CHEM 641   SECOND EXAM FALL 2005

YOUR NAME: _____

SECTION (circle one)   Morning   or   Afternoon

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.   A glycolysis chart is included at the back of this exam (on page 11).
      Detach (carefully) if you wish. Please make sure you have all 11 pages.

8.   Good luck

Question 1. (10 pts) What is the yield of ATP per molecule of the following converted to lactate. Insert a number from 0-100 in the space provided.

Fructose   _____

Sucrose in the diet   _____

Maltose in the diet   _____

Dihydroxyacetone-P in the presence of arsenate   _____

For a typical glucose molecule released from glycogen intracellularly   _____

Question 2. (6 pts.) Short problems. Show work, but most credit goes to the correct numerical answer.

a. an enzyme has a V_{max} of 0.55 μmol/min and a rate of 0.12 μmol/min
    as 260 μM substrate. What is the K_m for the substrate K_m = _____M

b. a reaction: A ↔ B + C   has a standard free energy of +4 kcal. If A is maintained at 10 mM, what concentration of B and C (assume [B] = [C]) would be in equilibrium with A? Assume 27 °C, R= 2 cal/mole/° .

[B]=[C]=_____M

Question 3 (6 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 2 minutes by the addition of 7 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 and the temperature 25 °C.

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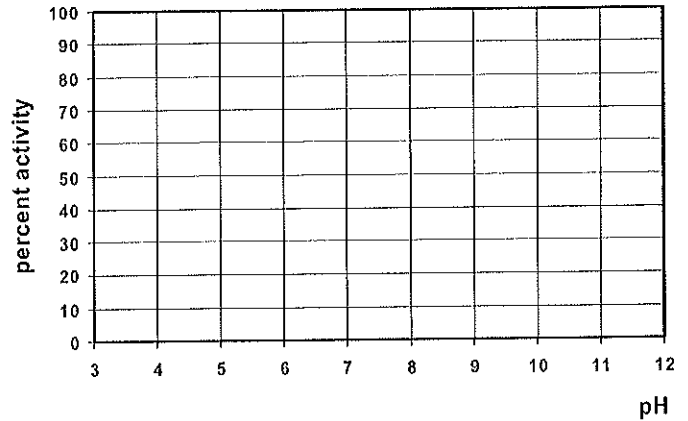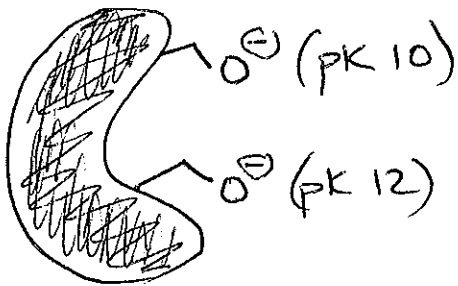

Substrate (micromoles) vs. Time (minutes)
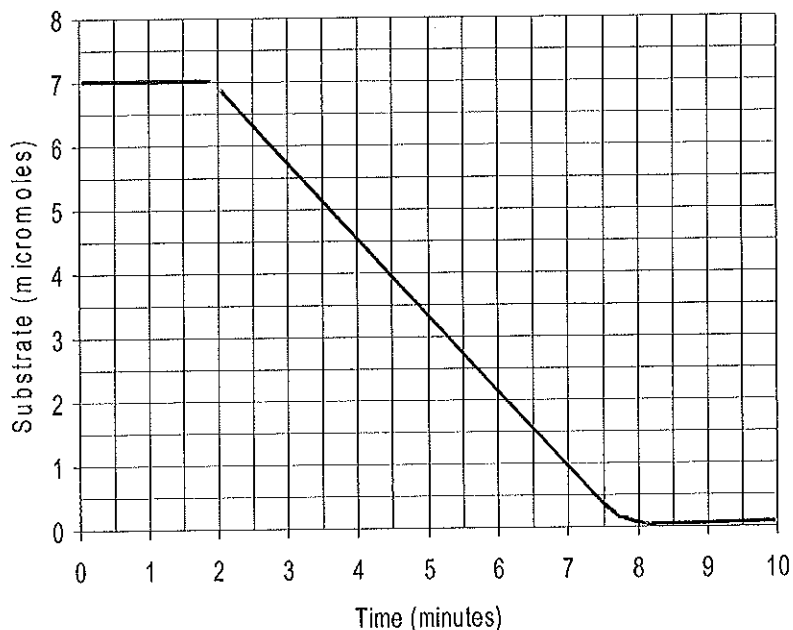
a. Calculate the rate of the enzyme assay _____ micromole substrate/min

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

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

**Question 4 (9 pts.)** Draw the pH activity curves for the following situations.

a. Only this protonic form is active.

