BIOCHEMISTRY FINAL EXAMINATION 641/10 FALL 1994

NAME KEY

Notes: There are 12 pages on this exam - please check.

You may detach the metabolic charts if you wish

The point value of questions varies widely - take note

Please make your answers brief and to the point

The final course grade is "curved"

Good luck

Course grades will be posted outside my office (identified by a partial social security number) **only if you agree by signing below:**

I agree that my course grade can be posted

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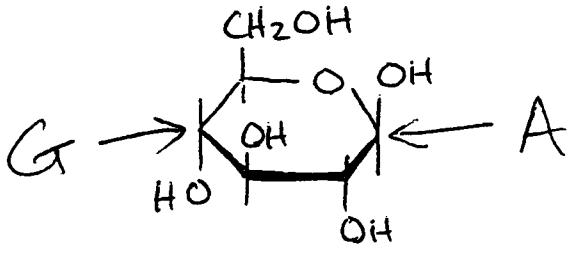
Grades will not be given over the phone.

Question 1 (~~8~~ 9 pts) The following refer to the monosaccharide on the left.



a) It is β-D-glucose

b) Label the anomeric carbon "A"

c) The equilibrium constant for isomerization at this position is likely to be? 1.0, or some other value
Some other value

d) How could isomerization at this position be prevented
in glycosidic linkage —OR (ether)?

1 pt e) Galactose is an epimer at which position? Show with a "G".

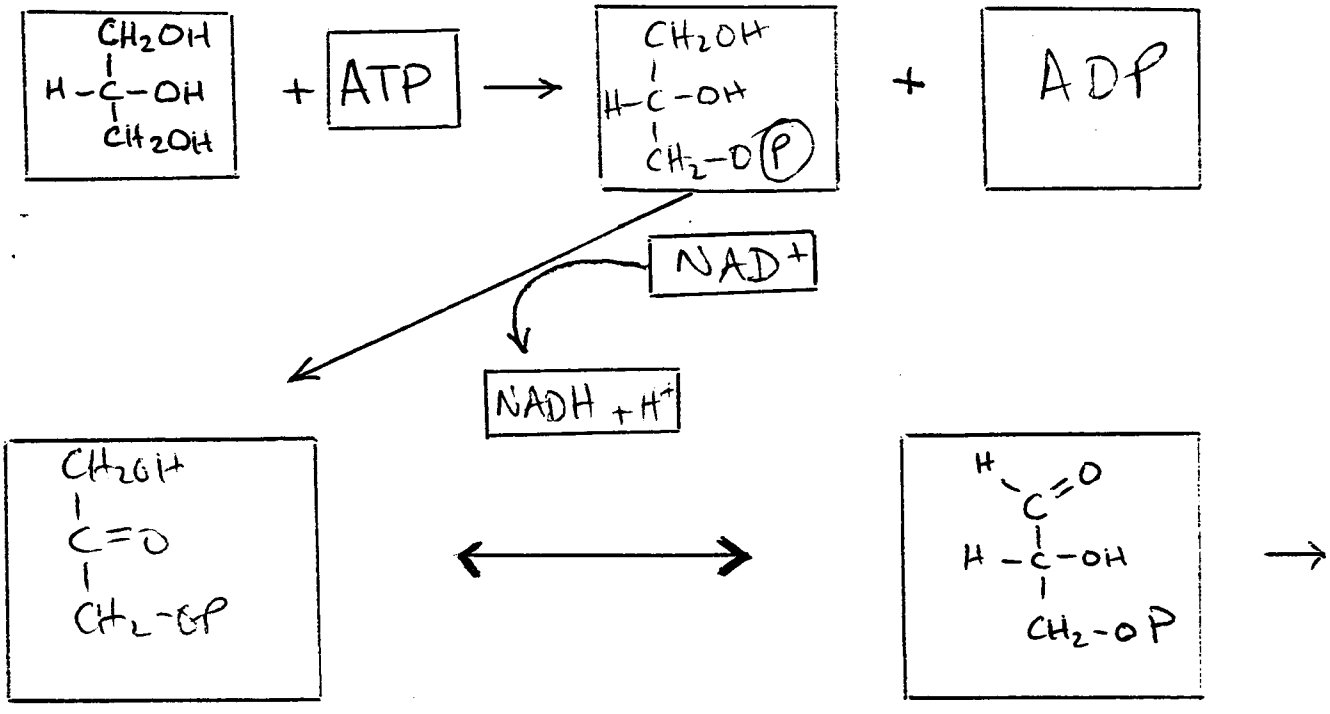
f) What cofactor is directly involved in galactose epimerization
NAD+ / NADH

