

Biochemistry 5700

Fall, 2004

Exam 1

INSTRUCTIONS:

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

table 5-1

Amino acid	Abbreviated names	M_r	pK_a values			pI	Hydropathy index*	Occurrence in proteins (%) [†]
			pK_1 (—COOH)	pK_2 (—NH ₃ ⁺)	pK_R (R group)			
Glycine		75	2.34	9.60				
Alanine		89	2.34	9.69				
Valine		117	2.32	9.62				
Leucine		131	2.36	9.60				
Isoleucine		131	2.36	9.68				
Methionine		149	2.28	9.21				
Phenylalanine		165	1.83	9.13				
Tyrosine		181	2.20	9.11	10.07			
Tryptophan		204	2.38	9.39				
Serine		105	2.21	9.15				
Proline		115	1.99	10.96				
Threonine		119	2.11	9.62				
Cysteine		121	1.96	10.28	8.18			
Asparagine		132	2.02	8.80				
Glutamine		146	2.17	9.13				
Lysine		146	2.18	8.95	10.53			
Histidine		155	1.82	9.17	6.00			
Arginine		174	2.17	9.04	12.48			
Aspartate		133	1.88	9.60	3.65			
Glutamate		147	2.19	9.67	4.25			

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (— values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105–132.

[†]Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad v = \frac{V_{\max} \cdot [S]}{K_m + [S]}$$

(1) (2 points) The formation of a chemical bond is an (circle one) **exothermic** / **endothermic** process.

(2) (15 points) For this problem you will need to use information from page 1 on the exam.

(a) Draw a complete titration curve, using NaOH as the titrant and starting at pH 0, for the amino acid lysine.

(b) Clearly label the positions, and indicate the values, of the pKa's and the pI on your titration curve (calculate the pI).

(c) Draw the structures of the two forms of lysine that predominate at pH 8.5.

(3) (6 points) You have a protein "P" containing 100 amino acids that binds a ligand "L". Binding of ligand "L" induces a conformational change in the protein that brings the side chain of Asp24 in close proximity to the side chain of Arg73. This conformational change will (circle one) **increase** / **decrease** the pKa for the side chain of Asp24 and (circle one) **increase** / **decrease** the pKa for the side chain of Arg73. Briefly explain why these changes occur.

(4) (10 points) Fill in the missing information in the following purification table:

Purification table for the new enzyme "professor hydrolase"						
step of purification	volume (ml)	total protein (mg)	total activity (units)	specific activity (units/mg)	% recovery	fold purification
lysate	1000	20,000	90,000		100	1
ion exchange	200		60,000	20		4.4
gel filtration	50	200	35,000	175	39	
hydrophobic interaction	25	25		800	22	178

(5) (5 points) Which state of hemoglobin, [R (oxy) or T(deoxy)] is most stabilized by the following:

(a) higher 2,3-BPG _____

(b) lower [CO₂] _____

(c) lower [H⁺] _____

(d) salt bridges _____

(e) O₂ binding to the first of the four sites _____

(6) (15 points) In the space provided, briefly describe how the following forces probably contribute to the proper folding of the protein myoglobin in aqueous solution. Use your knowledge of myoglobin structural features, and consider both protein-protein and protein-solvent interactions in answering the question. Circle the single force that is most important in the proper folding, and for that force, explain in thermodynamic terms why it is so important.