SH (pK 7)

NH$_3^{+}$ (pK 10)

percent activity vs. pH
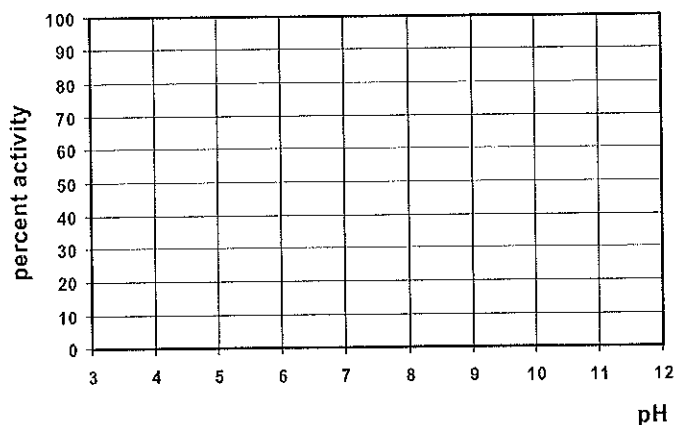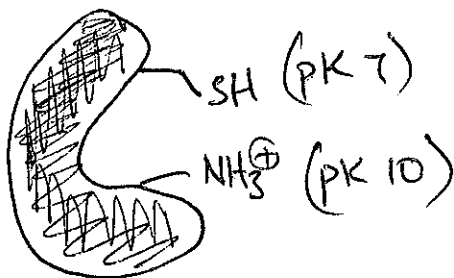
b. This enzyme is only active as shown

$O^{\ominus}$ (pK 10)

$O^{\ominus}$ (pK 12)

c. An enzyme has a lysine side chain (pK of 9 which solely determines the Km for the substrate. The protonated side chain shows a Km of 70 mM and the deprotonated form shows a Km of 10 mM. Complete the graph.
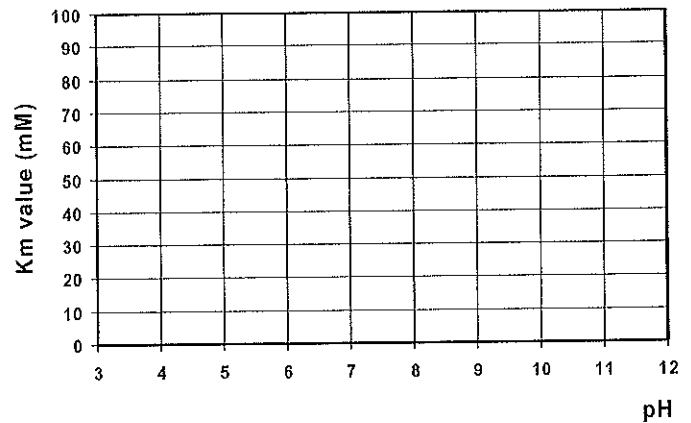
Question 5 (5 pts) Draw an equation to illustrate the reaction stoichiometry of adenylate kinase:

↔

You add phosphofructokinase to a tube containing these (initial) concentrations of substrates/products: [Compound #3] = 5 mM   [Mg ATP] = 5 mM

Why is the production of compound #4 accelerated as the reaction progresses by adding small amounts of adenylate kinase?

