

Professor Charles McKenna
Chemistry 519, Spring 2001
Monday, February 26, 2001 11:00 a.m.

NAME: _____

**Midterm
Take-Home Exam**

GOOD LUCK!

PROBLEM	POSSIBLE POINTS	SCORE
1	20	
2	15	
3	15	
4	20	
5	10	
6	30	
7	15	
8	10	
9	15	
TOTAL	150	

- 1 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, $\text{NAD}^+/\text{NADH} = 159$
 - (ii) lactate/pyruvate = 1000, $\text{NAD}^+/\text{NADH} = 1000$
 - (iii) Conclusion?
 - (iv) What is the ox-red potential of this solution before, and after the enzyme is added?

- 2 Using the equilibrium (Ks) scheme discussed in class, analyze the following enzyme kinetics situation: $\alpha < 1$, $\beta < 1$, $\beta < \alpha$. (construct L-B plot).

- 3 List factors contributing to the catalytic power and specificity of enzymes, giving particular examples not used in the lecture for each factor.

- 4 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). ROPO_2^{3-} species are hydrolyzed to $\text{ROH} + \text{Pi}$ by the *E. coli* alkaline phosphatase 10^9 to 10^{11} times faster than in the corresponding nonenzymatic hydrolysis. Incubation of the enzyme with either $[\text{32P}]\text{-P}_i$ or various $[\text{32P}]\text{-ROPO}_2^{3-}$ 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 ^{32}P -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 ($^3\text{-O}_3\text{POCH}_2\text{CH}_2\text{NH}_3^+$). Table 2 below

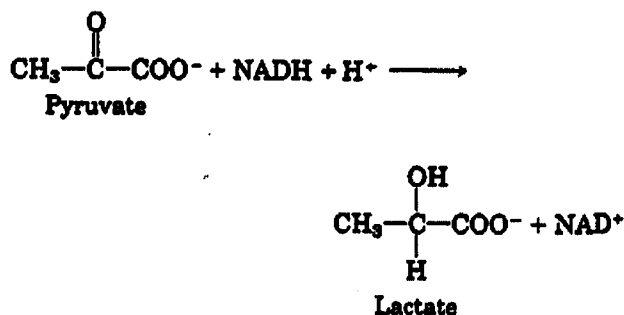
summarizes the data observed for the hydrolysis reaction $\text{ROPO}_3^{2-} \rightarrow \text{P}_i + \text{ROH}$ (7).

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 $\text{RCH}_2\text{PO}_3^{2-}$ 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. 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?

5. The 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.

6. Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. Prostaglandins are responsible for producing fever and inflammation and its associated pain. They are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme, a cyclooxygenase, uses oxygen to convert arachidonic acid to PGG₂, the immediate precursor of many different prostaglandins.

- (a) The kinetic data given below are for the reaction catalyzed by prostaglandin endoperoxide synthase. Focusing here on the first two columns, determine the V_{\max} and K_m of the enzyme, using the Hanes-Wolff graphical method.

[Arachidonic acid] (mM)	Rate of formation of PGG ₂ (mM/min)	Rate of formation of PGG ₂ with 10 mg/mL ibuprofen (mM/min)
0.5	23.5	16.67
1.0	32.2	25.25
1.5	36.9	30.49
2.5	41.8	37.04
3.5	44.0	38.91

- (b) Ibuprofen is an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. Using the data in the first and third columns of the table, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase (Eadie-Hofstee method).

7. The following experimental data were collected during a study of the catalytic activity of an intestinal peptidase with the substrate glycylglycine:



[S] (mM)	Product formed ($\mu\text{mol}/\text{min}$)
1.5	0.21
2.0	0.24
3.0	0.28
4.0	0.33
8.0	0.40
16.0	0.45

Use graphical analysis (L-B) to determine the V_{max} and K_m for this enzyme preparation and substrate.

8. Derive a steady state Michaelis-Menten equation for pure uncompetitive inhibition.
9. Explain supersecondary structure and give 3 examples of common protein motifs.