g) The epimer at the 2-position is called
MANNOSE

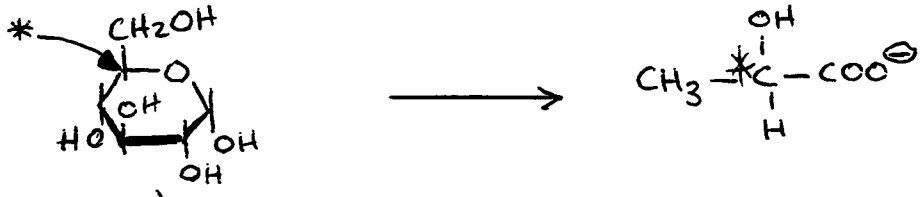
h) A disaccharide of two of the structures shown above is called
CELLOBIOSE
~~MALTOSE~~

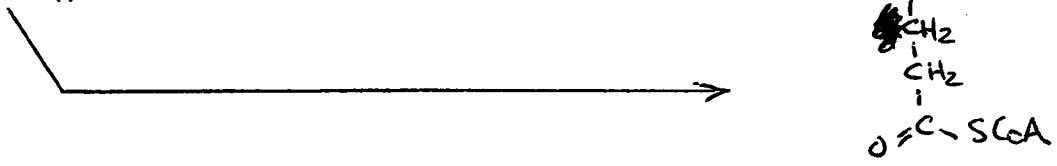
i) Name the glycosidic linkage in "h"
β(1→4)

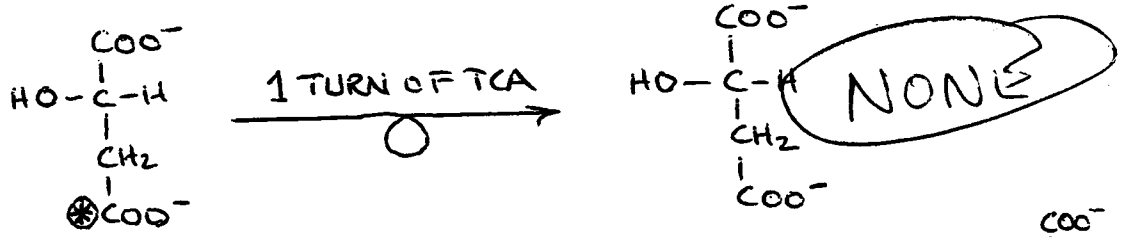
Question 2 (~~8~~ 5 pts) Glycerol can enter glycolysis by the pathway indicated. Complete the scheme with structures and/or the names of cofactors. Use reactions from the metabolic charts (or analogous ones) in your answer. Do not include the names of enzymes.



Question 3 (10 pts.) Trace the position of the radiolabel in the following transformations. If the product would not contain the label write "NONE"

A. 

B. 

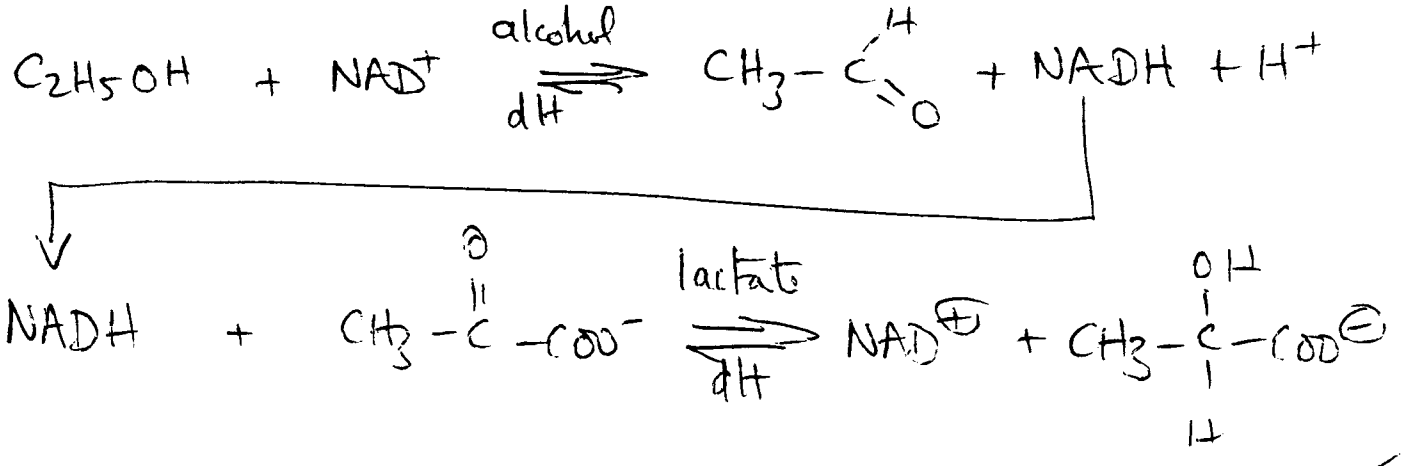
C. 

