BIOCHEMISTRY SECOND EXAMINATION 641/10 FALL 1994

NAME PARTIAL KEY

Notes: There are 10 pages on this exam - please check.

The point value of questions varies widely - take note

Please make your answers brief and to the point

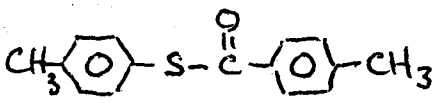
Please write LEGIBLY.

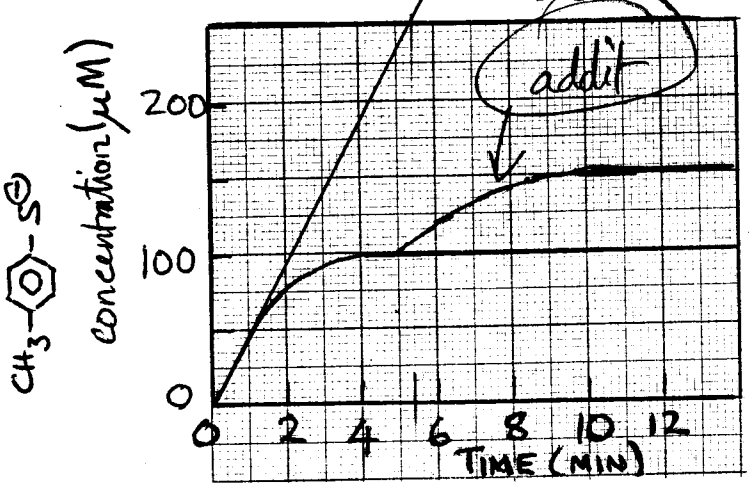
The course grade is "curved"

Good luck

Question 1 (17 pts.) Refer to the graph at the right in conjunction with the conditions shown to the left to answer the following questions.

A concentration of 0.01 μM chymotrypsin was added to 1 mL of phosphate buffer pH 7.8, containing 100 μM of the following thioester:





a. Explain the shape and amplitude of the curve.

This is conversion of S → two products. This is not a burst phase because of non correspondence of burst amplitude (100 μM vs. .01 μM)

b. Calculate an approximate turnover number for this substrate in units of turnover/min.

represents an initial rate of 46 μM/min

Enz conc = .01 μM

So TN = $\frac{46\ \mu M/min}{.01\ \mu M}$ = 4600/min

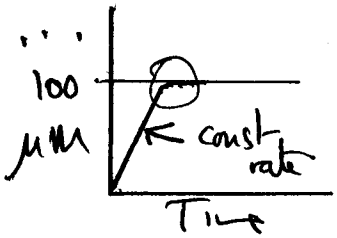
c. Draw **on the graph** what would happen if an additional 50 μM of substrate is added at 5 minutes.

d. Is the Km for the ester likely to be much less than, or considerably more than 1 μM.

LESS MORE CANNOT PREDICT

Explain your answer

If it was < 1 μM then it would remain at same rate almost until substrate depletion ...



e. Draw the time-line representation of one catalytic turnover of chymotrypsin with this substrate clearly showing the structures of EACH substrate and product

