

BCH 301 - Enzymology

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Introduction to Enzymes

- Living systems are shaped by an enormous variety of biochemical reactions, nearly all of which are mediated by a series of remarkable biological catalysts known as enzymes.
- **Enzymology**, the study of enzymes, has its roots in the early days of biochemistry; both disciplines evolved together from nineteenth-century investigations of fermentation and digestion. The name **"enzyme"** (Greek: en, in + zyme, yeast) was coined in 1878 in an effort to emphasize that there is something in yeast, that catalyses the reactions of fermentation.
- With the exception of a small group of catalytic RNA molecules, all enzymes are proteins. Their catalytic activity depends on the integrity of their native protein conformation, this means that if an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost. **Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.**
- Enzymes are the most remarkable and highly specialized protein that catalyses hundreds of stepwise reactions in biological systems. They regulate many different metabolic activities necessary to sustain life. **They are biological catalysts - A catalyst is defined as "a substance that increases the rate of a chemical reaction without being itself changed in the process."**

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Enzymes are classified into six (6) functional classes (EC number classification) by the International Union of Biochemists (I.U.B.) according to the type of reaction they catalyze:

Class

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

Reactions catalyzed

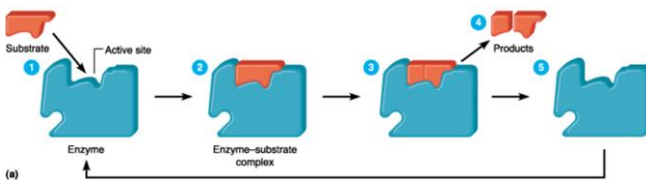
- Oxidation-reduction
- Transfer groups of atoms
- Hydrolysis
- Add/remove atoms to/from a double bond
- Rearrange atoms
- Use ATP to combine molecules

- Each enzyme has classification number consisting of four digits:
- Example, EC: (2.7.1.1) which indicate the following groups of enzymes:
 - 2. Class (Transferase)
 - 7. Subclass (Transfer of phosphate)
 - 1. Sub-sub class (Alcohol is phosphate acceptor)
 - 1. Specific name - ATP,D-hexose-6-phosphotransferase (**Hexokinase**)

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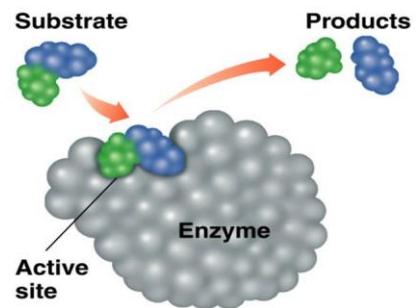
How Enzymes Work

- When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed: $E + S \rightleftharpoons ES$
- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P): $ES \rightarrow E + P$
- The products are then released, allowing another substrate molecule to bind the enzyme - this cycle can be repeated millions (or even more) times per minute. The overall reaction for the conversion of substrate to product can be written as follows: $E + S \rightleftharpoons ES \rightarrow E + P$



Active Site of Enzyme

The **active site** is a region within an enzyme that fits the shape of substrate molecules. Amino acid side-chains align to bind the substrate through H-bonding, salt-bridges, hydrophobic interactions, etc. Products are released when the reaction is complete because they no longer fit well in the active site.