D. 

E. 

Question 4 (7 pts) Draw a **clear scheme** to show how two NAD^+ dependent enzymes plus appropriate additional components could allow ethanol to reduce pyruvate. Explain concisely your strategy.

(3)



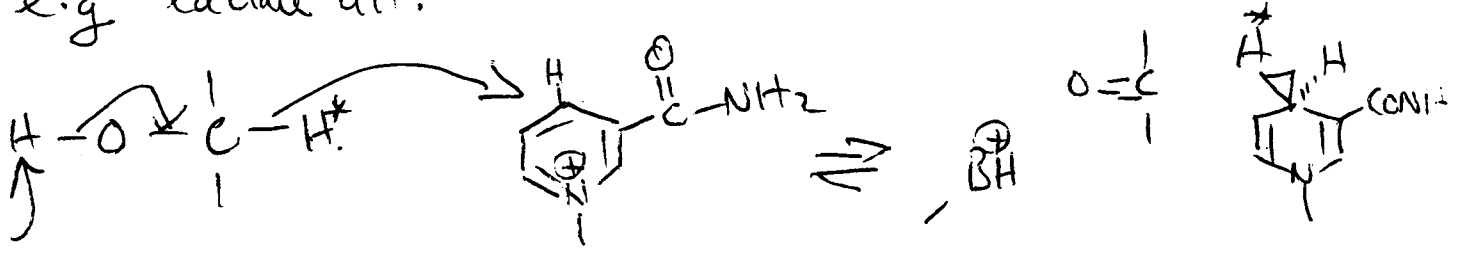
-0

How would you establish whether both enzymes showed the same NAD^+ specificity (A/B)?

2 e.g. use CH_3CT_2-OH & see whether T appears in lactate or etc !!!!

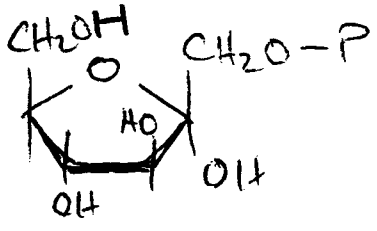
Draw a simple arrow pushing mechanism for **one enzyme** you select.

2 e.g. lactate dH:



<u>Question 5 (6 pts)</u> Fructose **may** enter glycolysis via a fructokinase which generates fructose-1-P. Draw fructose-1P:

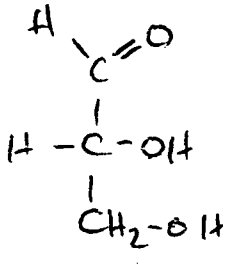
2

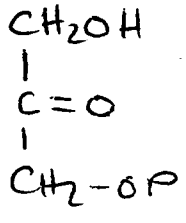


fructose 1 P

Fructose-1P is then a substrate of aldolase. Draw the structures of the two products of this reaction:

2





Identify how these fragments might enter the glycolytic system.

