CHEM 527 SECOND EXAM FALL 2002

YOUR NAME: _____

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 - unfocused, rambling answers won't receive as much credit as a few short appropriate phrases.
6. Please write CLEARLY - if I cannot read it - it is wrong.
7. Good luck

Question 1. (16 pts.) Short problems. Show work, but most credit goes to the correct numerical answer.

a. an enzyme has a Vmax of 0.2 µmol/min and a rate of 0.03 µmol/min at 10 µM substrate. What is the Km for the substrate Km = _____M

b. In "a" above the molecular weight of the enzyme was 25,000 g/mol and the amount used 10 µg. What is the maximal turnover number TN = _____/min

c. A competitive inhibitor of a enzyme is present at 6 mM and shows a Ki of 1 mM. If the Km of an enzyme for its substrate is 12 mM what is the apparent Km with the inhibitor present? Km = _____mM

d. A single subunit (monomeric) oxygen binding protein shows a K_p of 1 mm what is the fractional saturation at 2 mm partial pressure of oxygen?

Fractional saturation_____

e. The equilibrium constant for the reaction:

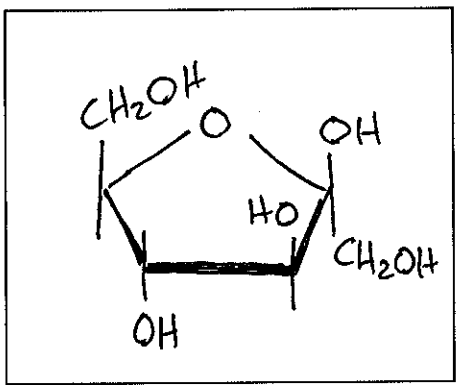
ATP + glucose ⇌ glucose-6P + ADP

Is **935** calculated from the standard free energy of the reaction.
In the cell, the concentrations were measured as:
ATP = 10 mM; ADP = 1 mM; glucose = 1 mM; Glucose-6P = 0.5 mM

Calculate the value of the equilibrium constant from these values: K= _____

Why are the numbers different?_____

Question 2 (5 pts) Regarding the monosaccharide shown to the left



- Label the anomeric carbon with an **A**
- This molecule is in the α– or β– configuration _____
- This molecule is: aldose ketose cannot say
- Label carbon atoms **1** and **4**

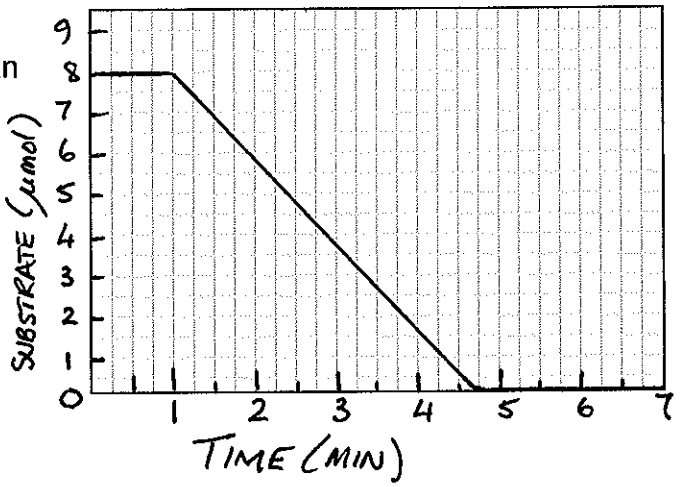
___Question 3 (3 pts.) Which is the most appropriate answer?

a. the rates of enzyme catalyzed reactions are usually limited by the rate of substrate binding
b. the Michaelis complex is never covalent
c. the Km is equal to $V_{max}/2$ for an enzyme-catalyzed reaction
d. mechanism-based inhibitors do not bind to their target enzymes
e. all of the above are false

___Question 4 (3 pts.) Which is the most appropriate answer. At constant E_T, if the rate of an enzyme assay almost doubled when the substrate concentration doubled:

a. the enzyme is saturated
b. the substrate concentration is well above the Km
c. the substrate concentration is well below the Km
d. more enzyme is needed
e. all of the above are false

Question 5 (10 pts.) The graph to the right shows an enzyme assay converting a single substrate into a single product (S → P). It was started at time 1 minute by the addition of 20 micrograms of enzyme to a solution of 1 mL of substrate containing the amount of substrate shown in the graph. The pH was 7.5 at 25 °C.



