Biochemistry 5700
Fall, 2002
Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam consists of 9 questions worth 100 points. In addition, a five point bonus question is included at the end of the exam for those of you who finish early and are up to the challenge. DO NOT WORK THE BONUS PROBLEM UNTIL YOU HAVE COMPLETED THE MAIN BODY OF THE EXAM. Please answer questions completely and legibly. The exam must be turned in by 11:20 AM. Good Luck!!

table 5–1

**Properties and Conventions Associated with the Standard Amino Acids**

| Amino acid | Abbreviated names | | $M_r$ | $pK_a$ values: $pK_1$ (—COOH) | $pK_2$ ($—NH_3^+$) | $pK_R$ (R group) |
|---|---|---|---|---|---|---|
| **Nonpolar, aliphatic R groups** | | | | | | |
| Glycine | Gly | G | 75 | 2.34 | 9.60 | |
| Alanine | Ala | A | 89 | 2.34 | 9.69 | |
| Valine | Val | V | 117 | 2.32 | 9.62 | |
| Leucine | Leu | L | 131 | 2.36 | 9.60 | |
| Isoleucine | Ile | I | 131 | 2.36 | 9.68 | |
| Methionine | Met | M | 149 | 2.28 | 9.21 | |
| **Aromatic R groups** | | | | | | |
| Phenylalanine | Phe | F | 165 | 1.83 | 9.13 | |
| Tyrosine | Tyr | Y | 181 | 2.20 | 9.11 | 10.07 |
| Tryptophan | Trp | W | 204 | 2.38 | 9.39 | |
| **Polar, uncharged R groups** | | | | | | |
| Serine | Ser | S | 105 | 2.21 | 9.15 | |
| Proline | Pro | P | 115 | 1.99 | 10.96 | |
| Threonine | Thr | T | 119 | 2.11 | 9.62 | |
| Cysteine | Cys | C | 121 | 1.96 | 10.28 | 8.18 |
| Asparagine | Asn | N | 132 | 2.02 | 8.80 | |
| Glutamine | Gln | Q | 146 | 2.17 | 9.13 | |
| **Positively charged R groups** | | | | | | |
| Lysine | Lys | K | 146 | 2.18 | 8.95 | 10.53 |
| Histidine | His | H | 155 | 1.82 | 9.17 | 6.00 |
| Arginine | Arg | R | 174 | 2.17 | 9.04 | 12.48 |
| **Negatively charged R groups** | | | | | | |
| Aspartate | Asp | D | 133 | 1.88 | 9.60 | 3.65 |
| Glutamate | Glu | E | 147 | 2.19 | 9.67 | 4.25 |

(1) 20 points. Answer the following questions about amino acid and protein structure/function:

(a) What two amino acids might you expect to encounter in a β-turn for a polypeptide?

Gly + Pro

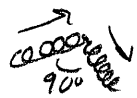
(b) You have samples of two fibrous proteins but their labels have fallen off. Amino acid analysis reveals that sample 1 has a high ratio of cysteine, while sample 2 has a high ratio of hydroxyproline and glycine. Identify samples 1 and 2.

1 = α-keratin

2 = collagen

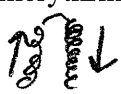
(c) What is the approximate orientation of adjacent α-helices in myoglobin?

perpendicular




(d) What is the approximate orientation of adjacent α-helices in hemerythrin, a protein built around the four helix bundle motif?

antiparallel



(e) What is the approximate orientation of adjacent β-strands in a protein built around the α/β barrel motif?

parallel

(f) What is the approximate orientation of adjacent β-strands in silk, which consists solely of β-stands and β-turns?

antiparallel

(g) What would be a "conservative" amino acid substitution for an internal valine residue in a protein?

