

Biochemistry 5700

Fall, 2007

Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam contains 12 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) 16 points.

- (a) Draw the tripeptide Cys-Glu-Pro at pH = 7.0, with the peptide bond between Cys and Glu in the trans conformation and the peptide bond between Glu and Pro being in the cis-conformation.
- (b) Use arrows with lettering (peptide, phi, or psi) to indicate all the peptide bonds and bonds defining phi and psi angles in this tripeptide
- (c) 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).
- (d) Briefly define pI, and use an arrow to indicate the position of the pI on the titration curve.

(2) (15 points) Pot pourri of definitions.

Name a fibrous protein that forms a triple helical structure
Name the fibrous protein that forms a two-chain coiled coil
Name a specific type of motif that contains both beta strands and alpha helices
Name the protein motif that contains 4 antiparallel alpha helices joined by short loops
Name a specific type of motif that contains 8 alpha helices that are roughly perpendicular to their neighbor helices
Provide the three letter abbreviations of two amino acids that are predominantly positively charged at pH 7
Provide the three letter abbreviations of two amino acids that absorb UV light
What type of bond is formed when two cysteine residues react and are oxidized?
Provide the three letter abbreviations of two amino acids that have an aliphatic side chain
The reaction between a primary amine and CO ₂ forms what new functional group?
Name a modified (nonstandard) amino acid that might be found in an extracellular structural protein
In a 3 strand β sheet, how many interchain H- bonds are formed, on average, per amino acid residue on the outer strand ?
In an alpha helix, an intrastrand hydrogen bond is formed between residue "n" and residue n+_____.
In a 3 strand β sheet, how many interchain H- bonds are formed, on average, per amino acid residue on the inner strand ?
The oxygen dissociation curve for myoglobin is hyperbolic while that for hemoglobin is _____.

(3) (9 points) Suppose you have a solution of hemoglobin (Hb). How will the following changes affect the following properties of hemoglobin? Complete the table appropriately by writing the correct **BOLD** word in each column.

condition	Stabilize T or R form?	Increased or decreased O ₂ affinity?	Direction hemoglobin curve is shifted (left or right)
higher 2,3-BPG			
lower [CO ₂]			
lower [H ⁺]			

