CHEM 641   SECOND EXAM FALL 2007

YOUR NAME: ____KEY_____

NOTES:

1. Where appropriate please show work – if in doubt show it anyway.

2. Pace yourself – you may want to do the easier questions first.

3. Please note the point value of questions – adjust your answers and effort accordingly.

4. Some questions may have more data than you need.

5. Please be brief – focus your answers to the space provided.

6. Please write CLEARLY – if I cannot read it – it is wrong.

7. You are welcome to detach the metabolic chart (carefully).

8. **Good luck.**

1

Question 1 (6 pts.). Draw clear accurate graphs to describe the behavior of the following systems. Clarity and accuracy rewarded.
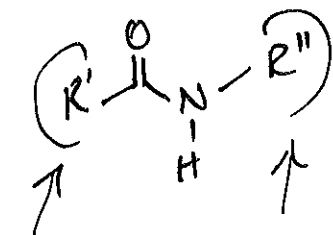
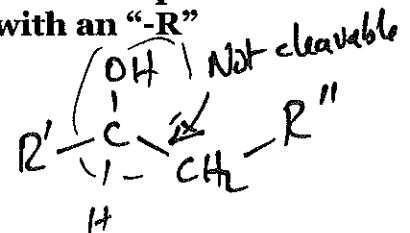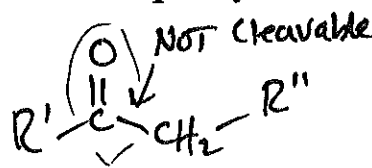**a.** only the form of the enzyme show below is active. Show its pH dependence at the right.

NH_2 (pK 9)

S^- (pK 5.0)

③
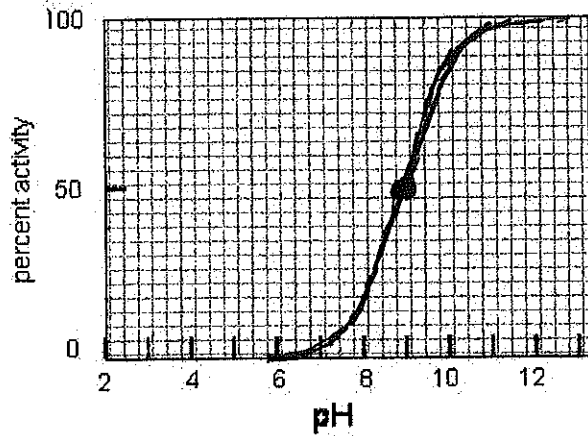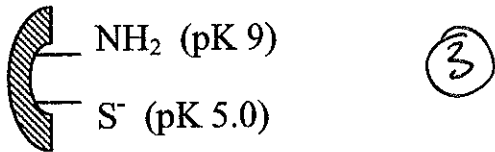
b. only the form of the enzyme show below is active. Show its pH dependence at the right.
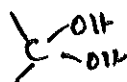
O^- (pK 10.0)

NH_3^+ (pK 12.0)

③
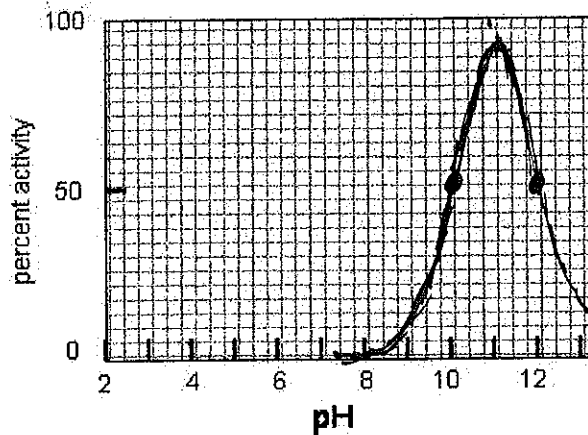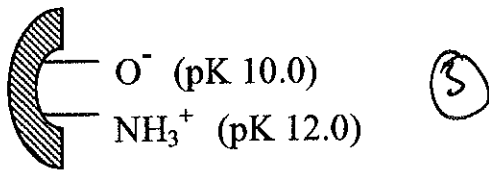
Question 2 (4 pts.). Draw the structure of a chemically-credible reversible inhibitor of an HIV-protease. Circle the functional group that is the key aspect of the anticipated inhibition. Chemical accuracy important. For parts of the structure that you don't want/need to specify ... denote with an "-R"

