Slide 1

Chapter 14

Enzyme Kinetics

Slide 2

Enzyme Characteristics

- Catalytic Power
  - Rate enhancements as much as 10^{14}
- Specificity
  - Enzymes can distinguish between closely related chemical species
    - D and L isomers
    - *cis* and *trans* isomers
    - Diastereomers (glucose and galactose)
- Regulation
  - Ability to activate or inhibit enzymes can control which reactions occur and when.

An example of specificity is illustrated by the enzyme **fumarase** which catalyzes addition of water to the double bond of **fumaric acid** to form **L-malic acid**. The *cis* isomer of fumarate, maleic acid, does not work. Neither does D-malic acid, the enantiomer of L-malic acid.

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Enzyme Terminology

- Substrates
  - Substances whose reaction is being catalyzed
- Products
  - End products of the catalyzed reaction
- For reversible reaction, designation depends on point of view.
  - A + B $\rightleftharpoons$ P + Q
  - A, B products, P, Q reactants, or vice versa

An enzyme is a catalyst, and as such does not influence the **equilibrium** position of the reaction—only its rate. So one can start with A and B and get P and Q formed, but one can also start with P and Q and get A and B formed.

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Enzyme Nomenclature

- Some names are historical common names
  - Trypsin, chymotrypsin,
- Some have common names related to the substrate(s), usually ending in **–ase**.
  - urease, protease, ribonuclease, ATPase, glucose-6-phosphatase
- All enzymes have a **systematic name** based on international agreement

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Systematic Classification of Enzymes

- 1. Oxidoreductases
- 2. Transferases
- 3. Hydrolases
- 4. Lyases
- 5. Isomerases
- 6. Ligases

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Oxidoreductases

- Catalyze transfer of electrons between species (i.e., oxidation and reduction)
  - Some are called **dehydrogenases** in their common name.
  - Some are called **oxidases** or **reductases**.
  - Often **coenzymes** such as NAD, NADP, FAD, or a metal ion is involved in the reaction.

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Transferases

- Catalyze transfer of a functional group from one molecule to another
  - e.g., phosphate groups, methyl groups, acyl groups, glycosyl groups, etc.
  - Enzymes catalyzing transfer of a phosphate from ATP to another acceptor are commonly referred to as **kinases**.

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Hydrolases and Lyases

- **Hydrolases** catalyze hydrolysis of a chemical bond
  - Includes proteases, esterases, phosphatases, etc.
- **Lyases** catalyze reversible addition of groups to a double bond.
  - Usually H_2O or NH_3 added
  - Interconversion of fumaric acid and L-malic acid which we alluded to earlier.

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Isomerases and Ligases

- **Isomerases** catalyze Conversion of one isomer to another.
  - The only enzyme class in which a single substrate and product is involved.
- **Ligases** catalyze the formation of a chemical bond at the expense of hydrolysis of ATP.

We will encounter a number of examples of ligases later.
Learn the general classification groups. As we study new enzymes, be able to fit an enzyme into one of these groups, but you won't be held for knowing the specific systematic name. We will usually refer to more common names—and sometimes there is more than one!

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Coenzymes

- Coenzymes are non-protein substances required for activity, sometimes called **cofactors**.
  - (See Table 14.2)
- Metal ions
  - Some metal ion cofactors are directly involved in the catalytic mechanism. Others are required simply for stabilization of protein structure.
- Organic cofactors
  - Non-amino acid compounds required for catalytic activity

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Organic Coenzymes

- Two classes of organic coenzymes
  - Coenzyme cosubstrates
    - Transformed in the reaction, regenerated in another reaction
    - Loosely bound to the enzyme, dissociates during course of reaction
  - Coenzyme prosthetic groups
    - Participates in the reaction, but original structure regenerated during catalytic cycle
    - Tightly bound, sometimes covalently, does not dissociate during course of reaction.

