Lecture 14 (10/15/25)

ENZYMES: Binding & Catalysis

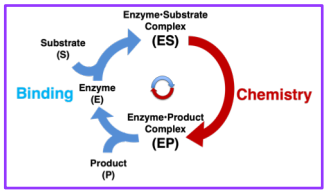
A. Binding; Binding curves;
B. Catalysis
   1. Catalytic power
   2. Nomenclature; Cofactors; Reaction; names
   3. Transition State Theory
      a. Energetics (thermodynamics) vs. kinetics
      b. Lower activation energy; negative $\Delta\Delta G^{\ddagger}$
   4. Catalytic strategies
      a. binding the transition state (What an enzyme must do)
         i. Position (proximity)
         ii. Polarization
         iii. Strain
         iv. desolvation
   5. Mechanistic strategies
      a. Howan enzyme does it
         i. Acid-base catalysis
         ii. Covalent catalysis
         iii. Metal-ion catalysis

C. Quantifying the Catalytic Power: Kinetics
   1. Review
   2. Enzyme Kinetics
      a. Rate vs. [S] for enzyme catalyzed reaction
         i. initial rate (v_0)
      b. ES complex
         i. Reactions
            - Binding reaction
            - Catalytic reaction
         ii. Meaning of rate curve: hyperbolic curve
         iii. Rate expression; Michaelis-Menten Kinetics
            - Assumptions
            - M-M equation derivation
            - V_{max} & K_m values
      c. Meaning of rate expression (M-M equation)
         i. [S] = K_m
         ii. [S] >> K_m
         iii. [S] << K_m

- Reading: **Ch6; 184-188**
  188-195
- Homework #13, 14

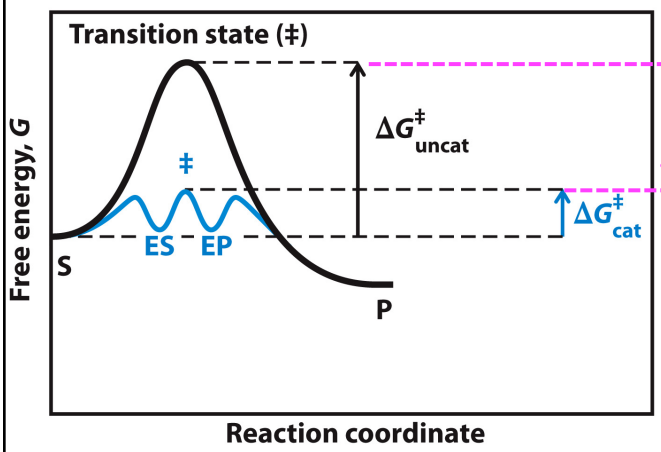
NEXT

- Reading: Ch6; 191, 197-200
- Homework #15



Enzymes

Enzymes Can Decrease $\Delta G^{\ddagger}$



Recall: Where do enzymes get the energy to do this?

$\Delta\Delta G^{\ddagger}$ ⊖

$\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{cat} - \Delta G^{\ddagger}_{uncat}$

There is always a difference in activation energies that yield a negative $\Delta\Delta G^{\ddagger}$. This is the amount of energy that must be supplied somehow to the reaction by the enzyme

ENZYMES

(The WHAT and the How)

What must ALL enzymes to to achieve these amazing rate enhancements?

How do enzymes do what they do…….. Mechanistically?

Enzymes

*Catalytic Strategies

versus

Mechanistic Strategies

WHAT must Enzymes do to lower Activation Energies?
-nearly all enzymes do these

HOW do Enzymes lower Activation Energies?
- enzymes may use none, one, or more of these

*Textbook uses this term a bit incorrectly. What they term Catalytic strategies are really those that answer HOW enzymes decrease the activation energy. The HOW-to strategies are really "Mechanistic" strategies.

