

- Possibly useful information is on the last page.
- There are a mix of short answer and longer problems: look over the exam before beginning, to optimize your use of time. Read carefully!!!
- SHOW YOUR WORK in order to receive partial credit.

**I. (3 points each)** Circle the letters of the correct answers.

1. Which of the following best approximates the pH of the cytosol in a living human cell?
  - a. 3
  - b. 5
  - c. 7
  - d. 9
  - e. 11
2. Which of the following best describes the arrangement of amino acid side chains in an alpha helix?
  - a. The side chains point outward away from the helical axis.
  - b. The side chains point inward toward the center of the helix.
  - c. The side chains point toward the N-terminal end of the helix.
  - d. The side chains point toward the C-terminal end of the helix.
  - e. The side chains point toward the nearest beta sheet.
3. Which of the following is least likely to be found in the hydrophobic core of a protein?
  - a. Alpha-helix
  - b. Beta sheet
  - c. Reverse turn or loop
  - d. disulfide bond
  - e. leucine
4. Which of the following is characteristic of a competitive inhibitor of an enzyme-catalyzed reaction?
  - a. The inhibitor binds covalently to the enzyme.
  - b. The inhibitor binds noncovalently to the substrate.
  - c. In the presence of the inhibitor, the reaction exhibits an apparent increase in  $K_M$ .
  - d. In the presence of the inhibitor, the reaction exhibits an apparent decrease in  $V_{max}$ .
  - e. Increasing the substrate concentration fails to overcome the effects of the inhibitor.
5. Binding of a ligand to protein X affects the binding properties of another site on X. This is an example of which of the following phenomena?
  - a. Allosteric regulation
  - b. Competitive inhibition
  - c. Noncompetitive inhibition
  - d. Induced fit
  - e. Transition state stabilization
6. What is the net charge on the following peptide at pH 7? Ala-Lys-Arg-Gly-Pro-Asn
  - a. +3
  - b. +2
  - c. +1
  - d. 0
  - e. -1

7. For weak acids and bases, including several amino acid sidechains, at  $\text{pH} = \text{pK}_a - 1$ :
- [protonated]=[deprotonated]
  - these groups are 90% deprotonated
  - these groups are 90% protonated
  - these groups are neutral

**II. Short Answer:** Write neatly in the space provided.

**8. (3 points)** Asp185 at the base of the specificity pocket in trypsin accounts for the preferred cleavage after Arg and Lys residues. Propose a change you could make in trypsin by site-directed mutagenesis that could lead to specific cleavage after Asp and Glu residues.

Asp185Lys mutation would replace the negative Asp that interacts favorably with positive Arg/Lys in the specificity pocket with a positive Lys to interact favorably with negative Asp/Glu.

**9. (14 points)** A graduate student is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present.

	pI (isoelectric point)	Size $M_r$	Bind to DNA?
protein A	7.4	82,000	yes
protein B	3.8	21,500	yes
protein C	7.9	23,000	no
protein X	7.8	22,000	yes

What type of protein separation techniques might she use to separate the following:

- (a) protein X from protein A? size exclusion or gel filtration chromatography or gel electrophoresis for analytical, not preparative, separation
- (b) protein X from protein B? ion exchange chromatography or isoelectric focusing for analytical, not preparative, separation
- (c) protein X from protein C? affinity chromatography, with immobilized DNA on column
- (d) If protein A is a protease, what chromatography step do you recommend performing first and why?

Start with size exclusion or gel filtration chromatography to rapidly remove the protease before it cleaves your protein.

(e) She analyzes the steps of her protein purification and final sample with SDS-PAGE. List two properties of her protein that she can deduce using this method and one that she cannot.

Can deduce

1.