2

need ATP dependent Kinase

Directly

Question 6 (14 pts) Calculate the total yield of ATP, or its equivalent, upon oxidation of one molecule of the following. Where appropriate, assume the involvement of glycolysis, TCA and oxidative phosphorylation.

a. Per glucose completely oxidized to CO_2 — 36

b. Per pyruvate in the presence of arsenite — 0

c. Per mannose in anaerobic yeast — 2 (2ea)

d. Per citrate in the presence of malonate — 7

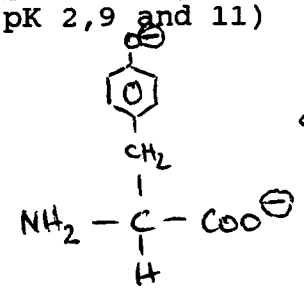
e. Per ethanol completely oxidized in liver — 14 (accept 17 didn't tell 'em about making Pyr)

f. Per 1,3-diphosphoglycerate aerobically — 17

g. Per acetyl-CoA in thiamine deficiency — 3

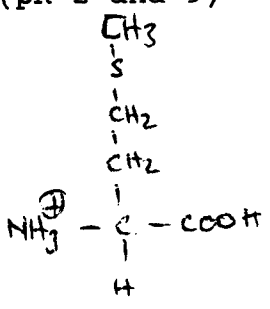
Question 7 (9 pts) Draw the following in the forms which predominate at the pH values indicated.

Tyrosine at pH 12
(pK 2,9 and 11)



(2)

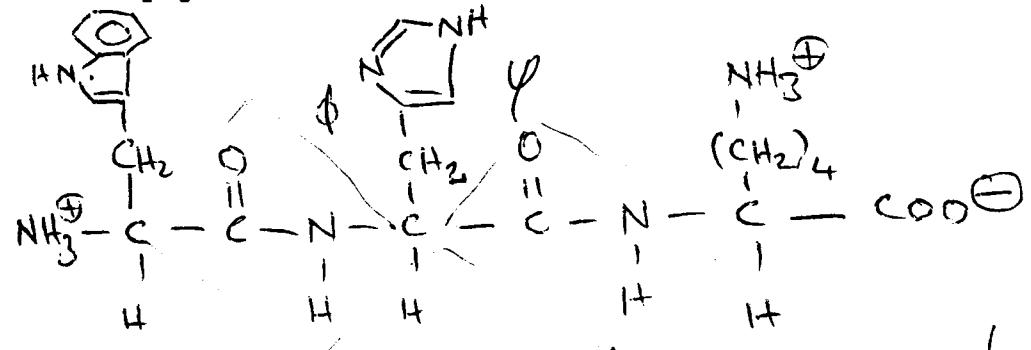
Methionine at pH 1
(pK 2 and 9)



2

The peptide: TRP-HIS-LYS at pH 7. pKs HIS_R, 6; LYS_R, 11
Indicate with arrows where the phi and psi angles are for HIS in this peptide.

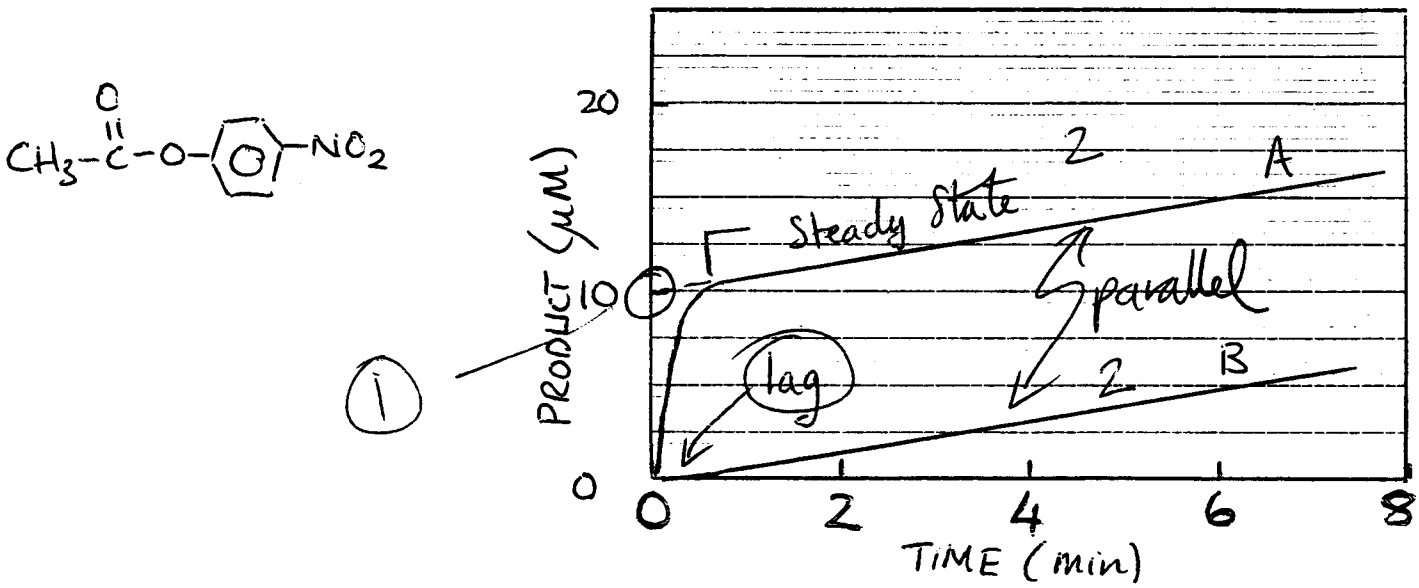
(5)



