Enzymes
Part III: Enzyme kinetics

Dr. Mamoun Ahram
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Kinetics

- Kinetics is deals with the rates of chemical reactions.
- Chemical kinetics is the study of the rates of chemical reactions.
- For the reaction (A → P), The velocity, *v*, or rate, of the reaction is the amount of P formed or the amount of A consumed per unit time, *t*. That is,

$$v = \frac{d[\mathrm{P}]}{dt} \quad \text{or} \quad v = \frac{-d[\mathrm{A}]}{dt}$$

Rate law

- The mathematical relationship between reaction rate and concentration of reactant(s) is the rate law (*the mathematical equation describing how the concentrations of reactants affect the rate of the reaction during a certain period*).
- For the reaction (A → P), the rate law is

$$v = \frac{-d[A]}{dt} = k[A]$$

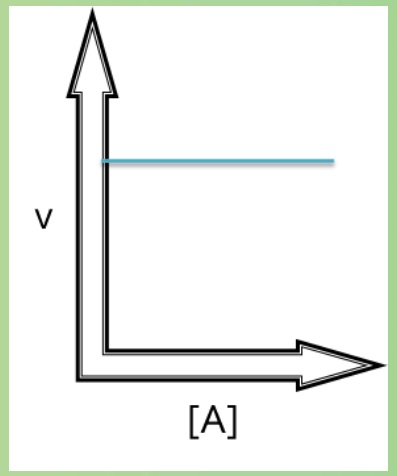
- From this expression, the rate is proportional to the concentration of A, and *k* is the rate constant.
  - *k* has the units of (time) $^{-1}$, usually sec^{-1}.

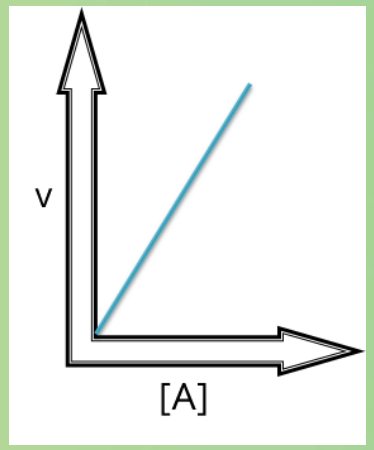
First-order reaction

- Since reaction order refers to the number of molecules involved in a reaction, the simple reaction of (A → P) is a first-order reaction.
- The rate of the reaction is directly proportional to the concentration of the reactant.
- Thus, as the concentration of A is reduced, the rate of the reaction slows down, and vice versa.

Zero- vs. first-order reaction

Overall order	V=	Dimentions of k
Zero	k	$(conc.)(time)^{-1}$
First	k(A)	$(time)^{-1}$





Enzyme kinetics

- The kinetics of the enzyme-catalyzed reactions are different than those of a typical chemical reaction,
  - Enzyme-catalyzed reactions have hyperbolic plots.
- The study of enzyme kinetics addresses the biological roles of enzymatic catalysts.

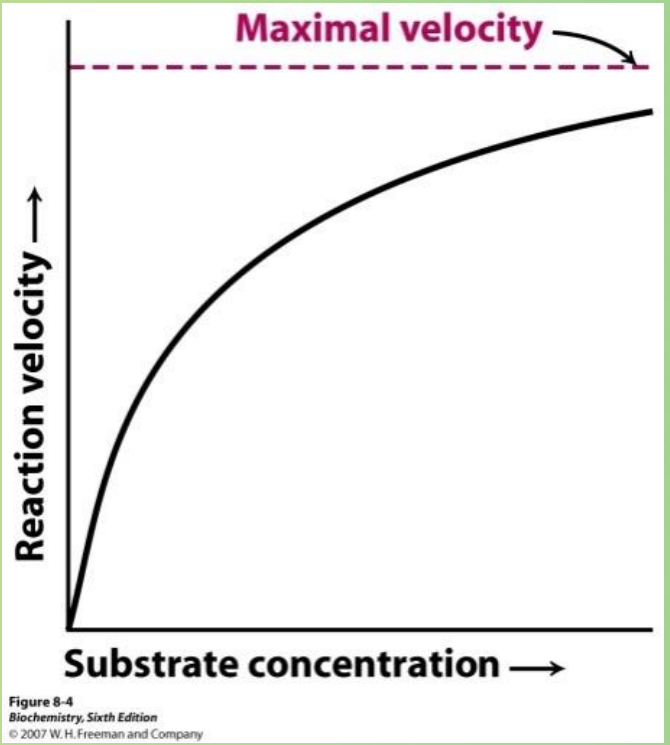
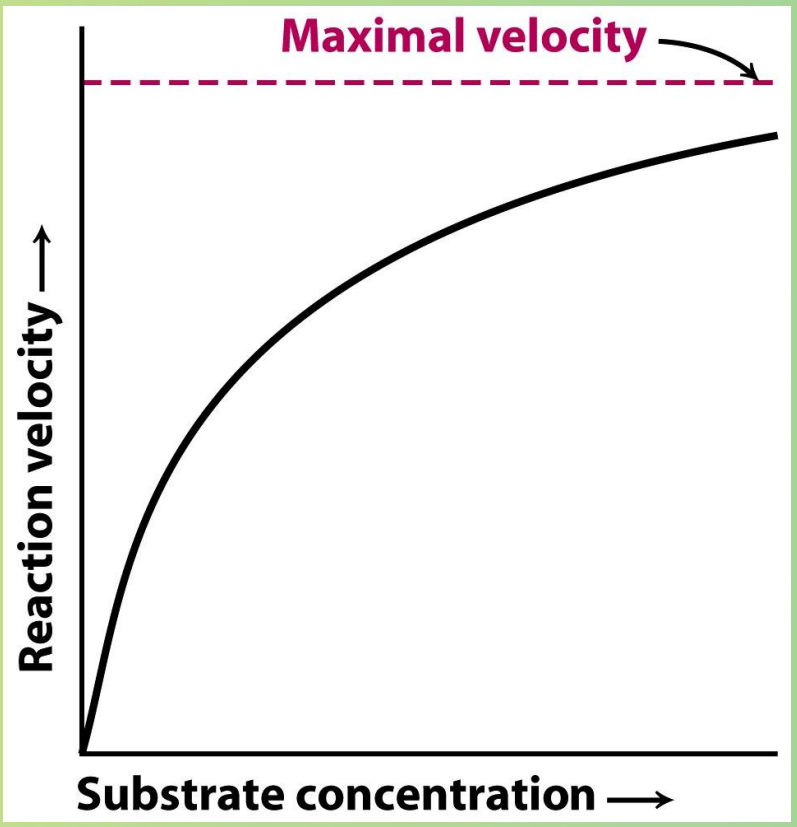


