10.E: Exercises

10.1: General Principles of Catalysis

Q10.1a

One day in class about enzyme kinetics, Jack comes over to you and asks, "I know enzymes are biological catalysts, but I do not understand how it works. Can you explain how enzymes make reactions go faster? And is it only faster in one direction?"

S10.1a

Because the activation energy is the energy hill between reactants and products, enzymes decreasing the size of the hill also decreases the amount of energy needed for reactions to go in either direction. A smaller energy hill allows reactants and products to overcome the barrier quicker, resulting a faster reaction rate.

Q10.1b

If your student colleagues argues that a catalysts affects only the rate of only one direction of a reaction. Explain why he is correct or not.

S10.1b

False. Catalysts affect rate by providing an alternative mechanism which has a lower transition state energy. It's impossible to lower transition state energy for only one direction of a reaction. (It'd be like making a hill shorter from the north, but keeping it the same height from the south.)

Q10.1c

How does the enzyme catalysis affect both forward and reverse reaction?

S10.1c

The enzyme catalyst lowers the Gibb energy of transition state, which reduces the activation energy of both reactions. Therefore, it makes reactions occur faster.

Q10.2a

Given enzyme-catalyzed reaction k_1 = 4x10^6 M^{-1} s^{-1} , k_{-1} =6x10^4 s^{-1} and k_2= 2.0x10^3 s^{-1}. Determine if the enzyme –substrate binding follow the equilibrium or not ?

S10.2a

The dissociation constant k_s = k_{-1} / k_1 =6x10^4 s^{-1}/ 4x10^6 M^{-1} s^{-1} = 0.15 M

The Michaelis constant k_M = k_{-1} +k_2 / k_1= 6x10^4 s^{-1}+ 2.0x10^3 s^{-1}/ 4x10^6 M^{-1} s^{-1} =0.155M

The two constant are not equal. Therefore, the binding does not follow the equilibrium scheme.

Q10.2b

Is it appropriate to use the rapid equilibrium scheme to model the kinetics of a catalyzed reaction with the following rate constants?

$$k_1 = 7 \times 10^7 \ M^{-1} \ s^{-1} \tag{10.E.1}$$

$$k_{-1} = 8 \times 10^5 \ s^{-1} \tag{10.E.2}$$

$$k_2 = 5 \times 10^4 \ s^{-1} \tag{10.E.3}$$

S10.2b

Assuming rapid equilibrium is appropriate when $k_{-1} \gg k_2$, so that K_M = K_S. In this case, the values of k_{-1} and k_2 are not dramatically different, so we can calculate K_S and K_M and compare.

1. Calculate K_M:

$$K_M = \frac{k_{-1} + k_2}{k_1} \tag{10.E.4}$$

$$K_M = \frac{8 \times 10^5 \ s^{-1} + 5 \times 10^4 \ s^{-1}}{7 \times 10^7 \ M^{-1} \ s^{-1}} \tag{10.E.5}$$

$$K_M = 0.01 \ M \tag{10.E.6}$$

2. Calculate K_S:

$$K_S = \frac{k_{-1}}{k_1} \tag{10.E.7}$$

$$K_M = \frac{8 \times 10^5 \ s^{-1}}{7 \times 10^7 \ M^{-1} \ s^{-1}} \tag{10.E.8}$$

$$K_M = 0.01 \ M \tag{10.E.9}$$

Since $K_M = K_S$, it is appropriate to assume rapid equilibrium.

Q10.2c

Given k_1= 7 x 10^6 M-1s-1, k_{-1}=6 x 10^4 s^{-1}, k_3= 2 x 10^3 s^{-1}, determine if the enzyme substance binding follow the equilibrium or steady state scheme?

$$K_s = \frac{k_{-1}}{k_1} \tag{10.E.10}$$

$$= \frac{6 \times 10^4 \ s^{-1}}{7 \times 10^6 \ M^{-1} s^{-1}} \tag{10.E.11}$$

$$= 8.8 \times 10^{-3} \ M \tag{10.E.12}$$

$$K_M = k_{-1} + k_2 \tag{10.E.13}$$

$$= \frac{6 \times 10^4 \ s^{-1} + 2 \times 10^3 \ s^{-1}}{7 \times 10^6 \ M^{-1} s^{-1}} \tag{10.E.14}$$

$$= 8.9 \times 10^{-3} \ M \tag{10.E.15}$$

Q10.3a

The substrate N-acetylglycine ethyl ester can be catalyzed by the enzyme carbonic anhydrase. This enzyme has a turnover rate of 30,000 s^{-1}. Determine how long it will take carbonic anhydrase to cleave the substrate.

