

# BMB 401 EXAM 3

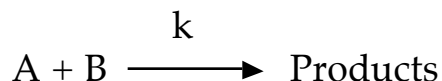
## April 10, 2003

Please write your name on your exam sheet and turn it in with your Scantron sheet!

Do not forget to include your student ID on the Scan Tron Sheet. It is not necessary to include a section number.

People whose last names begin with A–C should report to room 016 Ag Sci.

1. What are the units of the **rate constant** ( $k$ ) for the following elementary step?



- a.  $\text{sec}^{-1}$
  - b.  $\text{M}^{-1} \cdot \text{sec}^{-1}$
  - c.  $\text{M} \cdot \text{sec}^{-1}$
  - d.  $\text{M}^{-2} \cdot \text{sec}^{-1}$
  - e.  $\text{M} \cdot \text{sec}^{-2}$
2. A hypothetical enzyme in which the active site is more complementary to the substrate than the transition state will have which of the following effects on the reaction?
- a. It will increase the rate of the reaction
  - b. It will decrease the rate of the reaction
  - c. It will have no effect on the rate of the reaction
  - d. It will change the equilibrium constant for the reaction
  - e. both b and d

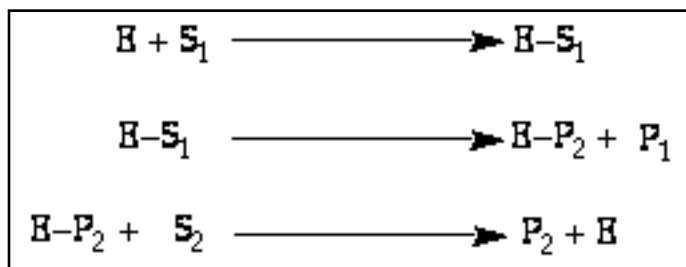
3. Stabilization of the transition state(s) in serine proteases is facilitated by the “oxyanion hole.” What is the oxyanion hole?

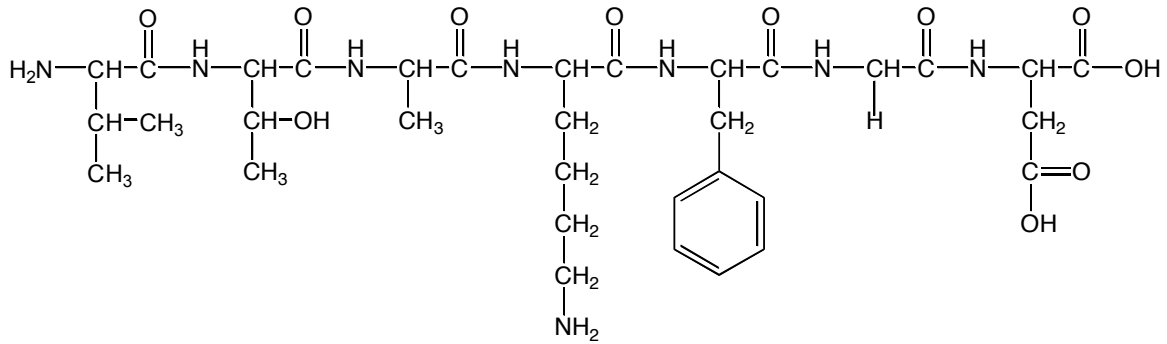
- a. It is a zinc atom.
- b. It is a histidine residue.
- c. It is a lysine residue.
- d. It is a hydrophobic pocket.
- e. It is the backbone amide protons of two amino acid residues.

4. What is the primary advantage of using a Lineweaver-Burk plot (double reciprocal plot) over a standard velocity versus substrate plot?

- a. it gives a more accurate estimation of [S]
- b. it gives a more accurate estimation of  $[E]_T$
- c. it gives a more accurate estimation of free [E]
- d. it gives a more accurate estimation of  $V_{\max}$
- e. it gives a more accurate estimation of [ES]

**For questions 5 through 8.** The mechanism of trypsin, a serine protease, can be described by the three following steps, wherein E stands for free enzyme, S stands for substrate or reactant, and P stands for product.





5. If  $S_1$  is the peptide shown above, then  $P_1$  would be which of the following?

- a. V-T-A-R
- b. Y-G-D
- c. F-G-D
- d. K-F-G-D
- e. V-T-A-K

6. After release of  $P_1$ , but before release of  $P_2$ , to which amino acid on **trypsin** would  $P_2$  be covalent bound.