Figure 8-4
Biochemistry, Sixth Edition

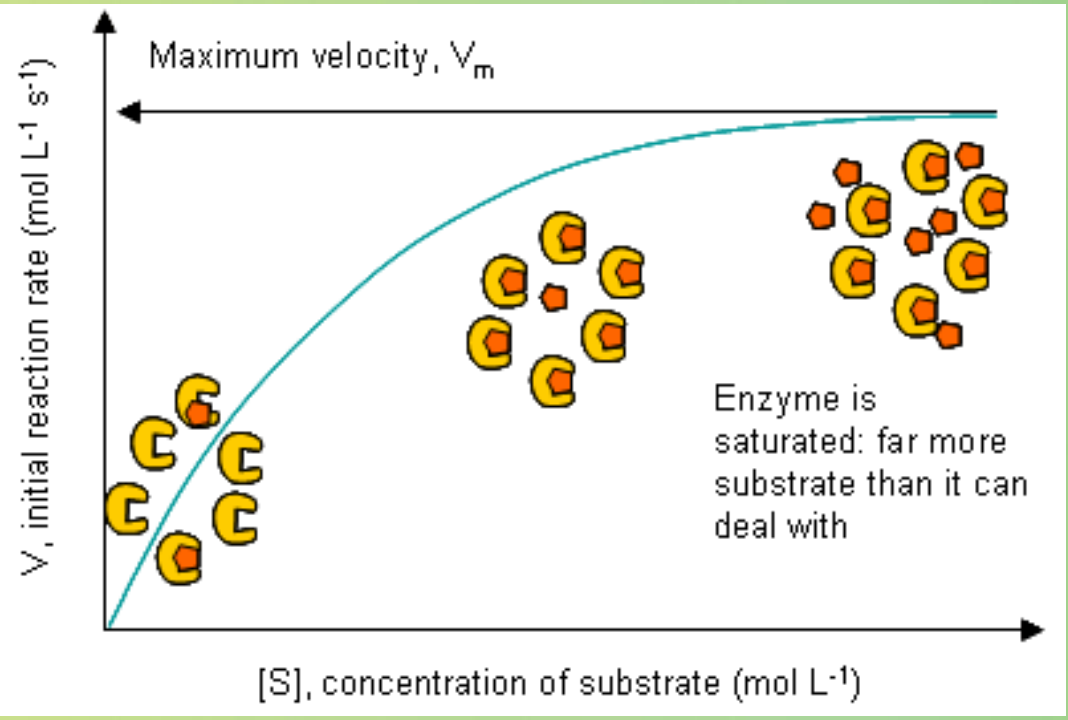
How?

- For many enzymes, initial velocity (V_0) varies with the substrate concentration [S].
- The rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations.



Why?

- **The hyperbolic plot is known as a saturation plot because the enzyme becomes "saturated" with substrate, i.e. each enzyme molecule has a substrate molecule associated with it.**



More explanation

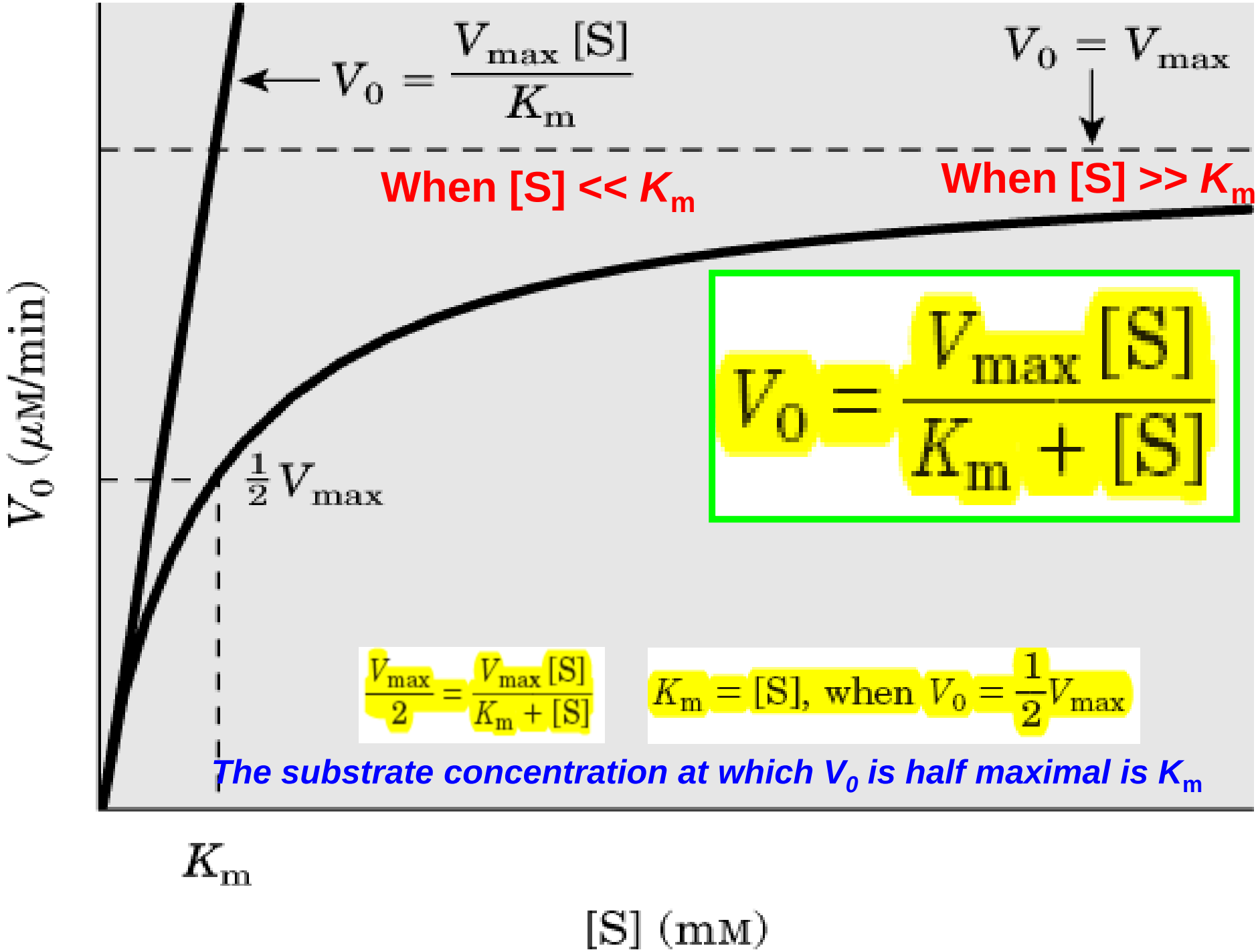
- At a fixed concentration of enzyme, V_0 is almost linearly proportional to [S] when [S] is small.
- However, V_0 is nearly independent of [S] when [S] is large
- The maximal rate, Vmax, is achieved when the catalytic sites on the enzyme are saturated with substrate.
- Vmax reveals the turnover number of an enzyme.
  - The number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.
- At Vmax, the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction.

The Michaelis-Menten equation

- Two scientists, Leonor Michaelis and Maud Menten, proposed a simple model to describe enzyme kinetics.

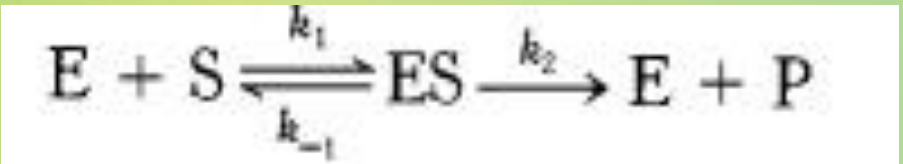
- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_0), substrate concentration [S], a rate constant (K_M) and maximal velocity (Vmax).

