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EXAM III
November 6, 2000
Biochemistry I

I. _____/45

II. _____/35

III. _____/25

TOTAL /100

I. **MULTIPLE CHOICE**. (45 points; 3 points each)
Choose the BEST answer to the question by circling the appropriate letter.

1. In competitive inhibition, an inhibitor:

   A. binds at several different sites on an enzyme.
   B. binds reversibly at the active site.
   C. binds only to the ES complex.
   D. binds covalently to the enzyme.
   E. lowers the characteristic V_{max} of the enzyme.

2. In a plot of 1/V against 1/[S] for an enzyme-catalyzed reaction, the presence of a competitive inhibitor will alter the:

   A. V_{max}.
   B. intercept on the 1/V axis.
   C. intercept on the 1/[S] axis.
   D. curvature of the plot.
   E. pK of the plot.

3. Enzyme X exhibits maximum activity at pH = 6.9. X shows a fairly sharp decrease in its V_{max} when the pH goes much lower than 6.4. One likely interpretation of this pH activity is that:

   A. a residue with a pK_a of 6.4 is involved in binding
   B. a His residue on the enzyme is involved in the reaction.
   C. a Glu residue on the enzyme is involved in the reaction.
   D. the enzyme is found in gastric secretions.
   E. the enzyme has a metallic cofactor.

4. An average protein will not be denatured by:

   A. sodium dodecyl sulfate.
   B. pH 10.
   C. heating to 90 °C.
   D. urea.
   E. iodoacetic acid.

5. Which of the following is false about the catalytic mechanism of acetoacetate decarboxylase?

   A. the mechanism involves general acid/base catalysis
   B. the mechanism involves covalent catalysis
   C. the mechanism involves metal-ion catalysis
   D. the mechanism involves a Schiff base
   E. the mechanism involves the stabilization of a carbanion

6. Protein S will fold into its native conformation only when protein Q is also present in solution. However, protein Q can fold into its native conformation without protein S. Protein Q therefore, may function as a _____ for protein S.

   A. molecular chaperone
   B. protein precursor
   C. ligand
   D. supersecondary structural unit
   E. structural motif

7. If a solution of equal amounts of hemoglobin and myoglobin is bubbled with a small amount of oxygen:

   A. the subunits of hemoglobin will be replaced by myoglobin.
   B. a polymer will form with alternating hemoglobin-myoglobin units.
   C. much more of the dissolved oxygen will bind to myoglobin than to hemoglobin.
   D. hemoglobin subunits will dissociate.
   E. hemoglobin will reach nearly 100% saturation with oxygen while myoglobin will remain essentially as deoxymyoglobin.

8. In an aqueous solution, protein conformation is determined by two major factors. One is the formation of the maximum number of hydrogen bonds. The other is the:

   A. formation of the maximum number of hydrophilic interactions.
   B. maximization of ionic interactions.
   C. minimization of entropy by the formation of a water solvent shell around the protein.
   D. placement of polar amino acid residues around the exterior of the protein.
   E. placement of hydrophobic amino acid residues within the interior of the protein.

9. Allosteric enzymes:

   A. usually have only one active site.
   B. are regulated primarily by covalent modification.
   C. usually have more than one polypeptide chain.
   D. usually show strict Michaelis-Menten kinetics.
   E. usually catalyze several different reactions within a metabolic pathway.

10. A transition-state analog:

    A. resembles the transition-state structure of the normal enzyme-substrate complex.
    B. typically reacts more rapidly with an enzyme than the normal substrate.
    C. is less stable when binding to an enzyme than the normal substrate.
    D. stabilizes the transition state for the normal enzyme-substrate complex.
    E. resembles the active site of general acid-base enzymes.

NAME _____

11. A small molecule that decreases the activity of an enzyme by binding to a site other than the catalytic site is termed a(n):

A. alternative inhibitor.
B. allosteric inhibitor.
C. homotropic activator (positive effector).
D. competitive inhibitor.
E. transition-state analog.

