

Biochemistry 5700

Fall, 2008

Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam contains 17 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!!

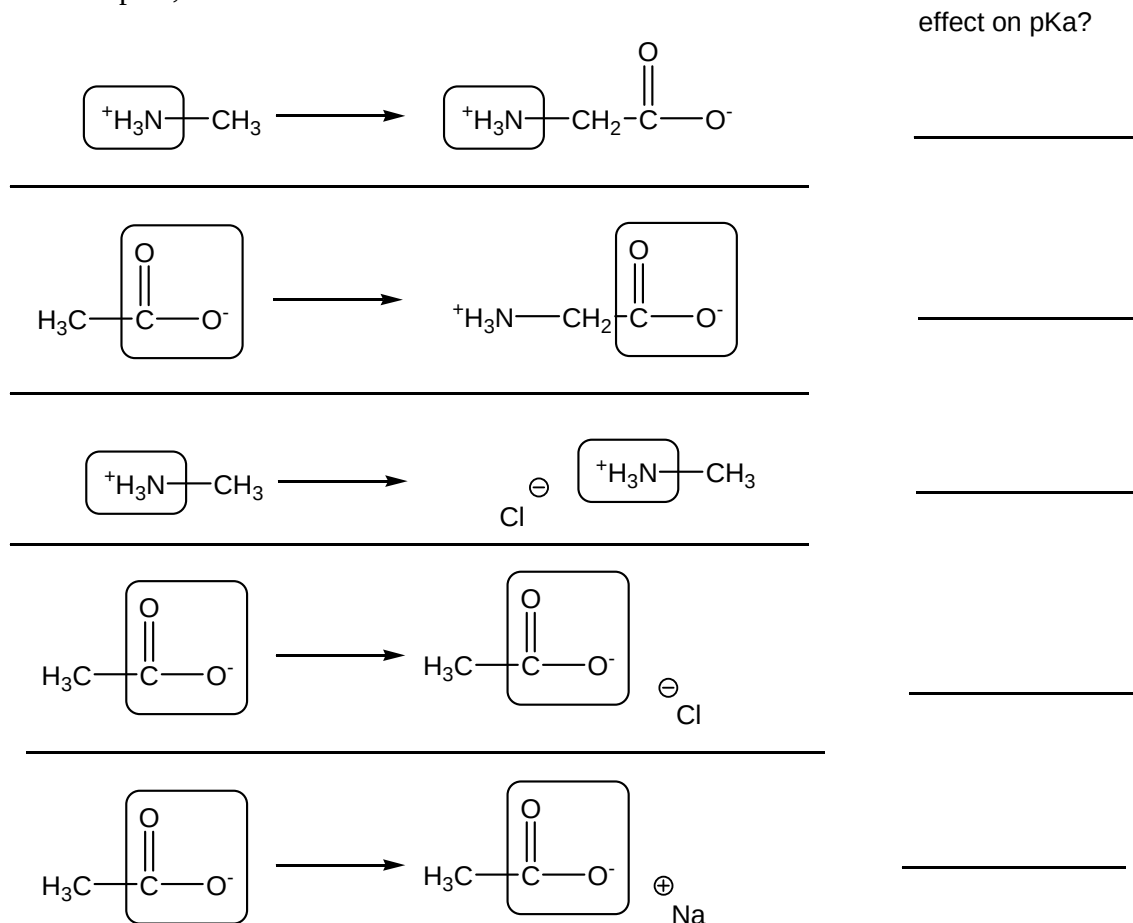
TABLE 3-1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values			pI	Hydropathy index*	Occurrence in proteins (%)†
			pK_1 (—COOH)	pK_2 (—NH ₃ ⁺)	pK_R (R group)			
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

