

- 1. d 8. d 15. e
- 2. b 9. e 16. f
- 3. c 10. a 17. c
- 4. a 11. e 18. b
- 5. a 12. b 19. d
- 6. c 13. d 20. c
- 7. b 14. c 21 (bonus). e

Bonus. Which of the following is not a nucleophile?

- a. alkoxide
- b. carbanion
- c. thiolate
- d. amine
- e. carbonyl

Short Answer:

1. Three enzyme-catalyzed reactions are shown below. For each reaction, you are given the active site and catalytic mechanism, the reaction proceeding from substrate to product through the rate-limiting transition state, and an inhibitor of the enzyme.

For all reactions, identify (1) the type of enzyme catalytic mechanisms used in the reaction in the left box provided, (2) and identify the type of irreversible inhibition for the given inhibitor in the box on the right. For mechanisms involving acid/base catalysis, (3) circle the acidic/basic residue(s) **directly interacting with the substrate** in the active site diagram and label as acidic/basic. (4) Circle and label the catalytic nucleophile(s) where they exist. (5) Where applicable, circle the cofactor(s) or coenzyme(s) and label each as cofactor or coenzyme.

(a) (4 marks)

Active Site

Reaction Mechanism

Inhibitor

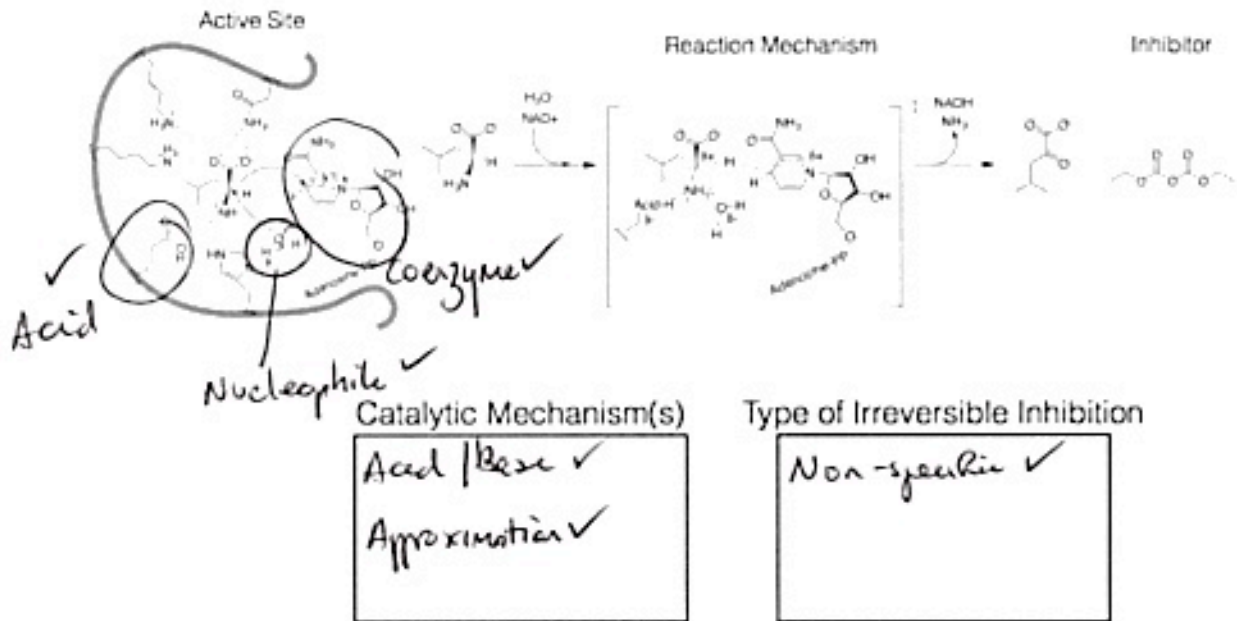
Catalytic Mechanism(s)