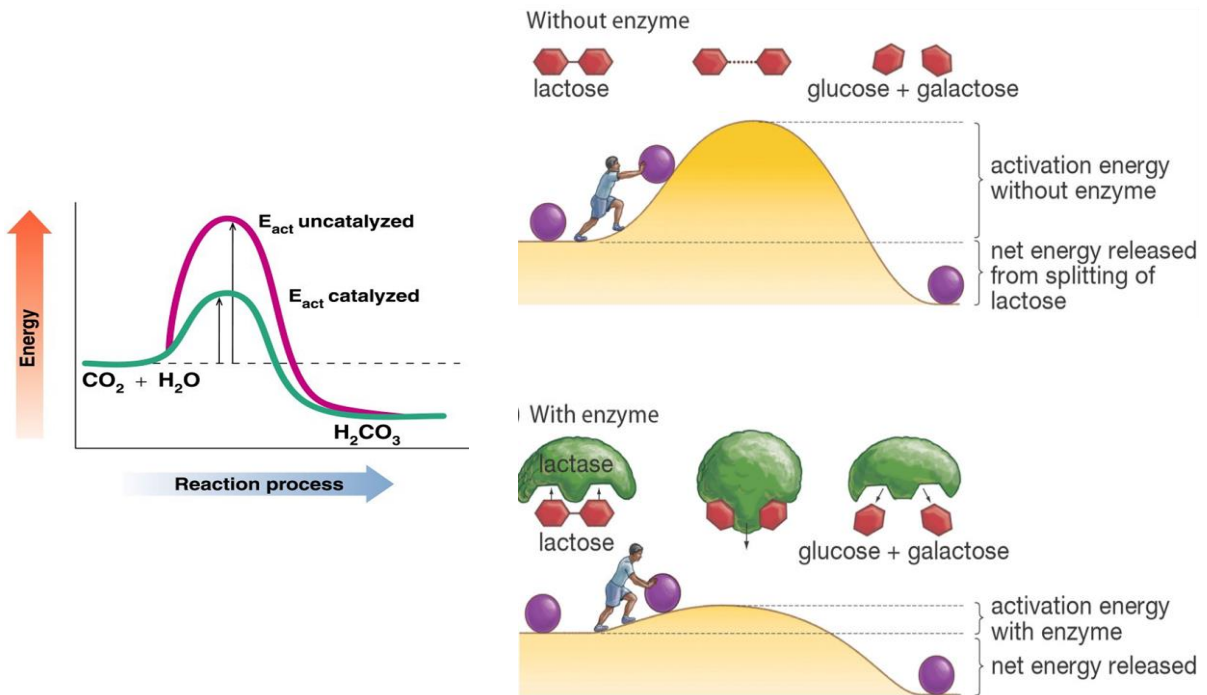
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How Enzymes work

Enzymes Affect Reaction Rates, Not Equilibria

- To undergo reaction, such as $E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$ the molecules must overcome the energy barrier and therefore must be raised to a higher energy level.
- At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way). This is called the **Transition state**.
- The transition state is not a chemical species with any significant stability and should not be confused with a reaction intermediate (such as ES or EP). It is simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally likely.
- The difference between the energy levels of the ground state and the transition state is the **Activation energy ($G^\ddagger$)**. The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction. Reaction rates can be increased by adding a catalyst thereby lowering the activation energy.

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Enzymes Act on Specific Substrates

- The noncovalent forces through which substrates and other molecules bind to enzymes are similar in character to the forces that dictate the conformations of the proteins themselves. Both involve van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions.
- In general, a substrate-binding site consists of an indentation or cleft on the surface of an enzyme molecule that is complementary in shape to the substrate (geometric complementarity).
- Moreover, the amino acid residues that form the binding site are arranged to specifically attract the substrate (electronic complementarity).
- Molecules that differ in shape or functional group distribution from the substrate cannot productively bind to the enzyme.
- The substrate-binding sites of most enzymes are largely pre-formed but undergo some conformational change on substrate binding (**induced fit**).
- The complementarity between enzymes and their substrates is the basis of the **“lock-and-key”** model of enzyme function first proposed by Emil Fischer in 1894. Such specific binding is necessary but not sufficient for efficient catalysis.

Some Enzymes Require Cofactors

- The functional groups of proteins can participate in acid–base reactions, form certain types of transient covalent bonds, and take part in charge–charge interactions. They are, however, less suitable for catalysing oxidation–reduction reactions and many types of group-transfer processes. Although enzymes catalyse such reactions, they can do so only in association with small **cofactors**, which essentially act as the enzymes' "chemical teeth".
- Cofactors may be metal ions, such as Cu^{2+} , Fe^{3+} , or Zn^{2+} . The essential nature of these cofactors explains why organisms require trace amounts of certain elements in their diets. It also explains, in part, the toxic effects of certain heavy metals. For example, Cd^{2+} and Hg^{2+} can replace Zn^{2+} in the active sites of certain enzymes and thereby render these enzymes inactive. Cofactors may also be organic molecules known as **coenzymes**.
- Some cofactors are only transiently associated with a given enzyme molecule, so that they function as **co-substrates**. Other cofactors, known as **prosthetic groups**, are permanently associated with their protein, often by covalent bonds.
- A catalytically active enzyme–cofactor complex is called a **holoenzyme**. The enzymatically inactive protein resulting from the removal of a holoenzyme's cofactor is referred to as an **apoenzyme**; that is, apoenzyme (inactive) + cofactor $\rightleftharpoons$ holoenzyme (active)

