ENZYMES: Binding & Catalysis

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
   1. Transition State Theory
   2. **Catalytic** strategies (**What**)
   3. **Mechanistic** strategies (**How**)

E. Quantifying the Catalytic Power: Kinetics
   1. Review
   2. Enzyme Kinetics
      a. Rate vs. [S] for enzyme catalyzed reaction
      b. ES complex;
         Rate expression; Michaelis-Menten
      c. Meaning of M-M equation
      d. Collection and manipulation of data
      e. Inhibition
         i. Irreversible: protein modification
         ii. Reversible; competitive, non-, un-
      f. Uses of Steady-state kinetics
      g. Energetics of Catalysis

Lecture 14 (10/16/24)

- Reading: Ch6; 188-195
- Homework #13 & #14

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

Enzyme Kinetics

What Is Enzyme Kinetics?

- **Kinetics** is the study of the rate at which compounds react to form products.
- 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)(Greek n; nu) 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}.

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 $A + B \rightarrow P$

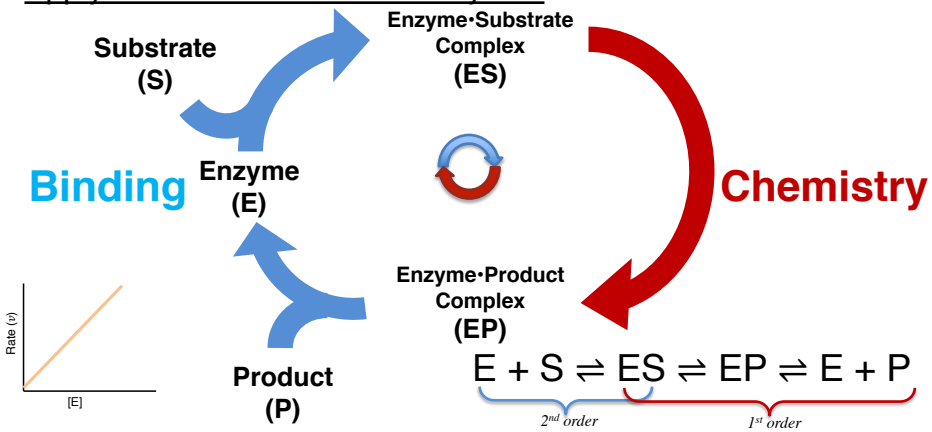
The rate equations for these reactions are:

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

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

Enzyme Kinetics

Apply Chemical Kinetics to Enzymes



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

(2^{nd} order; 1^{st} order)

- 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 1^{st} 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

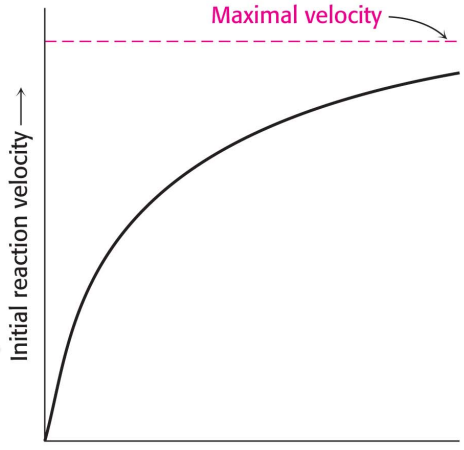
-If the velocity has a dependence on [S], or

$v \propto$ [S],

Then what is the proportionality constant in the rate equation?

$v =$ "k" [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?

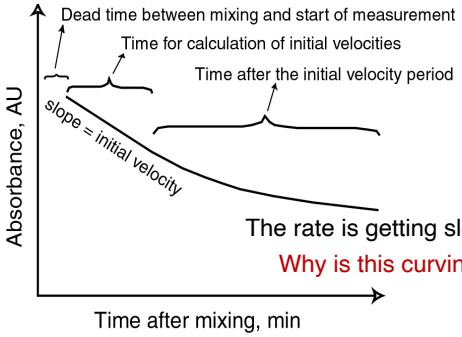
But first, let's recall what is meant by this "initial" velocity?

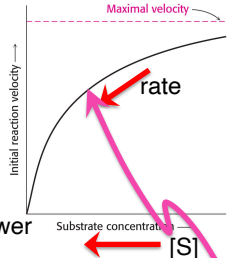
Enzyme Kinetics

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.





The rate is getting slower and slower

Why is this curving off?

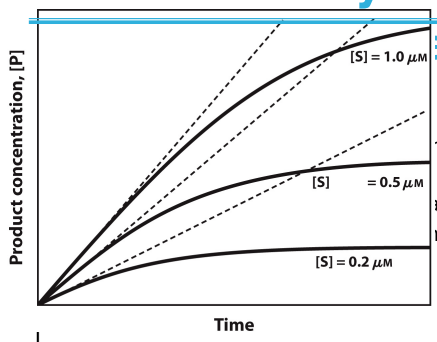
As S goes to P, the [S] decreases.