12. The binding of one O_2 to a molecule of hemoglobin results in:

A. the release of any other O_2 that may have bound earlier.
B. a decrease in hemoglobin's ability to bind a second O_2.
C. dissociation of the hemoglobin subunits.
D. the movement of hemoglobin to an organism's muscle tissue.
E. an increased affinity for O_2 in the remaining subunits (which have not yet bound O_2).

13. Experiments on denaturation and renaturation after the reduction and reoxidation of the -S-S- bonds in the enzyme ribonuclease (RNase) have shown that:

A. the primary sequence of RNase is sufficient to determine the formation of a specific secondary and tertiary structure.
B. the enzyme, dissolved in water, is thermodynamically stable relative to the mixture of amino acids whose residues are contained in RNase.
C. native ribonuclease does not have a unique secondary and tertiary structure.
D. the completely unfolded enzyme, with all -S-S- bonds broken, is still enzymatically active.
E. the folding of denatured RNase into the native, active conformation, requires the input of energy in the form of heat.

14. Which of the following statements about the Bohr effect is true?

A. Lowering the pH shifts the oxygen dissociation curve of hemoglobin to the right (lower affinity).
B. The acidic environment of an exercising muscle allows hemoglobin to bind O_2 more strongly.
C. The affinity of hemoglobin for O_2 is improved by high concentrations of CO_2.
D. In the lungs, the presence of higher concentrations of H^+ and CO_2 allows hemoglobin to become more oxygenated.
E. In the lungs, the presence of higher concentrations of O_2 promotes the binding of CO_2 and H^+.

15. Which of the following statements about allosteric control of enzymatic activity is false?

A. Allosteric proteins are generally composed of several subunits.
B. Heterotropic effectors compete with substrate for binding sites.
C. Binding of the effector changes the conformation of the enzyme.
D. An effector may either inhibit or activate an enzyme.
E. Allosteric proteins are generally have Hill coefficients greater than 1.0

NAME _____

II. **SHORT ANSWER** (35 points)
Give a brief answer or fill in the blanks as directed to each problem or question below.

16. Consider two proteins: myoglobin, a typical soluble protein, and rhodopsin, a typical membrane protein. Discuss briefly the probable arrangement of hydrophobic and hydrophilic amino acid residues in each of the proteins in its native conformation. It may be helpful to draw simple diagrams. (8 pts)

17. On the enzyme hexokinase, ATP reacts with glucose to produce glucose-6-phosphate and ADP five orders of magnitude faster than ATP reacts with H_2O to form phosphate and ADP. The intrinsic chemical reactivity of the -OH group in water is about the same as that of the glucose molecule, and water can certainly fit into the active site. Explain this rate differential in two sentences or less. (6 pts)

18. What is the difference between the data in a file or record from the GenBank database versus that from the Protein database? (5 pts)

19. Explain how a mutation in the regulatory (R) subunit of cAMP-dependent protein kinase (PKA), making R incapable of binding to the catalytic (C) subunit, might result in either loss of responsiveness to a given hormone or production of a continuous signal even in the absence of the hormone. (5 pts)

20. An enzyme follows Michaelis-Menten kinetics. Indicate (with an "x") which of the kinetic parameters at the left would be altered by the following factors. Give only one answer for each. (5 pts)

K_m	V_{max}	Neither	Both	
				(a) a competitive inhibitor
				(b) a noncompetitive inhibitor
				(c) a suicide inactivator
				(d) 6 M urea
				(e) doubling [S]

NAME _____

21. Hemoglobin (Hb) is an allosteric enzyme whose binding of oxygen can be increased by positive homotropic effects or decreased by negative heterotropic effectors. Draw a plot of fraction bound (Y) vs. $[O_2]$ for Hb as it might be found in the blood, then draw a curve for HB + a negative effector. Name one such negative heterotropic effector.(6 pts)

III. **MECHANISMS**. (25 points)

22. You have been working on the mechanism of a β-keto decarboxylase. You find that reaction of the β-keto substrate with the enzyme in $H_2{}^{18}O$ yields product with the keto group labeled with ^{18}O. This is evidence for a mechanism with what kind of intermediate? What further experiment could you do to confirm the mechanism? How do the results of either experiment argue against metal ion catalysis? (10 pts)

