 Name Key

Biochemistry 5700
Fall, 2000
Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The in-class portion of the exam must be turned in by 11:20 AM. The in-class portion should have 8 questions and 5 pages. **ANSWER ANY 7 OF 8 OF THE QUESTIONS- Mark "DO NOT GRADE" on the one question you choose to skip. DO NOT answer all eight questions-if you do, cross the one out which you don't want graded.** Each graded question is worth 10 points. The take home portion contains two questions, worth a total of 30 points. The take home portion is due beginning of class next Friday. Good Luck!!

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(1)

A	B	C	D	E
Tyr-Lys-Cys	Glu-Pro-Arg	Asp-Trp-Tyr	Asp-His-Glu	Leu-Val-Phe
(+)	(−) (+)	(−)	(−) (−)	

Which one of the above tripeptides:

+1 (a) Is most positively charged at pH 7? A

+1 (b) contains the largest number of nonpolar side chains? E

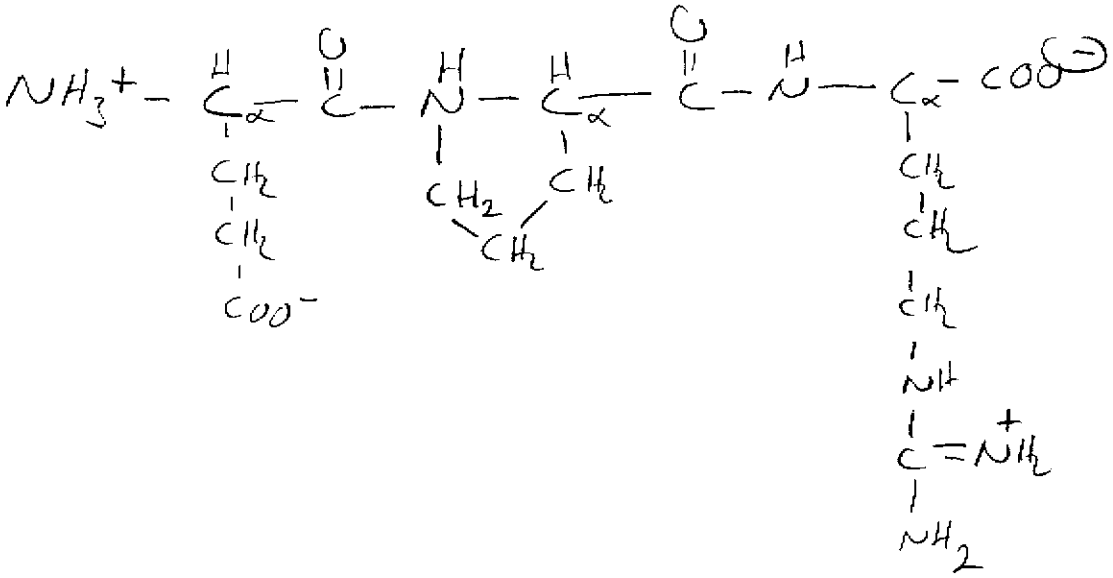
+1 (c) contains sulfur? A

+1 (d) will have the greatest light absorbance at 280 nm? C

+1 (e) contains an imino acid? B

+5 (f) Draw the structure of one of the above peptides as it would exist at pH 7; indicate which peptide you drew.

B =



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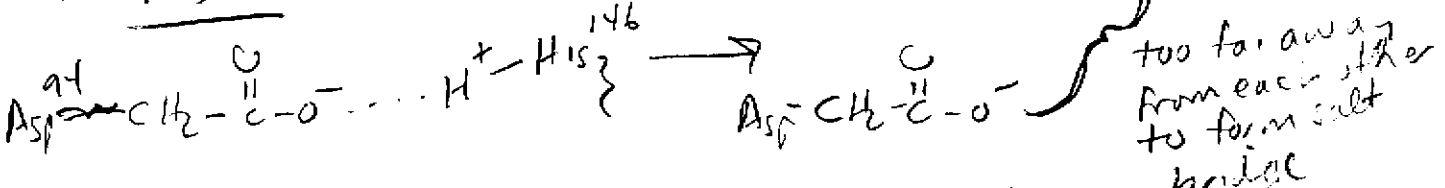
(2) In the protein hemoglobin, a protonated histidine residue (His146) forms a salt bridge with an aspartate residue (Asp94) on an adjacent strand in the T state. Upon binding O_2, this salt bridge is broken as the residues move away from each other to form the R state.

