

Biochemistry 5700

Fall, 2003

Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam contains 6 questions. You are to answer only five of the six questions. Each of the five questions you answer is worth 20 points. Mark in huge letters “do not grade” on the one question you choose not to answer. If you do not do this I will grade the first five problems I encounter and ignore the sixth one. Please answer questions completely and legibly. You may write on the back of a page if you need more space to answer a question. 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			pI	Hydropathy index*
			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.

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

(1) (20 points) You have an aqueous 0.1 M solution of the amino acid histidine at pH 6.8.

(a) (5 points) Draw the structure of the form of histidine that predominates (is present in highest amount) in this solution

(b) (10 points) Determine the actual concentrations of the protonated and nonprotonated forms of histidine in this solution.

(c) (5 points) Calculate the isoelectric point of histidine.

(2) Definitions. 20 points.

What structural protein forms a two chained coiled coil?
Which amino acid is often found in the cis confirmation in proteins
Which three atoms in a polypeptide chain define the bond angle phi?
Which three atoms in a polypeptide chain define the bond angle psi?
What structural protein forms a triple helix?
What type of inhibitor binds only to the ES complex?
Name a protein motif that contains parallel beta strands
Name a protein motif that contains alpha helices joined perpendicularly by loops or bends
What type of motif allows two adjacent alpha helices to be anti parallel to each other?
Globular proteins are built from the 2 ° elements called alpha helices, beta strands, and _____.
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 two uncharged polar amino acid residues containing a nitrogen heteroatom
Name an amino acid containing a thioether group
Name a modified (nonstandard) amino acid commonly found in proteins
Name an amino acid containing a side chain that might form a salt bridge with Glu at pH 7
The aggregation of nonpolar molecules in water is driven largely by which thermodynamic parameter?
Name an amino acid that is not chiral
Which amino acid is key to the chemistry of a hair perm?
“Catalytic efficiency” is the ratio of what two kinetic parameters?

(3) 5 points each

(a) Define “proximal” and “distal” histidine as they relate to the oxygen storage protein myoglobin and describe one role the distal histidine plays in the physiology of myoglobin and hemoglobin.

(b) Check the appropriate boxes in the table below:

Treatment	Effect on oxygen binding affinity of hemoglobin		
	No effect	Increase affinity	Decrease affinity
Increase $[H^+]$			
Increase $[CO_2]$			
Increase [DPG]			

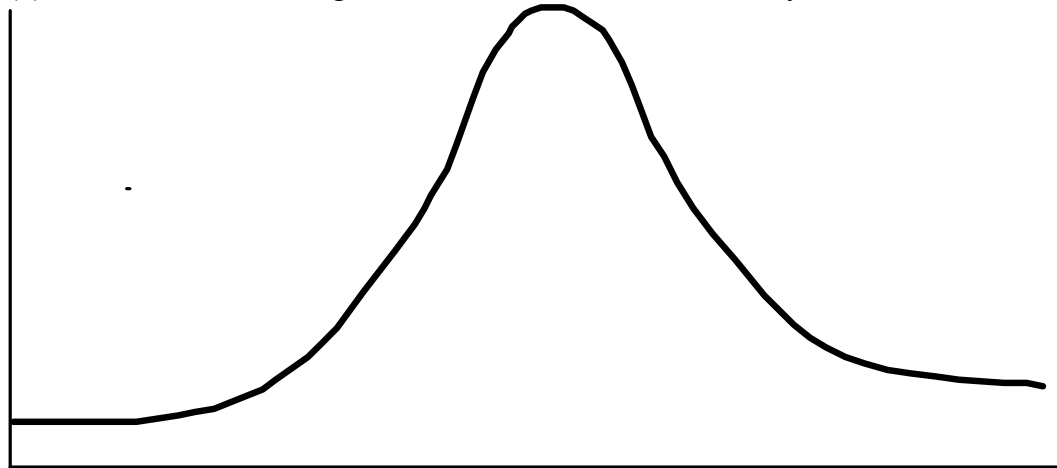
(c) Describe the biochemical/molecular basis for one of the effects indicated in (b) above.

(d) What is sickle cell anemia, and what is the biochemical/molecular basis for the disease?

(4) 4 points each. During the purification of a protein, you start with a crude extract (lysate) containing 50,000 units of enzyme activity and 3000 mg of protein. Your final purified enzyme preparation contains 10,000 units of activity and 15 mg of protein.