- a. H
- b. F
- c. K
- d. S
- e. D

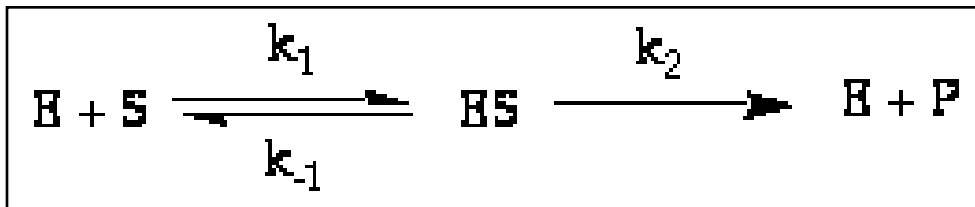
7. If  $S_1$  is the peptide shown above, then  $P_2$  would be which of the following?

- a. V-T-A-K
- b. Y-G-D
- c. F-G-D
- d. K-F-G-D
- e. V-T-A-F

8. If  $S_1$  is the peptide shown above, then  $S_2$  would be which of the following?

- V-T-A-K
- serine
- $\text{NH}_2\text{OH}$  (hydroxylamine)
- $\text{H}_2\text{O}$
- p*-nitrophenylacetate

9. For the kinetic scheme shown below, the steady-state equation for the change in  $[\text{ES}]$  as a function of time ( $d[\text{ES}]/dt$ ) would be equal to which of the following?



- $k_2[\text{ES}]$
- $k_1[\text{E}][\text{S}]$
- $k_2[\text{ES}] - k_{-1}[\text{ES}]$
- $k_1[\text{E}][\text{S}] - (k_2 + k_{-1})[\text{ES}]$
- $k_2[\text{E}][\text{P}] + k_1[\text{E}][\text{S}]$

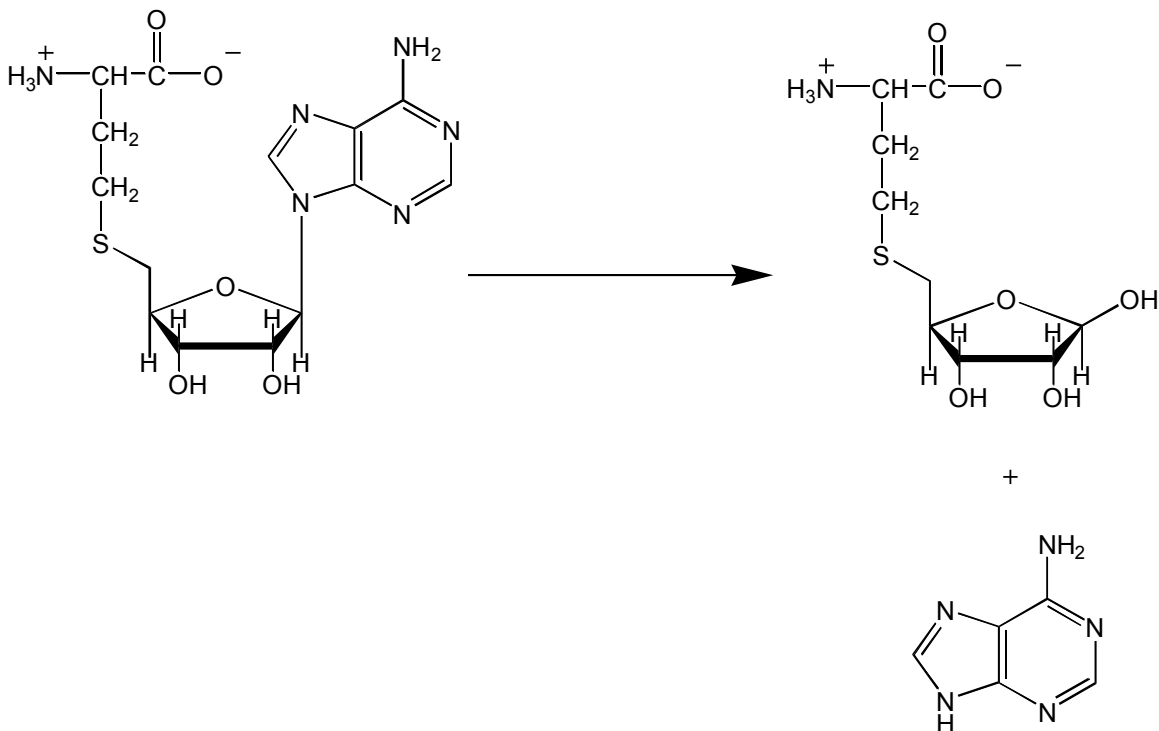
10. What reason **best** explains why the enzymatic activity of lysozyme is more than ten times slower at pH 7.0 than at pH 5.0?