Answer the following questions - there is more information than you need.

a. Calculate the rate of the enzyme assay _____micromole substrate/min

b. What is the rate in the absence of enzyme? _____micromoles/min

c. The enzyme is saturated with substrate over most of the assay - circle the appropriate:

YES NO Cannot say

d. the enzyme is operating at V_{max} over most of the assay - circle the most appropriate

YES NO Cannot say

e. the product (P) is a powerful competitive inhibitor of the enzyme - circle the most appropriate

YES NO Cannot say

f. the equilibrium constant for the conversion of S → P is greater than 10

YES NO Cannot say

g. what is the *concentration* of substrate in the assay before the addition of enzyme _____[M]

Question 6 (4 pts.) You take equal volumes of blood from a sickle cell patient and a normal individual and mix them carefully and then remove oxygen from the blood sample.

The RATE of sickling will: INCREASE DECREASE STAY THE SAME CANNOT PREDICT

Now in one line explain your answer:

_____

Question 7 (6 pts.) Given the following calculate $\Delta G^{o'}$ for equation 1:

1. ATP + H_2O = AMP + $P\text{-}P_i$ $\Delta G^{o'}$ = _____ kcal

2. ATP + H_2O = ADP + P_i $\Delta G^{o'}$ = -7.3 kcal

3. ADP + H_2O = AMP + P_i $\Delta G^{o'}$ = -7.3 kcal

4. $P\text{-}P_i$ + H_2O = P_i + P_i $\Delta G^{o'}$ = -6.9 kcal

Please show work:

Pyrophosphatase catalyzes reaction 4. The addition of this enzyme to equation 1 will (circle all answers that are appropriate)

a. make reaction more exergonic **b.** drive the reaction to the right

c. drive the reaction to the left **d.** have no effect on the equilibrium position

e. insufficient information to make choices

What metal ion would you expect to be involved in pyrophosphatase action? _____

Question 8 (15 pts.) Short answers (3 pts each) - a few phrases or a labelled diagram is sufficient.

a. Describe FRAP

b. Why is the affinity of oxygen for normal hemoglobin dependent on $[H^+]$?

c. Vitamin B12 is a large, polar, molecule that is concentrated from the environment into certain microbial cells. What type of process would be involved and why?

d. Glycogen and starch are stored within cells as granules. Why not store energy in the form glucose instead?

e. You hear of an enzyme whose kinetic constants were reported to be k_{cat} = 1000 /sec and Km = 0.1 μM. Why should you be suspicious?

Question 9 (6 pts.) An oxygen binding protein (OBP) from Europa exists in two states A and B that are in equilibrium.

A binds oxygen tighter than **B**
A has a higher pI than **B**
A binds CO_2 tighter than **B**
A binds Zn^{2+} tighter than **B**
A is a dimer, **B** is a monomer

a. Increasing CO_2 causes oxygen affinity of OBP to (circle):

increase decrease stays unchanged cannot predict

b. Increasing Zn causes oxygen affinity of OBP to (circle):

increase decrease stays unchanged cannot predict

c. Increasing total OBP concentration causes oxygen binding of OBP to (circle):

increase decrease stays unchanged cannot predict

d. Increasing total OBP concentration causes CO_2 binding of OBP to (circle):

increase decrease stays unchanged cannot predict

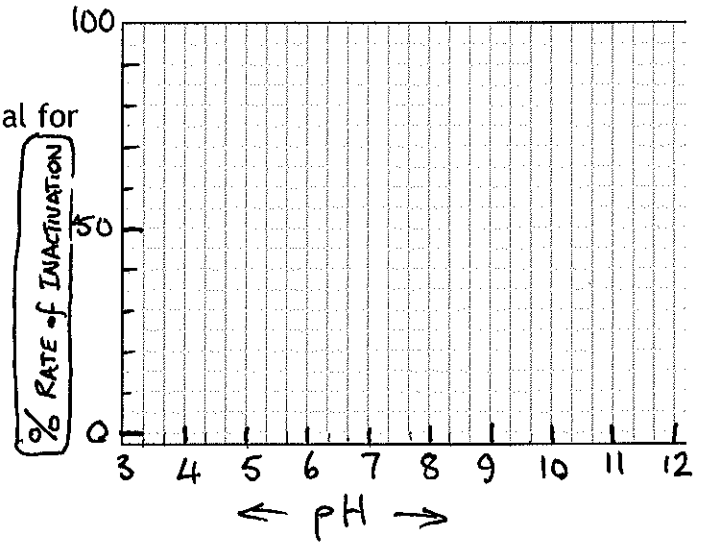
e. Lowering the pH causes oxygen binding of OBP to (circle):

increase decrease stays unchanged cannot predict

f. Lowering the pH causes the percentage of monomeric OBP to (circle):

increase decrease stays unchanged cannot predict

Question 10 (7 pts.) An enzyme has a single cysteine side chain (-CH_2-SH; pK = 8) which is essential for activity. The enzyme is inactivated by reacting with iodoacetate.



a. iodoacetate is what type of inhibitor?