Your textbook does not make this distinction clear between these two classes of coenzymes, yet keeping their differences straight is important in understanding some metabolic processes. As we study new enzymes involving coenzymes, be prepared to classify the type of coenzyme involved.
An enzyme lacking its prosthetic group is called an **apoenzyme**. When its prosthetic group is present, it is called a **holoenzyme.**

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Enzyme Kinetics

- Chemical **kinetics** refers to the **rate** of chemical reactions, and things which influence that rate.
- Enzymes as **catalysts** accelerate the rate, but not the equilibrium position.
- A **chemical rate law** describes the effect of variables, such as reactant concentration, on the rate of the reaction.

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Enzyme Kinetics--Applications

What can kinetics tell us?

- Kinetic studies can help us understand how metabolic pathways are controlled, and the conditions under which an enzyme is active.
- Kinetic studies can yield information about the **mechanism** of an enzymatic reaction.
  - However, kinetic studies can only **rule out** mechanistic models that do not fit the data. They cannot **prove** a mechanism.

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Reaction Rate

- The **rate** of a reaction is the **slope** of a **progress curve**. (See Figure 14.4)
- For the simple reaction:
  - $A \rightarrow P$

$$\text{rate} = \frac{d[P]}{dt} \text{ or } -\frac{d[A]}{dt}$$

Note that the slope of the progress curve changes with time. Most studies in enzyme kinetics try to deal with the **initial rate** of a reaction, that is the slope of the curve where t = 0. Many things can influence the catalytic ability of an enzyme, including the accumulation of products as well as denaturation of the protein itself. It is only at the initiation of the reaction that one is sure of the amount of active enzyme present and the other conditions of substrate and product concentration.

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Reaction Rate Theory: The Transition State

- For chemical bond breakage and formation to occur, the reactants must go through an intermediate **transition state**.
- The free energy of the transition state is higher than that of either the reactants or the products. (See Figure 14.1)
- Only a small fraction of the reactants have sufficient energy to achieve this "activation energy", referred to as $\Delta G^{\ddagger}$.

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Reaction Rate Theory: Effect of Temperature

- Increasing the temperature increases the fraction of reactants which have enough energy to achieve the transition state.
- This relationship is expressed in the **Arrhenius Equation:**

$$k = Ae^{-\frac{\Delta G}{RT}}$$

where ΔG is the activation energy

(see Figure 14.5a)

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Reaction Rate Theory: Effect of a catalyst

- A catalyst accelerates the rate of a reaction by **lowering the activation energy** of the reaction. (See Figure 14.5b)

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Rate Law

- Expresses relationship between rate and concentration of reactants.
  - e.g. $v = k[A]$, $v = k[A]^2$, $v = k[A][B]$
- k is the **rate constant** (a proportionality constant between rate and the concentration terms).
- Effect of **temperature** on reaction rate is an effect on k.

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Reaction Order

- The **order** of a reaction is given by the exponents of the reactant concentrations in the rate law.
  - $v = k[A]^n$
    - n=0, zero order (no dependence on [A]
    - n=1, first order
    - n=2, second order
  - $v = k[A]^2[B]$
    - second order in A, first order in B, third order overall

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Reaction Order, con't.

- Experimental reaction rate order
  - Determined by experimental measurements.
  - Need not be whole numbers
- Theoretical reaction rate order
  - Interpreted as the **molecularity** of **elementary steps** in the reaction.
  - The reaction $A + B \rightarrow P + Q$
    - Would have a molecularity of 2, and a theoretical rate law: $v = k[A][B]$

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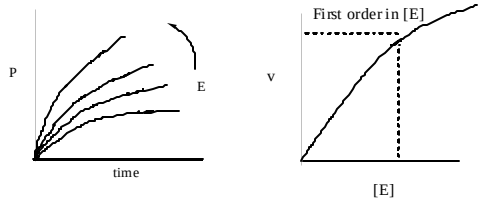
Effect of Enzyme Concentration on Rate

- First establish the effect of Enzyme concentration on reaction rate.
  - Try to work only in region where $v = k[E_t]$



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Effect of Substrate Concentration on Rate