$\left(R' \overset{O}{\underset{\underset{H}{|}}{\overset{||}{C}}} N - R'' \right)$

↑ peptidic   ↑

NORMAL

$\overset{-OH}{\underset{-OH}{\underset{\diagdown}{\diagup}}}$

NOT Cleavable

$R' - \overset{O}{\overset{||}{C}} - CH_2 - R''$

The $\diagdown C=O$ might form a hydrate too

OH Not cleavable

$R' - \overset{|}{\underset{\underset{H}{|}}{C}} \diagup CH_2 - R''$

③

Now explain the key feature of the proposed inhibitor:

So the cleavable amide (peptide bond) is replaced by a functional group that might resemble the tetrahedral intermediate. BUT it is not cleaved R' & R'' are peptide-like (peptidic)
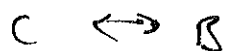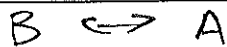
①

Question 3 (6 pts.). Given the following calculate $\Delta G^{o'}$ for equation 1:

1.   C $\leftrightarrow$ A $\Delta G^{o'} = $ ___+2___ kcal

2.   C $\leftrightarrow$ B $\Delta G^{o'} = +5$ kcal

3.   A $\leftrightarrow$ B $\Delta G^{o'} = +3$ kcal

B $\leftrightarrow$ A -3 kcal
C $\leftrightarrow$ B $+5$ kcal
                              _____
                              $+2$ kcal.

In equation 1 ... To make ΔG **more negative** I would: **circle all of the following which will definitely accomplish this:**

1. increase the concentration of **A**   ② decrease the concentration of **A**

③ increase the concentration of **C**   *4.* decrease the concentration of **C**

-1 for each incorrect to 0

5. Double the concentration of **both C** and **A**

6. Dilute the mixture of C and A with an equal volume of water.

Question 4 (6 pts.). Yield of ATP. In the space provided give the yield of ATP that would be formed in the following processes (enter a number from 0-10):

a. per molecule of glucose-1P phosphate to ethanol   ___3___ ②

b. per molecule of fructose 1,6-diP converted to lactate   ___4___ ②

c. per molecule of mannose converted to ethanol   ___2___ ②

Question 5 (12 pts.). Quick calculations. Please note that most of the points are given for the correct numerical answer.

a and b: The osmotic pressure of 1 mL of a 0.1 M solution of glucose is 2.4 atmospheres.   ③

a. Calculate the new osmotic pressure if the same amount of glucose were found in 1 mL of amylose (containing 10,000 glucose units per amylose molecule).

$= $ ___2.4×10^{-4}___ atmospheres

b. Calculate the new osmotic pressure if the same amount of glucose were found in 1 mL of glycogen (again containing 10,000 glucose units per glycogen molecule).

$$= \underline{2 \cdot 4 \times 10^{-4}} \text{ atmospheres}$$

c. What is the magnitude of the burst phase (i.e. the increase in absorbance at 405 nm when 30 µM of chymotrypsin is mixed with 5 mM of p-nitrophenylacetate at pH 8.0 in a 1 cm pathlength cell? The molar absorbtivity (extinction coefficient) of p-nitrophenol at this pH is 11,000 M⁻¹cm⁻¹

$$A = \left(11,000 \, M^{-1} cm^{-1}\right)\left(30 \times 10^{-6} \, M\right)\left(1 \, cm\right)$$

absorbance $\underline{0 \cdot 33}$

d. A competitive inhibitor shows a K_i of 2 mM. If the K_m for the substrate is originally 10 mM, what is the apparent K_m in the presence of 30 mM of the competitive inhibitor

