BIOCHEMISTRY SECOND EXAMINATION 641/10 FALL 1998

NAME _____ PARTIAL KEY

Notes: There are 9 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. Draw clear diagrams where appropriate.

The course grade is "curved".

Good luck.

Question 1 (16 pts.) Consider the following proteins:

protein	MW	pI
A	160,000	5
B	180,000	3
C	45,000	8
D	110,000	7
E	16,000	10

a. What is the expected order of elution on gel filtration

FIRST _____ _____ _____ _____ _____ LAST

b. Circle the proteins you would expect to stick on anion exchange chromatography in buffer at pH 6.0

A B C D E

c. Circle the proteins you would expect to stick on cation exchange chromatography in buffer at pH 6.0

A B C D E

d. Protein C is 50% salted-out by 4 M ammonium sulfate adjusted to pH 8. What would you expect if a solution of 4 M ammonium sulfate at pH 7 were used?

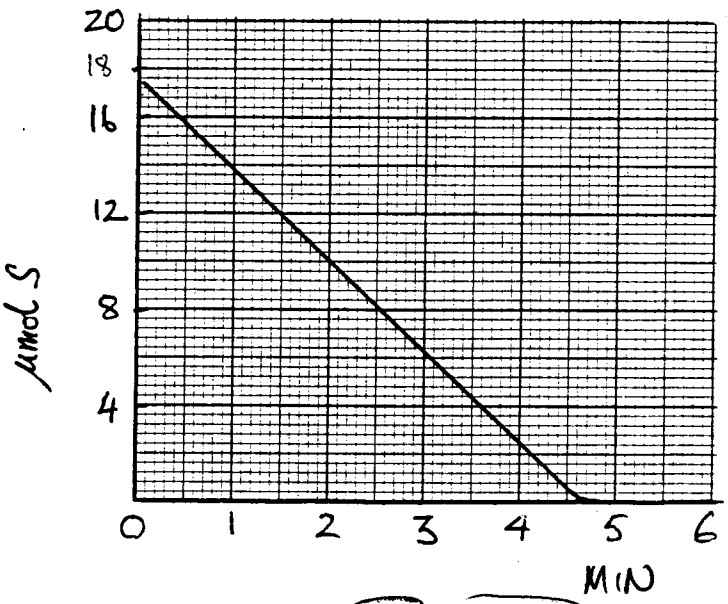
e. If samples of proteins A-E are stored for long periods of time, re-running gel filtration shows that peak C apparently diminishes and peak B apparently increases. Propose a specific single explanation.

then briefly explain how you would test the valididity of your proposal

f. What amino acid generally dominates the absorbance of a protein at 280 nm? _____

g. If protein A is an enzyme, how would you monitor the success of its purification from pig kidney?

Question 2 (8 pts) In the enzyme assay shown to the right, 25 µg of enzyme catalyzed the disappearance of substrate.



Answer the following questions:

Rate = $\frac{17.5\ \mu mol}{4.6\ MIN}$

a.	The enzyme has a high pK for its substrate	YES	NO	CANNOT SAY
b.	The product P is a strong competitive inhibitor	YES	NO	CANNOT SAY
c.	The enzyme does not obey Michaelis Menten kinetics	YES	NO	CANNOT SAY either OK
d.	The equilibrium constant for conversion from S to P is less than about 0.01 ($\frac{[P]}{[S]} \gg 100$)	YES	NO	CANNOT SAY

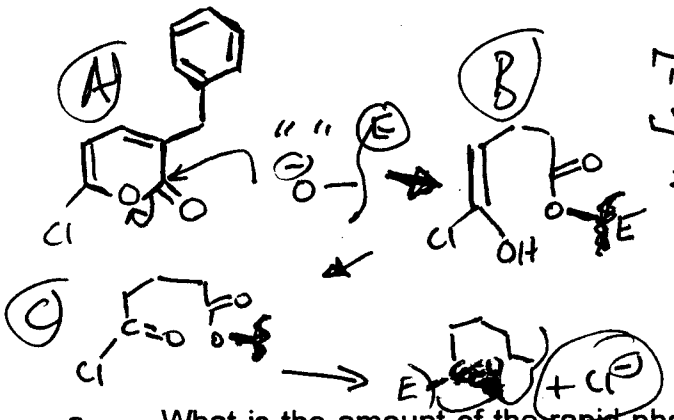
e. Calculate the initial rate of the enzyme catalyzed reaction

