 Name Key

Biochemistry 5700
Fall, 1998
Exam 1

Louse. pts
16

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam must be turned in by 11:20 AM. This exam should have 10 questions and 11 pages. **Answer any 7 of the 10 questions-I will only grade 7 questions. Mark "do not grade" under the two questions you choose not to answer. Each graded problem will count 14 points, giving a total of 98 graded points.** Please print your name at the top of each page of the exam-if you do so you will get an additional **2 points**, bringing the exam total to 100. If you do not put your name on each page you lose the 2 points!! Good Luck!!

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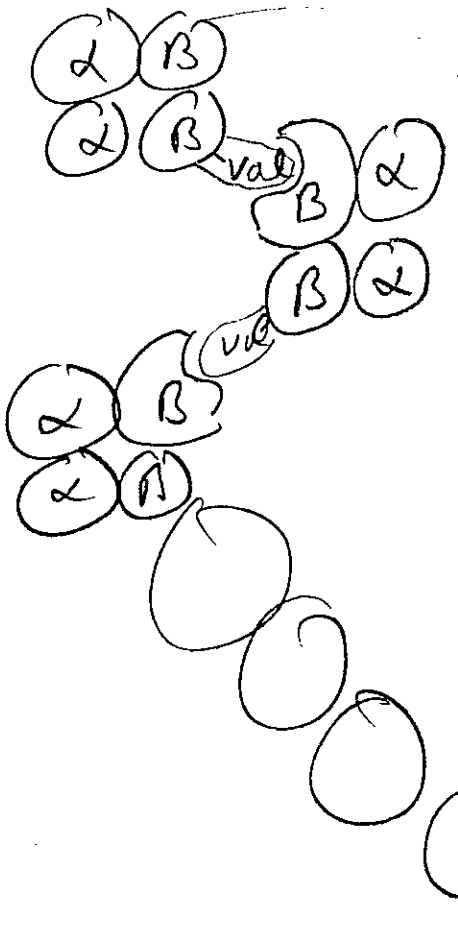
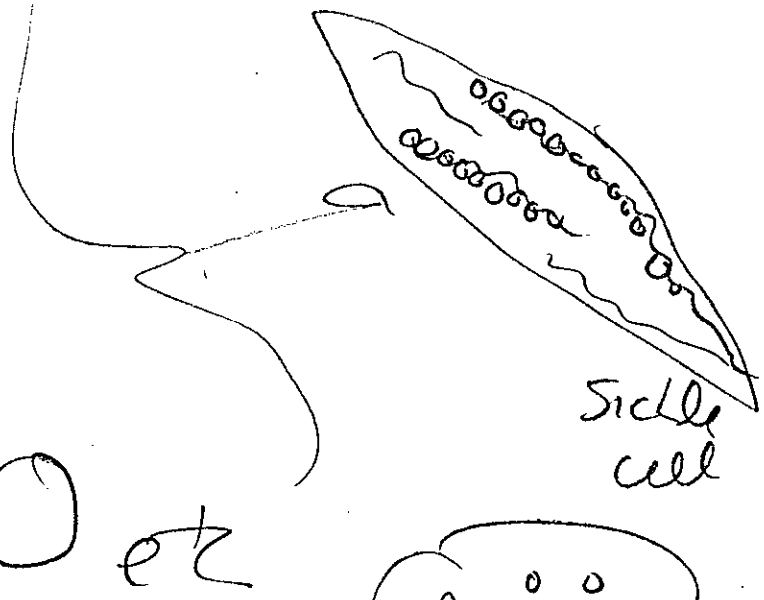
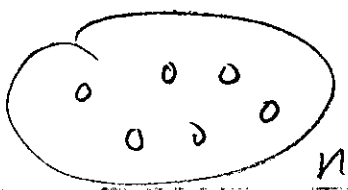
(1) Provide a detailed explanation for the molecular basis for the genetic disorder "sickle cell anemia".

A point mutation at the position of a surface glu residue on the β-chain of Hb occurs, changing the glu to val. This leads to an interaction between the val & hydrophobic residues on the surface of another β subunit, giving rise to a "intermolecular" association between distinct Hb tetramers. multiple Hb molecules thus aggregate into a long fibrous like structure, deforming the normal cell structure & causing a "sickled" appearance