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

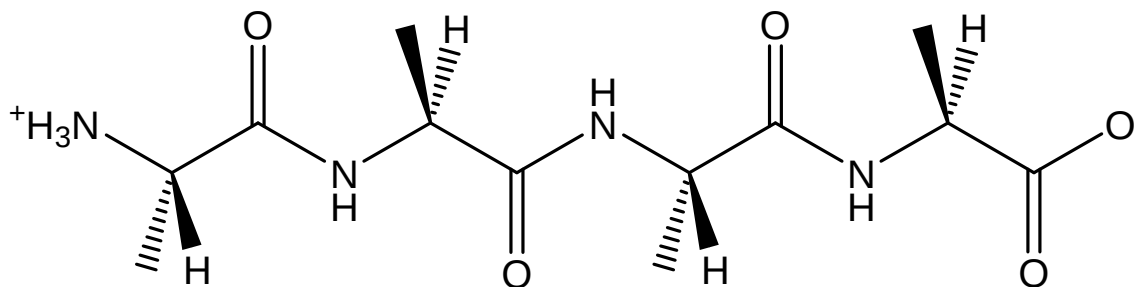
$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

(1) (5 points) Predict how the pKa of the ionizable group (inside the box) will be affected by the indicated change of environment (going from left to right side). The choices are: increase pKa, decrease pKa, no effect.



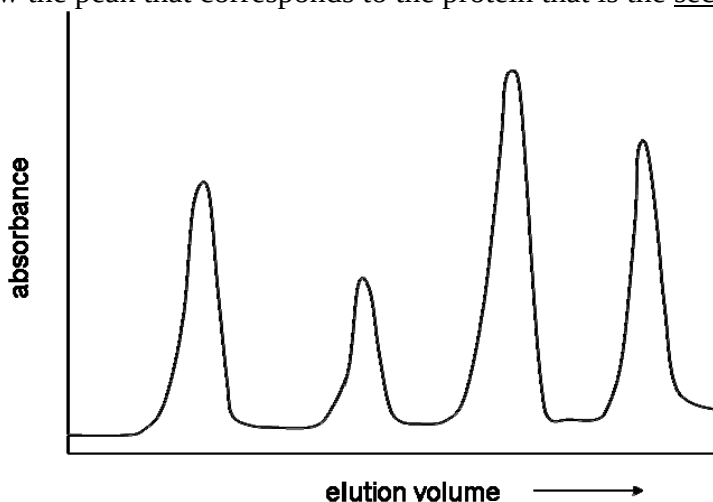
(2) (a) (4 points) Add the appropriate side chains (using the first “blank” bond provided as a template on each residue as a starting point) to the polypeptide shown below at pH 7.0 to give the sequence: VKRM.

(b) (3 points) label the innermost peptide, psi, and phi bonds on the polypeptide.



(3) (4 points) Using information from the front page of the exam, calculate the pI for lysine.

(4) (3 points) A mixture of 4 proteins is separated by gel filtration (size exclusion chromatography). Indicate with an arrow the peak that corresponds to the protein that is the second largest of the four proteins.



(5) (4 points) Properly name the following levels of protein structure.

a)	A regular, recurring arrangement in space of adjacent amino acid residues in a polypeptide chain
b)	spatial relationship between all amino acids in polypeptide; the 3-D structure of the polypeptide
c)	A simple combination of a few 2° elements with a specific geometric arrangement
d)	a polypeptide chain or part of a polypeptide chain that can independently fold into a stable tertiary structure

(6) (6 points) Give the 3 letter code for an amino acid that matches the indicated property. There may be more than one correct answer, but only name one amino acid for each question.