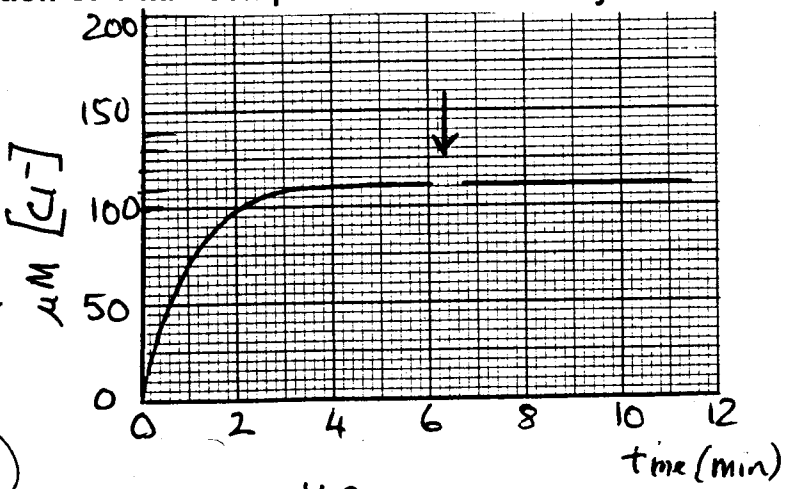
Rate= 3.8 µmol/min

f. Predict the rate if the initial substrate concentration were doubled.

Double (7.6 µmol/min)

Question 3 (7 pts) Chymotrypsin (10 μM) is treated with the 1 mM of the compound shown to the left (it was discussed in lecture). The resulting release of chloride ion was followed as in the graph to the right. A further addition of 1 mM compound is indicated by the arrow.





a. What is the amount of the ~~rapid~~ phase seen above. 110 μM

b. What is happening in this particular rapid phase. Explain carefully.

The mechanism based inhibitor is attacked by ser of catalytic triad. Acyl chloride "C" reacts with enzyme nucleophile to release Cl^- in BURST phase

c. Explain the behavior after the second addition of ~~substrate~~ compound.

The enzyme is INACTIVATED & cannot react with an additional aliquot of "A"

Question 4 (14 pts) Quick problems. Most of the credit goes to the correct numerical answer.

a. An enzyme has a Km of 9 mM for substrate S. What concentration of S would give a rate of 33% of the maximal rate? 4.43 mM

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \qquad 0.33 V_{max} = \frac{V_{max} \cdot [S]}{9\,mM + [S]}$$

$$9 + [S] = 3.03[S] \qquad [S] = 4.433\ mM$$

b. Calculate the amount of enzyme in grams added to a 1 mL assay if it catalyzes a rate of 4 μmol product formed per minute at a temperature of 25°C and a pH of 7.5. The molecular weight of substrate and enzyme are 200 and 20,000 g/mole respectively. _____ g

IGNORE Q

c. The Km for a substrate is observed to be 0.6 mM in the presence of 5 mM of a competitive inhibitor but 0.1 mM in its absence. Calculate the Ki value for the inhibitor. 1 mM

$K_m = 0.1\,mM$ $\quad K_{m\,app} = 0.6\,mM = 0.1\,mM\left(1 + \frac{5\,mM}{K_i}\right)$

$5 = \frac{5\,mM}{K_i}$ $\quad K_i = 1\,mM$

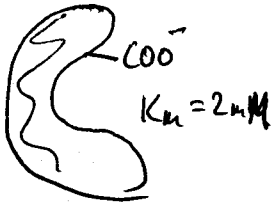
Now, write out the mathematical expression for Ki: $K_i = \frac{[E][I]}{[EI]}$

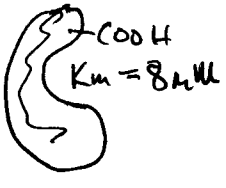
d. Calculate the ratio of the ionic strengths of 10 mM solutions of $MgSO_4$ and KCl. RATIO = _____