apparent $K_m = \underline{160}$ mM

$$K_{m_{app}} = K_{m_{real}}\left(1 + \frac{I}{K_i}\right)$$

Question 6 (6 pts). Tracing radiolabels and *etc*. Place asterisks indicating the position of the radiolabel in the molecules shown to the right – if the product contains no radiolabel write "NONE".
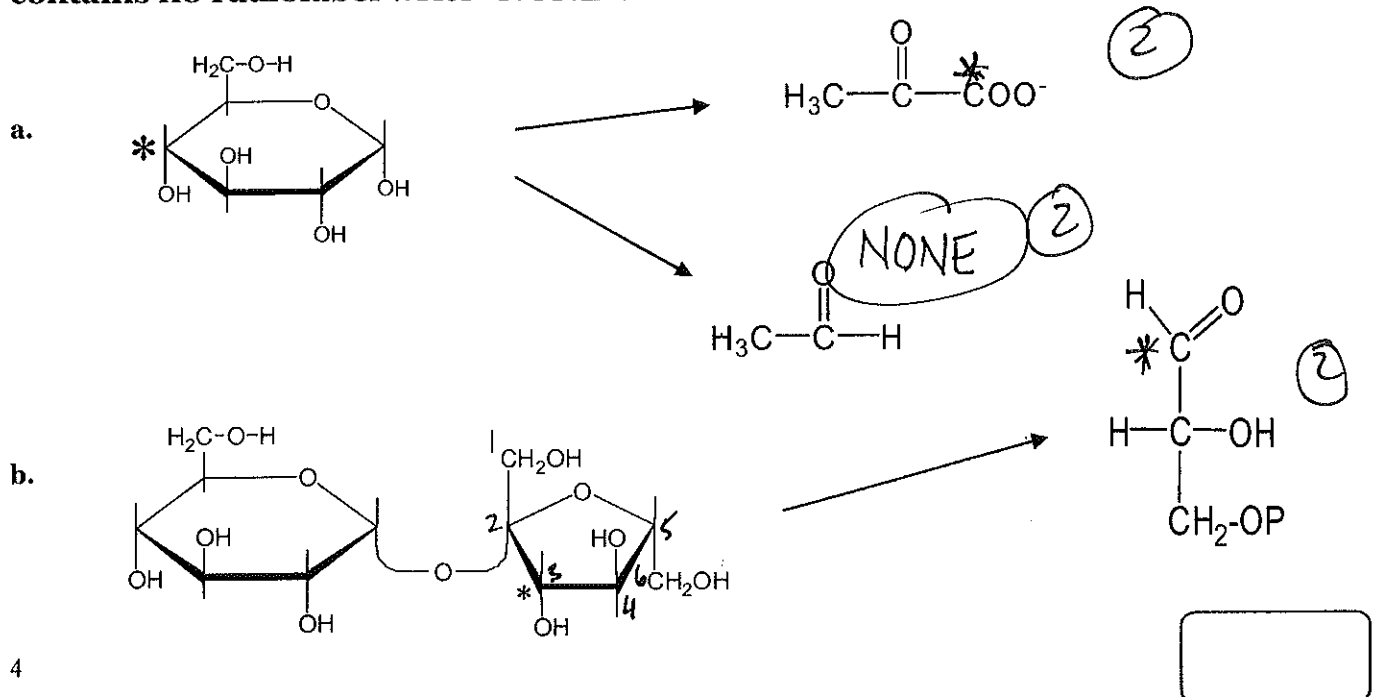
Question 7 (5 pts.) Trehalose is a major energy storage compound in the circulatory systems of many insects. Answer the following questions

Trehalose
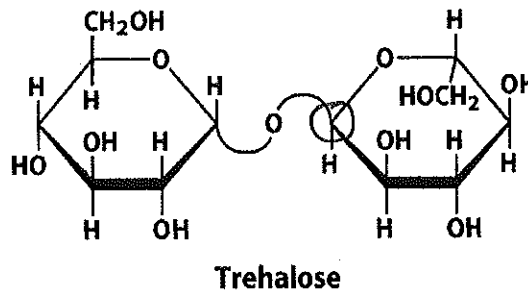
a. Name the glycosidic linkage:

$\alpha(1-1)$ ②

b. Name the monosaccharide comprising the LEFT ring: D - glucose ①

c. Name the monosaccharide comprising the RIGHT ring: D - glucose ①

d. circle the anomeric carbon atom in the RIGHT ring. ①

Question 8 (9 pts). Freeze-clamping a tissue gave the concentrations of A and B in a metabolic pathway (A→B→C→D→E→F) to be [A] = 10^{-3}M and [B] = 10^{-5} M.