Ile, leu, or ala

(h) What is the proper generic name for an organic cofactor that is covalently bound to a protein?

prosthetic group

(i) Write the formula for a multimeric protein consisting of 25 and 50 kDa subunits, present in a ratio of 1:2, which has a native molecular weight of [redacted] kDa (1 kDa = 1000 daltons or 1000 amu)

375

375/125 = 3

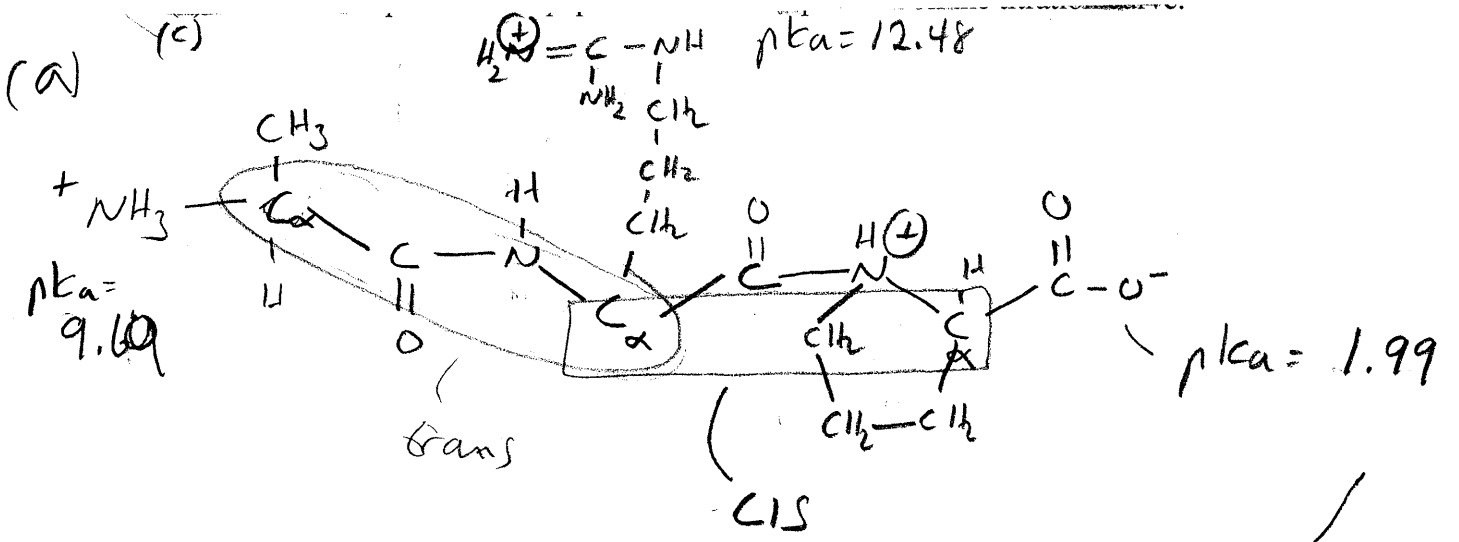
α2β1 = 125kDa

50 | α6β3

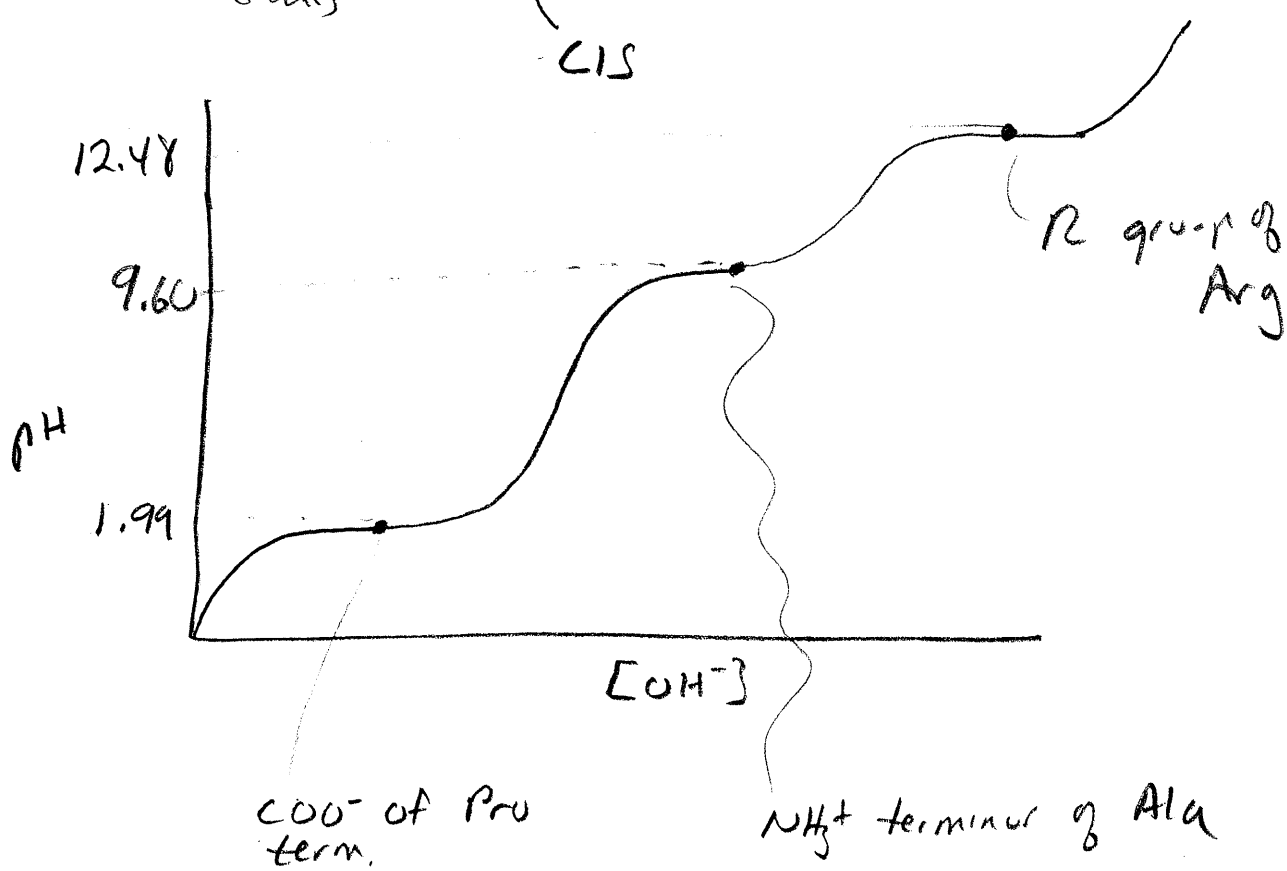
(j) All of the standard amino acids have the L configuration except gly, which is not chiral

(2) 15 points.
(a) Draw the tripeptide Ala-Arg-Pro at pH = 7.0, with Pro being in the cis-conformation.
(b) Draw a titration curve (with NaOH as the titrant; start at pH 0) for this tripeptide. Indicate on the titration curve the positions of and values for the $pK_a$'s of ionizable groups and which groups are being ionized (see the chart on page 1 for values).