charge 2

1 ea

Question 8 (5 pts) A serine protease (10 μM) is added to 20 mM p-nitro-phenylacetate (shown at left). On the graph at the right draw accurate and clear representations of the release of A: p-nitrophenolate and B: acetate ion in this experiment. Show where the steady state is attained.



Question 9 (3 pts) What is the most appropriate answer.

a. At a substrate concentration = 2Km, v = Vmax.
b. The higher the Km the higher the affinity.
c. Km is one-half maximal velocity.
d. At a substrate concentration = Km, doubling the enzyme concentration would exactly double the rate.
e. All of the above are false.

C

Question 10 (3 pts) What is the most appropriate answer concerning a regulatory enzyme in a catabolic pathway. The enzyme would be:

a. Inhibited by ADP and activated by ATP.
b. Inhibited by NAD^+ but activated by ATP.
c. Inhibited by ATP but activated by NAD^+.
d. Inhibited by AMP but activated by NADH.
e. None of the above are likely.

See

B

Question 11 (3 pts) The rate of an enzyme catalyzed reaction almost doubled when the substrate concentration was doubled without changing any other variable. Which is the most appropriate explanation.

a. The enzyme is saturated with substrate.
b. Both substrate concentrations are well below the Km.
c. The enzyme does not obey Michaelis Menten kinetics.
d. None of the above are true.

C

Question 12 (3 pts) Formation and cleavage of one of these bonds occurs by a general mechanism which is different from the others. Which one is it?

a. Peptide bond b. Ester bond c. Disulfide bond
d. Thioester bond e. Phosphate ester

D

Question 13 (3 pts) Which statement is false?

a. Glukokinase is involved in the removal of glucose from blood circulation.
b. Hexokinase and glucokinase catalyze the same reaction in liver.
c. Hexokinae can phosphorylate fructose.
d. Hexokinase has a higher Km for glucose than does glucokinase.

D

Question 14 (3 pts) Which of the following statements concerning hemoglobin is most appropriate?

a. All mutations result in a decrease in oxygen affinity.
b. All mutations in hemoglobin are eventually fatal.
c. There are 4 DPG sites in sickle cell hemoglobin.
d. The binding of CO to deoxyhemoglobin shows positive cooperativity.
e. All of the above are false.

A

Question 15 (3 pts) 50 mL of whole blood from both a normal individual and a sickle cell patient were gently mixed and the oxygen removed from the mixture quickly. Which is the most appropriate answer.

a. The rate of sickling will be about the same as with the sickle cell patient's blood.
b. Sickling will occur much slower in the mixture.
c. Sickling will occur much faster in the mixture than in the sickle cell patient.
d. All of the above are false.

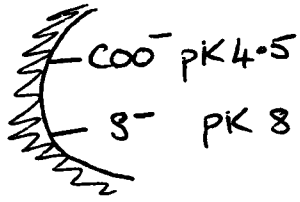
B

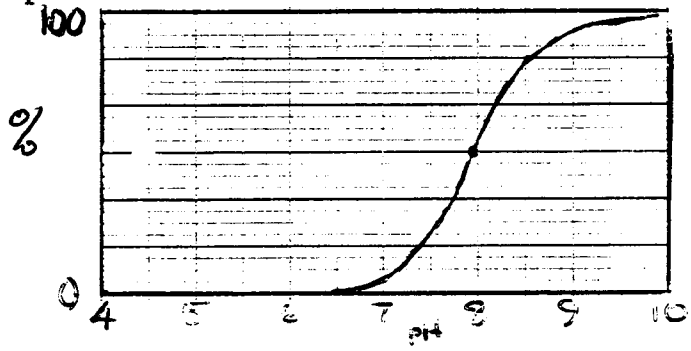
Question 16 (3 pts) Which statement is false.

a. Soft keratins can easily be extended because of their α-helix content and their relatively low disulfide content.
b. Fibroin contains a high proportion of CYS residues
c. The triple helix has never been found in globular proteins
d. Adjacent chains of fibroin are H-bonded together
e. Every other amino acid in fibroin is likely to be a GLY residue.