Calculate the mass action ratio for the reaction A↔B

$MAR = \dfrac{10^{-2}}{2 \text{ pts}}$ $\dfrac{[B]}{[A]}$

2

A and B are interconverted by E1.
Compound **A** is mixed with a catalytic amount of E1 in an appropriate buffer and the results shown to the right are obtained:

Calculate the equilibrium constant [B]/[A] =
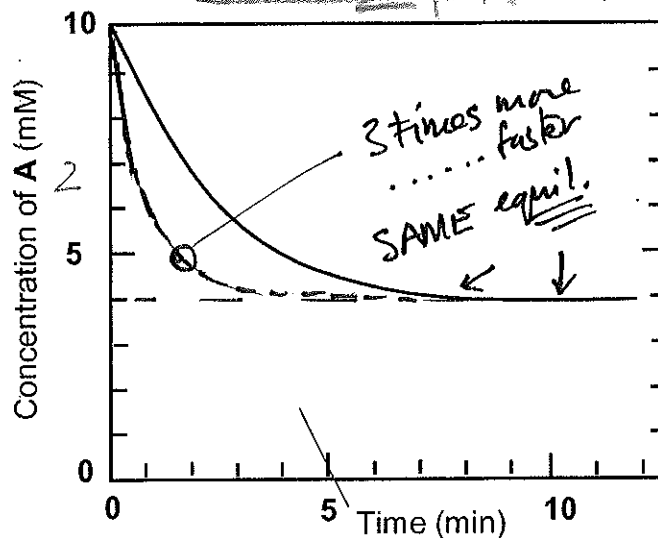
③ $K_{eq} = \sim 1.5$

$A_{init} \rightarrow A_{final} = 10mM - 4mM$

So $B_{final} = 6mM$ $\left(\dfrac{6 mM}{4 mM}\right)$

Is this reaction at equilibrium in the cell? - circle one answer: Yes   (No)   OR...   ②

**Cannot say** (why "cannot say"?_____)

Finally draw an accurate representation of the progress of the reaction **on the graph** when the concentration of E1 used was **increased by a factor of 3**.

②

3 times more faster
...... SAME equil.

5

Question 9 (12 pts) This question concerns glyceraldehyde-3P dehydrogenase.

This enzyme has a thioester intermediate that is shown incomplete in the top half of the box.
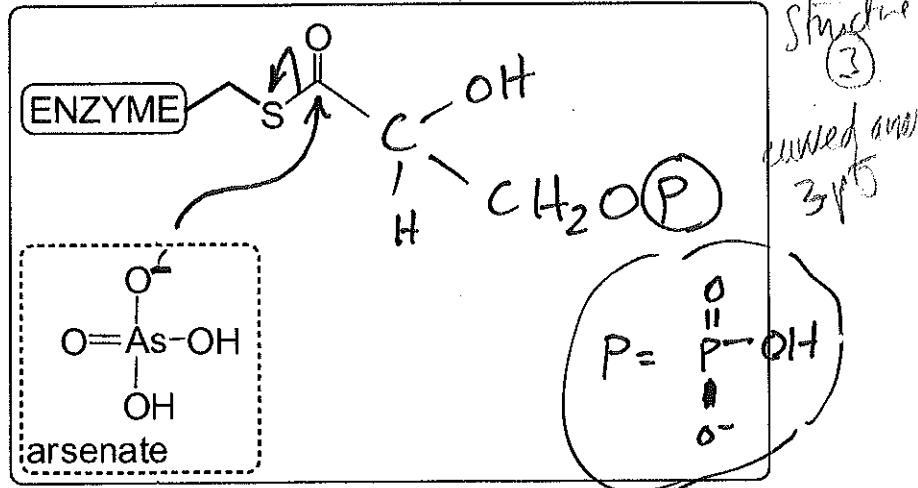
a. **complete the structure of the intermediate** found during catalysis by this dehydrogenase - put all the missing atoms in the box.

