CT part *75 pts.*

CHEM 641 THORPE part FINAL EXAM FALL 2005

YOUR NAME: KEY

SECTION (circle one) **Morning** **or** **Afternoon**

NOTES:

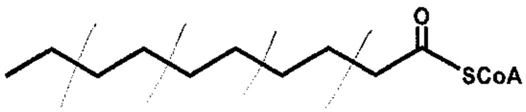
1. where appropriate please show work - if in doubt show it anyway.
2. pace yourself - you may want to do the easier questions first.
3. please note the point value of questions - adjust your answers and effort accordingly.
4. some questions may have more data than you need.
5. please be brief - unfocused, 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. Three metabolic charts are included at the back of this exam.
   Detach (carefully) if you wish.
8. Good luck

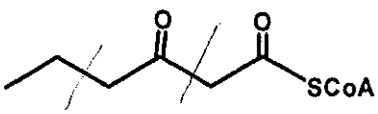
Question 1. (10 pts) What is the yield of ATP per molecule of the following. Insert a number from 0-100 in the space provided.

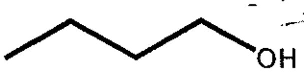
a. Pyruvate converted to CO_2 and water ____12.5____

b. Dietary maltose converted to CO_2 and water ____60____

c. Per isocitrate in the presence of malonate ____6____

d.  converted to CO_2 and water ____66____

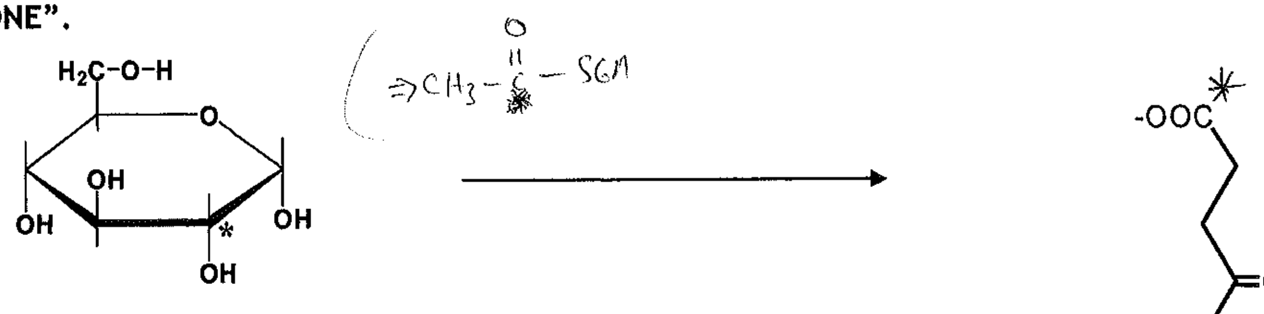
e.  converted to CO_2 and water ____34____

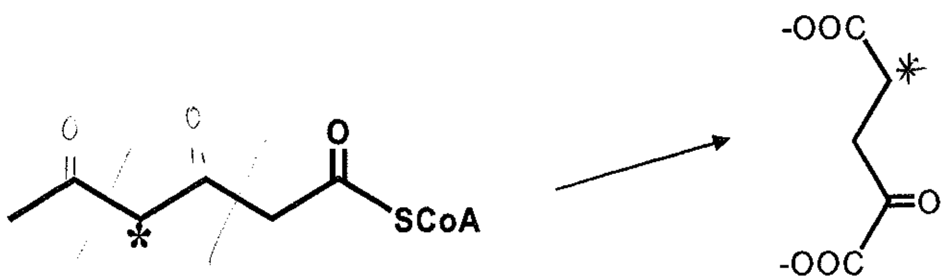
f.  converted to CO_2 and water ____25____

g. Dihydroxyacetone-P to lactate in the presence of arsenate ____1____

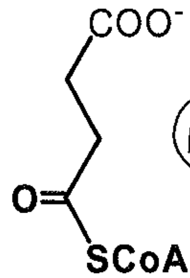
h. Glucose completely oxidized to CO_2 and water ____30____

Question 2 (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. 

b. 