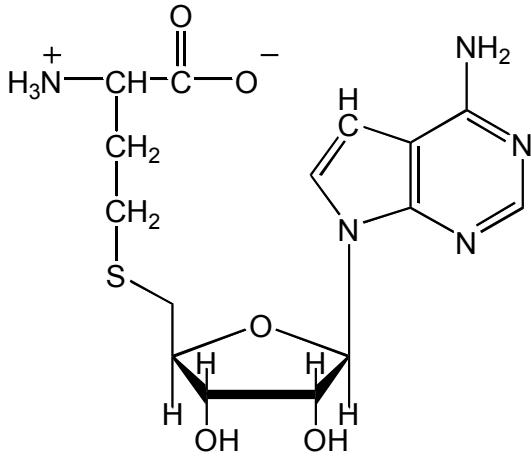
- A histidine, which acts as the nucleophile in the reaction is in the wrong protonation state at pH 7.0.
- An aspartate residue, which stabilizes the transition state of the reaction via electrostatic interactions, is in the wrong protonation state at pH 7.0.
- The substrate no longer binds to the enzyme at pH 7.0.
- A glutamic acid residue, which protonates the leaving group, can no longer function as a general acid catalyst at pH 7.0.
- A serine, which acts as the nucleophile in the reaction is in the wrong protonation state at pH 7.0.

### Questions 11 through 13

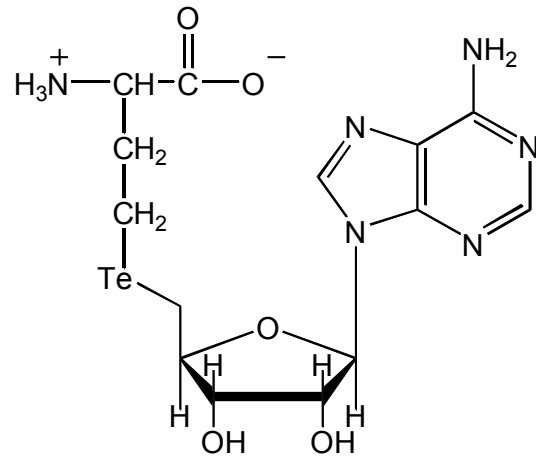
The enzyme S-adenosyl-L-homocysteine (SAH) nucleosidase catalyzes the reaction shown below. David Iwig, a graduate student in my laboratory, recently showed that Te-adenosyl-L-methionine (TeAdoMet, Compound A) is an alternate substrate for the enzyme. When 10  $\mu\text{M}$  SAH nucleosidase was treated with varying concentrations of TeAdoMet, the rate ( $\mu\text{M}\cdot\text{min}^{-1}$ ) as a function of substrate concentration ( $\mu\text{M}$ ) displayed hyperbolic (Michaelis-Menton) kinetics. Lineweaver-Burk analysis of the data produced a straight line, which is described by the equation:  $y = 126.7x + 0.855$

#### SAH Nucleosidase Reaction





Compound B



Compound A

11. What is the maximal velocity of the reaction ( $V_{\max}$ )?
- $126.7 \mu\text{M} \cdot \text{min}^{-1}$
  - $0.00789 \mu\text{M} \cdot \text{min}^{-1}$
  - $0.855 \mu\text{M} \cdot \text{min}^{-1}$
  - $148 \mu\text{M} \cdot \text{min}^{-1}$
  - $1.17 \mu\text{M} \cdot \text{min}^{-1}$
12. What is the  $K_m$  of SAH nucleosidase for TeAdoMet?
- $126.7 \mu\text{M}$
  - $0.00789 \mu\text{M}$
  - $0.855 \mu\text{M}$
  - $148 \mu\text{M}$
  - $1.17 \mu\text{M}$
13. What is the turnover number of SAH nucleosidase for TeAdoMet?
- $14.8 \text{ M}^{-1} \cdot \text{min}^{-1}$
  - $0.117 \text{ M}^{-1} \cdot \text{min}^{-1}$
  - $8.55 \text{ M}^{-1} \cdot \text{min}^{-1}$
  - $14.8 \text{ min}^{-1}$
  - $0.117 \text{ min}^{-1}$