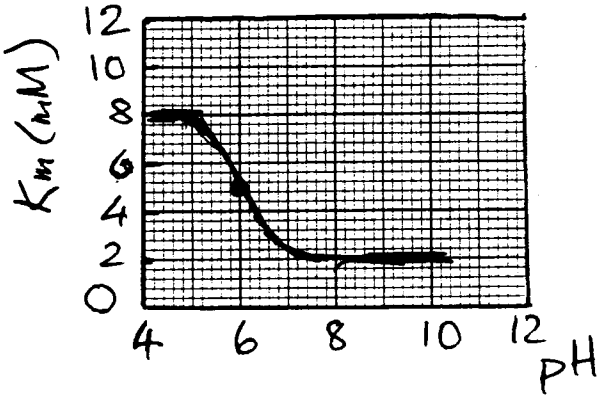
which is better at salting in?_____

Question 5 (12 pts) Draw clear and accurate graphs for the pH dependence of the following processes . Accuracy rewarded.

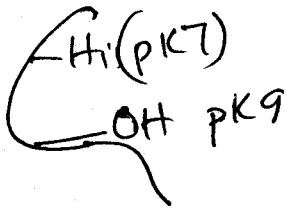
a. The Km of this enzyme is dependent only on the ionization of a single carboxyl residue (pK 6). At pH 8 and pH 4 the Km values are 2 and 8 mM respectively

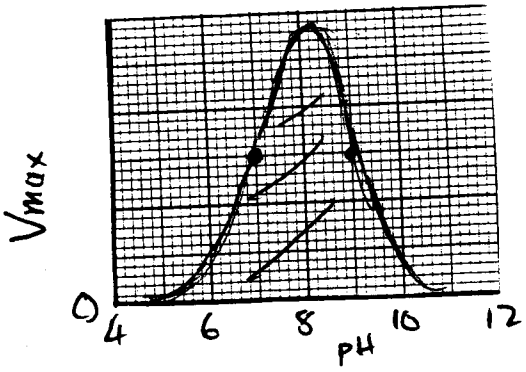




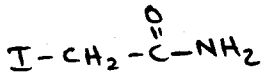


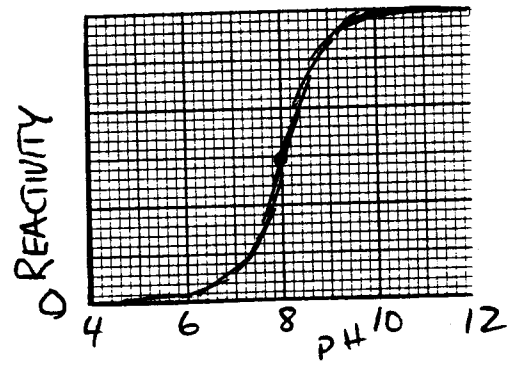
b. Vmax for an enzyme with a histidine (pK 7) as a general base and tyrosine (pK 9) as a general acid.



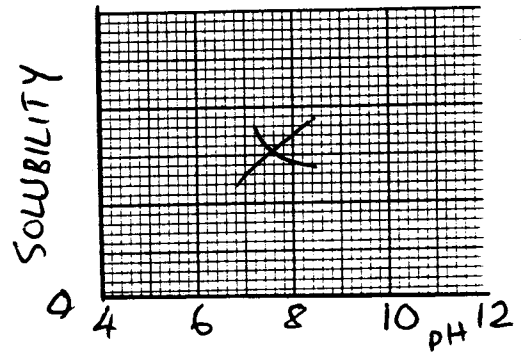


c. Draw the pH dependence for the reaction of iodoacetamide with a cysteine side chain (pK 8). Iodoacetamide =





d. The solubility of leucine (pK amino 2.3; carboxyl 9.7).



Question 6 (7 pts) What is the effect on human hemoglobin. Circle the most appropriate answer. (INCR. = increase; NC= no change; DECR. = decrease).

Lowering the pH on the oxygen affinity of hemoglobin	INCR.	NC	DECR.
Increasing CO on the oxygen affinity of hemoglobin	INCR.	NC	DECR.
Increasing oxygen on the binding of DPG to normal hemoglobin	INCR.	NC	DECR.
On the oxygen affinity - diluting hemoglobin until subunits dissociate	INCR.	NC	DECR.
Increasing sickle cell deoxy-hemoglobin concentration on the rate of fiber formation	INCR.	NC	DECR.

Adding CO to an unbuffered solution of normal deoxyhemoglobin does what to the pH?	INCR.	NC	DECR.
Increasing DPG concentration to the CO_2 binding of normal hemoglobin	INCR.	NC	DECR.

