

Professor Charles McKenna

NAME: _____

Chemistry 519, Spring 1996

Monday, March 4, 1996 11:00 a.m.

Due: Monday, March 18, 1996 11:00 a.m.

Midterm
Take-Home Exam

GOOD LUCK!

PROBLEM	POSSIBLE POINTS	SCORE
1	30	
2	30	
3	30	
4	30	
5	30	
TOTAL	150	

- 1a) A peptide glu-ser-gly-arg hydrochloride is titrated in H₂O with KOH.
- (i) Construct the titration curve for intervals of 0.5 eq. OH⁻ from pH = 1.5 (0 eq.) through pH = 13.
 - (ii) Identify the main species present at each inflection point.
 - (iii) What is the pK_I of this peptide?
 - (iv) If the Glu is changed to Gln, will the peptide mobility in an electrophoretic gel at pH = 10 increase or decrease (cathodic start point)? Would it be the same as at pH = 3? Why?
- 2a) The ATP/ADP ratio in an actively respiring yeast cell is about 10. What should the intracellular 3-phosphoglycerate/1,3-diphosphoglycerate ratio be to make the phosphoglycerate kinase reaction thermodynamically favorable in the direction of 1,3-diphosphoglycerate synthesis? Show your work.
- 2b) Write the spontaneous reaction that occurs and calculate the ΔG of the reaction when the enzyme lactic dehydrogenase is added to a solution containing pyruvate, lactate, NAD⁺, and NADH at the following concentration ratios:
- (i) lactate/pyruvate = 159, NAD⁺/NADH = 159
 - (ii) lactate/pyruvate = 1000, NAD⁺/NADH = 1000
 - (iii) Conclusion?
- 3a) The following data (Table 1) were obtained for an enzyme that catalyzes the reaction S → P. The substrate concentrations have been spaced to allow use of any of the linear plots.
- (i) Plot the data and determine K_m and V_{max} using an Eadie plot.
 - (ii) Show the plot for a competitive inhibitor I, [I] = K_i = 4.00 × 10⁻⁵ M. Assume partial competitive inhibition and K_m = K_s; with $\alpha = 5$, add the plot for [i] = 1 M.

Table 1

[S] (M)	v nmoles x liter ⁻¹ x min
8.33 x 10 ⁻⁶	13.8
1.00 x 10 ⁻⁵	16.0
1.25 x 10 ⁻⁵	19.0
1.67 x 10 ⁻⁵	23.6
2.00 x 10 ⁻⁵	26.7
2.50 x 10 ⁻⁵	30.8
3.33 x 10 ⁻⁵	36.3
4.00 x 10 ⁻⁵	40.0
5.00 x 10 ⁻⁵	44.4
6.00 x 10 ⁻⁵	48.0
8.00 x 10 ⁻⁵	53.4
1.00 x 10 ⁻⁴	57.1
2.00 x 10 ⁻⁴	66.7

- b) Using the equilibrium (Ks) scheme discussed in class, analyze the following situation: $K_1 < 1$, $K_2 < 1$, $K_3 < 1$. (construct L-B plot).
- 4a) Stryer, p.73 #1; explain / illustrate chemistry involved for each answer.
- 4b) List factors contributing to the catalytic power and specificity of enzymes, giving particular examples for each factor.
- 5 The nonspecific enzyme alkaline phosphatase shows an alkaline pH optimum for V_{max} (1) and is thus distinguished from a phosphomonoesterase activity. The enzyme is a zinc-containing dimer of 86,000 mol wt. Dialysis to remove the zinc abolishes activity, addition of Zn restores activity (2). ROPO₂³⁻ species are hydrolyzed to ROH+Pi by the E. coli alkaline phosphatase 10⁹ to 10¹¹ times faster than in the corresponding nonenzymatic hydrolysis. Incubation of the enzyme with either [32P]-Pi or various [32P]-ROPO₂³⁻ substrates at low pH leads to radiolabeled enzyme (3). Acid hydrolysis of the phosphoprotein yields a single phosphopeptide that is further degraded in acid to phosphoserine (4). A curious observation is that, although one can isolate the ³²P-serine enzyme at pH 4, one finds only

2% of this form at pH 7 (and even less at pH 8) on rapid quench into acid for isolation (5). The V_{max} for many substrates of structure $ROPO_3^{2-}$ or $RSPO_3^{2-}$ is identical (6). Transphosphorylation to an acceptor other than water is detectable; for example, the hydroxyl group of ethanolamine reacts with the phosphoryl enzyme to generate O-phosphoethanolamine ($^3-O_3POCH_2CH_2NH_3^+$). Table 2 below summarizes the data observed for the hydrolysis reaction $ROPO_3^{2-} \rightarrow P_i + ROH$ (7).

Table 2

Ethanolamine (nM)	ROH formed ($\mu\text{M min}^{-1} \text{mg}^{-1}$)	Inorganic phosphate formed ($\mu\text{M min}^{-1} \text{mg}^{-1}$)
0.0	0.86	0.87
0.114	0.96	0.85
0.343	1.19	0.86
0.572	1.35	0.86

With the use of p-nitrophenyl phosphate (PNP-phosphate) as substrate, a burst of p-nitrophenolate ion is seen at low pH (5.5), but not at high pH (such as pH 8, which is the pH optimum) (8). Phosphonates $RCH_2PO_3^{2-}$ are inhibitors but not substrates of phosphatase (9).

Devise or demonstrate a mechanism for alkaline phosphatase catalysis. Explain how your mechanism accounts for each of the observations (1) — (9). In the case of the serine and the Zn, discuss additional factors which might influence their contributions, if any, to the catalysis. Could the role of serine be tested stereochemically? Is PIX a relevant phenomenon? Why or why not? Are the data presented the whole story?