonuclease A (RNAase)

A. How many fragments would be expected to be produced when RNAase is digested with trypsin. Your rationale must be clear. (3 points)

B. Identify ONE of the dipeptides that would be found in such a tryptic digest. Estimate its isoelectric point. Your choice and rationale must be clear; be specific. (3 points)
VIII. [8 points]
The experimental curves for an enzyme catalyzed reaction, with initial velocity expressed as a percentage of $V_{\text{MAX}}$, plotted versus [S] are illustrated below. The lower curve is for results obtained in the presence of an inhibitor.

A. What type of inhibition is indicated? Explain

B. Write out an appropriate sequence of reactions that describe the mechanism of inhibition represented by these results. Explain
 IX. [9 points]
Some molecular interactions involved in enzymatic catalysis are associated with substrate binding while others are intimately involved in the mechanism that leads to conversion to products. As emphasized in class, as well as in the assigned reading, one approach to investigating and discriminating among specific residues with respect to their roles in these two types of interactions is site directed mutagenesis of various amino acids in the polypeptide(s) that comprise the enzyme.

A complementary approach involves synthesis of various substrate derivatives, followed by study of the kinetics of catalysis by the same (unmodified) enzyme preparation. The structures of three substrates for chymotrypsin are illustrated below. Chymotrypsin catalyzes the cleavage of each of these compounds at the bonds indicated by the arrows.

A. Circle the group on these substrates that is most involved in interaction with the substrate specificity pocket. Explain your choice. (3 points)

B. The table provides a summary of relevant kinetic data obtained for the chymotrypsin-catalyzed hydrolysis of these compounds. What do these data suggest as to the effect of the indicated substitutions on substrate binding and/or complementarity with (and stabilization of) the transition state?
Explain your rationale. (6 points)

<table>
<thead>
<tr>
<th></th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$K_m$ (mM)</th>
<th>$k_{cat}/K_m$ (M$^{-1}$s$^{-1}$)</th>
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<tbody>
<tr>
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<tr>
<td>Substrate B</td>
<td>0.14</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Substrate C</td>
<td>2.8</td>
<td>25</td>
<td>114</td>
</tr>
</tbody>
</table>
A. Circle the group on these substrates that is most involved in interaction with the substrate specificity pocket. Explain your choice. (3 points)

B. The table provides a summary of relevant kinetic data obtained for the chymotrypsin-catalyzed hydrolysis of these compounds. What do these data suggest as to the effect of the indicated substitutions on substrate binding and/or complementarity with (and stabilization of) the transition state? Explain your rationale. (6 points)

X. [12 points]
Answer A OR B; only your first answer will be graded. Full credit will be awarded for the most concise answer which provides all the relevant information.

A. Describe the rationale for the production of catalytic antibodies, including, among other things, relevant principles of catalysis.
B. A molecular graphics tutorial developed by Gale Rhodes (Prof. of Chemistry, University of Southern Maine) poses the following question. Are Ramachandran Plots Useful? It also offers the following answer.

During the last stages of structure determination of proteins by any method -- x-ray crystallography, NMR, or homology modeling -- structural biologists use a variety of tools, including Ramachandran plots, to call their attention to unrealistic conformations in their models. Such plots plainly signal residues that need further work before the entire model can be declared chemically realistic.

1. Describe a Ramachandran plot in as much detail as you are able. What was the experimental basis for the design of the plot?

2. What is the most stable configuration of the peptide bond?

3. A Ramachandran plot of the well established native structure of lysozyme reveals three non-trans peptide bonds at residues N19, W63 and P70. How do you explain this fact in the light of your answer to part 2?

XI. [16 points]


Part of the text for the following question is excerpted from the reference cited above. In addressing this
question, you will be called upon to recall several significant aspects of the structure and function of Hb; including: the subunit structure/composition of Hb from normal adult humans, the significance of the cooperative binding of oxygen, the nature and significance of Bohr effect, the regulation of oxygen binding by BPG, the molecular basis of BPG binding, all of which were discussed in class. You will also need to reflect on the structure, function, and regulatory properties of fetal Hb.

Read the question and then address the each of the questions below, in as much specific detail as you are able. Credit awarded will be proportional to the accuracy, specificity of relevant detail and clarity of your answers.

PART I

A. What is the subunit composition of normal Hb in adult humans? (2 points)

B. What is the Bohr effect? What is its physiological significance in the overall scheme of oxygen transport in animals? NOTE: I am NOT asking you to describe the molecular basis of the Bohr effect. (6 points)

C. What is the subunit composition of fetal Hb in normal humans prior to birth? (3 points)

D. How do the adult and fetal Hb's differ with respect to regulation of oxygen binding by BPG? Why? Be specific. (5 points)

For Extra Credit: [10 points]

PART II

In normal healthy humans, transcription and translation of the alpha-type and beta-type globin chains produces closely matched quantities of each type that spontaneously associate to form the functional molecule. In the conditions known as the thalassemias however, mis-matched
quantities of globin chains are produced. Hemoglobin (Hb) Bart's is present in the red blood cells of millions of people worldwide who suffer from "-thalassemia. "-Thalassemia is a disease in which there is a deletion of one or more of the four "-chain genes. The resulting excess ( and ß chains spontaneously form homotetramers; (4 (Hb Bart's) and $4 (HbH). Furthermore, since the globin chain is expressed at all stages of human development, two main varieties of development-stage linked homotetramers are formed in "-thalassemias. During the adult stage the predominant abnormal homotetramer is HbH (ß4), while at the fetal stage Hb Bart's (4) predominates.

Hb Bart's is a stable species that exhibits a higher affinity for O₂ than normal adult human Hb but binding is not cooperative and it does not exhibit a Bohr effect.

A. What do these characteristics tell you about (4 as a functional oxygen carrier? [Hint: Think about the overall physiological role of Hb.] Explain in detail. (5 points)

B. Relative to HbA (the major Hb in normal adults), what would you expect with respect to binding and regulation of Hb Bart's by BPG? Explain in detail. (5 points)

More details about "-thalassemia — for interest only — read later.
Alpha thalassemia is associated with four general clinical syndromes that correspond with the loss of increasing numbers of the four Hb " genes: (1) The silent-carrier state ("-thalassemia 2). One gene is missing. This condition is entirely symptomless with 1–2% Hb Bart's present at birth. (2) Classical "-thalassemia trait ("-thalassemia 1). Two genes are missing. About 5% Hb Bart's is present at birth. The symptoms, minor red blood cell abnormalities but no anemia, are the same for either homozygous or heterozygous individuals. (3) Hemoglobin H disease. Three " genes are missing, resulting in the presence of Hb Bart's in infancy and HbH in adulthood. (4) Hydrops fetalis. All four " genes are missing. This condition is fatal, leading to stillbirth in the last few weeks of pregnancy. Therefore, the presence of Hb Bart's is an important indicator of a major health disorder. Hb Bart's occurs in tens of millions of humans worldwide, and reaches a frequency of over 40% in the Laotian population of South-East Asia.

<table>
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<th>Three-letter</th>
<th>One-letter</th>
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17
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<tr>
<th></th>
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