	NAME	
EXAM II	I.	/ 45
October 19, 1998 Biochemistry I BI/CH421, BI601, BI/CH621	II.	/ 35
	III.	/ 20
	TOTAL	/100

## I. <u>MULTIPLE CHOICE</u>. (45 points)

Choose the BEST answer to the question by circling the appropriate letter.

- 1. A good transition-state analog:
  - A. is too unstable to isolate.
  - B. binds covalently to the enzyme.
  - C. binds to the enzyme more tightly than the substrate.
  - D. binds very weakly to the enzyme.
- 2. Below is a plot of  $V_0$  vs. [S] for a specific allosteric enzyme under different conditions.



Which of the following best describes the graph?

- A. Curve 3 represents the effect of a negative effector added to #2.
- B. Line 4 is valid exclusively for curves 1 and 2.
- C. Curve 1 represents maximum inhibition.
- D. Adding a positive effector to #2 would result in curve 3.
- E. Adding a positive effector to #1 would result in curve 2.

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- 3. Which of the following statements is true of enzyme catalysts?
  - A. To be effective, they must be present at the same concentration as their substrate.
  - B. They can increase the equilibrium constant for a given reaction by a thousand-fold or more.
  - C. They lower the activation energy for conversion of substrate to product.
  - D. Their catalytic activity is independent of pH.
  - E. They are generally equally active on D and L isomers of a given substrate.
- 4. A D-amino acid would interrupt an  $\alpha$  helix made of L-amino acids. Another naturally occurring constraint on the formation of an  $\alpha$  helix is the presence of:
  - A. a negatively charged Arg residue.
  - B. a positively charged Lys residue.
  - C. two Gly residues side by side.
  - D. a nonpolar residue near the carboxyl terminus.
  - E. a Pro residue.
- 5. The  $\alpha$ -keratin chains indicated by the diagram below have undergone one chemical step. To alter the shape of the  $\alpha$ -keratin chains--as in hair waving--what subsequent steps are required?



- A. chemical reduction and then chemical oxidation
- B. chemical oxidation and then shape remodeling
- C. shape remodeling and then chemical reduction
- D. shape remodeling and then chemical oxidation
- E. chemical reduction and then shape remodeling
- 6. Determination of the precise spacing of atoms within a large protein is possible only through the use of:
  - A. a Ramachandran plot.
  - B. molecular model building.
  - C. x-ray diffraction.
  - D. light microscopy.
  - E. electron microscopy.

- 7. One of the assumptions stated by Michaelis & Menten is called the steady state assumption. This assumption implies:
  - A.  $K_m = K_s$ .
  - B. the maximum velocity occurs when the enzyme is saturated.
  - C. the ES complex is formed and broken down at equivalent rates.
  - D. the  $\boldsymbol{K}_{m}$  is equivalent to the cellular substrate concentration.
  - E. the enzyme is regulated.
- 8. In an  $\alpha$  helix, the R groups on the amino acid residues:
  - A. are found on the outside of the helix spiral.
  - B. generate the hydrogen bonds that form the helix.
  - C. stack within the interior of the helix.
  - D. cause only right-handed helices to form.
  - E. alternate between the outside and the inside of the helix.
- 9. Which of the following statements about a plot of  $V_0$  vs. [S] for an enzyme that follows Michaelis-Menten kinetics is false?
  - A.  $K_m$  is the [S] at which  $V_0 = 1/2 V_{max}$ .
  - B. the shape of the curve is a hyperbola.
  - C. the y-axis is a rate term with units of  $\mu M/\text{min.}$
  - D. as [S] increases, the initial velocity of reaction,  ${\bf V}_{0},$  also increases.
  - E. at high [S], the velocity curve becomes superimposed on a horizontal line which intersects the y-axis at  ${\tt V}_{\max}.$
- 10. To calculate the turnover number of an enzyme you need to know the:
  - A. initial velocity of the catalyzed reaction at low [S].
  - B. initial velocity of the catalyzed reaction at [S] >>  $K_m$ .
  - C.  $K_{\rm m}$  for the substrate.
  - D. enzyme concentration.
  - E. both B and D.
- 11. Studies with mutated forms of tyrosyl-tRNA synthetase showed that changing some active-site amino acids decreased the enzyme's turnover number but not the  $K_m$  of the reaction. The best interpretation of these results is that the:
  - A. reaction's  $K_{\rm m}$  does not depend on which amino acid side chains are found in the active site.
  - B. two terms,  $K_m$  and turnover number, are inversely proportional.
  - C. transition state for this reaction is formed prior to the formation of the ES complex.
  - D. two substrates, tyrosine and ATP, must enter the active site in a specific order.
  - E. amino acids involved with the transition-state complex are different from the ones that affect enzyme-substrate association.