Principles of Enzyme Catalysis and Specificity

Rearrangement of covalent bonds during an enzyme-catalysed reaction

- Chemical reactions of many types take place between substrates and enzymes' functional groups (specific amino acid side chains, metal ions, and coenzymes).
- Catalytic functional groups on an enzyme may form a transient covalent bond with a substrate and activate it for reaction, or a group may be transiently transferred from the substrate to the enzyme. In many cases, these reactions occur only in the enzyme active site.
- Covalent interactions between enzymes and substrates lower the activation energy (and thereby accelerate the reaction) by providing an alternative, lower-energy reaction path.

Noncovalent interactions between enzyme and substrate

- Much of the energy required to lower activation energies is derived from weak, noncovalent interactions between substrate and enzyme in the form of a specific ES complex.
- The interaction between substrate and enzyme in this complex is mediated by the same forces that stabilize protein structure, including hydrogen bonds and hydrophobic and ionic interactions.
- Formation of each weak interaction in the ES complex is accompanied by release of a small amount of free energy that stabilizes the interaction.
- The energy derived from enzyme–substrate interaction is called binding energy, ΔG_{B} . Binding energy is a major source of free energy used by enzymes to lower the activation energies of reactions.

Catalytic Mechanisms of Enzymes

- Enzymes achieve their enormous rate accelerations via the same catalytic mechanisms used by chemical catalysts. Enzymes have simply been better designed through evolution. Enzymes, like other catalysts, reduce the free energy of the transition state ($\Delta G^\ddagger$); that is, they stabilize the transition state of the catalysed reaction.
- What makes enzymes such effective catalysts is their specificity of substrate binding combined with their arrangement of catalytic groups.
- The types of catalytic mechanisms that enzymes employ have been classified as:
 - Acid-base catalysis
 - Covalent catalysis
 - Metal ion catalysis
 - Proximity and orientation effects
 - Transition state stabilization

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Acid-Base Catalysis

- In acid–base catalysis, the acceleration of a reaction is achieved by catalytic transfer of a proton.
- Acid–base catalysis is the most common form of catalysis in organic chemistry and is also common in enzymatic reactions.
- Enzymes that employ acid–base catalysis rely on amino acid side chains that can donate and accept protons under the nearly neutral pH conditions of cells.
- This type of acid–base catalysis, involving proton-transferring agents, is termed general acid–base catalysis.
- Catalysis by H^+ or OH^- is termed specific acid or specific base catalysis.
- In effect, the active sites of these enzymes provide the biological equivalent of a solution of acid or base.

Covalent catalysis

- In covalent catalysis, a substrate is bound covalently to the enzyme to form a reactive intermediate.
- The reacting side chain of the enzyme can be either a nucleophile or an electrophile. Nucleophilic catalysis is more common.
- In the second step of the reaction, a portion of the substrate is transferred from the intermediate to a second substrate.
- This is a common mechanism for coupling two different reactions in biochemistry.
- Recall that the ability to couple reactions is one of the important properties of enzymes.

Metal ion Catalysis

- Metals, whether tightly bound to the enzyme or taken up from solution along with the substrate, can participate in catalysis in several ways.
- Ionic interactions between an enzyme-bound metal and a substrate can help orient the substrate for reaction or stabilize charged reaction transition states.
- This use of weak bonding interactions between metal and substrate is similar to some of the uses of enzyme-substrate binding energy described earlier.
- Metals can also mediate oxidation-reduction reactions by reversible changes in the metal ion's oxidation state.
- Nearly a third of all known enzymes require one or more metal ions for catalytic activity.