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

Enzyme Kinetics

"Initial" velocity

easurements



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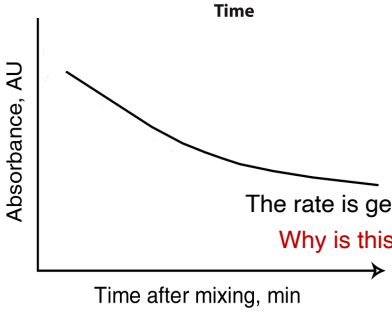

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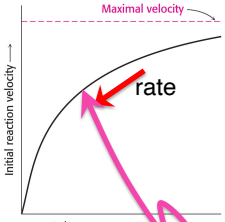
Why is this curving off?



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

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

Enzyme Kinetics

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

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

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

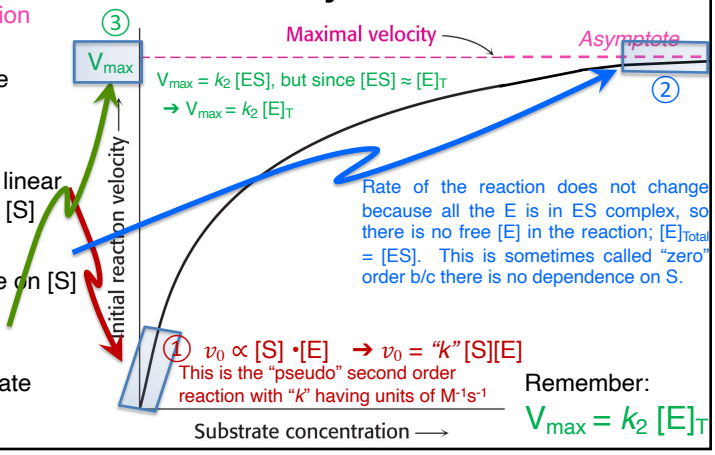
Binding Catalysis

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? The rate is called V_{max}



Remember:

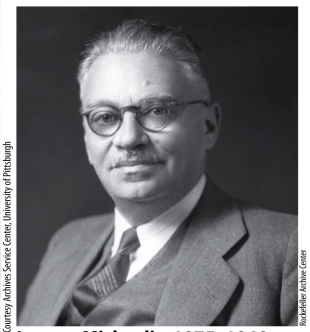
$V_{max} = k_2 [E]_T$

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

Maud Menten, 1879–1960



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 Kinetics

Derivation of Michaelis-Menten Equation:

2. Identify constraints and assumptions.

There are 4 important ASSUMPTIONS for this derivation.

1. Use only INITIAL rate (v_0). This means [P] = 0, so there is no back reaction and you can ignore k_{-2}. The mechanism simplifies further:

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

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

Units are $s^{-1}/M^{-1}s^{-1}$ or M

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 EQILIBRIUM 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?

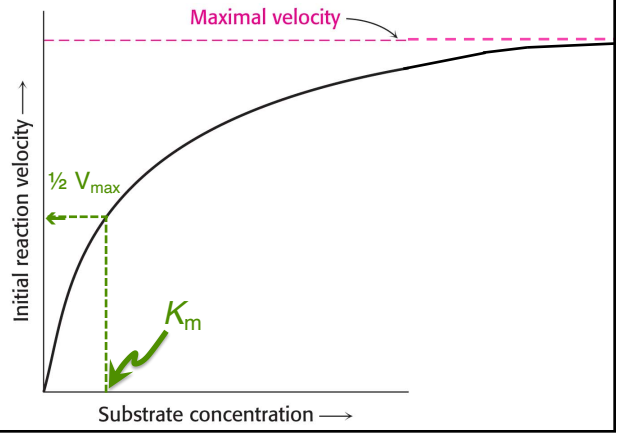
Michaelis-Menten Equation

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

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

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

Binding Catalysis

What is the rate equation for enzyme kinetics?

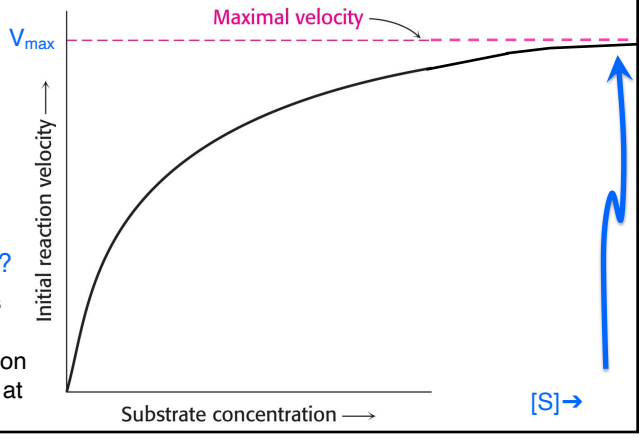
Michaelis-Menten Equation

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

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

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

Binding Catalysis

What is the rate equation for enzyme kinetics?