S10.3a

We already know the turnover number (k_{cat}). The amount of time necessary to cleave the substrate is the reciprocal of the turnover rate.

$$t = \frac{1}{k} = \frac{1}{30,000 \ s^{-1}} = 3.33 \times 10^{-5} \tag{10.E.16}$$

$$(5 \ minutes)(7.1 \times 10^{-6}) = 3.5 \times 10^{-5} \tag{10.E.17}$$

Q10.3b

RuBisCO is an enzyme in the Calvin cycle that fixes atmospheric carbon and has a turnover rate of 3.3 s^{-1}. How long does it take RuBisCO to fix one molecule of carbon dioxide?
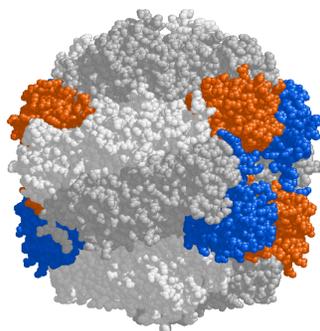
Spacefilling structure of RuBisCO created using Rasmol and the 8RUC file from the Protein Data Bank.

S10.3b

The turnover number is the number of molecules of substrate per unit time (when the enzyme is fully saturated). So simply take the reciprocal to find the time per molecule of substrate.

$$\frac{1}{3.3\ s^{-1}} = 0.30\ s \tag{10.E.18}$$

Note: RuBisCO is a notoriously slow enzyme.

Q10.3c

The catalyze of acetylcholine has a rate 50000 s^{-1} . Calculate the time for the enzyme to cleave one Ach molecule.

S10.3c

$$t = 1/k_2 = 1/\ 50000 = 2.0\ x10^{-5}s$$

Q10.3d

Carbonic anhydrase, an enzyme that catalyzes the dehydration of carbonic acid to form carbonic acid, has the turnover rate of k_{cat} 4.0x 10^5 s^{-1}. Calculate how long does it take does it take for the enzyme to cleave one molecule carbonic acid?

S10.3d

The time required for the enzyme to cleave one molecule carbonic acid:

$$t = 1/k_{cat} = 1/\ 4x10^5 s^{-1} = 2.5x10^{-6}s = 2.5\mu s$$

Q10.3e

p-nitrophenyl acetate(PNPA) is catalyzed by chymotrypsin to yield p-nitrophenolate ion and acetate ion. The turnover rate of that enzyme is 40,000 s^{-1}. How long will it take for the enzyme to produce 1 mole of Nitrophenyl acetate?

S10.3e

It takes 1 second to convert all 40,000 molecule substrate into the product, so: $t = \dfrac{40,000}{9.5x10^5} = 3.8$ sec.

10.2: The Equations of Enzyme Kinetics

Q10.4

What is plotted on the x and y axes on a Lineweaver-Burk plot? Show how to derive the equation for the plot from the equation

$$v_0 = \frac{V_{max}[S]}{K_M + [S]} \tag{10.E.19}$$

and explain how V_{max} and K_M can be found from the graph's intercepts. *Hint: A Lineweaver-Burk plot is also sometimes called a double reciprocal plot.*

S10.4

The x-axis is $1/v_0$, and the x-axis is $1/V_{max}$.