c. $^{14}CO_2$ + pyruvate + acetyl-CoA ⟶



(NONE) (2)

Question 3 (8 pts.) Suppose you have two enzymes (catalyzing steps A and B) involved in making acetyl-CoA by an unusual route:

STEP A: Acetate + ATP + CoASH ↔ Acetyl-CoA + ADP + Pi $\Delta G^{o'} = +3.6$ kcal

STEP B: ADP + H_2O ↔ AMP + Pi $\Delta G^{o'} = -7.3$ kcal

Calculate the standard free energy for the reaction as written below

Acetate + ATP + CoASH + H_2O ↔ acetyl-CoA +AMP+ 2 Pi **$\Delta G^{o'}$ =** -3.7 **kcal** 3

In class we discussed two enzymes (here, called enzyme 1 and enzyme 2) that, together, catalyze exactly this overall reaction. Name these enzymes below:

Enzyme 1 acetyl-CoA synthetase **Enzyme 2** pyrophosphatase

Write out the two reactions catalyzed by these two enzymes:

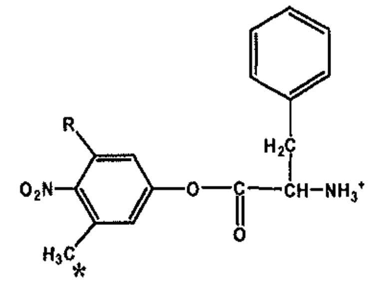
Enzyme 1: acetate + ATP + CoASH → acetyl-CoA + AMP + P-Pi (1)

Enzyme 2: P-Pi + H_2O → 2 Pi (1)

Explain, below, why the combination of steps A and B would not be used in Nature?

(1) Step B would lead to loss of energy charge ultimately catalyzing wasteful loss of ATP (via adenylate kinase). P-Pi release is only found in a certain restricted # of reactions & so hydrolysis does not have global effect 3

Question 4 (7 pts) Chymotrypsin is mixed with the radiolabeled ester substrate shown to the right (C^{14} at the asterisk) to give concentrations of 15 μM and 10 mM respectively.



The first product to be released shows a molar extinction coefficient of 8,000 $M^{-1}cm^{-1}$ at 410 nm under the conditions of the experiment (pH 8.5).

a. The burst phase is completed in less than 0.3 min. Calculate the **concentration** of first product released in the burst (1) 15 μM

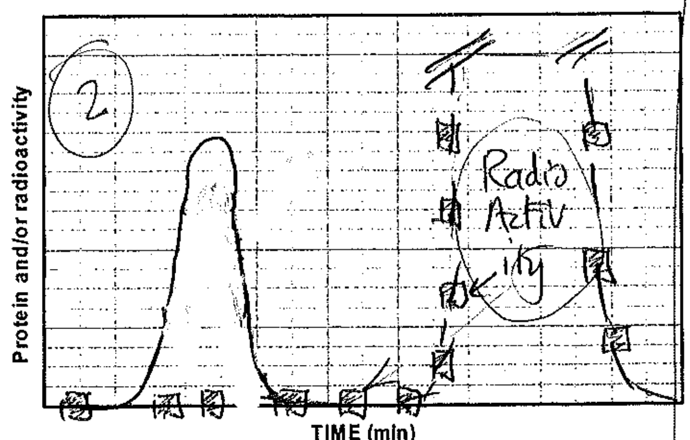
b. calculate the corresponding **absorbance** increase in a 1 cm pathlength (2) 0.12

c. Suppose the turnover number of the enzyme in the steady state was 5/min, what is the **increase in absorbance** at 410 nm that would be observed between 1 and 2 min after mixing?