12. The slight right-handed twist of  $\beta$ -sheets causes super-secondary structures such as the Rossmann-fold to \_\_\_\_\_ A. extend in a left-handed helix to make stronger fibers B. curve around making helical bundles possible C. curve back on themselves making  $\beta$ -barrels and  $\beta$ -saddles possible curve in a zig-zag way to make repeated structures with more tensile D. strength E. lay flat 13. For many enzymes, the slowest (rate-limiting) step is the reaction that  $k_2$ actually releases the product,  $\text{ES} \rightarrow \text{P}.$  Under these conditions  $k_2$  can be ignored, and  $\boldsymbol{K}_{m}$  becomes equivalent to: A. the [S] where  $V_0 = V_{max}$ . B. the turnover number. C. k<sub>cat</sub>. D. the dissociation constant,  $\boldsymbol{K}_{\mathrm{S}},$  for the ES complex. the maximal velocity. Ε. 14. Which of the following kinetic parameters remains the same for  $S \rightarrow P$ , whether the reaction is enzyme-catalyzed or uncatalyzed? A. k в. V c. v<sub>o</sub> D. K<sub>eq</sub>' 15. Enzyme X exhibits maximum activity at pH = 6.9. X shows a fairly sharp decrease in its activity when the pH goes much lower than 6.4. One likely interpretation of this pH activity is that: A. the reaction relies on specific acid-base catalysis. в. a His residue on the enzyme is involved in the reaction. C. a Glu residue on the enzyme is involved in the reaction. the enzyme is found in gastric secretions. D. the enzyme has a metallic cofactor. Е. II. **SHORT ANSWER** (35 points) Give a <u>brief</u> answer to each problem or question below. 16. What is the significance of  $k_{\mbox{cat}}/\mbox{K}_{\mbox{M}}\mbox{?}$  (3 pts)

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- 17. In normal people collagen provides a matrix for bone, which is then solidified by the deposition of crystals of calcium hydroxyapatite. A connective tissue disease called osteogenesis imperfecta is caused by defective collagen. Among the symptoms of this disorder are skeletal deformities caused by multiple fractures due to brittle bones. Analysis of the gene coding for one of the collagen chains in an affected person shows that the glycine residue normally located at position 988 in the primary sequence is changed to a cysteine residue. The alteration disrupts the collagen helix and causes it to unfold at room temperature. How could the substitution of cysteine for glycine cause helix disruption? (3 pts)
- 18. For each of these methods of separating proteins, describe the principle of the method, and tell what property of proteins allows their separation by this technique. (6 pts)
  - (a) ion-exchange chromatography
  - (b) size-exclusion (gel filtration) chromatography
  - (c) affinity chromatography

19. In purifying an enzyme that converts B into A in a single step, you discover that your enzyme activity disappears when you subject it to ion-exchange chromatography; none of the fractions from the ion-exchange column contains detectable enzyme activity. However, if you combine the material that passed straight through the column with a fraction that came off the column later, enzyme activity is again detectable. This is true even if you boil the first fraction (the "pass-through") before combining it with the second. Give a reasonable explanation for these observations. (3 pts)

20. Name and briefly define four types of noncovalent interactions that occur between a substrate and an enzyme (or a ligand and its binding protein). (8 pts)

21. Predict the pH of a 0.1 M HCl + 0.2 M glycine solution. The pKa' values for glycine are 2.4 and 9.9. How can you increase the buffer capacity of this solution? What is the average net change on the glycine molecule at this pH? (6 pts)

- 22. Suppose you are asked to evaluate a new scheme for the purification of an enzyme. What are your criteria for a "good" purification method? Be specific and concise. (2 pts)
- 23. Draw two ways in which parallel  $\beta$ -strands are connected and two ways in which antiparallel  $\beta$ -strands are connected. Be sure to label your drawings. (4 pts)

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## III. <u>MATCHING</u> (20 points)

- 24. Match each amino acid or class of amino acid residues in the right column with the secondary structure element in the left cloumn in which it is most likely to occur. (5 pts)
- 1. α-helix \_\_\_\_\_ A. Gly
- 2. β-sheet \_\_\_\_\_ B. Pro
- 3. β-turn \_\_\_\_\_
- D. Amino acids with branching at the  $\beta$  carbon
- E. Ala

C. Glu

- 25. Match each term or phrase in the left column with the most appropriate term or phrase in the right column. Items in the right column can be used more than once or not at all. (10 pts)
- \_\_ 1. Fructose 1,6-bisphosphate ==>
  glyceraldehyde 3-phosphate +
  dihydroxyacetone phosphate
- \_\_ 2. E + A ==> EA ==> F + P ==> F + B ==> FB ==> E + O
- 3. kinase
- \_\_\_\_ 4. alcohol dehydrogenase
- \_\_\_ 5. circular dichroism
- \_\_\_\_ 6. carboxypeptidase A
- \_\_\_\_7. 2D-NMR spectroscopy
- \_\_\_ 8. sedimentation equilibrium ultracentrifugation
- \_\_\_\_9. E + A ==> EA + B ==> EAB ==> E + P
- \_\_10. x-ray crystallography