x 4 (a) What effect does the structural rearrangement of going from the T to R state have on the pK_a of His146? Clearly explain why the pK_a changes in the manner it does.

x 4 (b) Explain how the increased concentrations of CO_2 present in the tissue facilitate conversion of R hemoglobin to T hemoglobin.

x 2 (c) Expound on your answers to (a) and (b) to explain how hemoglobin faciliates the net transport of protons and CO_2 from the tissue to the lungs.

a) T state



In T state, pka of His is raised because electrostatic interaction w/ Asp 94 stabilizes protonated His, meaning it is a weaker acid now. In R state pka is lowered back towards normal value of 6.0

b) ε-lys-NH_2 + CO_2 → R-$\overset{H}{N}$-$\overset{O}{\overset{\|}{C}}$-$O^-$ (+)-AA

amino group

amino acid

CO_2 reacts with a specific in tissue to form carbamylated which forms a salt bridge with a pos. AA, thereby stabilizing T state.

c) proximity of Asp 94 ↔ His 146 in T hemoglobin means proton can remain on His at pH of tissue (~7.2) Upon transport to lungs, H^+ is released as T → R & His pka drops back to 6.

∴ Hemoglobin containing carbamylated lys, travels to lung, where low [CO_2] results in decarbamylation & release of CO_2

 Name key

(3) Carbonic anhydrase, a zinc-metalloprotein, catalyzes the reversible hydration of CO_2 to bicarbonate:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

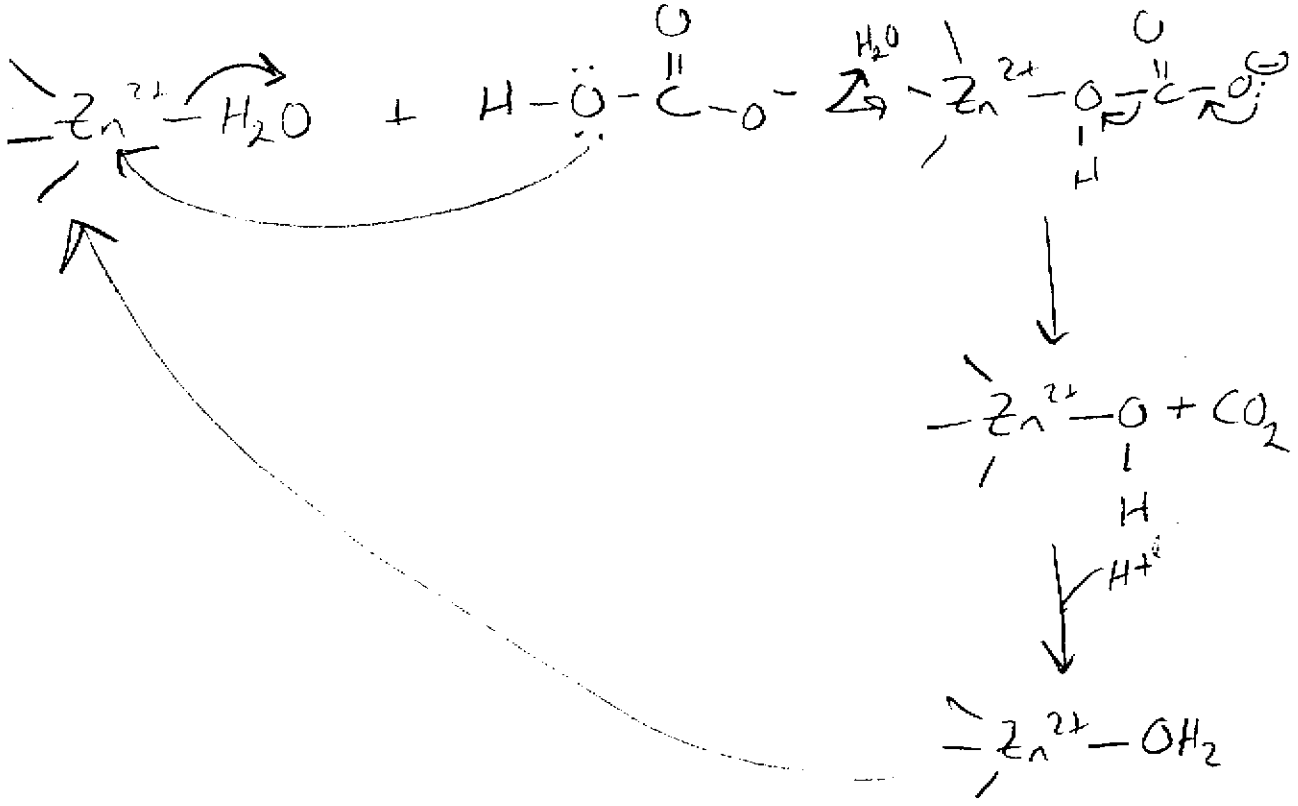
+4 (a) In which direction would you expect the above reaction to proceed in (i) the tissue and (ii) the lungs? Explain why.