as before :-

first product = $CH_3-C_6H_4-S^{\ominus}$

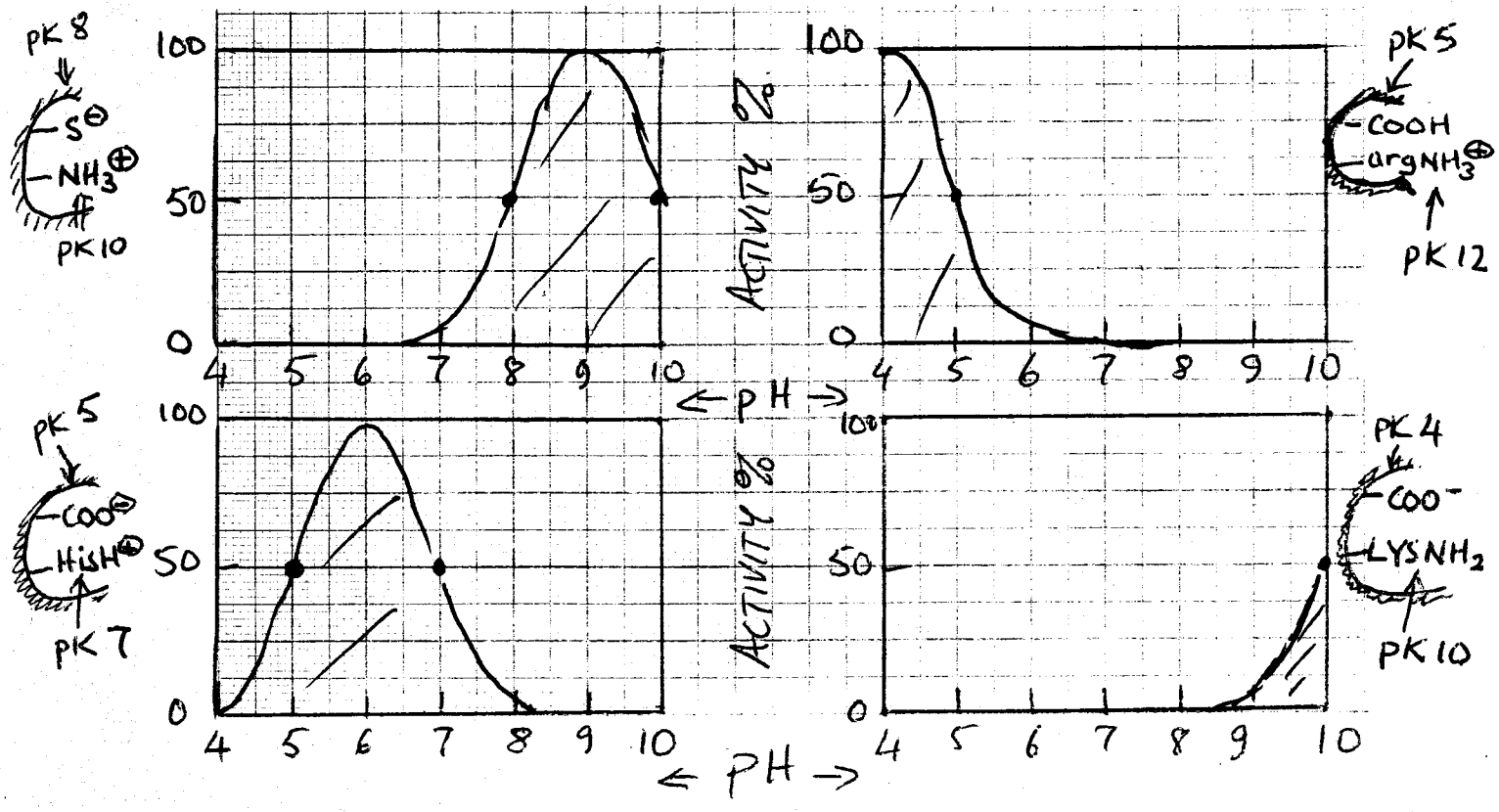
2nd .. $CH_3-C_6H_4-COO^{\ominus}$

f. Identify the slow step in catalysis on your diagram.

g. Chymotrypsin incubated with this substrate is protected against the action of di-isopropyl-fluorophosphate. Explain clearly why.

the acyl enzyme ($-O-C(=O)-C_6H_4-$) will protect the rxn with $F-P(=O)(OR)(OR)$

Question 2 (12 pts) Each of the following enzymes are only active in the ionic form shown. Draw clear and accurate Vmax versus pH curves.



Question 3 (12pts) Consider the following proteins.

protein	MW (daltons)	pI
A	78,000	7
B	158,000	4
C	58,000	3
D	20,000	5
E	29,000	6

a. What is the order of elution of A-E on gel-filtration

FIRST ___ ___ ___ ___ ___ LAST

b. CIRCLE which proteins you expect would stick to an anion exchange column at pH 5.5?