- For many enzymatic reactions, the **experimental rate law** is given by the equation for a **rectangular hyperbola**.

$$v = \frac{aS}{b+S}$$

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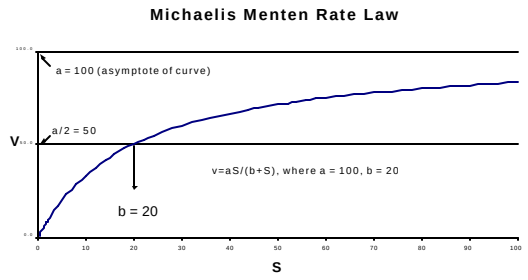

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Mixed Order Nature of the Experimental Rate Law

- At high S (S >>> b)
  - v = a
    - zero order in S
- At low S (S <<<b)
  - v = (a/b)S
    - first order in S

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Michaelis Menten Model to Explain Experimental Rate Law

- Terminology of M.M. equation
  - **a** is called $\mathbf{V_m}$, the **maximum velocity**
    - This is the asymptote of the saturation curve, the maximum velocity that can be achieved at a very high S concentration. It has the units of v.
  - **b** is called $\mathbf{K_m}$, the **Michaelis constant**
    - This is the substrate concentration at which v is one-half of V_m. It has the units of S.

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Michaelis Menten Model

- Michaelis and Menten proposed an **enzyme mechanism** (a model) to explain the rate law behavior.
- Features of their model give some insight into what is happening, nevertheless their model was overly simplistic.
- Deriving the rate law from their model shows it is **consistent** with the data, but does not **prove** the model.

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Postulates (Assumptions) of the M.M. Model

1. Enzyme and substrate combine to form an ES complex, which breaks down to form product.

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

Note the model assumes only one substrate. This is actually true only for isomerases.

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Postulates (Assumptions) of the M.M. Model, con't.

2. [S]>>[E], so $[S_t] = [S_{free}]$
   And [ES] can be ignored
3. Last step is irreversible
   either $k_{-2} = 0$, or [P] = 0
4. k_2,<<< k_{-1}, the **rapid equilibrium** assumption
   (The binding of S to E is rapid compared to the breakdown of the ES complex.)

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Derivations from the M.M. Postulates

(a) $K = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$ (b) $v = k_2[ES]$

(c) $[E_t] = [E] + [ES]$

where [E] is the free enzyme

- Algebraically eliminate [E] and [ES] from these equations to give an equation with v as a function of [S]

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Derivations from the M.M. Postulates, con't.

- Divide (b) by (c), and substitute (a)

$$\frac{v}{[E_t]} = \frac{k_2[ES]}{[E] + [ES]} \qquad \frac{v}{[E_t]} = \frac{k_2[ES]}{\frac{K[ES]}{[S]} + [ES]}$$

$$v = \frac{k_2[E_t][S]}{K + [S]} = \frac{V_m[S]}{K + [S]}$$

where $V_m = k_2[E_t]$

and $K = \frac{k_{-1}}{k_1}$

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Briggs-Haldane Refinement: The Steady State Assumption

- Assume that [ES] does not change over the course of the reaction.
  - i.e., it is being broken down as rapidly as it is being made. (See Figure 14.8)

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

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

Note the key difference between the results of the rapid equilibrium assumption and the steady state assumption is the interpretation of K_m. In the first case, it is the dissociation constant of the ES complex. In the second it is a more complicated assembly of rate constants.

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Michaelis-Menten Equation as a Theoretical Rate Law: Summary

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

Based on mechanism:

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

where $V_m = k_2[E_t]$ and

$K_m = \frac{k_{-1}}{k_1}$ (rapid equilibrium assumption) or $Km = \frac{k_{-1} + k_2}{k_1}$ (steady state assumption)

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Enzyme Units

- Quantity of an enzyme often measured in terms of its catalytic activity.
  - **International Unit**—amount that catalyzes formation of one micromole of product in one minute.
  - **katal**--amount that catalyzes conversion of one mole of substrate to product in one second
  - **turnover number**—substrate molecules converted per enzyme molecule per unit time.