+6 (b) Draw the reaction mechanism for carbonic anhydrase in the REVERSE direction, i.e., starting with bicarbonate and forming CO_2 and H_2O. Clearly show how Zn^{2+} facilitates catalysis.

a) In tissue reaction will proceed L → R, to solubilize CO_2 for transport in blood stream as HCO_3^-

In lungs, reaction will proceed R → L, so transported HCO_3^- can be dehydrated & expelled as insoluble CO_2.

b)



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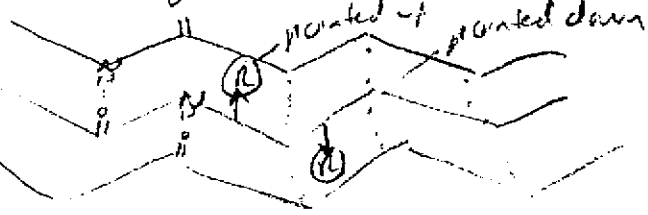
(4) Describe the features of the alpha-helix and beta sheet 2° structural elements.

α-helix - single polypeptide chain in a R-handed helical conformation. Polypeptide backbone winds about central axis. R groups (side chains) protrude outward. 1 helical turn / 3.6 AA residues. Helical structure stabilized by intrachain H-bonding - H atom of an amide N to carbonyl O of 4th AA on N-terminal side of helix

β-sheet: an extended conformation of parallel or antiparallel polypeptide chains. This structure does not have to span a continuous region of protein, different polypeptides can also assemble into β-sheet structure. Made up from H-bonding between adjacent strands typically 5-10 AA in length. Backbone polypeptides assume zig-zag structure rather than helix



Stabilized by H-bonding between carbonyl O on 1 strand & N-H on adjacent strand

AA side chains (R groups) protrude at right angle to sheet

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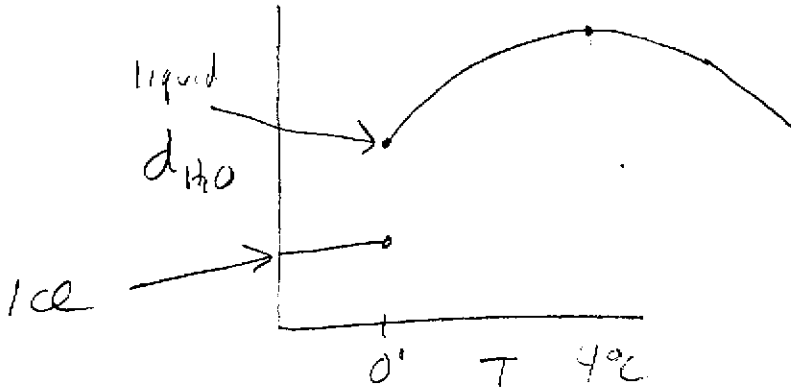
(5) Lakes that freeze in the winter and thaw in the spring "turnover" for each process, meaning that dissolved oxygen is delivered to the bottom of the lake while nutrients from decaying materials are delivered to the surface. Explain how the unique properties of water are responsible for these two turnover events in the yearly lake cycle.