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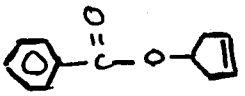
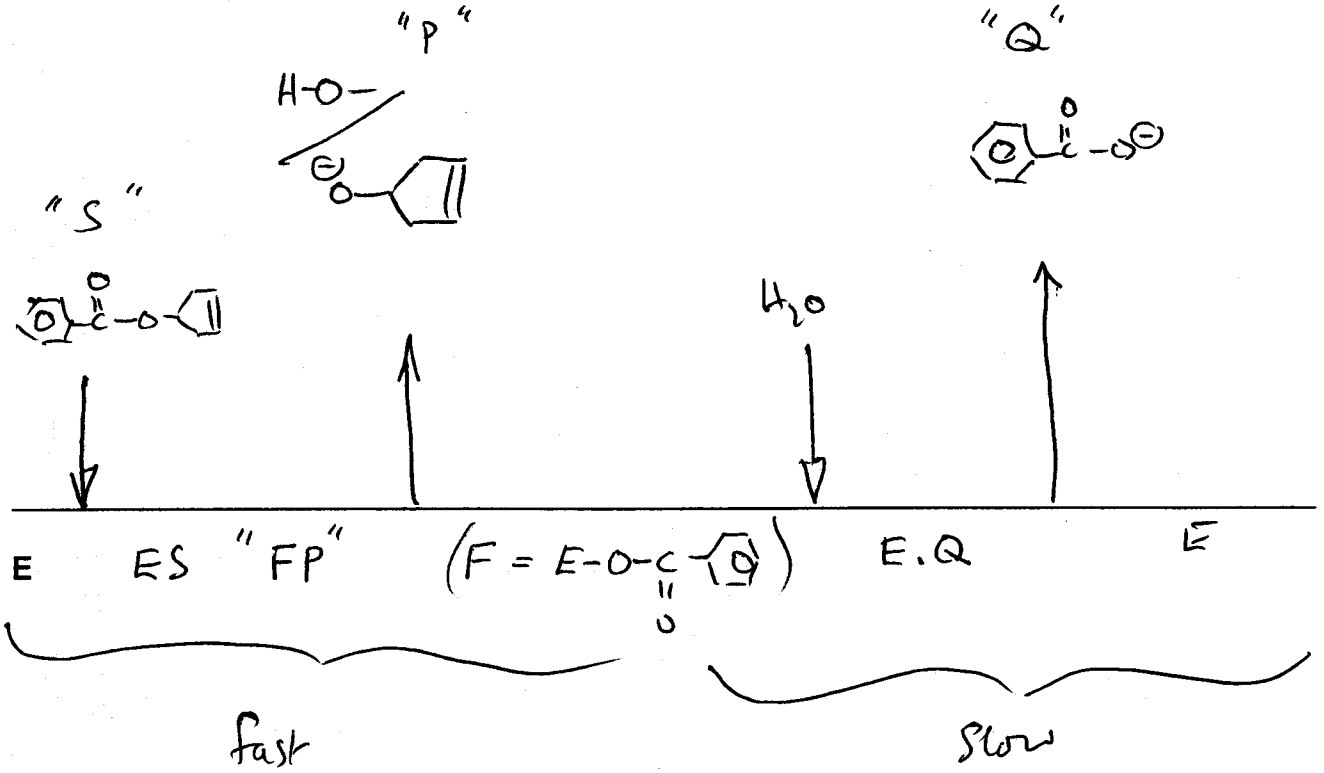
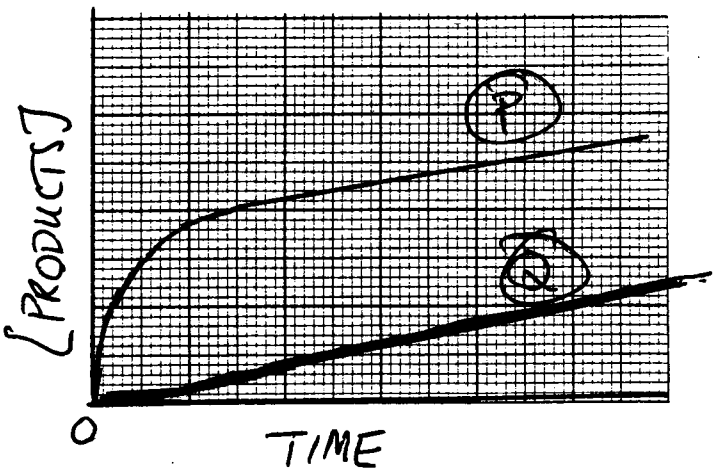
Question (10 pts) Draw a "timeline" representation of the hydrolysis of the compound shown to the right by chymotrypsin. Clearly show the structures of all substrates and products. Circle the form of the enzyme that would predominate in the steady state.