Proximity and Orientation Effects

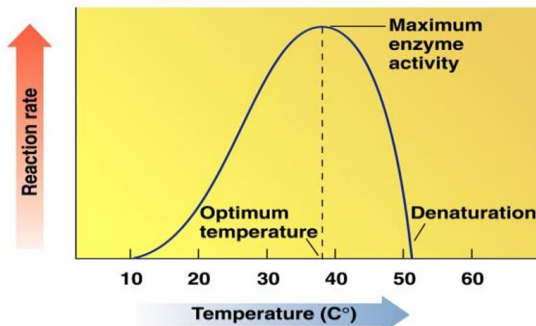
- The most obvious effects in the mechanisms of enzyme catalysis are proximity and orientation: Reactants must come together with the proper spatial relationship for a reaction to occur.
- By simply binding their substrates, enzymes facilitate their catalysed reactions in four ways:
 - I. Enzymes bring substrates into contact with their catalytic groups and, in reactions with more than one substrate, with each other.
 - II. Enzymes bind their substrates in the proper orientations for reaction. Molecules are not equally reactive in all directions. Rather, they react most readily if they have the proper relative orientation.
 - III. Charged groups may help stabilize the transition state of the reaction, a phenomenon termed electrostatic catalysis. The expulsion of water from the active site may enhance this effect. The charge distribution around the active sites of enzymes may also guide polar substrates toward their binding site.
 - IV. Enzymes freeze out the relative translational and rotational motions of their substrates and catalytic groups. This is an important aspect of catalysis because, in the transition state, the reacting groups have little relative motion. Indeed, experiments with model compounds suggest that this effect can promote rate enhancements of up to $\sim 10^7$.

Transition state stabilization effect

- The original concept of transition state binding proposed that enzymes mechanically strained their substrates toward the transition state geometry through binding sites into which undistorted substrates did not fit properly. Enzymes that preferentially bind the transition state structure increase its concentration and therefore proportionally increase the reaction rate.
- The more tightly an enzyme binds its reaction's transition state relative to the substrate, the greater is the rate of the catalysed reaction relative to that of the uncatalyzed reaction; that is, catalysis results from the preferential binding and therefore the stabilization of the transition state relative to the substrate. In other words, the free energy difference between an enzyme-substrate complex (ES) and an enzyme-transition state complex ($ES^\ddagger$) is less than the free energy difference between S and $S^\ddagger$ in an uncatalyzed reaction.
- Most enzymes combine several catalytic strategies to bring about a rate enhancement. A good example is the use of covalent catalysis, general acid-base catalysis, and transition-state stabilization in the reaction catalysed by chymotrypsin.

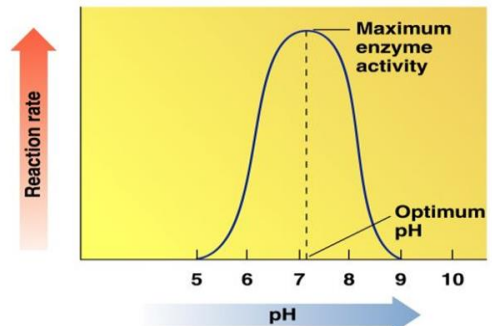
Factors affecting enzyme activity

Temperature - Enzymes are most active at an optimum temperature (usually 37°C in humans). They show little activity at low temperatures. Activity is lost at high temperatures as denaturation occurs.



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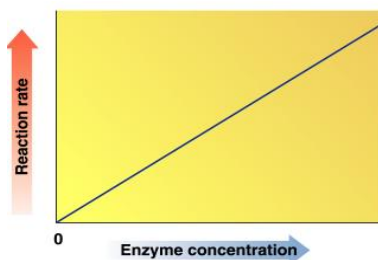
pH - Enzymes are most active at optimum pH. Amino acids with acidic or basic side-chains have the proper charges when the pH is optimum. Activity is lost at low or high pH as tertiary structure is disrupted.