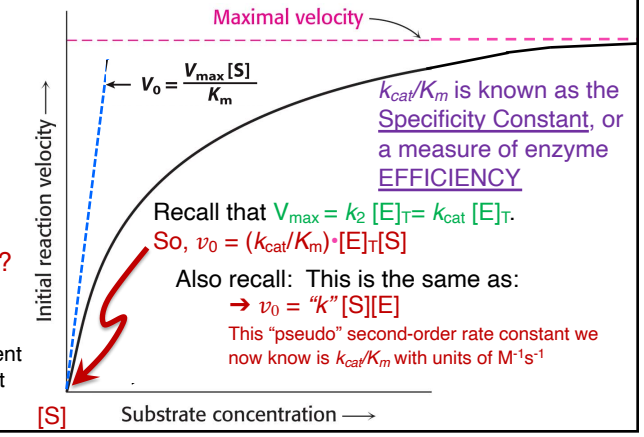
Michaelis-Menten Equation

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

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].



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 of the rate equation in the 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 an enzyme's affinity for a particular substrate; actually it is the ratio of rate constants for formation and loss of the enzyme intermediate involved in rate-limiting step.
- During steady state, the maximum velocity (V_{max}) occurs when all of 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

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

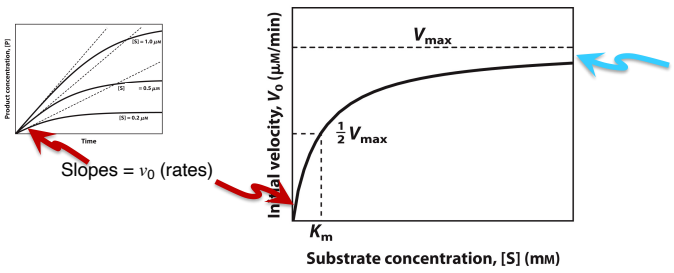
Some people respond to alcohol consumption with facial flushing and rapid heart beat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different acetaldehyde dehydrogenases in most people, one with a low K_M and one with a high K_M.

The low K_M enzyme is genetically inactivated in some individuals. The enzyme with the high K_M cannot process all of the acetaldehyde, 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?

Enzyme Kinetics

Determination of Steady-State Kinetic Parameters



A nonlinear Michaelis-Menten plot could be used to calculate parameters K_m and V_{max}.

Lineweaver-Burk derived a linear form of the M-M equation by taking the reciprocal of both sides. This is called the linearized double-reciprocal plot. Its good for analysis of enzyme kinetic data to get these kinetic parameters.

Enzyme Kinetics

Lineweaver-Burk Plot: Linearized, Double-Reciprocal

The Michaelis-Menten equation can be manipulated into one that yields a straight-line plot.

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

$$\frac{1}{v_0} = \frac{K_m + [S]}{V_{max}[S]}$$

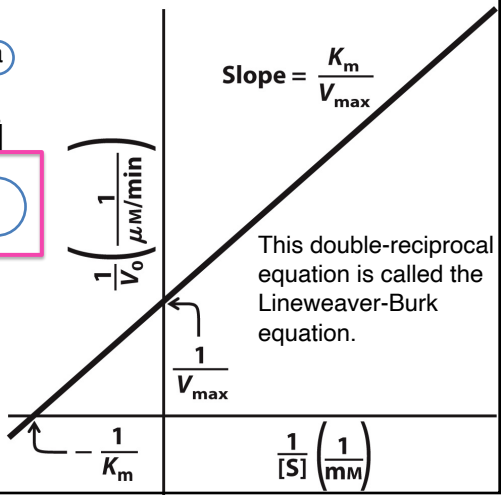
$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

y = bx + a

$y = \frac{1}{v_0}$ $x = \frac{1}{[S]}$

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



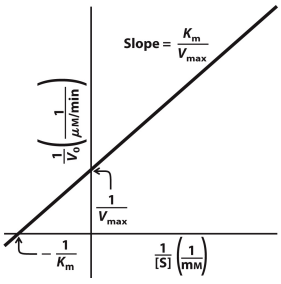
This double-reciprocal equation is called the Lineweaver-Burk equation.

Enzyme Kinetics

Linearized Derivations of the M-M Equation

Lineweaver-Burk

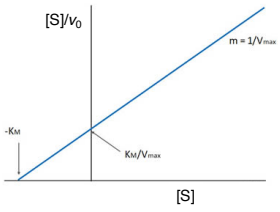
$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$



Hanes-Woolf

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

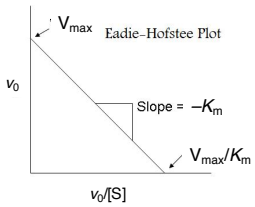
$\frac{[S]}{v_0}$ *vs.* [S]

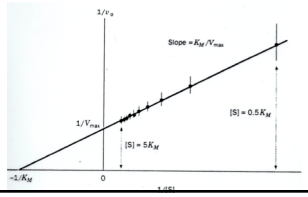


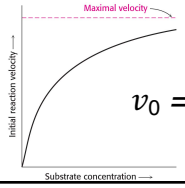
Eadie-Hofstee

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

v_0 *vs.* $v_0/[S]$







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