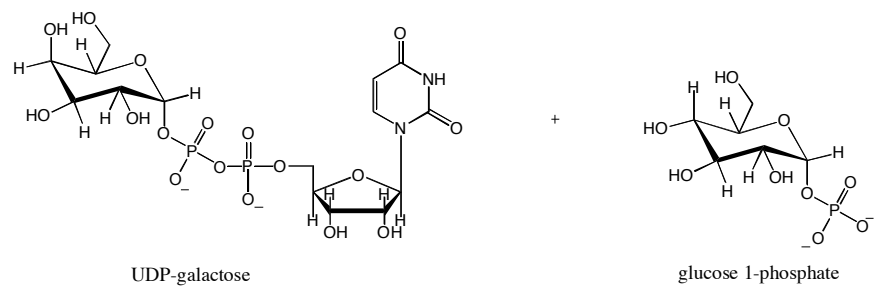
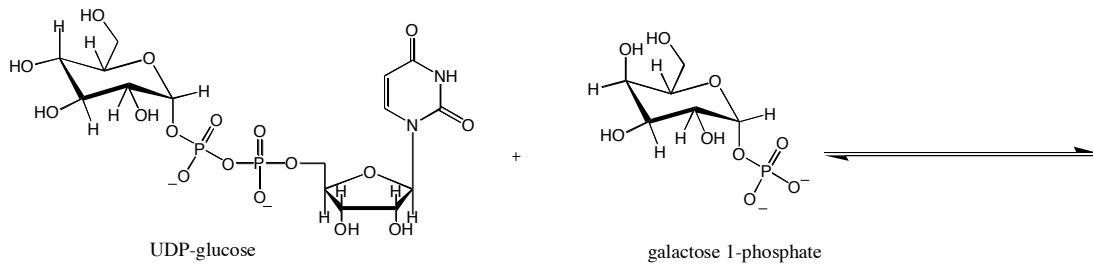
14. In contrast to the normal substrate, SAH nucleosidase cannot cleave Compound B to give the two products, because of a simple substitution of a carbon atom for a nitrogen atom in its adenine ring. What effect would the presence of Compound B would produce on the reaction with respect to the normal substrate?

- a. It would behave as an uncompetitive inhibitor.
- b. It would behave as a non-competitive inhibitor.
- c. It would behave as a competitive inhibitor.
- d. It would behave as an irreversible inhibitor.
- e. It would have no effect on the reaction.

15. Which of the following amino acids is capable of forming a Schiff's base (imine) for covalent catalysis, as in the case of acetoacetate decarboxylase?

- a. P
- b. E
- c. R
- d. K
- e. Y

**Galactose-1-phosphate uridylyltransferase catalyzes the interconversion of UDP-glucose and galactose-1-P with UDP-galactose and glucose-1-P (Shown below). It is a key enzyme in metabolism and is known to be defective in people who have the condition called galactosemia. From kinetic studies of the reaction (depicted in the following two figures), deduce the answers to the following two questions.**