4

Question 6 (6 pts) Fill in the initial series of curved arrows that start the reactions of the following enzymes. The curved arrows should make chemical sense. Don't draw any more detailed structures. (If you need to deprotonate or protonate something draw general base/acids as appropriate). Add any additional critical catalytic groups as appropriate.
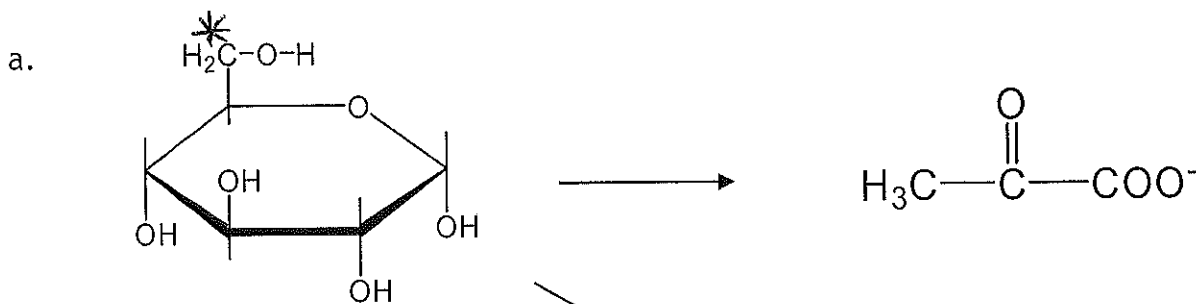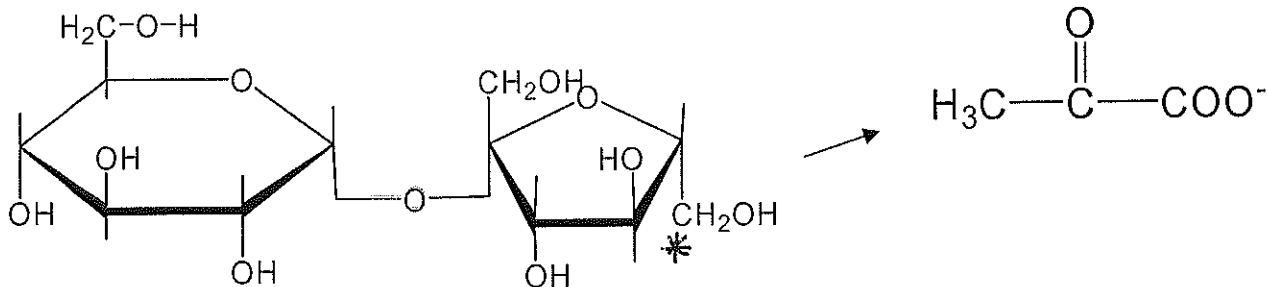
a. hexokinase
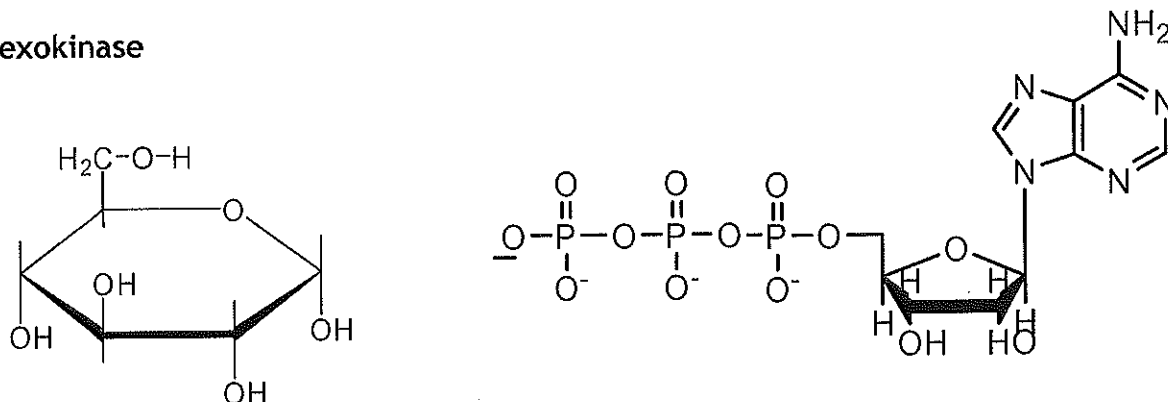
b. lactate dehydrogenase
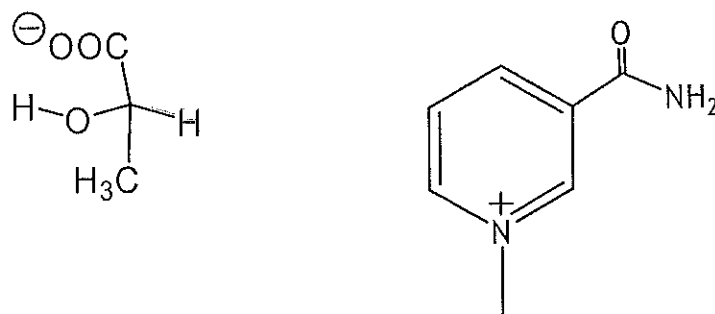
Question 7 (6 pts) Tracing radiolabels. Place asterisks indicating the position of the radiolabel in the molecules shown to the right - if the product contains no radiolabel write "NONE".
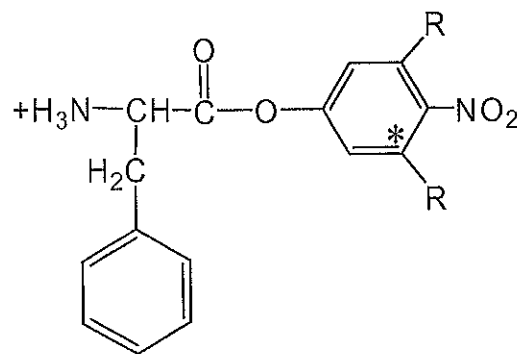
a.