The density of liq. water changes as a fxn of temperature, according to the following diagram:



In the fall, as air temp decreases, surface water cools, becomes more dense, & sinks to the bottom, delivering O_2. Warmer water at bottom rises bringing nutrients up. Once entire lake reaches 4°, colder surface water is now lighter & floats, until ice forms, which is yet less dense & floats on more dense liq. water

In spring ice melts. As surface water temp goes from 0 → 4°C water becomes more dense & sinks, while water at bottom which is colder, rises. Once temp of entire lake reaches 4°C, max. density is reached; further > T results in less dense, warmer H_2O at surface

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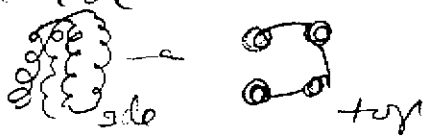
(6) Briefly explain the unique structural features of the following. In answering the question address what secondary structural elements they are built from and the orientations of adjacent elements (i.e. perpendicular, parallel, antiparallel; R-handed, left-handed, etc.)

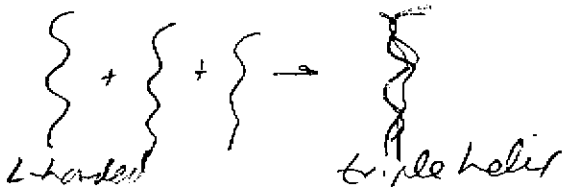
(a) four helix bundle

4 α-helices connected by 3 helix loops
adj α-helices are anti // 4 helices arranged about central axis



(b) collagen triple helix

Formed by the coiling, in a R-handed direction, of 3 L-handed collagen helices



(c) alpha-beta saddle

an α/β motif wherein sequential β-sheets are connected by α-helices in a // manner. The orientation of the β strands is offset such that they cross each other to form a central "saddle" conformation surrounded by the α-helices

(d) globin fold

a bundle of sequential α-helices that are largely arranged ⊥ or anti // to each other, connected by loops

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(7) You isolate a monomeric enzyme of 52,000 molecular weight (52 kDa; 52,000 grams/mol) that converts substrate "S" to product "P".

(a) You conduct kinetic studies of your enzyme, in assays that contain 0.1 mg protein, and determine that the K_m of the enzyme is 0.1 mM while V_{max} is 500 µmol product formed/min. Use this information to determine the turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_m) for your enzyme. [4] [in s^{-1}, M^{-1}]

(b) What concentration of substrate would be required to obtain an initial rate of 100 µmol/min in an assay set up as above? [3]

(c) The upper limit on k_{cat}/K_m for enzyme-catalyzed reactions is 10^8 to 10^9 $M^{-1}s^{-1}$. Explain why this upper limit exists and why an enzyme with a catalytic efficiency in this range is considered to be a "perfect" enzyme. [3]

a) $\left(\frac{500\ \mu mol\ P}{min}\right)\left(\frac{1}{0.1\ mg\ protein}\right)\left(\frac{52{,}000\ mg\ protein}{mmol\ protein}\right)\left(\frac{mmol}{1000\ \mu m}\right)$

$= 260{,}000/min$, or $\boxed{4{,}333/s}$

$\left(\frac{4333\ s^{-1}}{0.1\ mM}\right)\left(\frac{1000\ mM}{M}\right) = \boxed{4.33 \times 10^7\ M^{-1}s^{-1}}$

b) $v = \frac{V_{max}[S]}{K_m + [S]}$; $V_{max}[S] = v(K_m + [S])$

$[S] = \frac{v}{V_{max}}(K_m + [S])$

$[S] = \frac{v K_m}{V_{max}} + \frac{v}{V_{max}}[S]$

$[S] - \frac{v}{V_{max}}[S] = \frac{v K_m}{V_{max}}$

$[S] = \frac{\frac{v K_m}{V_{max}}}{\left(1 - \frac{v}{V_{max}}\right)}$

$[S] = \frac{(100\ \mu)(0.1\ mM)}{500\ \mu} = \frac{}{\left(1 - \frac{100}{500}\right)}$