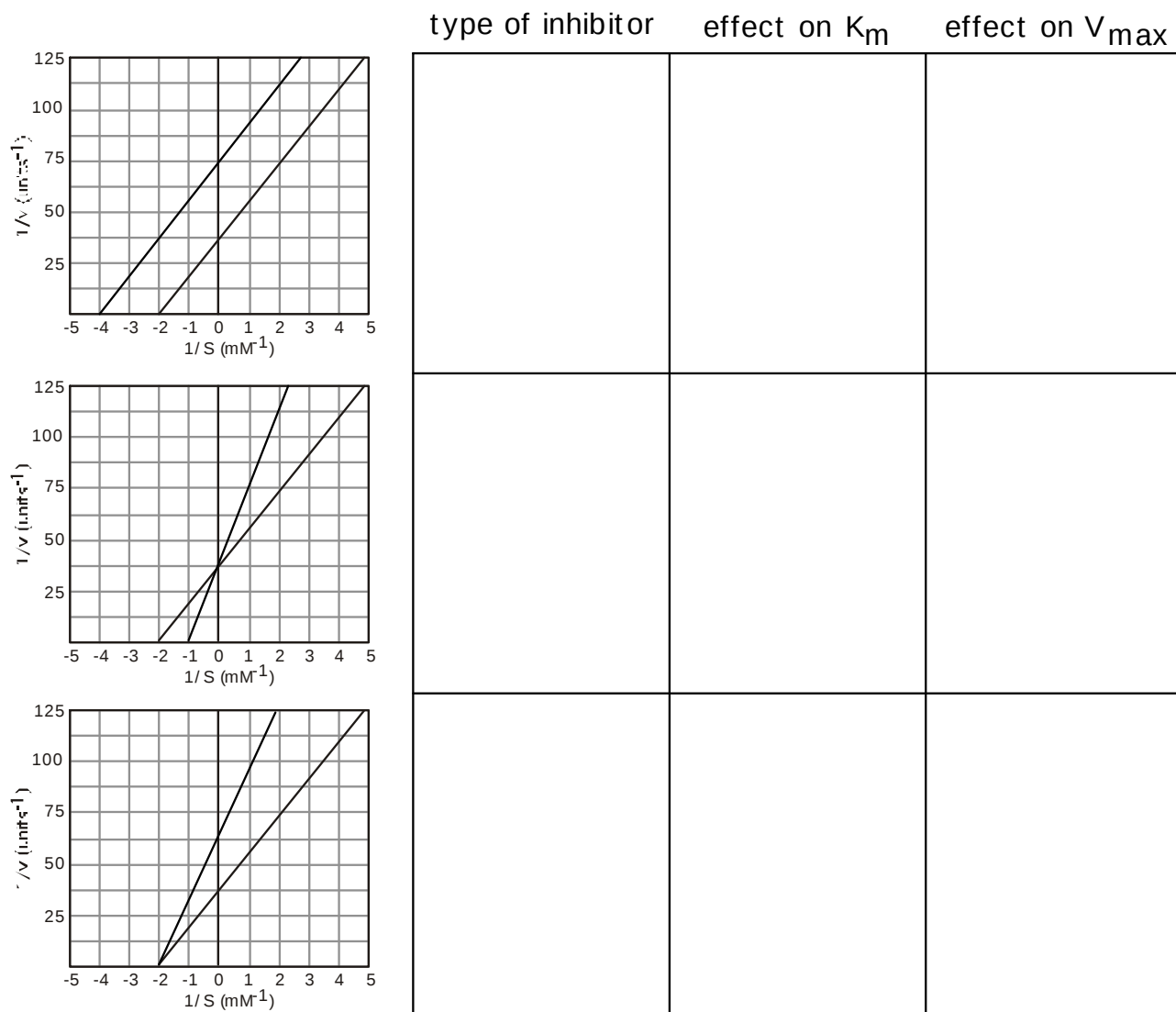
(4) (10 points) Fill in the missing information in the following purification table:

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		60,000	74.8	54.5	13.6
gel filtration	78.0		450		81.8
hydrophobic interaction	18.0		1500	24.5	

(5) (4 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 100,000, 60,000 and 30,000, 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 500,000. The quaternary structure of your protein is (fill in the blanks):

α _ β _ γ _

(6) (9 points) The following are Lineweaver-Burke plots for an enzyme-catalyzed reaction with and without an inhibitor. Use the plots to fill in the requested information:



(7) (12 points) Kinetic analysis of a homodimeric enzyme of 120,000 molecular weight (that is the M_r of the dimer) catalyzing conversion of substrate S to product P provides the following kinetic constants: $V_{\max} = 20,000$ units when 1 mg of enzyme is assayed, and $K_m = 0.3$ mM. Assume each subunit contains an active site; i.e. there are two active sites per dimer.

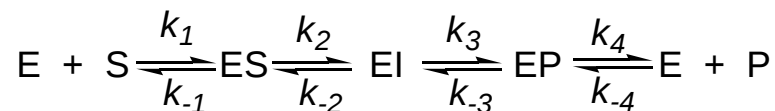
(a) (4 pts) Determine the turnover number of the enzyme in units of " s^{-1} "

(b) (4 pts) Determine the catalytic efficiency of the enzyme expressed in units of " $M^{-1}s^{-1}$ "