(c) Provide a one sentence definition for the term "pI".

(a)




b)

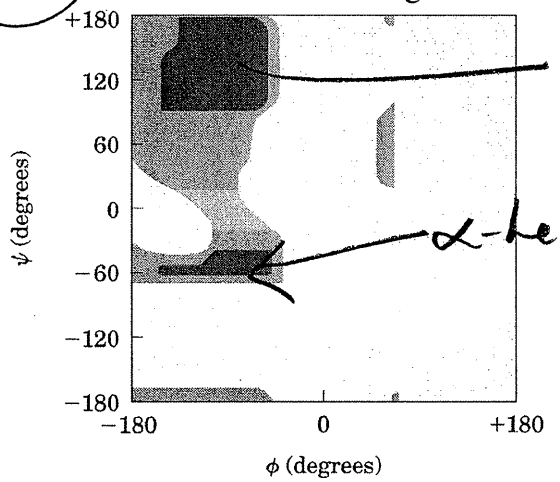



(c) the pH at which an amino acid or peptide bears a net neutral charge

(3) 10 points. Match the following terms with the appropriate partner. (Write the correct letter next to the term in the left hand column). 1 pt each

| | | | |
|---|---|---|---|
| Binding energy | c | a) | Uncompetitive inhibitor |
| proximity | e | b) | Noncompetitive inhibitor |
| Binds ES complex only | a | c) | enthalpy |
| Separation of proteins on basis of size | h | d) | isoleucine |
| separation of enzymes on basis of charge | g | e) | Entropy trap |
| Binds free enzyme only | i | f) | Charge stabilization |
| Binds E or ES complex | b | g) | Ion exchange chromatography |
| Metal ion | f | h) | Size exclusion chromatography |
| Aromatic amino acid | j | i) | Competitive inhibitor |
| Aliphatic amino acid | d | j) | tryptophan |

