Key

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

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			

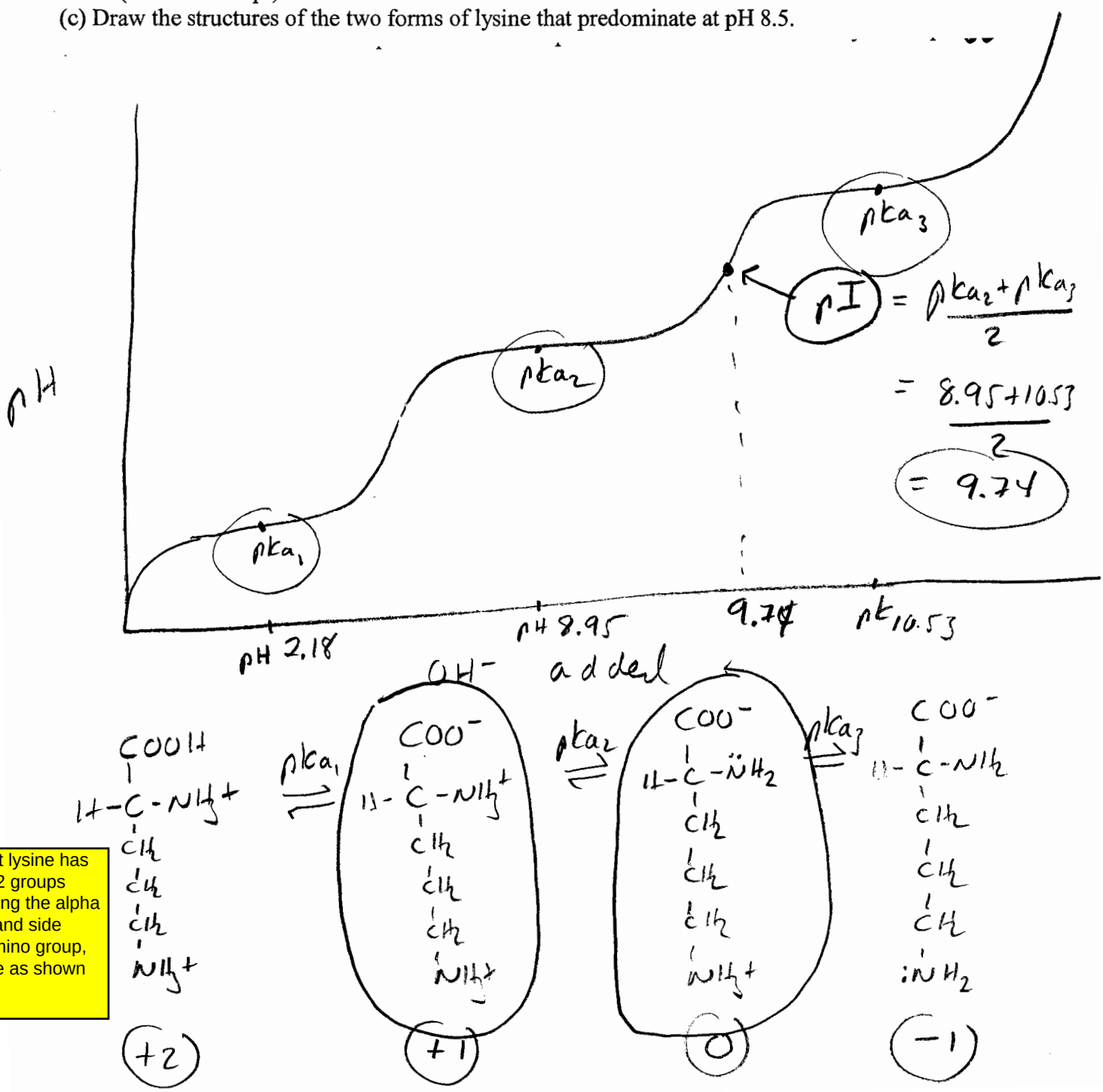
*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105–132.

†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.

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \qquad v = \frac{V_{\max} \cdot [S]}{K_m + [S]}$$

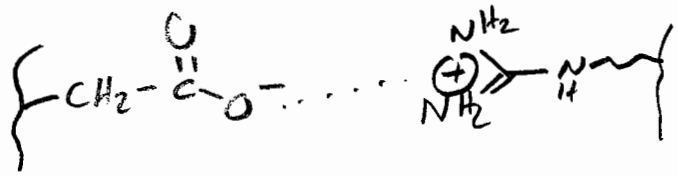
(1) (2 points) The formation of a chemical bond is an (circle one) **exothermic / endothermic** process.

