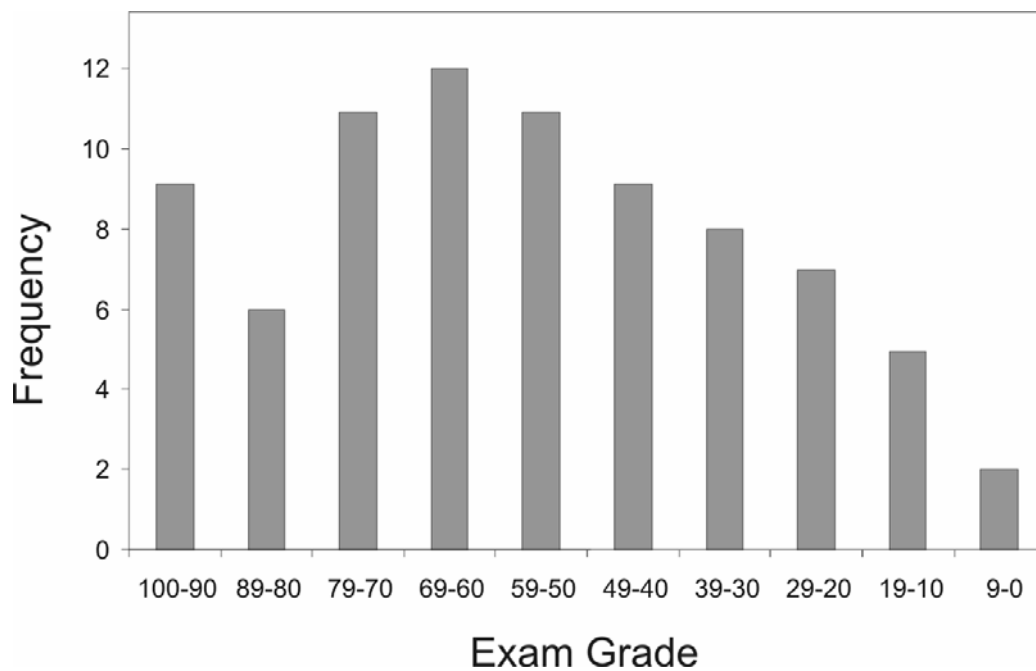
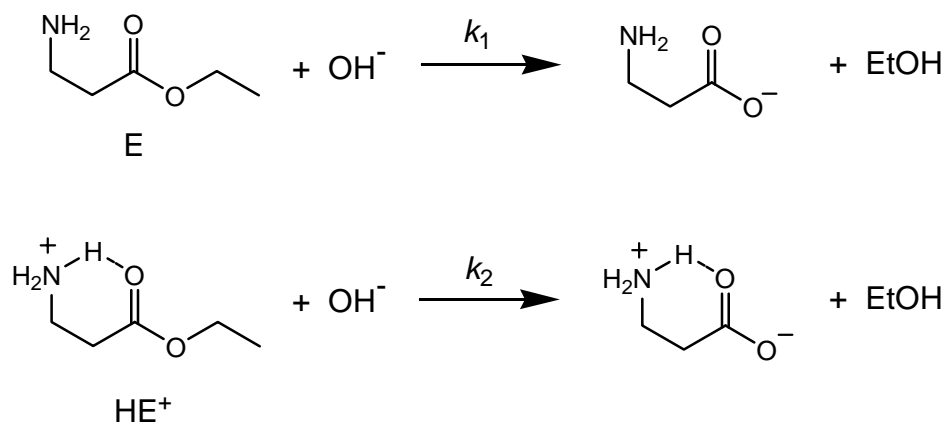


**Exam 1
Answer Key**

Exam 1 Mean: 52
Exam 1 Median: 50
Exam 1 St. Dev.: 28



1. The cleavage of γ -amino esters in aqueous base can take place either through the unprotonated (E) or protonated (HE^+) form of the ester.



