Name_________________________  

BIO 451  

KEY  

EXAM I  

23rd September, 2002  

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.**

Credit awarded will be proportional to the accuracy, relevancy, clarity, and legibility of the information you provide in your answers.

The process of grading and recording typically requires 7 to 10 days.

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I. [10 points]
Proteins are linear polymers of α-amino acids linked together by peptide bonds.

A. Draw a peptide bond; label all relevant components (keep part B in mind). (2 pts)

See text or class notes; proper linkages, as well as proper trans-orientation of relevant groups must be shown.

B. Describe the structural features of the peptide bond in as much detail as you are able, including all constraints that are relevant to the native conformation of polypeptides. (6 pts)

The following details must be included.
1. The $C_{\text{carbonyl}} - N$ bond has partial double bond character
2. The trans configuration is generally most stable
3. The relevant atoms must be coplanar
4. Only two degrees of freedom are allowed $C_\alpha - N$ and $C_\alpha - C$, along with designation of which torsional angle is $\phi$ and which is $\psi$.

C. How do these structural features relate to the stabilization of secondary structure of typical globular proteins? (2 pts)

When $\phi_1 = \phi_2 = \phi_3$, etc. AND $\psi_1 = \psi_2 = \psi_3$, etc., repeating hydrogen bonds are generated. This creates segments of $\alpha$-helix OR $\beta$-strands
II. [18 points]
This question is designed to test your familiarity with the molecular basis of the difference(s) between normal (HbA) and sickle cell (HbS), as well as several fundamental principles associated with the characterization of proteins. Recall that each of these proteins is comprised of four polypeptides, each of which has ~150 residues.

A. In what specific way(s) do the primary structures of HbA and HbS differ? (2 pts)

*E at position 5 in the β-strands of HbS are replaced by Val.*

Plots of Net Charge vs pH for HbA and HbS are virtually identical over the pH range from 1 to 13. Over the range from pH 6 to 10, however, significant differences are observed. (Please see next page) NOTE: if part of your answer is on the graph, do not forget to put your name on it and submit it with the rest of your exam.

B. Estimate the isoelectric point of each hemoglobin. Your rationale must be clear. (2 pts)

*Y has a pI of ~ 8.0  (net charge of 0)*

*X has a pI of ~ 8.5  (net charge of 0)*

C. Identify the set of data points that correspond to HbA and HbS, respectively. Your rationale must be clear. (3 pts)

*Y corresponds to HbA ---- lower pI due to E;  X corresponds to HbS*

D. Which of these hemoglobins is more acidic? Why? Explain. (2 pts)

*HbA  --- has two additional E's*
E. Sketch the relative positions of the native molecules if a mixture of the two Hb's was applied in the center of a gel, indicated by the rectangle below, and subjected to electrophoresis in the absence of a detergent at pH 7; assume that the positive pole is on the right. Assume that the sample is placed in the middle of the pattern and don't forget to label each of them. (3 pts)

```
          HbS    HbA
```

F. Assume that Hb’s A and S were heated separately with SDS and a reducing agent and then analyzed in separate lanes by SDS-PAGE.

1. How many protein bands would you expect to observe in each lane? Explain. (3 pts) NOTE: Reread the first paragraph on page 3.

   **SDS-PAGE separates on the basis of polypeptide chain size.**

   "Each chain has approximately the same number of residues, but alpha is not identical to beta. Therefore, one, or perhaps two closely spaced bands would be observed."

2. What would be the approximate size of each of the polypeptides? Explain. (3 pts)

   \[ 150 \times 110 = 16.5 \text{kDa} \]
III.  [ 9  points]

Each of the following is used in the determination of the primary structure of proteins. Describe the specific purpose of THREE of them. For full credit you must be specific and sufficiently detailed to illustrate your familiarity with the importance of these reagents. [For example, it is not sufficient to tell me that dansyl chloride reacts with primary amines.] ONLY YOUR FIRST THREE answers will be graded. Please DO NOT TRY TO ANSWER IN THE SPACE BESIDE THE INDIVIDUAL ITEMS.
A. Iodoacetic acid
B. 2- Mercaptoethanol
C. Cyanogen bromide 
D. Phenylisothiocyanate
E. Chymotrypsin
F. Dansyl chloride

Please see the text or class notes.
IV. [9 points]
Match the amino acids in the column on the left with the appropriate side chain type(s) in the column to the right. [1.5 points for each correct response]

a) R __3__
   1) Nonpolar aliphatic
b) D __4__
   2) Nonpolar aromatic
c) V __1__
   3) Basic
d) M __7__
   4) Acidic
e) F __2__
   5) Selenium-containing
f) S __6__
   6) Hydroxyl-containing
   7) Thioether-containing

V. [6 points]
Mutations in the polypeptide chains of collagen in which some of the G residues are replaced by C or S can be lethal. Explain in as much detail as you are able.