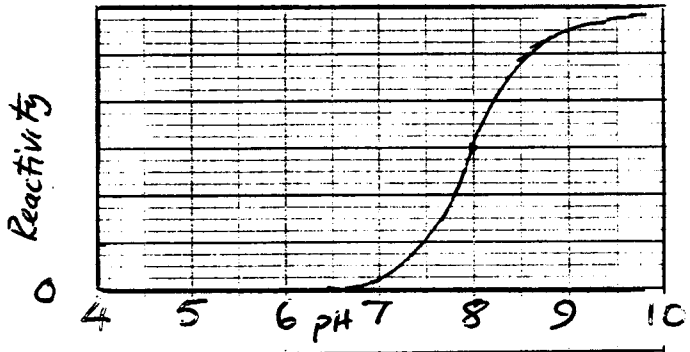
Question 17 (15 pts) Draw **accurate** graphs for the behavior of the following with pH.

a. An enzyme whose only active form is:

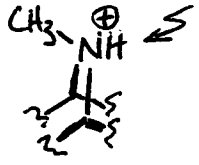


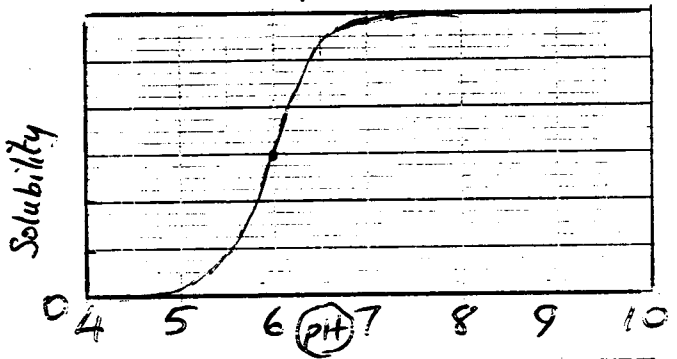


b. The reactivity of a cysteine side chain (pK=8) with iodo-acetate.

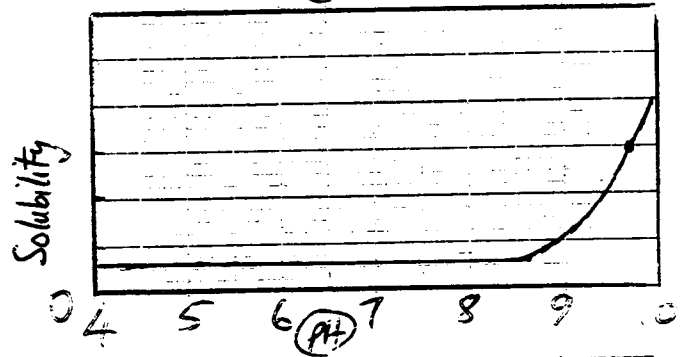


c. The solubility of cocaine in oil (indicated pK = 6)

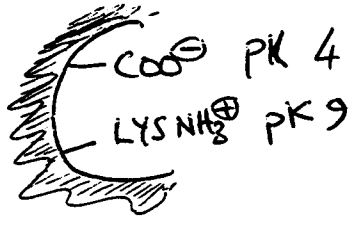


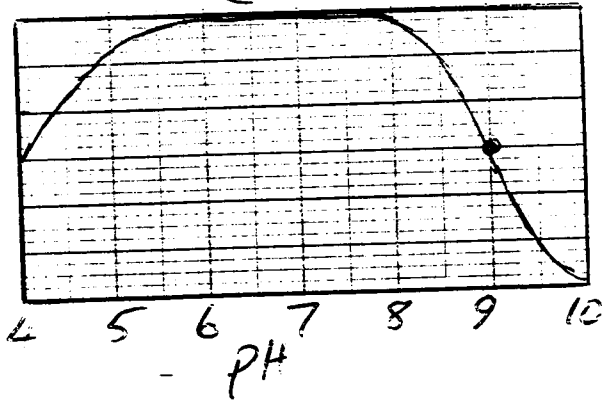


d. The solubility of isoleucine pK 2.3 and 9.7 in water.