23. For serine to work effectively as a nucleophile in covalent catalysis in chymotrypsin a nearby amino acid, histidine, must serve as general base catalyst. Briefly describe how these two amino acids work together. Draw the reaction of a active-site serine with a peptide to form the first enzymic intermediate (be sure to show the electron shifts). (15 pts)

Answers for Exam 3 11/6/2000

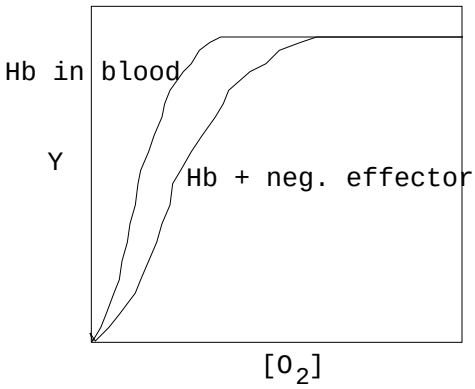
Test Question	Correct Answer
	The following questions are in section “Multiple Choice”
1	B
2	C
3	B
4	E
5	C
6	A
7	C
8	E
9	C
10	A
11	B
12	E
13	A
14	A
15	B
	The following questions are in section “Short Answer”
16	In a globular, water-soluble protein such as myoglobin, most of the hydrophobic side chains are inside the protein and away from the aqueous solvent, while most of the polar and charged residues have their side chains on the outside surface, in contact with the solvent. In a membrane protein such as rhodopsin, the hydrophobic residues occur on the outside, where they contact the lipid bilayer, and the polar residues tend to cluster on the two faces of the membrane bilayer, where they contact the polar, aqueous phase.

17	The binding of glucose to hexokinase induces a conformation change that brings the amino acid residues that facilitate the phosphoryl transfer into position in the active site. Binding of water alone does not induce this conformational change.

18	GenBank data is in the form of DNA sequences. Protein data is in the form of protein structures with a list of x, y, z coordinates.

19	When a mutation in the R subunit of cAMP-dependent protein kinase prevents R-C interaction, the inhibitory effect of R is lost, and the catalytic subunit continues to phosphorylate target proteins regardless of cAMP concentration.

20	(a) K_m; (b) V_{max}; (c) both; (d) both; (e) neither

Test Question	Correct Answer	
21		 Negative heterotropic effectors could be protons (lower pH), 2,3-bisphosphoglycerate(BPG), or carbon dioxide

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The following questions are in section “Mechanisms”

22	The appearance of ^{18}O in the product keto group indicates that the keto group exchanges with water during the mechanism. This is evidence for a Schiff base intermediate in the mechanism. This can be confirmed by reduction of this imine to the non-hydrolyzable amine using sodium borohydride ($NaBH_4$). Treatment of the enzyme with $NaBH_4$, in the presnece of substrate, will result in the inactivation of the enzyme. A control would be to do the same treatment in the absence of substrate, which should not affect enzyme activity. Metal ion catalysis would polarize the keto group such that the negative charge from the resulting carbanion formed following decarboxylation can be delocalized, and thus stabilized. This role does not require the exchange of the keto group with bulk water, nor the presence of an imine.

23	The serine has a polar hydroxyl, with the oxygen functioning as an electronegative nucleophile. A nearby histidine residue, with $pK_a \approx 6.0$, however, can function as a base to abstract the proton from the serine hydroxyl group. The result of transfering the proton from the serine hydroxyl to the histidine imidazole ring is to increase substantially the electronegativity of the serine oxygen, making it a much stronger nucleophile. This, in turn, lowers the activation energy of the covalent catalysis between serine and the carbonyl carbon of a peptide bond. The protonated His is further stabilized by delocalization of the positive charge by a low-barrier H-bond with the neighboring Asp-102 residue. The reaction would look like this:

Test Question	Correct Answer