Key

Biochemistry 5700
Fall, 2005
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 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)	pI	Hydropathy index*	Occurrence in proteins (%)†
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.

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

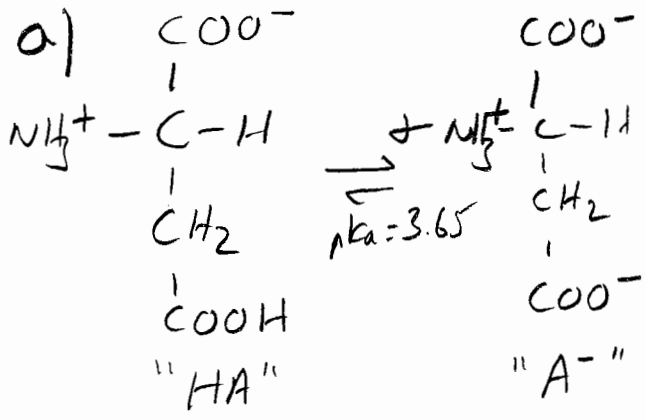
$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

Key

(1) You have an aqueous 0.1 M solution of the amino acid aspartate buffered at pH 4.3.

(a) (5 points) Draw the structures of the two forms of aspartate 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) Calculate the isoelectric point of aspartate.

a)



b) $pH = pK_a + \log \frac{[A^-]}{[HA]}$; $4.3 = 3.65 + \log \frac{[A^-]}{[HA]}$;

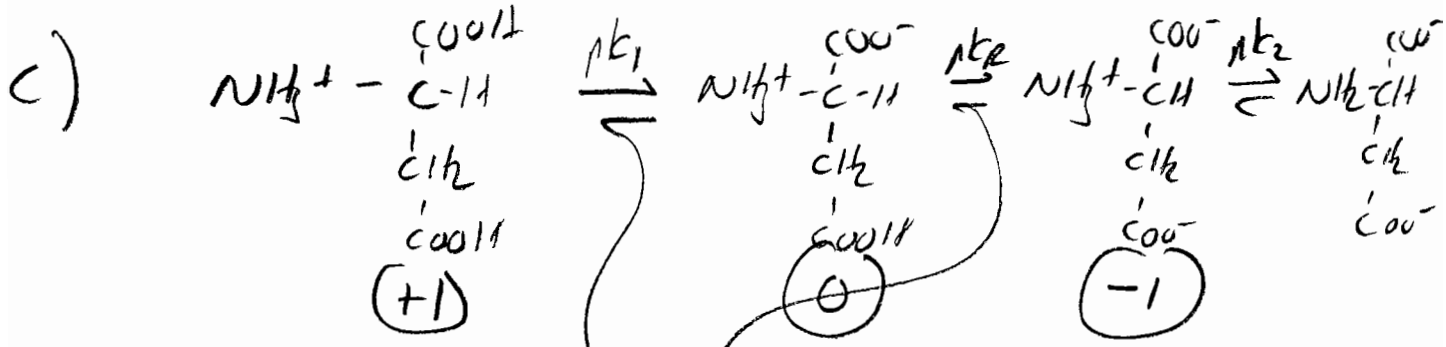
$\frac{[A^-]}{[HA]} = 10^{0.65} = 4.47$; $[A^-] = 4.47[HA]$