$$H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-COO^-$$

b.

$$H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-H$$

5

c.

H_2C-O-H

CH_2OH

OH

OH

OH

CH_2OH

HO

CH_2OH

OH

*

→

$H_3C-\overset{\overset{\displaystyle O}{\|}}{C}-COO^-$

Question 8 (12 pts) Chymotrypsin is mixed with the radiolabeled ester substrate shown to the right (C^{14} at the asterisk) to give concentrations of 30 µM and 10 mM respectively.

The first product to be released shows a molar extinction coefficient of 10,000 $M^{-1}cm^{-1}$ at 410 nm under the conditions of the experiments (pH 8.5).

$+H_3N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-O-$ [benzene ring with R, R substituents] $-NO_2$

H_2C

[phenyl ring]

*

Answer the following questions:

a. In the box at the right draw the second product to be released from the enzyme:

b. The burst phase is completed in less than 1 min. Calculate the absorbance increase at 410 nm in a 1 cm pathlength expected for the burst phase:

Absorbance increase_____

c. Suppose the turnover number of the enzyme in the steady state was 20/min what is the increase in absorbance at 410 nm that would be observed between 1 and 2 min after mixing?
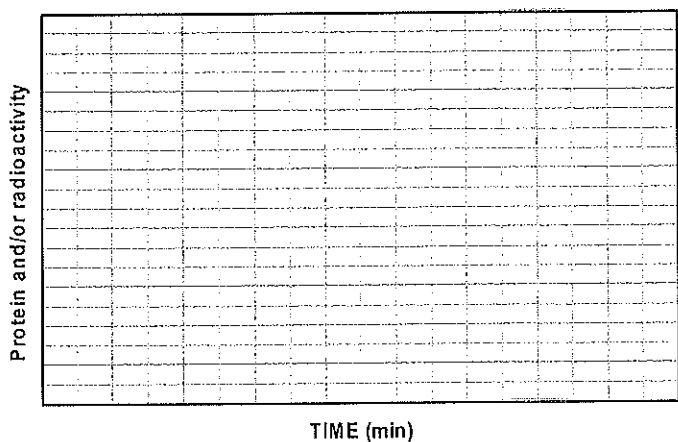
Absorbance increase_____

6

Question 8 (continued)

d. After 2 min the mixture is cooled rapidly and gel-filtered (size exclusion chromatography) at 4°C. Using the graph below **left** draw a representative trace of the chromatogram clearly indicating where protein and radioactivity would emerge.

e. The protein-containing fractions are collected and combined. They are allowed to warm to 25 C and then the chromatography is repeated. Again the fractions were followed for protein and radioactivity. Show the expected result at the **right** (above).
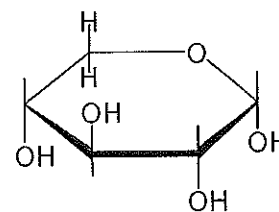
first chromatography   **second chromatography**

