

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!!

(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?

- (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 of adjacent α -helices in myoglobin?

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

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

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

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

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

- (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 375 kDa (1 kDa = 1000 daltons or 1000 amu)

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

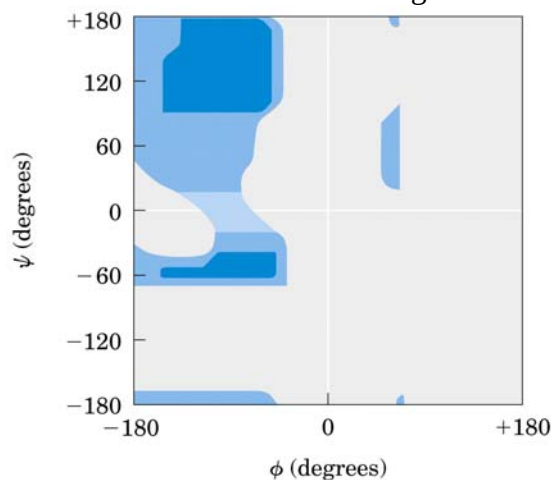
(2) 15 points.

- (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".

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

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

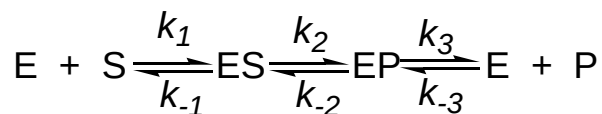
(4) 5 points. Indicate with arrows the approximate regions where (a) α -helices and (b) β -strands would be found on the following Ramachandran plot:



(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?

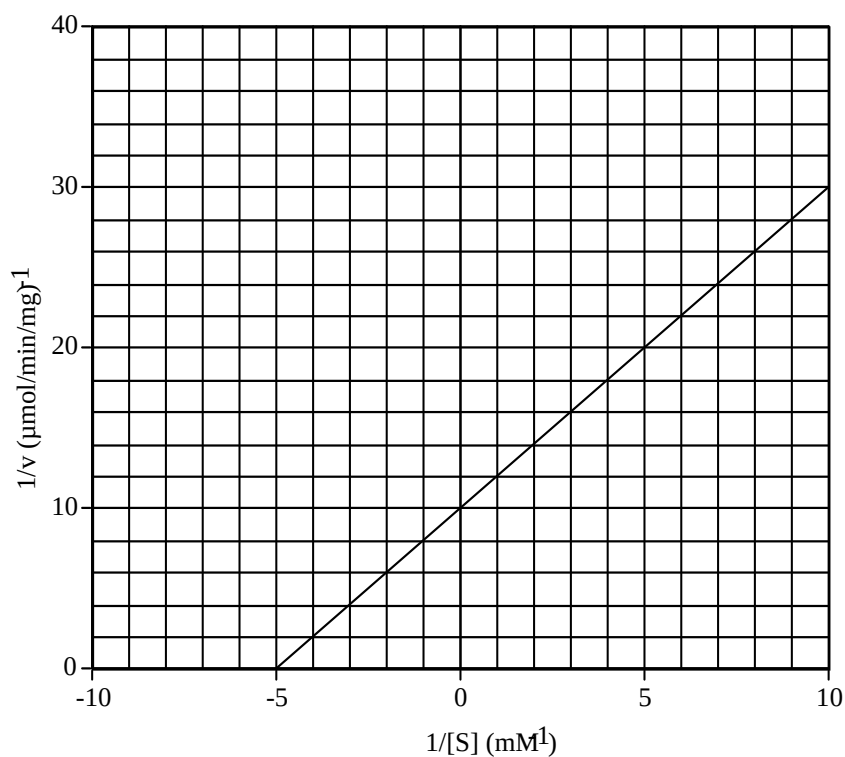


(b) Write the kinetic mechanism for a two substrate/two product sequential reaction, where substrate binding is ordered but product release is random.

(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 V_{max} by a factor of 2 while increasing K_m by a factor of 2.5.



(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-purification
1. Crude cellular extract	1,400	10,000	100,000	10	1
2. Precipitation with ammonium sulfate	280	3,000		32	
3. Ion-exchange chromatography	90		80,000	200	
4. Size-exclusion chromatography	80	100	60,000	600	
5. Affinity chromatography	6	3	45,000		

*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.

(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 $[\text{CO}_2]$; (b) the effect of increasing $[\text{H}^+]$; (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?