b. The next step in catalysis is **normally** attack of phosphate ion. At the bottom of the box is shown a phosphate analog (mimic) **arsenate**. Using the **arsenate drawn already**, show (by curved arrows) the next step of catalysis with release of a product.

c. In the box to the right show the structure of the **arsenate ester** released in 'b"
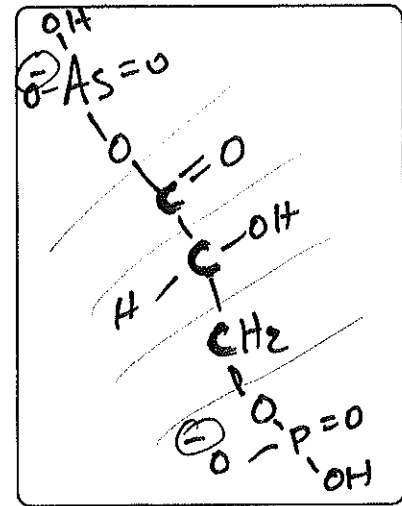
d. Such arsenate esters are very rapidly hydrolyzed in water to regenerate arsenate and the carboxylic acid (**before** the compound could get to the next enzyme in glycolysis. Using this information - what would the yield of ATP per glucose be if all of the thioester intermediate were captured by arsenate (not phosphate). Assume no other steps are affected.

··· by-pass from 6 ⟶ 8 "directly"

Yield of ATP from glucose to lactate in the presence of arsenate ___O___ ②

Question 10 (6 pts.) Numbers: the answer to these question is a number from 0-20. Do not write abbreviations or words.

a. This numbered glycolytic intermediate is the first common intermediate between glucose and glycogen breakdown ___2___

b. phosphoenolpyruvate is compound number ___ ___10___

c. how many phosphate atoms does a molecule of NADH have? ___2___

d. how many more electron does ethanol have than acetaldehyde? ___2___

e. conversion of one molecule of sucrose to ethanol releases ___ molecules of CO_2 ___4___

f. the number of different enzymes needed to convert glucose to lactate? ___11___

Question 11 (12 pts) This question deals with the inhibition of chymotrypsin by the compound shown to the right. Please pay attention to concentrations of each component listed below.
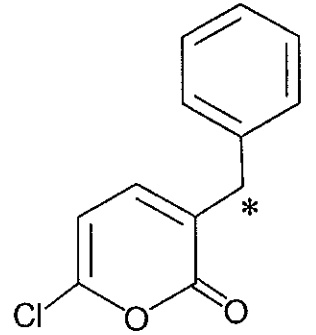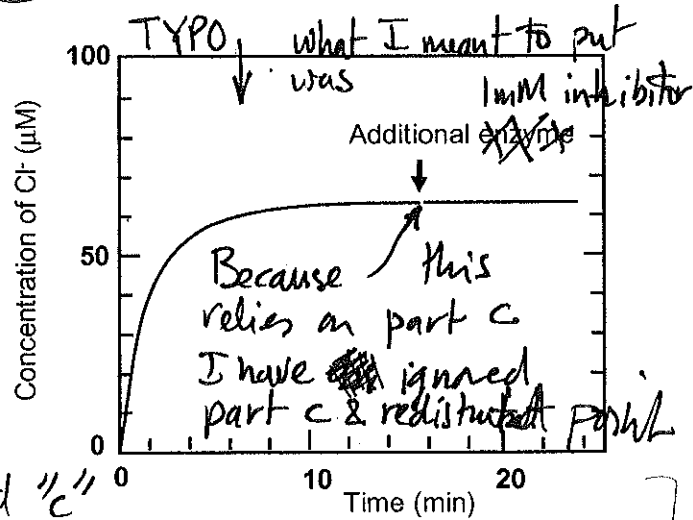
5 μM chymotrypsin was mixed with 1 mM of the inhibitor at time zero. The graph below follows the release of chloride ion. A second 5 μM addition of chymotrypsin was made at 15 min.

