Problem Set #5
BMB 401 Spring 2004

Problems from Lehninger: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,

Problem 1 – The k_{cat} for alkaline phosphatase-catalyzed hydrolysis of methylphosphate is approximately 14 sec^{-1} at pH 8 and 25°C. The rate constant for the uncatalyzed hydrolysis of methylphosphate under the same conditions is approximately 1 x 10^{-15} sec. What is the difference in the free energies of activation of these two reactions?

Problem 2 – For a Michaelis–Menten reaction (shown below), k_1 = 7 x 10^7 M^{-1} sec^{-1}, k_{-1} = 1 x 10^3 sec^{-1}, and k_2 = 2 x 10^4 sec^{-1}. What are the values of K_s and K_M? Does substrate binding approach equilibrium or does it behave more like a steady-state system?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Problem 3 – The following kinetic data were obtained for an enzyme in the absence of any inhibitor (**1**), and in the presence of two different inhibitors (**2**) and (**3**) at 5 mM concentration. Assume [E_T] is the same in each experiment.

[S] (mM)	(**1**) ν (μmol mL^{-1} sec^{-1})	(**2**) ν (μmol mL^{-1} sec^{-1})	(**3**) ν (μmol mL^{-1} sec^{-1})
1	12	4.3	5.5
2	20	8	9
4	29	14	13
8	35	21	16
12	40	26	18

a. Determine V_{max} and K_M for the enzyme.
b. Determine the type of inhibition and the K_i for each inhibitor.

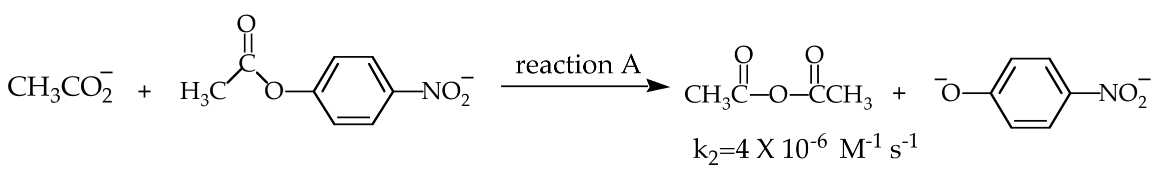
Problem 4 – The reaction of acetate anion and *p*-nitrophenolacetate to produce acetic anhydride and *p*-nitrophenolate (reaction A) proceeds with a rate constant of 4 x 10^{-6} M^{-1} s^{-1}. The analogous intramolecular acyl transfer reaction (reaction B) proceeds with a rate constant of 0.8 s^{-1}.

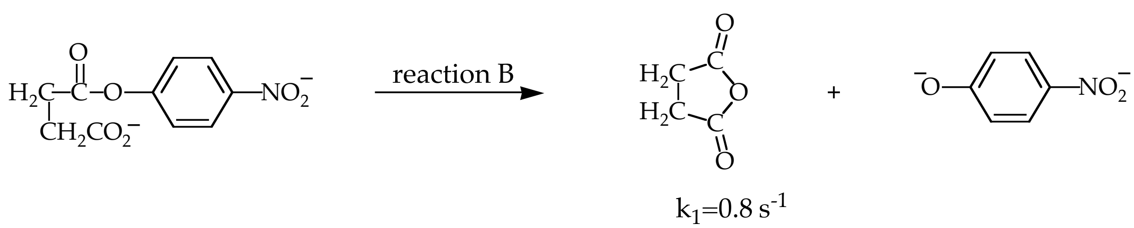
a. Write mechanisms for each of these reactions.

b. The rate of the reaction is a function of the concentration of the CH_3COO^- species. Sketch out the appropriate pH rate profile for reaction A, beginning at pH 2.

c. What are the orders of the rate constants in reactions A and B?