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$


$V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
When [S] << K_m
When [S] >> K_m
$V_0 = \frac{V_{max}[S]}{K_m + [S]}$
$\frac{1}{2} V_{max}$
$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$
$K_m = [S]$, when $V_0 = \frac{1}{2} V_{max}$
The substrate concentration at which V_0 is half maximal is K_m
V_0 (μM/min)
K_m
[S] (mM)

The Michaelis constant (Km)

- For a reaction:



- K_M, called the Michaelis constant is

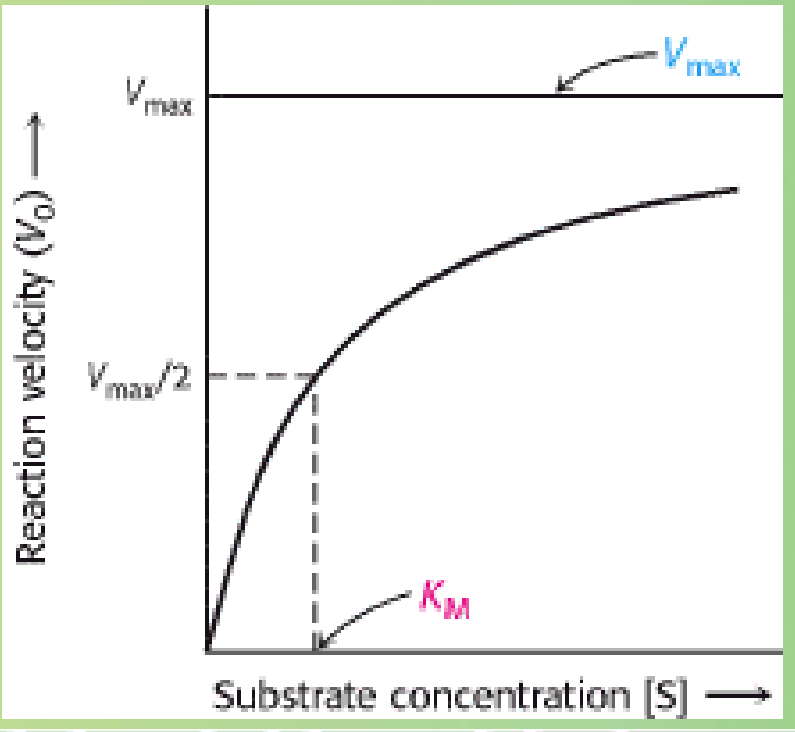
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

- In other words, Km is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex
- K_m describes the affinity of enzyme for the substrate.

Km

- K_M is the concentration of substrate at which half the active sites are filled.
- When [S] = K_M, then V_0 = Vmax/2
- Therefore, it provides a measure of enzyme affinity towards a substrate.
- The lower the K_M of an enzyme towards a substrate is, the higher its affinity to the same substrate is.

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$



$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

- At very low substrate concentration, when [S] is much less than K_M , $V_0 = (K_M)[S]$; that is, the rate is directly proportional to the substrate concentration.
- At high substrate concentration, when [S] is much greater than K_M , V_0 = Vmax; that is, the rate is maximal, independent of substrate concentration.

Note

- Each substrate will generate a unique K_M and Vmax for a given enzymatic process.
- The K_M values of enzymes range widely.
- For most enzymes, K_M lies between 10^{-1} and 10^{-7} M.

table 8–6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

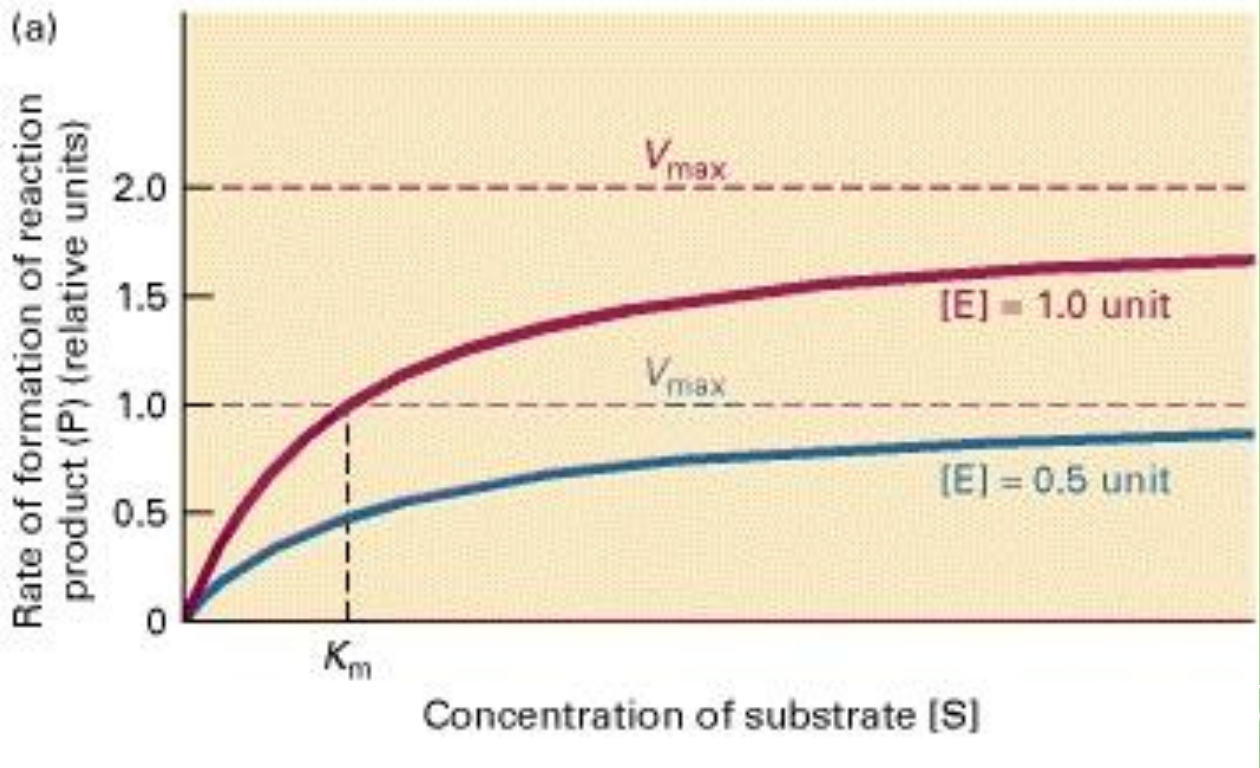
Example

- **A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, Vmax of this enzyme is … & K_M is …?**
  - **5000 & 699**
  - **699 & 5000**
  - **621 & 50**
  - **94 & 1**
  - **700 & 8**