Enzymes

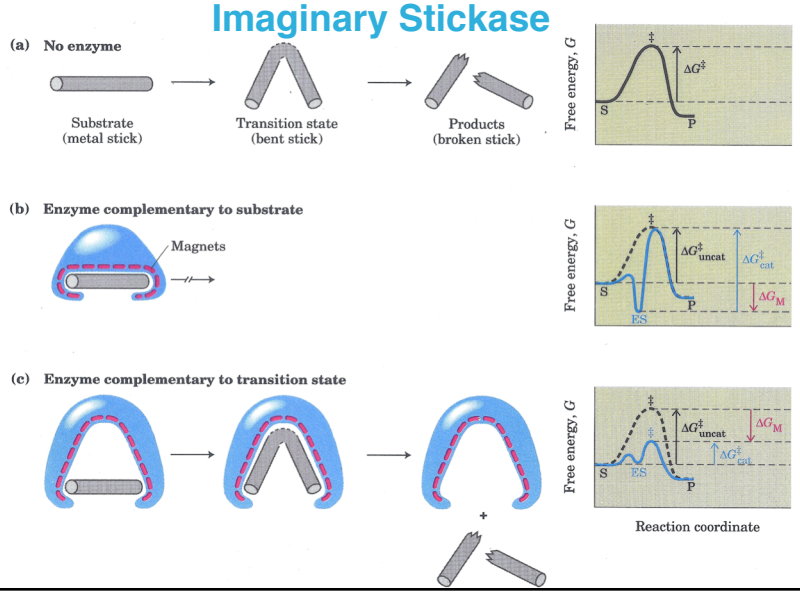
Catalytic Strategies

Effect on $\Delta\Delta G^{\ddagger}$

- Position Effects: bind substrates where they need to be for reaction (rather than depending on random collisions) — $\Delta S -$
- Polarization of bonds: make substrates more reactive by polarizing bonds (make better nucleophiles, electrophile, or leaving groups) (Electrostatics) — $\Delta H -$
- Strain of bonds: bind substrates in such a way that they "look" like products (put strain on bonds that are to be broken (sessile)) (Geometry) — $\Delta S -$
- De-solvation: assist in removal of water shell around substrates or adding to products upon release (S & P are usually in direct contact with residues at the active site (no water)) — $\Delta H -$

Enzymes

Illustration of TS Stabilization Idea: Imaginary Stickase



Enzymes

Catalytic Strategies

versus

Mechanistic Strategies

WHAT must Enzymes do to lower Activation Energies?
-nearly all enzymes do these

HOW do Enzymes lower Activation Energies?
- enzymes may use none, one, or more of these

Enzymes

Mechanistic Strategies

HOW do Enzymes lower Activation Energies?
- enzymes use may use none, one, or more of these

There are THREE major strategies used by enzymes:

- acid-base catalysis: give and take protons
- covalent catalysis: change reaction paths
- metal ion catalysis: use redox cofactors, pK_a shifters

Enzyme Kinetics

What Is Enzyme Kinetics?

- **Kinetics** is the study of the rate at which compounds react.
- The rate of enzymatic reaction is affected by:
  - enzyme
  - substrate
  - effectors
  - Temperature
  - Reaction conditions (salts, buffers, pH)

Enzyme Kinetics

Why Study Enzyme Kinetics?

- Quantitative description of biocatalysis
- Determine the order of binding of substrates
- Elucidate acid-base catalysis
- Understand catalytic mechanism
- Find effective inhibitors (drugs)
- Understand regulation of activity

Enzyme Kinetics

Review of Chemical Kinetics

Consider a simple reaction: $A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$

Thermodynamics tells us: $K_{eq} = \frac{[B]}{[A]}$ and $K_{eq} = \frac{k_1}{k_{-1}}$ making product / making reactant

Kinetics tells us: The rate of a chemical reaction (v) is proportional to the concentration of the specie(s) participating in the rate limiting step. $v \propto [A]$

Furthermore, the reaction rate (v) is determined by measuring how much A disappears **as a function of time** or how much B appears **as a function of time**. $v = -d[A]/dt = d[B]/dt$

Suppose that we can readily measure the disappearance of A. The velocity of the reaction is given by solving the differential equation above to get the formula below, where k_1 is a proportionality constant.

$$v = k_1[A]$$

When the velocity of a reaction is directly proportional to a single reactant concentration, the reaction is called a first-order reaction and the proportionality constant has the units s^{-1}.