Acid-Base ✓
Covalent Catalysis ✓

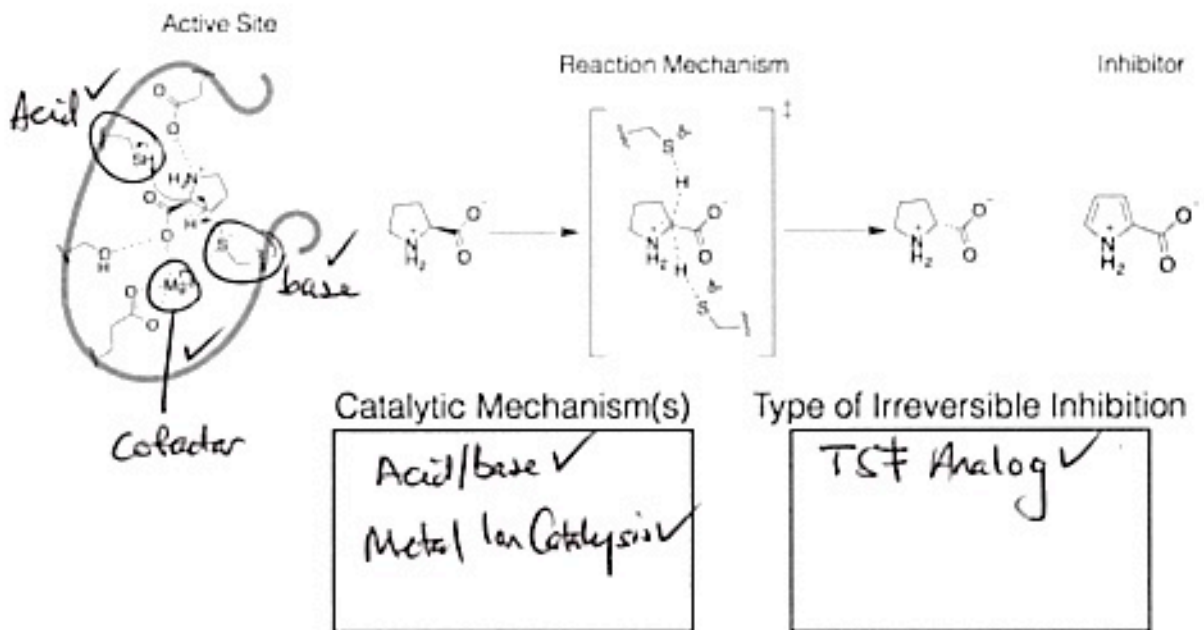
Type of Irreversible Inhibition

Affinity Label ✓

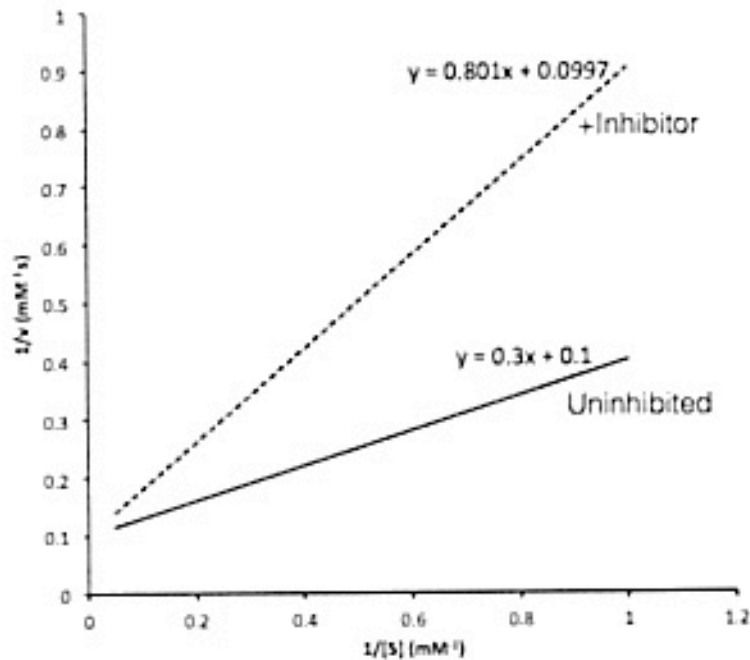
(b) (6 marks)



(c) (6 marks)



2. The initial velocity of an enzyme was measured at a range of substrate concentrations in the absence (solid line) and presence of a reversible inhibitor (dashed line), and the Lineweaver-Burk plot is given below. The enzyme amount was identical for both experiments.



(a) Calculate K_M , V_{max} , K_M^{app} , and V_{max}^{app} . (8 marks)

Out by orders of magnitude get points for units

$$\frac{1}{K_M^{app}} = -0.124$$

$$K_M^{app} = 8.06 \text{ mM}$$

$$V_{max}^{app} = 10.03 \text{ mM s}^{-1}$$

$$K_M = 3.03 \text{ mM}$$

$$V_{max} = 10 \text{ mM s}^{-1}$$

(b) What is the mechanism of inhibition? Can the effect of this inhibitor be overcome, and if so, how? (3 marks)

Mechanism is competitive ✓

Yes ✓ inhibitor can be overcome by increasing ✓ substrate concentration.

