BCH2333A **PS 6 2019**

Multiple Choice Questions. ***One correct answer per question. (1 mark each)***

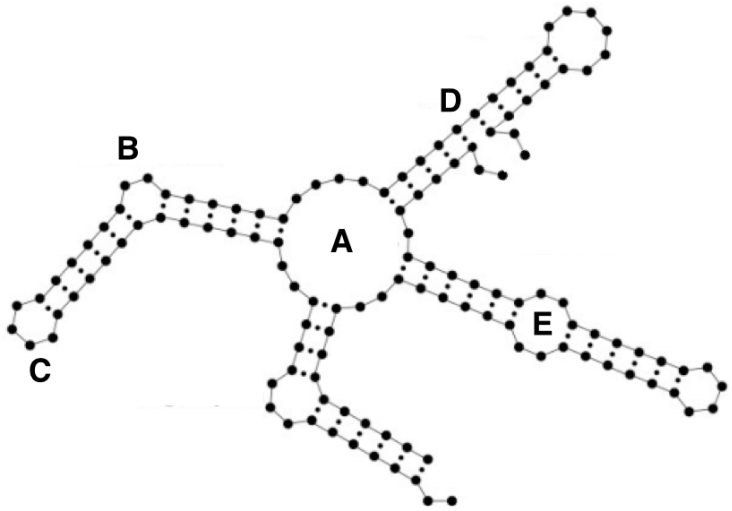
1. Serine proteases contain a catalytic triad made up of:

a. EKS
b. EHS
c. D(Zn^{+})S
d. DHS
e. D(H_2O)D

2. RNA is structurally diverse when compared to DNA, which is a result of:

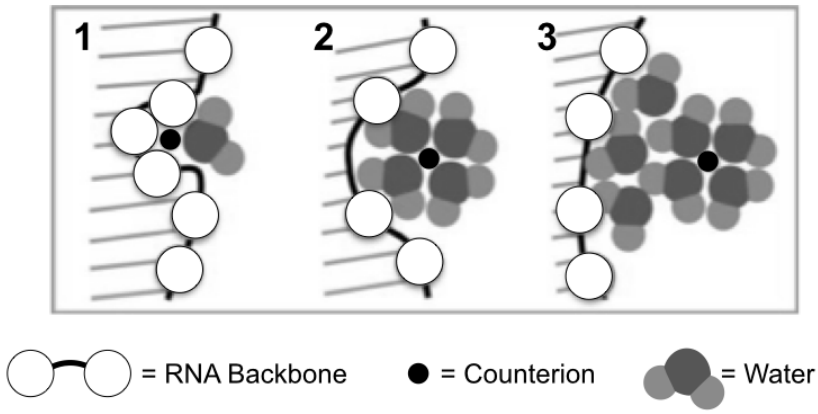
a. Expansion of the building block repertoire
b. Wobble
c. Introduction of chemical functionality into major and minor grooves
d. The non-coding nature of some of the cellular RNA population
e. All of the above

3. What are the correct identities of the RNA secondary structures in the non-coding RNA shown below?



a. A=internal loop, B=juntion, C=hairpin loop, D=bulge, E=internal loop
b. A=junction, B=bulge, C=hairpin loop, D=coaxial stack, E=internal loop
c. A=bulge, B=internal loop, C=hairpin loop, D=coaxial stack, E=internal loop
d. A=internal loop, B=coaxial stack, C=hairpin loop, D=bulge, E=junction
e. A=junction, B=internal loop, C=hairpin loop, D=coaxial stack, E=bulge

Questions 4 and 5 refer to the figure below.