*The tensile strength and general stability of collagen is critically dependent on the close approximation of the individual strands to form the triple helix. Normal collagen contains about 1/3 G residues; it is the only amino acid with an R group sufficiently small to allow proper folding.*

*Collagen is the most abundant animal protein. For this reason a defect in collagen structure would be expected to be lethal.*
VI. [11 points]
Demonstrate your familiarity with the fundamentals of structure/function relationships for the major oxygen transport proteins by matching the appropriate items on the left with the most relevant descriptive statements on the right. **Only your first 11 choices will be graded.**

| **A. O₂** |  | **F** 1. Binds to Hb at multiple non-Heme sites, causing an increase in $P_{50}$ |
| **B. His-F8** |  | **D** 2. Binds to a single site; in its absence, the O₂ saturation curve for Hb is much less sigmoidal. |
| **C. His-E7** |  | **A** 4. On binding, it induces a shift in electron distribution for the iron atom |
| **D. BPG** |  | **B** 5. Occupies the 5th coordination position of the Fe(II) in heme in Hb and Mb |
| **E. His 146** |  | **F,I,D** 6. Among the principal physiological regulators of the oxygenation of Hb. |
| **F. H⁺** |  | **I** 7. Forms a covalent derivative of the N-termini of the β-chains |
| **G. HbM** |  | 8. Occupies the 6th coordination position of the Fe(II) in heme in deoxyHb and deoxyMb. |
| **H. Hb_Hammersmith** |  |  |
| **I. CO₂** |  | **C** 9. Thought to be partially responsible for the “bent” orientation of the binding of O₂ to Mb and Hb. |
| **J. H₂O** |  | **E** 10. Undergoes a major shift in pK in the tissues; associated with the Bohr effect |
VII. [ 8 points]

A. Sketch a plot of velocity vs substrate concentration for an enzyme that follows Michaelis-Menten kinetics. (2 pts)

*Draw a simple hyperbolic curve; label the axes appropriately.*

B. Write a set of equations that represent the simplest mechanism that describes such kinetic behavior. (2 pts)

\[ E + S = ES \rightarrow E + P \]

The reaction catalyzed by lactic acid dehydrogenase (LDH) in muscle under anaerobic conditions is:

\[ \text{NADH} + \text{Pyruvate} \rightarrow \text{NAD}^+ + \text{Lactate} \]

C. The graph below (next page) summarizes the data obtained when the activity of purified LDH from cardiac muscle was measured as a function of pyruvate concentration, at constant (saturating) NADH.

Postulate a rational mechanism (with appropriate equations) for the shape of the curve. (NOTE: You may not invoke experimental artifacts, contaminants etc.) (4 pts)

*Substrate binds to for a proper ES complex, and then binds more substrate at a different or altered site. The ternary complex is not active.*

\[ E + S = ES \rightarrow E + P \]
\[ ES + S = ES_2 \rightarrow NR \]
VIII. [10 points] (1 point for each correct response)

For each description associated with enzyme kinetics or inhibition, select the corresponding constant, type of inhibition, or appropriate term/abbreviation.

___c___ parameter most closely associated with complementarity between and enzyme and the transition state of the reaction it catalyzes

___d___ best measure of the catalytic efficiency of an enzyme

a) d[ES]/dt ≠ 0

___l___ an expression of the steady state assumption

b) [E_t]

