Kinetics

Tuesday, October 18, 2016 9:45 AM

Enzyme Kinetics

Background/Review:

1. What is the difference between K and k?

   → Equilibrium constant

   → rate constant

2. For the following elementary reaction, explain why $K = \frac{k_1}{k_{-1}}$: $A + B \rightleftharpoons C$ $K = \frac{[C]}{[A][B]}$

   @ Equilibrium, rate for. = rate rev

   $k_1[A][B] = k_{-1}[C]$ $\frac{[C]}{[A][B]} = \frac{k_1}{k_{-1}} = K$ so an eq constant is a ratio of for + rev rate constants

Steady State Approximation: Theory

3. One main assumption of the steady state model of enzyme kinetics is that k_2 is the slowest rate constant. Explain why this is important.

   This allows us to state that rate = $k_2[ES]$

4. Consider the conversion of substrates to products according to the following mechanism. Which of these steps are combined into k_2 in the traditional Michaelis-Menten kinetics model?

   $E + S \rightleftharpoons ES \rightleftharpoons TS \rightleftharpoons EP \rightleftharpoons E + P$

   all part of k_2

5. The term used for the overall rate constant for multi-step reactions is **k_{cat}**. Discuss why it makes sense that $k_{cat}=k_2$ in simple Michaelis-Menten reactions.

   k_2 is the slow step, so the rate of forming P is only dependent on k_2 + [ES]

6. In the Michaelis-Menten model, the units of k_{cat} are s^{-1}. Discuss how the units are consistent with the name "turnover number". If k_{cat} is large, what does that imply about the enzyme?

   It can quickly convert ES → P

   2 s^{-1} → so each enzyme can create 2 products per second from an ES

7. The term efficiency is often used in describing enzymes. What does it mean for an enzyme to be efficient?

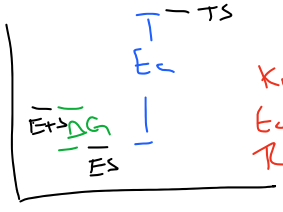
   can quickly convert S → P. This means a high affinity and fast turnover

8. How is your answer to the previous question consistent with the term for catalytic efficience (k_{cat}/K_M)? Discuss the contributions of k_{cat} and K_M (i.e. do efficient enzymes have large k_{cat}? How about K_M?)

   maximizing k_{cat} + minimizing K_M will result in an efficient enzyme.

   related to E_a

   related to ΔG: ↑ΔG = ↓K_M

   note that minimizing K_M actually increases E_a so decreases k_{cat}. The trick is to find a balance

9. Answer the following three questions using the information in the table to the right.

Enzyme	K_M (M)	K_{cat} (s^{-1})
A	9.5×10^{-5}	1.4×10^{4}
B	2.5×10^{-2}	1.0×10^{7}
C	5.0×10^{-6}	8.0×10^{2}

k_{cat}/K_m
1.5 e8
4 e8
1.6 e8

a. Which enzyme has the highest affinity for the substrate? How do you know?

C → lowest K_m (remember K_m is K_D)

b. Which enzyme can convert the most substrate to a product in 1 minute? How do you know?

B → largest turnover number

c. Which enzyme has the highest catalytic efficiency? How do you know?

