Biochemistry 5700
Fall, 2006
Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam contains 9 questions. Please answer questions completely and legibly. Show your work for partial credit. The exam must be turned in by 11:20 AM. Good Luck!!

TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Glycine		75	2.34	9.60				.2
Alanine		89	2.34	9.69				.8
Proline		115	1.99	10.96				.2
Valine		117	2.32	9.62				.6
Leucine		131	2.36	9.60				.1
Isoleucine		131	2.36	9.68				.3
Methionine		149	2.28	9.21				.3
Phenylalanine		165	1.83	9.13				.9
Tyrosine		181	2.20	9.11	10.07			.2
Tryptophan		204	2.38	9.39				.4
Serine		105	2.21	9.15				.8
Threonine		119	2.11	9.62				.9
Cysteine		121	1.96	10.28	8.18			.9
Asparagine		132	2.02	8.80				.3
Glutamine		146	2.17	9.13				.2
Lysine		146	2.18	8.95	10.53		−3.9	5.9
Histidine		155	1.82	9.17	6.00		−3.2	2.3
Arginine		174	2.17	9.04	12.48		−4.5	5.1
Aspartate		133	1.88	9.60	3.65		−3.5	5.3
Glutamate		147	2.19	9.67	4.25		−3.5	6.3

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

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

(1) You have an aqueous 0.1 M solution of the amino acid histidine buffered at pH 6.5.
(a) **(5 points)** Draw the structures of the two forms of histidine that predominate (are present in highest amount) in this solution
(b) **(5 points)** Determine the actual concentrations of these two species in this solution.
(c) **(5 points)** Draw a titration curve for histidine, beginning at pH 0. Clearly label all pK values on the curve. Label the position of the isoelectric point.

(2) (10 points)
(a) Draw the structure of the polypeptide with the abbreviated name MARK in the form it would predominantly exist as in solution at pH 7. Label a representative phi, psi, and peptide bonds in the peptide.

(3) For the purification table shown below:
(a) **(6 pts)** Calculate the values that belong in the cells labeled (i), (ii), and (iii)
(b) **(3 pts)** Based on the information in the purification table, which specific step of the purification is the most efficient at increasing fold purification relative to the previous step?
(c) **(3 pts)** Estimate what percentage of the protein in your original extract consisted of your enzyme of interest.

Purification table for a new enzyme

step of purification	volume (ml)	total protein (mg)	total activity (units)	specific activity (units/mg)	% recovery	fold purification
lysate	1000	20,000	15,000	(i)		
ammonium sulfate precipitation	100	6,000	10,000			
ion exchange	200	1,000	8,000			
gel filtration	25	200	4,000		(ii)	
hydrophobic interaction	100	50	3,000			(iii)
affinity chromatography	50	5	2,500			

(4) (**24 points: 2 points each**.) 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?

(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.

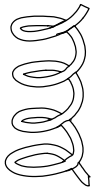
(c) What is the approximate orientation (e.g., perpendicular, parallel, antiparallel, etc) of adjacent α-helices in myoglobin?

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

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

(f) In an alpha helix, an intrastrand hydrogen bond is formed between residue "n" and residue _____.

(g) What is the "handedness" of the helix shown at right?



(h) In a mixture of the five proteins listed below, circle the protein that would elute second in size-exclusion (gel filtration) chromatography:
cytochrome c, MW= 13,000; immunoglobulin, MW = 145,000; ribonuclease, MW = 13,700; RNA polymerase, MW = 450,000; serum albumin, MW = 68,500

(i) Write the formula for a multimeric protein consisting of 50 and 25 kDa subunits, present in a ratio of 2:1, which has a native molecular weight of 375 kDa (a kDa = 1000 daltons or 1000 amu)

(j) In a 3 strand beta sheet, how many interchain hydrogen bonds are formed, on average, per amino acid residue on (i) the two outside strands and (ii) the inner strand ?

(k) On a reaction coordinate diagram, intermediates occupy positions represented by peaks/valleys (circle one) while transition states occupy positions represented by peaks/valleys (circle one)

(l) Define the term "binding energy" as it relates to enzymatic catalysis

(5) **(5 points)** Calculate k_{cat} (units of s^{-1}) for a homodimeric (α_2) enzyme of Mr = 126,000 exhibiting V_{max}=10,000 U when 1 mg enzyme is assayed. Assume one active site per subunit.