During an enzyme catalyzed reaction when the enzyme is saturated with substrate, it's first-order reaction….. only dependent on [E] with k_1 as k_{cat}

Enzyme Kinetics

Review of Chemical Kinetics

When the velocity of a reaction is directly proportional to the concentrations of **two** reactants, the reaction is called a second-order reaction and the proportionality constant has the units $M^{-1}s^{-1}$.

Many important biochemical reactions are biomolecular or second-order reactions.

$2\,A \rightarrow P$ or $A + B \rightarrow P$

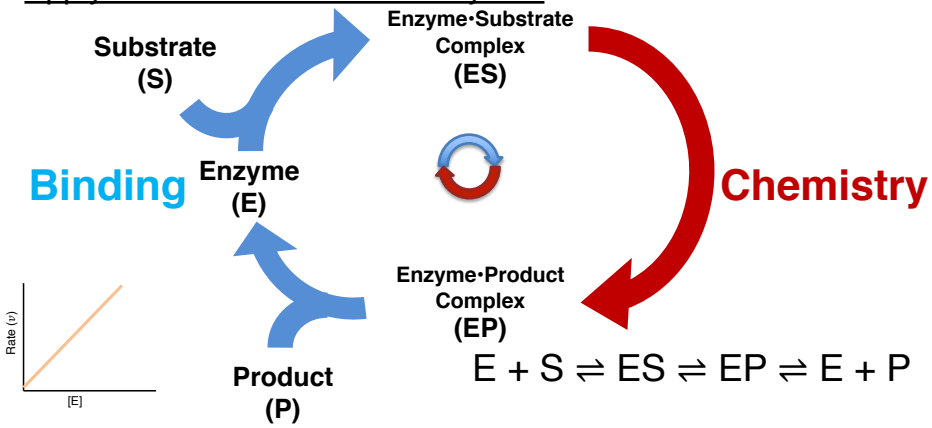
The rate equations for these reactions are:

$v = k_1[A]^2$ and $v = k_1[A][B]$

During an enzyme catalyzed reaction when the enzyme binds to substrate, it's a "pseudo" second-order reaction…..

Enzyme Kinetics

Apply Chemical Kinetics to Enzymes



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

- The rate that an individual enzyme can go around this cycle is called the "turnover number," or k_{cat}
- And, the rate depends on the [E] (recall)

Recall, this has units of a 1st order rate constant (sec^{-1})

- But, this is ONLY if there is AMPLE substrate.
- What if [S] is low?
- The velocity will now have a dependence on [S], or

$$v \propto [S]$$

Enzyme Kinetics

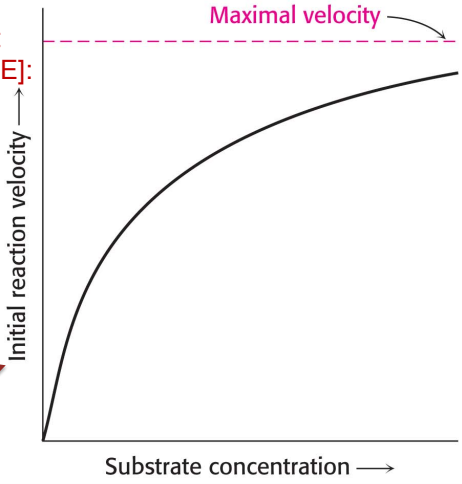
- If the velocity has a dependence on [S], or

$$v \propto [S],$$

Then what is the proportionality constant in the rate equation?