(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.



note that lysine has four CH2 groups connecting the alpha carbon and side chain amino group, not three as shown here.

(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.



Asp pKa decreases because close ⊕ charge stabilizes Asp in ionized form, hence it is more acidic

Arg pKa increases because close (−) charge stabilizes Arg in protonated form, hence it is more basic

(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	4.5	100	1
ion exchange	200	3000	60,000	20	67	4.4
gel filtration	50	200	35,000	175	39	39
hydrophobic interaction	25	25	20,000	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 T

(b) lower [CO_2] R

(c) lower [H^+] R

(d) salt bridges T

(e) O_2 binding to the first of the four sites R

(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

The 8 α-helices of the globin fold are stabilized by intrachain H-bonds of residues apart. Othe h-bonds between side chains/solvent may also be present

(b) ionic interactions

salt bridges between ⊕ & ⊖ side chains may stabilize structure in hydrophilic regions

(c) ion-dipole interactions

⊕ & ⊖ side chains on protein surface can interact w/ water in this manner

(d) hydrophobic interactions

nonpolar AA residues aggregate in the nonpolar interior of the protein

(e) most important force and why:

by aggregating nonpolar AAs, water entropy can be maximized

(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):

$\alpha_3\beta_6\gamma_3$

protomer is $\alpha_1\beta_2\gamma_1$

$(60)+(30)2+(10)1 = 130$ kD

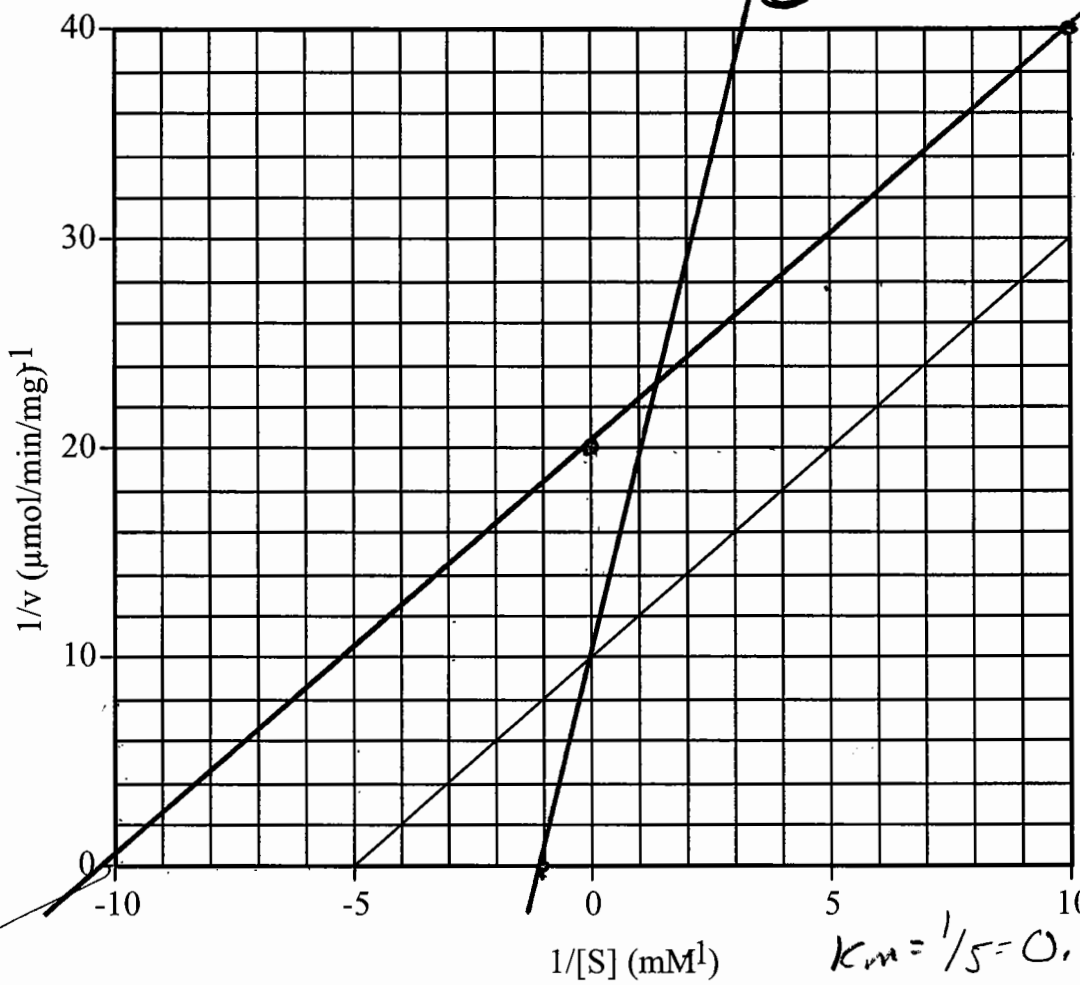
$390/130 = 3$

so $(\alpha_1\beta_2\gamma_1)3$

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

the enthalpy derived (released) from the formation of noncovalent interactions between E + S in the ES complex