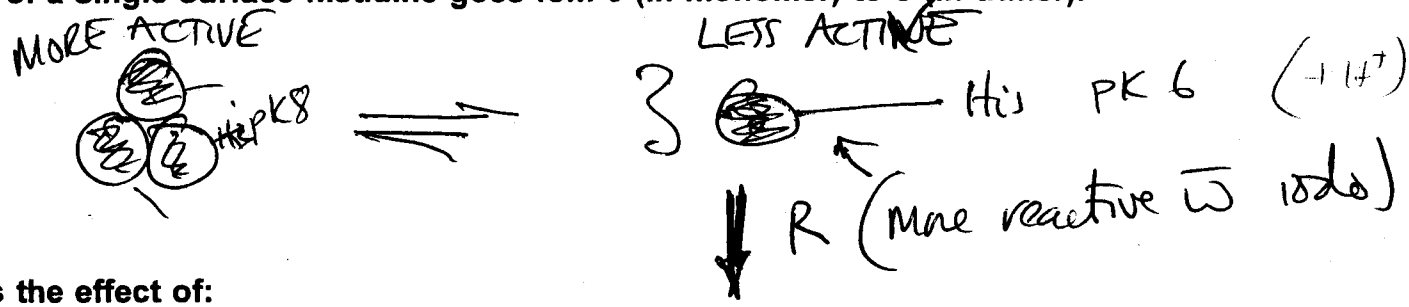


In the graph to the right, draw the appearance of both products of the enzyme following the initial addition of substrate. Indicate which is which.



8

Question (6 pts) Suppose that an enzyme is a trimer of identical subunits in equilibrium with monomers. The two substrates of the enzyme A and B bind 10-times more tighly to the trimer. The trimer is 5-times more active than the monomer. A regulatory molecule R binds preferentially to the monomer , Monomers react 100-times faster with iodoacetate. Finally the pK of a single surface histidine goes fom 6 (in monomer) to 8 (in trimer).



what is the effect of:

dilution on the enzyme activity	INCREASE	DECREASE	NC
increasing concentration of B on the extent of monomers	INCREASE	DECREASE	NC
lowering the pH on the concentration of trimer	INCREASE	DECREASE	NC
Increasing R on the reactivity with iodoacetate	INCREASE	DECREASE	NC
increasing the concentration of A on the affinity of B	INCREASE	DECREASE	NC
Decreasing protein concentration on the reactivity with iodoacetate	INCREASE	DECREASE	NC

9

Question (19 pts) Fill in the blanks with not more than three legible words

a. An affinity label for chymotrypsin TPCK.

b. A specific, potent, fluorophosphate inhibitor of actetylcholineesterase e.g. Sarin

c. A compound usually used for salting out in protein purifications —

d. Separates proteins only according to their pI values —

e. Elastase has three catalytically essential amino acid residues. Name them:

SER

[redacted] HIS

ASP

h. What interaction predominantly stabilizes the transition state in the oxyanion hole of chymotrypsin?

H-bonds

i. What limits catalysis in the most efficient enzymes

diffusion

j. Accumulation of heme in membranes is called

—

k. In one pathological mutation in hemoglobin, a direct iron ligand is replaced. It originally was what side chain

—

and it becomes __ in the mutant

—

m. This compound changes Km, but not Vmax

competitive I

n. Name a separation technique based on substrate recognition

—

o. The effect of pH on the oxygen affinity is called

—

p. Sickle cell disease has a geographically similar distribution to what other condition?

—

q. Sickle cell hemoglobin differs in charge from normal hemoglobin. By how much per tetramer?

—

r. A molecule that replaces DPG in non-mammalian hemoglobins

—

z. The single word that best describes this exam.

over