e. The activity of an enzyme whose only active form is:





Question 18 (25 pts) Simple calculations. Most of the credit goes to the correct numerical answer.

a. Freeze clamping of a tissue catalyzing this reaction:

A + B ⇌ 2C

gave the following concentrations A = 10 mM; B = 7 mM and C = 10 μM. Given that the standard free energy change for the reaction is -4 kcal, calculate the free energy change in vivo at 37°C (R = 2cal/°C). 275

$$\Delta G = \Delta G^{o\prime} + RT \ln K$$

$$\Delta G = -4{,}000 + 2 \times 310 \times \ln\left(\frac{[10^{-5}]^2}{(10\times10^{-3}\,M)(7\times10^{-3}\,M)}\right) \leftarrow 2 \qquad \frac{10^{-10}}{7\times10^{-5}} \equiv 1.43\times10$$

ΔG -12.3 kcal

b. What is the pH of a solution made by dissolving 0.6 moles of acetic acid (pK 4.7) with 0.6 moles of lithium acetate in a total volume of 1L of water

$$pH = pK + \log \frac{A^-}{HA}$$

pH = 4.7

c. 30 mL of 10 M HCl is added to solution b above. What is the new pH:

.6 moles acetate + (.03 L × 10 moles/L HCl ≡ .3 moles HCl)

Li^+A^- + HCl ⟶ Li^+Cl + HA

.6 moles .3 moles .3 moles

New HA = .9 moles $pH = 4.7 + \log \frac{.3}{.9}$

" A^- = .3 moles

new pH = 4.22

.05 moles

-10-

NH_3^+ R (12.5) Cl^-

(9.2) NH_3^+ — COOH (2.2)

Cl^-

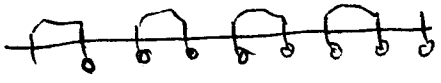
d. You have 0.5L of a 0.1M solution of arginine dihydrochloride (pK values 2.2, 9.2, and 12.5 (R-)). How many moles of KOH would you need to add to get the following pH values:

pH 12.5 .125 mol

pH 5.7 .05 mol

pH 2.2 .025 mol

e. A protein has 4 disulfide bridges and one free cysteine residue. How many different combinations of this arrangement are possible.



$8 \times 6 \times 4 \times 2$

384 combinations

f. If hair grows at a rate of 4 mm/week and the α-helix has a pitch of 5.4×10^{-8} cm, calculate the number of amino acids added to an α-helix per second

$0.4\ cm/week \equiv 6.61 \times 10^{-7}\ cm/sec$ No of turns of helix/sec $\equiv \frac{6.61 \times 10^{-7}\ cm/sec}{5.4 \times 10^{-8}\ cm}$

i.e. 12.25 turns/sec ; 3.6 AA/turn so

44 AA/sec

g. An enzyme shows a rate of 7 μmol/min at 2 mM substrate and a rate of 14 μmol/min at 5 mM substrate. What is the Vmax and Km for this substrate.

$V = \frac{V_{max} \cdot S}{K_m + S}$

$7\ \mu mol/min = \frac{V_{max} \cdot 2\ mM}{K_m + 2\ mM}$

$14\ \mu mol/min = \frac{V_{max} \cdot 5\ mM}{K_m + 5\ mM}$

$\frac{(7\ \mu mol/min)(K_m + 2\ mM)}{2\ mM} = \frac{(14\ \mu)(K_m + 5)}{5}$

$1.25(K_m + 2) = K_m + 5$

$1.25K_m + 2.5 = K_m + 5$

$.25K_m = 2.5$

$K_m = 10\ mM$

$V_{max} = 42$

Vmax 42 μmol/min

Km 10 mM

h. Splittase 100 µg catalyzes 1 µmol/min of substrate to product. The molecular weight of splittase is 10,000 and its substrate 300. What is the turnover number under these conditions.

$$\frac{10^{-6} \text{ /min}}{\frac{10^{-4}\ g}{10{,}000\ g/\text{mole}}}$$

$$TN = \underline{100 \text{ /min}}$$