International units and katals can be expressed even in a crude mixture where the purity of the enzyme or the actual quantity of enzyme protein present are not known. Turnover number, on the other hand, requires that one know the number of moles of enzyme present in the reaction.

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Turnover Number, con't.

- Called $\mathbf{k_{cat}}$
- Equivalent to $\mathbf{k_2}$ in the M.M. equation provided V_{max} and $[E_t]$ are in the same molar units.
  - For example,
    V_{max} in mmol-sec^{-1}-ml^{-1} and $[E_t]$ in mmol-ml^{-1}
- Varies over a wide range (See Table 14.4)

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Catalytic Efficiency

- k_{cat} measures *kinetic* efficiency, and K_m is inversely related to the binding affinity
- $\mathbf{k_{cat}/K_m}$ measures the *catalytic* efficiency of an enzyme—how well it has evolved to do its job.
- It is the first order rate constant at low substrate concentration:

$$\frac{v}{[E_t]} = \frac{k_{cat}}{K_m}[S]$$

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Catalytic Efficiency, con't.

- Using the M.M. model and the steady state assumption:

$$\frac{k_{cat}}{K_m} = \frac{k_2}{\frac{(k_{-1}+k_2)}{k_1}} = \frac{k_1 k_2}{(k_{-1}+k_2)}$$

when $k_2 >>> k_{-1}$ and the reaction is

$$\frac{k_{cat}}{K_m} = \frac{k_1 k_2}{k_2} = k_1$$ **Diffusion Controlled**

The reaction can never proceed faster than the time it takes the substrate to bind to the enzyme in the first place. In the extreme where every substrate binding event leads to product, the reaction is said to be **diffusion controlled**. Theoretically, the maximum rate for these collisions is about 10^8 sec^{-1} M^{-1}. See Table 14.5 for some enzymes that seem to have achieved this "perfection".

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Validity of M.M. Model Assumptions

1. (a) Enzyme and substrate combine to form an ES complex, which breaks down to form product.
   - This basic assumption must be true in any model.

   (b) One substrate, one product:
   - Seldom true; approximation if everything else is held constant.

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

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Validity of M.M. Model Assumptions, con't.

2. [S]>>[E], so $[S_t] = [S_{free}]$
   And [ES] can be ignored
   - Usually true in the test tube, not always in the cell.
3. Last step is irreversible
   either $k_2 = 0$, or [P] = 0
   - Usually only artificially when [P] = 0
4) Rapid equilibrium, or steady state assumption.
   - Steady state assumption is more general

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Expanding the M.M. Model Assumptions

- Adding additional steps in the mechanism
- Making the last step reversible
- More than one substrate or product

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Additional Steps in the Mechanism

- Suppose there is a "rearrangement" step added to the mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EP \xrightarrow{k_3} E + P$$

The rate law becomes:

$$v = \frac{\frac{k_2 k_3}{k_2 + k_{-2} + k_3}[E_t][S]}{\frac{k_{-1}k_{-2} + k_{-1}k_3 + k_2 k_3}{k_1(k_2 + k_{-2} + k_3)} + S}$$

You can derive this equation if you wish. Just note there are now three forms of the enzyme (E + ES + EP), and there are two steady state assumption statements: $d[ES]/dt = 0 = k_1[E][S] + k_{-2}[EP] - (k_{-1}+k_2)[ES]$ and $d[EP]/dt = 0 = k_2[ES] - (k_{-2} + k_3)[EP]$. And $v = k_3[EP]$.

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Additional Steps in the Mechanism, con't.

- This equation still has the same form as the M.M. equation, but the **interpretation** of V_{max} and K_m are different:

$$V_m = \frac{k_2 k_3}{k_2 + k_{-1} + k_3}[E_t]$$

$$K_m = \frac{k_{-1}k_{-2} + k_{-1}k_3 + k_2 k_3}{k_1(k_2 + k_{-2} + k_3)}$$

Therefore "steady state" kinetics cannot distinguish between mechanisms that just involve different numbers of rearrangement steps. The more complicated the mechanism, the more complicated the interpretation of K_m in terms of the parameters of the model.