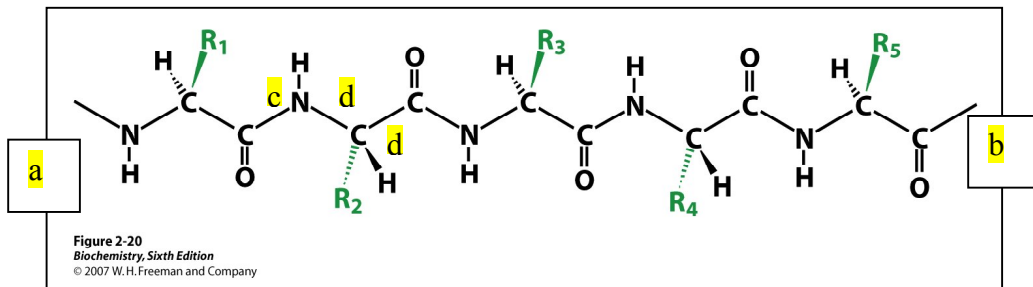
2.

Cannot deduce

1.

10. (8 points). Neatly label with letters a-d the following on the polypeptide chain below:

- amino terminal side
- carboxy terminal side
- 3 backbone bonds with restricted rotation due to partial double bond character
- 3 backbone bonds with free rotation whose conformation is plotted on a Ramachandran plot



11. (6 points) An enzyme catalyzes the reaction  $A \rightarrow B$ . The initial rate of the reaction was measured as a function of the concentration of A. The following data were obtained:

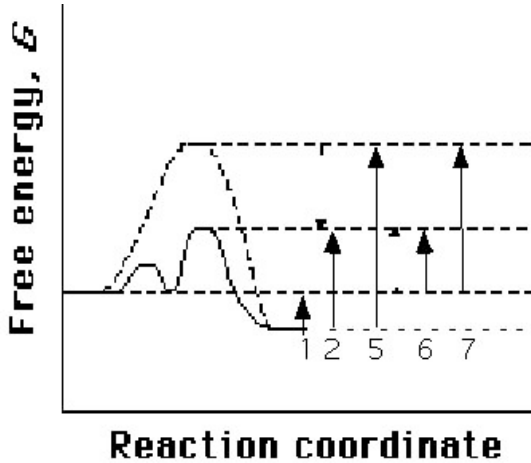
<u>[A], micromolar</u>	<u><math>V_0</math>, nmoles/min</u>
0.05	0.08
0.1	0.16
0.5	0.79
1	1.6
5	7.3
10	13
50	40
100	53
500	73
1,000	76
5,000	79
10,000	80
20,000	80

- What is the  $K_m$  of the enzyme for the substrate A?
- What is the value of  $V_0$  under these conditions when  $[A] = 43$ ?

13. (15 pts) The figure below shows a reaction coordinate diagram for a favorable conversion of S to P, uncatalyzed (dashed line) and catalyzed by an enzyme (solid line). For the numbered free energy differences on the diagram, fill in the blanks to indicate the corresponding  $\Delta G$  and the kinetic or thermodynamic constants that it determines (use those listed below):

$$\Delta G^{\ddagger}_{\text{for(cat)}}, \Delta G^{\ddagger}_{\text{rev(cat)}}, \Delta G^{\ddagger}_{\text{for(uncat)}}, \Delta G^{\ddagger}_{\text{rev(uncat)}}, \Delta G_{\text{(cat)}}, \Delta G_{\text{(uncat)}}$$

$$K_{\text{cat}}, K_{\text{uncat}}, k_{\text{rev(uncat)}}, k_{\text{for(uncat)}}, k_{\text{rev(cat)}}, k_{\text{for(cat)}}$$



1  $\Delta G_{\text{(cat)}}, \Delta G_{\text{(uncat)}}, K_{\text{cat}}, K_{\text{uncat}}$

2  $\Delta G^{\ddagger}_{\text{rev(cat)}}, k_{\text{rev(cat)}}$

5  $\Delta G^{\ddagger}_{\text{rev(uncat)}}, k_{\text{rev(uncat)}}$

6  $\Delta G^{\ddagger}_{\text{for(cat)}}, k_{\text{for(cat)}}$

7  $\Delta G^{\ddagger}_{\text{for(uncat)}}, k_{\text{for(uncat)}}$

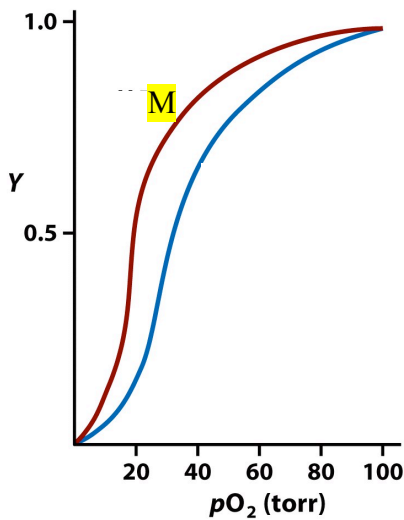


Figure 4-50 Principles of Biochemistry, 4/e  
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14. (11 points) The figure shows fractional binding curves for an oxygen-binding protein. Circle from the following list the properties of this protein that you can deduce from the figure.