Both of these are elementary reactions, and rates of each relate to the rate constants and reactant concentrations in the usual way. As you might imagine, the concentration of HE^+ is low under basic conditions; for the reactants shown above, the equilibrium constant

$$K_b = \frac{[\text{HE}^+][\text{OH}^-]}{[\text{E}]} = 5.01 \times 10^{-7} \text{ M}.$$

As a result, $[\text{E}] \gg [\text{HE}^+]$. However, if $k_2 \gg k_1$, then the second mechanism could still predominate.

- a. (25 pts) Kinetics experiments show that, as long as OH^- is in vast excess (which is usually true under basic conditions), the disappearance of E is pseudo-first order in the total concentration of E:

$$-\frac{\partial[\text{E}]_{\text{tot}}}{\partial t} = k_{\text{obs}}[\text{E}]_{\text{tot}},$$

where $[\text{E}]_{\text{tot}} = [\text{E}] + [\text{HE}^+] \approx [\text{E}]$. (This assumption must be made because neither $[\text{E}]$ nor $[\text{HE}^+]$ can be measured directly.) **Derive a rate expression for $-\partial[\text{E}]_{\text{tot}}/\partial t$ in terms of measurable concentrations, known constants and the individual rate constants k_1 and k_2 .** Do not use $[\text{E}]$ or $[\text{HE}^+]$ in your expression, and make sure your answer is consistent with the pseudo-first-order kinetics described above. (Write your answer on the next page.)

(answer, part a)

$$\begin{aligned} \frac{-d[E]_{\text{tot}}}{dt} &= \text{SUM OF ALL REACTION RATES THAT} \\ &\quad \text{CONSUME "E" SPECIES} \\ &= k_1[E][OH^-] + k_2[HE^+][OH^-]. \end{aligned}$$

$$[HE^+][OH^-] = K_b[E], \text{ so}$$

$$\frac{-d[E]_{\text{tot}}}{dt} = k_1[E][OH^-] + k_2K_b[E].$$

$$[E] = [E]_{\text{tot}} - [HE^+] \approx [E]_{\text{tot}}; \text{ substituting,}$$

$$\begin{aligned} \frac{-d[E]_{\text{tot}}}{dt} &= k_1[E]_{\text{tot}}[OH^-] + k_2K_b[E]_{\text{tot}} \\ &= [E]_{\text{tot}}(k_1[OH^-] + k_2K_b) \end{aligned}$$