$$v = \text{“}k\text{”}\ [S]$$

The curve for v *versus* [S] is not linear, like it is for v *versus* [E]:



OMG!! The relationship between reaction velocity and [S] is the same as between fraction bound (Y) and [L]……

Hyperbolic Curve

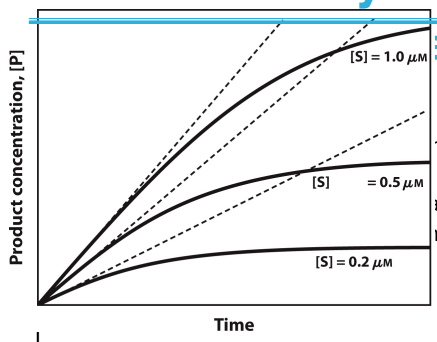
So, what is the equation that describes this behavior, i.e., the rate equation?

But first, let's recall what is meant by this "initial" velocity?

Enzyme Kinetics

"Initial" velocity

easurements



nce and/or product formation as a function of

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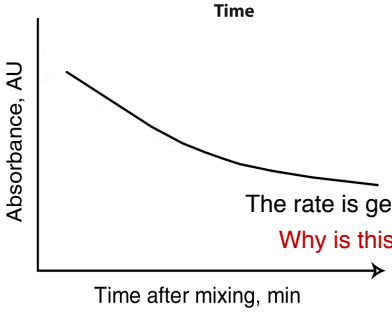
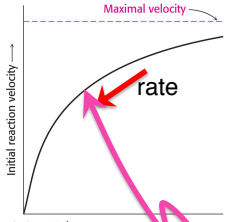
tration and repeat to get the best slopes.





The rate is getting slower and slower

Why is this curving off?

As S goes to P, the [S] decreases.

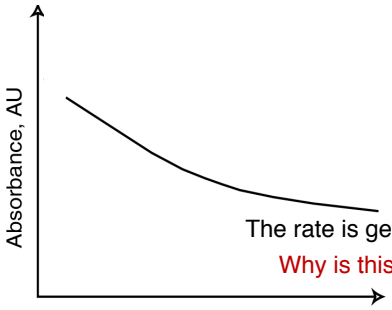
OK, back to the question: what is the equation that describes this behavior?

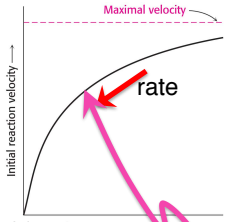
Enzyme Kinetics

How to Do Kinetic Measurements

Experiment:

1. Mix enzyme + substrate.
2. Record rate of substrate disappearance and/or product formation as a function of time (the velocity of reaction).
3. Plot initial velocity (v_0) *at that* substrate concentration [S].
4. Change substrate or enzyme concentration and repeat to get the best slopes.





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Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

So, what is the equation that describes this behavior, i.e., the rate equation?

Lets focus on binding:

$$K_{eq} = \frac{[ES]}{[E][S]} = \frac{k_1}{k_{-1}}$$

This is just like any other binding reaction, which we already discussed…

OR

$$K_d = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$

Remember:

$$K_d = \frac{k_{-1}}{k_1}$$

What about catalysis?

Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

What about the rate expression at ANY [S]?

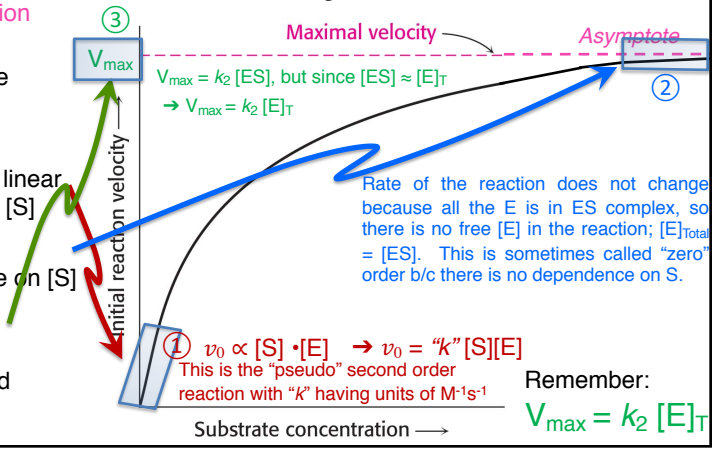
What is the rate equation for enzyme kinetics?