1. Take the reciprocal of both sides of the equation.

$$\frac{1}{v_0} = \frac{K_M + [S]}{V_{max}[S]} \tag{10.E.20}$$

$$\frac{1}{v_0} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \tag{10.E.21}$$

2. Set 1/[S] = 0 to find the y-intercept, and show that it relates to V_{max}.

$$Yint = \frac{1}{V_{max}} \tag{10.E.22}$$

$$V_{max} = \frac{1}{Yint} \tag{10.E.23}$$

3. Set $1/v_0$ = 0 to find the x-intercept, and show that it relates to K_M.

$$0 = \frac{K_M}{V_{max}}Xint + \frac{1}{V_{max}} \tag{10.E.24}$$

$$\frac{K_M}{V_{max}}Xint = -\frac{1}{V_{max}} \tag{10.E.25}$$

$$Xint = -\frac{1}{K_M} \tag{10.E.26}$$

$$K_M = -\frac{1}{Xint} \tag{10.E.27}$$

Q10.4a

Derive the Michaelis-Menten equation by assuming rapid equilibrium.

$$E + S \underset{}{\overset{K_1}{\rightleftharpoons}} ES \overset{K_2}{\rightarrow} E + P \tag{10.E.28}$$
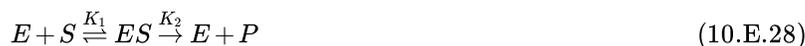
$$v_0 = \frac{V_{max}[S]}{K_M + [S]} \tag{10.E.29}$$

S10.4a

$$\frac{[E][S]}{dt} = K_{-1}[ES] \quad \frac{[ES]}{dt} = K_1[E][S] \quad \frac{[E][S]}{dt} = \frac{[ES]}{dt} \quad K_{-1}[ES] = K_1[E][S] \quad K_M\frac{k_{-1}}{K_1} = \frac{[E][S]}{[ES]}$$

given that $[E]_0 = [E] + [ES]$

$$K_M = \frac{[E]_0 - [ES][S]}{[ES]} \quad K_M = \frac{[E]_0[S] - [ES][S]}{[ES]} \quad K_M \times [ES] = [E]_0[S] - [ES][S] \quad K_M \times [ES] + [ES][S] = [E]_0[S]$$

$$(K_M + [S])[ES] = [E_0][S] \quad [ES] = \frac{[E_0][S]}{K_M + [S]}$$

since $\dfrac{d[P]}{dt} = k_2[ES]$

$$v_0 = \frac{K_2[E_0][S]}{K_M + [S]}$$

since $v_{max} = k_2[E]_0$

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Q10.4b

We know that the Michaelis Menten derivation for the following reaction:

$$E + S \rightleftharpoons ES \longrightarrow E + P \tag{10.E.30}$$

However, what if the reaction took place in a different scenario whereby:

$$E + S \rightleftharpoons ES_1 \longrightarrow ES_2 \longrightarrow E + P \tag{10.E.31}$$

What would be the corresponding Michaelis-Menten Ebe quation now?

S10.4b

This is the an outline for determining an expression for the rate of substrate conversion in the given case:

1. Set up the reaction with rate constants, assuming $k_{-2} \approx k_{-3} \approx 0$:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1 \overset{k_2}{\longrightarrow} ES_2 \overset{k_3}{\longrightarrow} E + P \tag{10.E.32}$$

2. Set up the differential equations describing the reaction, i.e. the rate of change for each component with time. The rate of substrate change, for example, will be

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES_1] \tag{10.E.33}$$

3. Choose initial conditions and set up two equations for conservation of mass. For example, the initial concentration of enzyme must equal the sum of the concentrations of E, ES_1 and ES_2.
4. Make the *steady-state assumpt*: assume that the concentrations of the intermediate complexes do not change on the time-scale of product formation, i.e.

$$\frac{d[ES_1]}{dt} \approx \frac{d[ES_2]}{dt} \approx 0 \tag{10.E.34}$$

5. Solve for $-r_S$, the negative rate of substrate conversion, obtaining the Michaelis-Menten expression describing the kinetics of the given situtation.

Q10.4c

Prove that K_s equals the concentration S when the initial rate is half its maximum value.