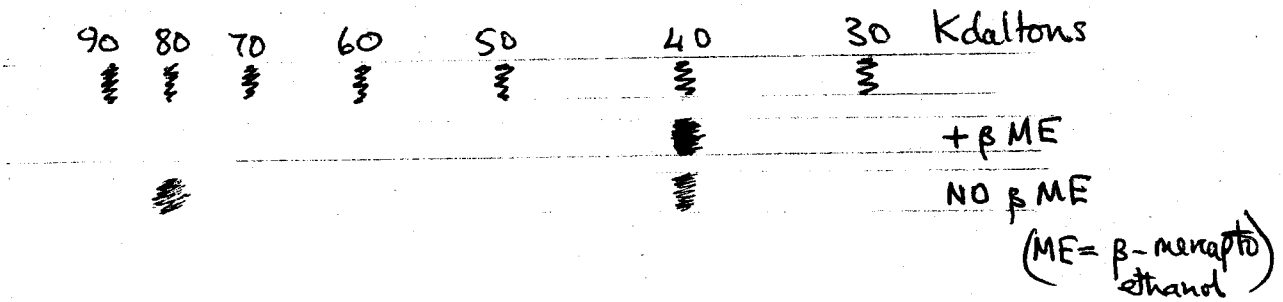
A B C D E

c. CIRCLE which proteins you expect would stick to a cation exchange column at pH 6.5?

A B C D E

d. Protein E when stored in buffer at 4 C for a long period co-eluted with protein C on gel filtration. Explain why this might happen.

e. SDS polyacrylamide gel electrophoresis of protein B in the presence and absence of ß-mercaptoethanol are shown below. Sketch a likely quaternary structure for the protein.



Question 4 (9 pts) What is the effect of the following on hemoglobin and myoglobin. Circle the most appropriate answer (NC = no change).

Decreasing pH on the oxygen affinity of normal hemoglobin	**increase**	**NC**	**decrease**
Decreasing pH on the oxygen affinity of sickle cell hemoglobin	**increase**	**NC**	**decrease**
Increasing the pH on DPG binding in hemoglobin	**increase**	**NC**	**decrease**
Increasing the pH on CO_2 binding to hemoglobin	**increase**	**NC**	**decrease**
Increasing the pH on CO binding to hemoglobin	**increase**	**NC**	**decrease**
Increasing the CO_2 concentration on the oxygen binding of myoglobin	**increase**	**NC**	**decrease**
The effect of low concentrations of CO on the oxygen affinity of hemoglobin	**increase**	**NC**	**decrease**
The effect of CO_2 on the oxygen affinity of hemoglobin	**increase**	**NC**	**decrease**
The effect of oxygen on the pH of an unbuffered solution of deoxyhemoglobin	**increase**	**NC**	**decrease**

Question 5 (21 pts) Quick problems. Most of the credit goes to the correct numerical answer.

a. If a monomeric oxygen binding protein has a K_p value of 30 mm of mercury (torr) what is the fractional saturation at a partial pressure of 15 torr?

Fractional saturation _____

b. A 1,000,000-fold increase in a reaction rate corresponds to what change in the standard free energy of activation? R = 2 cal/°; T = 300°K.

$2.3 \times 2 \times 300 \times \log 10^6$

$\Delta G^{\circ\prime}$ = 8.28 kcal

c. An enzyme shows a maximal rate of 25 umol/min. What substrate concentration will yield a rate of 15 umol/min if the Km is 9 mM?

$v = \frac{V_{max}[S]}{K_m + [S]}$ $\quad 15 = \frac{25[S]}{9+S}$ $\quad 15(9+S) = 25S$

$S = 13.5\,mM$

[S] = 13.5 mM

d. If a competitive inhibitor I (K_i = 3 mM) is added at a concentration of 27 mM to the enzyme in "c" what rate will be observed with 20 mM substrate?

$K_{m\,app} = 9\left(1 + \frac{27}{3}\right) = 90\,mM$

$v = \frac{25 \cdot 20}{90+20}$

Rate = 4.5 μmol/min

e. Calculate the free energy change for the attack of water (hydrolysis) on acetyl-P. Given:

ATP	+	Acetate	=	ADP	+	Acetyl-P	$\Delta G^{\circ\prime}$ = +3.3 kcal
ATP	+	H_2O	=	ADP	+	P_i	$\Delta G^{\circ\prime}$ = -7.3 kcal