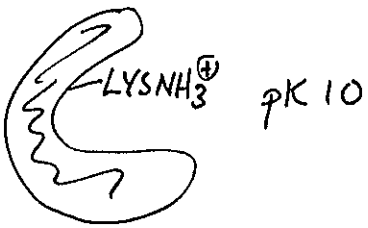
_____

b. Draw an accurate curve to represent the **rate of inactivation** with pH

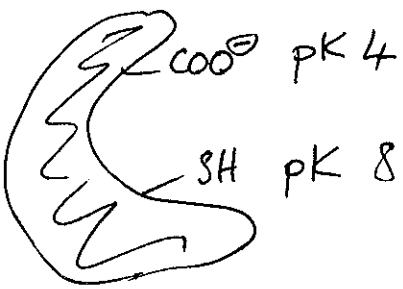
c. Draw an accurate representation of the chemistry of this inactivation reaction

d. Inactivation of the enzyme was slowed 100-fold by the substrate of the enzyme. Suggest an explanation for this (one sentence)

Question (9 pts.) Draw the pH activity curves for the enzymes shown to the left. Only these protonic forms are active.

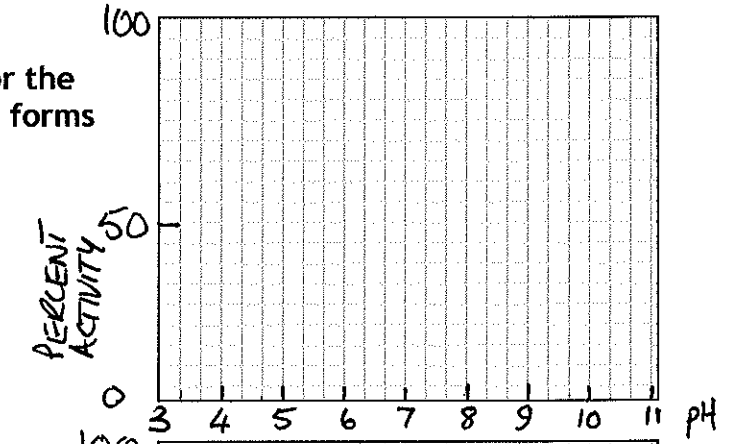


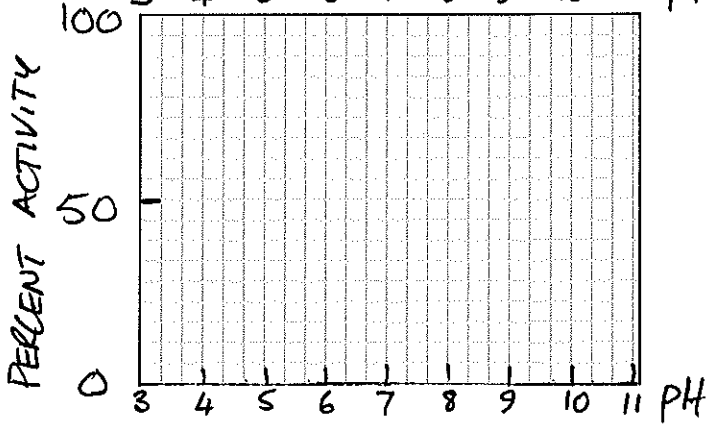
pK 10

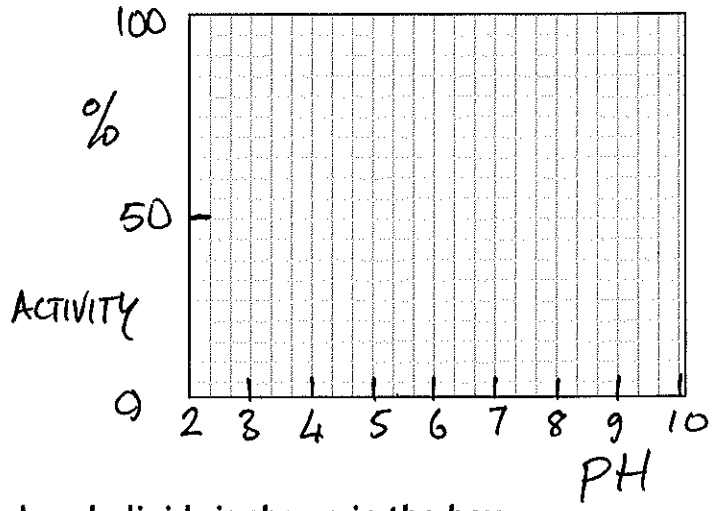


pK 4

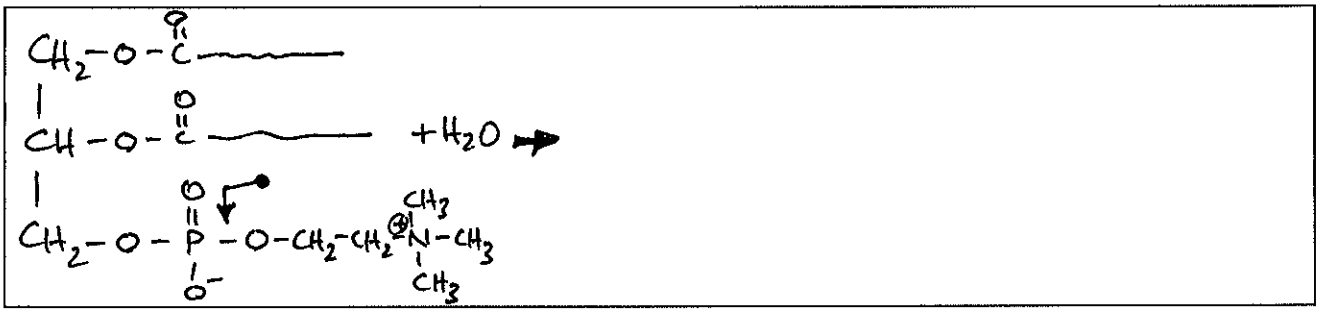
pK 8



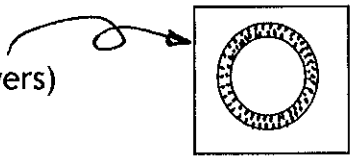




Question (10 pts). A schematic structure of a phospholipids is shown in the box. Phospholipase D hydrolyzes the bond shown. A. Complete the structure of the equation.