Lets first look at the hyperbolic curve in more detail:

① At low [S] – nearly linear function of rate vs. [S]

② At high [S] – no dependence of rate on [S]

③ At this asymptote, what is the rate expression? Called V_{max}



Remember:

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

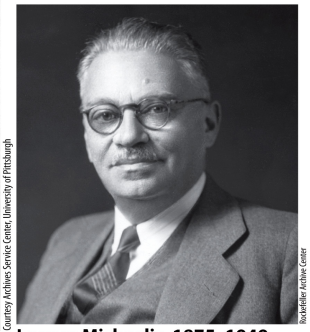
Enzyme Kinetics

This question was answered in 1913 by Michaelis & Menten

They used these principles and derived the equation that describes the relationship between v_0 and [S] for enzyme-catalyzed reactions that show "hyperbolic" behavior…..... The famous Michaelis-Menten Equation

Courtesy Archives Service Center, University of Pittsburgh

Maud Menten, 1879–1960



Rockefeller Archive Center

Leonor Michaelis, 1875–1949

Enzyme Kinetics

Derivation of Michaelis-Menten Equation:

1. Start with a model mechanism.
2. Identify constraints and assumptions.
3. Carry out algebra

1. Simplest Model Mechanism: one reactant, one product, no inhibitors

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

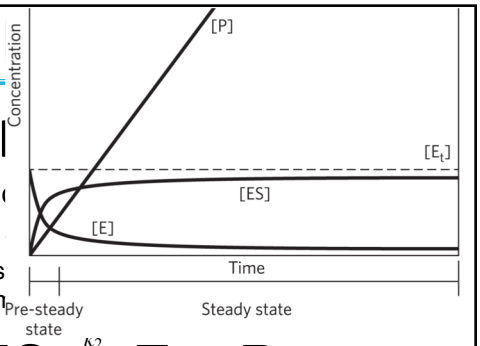
Enzyme

Derivation of Michael

2. Identify constraints and assumpti

There are 4 important ASSUMPTIONS for

1. Use only INITIAL rate (v_0). This means you can ignore k_{-2}. The mechanism sin



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

2. The slow step is AFTER binding. In other words, binding is a rapid equilibrium. This is the so-called RAPID EQUILIBRIUM ASSUMPTION.

$$v_0 = k_2\,[ES]$$

3. The substrate is in vast excess of the enzyme (a true catalyst). This means you can ignore the amount of substrate in the ES complex, and $[S]_{free} = [S]_{Total}$. We can get everything in easily measured quantities: $[E]_T$, $[S]_T$, and v_0, but not [ES].
4. The rate of formation of ES and the rate of breakdown are are equal. This is the so-called STEADY-STATE ASSUMPTION. Therefore, the [ES] does not change.

Enzyme Kinetics

Derivation of Michaelis-Menten Equation:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

3. Carry out the algebra.

- Starting with the Steady-State assumption

$$\frac{d[ES]}{dt} = \text{rate of formation of ES} - \text{rate of breakdown of ES} = 0$$

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

Collect [ES] terms: $k_1\,[E][S] = (k_2 + k_{-1})[ES]$

Substitute the expression $[E] = [E]_T - [ES]$, because free [E] is difficult to determine:

$$k_1\,([E]_T - [ES])[S] = (k_2 + k_{-1})[ES]$$

Collect [ES] terms:

$$k_1\,[E]_T[S] - k_1[ES][S] = (k_2 + k_{-1})[ES]$$

$$k_1\,[E]_T[S] = (k_2 + k_{-1} + k_1[S])[ES]$$

Enzyme Kinetics

Derivation of Michaelis-Menten Equation:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