5/min ≡ 5x15 μM /min = 75 μM (2)

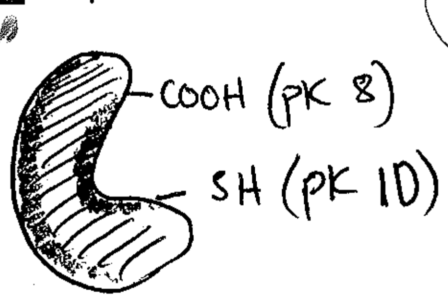
Absorbance increase 0.6

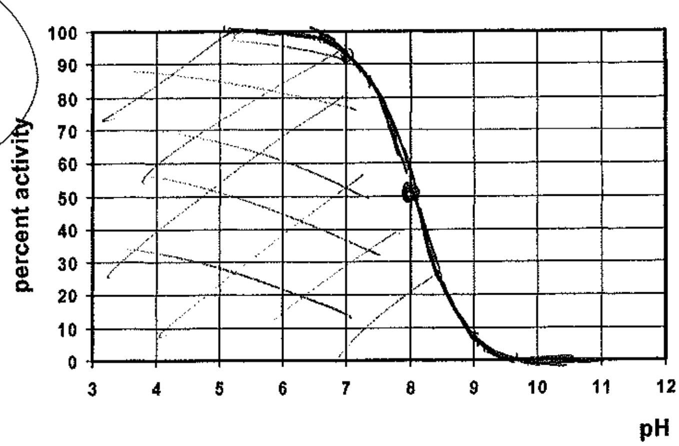
d. After 1 min the mixture is cooled rapidly and gel-filtered (size exclusion chromatography) at 4°C. Using the graph draw a representative trace of the chromatogram clearly LABEL where protein and radioactivity would emerge.



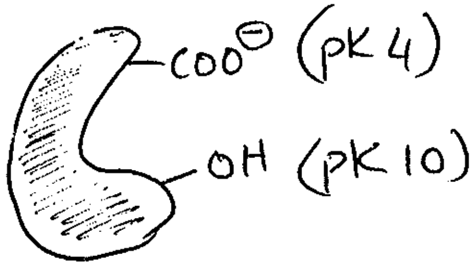
Question 5 (6 pts.) Draw the pH activity curves for the following situations. Accuracy matters.

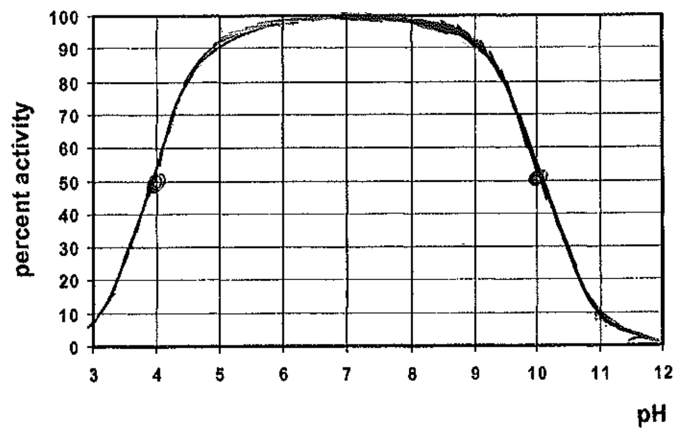
a. **Only this protonic form is active.** (2)