(c) Derive the equation for the kinetics of this enzyme-catalyzed reaction that takes into account the effect of this inhibitor. (6 marks)

$$[E]_T = [E] + [ES] + [EI] \quad \checkmark$$

$$\left. \begin{aligned} K_m &= \frac{[E][S]}{[ES]} & [ES] &= \frac{[E][S]}{K_m} \\ K_i &= \frac{[E][I]}{[EI]} & [EI] &= \frac{[E][I]}{K_i} \end{aligned} \right\} \checkmark$$

$$v = k_{cat}[ES] \quad \checkmark$$

$$[E]_T = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_i} \quad \checkmark$$

⋮

$$v = \frac{k_{cat}[E]_T[S]}{K_m \left(1 + \frac{[I]}{K_i}\right) + [S]} \quad \checkmark$$

$$= \frac{V_{max}[S]}{K_m \left(1 + \frac{[I]}{K_i}\right) + [S]} \quad \checkmark$$

(d) Calculate K_i given that 0.5 mM inhibitor was used in the experiment. (3 marks)

$$\alpha = 1 + \frac{[I]}{K_i} \quad \left. \vphantom{\alpha} \right\} \text{either or, or both}$$

$$K_m^{app} = \alpha K_m$$

$$\alpha = \frac{K_m^{app}}{K_m} = \frac{8.06 \mu\text{M}}{3.03 \mu\text{M}} = 2.66$$

$$2.66 = 1 + \frac{[I]}{K_i} = 1 + \frac{0.5 \mu\text{M}}{K_i}$$

$$K_i = 0.30 \mu\text{M}$$

3. A research group has discovered a new enzyme they denote XC-95, which catalyzes the conversion of its substrate, propanol, to propionic acid, the product. The researchers begin to characterize the enzyme.

(a) In the first experiment, with $[E]_T = 4 \text{ nM}$, they find that $V_{max} = 1.6 \mu\text{M s}^{-1}$. Based on this experiment, what is the k_{cat} for XC-95? (2 marks)

$$[E]_T = 4 \text{ nM} = 0.004 \mu\text{M}$$

$$V_{max} = k_{cat} [E]_T$$

$$\therefore k_{cat} = \frac{V_{max}}{[E]_T}$$

$$= \frac{1.6 \mu\text{M s}^{-1}}{0.004 \mu\text{M}}$$

$$= 400 \text{ s}^{-1}$$

(b) In their second experiment, with $[E]_T = 1 \text{ nM}$ and $[\text{propanol}] = 30 \text{ }\mu\text{M}$, the researchers find that $v_0 = 300 \text{ nM s}^{-1}$. What is the measured K_M of XC-95 for propanol? (2 marks)

$$v = \frac{k_{cat} [E]_T [S]}{K_M + [S]}$$

$$K_M = \left(\frac{k_{cat} [E]_T [S]}{v} \right) - [S]$$

$$= \left(\frac{400 \text{ s}^{-1} (0.001 \text{ }\mu\text{M}) (30 \text{ }\mu\text{M})}{0.3 \text{ }\mu\text{M s}^{-1}} \right) - 30 \text{ }\mu\text{M}$$

$$= 10 \text{ }\mu\text{M}$$

(c) Further research showed that the purified XC-95 used in the first two experiments was actually contaminated with a reversible inhibitor of the enzyme, butanol. When butanol is removed from the purified enzyme and the first two experiments are repeated, V_{max} increases to $4.8 \text{ }\mu\text{M s}^{-1}$, and the measured K_M becomes $15 \text{ }\mu\text{M}$. Based on this new data, calculate the values of α and α' for butanol. (4 marks)

$$V_{max} = 4.8 \text{ }\mu\text{M s}^{-1}$$

$$K_M = 15 \text{ }\mu\text{M}$$

$$V_{max}^{app} = 1.6 \text{ }\mu\text{M s}^{-1}$$

$$K_M^{app} = 10 \text{ }\mu\text{M}$$

$$V_{max}^{app} = \frac{V_{max}}{\alpha'} \checkmark$$

$$K_M^{app} = \frac{\alpha K_M}{\alpha'} \checkmark$$

$$\alpha' = 3 \checkmark$$

$$\alpha = \frac{\alpha' K_M^{app}}{K_M}$$

$$= 2 \checkmark$$

(d) What mechanism of reversible inhibition is employed by butanol? (1 mark)

~~Non~~ Mixed inhibition ✓