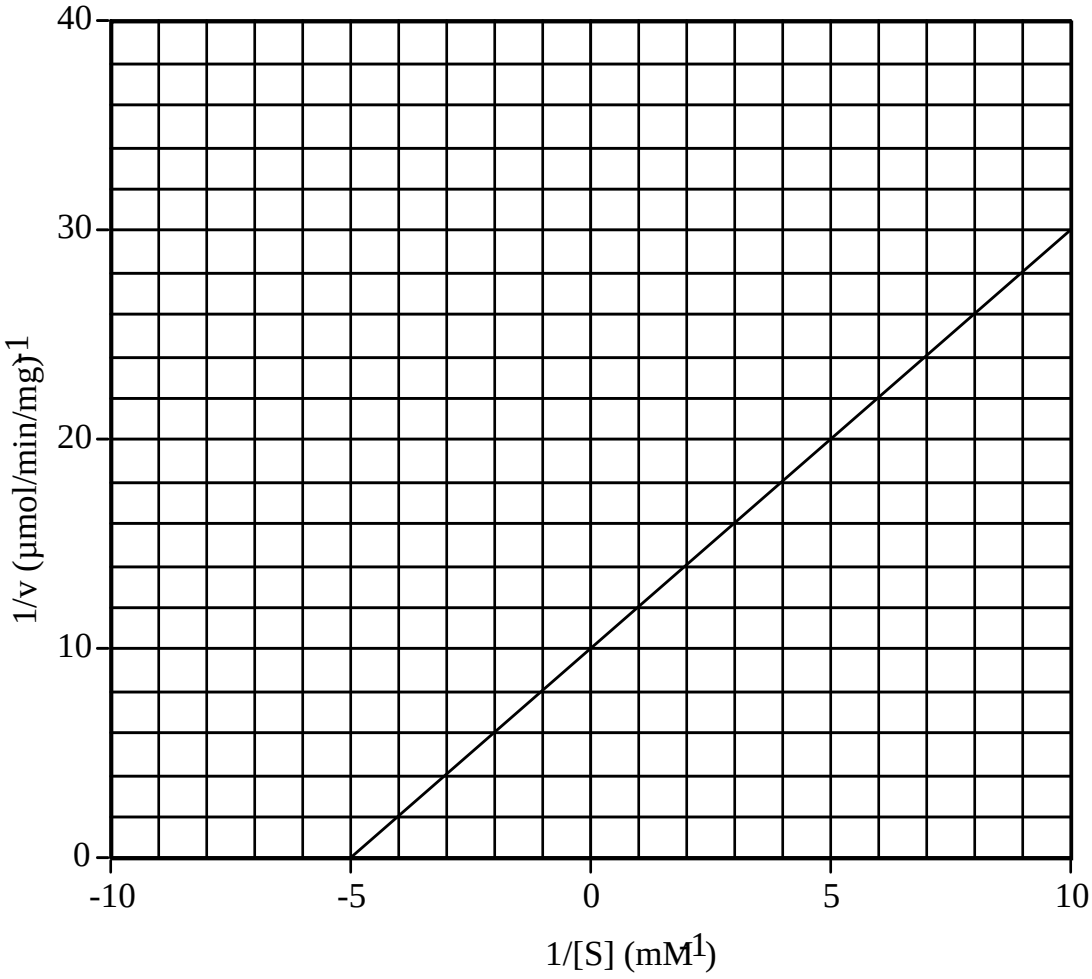
(6) **(4 points)** In the kinetic mechanism shown below, which rate constants are first order and which are second order?

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

(7) **(4 points)** Write the kinetic mechanism for a sequential bisubstrate reaction forming two products where substrate binding is random but product release is ordered.

(8) **(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 decreasing pH; (c) increasing [2,3-DPG] by a factor of two; (d) dissociating the Hb subunits into monomers.

(9) **(16 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) **(4 points)** Draw and label a line on the above graph representing the effect of a competitive inhibitor present at a concentration that would increase the apparent K_m by 5-fold (i.e., K_m is five times greater).

(b) **(4 points)** Draw and label a line on the above graph representing the effect of a noncompetitive inhibitor present at a concentration that would decrease the apparent V_{max} by 2-fold while increasing the apparent K_m by 2-fold.

(c) **(4 points)** Draw and label a line on the above graph representing the effect of an uncompetitive inhibitor present at a concentration that would decrease the apparent V_{max} by 1.5-fold.

(d) **(4 points)** For this enzyme, what would be the initial rate of reaction if the enzyme were assayed with a concentration of substrate of 0.5 mM?