TIME (min)   TIME (min)
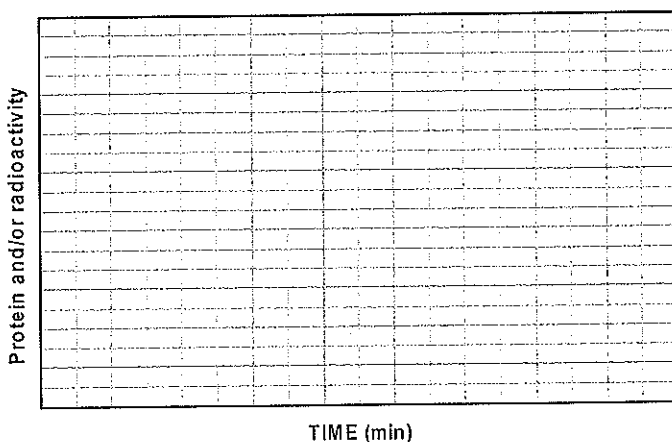
Question 9 (9 pts) Alpha-D-xylose (shown) is dissolved in buffer and mixed with ATP/Mg^{2+} and hexokinase. The concentration of selected compounds is shown below at time zero and after 5 min

Time	xylose	ATP	ADP	AMP	Pi
0 min	1mM	10 mM	0 mM	0 mM	0 mM
5 min	1 mM	6 mM	4 mM	0 mM	4 mM

a. show a chemical equation to describe this overall reaction in the presence of xylose:

b. Explain concisely what is happening in this example:

c. Finally, draw D-xylose in a <u>furanose</u> ring form. Label the configuration of the anomeric carbon atom that you chose and circle the C atom in this furanose form that designates this as a D-sugar.

Question 10 (7 pts) Draw the catalytic triad of a thiol protease. Include side chain structures and interactions.

Suggest a simple covalent inhibitor (less than 12 atoms) of the enzyme that you depict.

Name_____   Chemical structure

Then in the space below draw the essential chemistry using appropriate curved arrows.
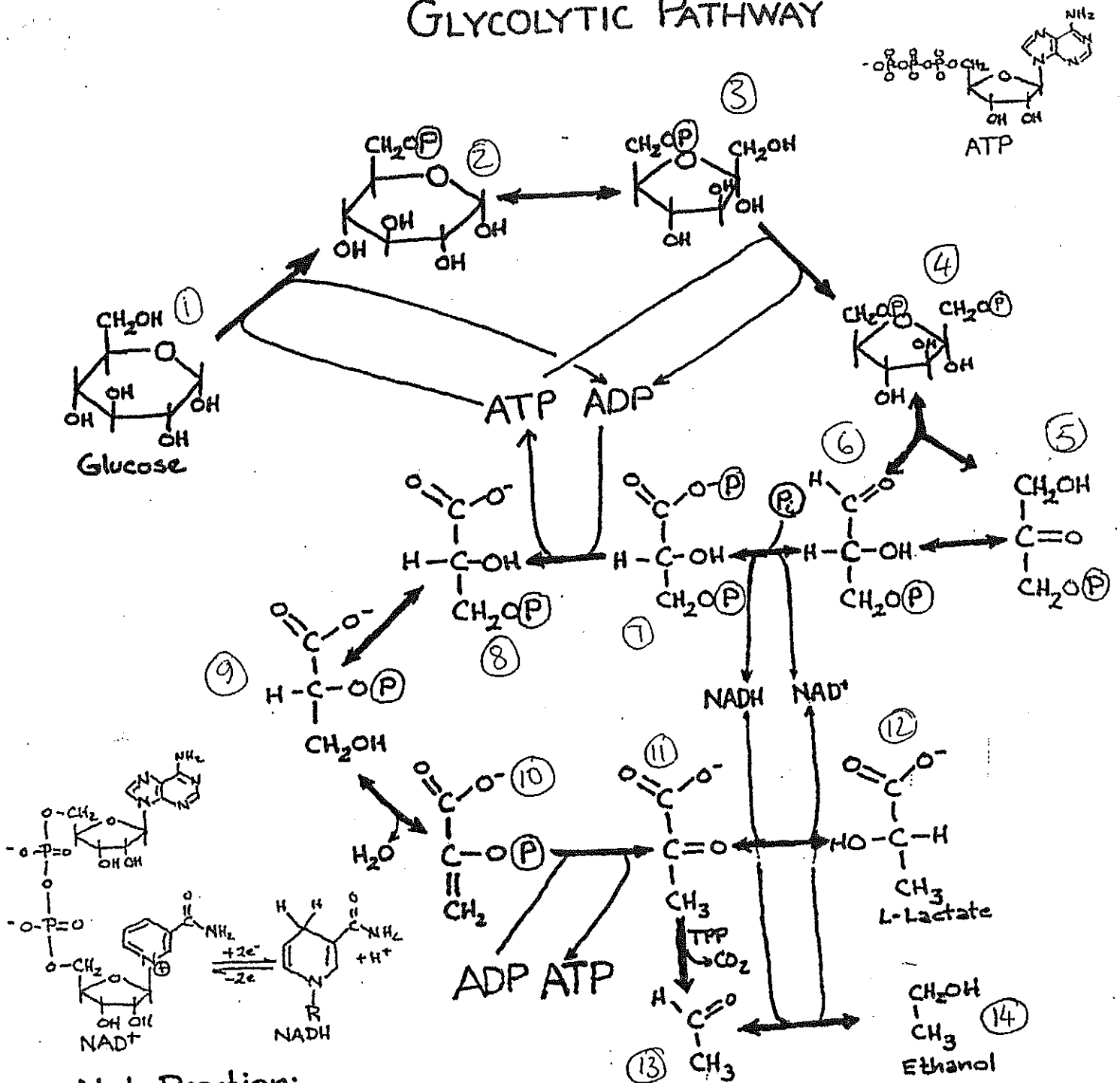
Question 11 (24 pts.) Fill in the blanks with not more than 3 legible words.