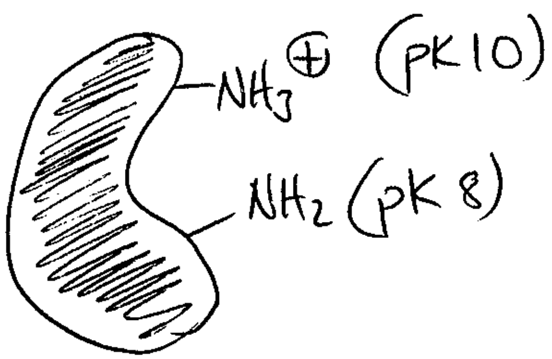
b. This enzyme is only active as shown

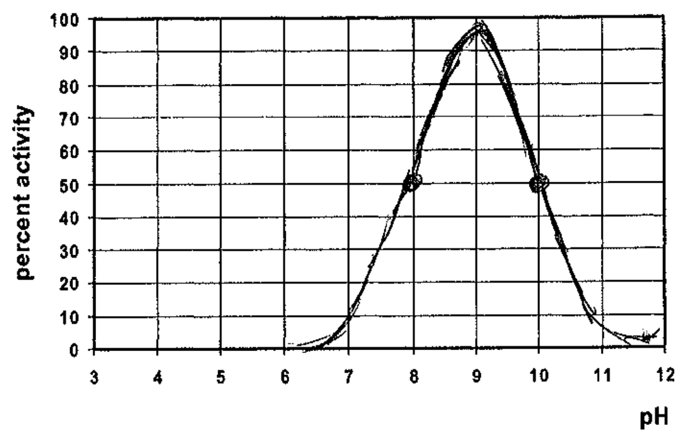




2

c. This enzyme is only active as shown





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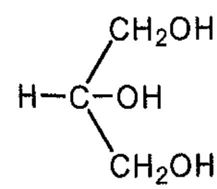
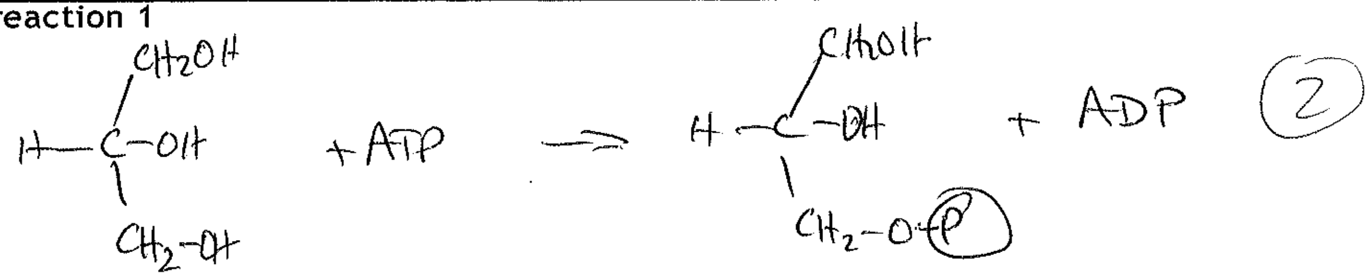


Question 6 (8 pts) Draw two equations to show clearly how glycerol (right) could enter the glycolytic pathway. The equations should be: compound A + B → C + D ..... Draw structures of A, B and etc, or provide acceptable names/abbreviations. Don't draw curved arrows or mechanisms. (Full credit given to any reasonable sets of equations - it is not necessary to reproduce the actual equations that we use to metabolize glycerol)

ANY REASONABLE COMBO (e.g.)

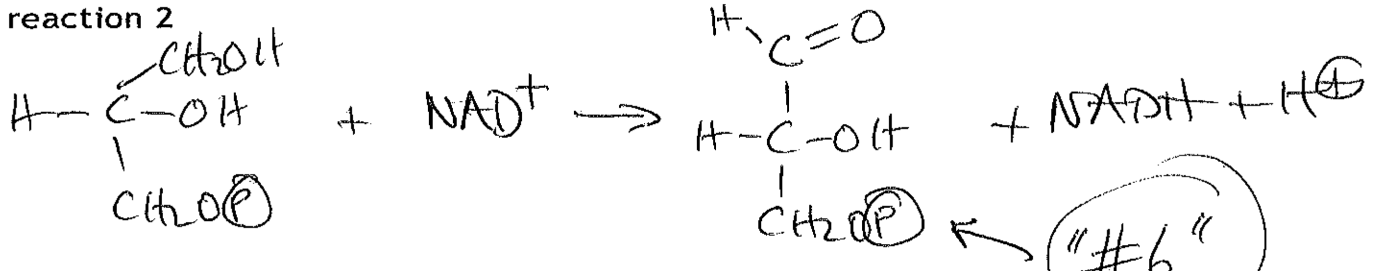
Reaction 1 (below) will be catalyzed by what class of enzyme? KINASE (1)

reaction 1



Reaction 2 (below) will be catalyzed by what class of enzyme? dehydrogenase (1)