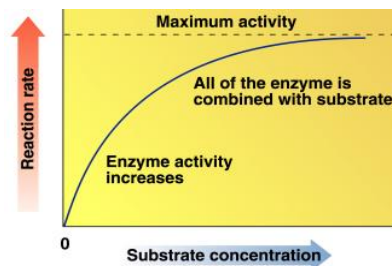
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Factors affecting enzyme activity

Enzyme concentration - At higher enzyme concentrations, more enzymes are available to catalyse the reaction (more reactions at once). There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration).



Substrate concentration - The rate of reaction increases as substrate concentration increases (at constant enzyme concentration). Maximum activity occurs when the enzyme is saturated. The relationship between reaction rate and substrate concentration is exponential, and asymptotes (levels off) when the enzyme is saturated.



Enzyme kinetics

- Enzyme kinetics is the study of chemical reaction that are catalyzed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Enzyme kinetics differs from simple chemical kinetics because the rates of enzyme-catalysed reactions depend on the concentration of enzyme and the enzyme is neither a product nor a substrate of the reaction. The rates also differ because substrate has to bind to enzyme before it can be converted to product.
- In an enzyme-catalysed reaction, the initial velocities are obtained from progress curves, just as they are in chemical reactions. Enzyme-catalysed reactions, like any chemical reaction, can be described mathematically by rate equations. Several constants in the equations indicate the efficiency and specificity of an enzyme and are therefore useful for comparing the activities of several enzymes or for assessing the physiological importance of a given enzyme.
- The first rate equations were derived in the early 1900s by examining the effects of variations in substrate concentration. The true value of maximum velocity of enzyme is precisely and correctly determined by fitting the data to the general equation for a rectangular hyperbola.
- The Michaelis-Menten equation describes the relationship between the initial velocity of a reaction and the substrate concentration.**

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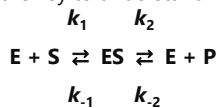
Rates of Reaction

- The rate of any reaction is determined by the concentration of the reactant and a **rate constant**, usually denoted by **k** . For the unimolecular reaction $S \rightarrow P$, the rate (or velocity) of the reaction (V), representing the amount of S that reacts per unit time—is expressed by a rate equation: $V = k[S]$. In this reaction, the rate depends only on the concentration of S and it is called a first-order reaction. The factor k is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, and so forth). Here, k is a first-order rate constant and has units of reciprocal time, such as s^{-1} .
- If a reaction rate depends on the concentration of two different compounds, or if the reaction is between two molecules of the same compound, the reaction is second order and k is a second-order rate constant, with units of $M^{-1}s^{-1}$. The rate equation then becomes $V = k[S_1][S_2]$.
- The relationship between substrate concentration and reaction rate can be expressed quantitatively**
- The curve expressing the relationship between $[S]$ and V_0 has the same general shape for most enzymes (it approaches a rectangular hyperbola), which can be expressed algebraically by the Michaelis-Menten equation.
- Michaelis and Menten derived this equation starting from their basic hypothesis that the rate-limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme.
- The important terms are $[S]$, V_0 , V_{max} and a constant called the Michaelis constant, K_m . All these terms are readily measured experimentally.

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Derivation of the Michaelis–Menten Equation...

▪The basic logic and the algebraic steps in a modern derivation of the Michaelis-Menten equation includes the steady-state assumption introduced by Briggs and Haldane. The derivation starts with the two basic steps of the formation and breakdown of ES. The ES complex is the key to understanding the kinetic behavior of an enzyme.



▪Early in the reaction, the concentration of the product, [P], is negligible, and the simplifying assumption is that the reverse reaction, $\text{P} \rightarrow \text{S}$ (described by k_{-2}) can be ignored.

▪The overall reaction then reduces to $\text{E} + \text{S} \xrightleftharpoons[k_{-1}]{k_1} \text{ES} \xrightarrow{k_2} \text{E} + \text{P}$

- V_0 is determined by the breakdown of ES to form product, which is determined by [ES]: $V_0 = k_2[\text{ES}] \dots\dots 1$
- Because [ES] in Equation 1 is not easily measured experimentally, the term $[\text{E}_t]$ is introduced to represent the total enzyme concentration (the sum of free and substrate-bound enzyme). Free enzyme can then be represented by $[\text{E}_t] - [\text{ES}]$.
- Also, because [S] is ordinarily far greater than $[\text{E}_t]$, the amount of substrate bound by the enzyme at any given time is negligible compared with the total [S]. With these conditions in mind, the following steps lead us to an expression for V_0 in terms of easily measurable parameters.

