

ENZYMES - EXTRA QUESTIONS

1. A chemical reaction has a $G^\circ = -60$ kJ/mol. If this were an enzyme-catalyzed reaction what can you predict about the kinetics?
 - A. It will exhibit very rapid kinetics.
 - B. It will exhibit very slow kinetics.
 - C. The kinetics of the reaction can not be predicted.
 - D. The kinetics depend on the nature of the reactants.
 - E. The kinetics depend on the nature of the products.
2. Suppose that the standard free energy change for an uncatalyzed reaction (S \rightarrow P) is +60 kJ/mol, and a specific enzyme enhances the rate of the reaction by 10^7 -fold. Under standard conditions in the presence of the enzyme, in which direction will the reaction go?
3. If the activation energy for an uncatalyzed reaction is + 25 kJ/mol, then what can you say about the activation energy for the enzyme catalyzed reaction?
4. Is it possible for pH to affect the activity of an enzyme in a range where none of the side chain groups of the protein are titrated? Explain.
5. An approximate "rule of thumb" is that the rate of a chemical reaction will double (reaction will go twice as fast) for every 10° increase in temperature. This observation holds for enzyme-catalyzed reactions up to a certain temperature range (different for each enzyme), at which the rate stops increasing and then decreases sharply as the temperature increases. Explain.
6. Assuming they have equal affinity for the enzyme, why would a noncompetitive inhibitor be a more effective drug than a competitive inhibitor?
7. Why is it advantageous to the cell to produce proteolytic enzymes in the form of zymogens, which are only activated after they are secreted?
8. Why does V_{\max} but not K_m depend on the amount of enzyme used in an enzyme reaction?
9. K_m and V_{\max} for two alternative substrates A and B for the same enzyme are:
for substrate A: 4.0 mM, 25 μ mol/sec and
for substrate B: 0.5 mM, 15 μ mol/sec.
At **low** substrate concentration ($\ll K_m$) which substrate will react most rapidly?
10. Tosyl-L-phenylalanine chloromethyl ketone is a specific inhibitor of chymotrypsin.
 - a) Why is this compound specific for chymotrypsin?
 - b) Can you suggest a way to make a similar compound that would be specific for trypsin?

11. The following table presents the rates, v (μ mol/sec), at which an enzyme converts its substrate to product. **Reaction 1** represents data for the **uninhibited** reaction and **Reactions 2 and 3** represent data collected in the presence of **two different inhibitors**, each present at **10mM**. Assuming the amount of enzyme is the same in each case, **determine K_m and V_{max} for the enzyme** (in absence of inhibitors) and **for each inhibitor determine K_i and the type of inhibition**.

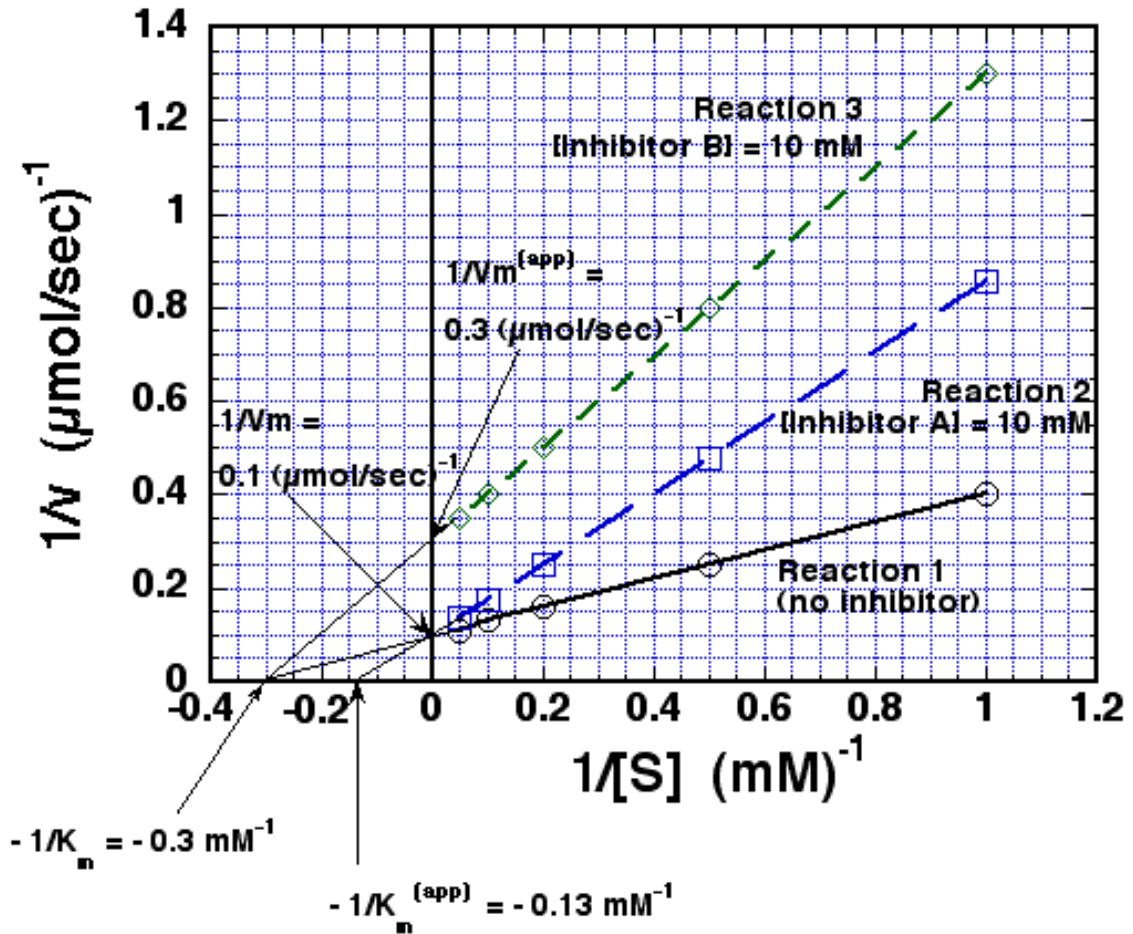
[S] mM	v , reaction 1 (μ mol/sec)	v , reaction 2 (inhibitor A) (μ mol/sec)	v , reaction 3 (inhibitor B) (μ mol/sec)
1	2.5	1.17	0.77
2	4.0	2.1	1.25
5	6.3	4.0	2.0
10	7.6	5.7	2.5
20	9.0	7.2	2.86