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

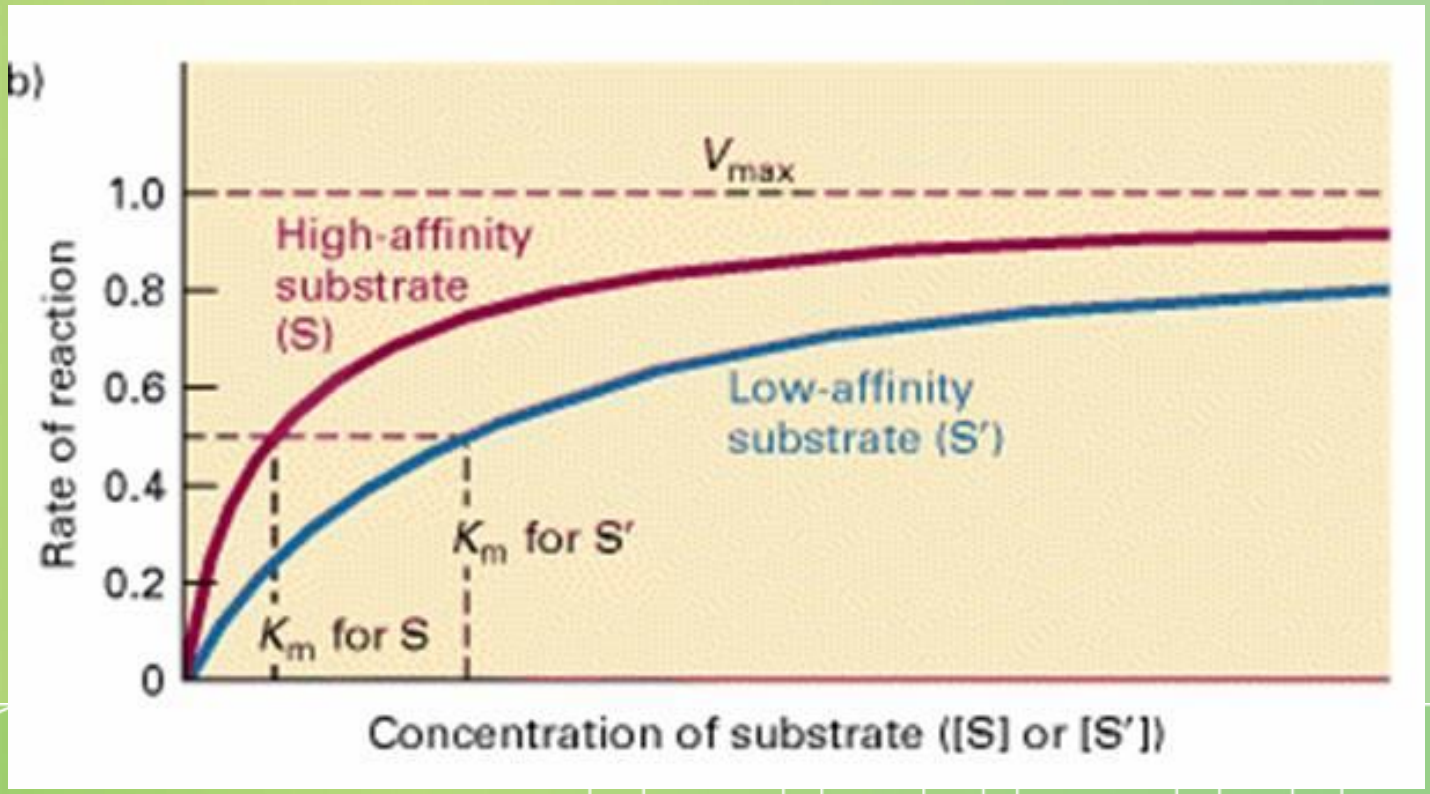
Example 1 of K_M

- **Doubling the concentration of enzyme causes a proportional increase in the reaction rate, so that the maximal velocity V_{max} is doubled; the K_M , however, is unaltered**



Example 2 of K_M

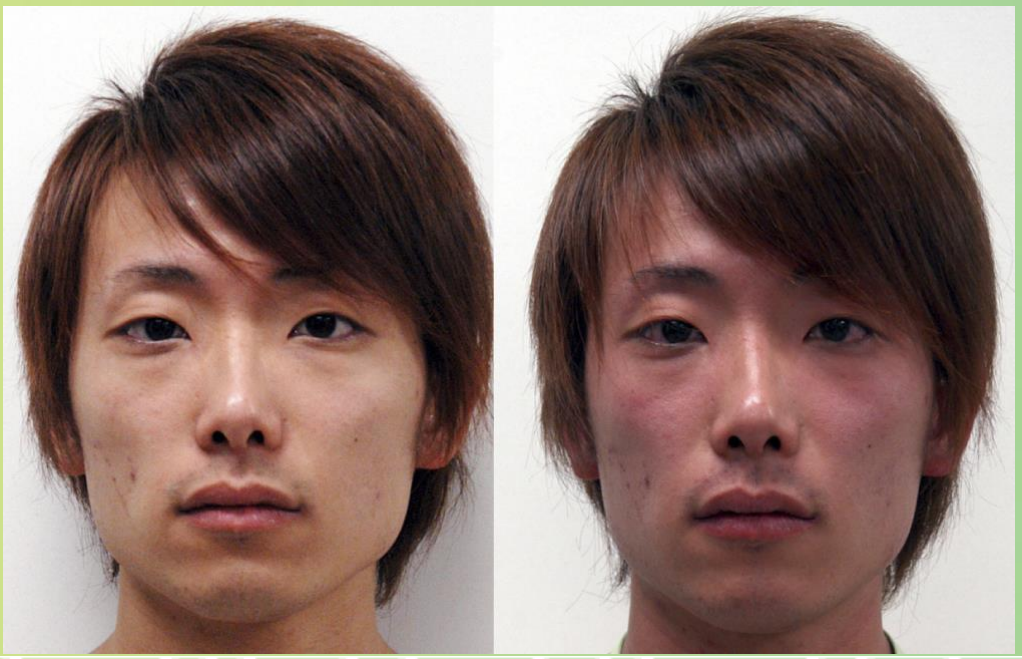
- A reactions is catalyzed by an enzyme with substrate S (high affinity) and with substrate S′ (low affinity).
- Vmax is the same with both substrates, but K_M is higher for S′, the low-affinity substrate.



Biological significance of Km

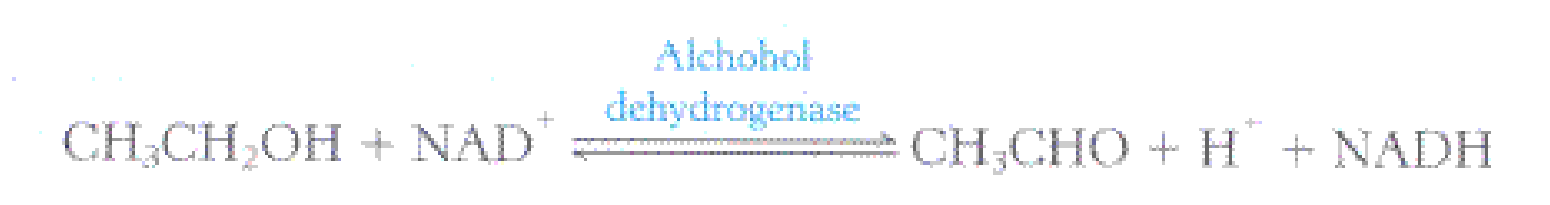
Effect of alcohol

- The physiological consequence of K_M is illustrated by the sensitivity of some individuals to ethanol.
- Such persons exhibit facial flushing and rapid heart rate (tachycardia) after ingesting even small amounts of alcohol.