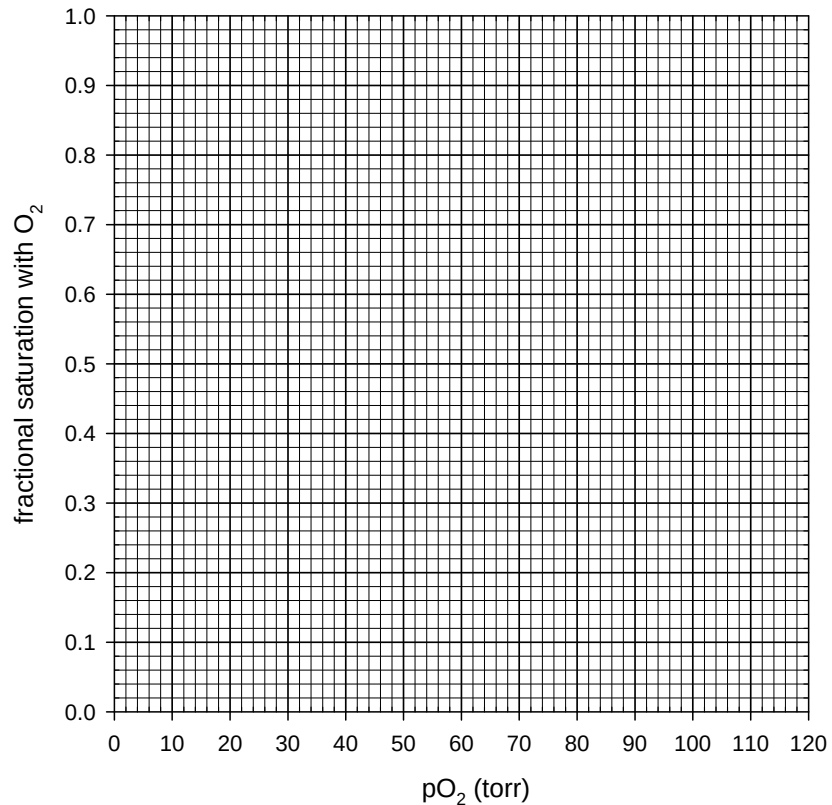
(a)	posttranslationally modified by hydroxylation in collagen
(b)	achiral amino acid
(c)	crosslinks chains of alpha-keratin
(d)	absorbs significant UV light
(e)	has a side chain containing a thioether group
(f)	has a side chain containing a guanidinium group

(7) (6 points) On the graph shown to the right, add plots for oxygen binding to:

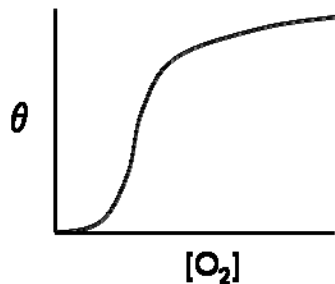
(a) myoglobin, where myoglobin exhibits 98% saturation with O_2 at $PO_2 = 120$ torr, and exhibits a P_{50} of 4 torr, and

(b) hemoglobin, which exhibits 90% saturation with O_2 at $PO_2 = 120$ torr, and exhibits a P_{50} of 20 torr

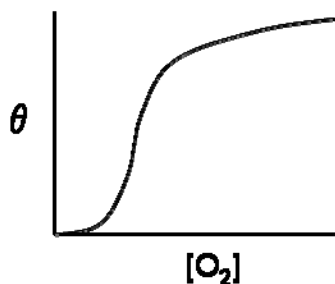
(c) Based on the plots you provided, what percentage of oxygen will be transferred to myoglobin from hemoglobin at a venous pressure of 30 torr?



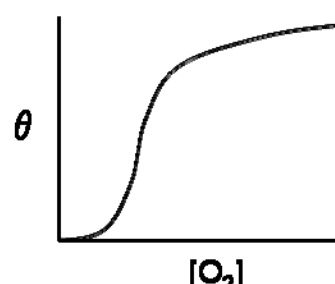
(7) (5 points) Add a second curve to each of the oxygen dissociation curves for hemoglobin to indicate what effect the indicated change has on the curve:



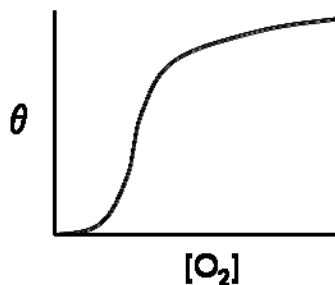
(a) Increase $[CO_2]$



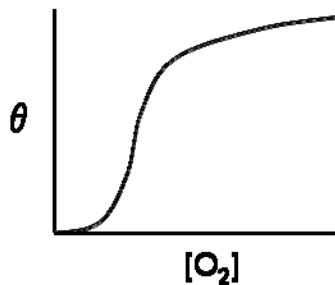
(b) Increase pH



(c) completely remove DPG



(d) dissociate subunits and isolate α monomers



(e) Increase $[DPG]$

(8) For the purification table shown below:

(a) **(6 pts)** Calculate the values that belong in the cells labeled (i), (ii), and (iii)