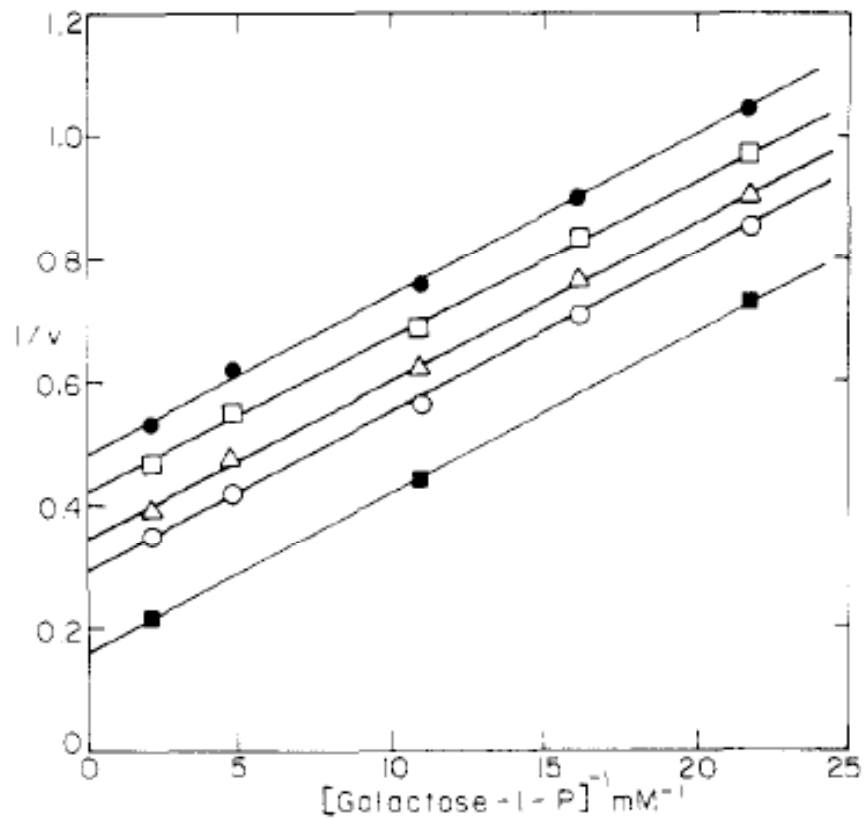


FIGURE 1: Double reciprocal plots of initial velocities of glucose-1-P formation at varying [galactose-1-P] and several fixed [UDP-glucose]. The rates were measured by method A. The UDP-glucose concentrations were: (●) 0.042 mM; (□) 0.051 mM; (△) 0.062 mM; (○) 0.082 mM; (■) 0.2 mM. The velocity units are  $\mu\text{mol}$  of glucose-1-P per min produced by 1.8 milliunits of enzyme.

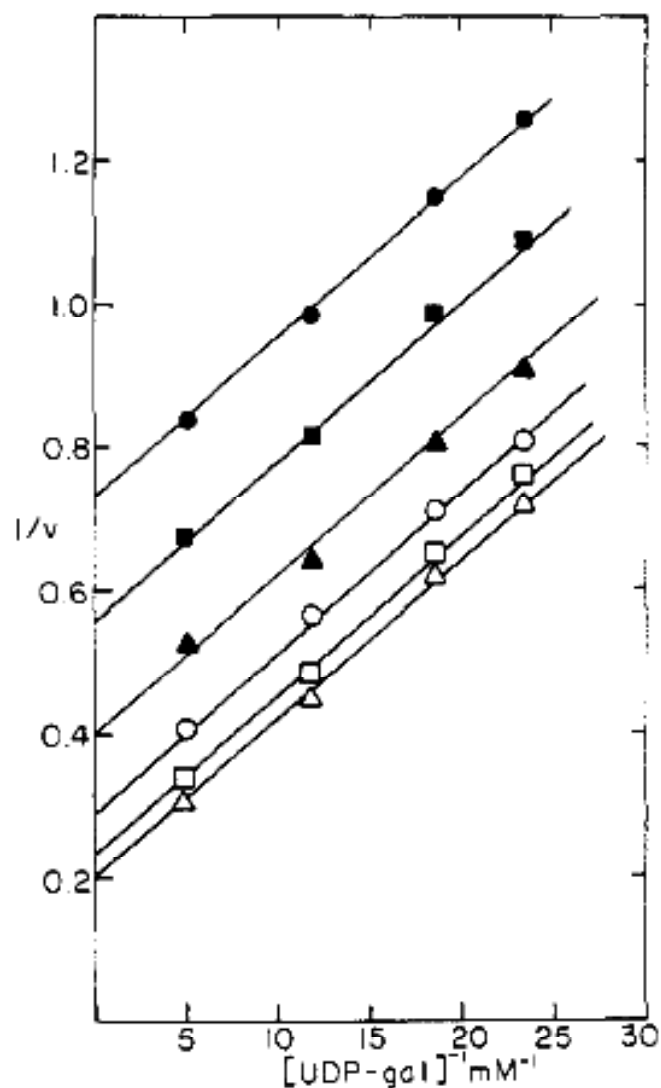


FIGURE 2: Double reciprocal plots of initial velocities of UDP-glucose formation at varying [UDP-galactose] and several fixed [glucose-1-P]. The rates were measured by method B. The glucose-1-P concentrations were: (●) 0.5 mM; (■) 0.073 mM; (▲) 0.125 mM; (○) 0.24 mM; (□) 0.49 mM; (△) 1.1 mM. The velocity units are  $m\mu\text{mol}$  of UDP-glucose per min produced by 2.4 milliunits of enzyme.

16. Which of the following kinetic schemes best describes the reaction catalyzed by galactose-1-phosphate uridylyltransferase?

- a. It is a bimolecular reaction showing ping-pong kinetics
- b. It is a bimolecular reaction showing ordered sequential kinetics
- c. It is a bimolecular reaction showing random sequential kinetics
- d. It is a bimolecular reaction in which each substrate exhibits uncompetitive inhibition with respect to each other.
- e. It is a bimolecular reaction in which each substrate exhibits noncompetitive inhibition with respect to each other.