(a) hydrogen bonds

(b) ionic interactions

(c) ion-dipole interactions

(d) hydrophobic interactions

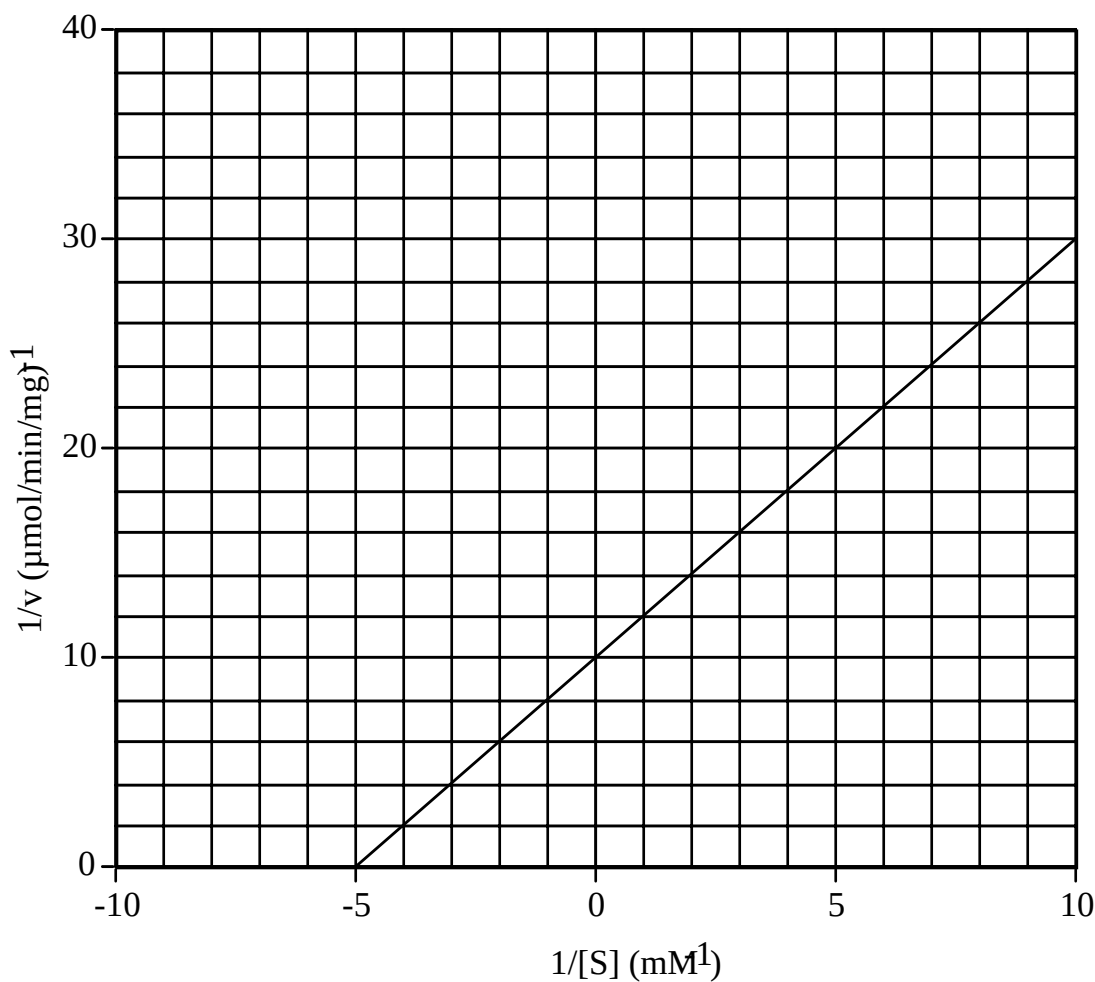
(e) most important force and why:

(7) (5 points) You purify an enzyme from a crude bacterial lysate. 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 60,000, 30,000 and 10,000, and the staining intensities of the proteins indicate they are present in a stoichiometric ratio of 1:2:1, respectively. Your protein elutes from a size exclusion (gel permeation) column with an apparent molecular weight of 390,000. The quaternary structure of your protein is (fill in the blanks):

$\alpha_ \beta_ \gamma_$

(8) (4 points) In one sentence define “binding energy”.

(9) (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) 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.
- (b) 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 2-fold.
- (c) Calculate the actual value for the apparent K_m observed for the enzyme under the conditions of part (b) above.

(10) Definitions. 20 points.

What structural protein forms a two chained coiled coil?
Which amino acid is often found in the cis confirmation in proteins
The rotation about which two atoms in a polypeptide chain defines the bond angle phi?
The rotation about which two atoms in a polypeptide chain defines the bond angle psi?
What structural protein forms a triple helix?
What type of inhibitor binds both to free E and the ES complex?
Name a protein motif that contains parallel beta strands
Name the protein motif that contains 4 antiparallel alpha helices joined by short loops
The reaction between a primary amine and CO ₂ forms what new functional group?
The oxygen dissociation curve for myoglobin is hyperbolic while that for hemoglobin is
A molecule containing both polar and nonpolar moieties is called what?
Provide the one letter abbreviations for three amino acids that absorb significant UV light
Name an amino acid containing a thioether functional group
Name an amino acid containing a thioalcohol functional group
Name a modified (nonstandard) amino acid commonly found in proteins
Name an amino acid that can serve as an effective nucleophile at physiological pH
What type of bond is formed when two cysteine residues react and are oxidized?
Name an amino acid that is not chiral
What is a chromatographic technique used to separate proteins on the basis of differences in charge?
“Catalytic efficiency” is the ratio of what two kinetic parameters?

(11) (8 points) The following shows the reaction coordinate for conversion of substrate S to product P, in the absence or presence of an enzyme. Use the information from this diagram to write the kinetic mechanism ($E + S \rightleftharpoons E + P$) for this reaction. Include rate constants above/below the arrows in your kinetic mechanism. Circle the rate constants that are first order and box (in a square) the rate constants that are second order.