3. Carry out the algebra.

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

Solve for [ES]:

$$\frac{k_1 [E]_T[S]}{(k_2 + k_{-1} + k_1[S])} = [ES]$$

Divide by $k_1/k_1 = 1$):

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

Define K_m as this collection or rate constants:
Substitute in K_m:

$$\frac{[E]_T[S]}{K_m + [S]} = [ES]$$

$K_m = \left(\frac{k_2 + k_{-1}}{k_1}\right)$ — Rate constants governing ES BREAKDOWN

Rate constant governing ES FORMATION

Recall Assumption #2: k_2 is slow (small number)

$K_m = \frac{k_{-1}}{k_1} \approx K_d$

Enzyme Kinetics

Derivation of Michaelis-Menten Equation:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

3. Carry out the algebra.

$$\frac{[E]_T[S]}{K_m + [S]} = [ES]$$

Use RAPID EQUILIBRIUM ASSUMPTION (#2): $v_0 = k_2 [ES]$

$$\frac{[E]_T[S]}{K_m + [S]} = v_0 / k_2$$

Solve for v_0 and make the rate equation:

$$\frac{k_2[E]_T[S]}{K_m + [S]} = v_0$$

Michaelis-Menten Equation

Recall definition of V_{max}:

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

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

Recall:

hyperbola → y = x/(b+x)

$$Y = \frac{[L]}{K_D + [L]} \qquad \frac{v_0}{V_{max}} = \frac{[S]}{K_m + [S]}$$

Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

What is the rate equation for enzyme kinetics?

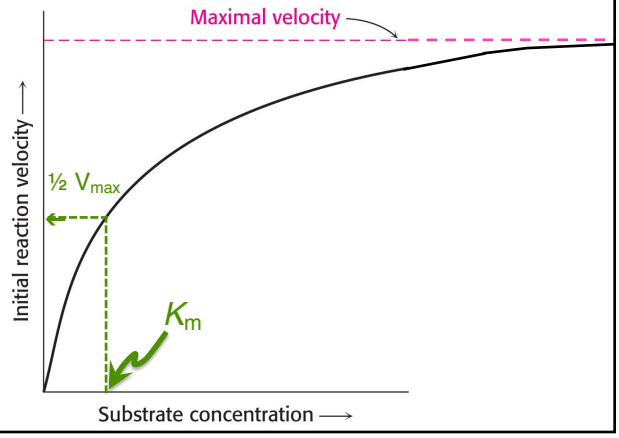
Michaelis-Menten Equation

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

① Special cases:

What is v_0 when [S] = K_m?

When K_M = [S], v_0 = ½ V_{max}. Thus, K_M is the substrate concentration that yields ½ V_{max}.



Enzyme Kinetics

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Binding Catalysis

What is the rate equation for enzyme kinetics?

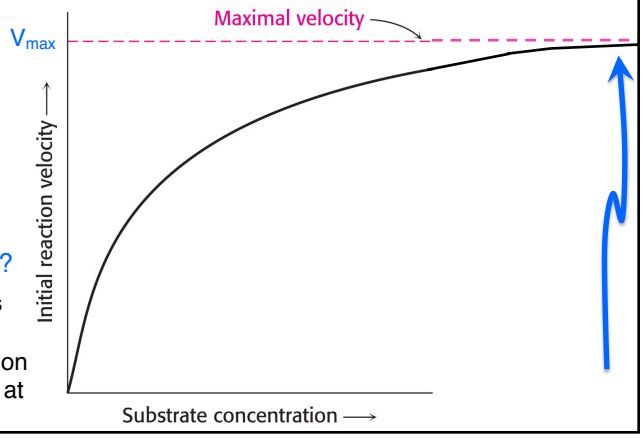
Michaelis-Menten Equation

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

② Special cases:

What is v_0 when [S] >> K_m?