Enzymatic reactions

- **In the liver, alcohol dehydrogenase converts ethanol into acetaldehyde.**



- **Normally, the acetaldehyde, which is the cause of the symptoms when present at high concentrations, is processed to acetate by acetaldehyde dehydrogenase.**

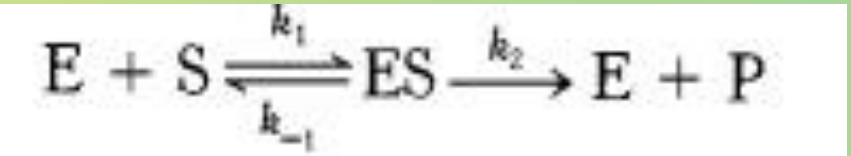
The effect

- Most people have two forms of the acetaldehyde dehydrogenase, a low K_M mitochondrial form and a high K_M cytosolic form.
  - These are called isozymes (keep that in mind)
- In vulnerable persons, the mitochondrial enzyme is less active due to the substitution of a single amino acid, and acetaldehyde is processed only by the cytosolic enzyme.
- Because the cytosolic enzyme has a high K_M, less acetaldehyde is converted into acetate; excess acetaldehyde escapes into the blood and causes the physiological effects.

Measurements of enzyme kinetics

Vmax & kcat

- For the enzymatic reaction



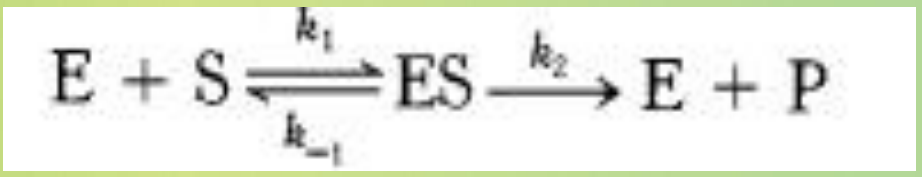
- The maximal rate, V_{max}, is equal to the product of k_2, also known as k_{cat}, and the total concentration of enzyme.

$$V_{max} = k_2 [E]_T$$

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Kcat



- kcat, the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated.
- It describes how quickly an enzyme acts, i.e. how fast the ES complex proceeds to E + P.
- In other words, the maximal rate, Vmax, reveals the turnover number of an enzyme if the total concentration of active sites [E]T is known.
- kcat is a constant you can look up for any given enzyme.

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

Example

- You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 µg of the enzyme in an experiment and the results show that the enzyme at best converts 9.6 µmol of the substrate per min at 25˚C. The turnover number (kcat) for the enzyme is:

A. 9.6 s^{-1} B. 48 s^{-1} C. 800 s^{-1}

D. 960 s^{-1} E. 1920 s^{-1}

Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate.
  - ✓ Hence, k_{cat} is 6×10^5 s^{-1}
  - ✓ 10^4 min^{-1}
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 µs for carbonic anhydrase.
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second.

40,000,000 molecules of H_2O_2 are converted to H_2O and O_2 by ONE catalase molecule within one second

table 8–7

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Kcat vs. Km

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a range of nearly $2X10^7$

K_M values vary over a range of nearly 4000

K_{cat}/K_M, the range is near 10^3

Specificity & Efficiency: Physiological [S]/ K_M

- Most enzymes are not normally fully saturated.
- Under physiological conditions, the [S]/K_M ratio is typically between 0.01 and 1.0
  - Specificity constant (k_{cat}/K_M): is also called catalytic efficiency tells us how rapidly the enzyme binds and how quickly it turns over.

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]}$$

$$V = (k_{cat}/K_M)[E][S]$$

- k_{cat}/K_M (M^{-1} min^{-1}) is indicative of:
  - ✓ Enzyme's substrate specificity: the higher the ratio, the higher the specificity.
  - ✓ Enzyme's catalytic efficiency:

    the higher the ratio, the more efficient the enzyme.

Rate of reaction (velocity)

- Rate of reaction is calculated as <u>concentration</u> of substrate disappearing (or concentration of product appearing) per unit time (mol L^{-1} . sec^{-1} or M . sec^{-1}).

Enzyme activity

- In order to measure enzyme activity, we measure the number of moles of substrate disappearing (or products appearing) per unit time (mol·. sec^{-1})

- In other words,

  enzyme activity = rate of reaction × reaction volume

Specific activity

- Specific activity is a measure of enzyme purity and quality.
- It is calculated as moles of substrate converted per unit time per unit mass of enzyme (mol . sec^{-1} . g^{-1}).
- In other words,

Specific activity =enzyme activity / mass of enzyme (grams)

- This is useful in determining enzyme purity after purification.

Turnover number

- **Turnover number (kcat) is related to the specific activity of the enzyme where it is**

  Turnover number = specific activity × molecular weight of enzyme

  - **It is expressed as moles of substrate converted per unit time (usually per second)/moles of enzyme (min^{-1} or sec^{-1})**

- **Remember: $k_{cat} = V_{max} / [E]_T$**

Sample calculations:

A solution contains initially **25.0×10^{-4} mol L^{-1}** of peptide substrate and **1.50 µg chymotrypsin**, in **2.5 mL** volume. **After 10 minutes, 18.6×10^{-4} mol L^{-1}** of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol^{-1}**.

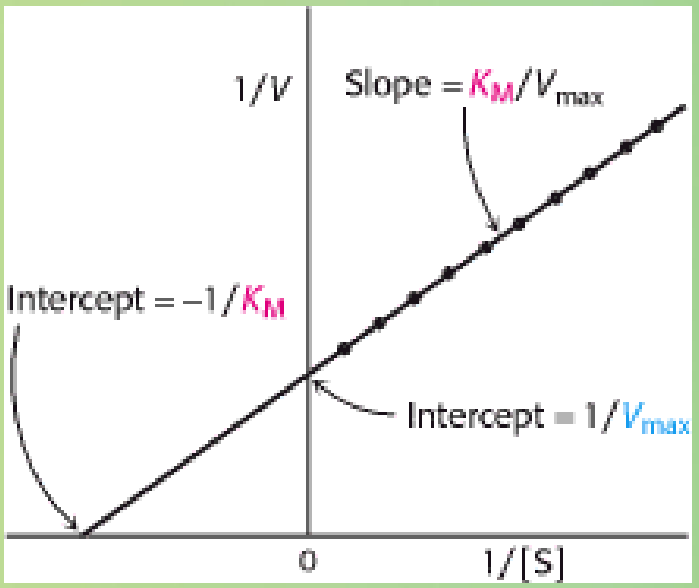
peptide substrate consumed	= 6.4 x 10^{-4} mol L^{-1} in 10 minutes
Rate of reaction	= **6.4 x 10^{-5} mol L^{-1} min^{-1}**
Enzyme activity	= 6.4 x 10^{-5} mol L^{-1} min^{-1} x 2.5 x 10^{-3} L
(rate × volume)	= **1.6 x 10^{-7} mol min^{-1}**
Specific activity	= 1.6 x 10^{-7} mol min^{-1} / 1.50 µg
(activity / mass)	= **1.1 x 10^{-7} mol $µg^{-1}$ min^{-1}**
Turnover number	= 1.1 x 10^{-7} mol $µg^{-1}$ min^{-1} x 25,000 g mol^{-1} x 10^{6} µg g^{-1}
(sp. act. × molar mass)	= 2.7 x 10^{3} min^{-1} = **45 s^{-1}**

Disadvantage of Michaelis-Menten equation

- Determination of Km from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach Vmax.
- This prevents the calculation of both Vmax and K_M.