(4) 5 points. Indicate with arrows the approximate regions where (a) α-helices and (b) β-strands would be found on the following Ramachandran plot:

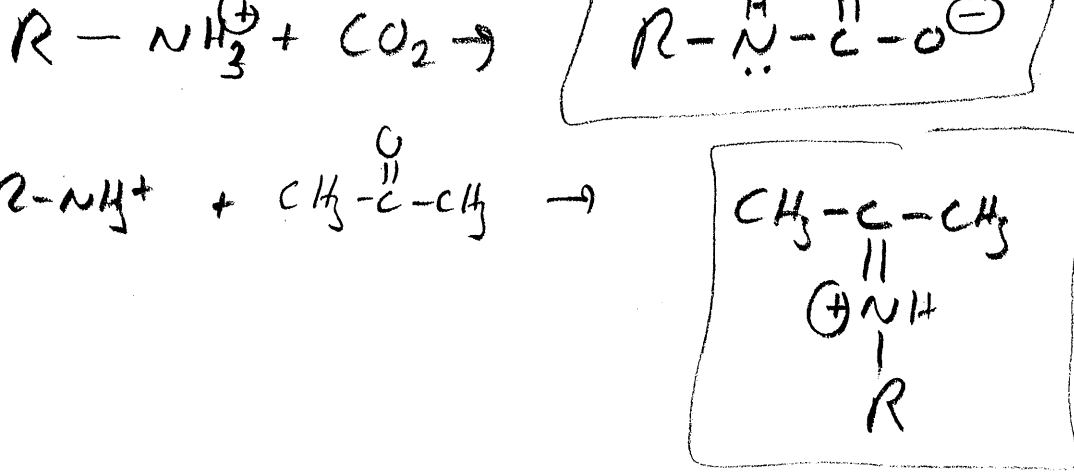



β-strand

α-helix

2.5 pt each

(5) 10 points. Draw the products of the reaction of the side chain of lysine with (a) $CO_2$ and ~~(b)~~ acetone



(6) 10 points.
(a) In the kinetic mechanism shown below, which rate constants are first order, and which are second order?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

1st order: $k_2$, $k_3$, $k_{-2}$, $k_{-1}$

2nd order: $k_1$, $k_{-3}$

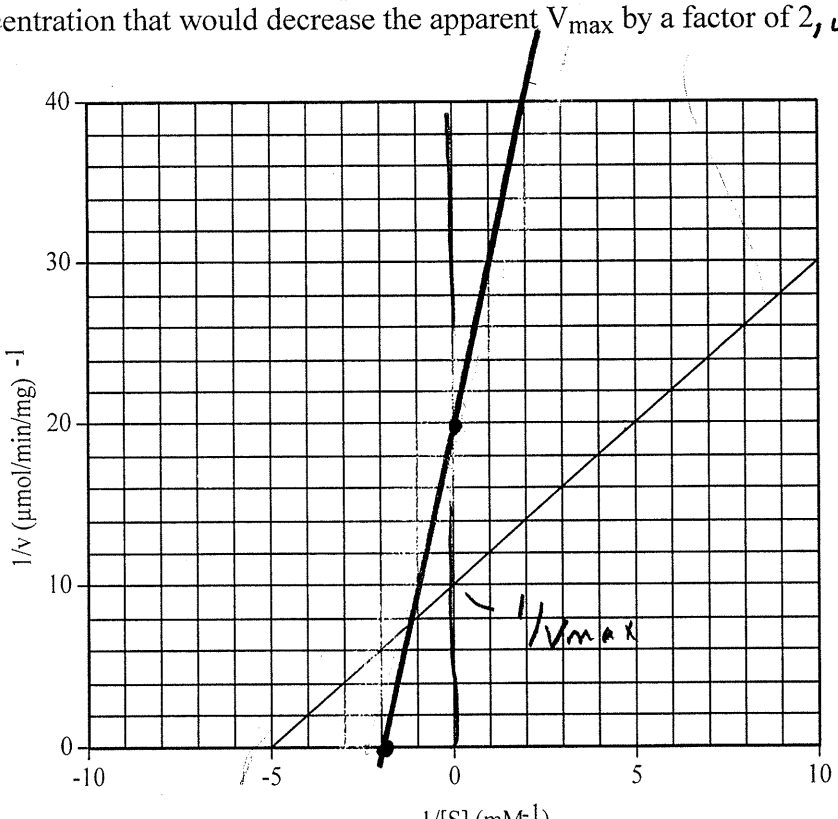
(b) Write the kinetic mechanism for a two substrate/two product sequential reaction, where substrate binding is ordered but product release is random.

