Name: _____

Biological Sciences 4087
Exam I
9/20/11

Total: 100 points
Be sure to include units where appropriate. Show all calculations. There are 5 pages and 11 questions.

1.(20pts)A. If pH = 4.6, $[H^+]$ = 2.5 x 10^{-5} M.

B. Draw the complete structure of the amino acid aspartate at pH 7.0.
See Fig. 3-5

C. The pK_a for the side chain of aspartate is 3.65. What is the ratio of the concentrations of the base to acid forms of the side chain of aspartate at pH 4.6? Show your work.

$pH = pK_a + \log [A^-]/[HA]$
$4.6 = 3.65 + \log [A^-]/[HA]$
$0.95 = \log [A^-]/[HA]$
$8.9 = [A^-]/[HA]$

D. Write out the amino acid sequence of the following peptide. Write the complete name for each amino acid.

NCIS asparagine-cysteine-isoleucine-serine

E. Circle the N-terminal amino acid of the peptide in part D. asparagine

F. Which amino acid side chains in the peptide in part D can form hydrogen bonds?

asparagine, serine

2.(8pts) Name the technique (write out the complete name) which can be used to

A. determine the primary structure of a protein Edman degradation

B. separate myglobin (molecular weight 16,700 daltons) from hemoglobin (molecular weight 64,500 daltons), keeping them in their native, active form

size-exclusion chromatography

C. get a good estimate of the molecular weight of the subunits of a protein

sodium dodecyl sulfate polyacrylamide gel electrophoresis

D. carry out a one-step purification of a His-tagged protein

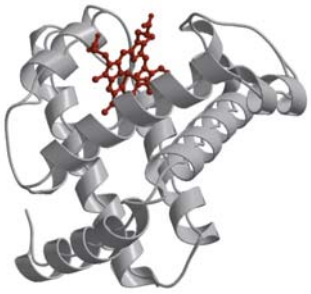
affinity chromatography

3.(8pts) At right is a ribbon diagram of myoglobin.

A. Name the prosthetic group in myoglobin heme or iron protophorphyrin IX

B. Name and **define** the type of secondary structure in myoglobin.

α helix-tightly coiled structure stabilized by hydrogen bonds between main chain carbonyl O and main chain N-H



4.(6pts) Fill in the blanks:

A. The structure on the antigen to which an antibody binds is called the

epitope

B. The structure on the antibody which binds to the antigen is called the

complementarity determining region

C. The tertiary structural motif found in antibodies is called the

immunoglobulin fold

5.(10pts) Discuss the importance of quaternary structure in the physiological function of hemoglobin. Include in your answer the specific structures involved in cooperative O_2 binding and the names of the 3 heterotropic allosteric inhibitors of O_2 binding and how they affect the structure.

Hemoglobin has 4 subunits, each with an O_2- binding heme prosthetic group. Electrostatic interactions in the structure of deoxyhemoglobin hold it in a tense T state that has low affinity for O_2. These quaternary structure interactions at the subunit interfaces pull on the F helix of each subunit so that the heme Fe is out of the plane of the porphyrin. This decreases the affinity for O_2 at the hemes. When O_2 binds to one heme, this pulls the Fe into the heme plane. This in turn pulls on the F helix, and breaks the electrostatic bonds at the subunit interfaces, increasing the affinity for O_2 at the other hemes. Hemoglobin switches to the high affinity R state at high O_2 concentrations. The change in quaternary structure allows hemoglobin to efficiently take up O_2 at high concentrations in the lungs and release it at low O_2 concentrations in peripheral tissues.

The three heterotropic allosteric effectors of hemoglobin are H^+, CO_2, and 2,3-bisphosphoglycerate (BPG). All three decrease the affinity of hemoglobin for O_2 by forming electrostatic interactions that stabilize the T state. H^+ protonates histidines, CO_2 forms carbamates on amino terminal residues, and BPG binds to a pocket of positively charged amino acids on the β chains.