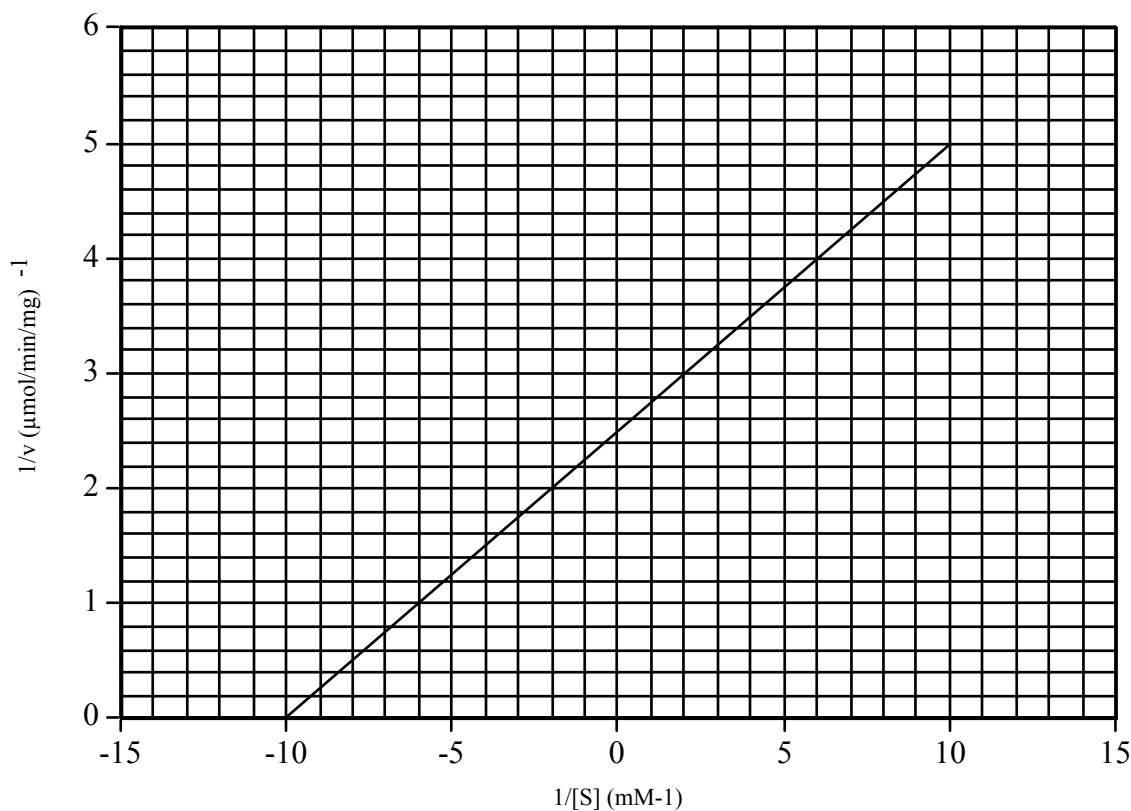
- (a) What is the specific activity of your purified enzyme?
- (b) What is the fold-purification of your purified enzyme?
- (c) What is the percent recovery of activity for your purification?
- (d) Approximately what percentage of all the proteins in the cell extract consisted of your enzyme?
- (e) If your protein is a monomer with molecular weight of 50,000, what is the turnover number for the enzyme in units of s^{-1} ?

(5) Consider the following reaction coordinate for an uncatalyzed chemical reaction:



- (a) (3 points) What is the sign of ΔG° for this reaction?
- (b) (6 points) Draw a new reaction pathway for this transformation on the above diagram that represents an enzyme-catalyzed reaction with a sequence that involves two intermediates. Label the positions of substrate, intermediates, and product on your path.
- (c) (3 points) Indicate where $\Delta G^\ddagger$ is found on your new pathway.
- (d) (8 points) Define “binding energy” and “entropy trap”

(6) 5 points each part. The following graph shows a Lineweaver-Burke ($1/v$ vs. $1/[S]$) plot for an enzymatic reaction transforming substrate S to product P.



- Determine the values of K_m and V_{max} for this enzyme (remember to include proper units in your answer).
- On the next page, draw a new graph replotting the data as v vs. $[S]$. Label V_{max} and K_m on your new graph.
- 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.
- Write the complete kinetic mechanism for this enzyme in the presence of a noncompetitive (i.e. mixed) inhibitor "I".

(6) continued