$$E + S_1 \rightleftharpoons E{\cdot}S_1 \overset{S_2}{\rightleftharpoons} E{\cdot}S_1{\cdot}S_2 \rightleftharpoons E{\cdot}P_1{\cdot}P_2$$

$E{\cdot}P_2 \rightleftharpoons E + P_2$ ($P_2$ release)

$E{\cdot}P_1 \rightleftharpoons E + P_1$ ($P_1$ release)

(7) (10 points) The following graph shows a Lineweaver-Burke ( **1/v** vs. **1/[S]** ) plot for an enzymatic reaction transforming substrate S to product P.
(a) What are $K_m$ and $V_{max}$ for this enzyme?
(b) Draw and label a line on the graph representing the effect of a noncompetitive inhibitor present at a concentration that would decrease the apparent $V_{max}$ by a factor of 2, while increasing $K_m$ by a factor of 2.5




a) $1/V_{max} = 10$ so $V_{max} = 0.1$ u/mg

$-1/K_m = -5$ so $K_m = 0.2$ mM

b) want new $V_{max} = 0.05$ so $1/V_{max} = 20$

want new $K_m = 0.5$ so $1/K_m = 2$

(8) 10 points. Fill in the missing information that is indicated in the boxes for this purification table. What is the percent recovery of activity for the overall purification?

Table 5–5

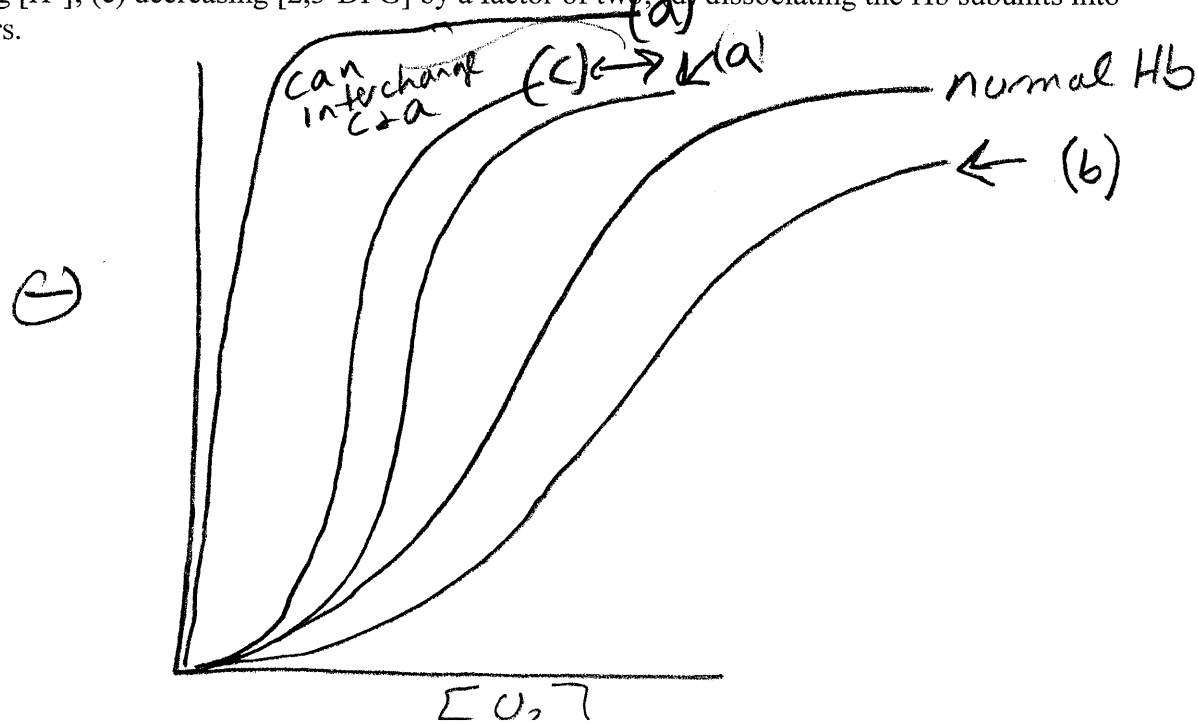
**A Purification Table for a Hypothetical Enzyme***