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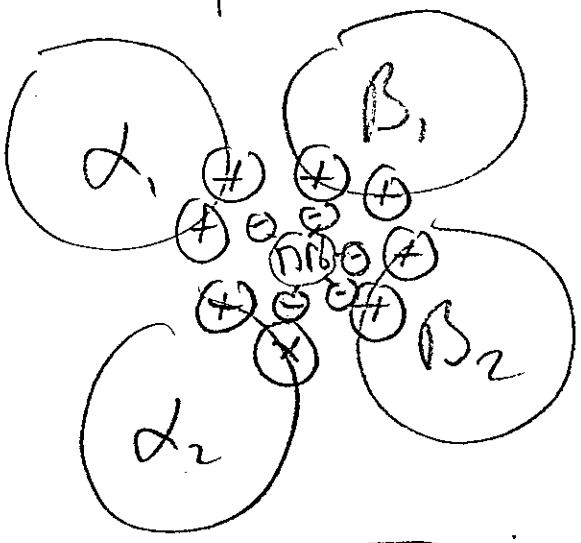
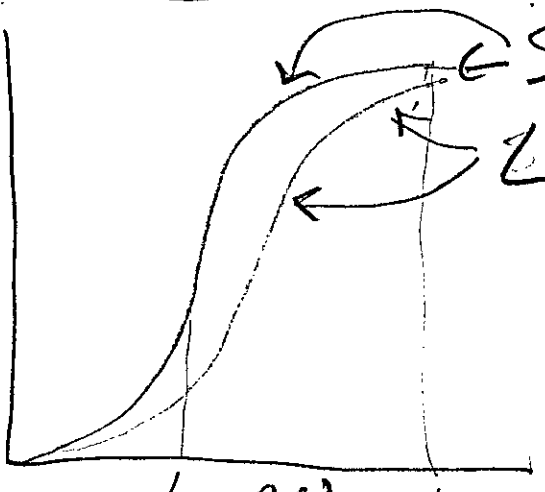
(2) How will the concentration of 2,3-diphosphoglycerate (DPG) in your blood stream change (i.e. go up, go down, stay the same) if you move from Logan, Utah to San Diego California? Describe the molecular and physiological consequences of this change in DPG concentration.

2,3-DPG levels will decrease. With lower DPG available, less of Hb molecules will contain this molecule bound in the Hb central cavity. The fxn of DPG is to form ionic interactions (salt bridges) w/ pos. residues on Hb monomers, stabilizing the deoxy "T" state of the protein:



w/ less DPG, more Hb will shift to R, oxy form. Thus, O_2-binding affinity of Hb increases at lower altitude in San Diego. This actually results in less net O_2 delivery due to Hb holding O_2 tighter at the tissue PO_2 (see graph)



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(3) What is the "Haldane relationship" and what important information does it provide concerning enzymatic reactions?

(Did anyone attempt this problem)?!

Haldane relationship says that, for a reversible enzymatic rxn described by the scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

the equilibrium constant for the overall reaction of $S \rightarrow P$ is related to the kinetic parameter K_m^S, K_m^P, V_{max}^S, & V_{max}^P (parameter for forward & reverse reactions)

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(4) An enzyme catalyzes a reaction at a velocity of 20 µmol/min/mg protein when the concentration of substrate (S) is 0.01 M. The K_m for the substrate is 1×10^{-5} M. Assuming that Michaelis-Menten kinetics are followed, what will the reaction velocity be when the concentration of substrate is 0.5×10^{-5} M? What concentration of substrate will give a velocity of 17.5 µmol/min/mg?

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

v at $1000 \times K_m$ is $\sim V_{max}$, so $V_{max} \approx 20$ u/mg

$$v = \frac{(20\ u/mg)(0.5 \times 10^{-5}\ \cancel{M})}{(1 \times 10^{-5}\ \cancel{M}) + (0.5 \times 10^{-5}\ \cancel{M})} = \boxed{6.7\ u/mg}$$

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

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

$$[S] = \frac{vK_m}{V_{max} - v} = \frac{(17.5\ \cancel{U/mg})(1 \times 10^{-5}\ \underline{M})}{(20 - 17.5)\ \cancel{U/mg}}$$

$$\boxed{[S] = 7.0 \times 10^{-5}\ M}$$

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(5) In the space provided, define the following terms related to enzyme kinetics:

(a) Michaelis Constant or "conc. of substrate giving 1/2 Vmax"
the K_m is the ratio of the sum of the rate constants for dissociation of the ES complex divided by the rate constant for formation of the complex from E + S

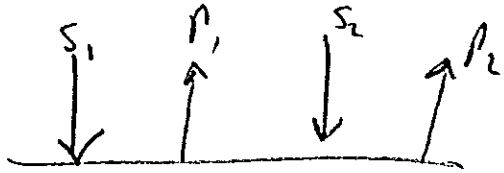
$K_m = \frac{k_{-1} + k_2}{k_1}$

(b) Turnover number
the number of reaction processes that each active site catalyzes per unit time. A 1st order rate constant.

(c) Catalytic efficiency
$\frac{k_{cat}}{K_m}$ - A 2nd order rate constant

(d) uncompetitive inhibitor
A molecule that reversibly binds to the ES complex to form a catalytically inactive ESI complex

(e) ping-pong mechanism
A bisubstrate rxn where product release occurs between the 2 substrate addition steps.