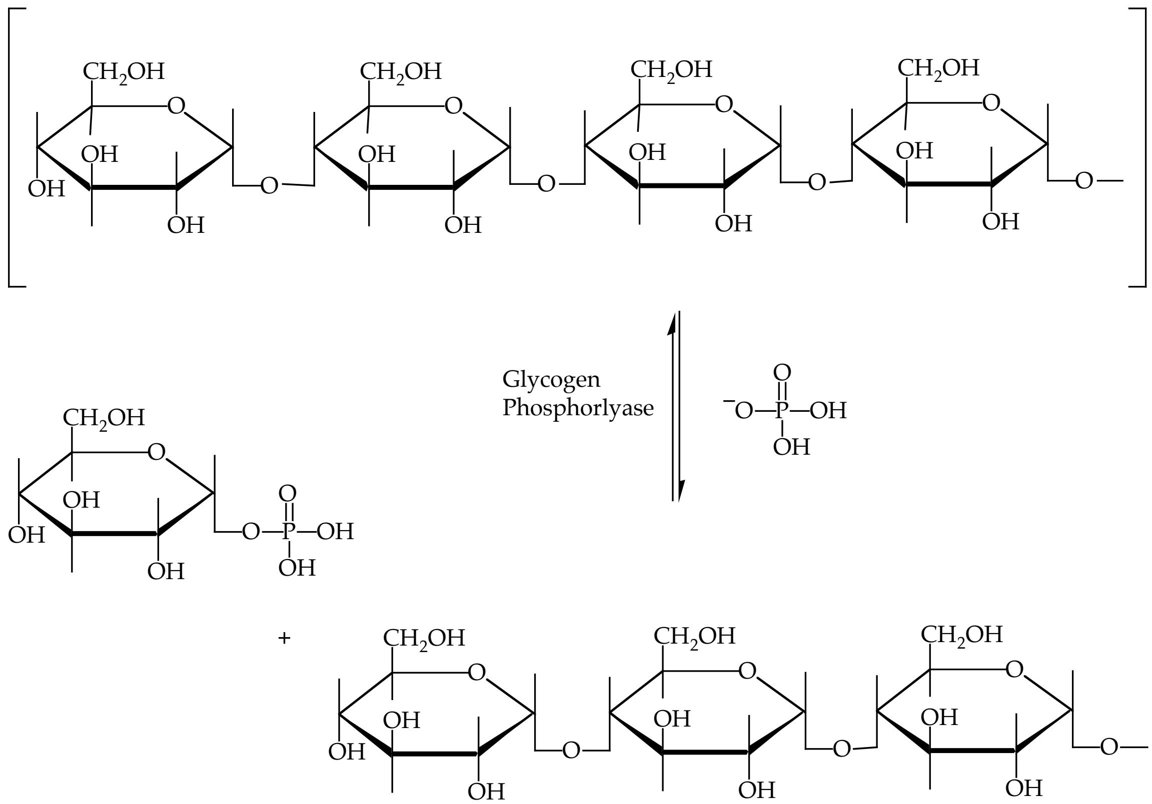
d. What is the effective concentration of $–CO_2^-$ in reaction B?





Problem 5 – The enzyme, thermolysin, is a zinc-dependent metalloprotease that does **not** use covalent catalysis in its reaction mechanism. Other than the zinc ion, a deprotonated glutamic acid and a protonated histidine are known to participate in catalysis. Sketch out a plausible reaction mechanism for thermolysin using a generic peptide. Clearly show the roles of the zinc ion and the active-site amino acids.

Problem 6 – Glycogen phosphorylase catalyzes the cleavage of glucose units from the nonreducing ends of glycogen molecules. The reaction is a phosphorolysis rather than a hydrolysis, since phosphate rather than water is the acceptor. Based on what you've learned from the mechanism of lysozyme, propose a plausible reaction mechanism for glycogen phosphorylase. How might any high-energy intermediates be stabilized?



Problem 7 – An enzyme that catalyzes a unimolecular reaction with a K_M of 2.4×10^{-4} M was assayed at the following substrate concentrations (a) 2×10^{-7} M, (b) 6.3×10^{-5} M, (c) 1×10^{-4} M, (d) 2×10^{-3} M, and (e) 0.05 M. The velocity observed at 0.05 M was 128 nM min^{-1}. Calculate the initial velocities at the other substrate concentrations.

Problem 8 – The following table indicates the rates at which a substrate reacts as catalyzed by an enzyme that follows the Michaelis–Menten mechanism: (1) in the absence of inhibitor; (2) and (3) in the presence of 10 mM concentration, respectively, of each of two different inhibitors. Assume $[E_T]$ is the same for all reactions.

a. Determine K_M and V_{max} for the enzyme.

b. For each of the two inhibitors, determine what type of inhibition is seen.

c. What additional information would be necessary to determine the turnover number (k_{cat}) for the enzyme?

[substrate] (mM)	rate (μM • s^{-1}) (1)	rate (μM • s^{-1}) (2)	rate (μM • s^{-1}) (3)
1	2.5	1.17	0.77
2	4.0	2.10	1.25
5	6.3	4.00	2.00
10	7.6	5.7	2.50
20	9.0	7.2	2.86

Problem 9 – An enzyme has a K_M of 4.7 x 10^{-5} M. If the V_{max} of the preparation is 22 μM min^{-1}, what velocity would be observed in the presence of 2 x 10^{-4} M substrate and 5 x 10^{-4} M of (a) a competitive inhibitor, (b) a noncompetitive inhibitor. K_i in both cases is 3 x 10^{-4} M.

Problem 10 – In solution chemistry, the decarboxylation of acetoacetate is facilitated by aniline. Write a detailed chemical mechanism for this reaction, showing clearly how the aniline functions.