$\boxed{[S] = 0.025\ mM}$

easier derivation →

or:

$V_{max}[S] = v K_m + v[S]$

$(V_{max} - v)[S] = v K_m$

$[S] = \frac{v K_m}{V_{max} - v} = \frac{(100\ \mu)(0.1\ mM)}{500 - 100}$

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(8) You purify an enzyme from a crude bacterial lysate. The purification of the enzyme leads to a 200-fold increase in specific activity when going from the crude extract to the purified protein. The protein runs as a single band on nondenaturing polyacrylamide gel electrophoresis (PAGE) and as three distinct bands on SDS-PAGE. The sizes of the three protein bands on SDS-PAGE are 10,000, 30,000 and 60,000, and the staining intensities of the proteins indicate they are present in a stoichiometric ratio of 1:2:2, respectively. Your protein elutes from a size exclusion (gel permeation) column with an apparent molecular weight of 570,000.

(a) (3 pts) Approximately what percentage of proteins in the lysate did your enzyme represent?

(b) (7 pts) What is the quaternary structure of your protein α_β_γ_). Explain how the information in the problem allowed you to figure this out.

(biggest smallest)

(a) 200-fold purification means protein was ~1/200, or 0.5%

b) empirical formula = $\alpha_2\beta_2\gamma_1$ for 60kDa, 30kDa, & 10kDa subunits

$1(10) + 2(30) + 2(60) = 190\text{kDa}$

$\frac{570\text{kDa}}{190\text{kDa}} = 3$

so mol. formula = 3x emp formula:

$3(\alpha_2\beta_2\gamma_1) = \boxed{\alpha_6\beta_6\gamma_3}$

α subunit is biggest,

γ subunit smallest

by standard nomenclature

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Take home portion of Exam 1: You may consult any reference texts you like when working this portion. You are to work the problems individually- do not discuss with fellow students, faculty, etc. Turn in your solutions to me at beginning of class Wednesday.

(1) You wish to prepare 2 liters of a 50 mM solution of potassium phosphate buffer at pH 7.3. Determine what masses of KH_2PO_4 and K_2HPO_4 solid could be combined in water to obtain the desired buffer concentration, volume, and pH. Note- the pKa values for phosphoric acid ionization are 2.14, 6.86, and 12.4 (see Fig. 4-14 in text)

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(2) 15 points. Draw two different complete mechanisms for acetoacetate decarboxylation that involve:
(a) covalent catalysis (via Schiff base)
(b) metal ion catalysis.

Use general acid-base catalysis as needed in each mechanism to facilitate catalysis. Indicate what "intermediates" in your three mechanisms represent transition states, and briefly discuss how the catalytic group(s) stabilize these states.

$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$ ($H_2PO_4^-$ = HA; HPO_4^{2-} = A^-)

(1) $pH = pK_a + \log\frac{[A^-]}{[HA]}$; $[A^-] + [HA] = 0.050\,M$

$\log\frac{A^-}{HA} = pH - pK_a = 7.3 - 6.86 = 0.44$; $\frac{A^-}{HA} = 10^{0.44} = 2.754$

$A^- = 2.754\,HA$; $2.754\,HA + HA = 0.05$; $[HA] = 0.01$

So $A^- = 0.05 - 0.0133 = 0.$

$HA = H_2PO_4^-$. For 2 L of solution, need:

$$\left(0.0133\ \frac{\text{mol HA}}{\text{L}}\right)(2\text{ L})\left(\frac{1\text{ mol } KH_2PO_4}{1\text{ mol HA}}\right)\left(\frac{136.09\text{ g } KH_2PO_4}{\text{mol } KH_2PO_4}\right)$$