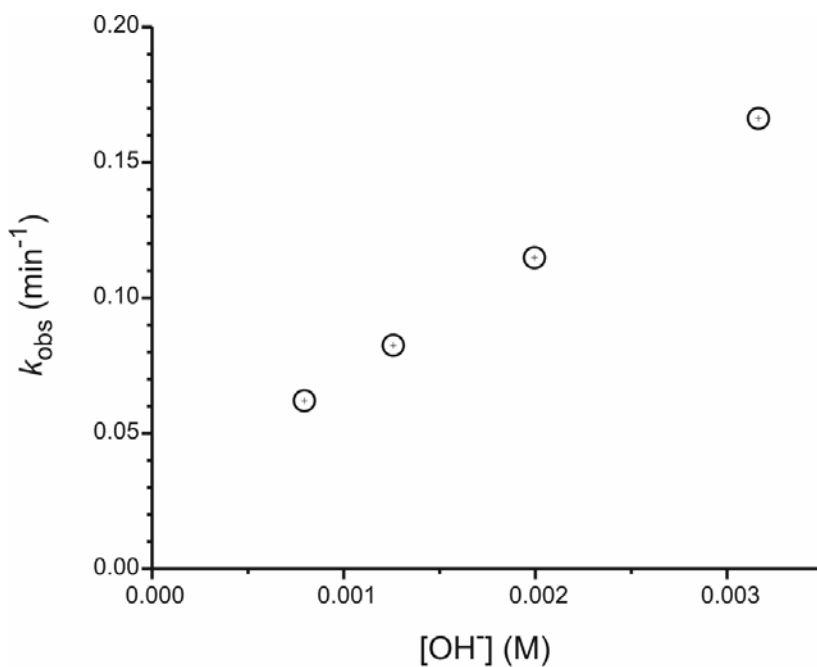
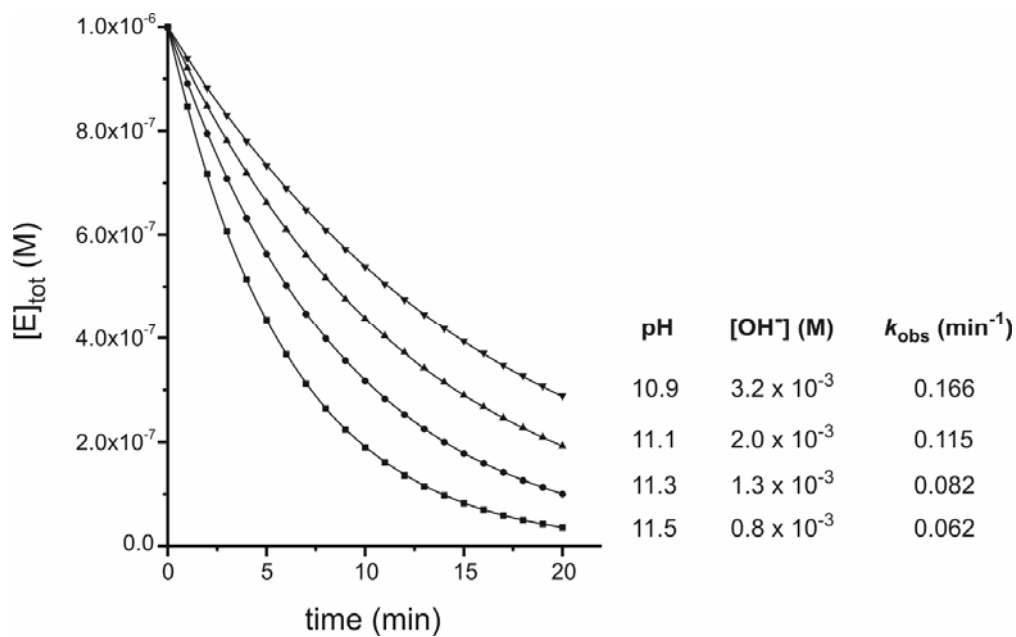
5 POINTS FOR EACH HALF OF INITIAL k_1, k_2
COMPONENT EQN (10 TOTAL);

5 POINTS FOR ANY EQUATION IN TERMS ONLY OF
 $[OH^-], k_1, k_2, K_b$ &/OR $[E]_{\text{tot}}$

10 POINTS FOR CORRECT EQN AT END;

5 POINTS FOR ANSWER THAT HAS MINOR ERROR

- b. (10 pts) A series of measurements were made in which pseudo-first-order rate constants k_{obs} were evaluated at different pH's (and thus different concentrations $[\text{OH}^-]$). The decays are shown in the graph below, and the evaluated k_{obs} values are plotted vs. $[\text{OH}^-]$ in the following graph.



From these graphs, estimate k_1 and k_2 . Include units in your answer. You might solve this problem by drawing a function onto the plotted points, or by setting up your rate expression for two of the points as a set of simultaneous equations.