- A. determine secondary structure in proteins
- B. determine molecular weight of proteins
- C. EC 3.4.17.1
- D. ping-pong, ordered, uni, bi
- E. sequential, ordered, uni, bi
- F. ping-pong, ordered, bi, bi
- G. transferase
- H. determine the primary structure of proteins
- I. EC 1.1.1.1
- J. sequential, ordered bi, uni
- K. sequential, random, bi, uni
- L. determine teritary structure of proteins

26. Match the levels of protein structure in the left column with the best descriptions in the right column. Use each item once. (5 pts)

- \_\_\_\_ 1. primary
- A. association of protein subunits
- B. aggregates of  $\alpha$ -helical and  $\beta$ -sheet structures C. linear amino acid sequence
- \_\_\_\_4. super-secondary
- D. spatial arrangement of amino acids that are near each other in the linear sequence
- \_\_\_\_ 5. quaternary

3. tertiary

E. necessary for the catalytic activity of an enzyme

Test Question	Correct Answer	
	Multiple (	Choice
1		C
2		А
3		C
4		Ε
5		D
б		С
7		С
8		A
9		Ε
10		Ε
11		Ε
12		C
13		D
14		D
15		В
	Short An	lswer
16		The ratio of $\mathbf{k}_{cat}/\mathtt{K}_{\mathtt{M}}$ allows one to estimate the catalytic
		efficiency of an enzyme. The better enzymes have higher $k_{cat}$ and lower $K_M$ , which means they achieve their maximal
		concentration than other enzymes with larger $K_M$ . The
		upper limit for this value is $10^9 \text{ M}^{-1}\text{s}^{-1}$ , and is set by the rate of diffusion of the substrate in the solution, which limitws the rate at which it encounters the
		enzyme.
17		In the helical region of collagen, glycine occupies every third position in a sequence of just over 1000 amino acids that constitute the typical collagen chanin. Each glycine residue is loacted on the inside of the three-chain helix, where there is no room for larger side cahins. The substitution of a cysteine residue at one loaction probably disrupts the helix because the larger side chain $(-CH_2-SH)$ cannot fit inside.
18		(a) Ion-exchange chromatography separates proteins on the basis of their charges. Proteins have a pH-dependent charge that varies for each. (b) Size-exclusion or gel filtration chromatography separates on the basis of size. Proteins vary greatly by size. (c) Affinity chromatography separates proteins with specific, high affinity for some ligand (attached to an inert support) from other proteins with no such affinity.
19		The "pass-through" contains a heat-stable coenzyme or cofactor, and the later-eluting fraction contains the enzyme itself. Neither is active without the other.
20		(1) hydrogen bonds: weak electrostatic attractions between one electronegative atom (such as oxygen or

Answers for Exam 2 10/18/98

Test Question	Correct Answer	
Queberon		nitrogen) (acceptor) and a hydrogen atom covalently linked to a second electronegative atom (donor). (2) van der Waals interactions: weak ineractions between the electric dipoles that two close-spaced atoms induce in each other. (3) electrostatic/ionic interactions: relatively weak charge-charge interactions (attractions of opposite charges, repulsions of like charges) between two ionized groups. (4) hydrophobic interactions: the forces that tend to bring two hydrophobic groups together reducing
		the total area of the two groups that is exposed to
	surr	ounding molecules of the polar solvent- gain of entropy for water.
21		-The addition of 0.1 M HCl to the 0.2 M glycine titrates
		the zwitterionic form half way back to the fully
		HA (net charge $+1$ ) is then 0.1 M and the concentration
		of A- (net charge 0) is then 0.1 M. In this case pH =
		pKa' + log 0.1M/0.1M; or $pH = pKa' + 0$ , or 2.4.
		-This is at the pKa' and the buffer capacity cannot be
		Increased.
2.2		Answers will vary, but the key criteria are that each
		step must result in increased specific activity and
		reasonably good recovery of enzyme activity.
23		Two antiparallel arrows connected by a loop, $\beta$ -turn, or
		lpha-helix for the antiparallel $eta$ -strands and two parallel
		arrows connected by a loop or $\alpha$ -helix for the parallel
	Matchin	p-strands.
2.4	Matcilli	Ч 1) С.Е. 2) D. 3) А.В.
25		E, F, G, I, A, C, L, B, J, L
26		C, D, E, B, A