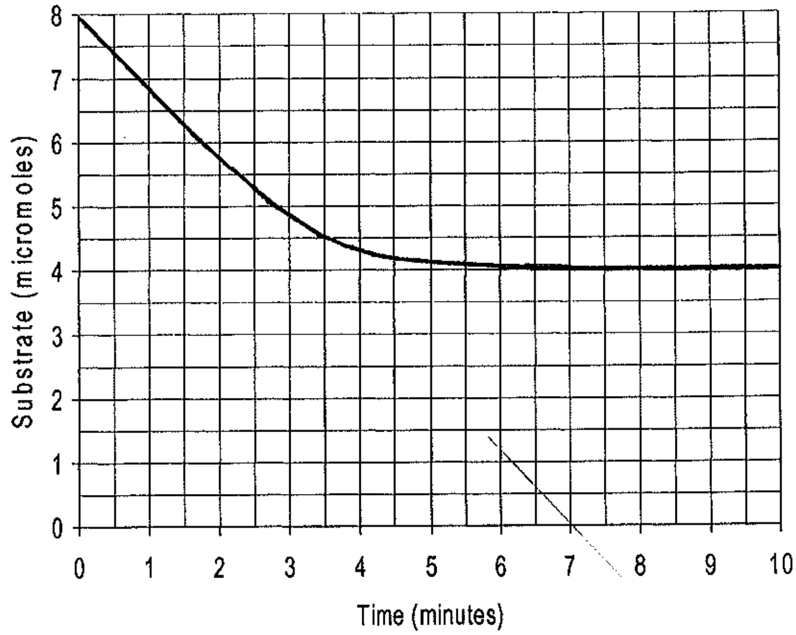
reaction 2



How many molecules of ATP would you get in the oxidation of one molecule of glycerol to CO_2 and water? 16.5 (2)

Question 7 (7 pts.) The graph to the right shows an enzyme assay converting a single substrate (L-alanine) into a single product (D-alanine). It was started at time 0 minutes by the addition of 7 nM enzyme to a solution of 1 mL of L-alanine. The pH was 7.5 and the temperature 25 °C.

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



a. what is the concentration of enzyme in the assay?

7 nM (1)

b. what is the concentration of L-alanine at time zero? 8 mM (1)

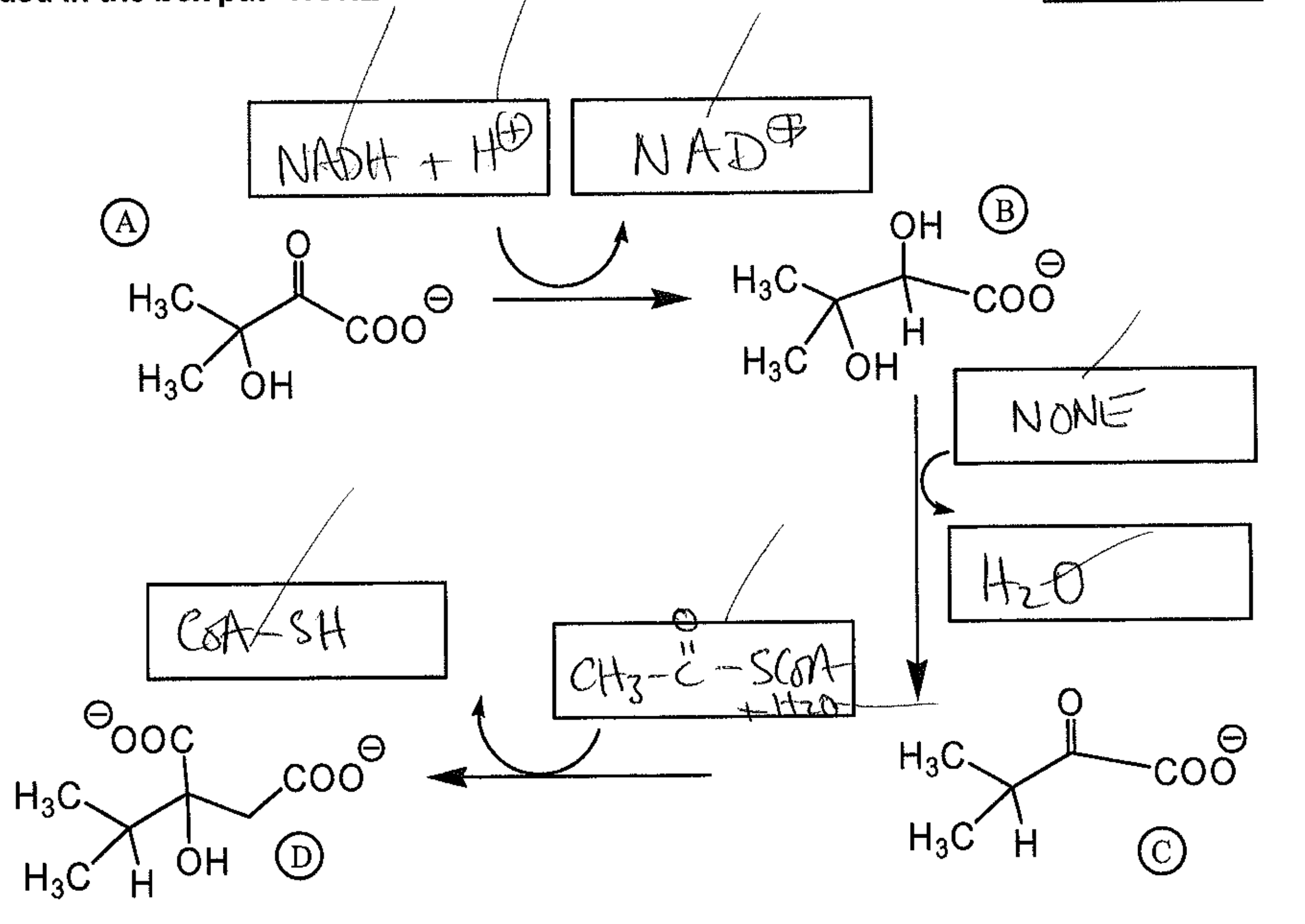
c. what is the initial rate of the assay? 8/7 1.14 µmoles/min (1)