4. What is the correct definition of each type of interaction of counterion with RNA backbone?

a. 1=diffuse, 2=outer-sphere, 3=inner-sphere
b. 1=outer-sphere, 2=inner-sphere, 3=clathrate
c. 1=clathrate, 2=outer-sphere, 3=diffuse
d. 1=inner-sphere, 2=outer-sphere, 3=diffuse
e. 1=diffuse, 2=clathrate, 3=outer-sphere

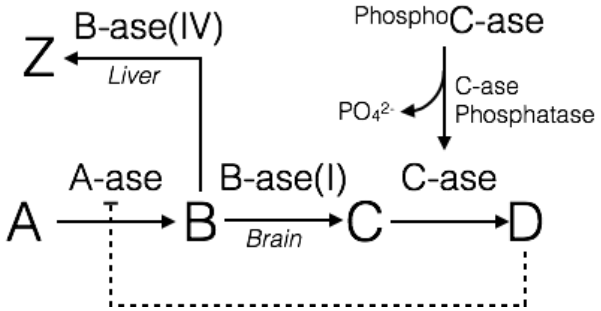
5. Which of the three types of counterion-RNA interactions is more likely to have a negligible ΔG_{hyd} and the smallest magnitude $\Delta G_{ion\text{-}RNA}$?

a. 1
b. 2
c. 3
d. 2 & 3 would have the same ΔG_{hyd} and $\Delta G_{ion\text{-}RNA}$
e. All counterion interaction types would have the same magnitude $\Delta G_{ion\text{-}RNA}$

6. Which of the following is *not* a difference between protein and RNA folding processes?

a. Counterions are necessary for RNA folding due to the anionic RNA backbone, where the protein backbone is not formally charged
b. The thermodynamics of protein folding requires protein aggregation to achieve stable misfolded structures, whereas the base pairing in RNA allows misfolded structures to readily form with lower free energy than the native structure.
c. Some protein folding can occur through 2-state kinetic models, where RNA necessarily folds through multistate pathways with several interchangeable stable intermediates.
d. Backbone interactions drive protein folding, but limit RNA folding unless adequately neutralized
e. Protein and RNA folding is driven by a hydrophobic collapse event that precedes achievement of the native folded structure.

For questions 7 to 9, consider the metabolic pathway show below that converts substrate A to B with the enzyme A-ase, B to C with B-ase(I), and so forth.



7. What is the mechanism of regulation of A-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

8. What is the mechanism of regulation of C-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

9. What is the mechanism of regulation of B-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

10. Which of the following is *not* a nucleophile?

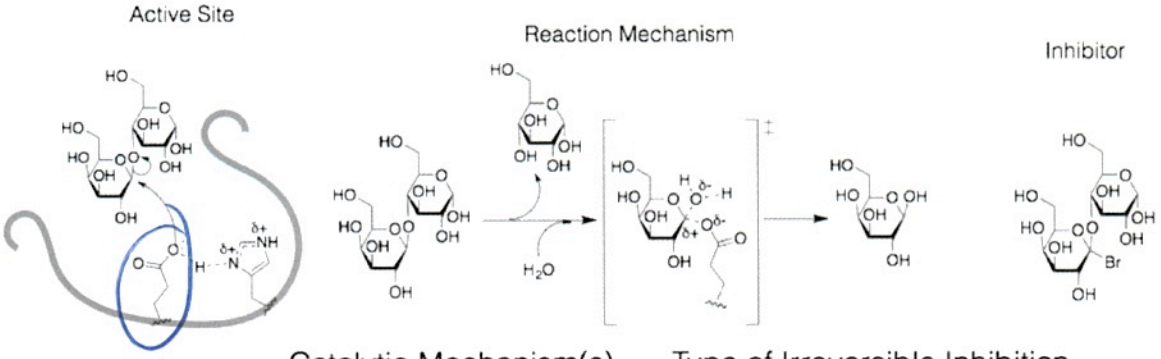
a. alkoxide
b. carbanion
c. thiolate
d. amine
e. carbonyl

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.