6.(8pts) A. Given the information below, calculate $\Delta G'^{o}$ for the reaction. SHOW YOUR CALCULATIONS.

dihydroxyacetone phosphate ↔ glyceraldehyde 3-phosphate

$K'_{eq} = 0.0475$
$R = 8.315 \times 10^{-3}$ kJ/degree-mole
$T = 310\ ^{o}K$

$\Delta G'^{o} = - RT \ln K'_{eq}$
$\Delta G'^{o} = - (8.315 \times 10^{-3}\ kJ/degree\text{-}mole)(310\ ^{o}K) \ln(0.0475)$
$\Delta G'^{o} = 7.9\ kJ/mole$

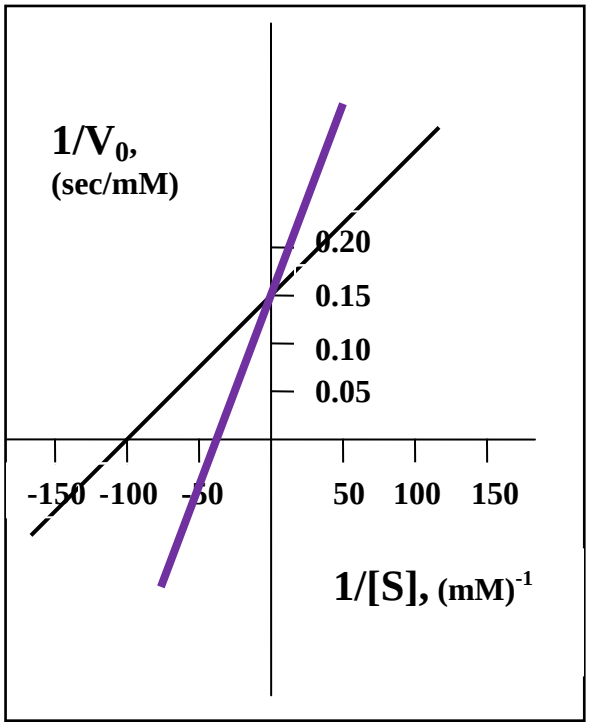
B. Under standard conditions, this reaction (CIRCLE ONE):

WILL PROCEED AS WRITTEN IS AT EQUILIBRIUM **IS UNFAVORABLE**

7.(3pts) A V_0 vs. [S] plot for an allosteric enzyme is typically (CIRCLE ONE):

HYPERBOLIC **SIGMOIDAL** SINUSOIDAL

8.(8pts) Answer the following questions for the graph below. Show your calculations and **include units**.



A. Calculate K_m

$-1/K_m = -100\ (mM)^{-1}$
$K_m = 1/100$ mM
$K_m = 0.01$ mM

B. Calculate V_{max}

$1/V_{max} = 0.15$ sec/mM
$V_{max} = 1/0.15$ mM/sec
$V_{max} = 6.7$ mM/sec

C. If the total enzyme concentration in this assay is 5 μM, what is k_{cat}?

$V_{max} = k_{cat}\ [E_t]$
$k_{cat} = V_{max}/[E_t]$
$k_{cat} = (6.7 \times 10^{-3}\ M/sec)/(5 \times 10^{-6}\ M)$
$k_{cat} = 1300\ sec^{-1}$

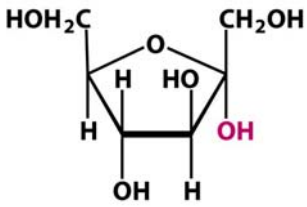
D. Draw a line on the graph to indicate the expected kinetics in the presence of a competitive inhibitor.

9.(9pts) For each of the following methods of regulating enzyme activity, write a definition and give a specific example of an enzyme regulated that way.

A. allosteric- an enzyme with multiple subunits that exists in a tense, low affinity state for substrate (T state) and a relaxed, high affinity state for substrate such that exhibits cooperative substrate binding. Allosteric effectors bind at sites other than the active sites to influence the equilibrium between the R and T states. Aspartate transcarbamoylase is an example.

B. proteolytic activation- conversion of an enzyme from an inactive precursor to an active enzyme by hydrolyzing a peptide bond. An example is the conversion of chymotrypsinogen to chymotrypsin.

C. reversible covalent modification-covalent addition of a group to an enzyme to change its activity. An example is phosphorylation of glycogen phosphorylase



10.(14pts) Fill in the blanks.

A. The carbohydrate shown at right is fructose

B. Lactose is composed of the monosaccharides

glucose and galactose

C. The configuration of the O-glycosidic bond in cellulose is β1-4

D. The enzymes that attach carbohydrate residues to glycoproteins, for example to make the A, B, and O blood types, are called

glycosyltransferases

E. N-linked sugars are attached to the amino acid asparagine

F. Influenza virus hemagglutinin binds to negatively charged modified carbohydrate residues called

sialic acid

11.(6pts) Define:

A. β-mercaptoethanol-thiol reagent that can reduced cystine to make 2 cysteines; reduces disulfide bonds

B. peptide bond-bond between the carbonyl group of one amino acid and the amine group of a second one; links amino acids together to make proteins.