17. Which of the following statements represents the most plausible mechanistic scenario in the forward direction based on the kinetic data?

- a. Galactose-1-phosphate and UDP-glucose both bind to the enzyme at the same time to form a ternary complex, before the first product is released.
- b. Galactose-1-phosphate becomes covalently attached to the enzyme during the reaction.
- c. Uridylyl monophosphate becomes covalently attached to the enzyme during the reaction.
- d. Uridylyl diphosphate becomes covalently attached to the enzyme during the reaction.
- e. galactose becomes covalently attached to the enzyme during the reaction.

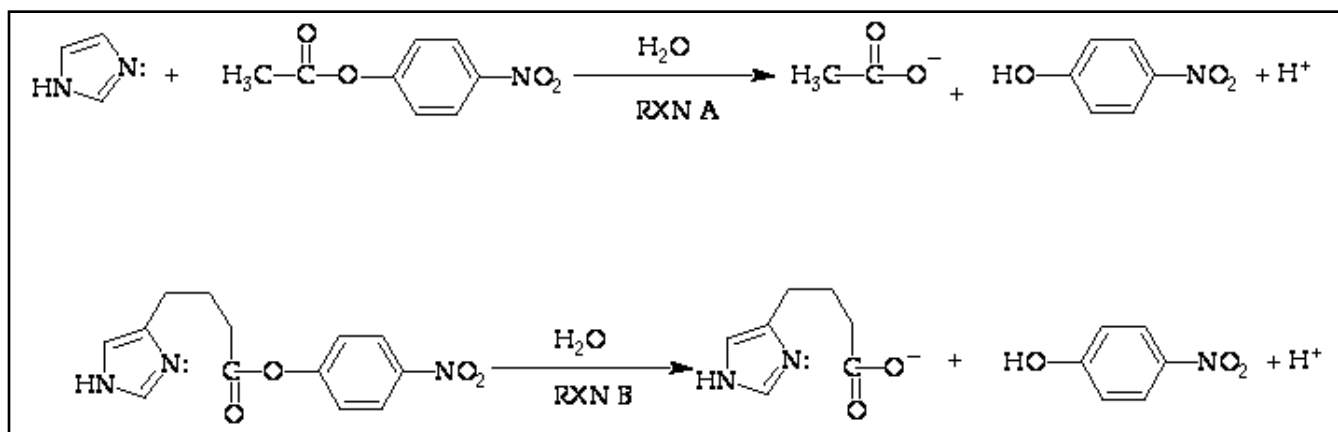
18. Because of potential problems that relate to inhibition of the reaction by too much substrate, enzymologists frequently consider that their reactions are “saturated” when the substrate concentration is 10 times its  $K_m$  value. At what percent of  $V_{max}$  is the rate when the substrate concentration is 10 times the  $K_m$  value?

- a. 99%
- b. 91%
- c. 99.9%
- d. 95%
- e. 80%

19. For an enzyme which obeys Michaelis-Menten kinetics, what is the  $V_{max}$  value in  $\mu\text{mol}\cdot\text{min}^{-1}$  if  $v = 35 \mu\text{mol}\cdot\text{min}^{-1}$  when  $[S] = K_m$ ?

- a. 50
- b. 70
- c. 45
- d. 95
- e. 35

20. An example of catalysis by approximation from physical organic chemistry is the hydrolysis of paranitrophenyl ester catalyzed by imidazole (Rxn A). It proceeds with a second-order rate constant of  $35 \text{ M}^{-1}\cdot\text{min}^{-1}$ . If the effective molarity of imidazole in Rxn B is 26 M, what must the first order rate constant be for Rxn B?



- a.  $35 \text{ sec}^{-1}$
- b.  $15 \text{ sec}^{-1}$
- c.  $0.013 \text{ min}^{-1}$
- d.  $910 \text{ min}^{-1}$
- e.  $910 \text{ sec}^{-1}$

Equations:

Michaelis–Menton

$$v = V_{\max}[S] / (K_m + [S])$$

Competitive Inhibition

$$v = V_{\max}[S] / (\alpha K_m + [S])$$

Noncompetitive Inhibition

$$v = V_{\max}[S] / (\alpha K_m + \alpha'[S])$$

Uncompetitive Inhibition

$$v = V_{\max}[S] / (K_m + \alpha'[S])$$

In all cases,  $\alpha$  or  $\alpha' = 1 + [I]/K_i$