(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. new K_m (0.2) 5 = 1 mM $-1/K_m = -1$

(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. $V_{max} = 1/10 = 0.1$, new $V_{max} = 0.05$, so new $1/V_{max} = 20$

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

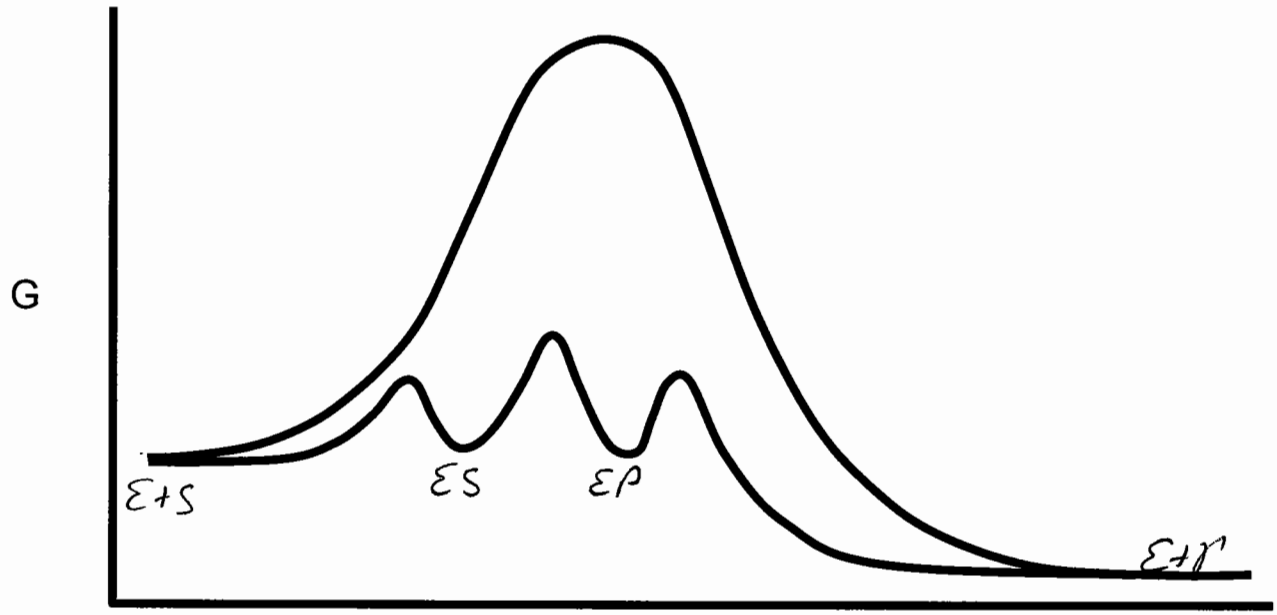
$-1/K_m = -10$, $K_{m_{app}} = 0.1$ mM

(10) 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
The rotation about which two atoms in a polypeptide chain defines the bond angle phi?	N - C_α
The rotation about which two atoms in a polypeptide chain defines the bond angle psi?	$C_{carbonyl}$ - C_α
What structural protein forms a triple helix?	collagen
What type of inhibitor binds both to free E and the ES complex?	noncompetitive
Name a protein motif that contains parallel beta strands	α/β saddle or barrel
Name the protein motif that contains 4 antiparallel alpha helices joined by short loops	4 helix bundle
The reaction between a primary amine and CO_2 forms what new functional group?	carbamate
The oxygen dissociation curve for myoglobin is hyperbolic while that for hemoglobin is	sigmoidal
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
Name an amino acid containing a thioether functional group	Methionine
Name an amino acid containing a thioalcohol functional group	cysteine
Name a modified (nonstandard) amino acid commonly found in proteins	hydroxy-pro, -lys
Name an amino acid that can serve as an effective nucleophile at physiological pH	Histidine
What type of bond is formed when two cysteine residues react and are oxidized?	disulfide
Name an amino acid that is not chiral	glycine
What is a chromatographic technique used to separate proteins on the basis of differences in charge?	ion exchange chromat.
"Catalytic efficiency" is the ratio of what two kinetic parameters?	k_{cat} / K_m

Key

(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 ---------- 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.



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

(not necessary if assume irreversible)