(acetyl-P + H_2O → acetate + P_i)

acetyl P + ~~ADP~~ → ATP + acetate −3.3 kcal

~~ATP~~ + ~~H_2O~~ → ~~ADP~~ + P_i −7.3

acetyl P + H_2O → acetate + P_i −10.6 kcal

f. In this reaction: A = B + C

The standard free energy change is +6 Kcal. Calculate the free energy change if A were 10^{-1} M and B and C were 10^{-4} M. [R=2 cal/mole/°K; temperature = 37°C]

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[B][C]}{[A]}$$

$$\Delta G = 6000 + 2.3 \times 2 \times 310 \times \log \left(\frac{10^{-4} \times 10^{-4}}{10^{-1}} \right)$$

$$= 6000 - 9977$$

$\Delta G^{\circ\prime}$ = −3.98 kcal

g. If the concentration of B and C were **maintained at 10^{-4} M** in question "f" above, what would the concentration of the reactant A need to become for the reaction to be at equilibrium?

$\Delta G^{\circ\prime} = -RT \ln K_{eq}$ so $K_{eq} = 6.28 \times 10^{-5}$ (M)

$$6.28 \times 10^{-5}\ M = \frac{10^{-4} \times 10^{-4}}{[A]}$$

[A] = 0.159 mM

$$A = \frac{10^{-8}}{6.28 \times 10^{-5}}$$

A

Question 6 (3 pts) Which statement is most appropriate?

a. Transition state analogues are likely to be competitive inhibitors of an enzyme catalyzed reaction.

b. In Michaelis-Menten kinetics, the rate of conversion of S to P and P to S are equal in the steady state.

c. The slowest step of an enzyme catalyzed reaction is always the diffusion of substrate into the active site.

d. The more tightly a product is bound the higher the Vmax.

e. All of the above are false.

____

Question 7 (3 pts) Which of the following statements concerning aspartate transcarbamylase is false?

a. The enzyme normally shows sigmoidal kinetics with aspartate as the varied substrate.

b. CTP binds to the regulatory subunit.

c. ATP binds to the catalytic subunit.

d. Catalytic trimers have enzyme activity.

e. p-OH-mercuribenzoate treatment does not abolish enzyme activity.

E

Question 8 (3 pts) Which is the most appropriate answer?

a. Spontaneous reactions are always exothermic

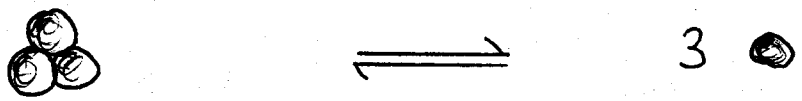
b. Spontaneous reactions are never endothermic

c. Spontaneous reactions always involve an increase in entropy

d. Spontaneous reactions are never exergonic

e. All of the above are false.

Question 9 (5 pts) The following hypothetical enzyme exists as an equilibrium between inactive monomers and active trimers. Monomers and trimers have the properties listed below:



Trimer	**Monomer**
Active	Inactive
Binds L strongly	Binds L weakly
pK of exposed HIS is 8	pK of exposed HIS is 6

Circle what will be the effect of **increasing** ..... on .....

a.	**L** concentration on enzyme activity	**Increase**	**N.C.**	**Decrease**
b.	**pH** on the concentration of trimer	**Increase**	**N.C.**	**Decrease**
c.	[**Enzyme**] on percentage of monomer	**Increase**	**N.C.**	**Decrease**
d.	**L** concentration on % monomer	**Increase**	**N.C.**	**Decrease**
e.	[H^+] on L binding	**Increase**	**N.C.**	**Decrease**

Question 10 (pts) Fill in the blanks with not more than three legible words.

a. Inactive precursors of proteolytic enzymes _____

b. Inhibitors which affect Km and Vmax are termed _____

c. Enzyme kinetics in which the enzyme alternates between two covalent states _____

d. A spectrophotometric method for studying rapid reactions in enzymology _____

e. Sickle cell trait confers resistance to what disease _____

f. The increase in solubility of a protein with increasing ionic strength is called _____

g. Name a serine protease with no sequence similarity with chymotrypsin _____

h-j. In the enzyme named in g list three catalytically essential amino acids in its active site _____

_____

_____

k. Another name for mechanism-based inhibition _____

l. A separation method based on pI _____

m. One method routinely used to disrupt cells _____

z. The word that best describes this exam _____