$[A^-] + [HA] = 0.1$; $4.47[HA] + [HA] = 0.1$

$[HA] = 0.0183\,M$

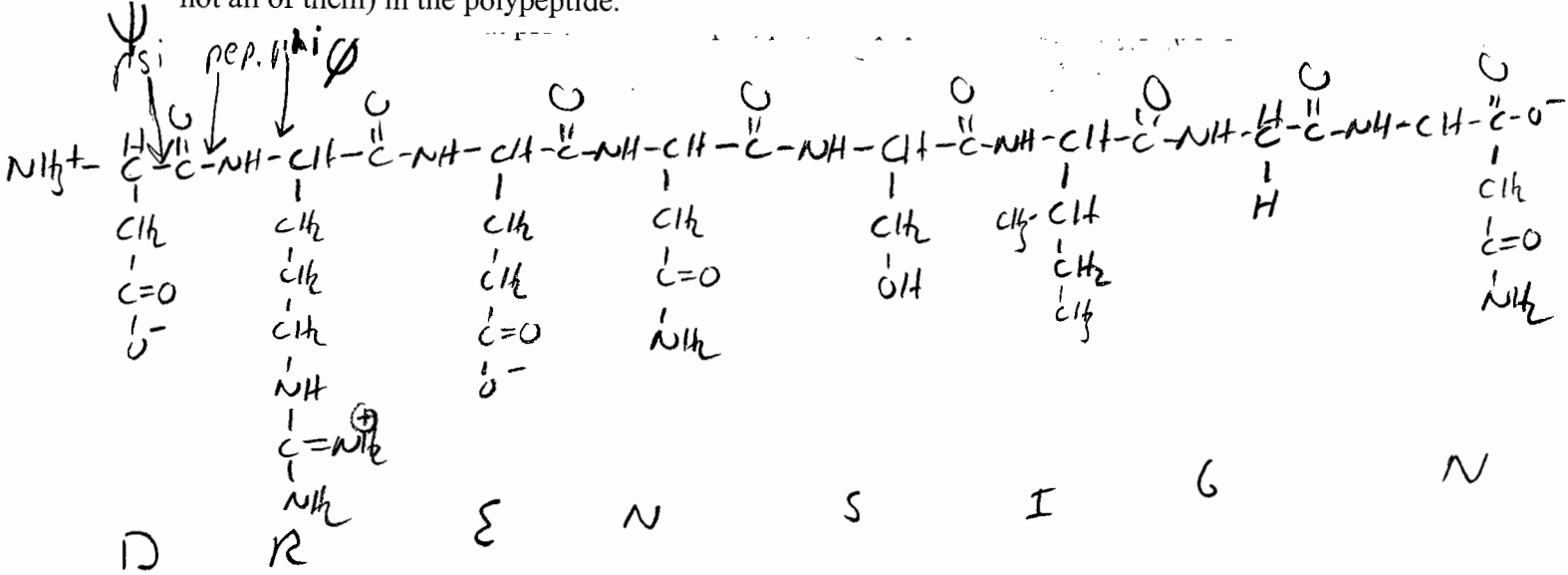
$[A^-] = 0.0817\,M$

c)



$pI = \frac{pK_1 + pK_R}{2} = \frac{1.88 + 3.65}{2} = 2.76$

(2) (10 points) Draw the structure of the polypeptide with the abbreviated name DRENSIGN as it would exist in solution at pH 7. Label a representative phi, psi, and peptide bond (just one of each, not all of them) in the polypeptide.



(3) (10 points) Fill in the missing information in the following purification table:

step of purification	total protein (mg)	total activity (units)	specific activity (units/mg)	% recovery	fold purification
lysate	20,000	110,000	5.50	100	1
ion exchange	802	60,000	74.8	54.5	13.6
gel filtration	78.0	35,100	450	31.9	81.8
hydrophobic interaction	18.0	26,950	1500	24.5	273

(4 points) (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 70,000, 60,000 and 20,000, and the staining intensities of the proteins indicate they are present in a stoichiometric ratio of 2:2:1, respectively. Your protein elutes from a size exclusion (gel permeation) column with an apparent molecular weight of 840,000. The quaternary structure of your protein is (fill in the blanks):