The Lineweaver-Burk or double-reciprocal plot

- A plot of 1/ V_0 versus 1/[S], called a Lineweaver-Burk or double-reciprocal plot, yields a straight line with an intercept of 1/Vmax and a slope of K_M /Vmax.
- The intercept on the x-axis is -1/ K_M.

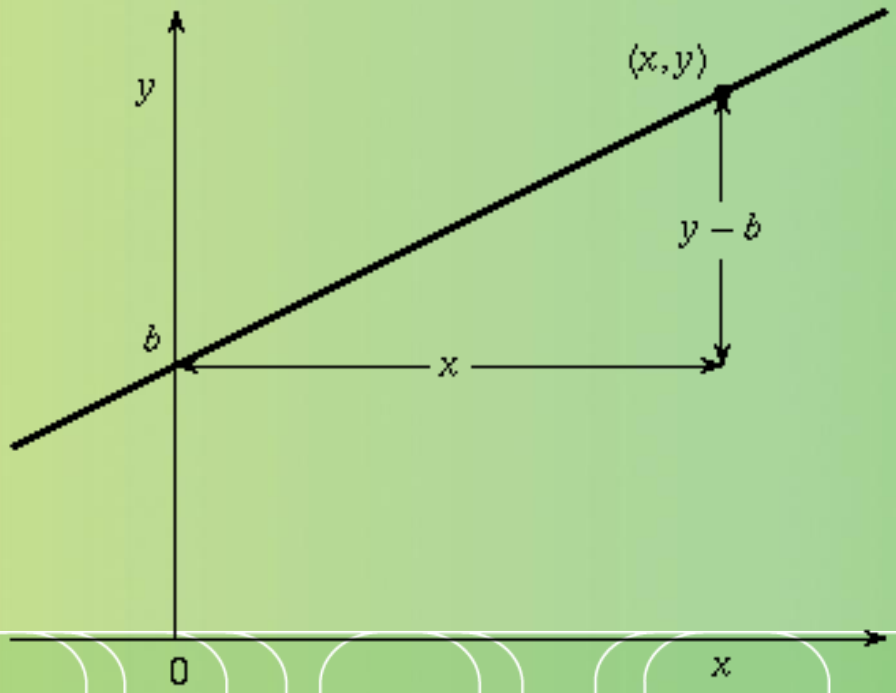


$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

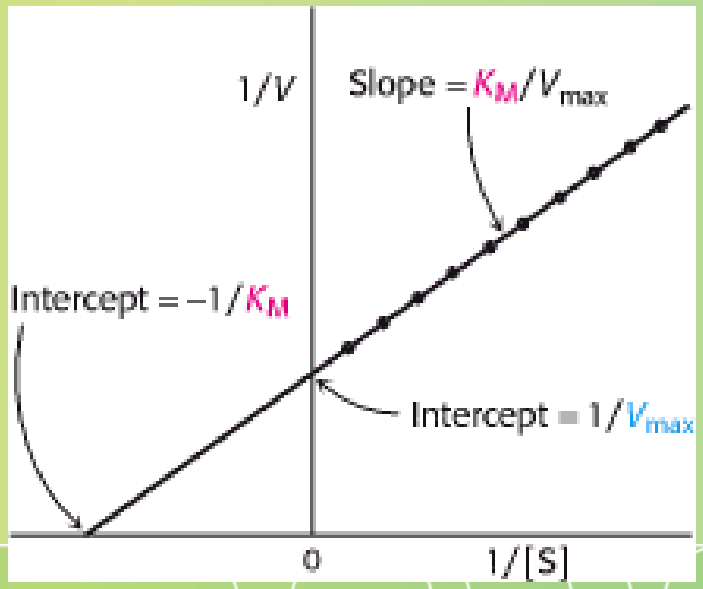
- y is y-axis = 1/ V_0
- x is x-axis = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

- If x = 0, then y = b (x-axis is 0, then y-intercept = 1/Vmax)



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

If y = 0, then mx = -b (y-axis is 0, then x-intercept = -1/Km)

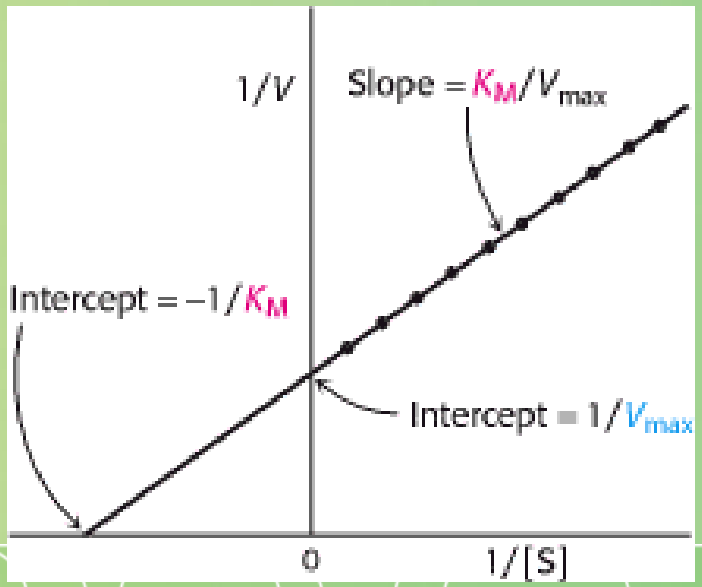
How?

0 = 1/Vmax + (K_M/Vax) . (1/[S])

-1/Vmax = (K_M/Vax) . (1/[S])

-1 = K_M . (1/[S])

-1/ K_M= 1/[S]