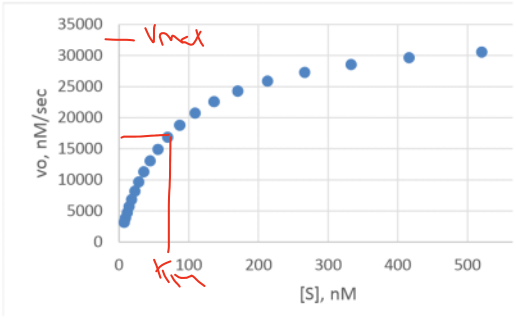
B → largest k_{cat}/K_m

10. Using the image to the right and E_{tot} = 1 nM, estimate:

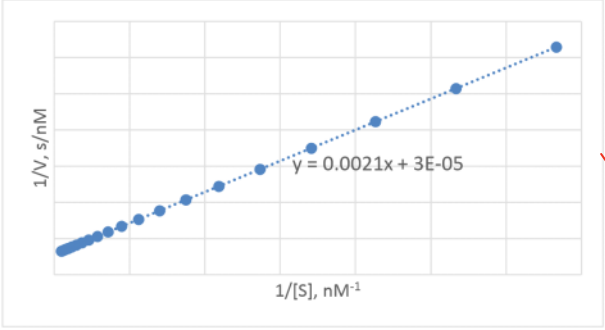
a. V_{max} ≈ 33000 nM/s
b. K_M ≈ 70 nM
c. k_{cat} = 33000 s^{-1}
d. Catalytic efficiency 471 $nM^{-1}s^{-1}$
e. The dissociation constant

70 nM

$K_m = K_D$



11. Below is the double reciprocal plot of the data in problem 10. Determine Km and Vmax and compare your estimate from the previous problem.



$\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$

$V_{max} = \frac{1}{3 \times 10^{-5}} = 33{,}333 \frac{nM}{s}$

$K_m = 0.0021(33{,}333)$

$K_m = 70$ nM

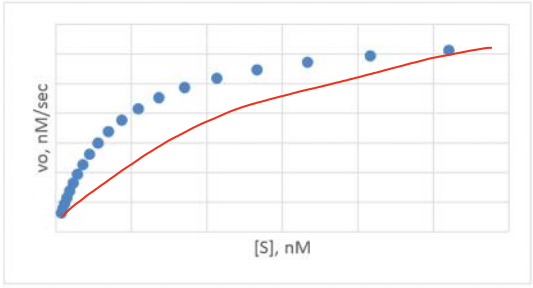
Enzyme Inhibition

12. For each type of inhibition, determine how K_M and Vmax will change (increase or decrease) upon addition of an inhibitor. On each graph, sketch what you would expect the plot to look like when an inhibitor is present.

Competitive:

K_M	Increase	Decrease
Vmax	Increase	Decrease

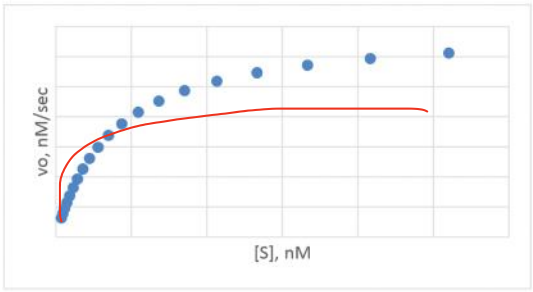
stay the same



$E + S \rightleftharpoons ES \rightarrow E + P$
$+$
I
$\downarrow$
EI

Uncompetitive:

K_M	Increase	Decrease
Vmax	Increase	Decrease

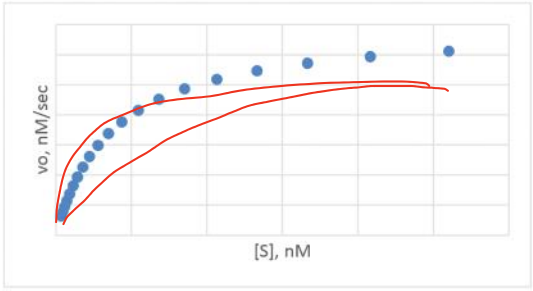


$E + S \rightleftharpoons ES \rightarrow E + P$
$+$
I
$\downarrow$
ESI

Mixed:

K_M	Increase	Decrease
Vmax	Increase	Decrease

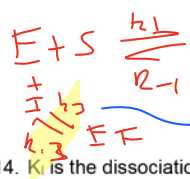
could be either depending which K_I is bigger (K_{I_1} or K_{I_2})



$E + S \rightleftharpoons ES \rightarrow E + P$

$+$	$+$
I	I
$\downarrow K_{I_1}$	$\downarrow K_{I_2}$
EI	ESI

13. Recall that K_M is the ratio rate constants: $\frac{ES_{decomposition}}{ES_{formation}} = \frac{k_2+k_{-1}}{k_1}$. Based on this, what would the K_M expression be if a competitive inhibitor was present?