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Making the Last Step Reversible

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P$$

the rate law takes the form

$$v = \frac{a[S] - b[P]}{c + d[S] + e[P]}$$

where a, b, c, d, and e are assemblies of rate constant

more complicated, but not impossible, to analyze

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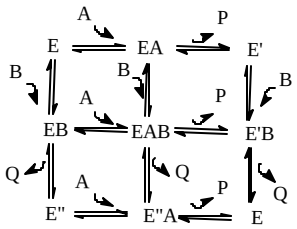
More Than One Substrate or Product

- Even simplest mechanism is much more complex. Several pathways are possible:



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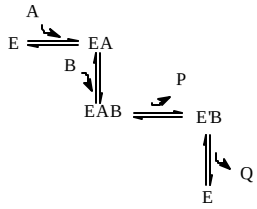
Ordered Sequence Mechanisms

- Ordered, Single-Displacement. Substrates add in a definite order.



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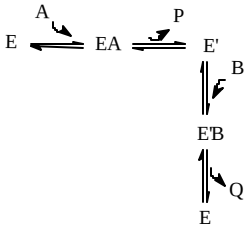
Ordered Sequence Mechanisms

- Ordered. Double-Displacement (Ping-Pong) First substrate is converted to product before second substrate adds. Requires two forms of "free" enzyme.



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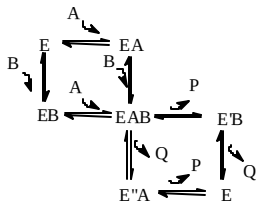
Random Addition Mechanisms

- A and B can add in any order, P and Q can be released in any order.



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Kinetic Consequences of Multi-substrate Mechanisms

- Hold one substrate constant, and vary the other one:
- M.M. kinetics (rectangular hyperbola) shown **only** for:
  - Ordered sequence mechanisms
  - Random mechanisms **if** the rapid equilibrium assumption holds.

Only skim the textbook analysis of two-substrate reactions. Their treatment is incomplete, and an adequate treatment would take more time than we have available.

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Linear Transformations of the M.M. Equation

- Before the days of computers, linear plots of the M.M. equation were used to calculate values for the kinetic constants V_{max} and K_m
- Linear plots are still useful in "visualizing" the data.
- Three common linear plots:
  - Lineweaver-Burk plot
  - Haynes-Woolf plot
  - Eadie-Hofstee plot

Mechanisms which give true rectangular hyperbola give straight lines in these plots. Such mechanisms are referred to as "linear" mechanisms. Hence ordered sequence mechanisms are "linear", while only "rapid equilibrium" random mechanisms are linear. In all cases, the assumption that the reverse reaction is insignificant must be correct for the results to be linear.

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Lineweaver-Burk Plot

- Take the reciprocal of the M.M. equation, and rearrange it: plot 1/v versus 1/[S]

$$\frac{1}{v} = \frac{K_m + [S]}{Vm[S]} \quad \text{or} \quad \frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} x \frac{1}{[S]}$$

$y = mx + b$ slope = $\frac{K_m}{V_m}$; intercept = $\frac{1}{V_m}$

see Figure 14.9

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Hanes-Woolf Plot

- Multiply Lineweaver-Burk equation through by [S]: plot [S]/v versus [S]

$$\frac{[S]}{v} = \frac{1}{V_m}[S] + \frac{K_m}{V_m}$$

See Figure 14.10

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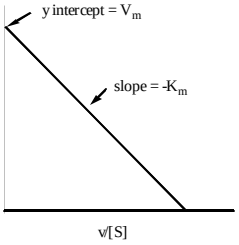
Eadie-Hofstee Plot

- Multiply Lineweaver-Burk equation through by v and V_m: plot v versus v/[S]