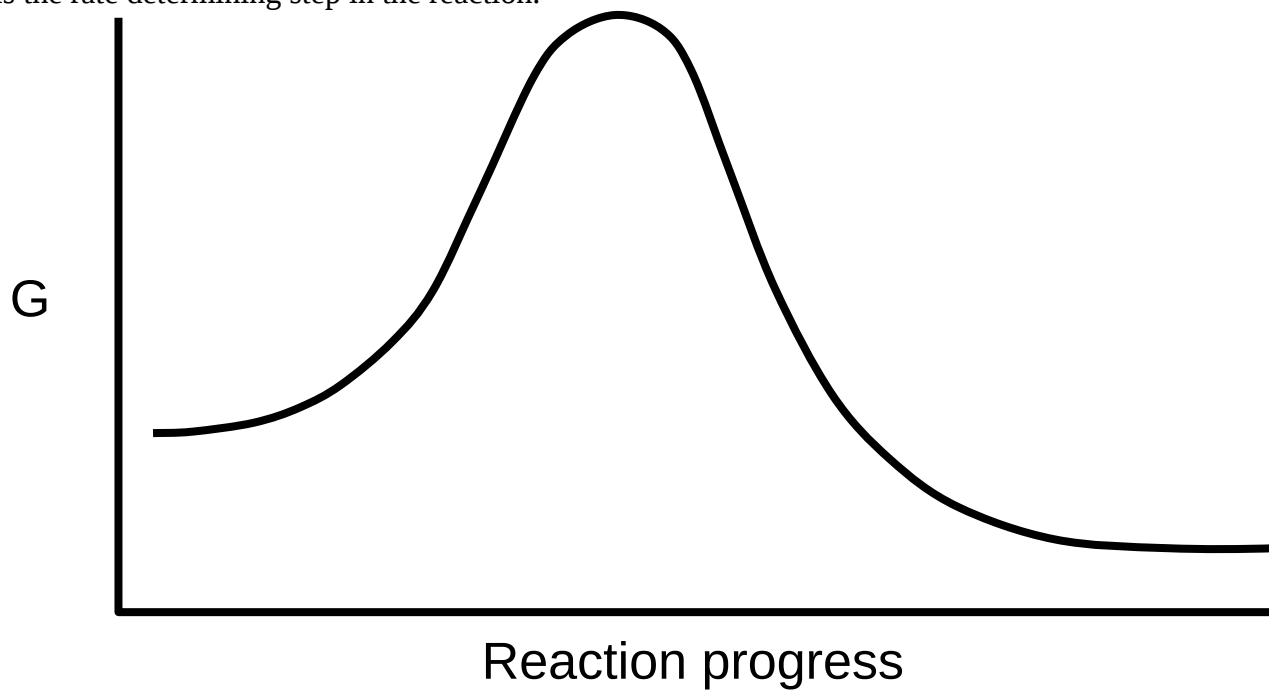
(c) (4 pts) What would the initial velocity be for the enzyme if 1 mg of enzyme were assayed with a concentration of substrate equal to 0.10 mM?

(8) (4 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 Glu24 in close proximity to the side chain of Lys73. This conformational change will (circle one) **increase** / **decrease** the pK_a for the side chain of Glu24 and (circle one) **increase** / **decrease** the pK_a for the side chain of Lys73.

(9) (10 points) (a) In the kinetic mechanism shown below, which rate constants are first order and which are second order?



(b) The following figure shows the progress curve for conversion of S to P in absence of E. Draw a progress curve on this figure that corresponds to the kinetic mechanism shown above, and where k_3 is the rate determining step in the reaction.



(10) (3 points) In one or two sentences, what is the primary thermodynamic driving force for the aggregation of nonpolar groups in water?

(11) (2 points) Which is true about the side chains of residues in an alpha-helix?

- a. They extend above or below the pleats
- b. they extend radially outward from the helix axis
- c. they point towards the center of the helix
- d. they hydrogen bond extensively with each other

(12) 6 points. Match the following levels of protein structure with the appropriate partner. (Write the correct letter next to the term in the left hand column).

Primary	a) A regular, recurring arrangement in space of adjacent amino acid residues in a polypeptide chain
Secondary	b) The spatial relationship of polypeptides in multisubunit proteins
tertiary	c) spatial relationship between all amino acids in polypeptide; the 3-D structure of the polypeptide
quaternary	d) A simple combination of a few 2° elements with a specific geometric arrangement
Motif	e) a polypeptide chain or part of a polypeptide chain that can independently fold into a stable tertiary structure
Domain	f) The linear sequence of amino acids and disulfide bonds in a protein