$k_1 =$

$$47 \text{ M}^{-1} \text{ min}^{-1}$$

$k_2 =$

$$6 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$$

5 POINTS EACH.

FULL CREDIT FOR

ANSWERS CONSISTENT

WITH (a), AS

LONG AS RELATIONSHIP

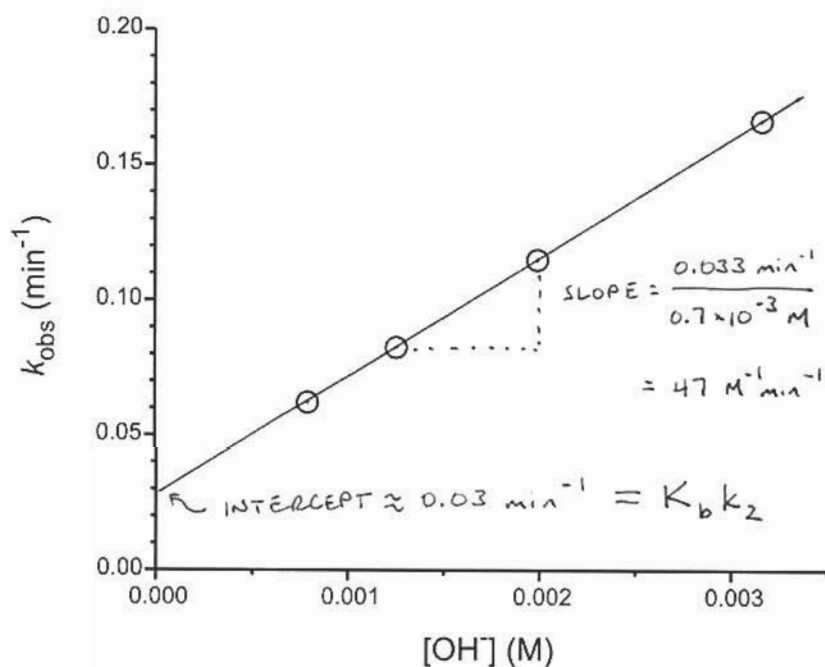
BETWEEN $[\text{OH}^-]$ AND

k_{obs} WAS DERIVED.

(put any work you'd like us to see below, or on graphs on previous page)

FROM PART (a), $k_{\text{obs}} = k_1 [\text{OH}^-] + k_2 K_b$

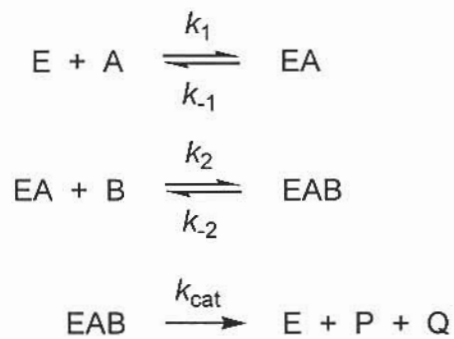
SO, PLOT OF k_{obs} vs. $[\text{OH}^-]$ SHOULD GIVE LINE w/
SLOPE = k_1 , INTERCEPT = $k_2 K_b$



$$k_2 = \frac{0.03 \text{ min}^{-1}}{5.01 \times 10^{-7} \text{ M}}$$

$$= 6 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$$

2. Glycerol kinase couples the phosphorylation of 1,2-diols into phosphono-1,2-diols with the dephosphorylation of MgATP into MgADP. The enzyme, in other words, converts two substrates into two products. Like many other enzymes that do this, glycerol kinase binds the two substrates sequentially in a specific order:



In this problem, you will determine which of "A" or "B" corresponds to which of 1,2-diol or MgATP.

- a. (10 pts) *For this part only*, assume that EAB is a steady-state intermediate. Write an expression that relates $[EAB]_{ss}$ to any other species in the kinetic scheme above.

$[EAB]_{ss} =$ $\frac{k_2 [EA][B]}{k_{-2} + k_{cat}}$

5 for numerator

5 for denominator

$$[X]_{ss} = \frac{\sum \text{rates that create } X}{\sum k_{obs} \text{ that destroy } X}$$

- b. (25 pts) For the rest of this problem, assume instead that the first two steps of the mechanism are in pre-equilibrium, with $K_A = k_{-1}/k_1$ and $K_{AB} = k_{-2}/k_2$. (These equilibrium constants are the inverse of what you might ordinarily express from the reactions as written.) Using the additional simplification $[E]_{tot} = [E] + [EA] + [EAB]$, derive an expression for the initial rate $\partial[P]/\partial t$ in terms of $[E]_{tot}$, $[A]$, $[B]$, and constants.

