Chem 4540 Enzymology Winter 2005
Problem Set #1

1. The taste of freshly picked Ontario corn is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet because about 50% of the free sugar is converted into starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water. Corn processed in this way and stored in a freezer maintains its sweetness. What is the biochemical basis for this procedure?

2. To approximate the actual concentration of enzymes in a bacterial cell, assume that the cell contains equal concentrations of 1,000 different enzymes in solution in the cytoplasm and that each protein has a molecular weight of 100,000. Assume also that the bacterial cell is a cylinder (diameter 1.0 μm, height 2.0 μm), that the cytoplasm (specific gravity 1.20) is 20% soluble protein by weight, and that the soluble protein consists entirely of enzymes. Calculate the *average* molar concentration of each enzyme in this hypothetical cell.

3. The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20 °C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5 min at 20 °C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

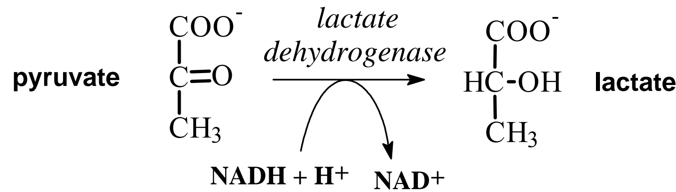
4. When enzyme solutions are heated, there is a progressive loss of catalytic activity over time due to denaturation of the enzyme. A solution of the enzyme hexokinase incubated at 45°C lost 50% of its activity in 12 min, but when incubated at 45°C in the presence of a very large concentration of one of its substrates, it lost only 3% of its activity in 12 min. Suggest why thermal denaturation of hexokinase was retarded in the presence of one of its substrates.

5. Carboxypeptidase, which sequentially removes carboxyl-terminal amino acid residues from its peptide substrates, is a single polypeptide of 307 amino acids. The two essential catalytic groups in the active site are furnished by Arg^{145} and Glu^{270}.

    (a) If the carboxypeptidase chain were a perfect α-helix, how far apart (in Ångstroms) would Arg^{145} and Glu^{270} be? (Recall that there are 3.6 residues in one α-helical turn with a translation distance of 5.4 Å).

    (b) Explain how two amino acids separated by this distance can catalyze a reaction occurring in the space of a few Ångstroms.

6. The muscle enzyme lactate dehydrogenase catalyzes the reaction:



NADH and NAD^+ are the reduced and oxidized forms, respectively, of the coenzyme NAD^+. Solutions of NADH, but not NAD^+, absorb light at 340 nm. This property is used to determine the concentration of NADH in solution by measuring spectrophotometrically the amount of light absorbed at 340 nm by the solution. Explain how these properties of NADH can be used to design a quantitative assay for lactate dehydrogenase.

7. A cell-free extract of *E. coli* contains 24 mg protein per mL. Twenty µL of this extract in a standard incubation volume of 0.1 mL catalyzed the incorporation of [^{14}C]-glucose from [^{14}C]-glucose-phosphate into glycogen at a rate of 1.6 nmol/min. Calculate the velocity of the reaction in terms of: (a) µmoles/min, (b) µmoles/L-min, (c) µmoles/mg protein-min. Also calculate the synthase activity of the extract in terms of (d) units/mL and (e) units/mg protein.

8. Fifty mL of the cell-free extract described in Question #7 (above) was fractionated by ammonium sulfate precipitation. The fraction precipitating between 30% and 50% saturation was redissolved in a total volume of 10 mL and dialyzed. The solution after dialysis occupied 12 mL and contained 30 mg protein/mL. Twenty µL of the purified fraction catalyzed the glycogen synthase reaction at a rate of 5.9 nmoles/min. Calculate (a) the recovery of the enzyme and (b) the degree of purification obtained in the ammonium sulfate step.

9. A 1% (w/v) solution of starch at pH 6.7 is digested by 15 µg of β-amylase (MW 152,000). The rate of maltose (MW 342) liberation was determined to have a maximal initial velocity of 8.5 mg formed per minute. What is the specific activity in units per mg of the enzyme β-amylase? (Note: for every maltose residue liberated, one glucosidic bond in the starch molecule is hydrolyzed). What is the specific activity in katal per kg enzyme?

10. You are given an unknown enzyme and told that it is 70% protein and possesses phosphohydrolase activity. The dried enzyme material is sealed in a small glass ampoule. You reconstitute the sample by adding 3 mL of 0.1 M Tris buffer pH 7.5. From this stock solution you remove 12.5 µL and dilute with Tris buffer to 1 mL. You pipet 5.5 µL from this secondary stock solution into your assay tubes (n=3) and find that this amount of enzyme yields 5.5, 5.9, and 5.75 µg of dephosphorylated product, p-nitrophenol (MW 139.11) in a final reaction volume of 2 mL. The time for each assay was 125 sec. You perform a Bradford protein assay of the secondary stock solution and determine that the concentration is 0.23 mg/mL. Calculate the average specific activity for the phosphohydrolase activity in Units/mg and katal/kg for the original enzyme stock.