- a) Single binding site
- b) Multiple binding sites
- c) Identical and independent binding sites
- d) Cooperative binding
- e) Single  $K_d$  for binding

You obtain these two curves in the presence and absence of an allosteric modulator that binds only to the R (oxy) state:

- f) Label with an "M" the curve in the presence of the allosteric modulator.
- g) Sketch a curve you might observe if you increase the concentration of the modulator.

Sigmoidal curve between red curve and y axis

15. (12 points) Use the figure to answer the following questions about the mechanism of lactate dehydrogenase.

- (a) What mode of rate enhancement is illustrated by the proposed role of His<sub>195</sub>?

General acid/base

- (b) Suppose you mutate Arg171 to Glu. Assuming that your enzyme follows Michaelis-Menten kinetics, predict which kinetic parameter would change most and how it would change. Explain briefly.

Increase  $K_M$  – because affects binding more than catalysis. Worse binding because of repulsion of negative Glu vs negative substrate, increases  $K_M$  (need higher [S] to get full binding).

- (c) On the plot shown for the kinetics of the wild type enzyme, sketch a predicted curve for the mutant enzyme.

Curve of similar shape, below red curve – reaches same max but at higher [S]

- (d) Sketch a pH profile (reaction velocity vs pH) you predict for this mechanism, indicating any important pK<sub>a</sub>'s on your diagram. (assume you are measuring at low [S]  $\ll$   $K_m$ , ignore the rest of the enzyme beyond the groups shown, and use the table of pK<sub>a</sub> values)

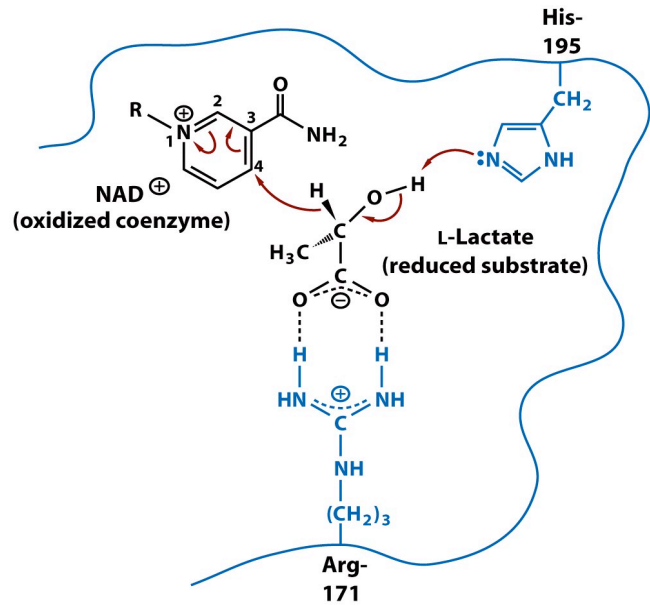
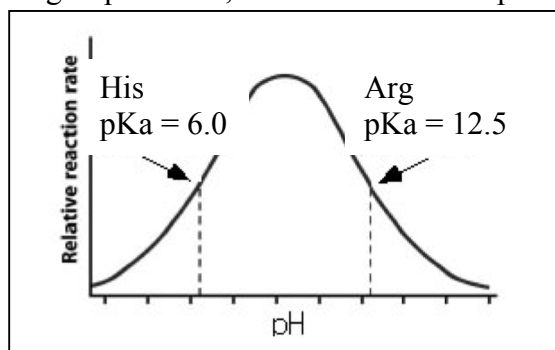


Figure 7-9 Principles of Biochemistry, 4/e  
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