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(6) Using thermodynamic considerations, explain why: (a) sodium hydroxide dissolves in water with an increase in the temperature of the solution; (b) ammonium acetate dissolves in water with a decrease in the temperature of the solution; and (c) sodium chloride dissolves in water with no change in the temperature of the solution.

In order for a solute to dissolve ΔG must be negative. $\Delta G = \Delta H - T\Delta S$. Thus a reaction is favored by heat release ($-\Delta H$) and/or increase in entropy ($+\Delta S$) which sum to give $-\Delta G$. ΔH can be (+) if ΔS is sufficiently (+) to give $-\Delta G$; conversely, ΔS can be (−) if ΔH is sufficiently positive.

(A) NaOH dissolving is enthalpically favored ΔH is (−) because heat was given off. ΔS is probably (+).

(B) NH_4OAc dissolving is enthalpically disfavored heat had to be transfered into dissolving process. However at room temp entropy increase was sufficiently pos that $|T\Delta S| > |\Delta H|$

(C) No significant enthalpic change has entropy driver dissolving.

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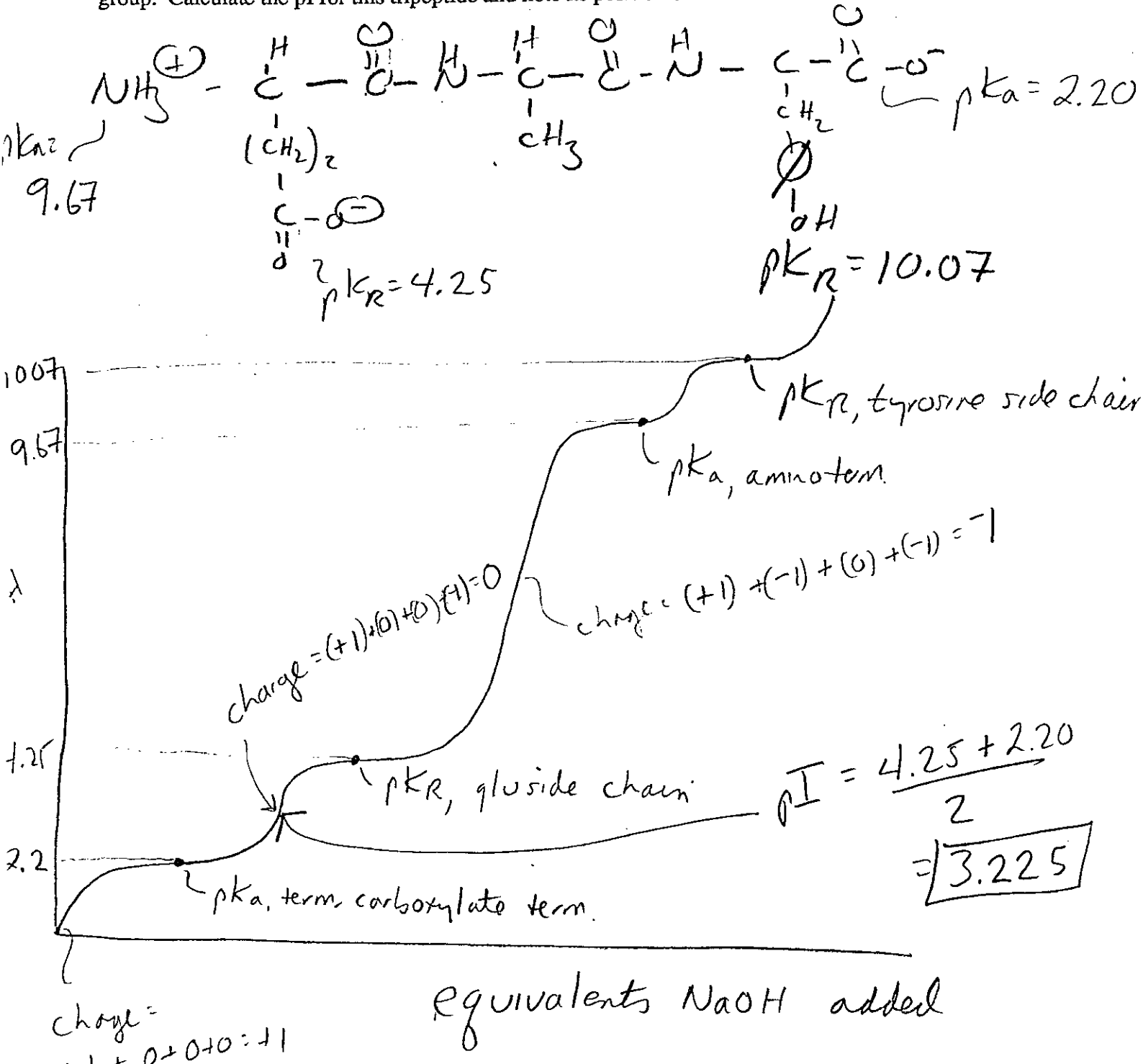
(7) Draw a titration curve (with NaOH as the titrant; start at pH 0))for the tripeptide (Glu-Ala-Tyr). Indicate on the titration curve the positions of and values for the pKa's of any ionizable groups (see the chart on page 1 for values). Also indicate on the curve which pKa corresponds to which ionizable group. Calculate the pI for this tripeptide and note its position on the titration curve.