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

or $v = V_m - K_m \frac{v}{[S]}$



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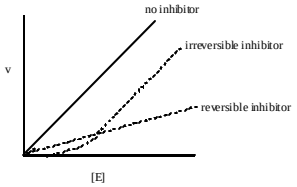
Enzyme Inhibition

- Two general classes of inhibitors
  - Reversible—inhibitors can dissociate and be removed through dilution or dialysis
  - Irreversible—inhibitors form a covalent alteration of the enzyme



Reversible inhibition is usually immediate, whereas irreversible inhibition may vary with time of incubation of the enzyme with the inhibitor. Some irreversible inhibitors are called "suicide" inhibitors, in that the enzyme converts the inhibitor into something which covalently binds to it and destroys its activity. Penicillin is an example. See Figure 14.17.

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Classes of Inhibition

- Based on reversible inhibition.
- Based on simple M.M. one-substrate model
- Four types: (Your text only describes three)
  1) Competitive
  2) Uncompetitive
  3) Pure noncompetitive
  4) Mixed noncompetitive

  (2, 3, and 4 also called "not-competitive")

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Competitive Inhibition

- Assumes that the inhibitor binds only to the free enzyme.

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

$$E + I \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} EI; \quad K_I = \frac{[E][I]}{[EI]}$$

gives the following rate law:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]}$$

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Competitive Inhibition, con't.

- Effect is only on **slope** and not the **intercept** of the Lineweaver-Burk plot.
  - See Figure 14.13
- Interpretation is that the inhibitor binds at the same site as the substrate. Both cannot bind to the free enzyme.
  - Example is succinate dehydrogenase, inhibited by malonate.

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Uncompetitive Inhibition

- Assumes that the inhibitor binds only to the ES complex.

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

$$ES + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ESI; \quad K'_I = \frac{[ES][I]}{[ESI]}$$

gives the following rate law:

$$\frac{1}{v} = \frac{1}{V_m}\left(1 + \frac{[I]}{K'_I}\right) + \frac{K_m}{V_m}\frac{1}{[S]}$$

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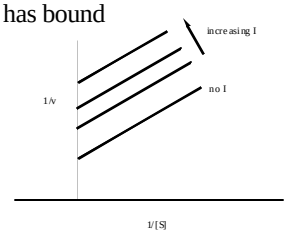
Uncompetitive Inhibition, con't.

- Effect is on intercept and not slope of the Lineweaver-Burk plot. Gives parallel lines.
- Interpretation is that inhibitor can only bind after substrate has bound



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Noncompetitive Inhibition

- Assumes inhibitor binds to either the free enzyme, or the ES compex.

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

$$E + I \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} EI; \quad ES + I \underset{k'_{-i}}{\overset{k'_i}{\rightleftarrows}} ESI$$

$$K_I = \frac{[E][I]}{[EI]}; \quad K'_I = \frac{[ES][I]}{[ESI]}$$

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Noncompetitive Inhibition, con't.

Gives the following rate law:

$$\frac{1}{v} = \frac{1}{V_m}\left(1 + \frac{[I]}{K'_I}\right) + \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]}$$

- Both intercept and slope of Lineweaver-Burk plot are affected.
- **Pure Noncompetitive**, when $K_I = K'_I$
  - Lines cross at x axis. (See Figure 14.15)
- **Mixed Noncompetitive**, when $K_I \neq K'_I$
  - Lines do not cross at x axis. (See Figure 14.16)

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Ribozymes

Several cases are now known where RNA has catalytic activity like an enzyme.

- RNase P, involved in splicing out a piece of tRNA, has an RNA component.
- Self-splicing RNA from several sources.
- Peptidyl transferase of protein synthesis is an RNA component of the ribosome.

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Abzymes

- Enzymes work by lowering the energy of the transition state. They bind the transition state better than the substrate.
- Antibodies raised by using transition state analogues as antigens show catalytic activity.
- So far the reaction types are limited and catalytic enhancement is low.