However, [E] would decrease, so $k_1[E][S]$ would decrease making K_M appear smaller

none of those effect the [ES], so the expression would be the same

14. K_I is the dissociation constant for an inhibitor. Which of the following would be better competitive inhibitor of an enzyme? Explain your answer.

A: K_I = 100 nM B: K_I = 10 μM C: K_I = 10 nM

low K_I is better inhibitor

If you care, the derivation is shown below

15. When a competitive inhibitor is added to a reaction mixture, the apparent K_M' is related to the uninhibited KM according to the following equation. If KM is determined to be 100 nM without and inhibitor present and 250 nM with 5 nM inhibitor, what is K_I?

$$K_M' = K_M\left(1 + \frac{[I]}{K_I}\right)$$

$$250\ nM = 100\ nM\left(1 + \frac{5\ nM}{K_I}\right)$$

$$2.5 = 1 + \frac{5}{K_I}$$

$$1.5\ nM = \frac{5}{K_I}$$

$K_I = 3.33$ nM

16. For uncompetitive inhibition, V_{max} and K_M are both changed by a factor of $\frac{1}{1+\frac{[I]}{K_I}}$. If K_M is normally 10 nM but is determined to be 8 nM in the presence of 50 nM inhibitor, determine:

a. K_I.

$K_M' = 8$, $K_M = 10$

$K_M' = K_M\left(\frac{1}{1+\frac{I}{K_I}}\right)$

$8 = 10\ (X)$

$X = 0.8 = \frac{1}{1+I/K_I}$

$1 + \frac{I}{K_I} = 1.25$

$\frac{I}{K_I} = 0.25 = \frac{50\ nM}{K_I}$

$K_I = 200$ nM

b. The uninhibited Vmax if the inhibited Vmax (Vmax') is determined to be 108 nM min^{-1}.

$$V_{max}' = V_{max}\left(\frac{1}{1+\frac{I}{K_I}}\right)$$

$$\frac{1}{1+\frac{50}{200}} = 0.8$$

$$\frac{108\ nM}{min} = V_{max}(0.8)$$

$$V_{max} = 135\ \frac{nM}{min}$$

Competitive

Changes from normal Enzyme Kinetics: $E_{tot} = E + ES + EI$

- still need to solve for ES because rate $= k_2[ES]$
- still true: $[ES]_{formation} = [ES]_{decomposition}$

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

this will be different b/c $E_{TOT} = E + ES + EI$

- Still true: need to solve E_{TOT} expression for $[E]$

$$EI \rightleftharpoons E + I \qquad K_I = \frac{[E][I]}{[EI]} \qquad [EI] = \frac{[E][I]}{K_I}$$

$$E_{TOT} = [ES] + [E] + \frac{[E][I]}{K_I} = [ES] + [E]\left(1 + \frac{[I]}{K_I}\right)$$

$$E_{TOT} = [ES] + [E] + \frac{[E][I]}{K_I} = [ES] + [E]\underbrace{\left(1 + \frac{[I]}{K_I}\right)}_{\alpha}$$

$$E_{TOT} = [ES] + \alpha[E]$$

$$[E] = \frac{E_{TOT} - [ES]}{\alpha}$$

$$k_1[S]\left(\frac{E_{TOT} - [ES]}{\alpha}\right) = k_{-1}[ES] + k_2[ES]$$

$$\frac{k_1[S]E_{TOT} - k_1[S][ES]}{\alpha} = [ES](k_{-1} + k_2)$$

$$k_1[S]E_{TOT} - k_1[S][ES] = \alpha\left(\frac{k_{-1} + k_2}{k_1}\right)[ES] = \alpha K_M[ES]$$

$$[S]E_{TOT} = (\alpha K_M + [S])[ES]$$

$$[ES] = \frac{[S]E_{TOT}}{\alpha K_M + [S]}$$

$$\text{rate} = \frac{k_2 E_{TOT}[S]}{\alpha K_M + [S]} = \frac{V_{max}[S]}{\alpha K_M + [S]}$$