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(8) Describe the steps involved in determining the primary sequence of a short (i.e. less than 20 amino acid residues) polypeptide. What additional procedure(s) must be done to determine the sequence of larger (i.e. 50 amino acids) polypeptides? How are the sequences of most proteins (10,000 molecular weight and up in size) determined and why?

(1) reduce and acetylate or oxidize disulfide bonds.

(2) Determine total AA composition - acid hydolysis & separation & quantitation of AAs

(3) Determine N-terminal residue w/ Sanger's reagent, which reacts w/ free amino group

(4) determine C-terminus, if possible, w/ Carboxypeptidase

(5) Sequence the peptide using "Edman degradation" - a recycling procedure that sequentially removes N-term residues.

Large pp - use endoproteases to generate smaller, overlapping peptides. Subject these to steps 1-5, then deduce overall sequence from overlaps

Protein - deduce AA sequence from DNA

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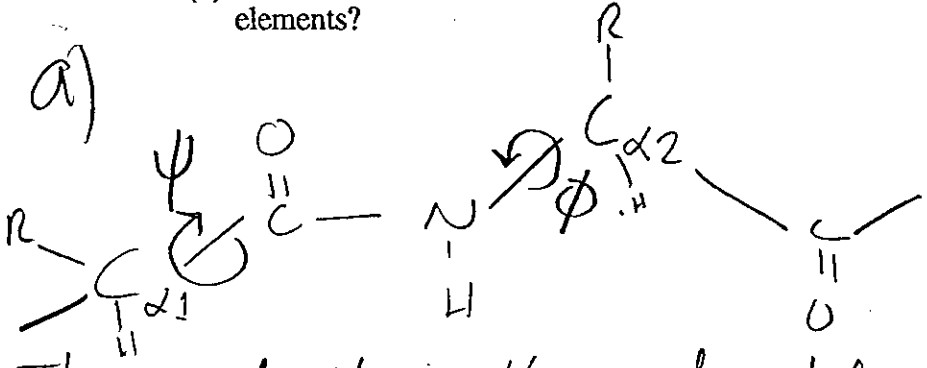
(9)

(a) Define and explain the significance of phi and psi angles in the alpha helical and beta sheet secondary structural elements.
(b) How do intra and/or interchain hydrogen bonds contribute to the stability of these 2° elements?

a)



The angle ψ is the angle defined by rotation about the $C_\alpha - C_{carbonyl}$ bond

The angle φ is the angle defined by rotation about the $N - C_\alpha$ bond.

The value of 0° for φ & ψ is the conformation in which the 2 peptide bonds connected to an αC atom are in the same plane. This is sterically not allowed. φ & ψ adjust to form sterically allowed, favored conformations. These include the fixed ∠s of φ & ψ that give rise to the α-helix & β-sheet. The ∠s of φ & ψ are greater for β sheet, giving rise to more extended conf.

b) The α-helix is stabilized by intrachain H-bonding between the H atom of an amide N & the carbonyl O of the 4th AA residue in the amino terminal direction.

The β-pleated sheet is stabilized by interchain or intrachain H-bonds between amide H's

stuff you weigh out is a mix of HA + A⁻ or all HA or all A⁻ ie pH ≠ is not pka

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(10) ~~You wish to prepare~~ four liters of 50 mM HEPES buffer with a pH of 7.8. A four-liter solution of ~~50 mM HEPES buffer~~ to which no acid or base has been added has a pH of 4.0. What volume of 6 M ~~KOH~~ will you need to add in preparing your four liters of HEPES buffer adjusted to pH 7.8?

$$Hepes\text{-}H^+ \rightleftharpoons Hepes^- + H^+$$

$$pH = pKa + \log \frac{[Hepes^-]}{[Hepes\text{-}H^+]}$$

Aaaugh! I didn't give you a pKa for Hepes!? Why didn't someone inform me of my error? That's what I get for writing an exam at 2AM.

$pKa_{Hepes} = 7.5$

ie it's all your fault not mine. ☺

For those of you that worked this problem - I will grade it based on how you set it up & solved it w/ the info I gave. Many of you assumed the pKa was 4.0 - for those who did, you would get an answer of 16.7 ml NaOH

So - as my punishment for messing this up - I guarantee this problem will reappear on the final with the pKa given!