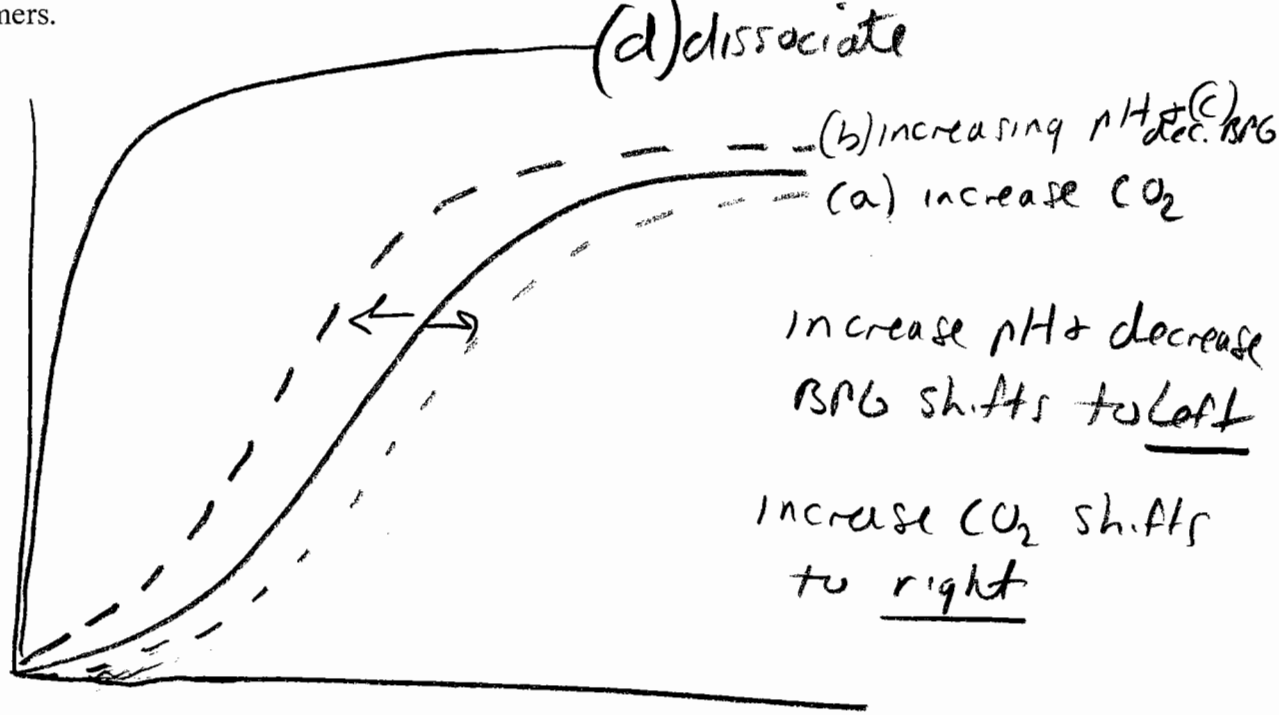
$\alpha_6\beta_6\gamma_3$

$70 \times 2 + 60 \times 2 + 20 \times 1 = 280$ kDa for protomer

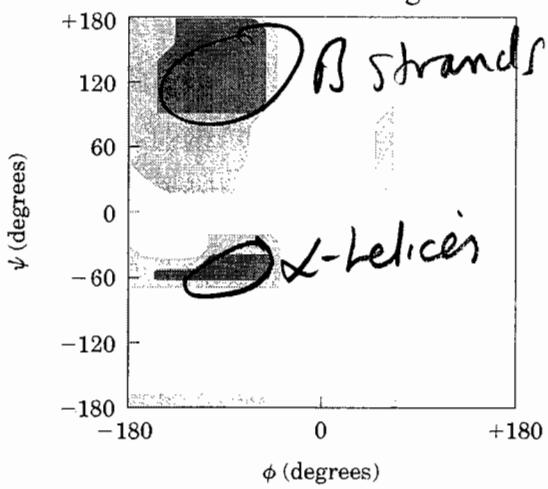
$840/280 = 3$

$(\alpha_2\beta_2\gamma_1) \times 3$

(5) (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 increasing [CO_2]; (b) the effect of increasing pH; (c) decreasing [2,3-DPG] by a factor of two; (d) dissociating the Hb subunits into monomers.