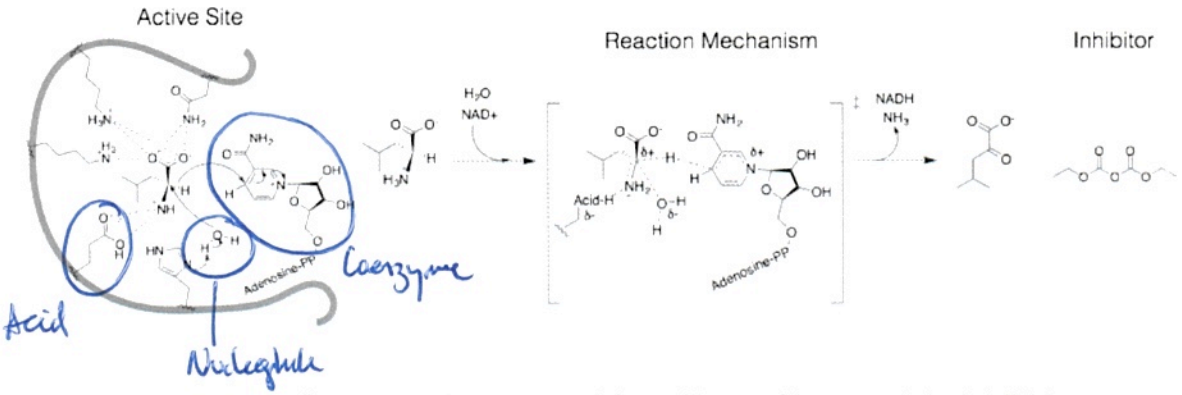
For all reactions, identify (**1**) the type of enzyme catalytic mechanisms used in the reaction in the left box provided, (**2**) and identify the type of irreversible inhibition for the given inhibitor in the box on the right. For mechanisms involving acid/base catalysis, (**3**) circle the acidic/basic 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**)



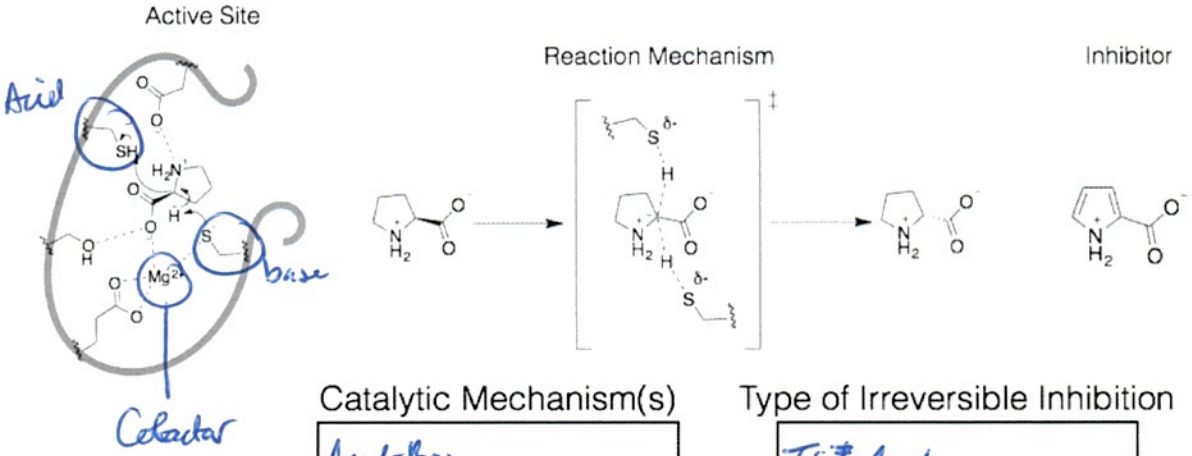
Catalytic Mechanism(s)	Type of Irreversible Inhibition
Acid-Base Covalent Catalysis	Affinity Label

(b) (**6 marks**)