a. what is the amount of the rapid phase seen below ⟨2⟩ ~ 62 μM

**b.** explain the significance of the rapid phase seen to right. *because 62 μM ≠ 5 μM this ⟨4⟩ is NOT a burst phase...! The enz. turns the inhibitor over about 12 times (on average) before this mechanism based inhibitor inactivates all enzyme. Inactivation is associated w/ release of Cl⁻*

TYPO, what I meant to put was 1mM inhibitor

Additional ~~enzyme~~

Because this relies on part C I have ~~x~~ ignored part c & redistributed points

100

50

0

Concentration of Cl⁻ (μM)

0   10   20
Time (min)

c. explain what happens at the second addition of enzyme ... and why NOT GRADED

NOT Graded "c"

X 0 pts

The enzyme is inactivated already so no further processing of inhibitor ✓

d. the inhibitor is radiolabelled at the asterisk * (top right). After 25 min (see graph) the mixture was subject to size exclusion chromatography. Draw clear curves to illustrate the elution profile for both protein and radioactivity.

⟨4 pts⟩

Protein ——
Radioactivity ┊ ┊ ┊

Radioactivity

0
Time (min)
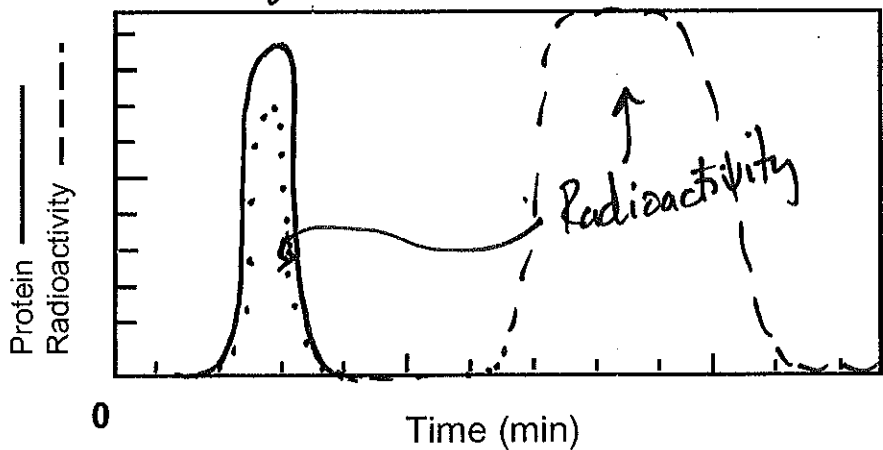
e. after size-exclusion (gel-filtration) chromatography, the enzyme is assayed with a suitable substrate. What do you expect to see?

⟨2 pts⟩

The enzyme is covalently modified so should still be inactive

⟨2 pts⟩

7

Question 13 (16 pts.). Fill in the blanks with not more than 3 legible words.

a. name an irreversible inhibitor of an enzyme

many

b. and the enzyme that is the target of your answer in "a"

many

c. these enzymes do not follow Michaelis-Menten kinetics

allosteric

d. the water-soluble vitamin incorporated into NAD^+ is called

NIACIN

e. a negative allosteric regulator of glycolysis

ATP, NADH

f. a major regulatory enzyme in glycolysis

hexokinase, Phosphofructokinase

g. the monosaccharides D-glucose and D-galactose are ___ of one another

EPIMERS

h. a metal frequently associated with kinases

Mg^{2+}

i. a hormone that indirectly activates phosphoryase

EPINEPHRINE

j. name an an acid protease

Pepsin, HIV protease · →

k. a thiol protease

papain / cathepsin

l. inactive precursors of proteolytic enzymes are called

zymogen

m. a protease containing a bound zinc ion

carboxypeptidase

n. a technique used for studying rapid reactions in enzymology

stopped flow

z. how many times have you been taught "glycolysis"

—

zz. the word that best describes this exam

——