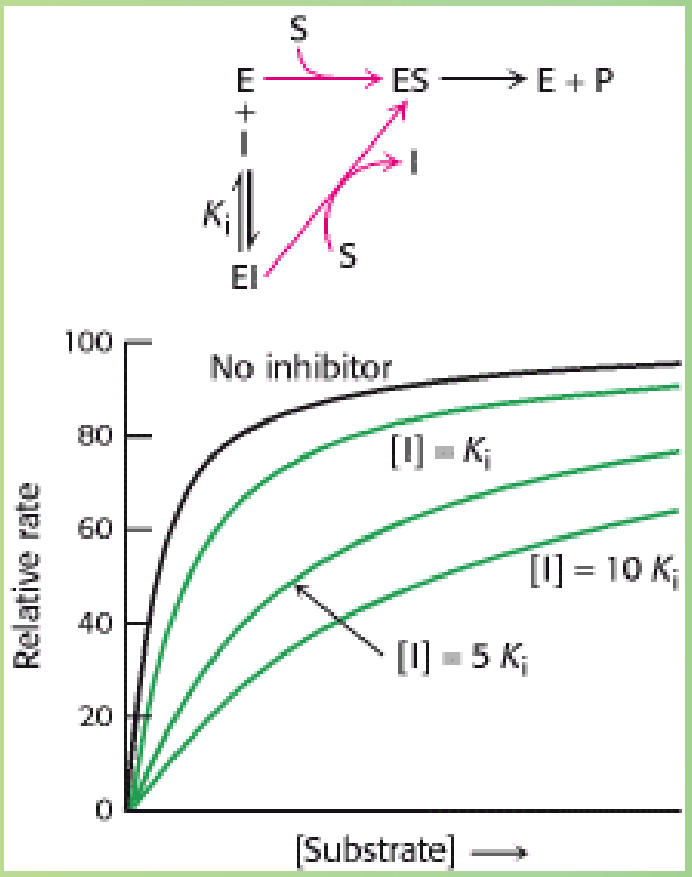


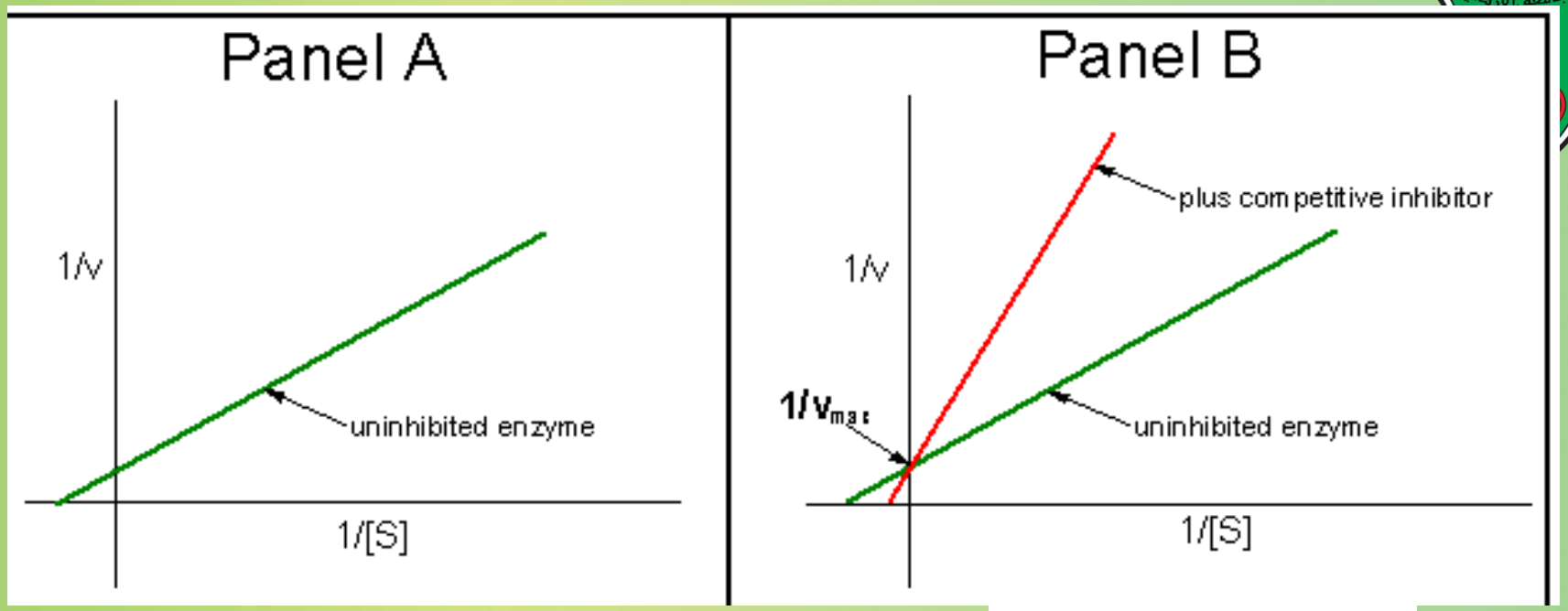
Enzyme inhibitors

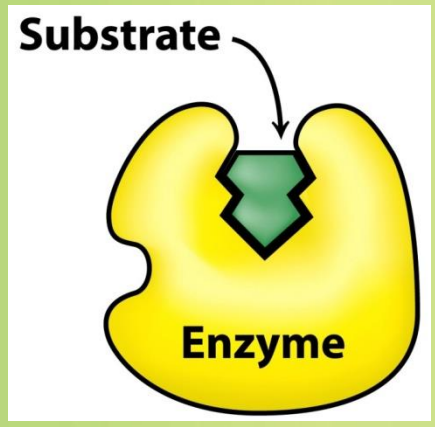
- Enzyme inhibition can be either reversible or irreversible.
- An irreversible inhibitor dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme, mainly covalently.
  - The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme.
- Reversible inhibition is characterized by a rapid dissociation of the enzyme-inhibitor complex.
  - Usually these inhibitors bind to enzymes by non-covalent forces and the inhibitor maintains a reversible equilibrium with the enzyme.
    - Reversible inhibitors can be competitive, noncompetitive, and uncompetitive inhibitors.

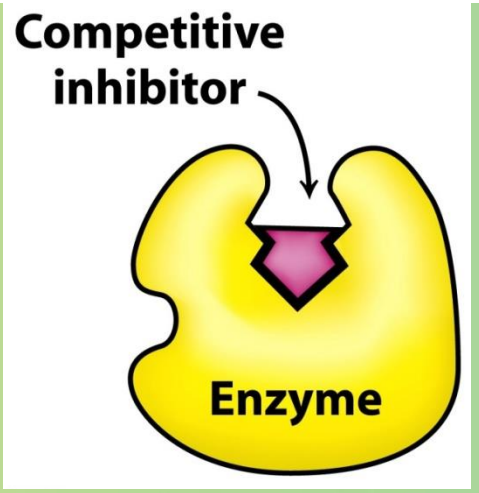
Competitive inhibition

- In competitive inhibition, the inhibitor competes with the substrate for the active site.
- Because increasing the amount of substrate can overcome the inhibition, Vmax can be reached in the presence of a competitive inhibitor.
- In the presence of a competitive inhibitor, an enzyme will have the same Vmax as in the absence of an inhibitor, but the value of K_M is increased.



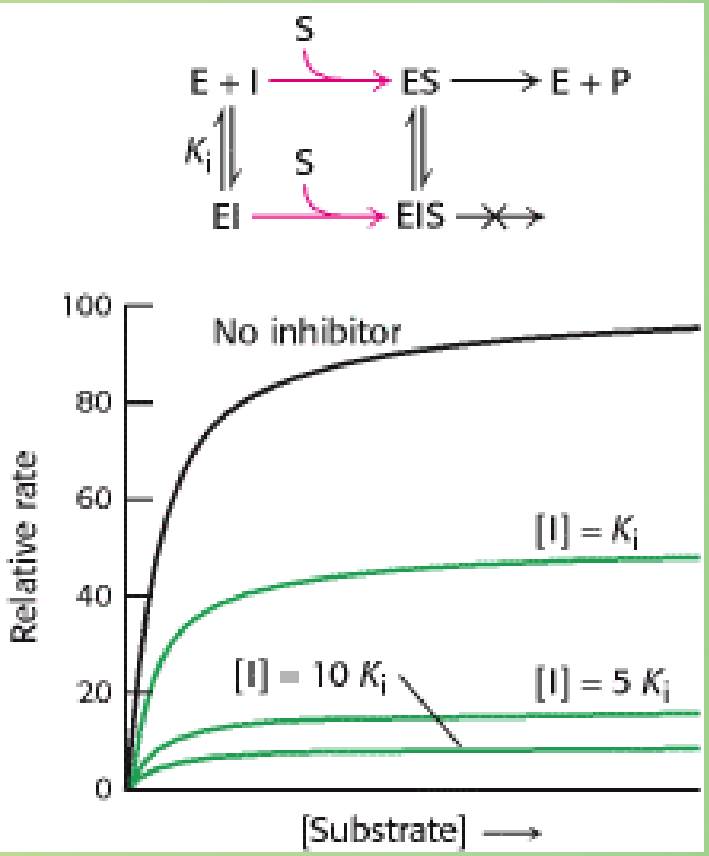

Panel A
1/v
uninhibited enzyme
1/[S]
Panel B
plus competitive inhibitor
1/v
1/v
uninhibited enzyme
1/[S]