S10.4c

We have: $v_o = \dfrac{V_{max}[S]}{K_M + [S]}$

Divided both said by 1 $\dfrac{1}{v_o} = \dfrac{K_M + [S]}{V_{max}[S]}$

$$\frac{1}{v_o} = \frac{K_M}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v_o} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$

When the initial rate is half its maximum value: $v_o = \dfrac{V_{max}}{2}$

$$\frac{2}{V_{max}} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$

$$\frac{2}{V_{max}} - \frac{1}{V_{max}} = \frac{K_M}{V_{max}[S]}$$

$$\frac{1}{V_{max}} = \frac{K_M}{V_{max}[S]}$$

$$\frac{V_{max}}{V_{max}} = \frac{K_M}{[S]}$$

$$[S] = K_M$$

Q10.5a

An enzyme that has a K_m value of 4.6×10^{-5} M is studied at an initial substrate concentration of 0.041 M. After a minute, it is found that 7.3 uM of product has been produced. Calculate the value of Vmax and the amount of product formed after 4.5 minutes.

Q10.5b

An solution initially contains a catalytic amount of an enzyme with K_M = 1.5 mM, 0.25 M of substrate, and no product. After 45 seconds, the solution contains 25 µM of product. Find V_{max} and the concentration of product after 2.0 minutes.

Hint: [S] >> K_M

S10.5b

1. Find the initial velocity.

$$v_0 = \frac{25\ \mu M}{0.75\ min} \tag{10.E.35}$$

$$v_0 = 33.3\ \mu M/min \tag{10.E.36}$$

2. Use v_0, [S], and K_M to solve for V_{max}.

$$v_0 = \frac{V_{max}[S]}{K_M + [S]} \tag{10.E.37}$$

$$V_{max} = v_0 \left(\frac{K_M}{[S]} + 1 \right) \tag{10.E.38}$$

$$V_{max} = 33.3\ \mu M/min \left(\frac{1.5\ mM}{0.25\ M} \times \frac{M}{1000\ mM} + 1 \right) \tag{10.E.39}$$

$$V_{max} = 33.3\ \mu M/min \tag{10.E.40}$$

Notice that v_0 = V_{max}. Since [S] >>K_M, the reaction will continue with a velocity of V_{max} for the remainder of the two minutes.

3. Predict [P] after 2.0 minutes at the rate V_{max}.

$$[P] = V_{max} \times t \tag{10.E.41}$$

$$[P] = 33.3\ \mu M/min \times 2.0\ min \tag{10.E.42}$$

$$[P] = 66.6\ \mu M \tag{10.E.43}$$

Q10.5c

A particular enzyme at a research facility is being studied by a group of graduate students. This enzyme has a K_m value of 5.0 X 10^{-6} M. The students study this enzyme with an initial substrate concentration of 0.055 M. At one minute, 7 µM of product was made. What is the amount of product produced after 5 minutes. What is the V_{max} ?

S10.5c

$V_o = V_{max}[substrate]/K_m + [substrate]$ 7.0 X 10^{-6} M = V_{max} (0.055 M) / (5.0 X 10^{-6} M + .055 M) V_{max} = 7.1 X 10^{-6} M/min At 5 minutes the amount of product formed is:

Q10.5d

Calculate the value v_o if an enzyme has $K_M = 5.4 * 10^{-4}$, value $V_{max} = 48 \mu M min^{-1}$ and $[S] = 0.001M$

S10.5d

We have: $v_o = \frac{V_{max}[S]}{K_M + [S]}$

$$v_o = \frac{48 * 0.001}{5.4 * 10^{-4} + 0.001} = 31 \mu M min^{-1}$$

Q10.6a

Given the values, [S]/10^-4 M 3.0 4.6 10.5 16.5

vo/10^-6 M * min^-1. 2.64 3.5 6.2 7.8

construct a Lineweaver-Burk plot, and assuming Michaelis-Menten kinetics, calculate the values of Vmax, Km, and k2 using the constructed plot.

Q10.6b

The data below represents the data recorded after the hydrolysis of a substrate by an enzyme.