(6) 5 points. Indicate with arrows the approximate regions where (a) α-helices and (b) β-strands would be found on the following Ramachandran plot:



(7) Definitions. 20 points.

What structural protein forms a two chained coiled coil?	α-keratin
Which amino acid is often found in the cis confirmation in proteins	Proline, (Pro, P)
The time period in which an equilibrium between E, S and ES is established is called what?	pre-steady state
Which amino acid contains a guanidinium group in the R side chain?	Arginine (Arg, R)
What structural protein forms a triple helix?	collagen
What type of inhibitor binds only to the ES complex?	Uncompetitive
The reaction of an N-terminal lysine in hemoglobin with CO2 forms what modified residue?	carbamino (carbamate)
The histidine that coordinates iron in myoglobin and hemoglobin is called the _____ histidine	proximal
Name two amino acid substitutions for valine that would be considered conservative changes in a protein interior.	Ile, Leu, Ala, Gly
What two residues are most likely to be found in a beta turn?	Pro, Gly
A molecule containing both polar and nonpolar moieties is called what?	amphipathic
Provide the one letter abbreviations for three amino acids that absorb significant UV light	Y F W
How many of the standard amino acids contain the heteroatom nitrogen in the side chain "R"?	6
Name an amino acid containing a thioether group	Met (M)
Name a modified (nonstandard) amino acid commonly found in proteins	hydroxyPro, hydroxyLys
Name an amino acid containing a side chain that might form a salt bridge with Lys at pH 7	Glu, Asp (E, D)
The aggregation of nonpolar molecules in water is driven largely by which thermodynamic parameter?	entropy
Name an amino acid that is not chiral	Gly (G)
Which amino acid is key to the chemistry of a hair perm?	Cys (C)
A nonprotein component of an enzyme that is organic and covalently bound is called what?	prosthetic groups

(8) (15 points) Kinetic analysis of a homodimeric enzyme of 150,000 molecular weight (that is the Mr of the dimer) catalyzing conversion of substrate S to product P provides the following kinetic constants: V_{max} = 10,000 units/mg and K_m = 0.1 mM. Assume each subunit contains an active site; i.e. there are two active sites per dimer.

(a) (5 pts) Determine the turnover number of the enzyme in units of "s^{-1}"
(b) (4 pts)Determine the catalytic efficiency of the enzyme expressed in units of "$M^{-1}s^{-1}$"
(c) (3 pts) What would be the specific activity of the enzyme when assayed with [substrate] = 0.02 mM?
(d) (3 pts) The presence of inhibitor I decreases the apparent V_{max} of the enzyme and increases K_m. What type of inhibitor is it? Briefly explain how you know what type of inhibitor it is.

a) $\left(10{,}000 \frac{\mu mol\ P}{min \cdot mg}\right)\left(\frac{150{,}000\ mg}{mmol\ E}\right)\left(\frac{1\ mol}{1000\ \mu mol}\right)\left(\frac{1\ min}{60\ s}\right)\left(\frac{1\ \mu mol\ E}{2\ \mu mol\ active\ sites}\right)$

$k_{cat} = 12{,}500\ s^{-1}$

c) $v = \frac{V_{max}[S]}{K_m + [S]} = \frac{(10{,}000\ U/mg)(0.02\ mM)}{0.1\ mM + 0.02\ mM} = 1{,}666\ U/mg$

b) $cat.\ eff = \frac{k_{cat}}{K_m} = \left(\frac{12{,}500^{-1}}{0.1\ mM}\right)\left(\frac{1000\ mM}{M}\right) = 1.25 \times 10^8\ M^{-1}s^{-1}$

d) noncompetitive - binds both free E & ES complex, changing both Vmax & Km

(9) (10 points) Name the structural motifs present in each of the four proteins shown below. Then, use arrows to highlight representative important secondary structural elements of each protein, and name the secondary structural elements. You don't need to draw arrows to each individual secondary element if it is repeated several times, but be sure to indicate all different types of secondary ~~structural~~ elements present in each protein.