___c___ turnover number

___m___ type of inhibition characterized by lower V_m and higher K_m

c) k_{CAT}

___f___ [S] at ½ V_M

___i___ a mechanism of inhibition in which an inhibitor binds with equal affinity to both E_free and ES

d) k_{CAT}/ K_M

e) v_0

___k___ type of inhibition associated with diminished K_M and V_M

___h___ type of inhibition that is overcome at saturating [S]

f) K_M

___b___ initial velocity at saturating [S] is first order in this variable.

g) V_M

h) Competitive inhibition

_____ type of inhibition characterized by unchanged K_M and higher V_M

i) Noncompetitive inhibition

j) Suicide inhibition

k) Uncompetitive inhibition

l) d[ES]/dt = 0

m) Mixed
IX. [6 points]
Papain is a plant cysteine protease isolated from papaya latex. It is a 212-amino acid polypeptide with well-known biological and structural properties. Its catalytic mechanism involves the following residues: C25, H159, and N175, that reside in a cleft between two domains. During catalysis the reactive thiol of C25 forms a covalent acyl enzyme intermediate with the carbonyl carbon of the scissile bond.

Compare and contrast the active site residues in this cysteine protease, and its mechanism, with that of typical serine proteases in as much detail as you are able. [NOTE: I am NOT asking you for a detailed analysis, with structures.]

Describe the catalytic triad for a serine protease $D,H,S$. Designate the residue that forms and acyl intermediate with product 2. Compare with the C protease; e.g., both have an imidazole, one has $D$ and the other has $N$. The mechanisms of both involve covalent catalysis; one involves an acyl-$S$ while the other involves an acyl-$C$. 
The primary structure (one continuous polypeptide) of the \( \alpha \)-chain of human hemoglobin is presented in the figure below.

\[
\begin{align*}
\text{VLSPADK} & /\text{TNVK} / \text{K} / \text{AWGK} / \text{VGAHAGEYGAEARL} / \text{MFLSFPTTK} / \text{TYFP} \\
\text{HFDLSHGSAQVK} & / \text{GHGK} / \text{K} / \text{VADALTNAHVDDMPNALSLSDLHAAH} \\
\text{LR} & / \text{VDPPNFK} / \text{LLSHCLLVTLAALPAPAEFTPAVHASLDKFLASVSTVLSK} / \\
\text{YR}
\end{align*}
\]

For full credit, in each instance, the rationale for your response must be clear and explicit. You must also demonstrate that your answer is consistent with ALL of the information provided.

a. Mark ALL potential trypsin cleavage sites. Be sure to state why you chose these sites. (2pts)

*Trypsin cleaves on the carboxyl side where there are K or R residues.*

d. Verify that you predict THREE tetrapeptides; circle them. (1 pt)

c. The titration curve for one of the three tetrapeptides, isolated from the tryptic digest, exhibits a clear inflection point at pH 6.1. This tetrapeptide does not exhibit UV absorbance in the 280nm region, and is not cleaved by chymotrypsin.

Write the sequence for the tetrapeptide; the rationale for your choice must be clear and consistent with all the information provided. (3 pts)

*The peptide is GHGK; it is the only one of the tetrapeptides having an imidazole groups (H residue) that ionizes in the indicated pH range. Only W, F, and Y absorb in the 280nm region.*

CONTINUED
The α-chains of a mutant hemoglobin, (β-chains are the same as for normal Hb), are identical in every way to the α-chains of normal hemoglobin, including cleavage sites for trypsin, except for a single amino acid replacement. This hemoglobin exhibits a strong tendency toward conversion to methemoglobin.

d. A tryptic digest is prepared of the mutant α-chains; the individual fragments were isolated. The titration curves for none of the three tetrapeptides from the mutant α-chain exhibits an inflection point in the range of pH 6.1. One tetrapeptide, however, exhibits significant UV absorbance in the 280nm region; this spectrum shifts dramatically above pH 10. It is also subject to cleavage by chymotrypsin.

Write the sequence for this peptide and show that this structure is consistent with the information provided. (3pts)

only chain in 6H6L → 6Y6K
Chymotryptic cut after Y, F, W
280nm absorbance → Y, F, W
PK & Y = 10.46

4. Estimation of the pI of the mutant tetrapeptide for which you gave the sequence in part d. For full credit your rationale must be clear. (4pts)

\[
\text{pI} = \frac{\varepsilon + 10.46}{2} = 9.68
\]
<table>
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<th>Amino Acid</th>
<th>Three-letter abbreviation</th>
<th>One-letter symbol</th>
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<tr>
<td>Alanine</td>
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<tr>
<td>Arginine</td>
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<td>R</td>
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<td>Aspartic Acid</td>
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<td>D</td>
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<td>Aspargine or Aspartic Acid</td>
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