a. name the glycosidic bond formed in amylose _____

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

c. These compounds bind equally to both free enzyme and the enzyme substrate complex _____

d. This process sets a physical upper limit on an enzymes catalytic efficiency _____

e. The reagent used to diagnose *Helicobacter pylori* infections discussed in class _____

f. The method for determining the in vivo concentrations of metabolites discussed in class _____

g. A chemical inhibitor of glycolysis discussed in class _____

h. And the enzyme it inhibits _____

i. Inactive precursors of proteolytic enzymes _____

j. Give an example of an affinity label for an enzyme _____

k. And the enzyme it inhibits _____

l. The epimerization step converting a galactose derivative to a glucose derivative utilizes what coenzyme _____

m. this glycolytic enzyme generates a thioester intermediate _____

n. One form of this enzyme uses a Schiff base intermediate   _____

o. if you could make yeast thiamine deficient, what compound
    would accumulate when yeast are fed fructose   _____

p. Name a metalloprotease   _____

q. Name an aspartyl protease   _____

r. Name a thiol protease   _____

s. reactions with positive free energies are called   _____

t. Name one component responsible for the brown color of
    aerobic avian muscle   _____

u. the absence of which enzyme in humans prevents us
    from converting dietary starch to ethanol   _____

v. the vitamin incorporated into NAD$^+$ is called   _____

w. The human deficiency disease caused by the vitamin in "v"   _____

zz. the word that best describes this exam   _____

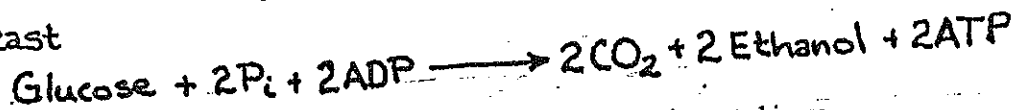*"Life is a struggle with equilibrium that we all eventually lose"*

GLYCOLYTIC PATHWAY

ATP

Glucose

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫ ⑬ ⑭

ATP ADP

P_i

NADH NAD$^+$

NAD$^+$ NADH

$+2e^-$ / $-2e$ $+H^+$

R

H$_2$O

ADP ATP

TPP CO$_2$

CH$_3$

L-Lactate

Ethanol

Net Reaction:

in vertebrates

Glucose + 2P_i + 2ADP $\longrightarrow$ 2 Lactate + 2 ATP

in yeast

Glucose + 2P_i + 2ADP $\longrightarrow$ 2CO$_2$ + 2 Ethanol + 2 ATP

1/2	hexokinase
2/3	phosphoglucoisomerase
3/4	phosphofructokinase
4/5+6	aldolase
5/6	triosephosphate isomerase
6/7	glyceraldehyde 3P dehydrogenase

7/8	phosphoglycerate kinase
8/9	phosphoglyceromutase
9/10	enolase
10/11	pyruvate kinase
11/12	lactate dehydrogenase
11/13	pyruvate decarboxylase
13/14	alcohol dehydrogenase

HB

⑪