This phospholipid can form either vesicles (self-sealed spherical bilayers) or micelles.

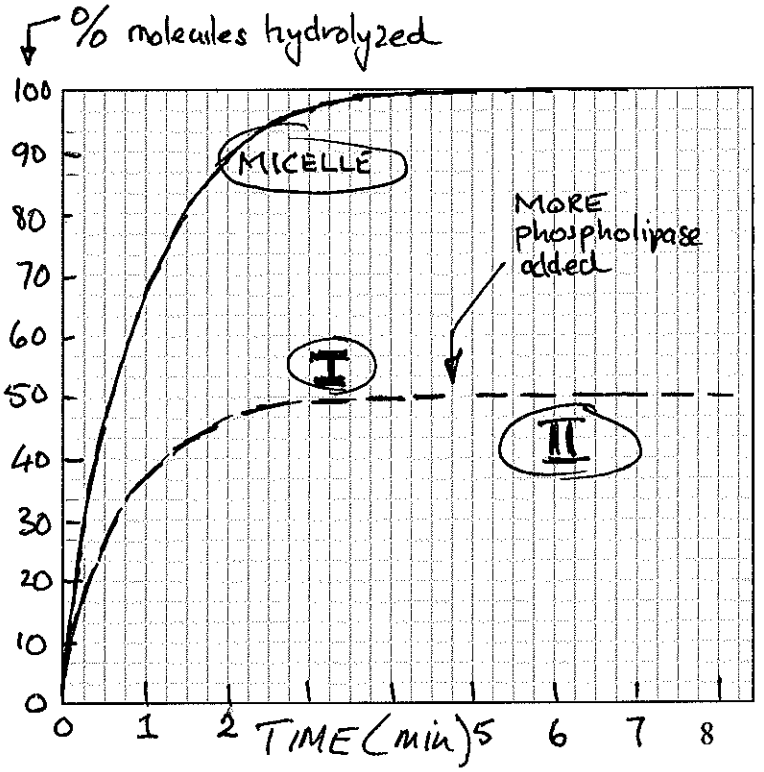


Phospholipase D is added to either a vesicles or a micelle preparation of the phospholipids. The extent of hydrolysis is shown in the graph.

B. Explain the behavior in region I

C. Explain the behavior in region II

D. Explain the behavior for the micelle



Question 10 (pts.) Fill in the blanks with not more than 3 legible words.

a. Name an irreversible inhibitor of an enzyme _____

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

c. name the glycosidic bond formed in cellulose _____

d. name the glycosidic bond at the branch points in amylopectin _____

e. these enzymes do not follow Michaelis Menten Kinetics _____

f. name a non-saponifiable lipid _____

g. another name for biosynthesis _____

h. a small molecule binding to the central intersubunit cavity in hemoglobin _____

zz. the word that best describes this exam _____

the end