When K_M << [S], its value is negligible and v_0 = V_{max}. Thus, at high [S], the equation tells us the obvious; we are at V_{max}.



Enzyme Kinetics

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Binding Catalysis

What is the rate equation for enzyme kinetics?

Michaelis-Menten Equation

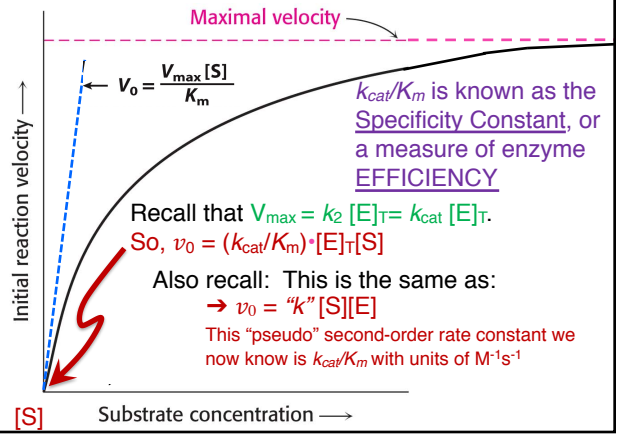
$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

③ Special cases:

What is v_0 when [S] << K_m?

When K_M >> [S], v_0 = (V_{max}/K_m)•[S].

Thus, the rate is strictly dependent on the [S]. But, remember that it also depends on [E].



k_{cat}/K_m is known as the Specificity Constant, or a measure of enzyme EFFICIENCY

Recall that $V_{max} = k_2 [E]_T = k_{cat} [E]_T$.

So, $v_0 = (k_{cat}/K_m)•[E]_T[S]$

Also recall: This is the same as:

→ v_0 = "k" [S][E]

This "pseudo" second-order rate constant we now know is k_{cat}/K_m with units of $M^{-1}s^{-1}$

Enzyme Kinetics

Enzyme Efficiency is Limited by Specificity: k_{cat}/K_M

- Diffusion from the active site limits the maximum value for specificity/efficiency.
- Can gain efficiency by having high velocity or affinity for substrate
  - catalase vs. acetylcholinesterase

TABLE 6-8 Enzymes for Which k_{cat}/K_m is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	1×10^7	2.5×10^{-2}	4×10^8
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: A. Fersht, *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, 1999.

Enzyme Kinetics

SUMMARY:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- The final form in case of a single substrate is the Michaelis-Menten equation:

$$v_0 = \frac{k_{cat}[E_{tot}][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- k_{cat} (turnover number): how many substrate molecules one enzyme molecule can convert per second
- K_m (Michaelis constant): an approximate measure of a substrate's affinity for an enzyme; actually, it is the **ratio of rate constants for formation and loss intermediate involved in rate-limiting step**.
- During steady state, the maximum velocity (V_{max}) occurs when all the enzyme is in the ES complex and is dependent on the breakdown of that complex (k[ES]).
- The microscopic meaning of K_m and k_{cat} depends on the details of the mechanism.

Enzyme Kinetics

CLINICAL INSIGHT

Variations in K_M Can Have Physiological Consequences

Two enzymes play a key role in the metabolism of alcohol.

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{Alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

Ethanol — Acetaldehyde

$$CH_3CHO + NAD^+ + H_2O \xrightleftharpoons{\text{Aldehyde dehydrogenase}} CH_3COO^- + NADH + 2H^+$$

Some people respond to alcohol consumption with facial flushing and rapid heart beat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different acetaldehyde dehydrogenases in most people, one with a low K_M and one with a high K_M.

The low K_M enzyme is genetically inactivated in some individuals. The enzyme with the high K_M cannot process the acetaldehyde fast enough (less efficient), and so some acetaldehyde appears in the blood.

So, if knowing the values of the constants, K_m and V_{max}, for enzymes and their substrates is important, how are they determined?