[S]/10-4 M	2.1	4.2	9.3	14.2
vo / 10-6 M · min-1	1.2	3.1	6.3	9.1

Calculate V_{max}, K_m and K_2 using a Lineweaver-Burk plot. Assume Michaelis. Given $[E}_0$ is 5.0 X 10^{-6} M

S10.6b

| (1/[S])/10-3 M-1	(1/vo)/ 10-5 M-1 · min
4.8	8.3
2.4	3.2
1.1	1.6
0.7	1.1
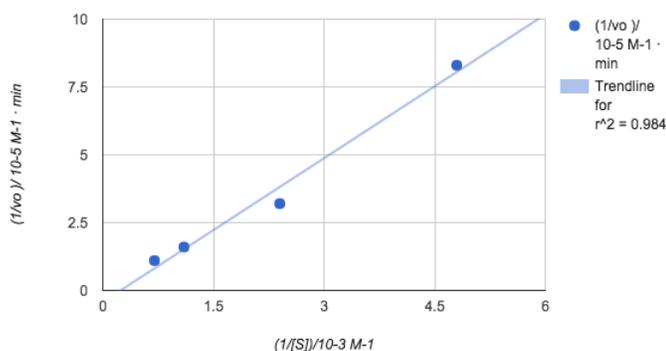
Figure: Lineweaver-Burk Plot

The linear equation for the graph above is: y=176x+4.23X10^4 To solve for V_{max} use: Intercept= 1/V_{max} V_{max} = 1/(.4.23X10^4) = 2.36 X 10^{-5} To solve for K_m use Slope= K_m/V_{max} (176)(2.36 X 10^{-5}) = 4.2 X 10^{-5} To solve for K_2 use K_2 = V_{max}/ $[E}_0$ = (2.36 X 10^{-5})(5.0 X 10^{-6} M)= 4.72 min^{-1}

Q10.6c

Using the table below, calculate the K_M, V_{max}, and slope.

$\frac{1}{V_0}(10^{-3}\frac{sec}{M^{-1}})$	(10.E.44)	$\frac{1}{[S]}(10^2 M^{-1})$	(10.E.45)
2.9		0.3	
3.2		1.2	

$\frac{1}{V_0}\,(10^{-3}\,\frac{sec}{M^{-1}})$	(10.E.44)	$\frac{1}{[S]}\,(10^2 M^{-1})$	(10.E.45)
4.4		1.8	
5.1		3.3	

S10.6c

$\text{slope} = \dfrac{5.1 \times 10^{-3} - 2.9 \times 10^{-3}}{3.3 \times 10^2 - 0.3 \times 10^2} = 6.73 \times 10^{-6}$ $6.73 \times 10^{-6} \times 0.3 \times 10^2 + \dfrac{1}{V_{max}} = 2.9 \times 10^{-3}$ $\dfrac{1}{V_{max}} = 0.00270$

$V_{max} = 370.62 Ms^{-1}$ $6.73 \times 10^{-6} \times \dfrac{-1}{K_M} + 0.00270 = 0$ $\dfrac{-1}{K_M} = -0.00943$ $K_M = 106.044$

Q10.6d

Given the value $[S]$= 0.00032 and $K_M = 3.5 * 10^{-5}$. Find the ratio between v_o and V_{max}

S10.6d

$v_o = \dfrac{V_{max}\,[S]}{K_M + [S]}$

Divided both side by 1: $\dfrac{1}{v_o} = \dfrac{K_M}{V_{max}\,[S]} + \dfrac{1}{V_{max}}$

Divided both side by 1 again: $v_o = \dfrac{V_{max}\,[S]}{K_{max}} + V_{max}$

$v_o = V_{max} \left(\dfrac{[S]}{K_{max}} + 1 \right)$

$\dfrac{v_o}{V_{max}} = \dfrac{[S]}{K_M} + 1$

$\dfrac{v_o}{V_{max}} = \dfrac{0.00032}{3.5 * 10^{-5}} + 1 = \dfrac{71}{7} = 10.14$

Q10.7a

From this graph determine the K_M and V_{max}?
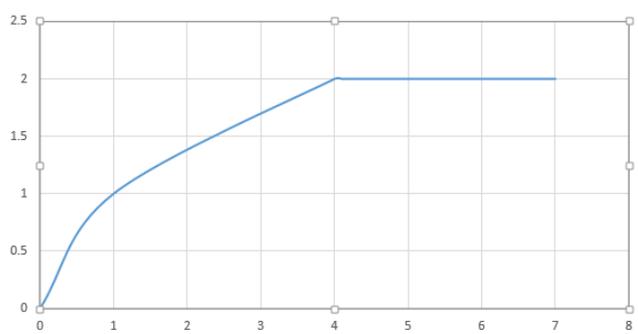
S10.7b

From his graph we can see that the value K_M is 2. Then we look to see where K_M is half. At that point, we see that $K_M/2$ is 1 and the x-value for that coordinate is 1. This means V_{max} is 1.