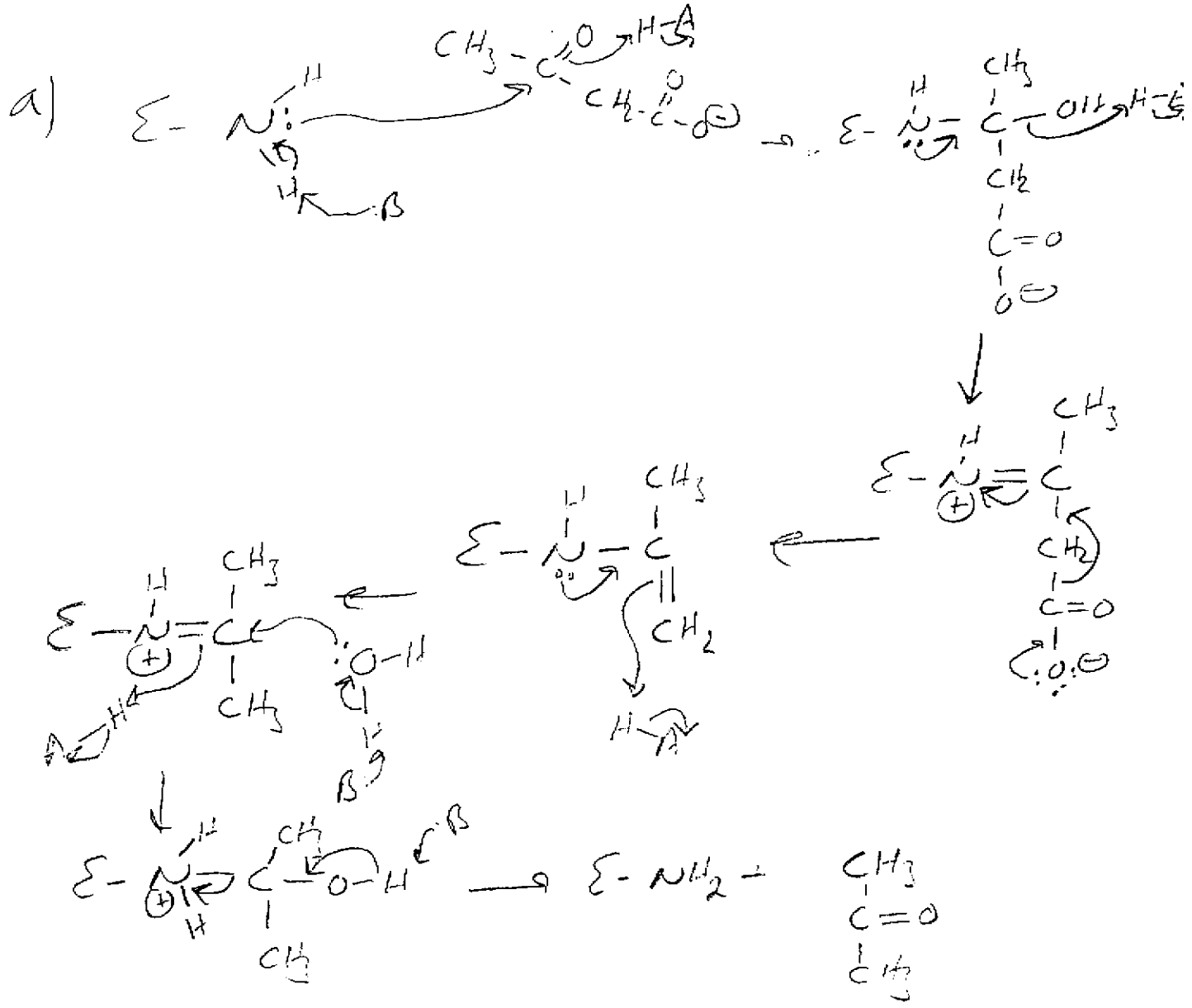
$$= 3.62\text{ g } KH_2P$$

$A^- = HPO_4^{2-}$

$$\left(0.0367\ \frac{\text{mol } A^-}{\text{L}}\right)(2\text{ L})\left(\frac{1\text{ mol } K_2HPO_4}{\text{mol } A^-}\right)\left(\frac{174.18\text{ g } K_2HPO_4}{\text{mol } K_2HPO_4}\right)$$

$$= 12.78\text{ g } K_2H$$

a)



b)