5 POINTS FOR
STARTING W/
 $\frac{\partial P}{\partial t} = k_{cat}[EAB]$

5 POINTS FOR
EQUIL. K SUBST.

5 POINTS FOR
USING $[E]_{tot}$
~~IN~~
TO ELIMINATE
VARIABLES

5 POINTS FOR
SOLVING FOR $[EAB]$
CORRECTLY (OR
GOING THROUGH
OTHER ROUTE)

5 POINTS FOR
ANSWER.

$$\frac{\partial [P]}{\partial t} = k_{cat} [EAB]$$

NEED TO CONVERT
[EAB] INTO $[E]_{tot}$
SOMEHOW.

$$K_{AB} = \frac{k_{-2}}{k_2} = \frac{[EA][B]}{[EAB]}$$

$$K_A = \frac{k_{-1}}{k_1} = \frac{[E][A]}{[EA]}$$

$$[EA] = \frac{K_{AB} [EAB]}{[B]}$$

$$[E] = \frac{K_A [EA]}{[A]}$$

$$[E]_{tot} = [E] + [EA] + [EAB]$$

} substitute
for [E]

$$= \frac{K_A [EA]}{[A]} + [EA] + [EAB]$$

$$= \frac{K_A K_{AB} [EAB]}{[A][B]} + \frac{K_{AB} [EAB]}{[B]} + [EAB]$$

$$= \frac{K_A K_{AB} [EAB] + K_{AB} [A][EAB] + [EAB][A][B]}{[A][B]}$$

$$[EAB] = \frac{[E]_{tot} [A][B]}{K_A K_{AB} + K_{AB} [A] + [A][B]}$$

$$\frac{\partial [P]}{\partial t} = k_{cat} [EAB] = \frac{k_{cat} [E]_{tot} [A][B]}{K_A K_{AB} + K_{AB} [A] + [A][B]}$$

- c. (10 pts) Your answer to part (b) should not have been commutative; in other words, if you switched the variables [A] and [B] in your rate equation, you would not get the same equation back. To put it another way, the order of addition of A and B to the enzyme active site matters in this case.

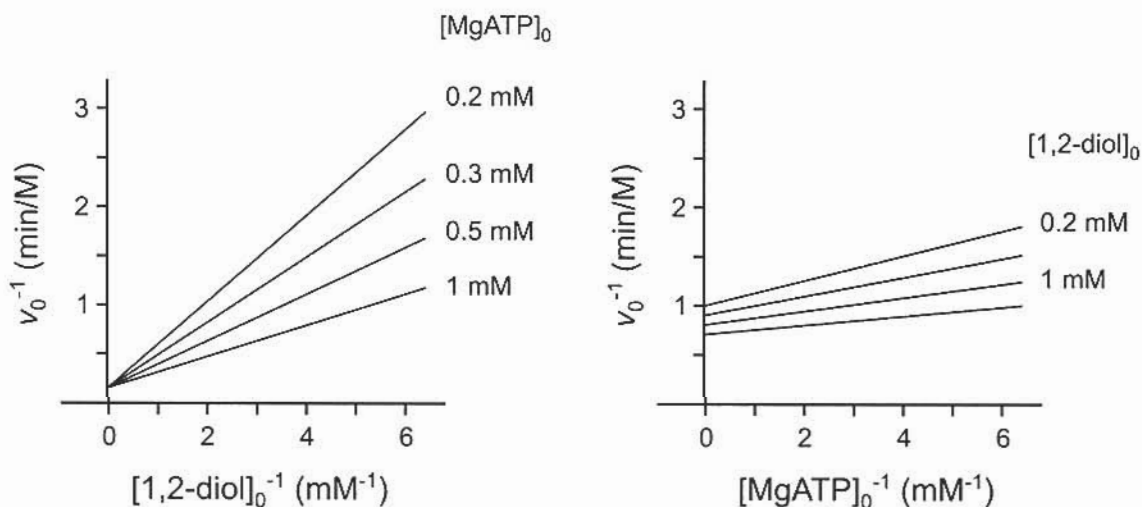
Re-write your answer to part (b) in the form of a Michaelis-Menten expression (as $1/\text{RATE}_0$), using the substitution $V_{\max} = k_{\text{cat}}[E]_{\text{tot}}$.