12. Methanol is oxidized by alcohol dehydrogenase (ADH, found in the liver and other tissues) to the highly toxic compound formaldehyde. Drinking methanol is fatal because of the production of formaldehyde; methanol itself is harmless and is excreted by the kidneys. ADH will also oxidized other alcohols, such as ethanol.
- Based on this information propose a way to treat an individual who has ingested methanol.
 - What additional information would you need before such a treatment could be tried?

ANSWERS

1. (C) You can not make any predictions about kinetics from thermodynamic data alone because G° does not depend on the pathway from reactants to products, whereas the kinetics does depend on the pathway.
2. The direction will be the same \pm a catalyst. The catalyst does not change K_{eq} , and under standard conditions the equilibrium for this reaction would lie far to the LEFT. Enzymes only accelerate the rate at which a chemical reaction reaches equilibrium, not the position of the equilibrium.
3. It must be lower. Enzymes accelerate reaction rates by decreasing the activation energy of the reaction.
4. Yes, if the substrate must be in a particular ionic form in order to bind to the enzyme.
5. Enzymes are proteins and at high temperature the enzyme is denatured.
6. The presence of the substrate would not alter the effectiveness of the noncompetitive inhibitor.
7. Because the zymogens are inactive, the cell is protected from proteolytic degradation (digesting itself from inside).
8. The velocity equation for V_{max} includes the concentration of enzyme (actually, the concentration of enzyme *active sites*): $V_{max} = k_2[E_t] = k_{cat}[E_t]$; K_m is a substrate concentration, and is independent of the concentration of enzyme.
9. (B) At low $[S]$, k_{cat}/K_m (an apparent second order rate constant) is an indication of how fast $E+S$ can be converted to $E+P$. $V_{max} = k_{cat} [E_t]$. Although we don't know k_{cat} (because we don't know the enzyme/active site concentration), we have V_{max} , which is proportional to k_{cat} at a given enzyme concentration, so we can use V_{max}/K_m to compare the enzyme's efficiency with substrates A and B. As long as the units are the same for our comparison, we don't need to worry about the units. Because V_{max}/K_m is 30 ($\mu\text{mol}/\text{sec}/\text{mM}$) for substrate B and 6.25 ($\mu\text{mol}/\text{sec}/\text{mM}$) for substrate A, we can say that at low concentration substrate B will be used more efficiently.
10. a) This compound binds in the specificity pocket of chymotrypsin via the Phe group.
b) Replace the Phe with an Arg, which would bind in trypsin's specificity pocket.

11. $K_m = 3.3 \text{ mM}$, $V_{\max} = 10 \text{ } \mu\text{mol/sec}$; in reaction 2, the inhibitor (A) is competitive, with $K_i = 7.5 \text{ mM}$; in reaction 3, the inhibitor (B) is noncompetitive, with $K_i = 5 \text{ mM}$.



Algebra for calculations of K_I for the two inhibitors:

Inhibitor A (competitive -- no change in V_{\max} , but $K_m^{app} = K_m \{1 + ([I]/K_I)\}$)

$$-\frac{1}{K_m^{app}} = -0.13 \text{ mM}^{-1}$$

$$K_m^{app} = 7.7 \text{ mM}$$

$$\frac{K_m^{app}}{K_m} = \frac{7.7 \text{ mM}}{3.3 \text{ mM}} = 2.33 = 1 + \frac{[I]}{K_I} = 1 + \frac{[10 \text{ mM}]}{K_I}$$

$$K_I(\text{inhibitor A}) = \frac{10 \text{ mM}}{2.33 - 1} = \frac{10 \text{ mM}}{1.33} = 7.5 \text{ mM}$$

Inhibitor B (pure noncompetitive -- no change in K_m , but $V_{max}^{app} = V_{max} / \{1 + ([I]/K_I)\}$)

$$\frac{1}{V_{max}^{app}} = 0.3(\mu\text{mol}/\text{sec})^{-1}$$

$$V_{max}^{app} = 3.33\mu\text{mol}/\text{sec}$$

$$\frac{V_{max}}{V_{max}^{app}} = \frac{10\mu\text{mol}/\text{sec}}{3.33\mu\text{mol}/\text{sec}} = 3 = 1 + \frac{[I]}{K_I} = 1 + \frac{[10\text{mM}]}{K_I}$$

$$K_I(\text{inhibitor B}) = \frac{10\text{mM}}{3-1} = \frac{10\text{mM}}{2} = 5\text{mM}$$

12. a) If the individual were given ethanol, the ethanol would act as a competitive inhibitor of methanol oxidation, allowing the kidneys to excrete the methanol.
- b) You would need to know the relative K_m s of ADH for ethanol and methanol in order to know how much ethanol to give so as to prevent methanol oxidation. You should also know something about how much *ethanol* would be toxic, so you don't kill the patient with the treatment.
- NOTE: EMTs and hospital emergency room personnel actually use ethanol as a treatment for methanol poisoning, and also for ethylene glycol (antifreeze) poisoning.