(b) **(2 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	10,000			
ammonium sulfate precipitation	100	6,000	10,000	(i)		
ion exchange	200	1,000	8,000			(iii)
gel filtration	25	200	4,000			
hydrophobic interaction	100	50	3,000		(ii)	
affinity chromatography	50	5	2,500			

(9) **(4 points)** Write the formula for a multimeric protein consisting of 75, 50 and 25 kDa subunits, present in a ratio of 1:1:2, respectively, which has a native molecular weight of 525 kDa (a kDa = 1000 daltons or 1000 amu)

(10) **(6 points)** Kinetic analysis of a homotetrameric enzyme of 200,000 molecular weight (that is the M_r of the α_4 tetramer) catalyzing conversion of substrate S to product P provides the following kinetic constants: $V_{\max} = 4,000$ U when 1 mg of protein is assayed, and $K_m = 0.020$ mM. Assume each subunit contains an active site; i.e. there are four active sites per tetramer. Calculate the catalytic efficiency (units of $M^{-1}\cdot s^{-1}$) of the enzyme on the basis of monomeric units (i.e. per active site).

(11) **(4 points)** You have an aqueous solution of the amino acid histidine at pH 6.4. What is the ratio of the deprotonated to protonated forms of histidine at this pH?

(12) **(9 points)** Predict the effects of the following inhibitors on the values of $V_{\max}$ and K_m observed in the presence of the inhibitor (increase, decrease, no change, or possibly a combination of two), and indicate what form(s) of the enzyme the inhibitor binds to (E and/or ES-may be one form, or both)

Inhibitor	Effect on $V_{\max}$	Effect on K_m	Binds to:
Competitive			
Noncompetitive (mixed)			
uncompetitive			

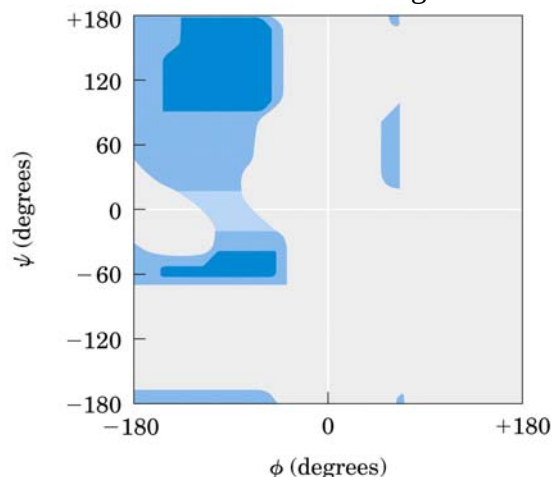
(13) **(5 points)** Write the kinetic mechanism for a sequential bisubstrate reaction forming two products where substrate binding is random but product release is ordered.

(14) **(5 points)** Are the following statements correct descriptions of the steady state assumption used to derive the Michaelis-Menten equation? Mark T (true) or F (false) next to each statement.

- (a) the equilibrium between E, S, and ES is rapidly established
- (b) ES breaks down faster towards E and P than towards E and S
- (c) The concentration of the ES complex changes during the time frame where enzyme activity is being measured
- (d) The concentration of enzyme in an assay is much lower than the concentration of substrate
- (e) product formed during the reaction reacts with E to reform ES at a significant rate

(15) **(5 points)** An enzyme exhibits a K_m of 0.1 mM and $V_{\max}$ of 5,000 U when 1 mg of enzyme is assayed. What concentration of substrate will give a rate of 3,500 U?

(16) **4 points.** Indicate with arrows the approximate regions where (a) α -helices and (b) β -strands would be found on the following Ramachandran plot:



(17) **(10 points)**

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

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

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

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

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

(f) In a 3 strand beta sheet, how many interchain hydrogen bonds are formed, on average, per amino acid residue on a the inner strand ?

(g) An alpha helix containing polar residues on one side and nonpolar residues on the other is called _____.

(h) The histidine that does not coordinate iron in myoglobin and hemoglobin is called the _____ histidine

(i) The reaction of an amino group and CO_2 is called _____

(j) What amino acid residue is most commonly associated with formation of a cis peptide bond in a polypeptide?