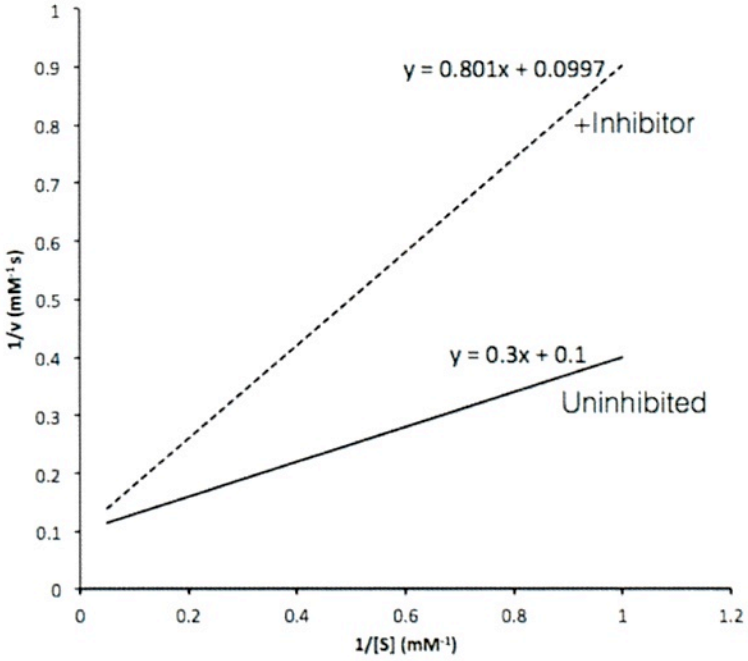
Catalytic Mechanism(s)	Type of Irreversible Inhibition
Acid-base Approximation	Non-specific

(c) (**6 marks**)



Catalytic Mechanism(s)	Type of Irreversible Inhibition
Acid-Base Metal Ion Catalysis	TS# Analog

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**)

$\frac{1}{K_{M}^{app}} = -0.124$

$K_M^{app} = 8.06$ mM $K_M = 3.03$ mM

$V_{max}^{app} = 10.03$ mM s^{-1} $V_{max} = 10$ 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]

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

$$[E_T] = [E] + [ES] + [EI]$$

$$k_m = \frac{[E][S]}{[ES]} \qquad [ES] = \frac{[E][S]}{k_m}$$

$$k_i = \frac{[E][I]}{[EI]} \qquad [EI] = \frac{[E][I]}{k_i}$$

$$v = k_{cat}[ES]$$

$$[E]_T = [E] + \frac{[E][S]}{k_m} + \frac{[E][I]}{k_i}$$

$$\vdots$$

$$v = \frac{k_{cat}[E]_T[S]}{k_m\left(1 + \frac{[I]}{k_i}\right) + [S]}$$

$$= \frac{V_{max}[S]}{\alpha k_m + [S]} \qquad \alpha = 1 + \frac{[I]}{k_i}$$

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

$\alpha = 1 + \frac{[I]}{K_i}$ $\quad K_m^{app} = \alpha K_m$

$\alpha = \frac{K_m^{app}}{K_m} = \frac{8.06\ mM}{3.03\ mM} = 2.66$

$2.66 = 1 + \frac{[I]}{K_i} = 1 + \frac{0.5\ mM}{K_i}$

$K_i = 0.30\ mM.$

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 nM, they find that V_{max}=1.6 μM s^{-1}. Based on this experiment, what is the k_{cat} for XC-95? (**2 marks**)

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

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

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

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

$= 400\ s^{-1}$

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

$$v_0 = \frac{k_{cat}[E_T][S]}{K_M + [S]}$$

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

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

$$= 10\,\mu M.$$

(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 μM s^{-1}, and the measured K_M becomes 15 μM. Based on this new data, calculate the values of α and α' for butanol. (**4 marks**)

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

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

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

$\alpha' = 3$

$K_M = 15\,\mu M$

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

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

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

$\alpha = 2.$

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

Mixed Inhibition.