Q10.7b

Neutral sphingomyelinase 2 converts sphingomyelin into ceramide and phosphocholine. Assume its V_{max} is 35 µM min^{-1}. When you provide 3 x 10^{-5} M of sphingomyelin, you observe an initial velocity of 6.0 µM min^{-1}. Calculate the K_M for this reaction, rounding to 3 significant figures.

https://chem.libretexts.org/@go/page/41336

S10.7b

$$\frac{1}{V_o} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}} \tag{10.E.46}$$

$$\frac{1}{6.0} = \frac{K_M}{(35)(30)} + \frac{1}{35} \tag{10.E.47}$$

$$\frac{1}{6} - \frac{1}{35} = \frac{K_M}{1050} \tag{10.E.48}$$

$$K_M = 145 \text{ µM}$$

Q10.8a

We are given a second order equation: r=k[A][B]. The concentration of A is 0.05g and the concentration of B is 2.5g. We decide this difference is great enough to treat this as a pseudo first reaction? What concentration is held constant and why? Write the new equation.

S10.8a

We hold B constant because the concentration is so much larger, so it should be close to constant for the reaction. The new equation would be r=k'[a]

Q10.9a

Name and briefly describe two types of reactions that do not follow Michaelis-Menten kinetics.

S10.9a

Irreversible inhibition - the inhibitor binds covalently and irreversibly to the enzyme Allosteric interactions - the binding of effectors at allosteric sites (away from the active site) influence substrate binding.

Q10.9b

For the reaction mechanism below, how does the concentration of C affect the concentration of B?

$$A \rightleftharpoons B \rightarrow C \tag{10.E.49}$$

S10.9b

Because the second state is *irreversible* (i.e., since arrow), it does not matter if you have a large or little concentration of C, it would not affect B and hence the kinetics of the reaction. However, the concentration of $A\backslash 0 would clearly affect the concentration of \backslash(B$.

10.3: Chymotrypsin: A Case Study

Q1

1. Speculate on how the catalytic rate constant can be determined from the spectrophotogram.
2. How can product be consistently produced if the rate of change of the ES complex is 0?
3. How would the rate of product formation change if:
   a. the substrate concentration were doubled?
   b. the enzyme concentration were doubled?
   c. The reaction was carried out in mono-deuterated water instead of H_2O (comment qualitatively)?
4. Explain the role of hydrogen bonding in protein hydrolysis catalyzed by chymotrypsin.
5. What would the spectrophotogram look like if the reaction proceeded via a steady-state mechanism instead of pre-equilibrium.

S2

1. The catalytic rate constant can be deduced from the graph by simply determining the slope of the line where the reaction demonstrates 0-order kinetics (the linear part).
2. This is pre-equilibrium kinetics in action. The ES complex is formed from E and S at a faster rate than any other step in the reaction. As soon as ES is converted to *ES, another mole of ES is produced from an infinite supply of E + S. This means that the amount of ES and E + S is constantly at equilibrium, and thus the change of either with respect to time is 0.

   a. No change.
   b. Two-fold increase.
   c. Because water is involved in the final, slowest step of the mechanism, deuterating the water would decrease the rate of the overall reaction from 5- to 30-fold.
3. Initially, hydrogen bonding between the enzymes histidine and serine side chains weakens the bond of serine's O-H. This allows a facilitated nucleophilic attack of the hydroxyl oxygen on the substrates carbonyl group. Conversely, in the final step of the reaction, the bound serine oxygen forms a hydrogen bond with a protonated histidine, which allows for easier cleavage from the substrate.
4. The graph would show similar 0-order kinetics, but the line would intercept the Y-axis at an absorbance of 0 instead of the 1:1 mole ratio of nitrophenolate to enzyme.

10.4: Multisubstrate Systems

10.5: Enzyme Inhibition

10.6: Allosteric Interactions

10.7: The Effect of pH on Enzyme Kinetics

10.4: Multisubstrate Systems

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10.7: The Effect of pH on Enzyme Kinetics