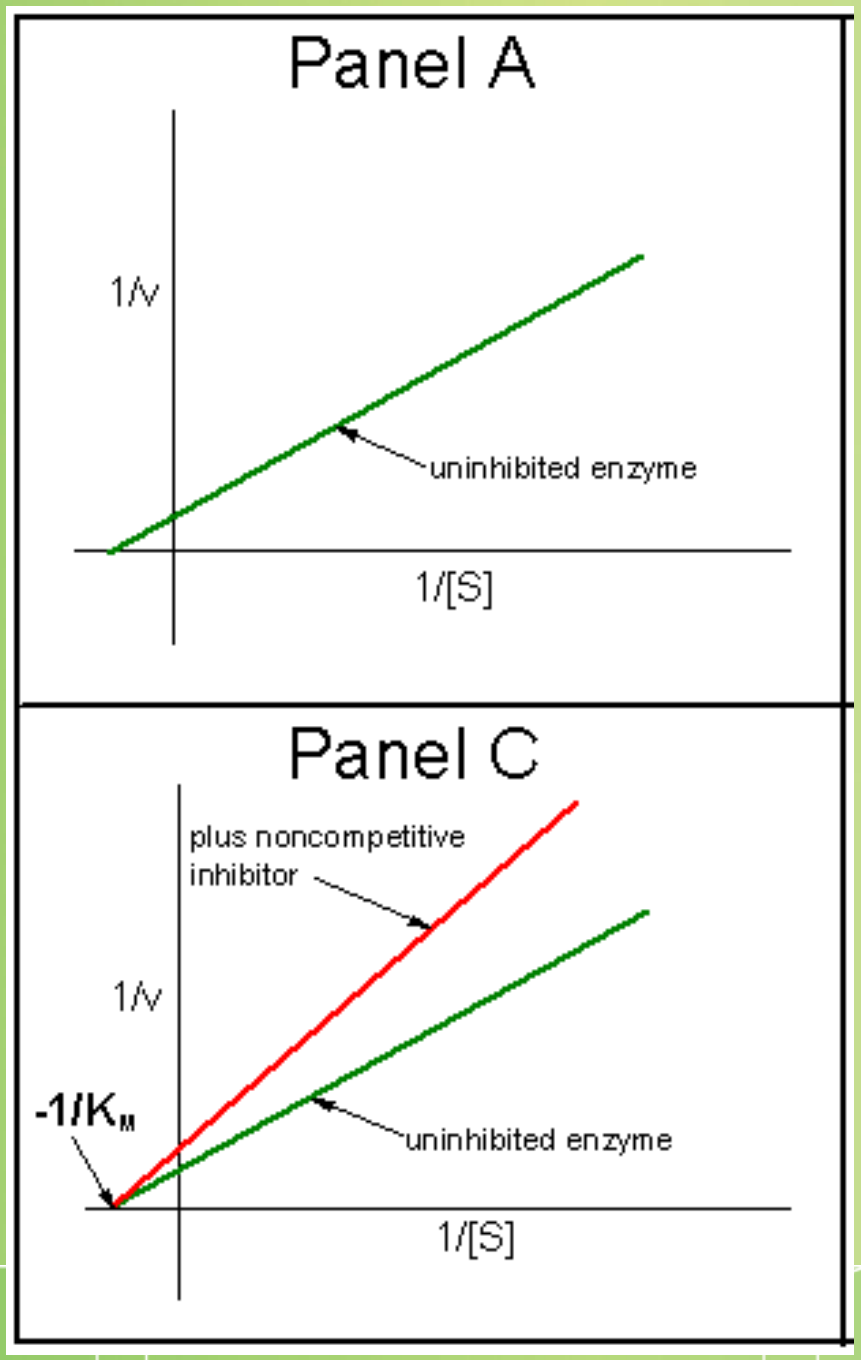

Substrate
Enzyme

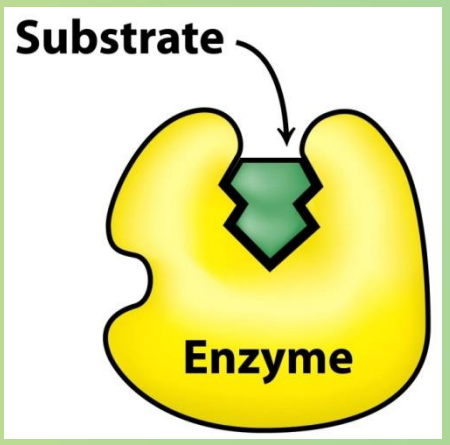

Competitive inhibitor
Enzyme

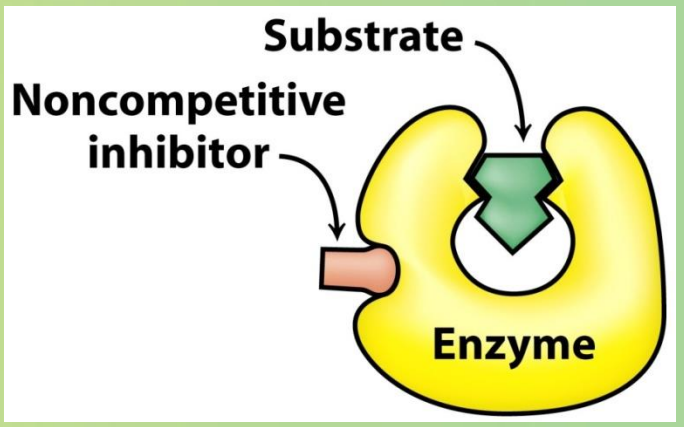
Noncompetitive inhibition

- In noncompetitive inhibition, the inhibitor binds E or ES complex at a site other than the catalytic site
- Substrate can also bind to the enzyme-inhibitor complex
- However, the enzyme-inhibitor-substrate complex does not proceed to form product
- The value of Vmax is decreased while the value of K_M is unchanged
- Noncompetitive inhibition cannot be overcome by increasing the substrate concentration




Panel A
1/v
uninhibited enzyme
1/[S]
Panel C
plus noncompetitive
inhibitor
1/v
-1/K_M
uninhibited enzyme
1/[S]


Substrate
Enzyme


Substrate
Noncompetitive
inhibitor
Enzyme