d. what is the corresponding turnover number? 10^{-3} 1.14 M/min, 7×10^{-9} M 143,000 /min (2)

e. what is the value of K_{eq} = **[D-ala]/[L-ala]?** 1.000 (1)

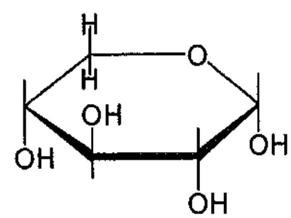
Question 8 (8 pts) The following is part of a metabolic pathway (here, converting A to D) that we did not discuss. Reason by analogy using your metabolic charts to **clearly indicate in the boxes every substrate and product missing for each reaction. Don't put enzyme names - a hypothetical example for one box is shown at right). If nothing is needed in the box put "NONE".**

NADH, H_2O, CO_2



Finally put an asterisk (indicating C-14) on the carboxylate carbon of compound A and trace it through to compound D. If there is no radiolabel on D write "**none**" on the line by the structure

Question 9 (3 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 3 min



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

Below, show a chemical equation to describe this overall reaction in the presence of xylose

$ATP + H_2O \rightarrow ADP + P_i$

Questions 10 (6 pts). Fill in the blanks with a number from 0-20 - no words allowed

a. the number of <u>electrons</u> removed during the oxidation of lactate to CO_2 12

b. the number of electrons removed during the complete oxidation of glucose 24

c. the number of electrons required to reduce one molecule of oxygen to water 4

Question 11 (8 pts) Fill in the blanks with not more than three legible words.

a. give the name of a naturally occurring inhibitor of fatty acid oxidation hypoglycin

b. the name of a direct inhibitor of ATP synthase oligomycin

c. the name of the compound that might be expected to accumulate during fatty acid oxidation in thiamine deficiency α-ketoglutarate

d. this <u>class</u> of compounds inhibits ATP formation but not electron transport uncouplers

e. an inhibitor of cytochrome oxidase CN^-, CO, H_2S ~~etc~~

f. what is/are the <u>product(s)</u> of the cytochrome oxidase enzyme reaction Water

g. reactions with positive free energy changes are called endergonic

h. the water soluble vitamin incorporated into NAD^+ is called Niacin

zzz. The word that best describes today ok, cold