| Procedure or step | Fraction volume (ml) | Total protein (mg) | Activity (units) | Specific activity (units/mg) | Fold-purificatio |
|---|---|---|---|---|---|
| 1. Crude cellular extract | 1,400 | 10,000 | 100,000 | 10 | 1 |
| 2. Precipitation with ammonium sulfate | 280 | 3,000 | 96,000 | 32 | 3.2 |
| 3. Ion-exchange chromatography | 90 | 400 | 80,000 | 200 | 20 |
| 4. Size-exclusion chromatography | 80 | 100 | 60,000 | 600 | 60 |
| 5. Affinity chromatog-raphy | 6 | 3 | 45,000 | 15,000 | 1500 |

*All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 137.

$$\%\ \text{recovery} = \frac{45{,}000}{100{,}000} \times 100 = 45\%$$

(9) (10 points) Draw and label a representative oxygen dissociation curve for hemoglobin. Then, add clearly labeled lines to your plot representing (a) the effect of decreasing $[CO_2]$; (b) the effect of increasing $[H^+]$; (c) decreasing [2,3-DPG] by a factor of two; (d) dissociating the Hb subunits into monomers.

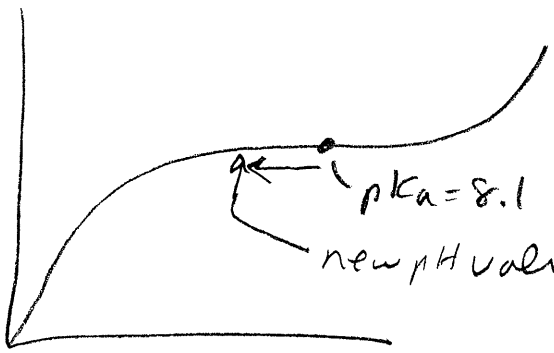


BONUS QUESTION FOR THE FAST WORKER AND THINKER: EARN 5 POINTS EXTRA CREDIT!!! NO PARTIAL CREDIT HERE BUT SHOW YOUR WORK

An enzyme produces protons as the product with specific activity 4 U/mg. If the reaction was carried out with 10 mg protein in 0.1 liter of 0.100 M Tris buffer ($pK_a = 8.1$) starting at pH 8.1, what would be the pH of the solution after the 15 minutes of reaction?

$$\left(\frac{4\ \mu mol}{min \cdot mg}\right)(10\ mg)(15\ min) = 600\ \mu mol\ H^+\ formed$$

$$\left(\frac{600\ \mu mol\ H^+\ formed}{0.1\ L}\right)\left(\frac{mol}{10^6\ \mu mol}\right) = 0.006\ \underline{M}\ H^+\ formed$$




$[HA] = 0.05 + 0.006 = 0.056\underline{M}$

$[A^-] = 0.05 - 0.006 = 0.044\underline{M}$

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

$$pH = 8.1 + \log\frac{0.044}{0.056} = \boxed{8.0}$$