$$\frac{1}{\frac{\partial [P]}{\partial t}} = \frac{1}{\text{RATE}_0} = \frac{K_A K_{AB} + K_{AB} [A]_0 + [A]_0 [B]_0}{k_{\text{cat}} [E]_{\text{tot}} [A]_0 [B]_0}$$

$$= \frac{K_A K_{AB}}{V_{\max} [A]_0 [B]_0} + \frac{K_{AB}}{V_{\max} [B]_0} + \frac{1}{V_{\max}}$$

5 POINTS FOR CONSISTENT w/ (b) IN ANY FORM
5 POINTS FOR CORRECT FORM

- d. (20 pts) Initial reaction rates were measured for a variety of concentrations [1,2-diol] and [MgATP]. The data could be plotted either as $1/\text{RATE}_0$ vs. $1/[1,2\text{-diol}]$ or as $1/\text{RATE}_0$ vs. $1/[\text{MgATP}]$:



Based on the relative values of $(1/V_{\max})_{\text{app}}$ and $(K_m/V_{\max})_{\text{app}}$ that might be measured from these graphs, **which starting material is A and which is B** according to the reaction scheme at the beginning of the problem? **Why?** (Answer on next page.)

1,2-diol: A or **B** ? (circle one)

5 POINTS FOR (BOTH)
CORRECT CIRCLES.

MgATP: **A** or B ? (circle one)

Explain.

15 POINTS
TOTAL.

5 POINTS FOR
ANY ANSWER
CONCERNING
SLOPE AND
INTERCEPT

5 POINTS FOR
DIFFERENTIATING
[B] AND [A] IN
MICHAELIS-MENTEN
IN TERMS OF
SLOPE & INTERCEPT

GRAPHS OF $\frac{1}{\text{RATE}_0}$ VS. $\frac{1}{[A]_0}$ AND $\frac{1}{\text{RATE}_0}$ VS. $\frac{1}{[B]_0}$,

GIVEN ANSWER TO (C), SHOULD GIVE DIFFERENT SLOPES
& INTERCEPTS.

$$\frac{1}{\text{RATE}_0} = \frac{1}{[A]_0} \left(\frac{K_A K_{AB}}{V_{\max} [B]_0} \right) + \left(\frac{K_{AB}}{V_{\max} [B]_0} + \frac{1}{V_{\max}} \right)$$

slope intercept

$$\frac{1}{\text{RATE}_0} = \frac{1}{[B]_0} \left(\frac{K_A K_{AB}}{V_{\max} [A]_0} + \frac{K_{AB}}{V_{\max}} \right) + \left(\frac{1}{V_{\max}} \right)$$

slope intercept

PLOT OF $\frac{1}{\text{RATE}_0}$ VS. $\frac{1}{[A]_0}$

SHOULD GIVE LOWER SLOPE,
HIGHER INTERCEPT THAT
DEPENDS ON $[B]_0$.

GRAPH ON RIGHT. (A = MgATP)

PLOT OF $\frac{1}{\text{RATE}_0}$ VS. $\frac{1}{[B]_0}$

SHOULD GIVE HIGHER SLOPE,
LOWER INTERCEPT THAT IS
INDEPENDENT OF $[A]_0$ OR $[B]_0$.
GRAPH ON LEFT. (B = 1,2-diol)