Derivation of the Michaelis–Menten Equation...

Step 1 - The rates of formation and breakdown of **ES** are determined by the steps governed by the rate constants k_1 (formation) and $k_{-1} + k_2$ (breakdown), according to the expressions:

$$\text{Rate of ES formation} = k_1([\text{E}_t] - [\text{ES}])[\text{S}] \dots\dots 2$$

$$\text{Rate of ES breakdown} = k_{-1}[\text{ES}] + k_2[\text{ES}] \dots\dots 3$$

Step 2 - Assume that the initial rate of reaction reflects a steady state in which **[ES]** is constant; that is, the rate of formation of ES is equal to the rate of its breakdown. This is called the **steady-state assumption**.

The expressions in Equations 2 and 3 can be equated for the steady state giving

$$k_1([\text{E}_t] - [\text{ES}])[\text{S}] = k_{-1}[\text{ES}] + k_2[\text{ES}] \dots\dots 4$$

Derivation of the Michaelis–Menten Equation...

Step 3 – Solve Equation 4 for $[ES]$.

First, the left side is multiplied out and the right side simplified to give

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES] \text{ 5}$$

Adding the term $k_1[ES][S]$ to both sides of the equation and simplifying gives

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES] \text{ 6}$$

Then solve this equation for $[ES]$:

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_{-1} + k_2} \text{ 7}$$

This can now be simplified further, combining the rate constants into one expression:

$$[ES] = \frac{[E_t][S]}{[S] + (k_2 + k_{-1})/k_1} \text{ 8}$$

The term $(k_2 + k_{-1})/k_1$ is defined as the **Michaelis constant, K_m** .

Substituting this into Equation 8 simplifies the expression to

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \text{ 9}$$

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Derivation of the Michaelis–Menten Equation...

Step 4 - V_0 can now be express in terms of $[ES]$.

Substituting the right side of Equation 9 for $[ES]$ in Equation 1 gives

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]} \text{ 10}$$

This equation can be further simplified. Because the maximum velocity occurs when the enzyme is saturated (that is, with $[ES] = [E_t]$), V_{max} can be defined as $k_2[E_t]$.

Substituting this in Equation 10 gives: $V_o = \frac{V_{max}[S]}{K_m + [S]}$

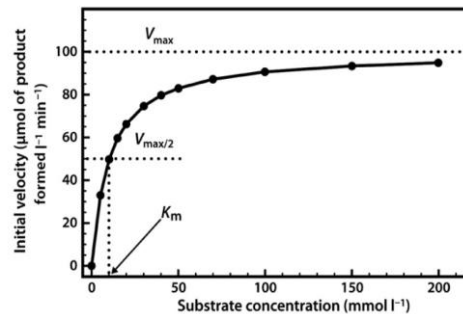
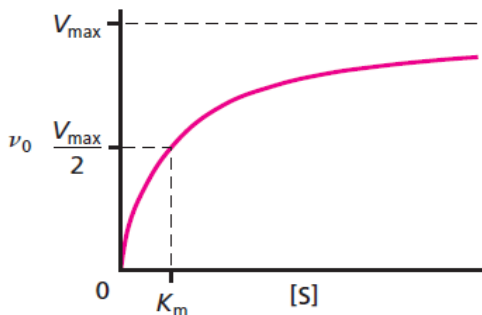
This is the **Michaelis-Menten equation (rate equation)**, for a one-substrate enzyme-catalysed reaction.

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Rectangular Hyperbola Curve

The **Michaelis-Menten equation (rate equation)**, for a one-substrate enzyme-catalysed reaction, is a statement of the quantitative relationship between the initial velocity V_0 , the maximum velocity $V_{\max}$, and the initial substrate concentration $[S]$, all related through the Michaelis constant K_m .

The Michaelis-Menten equation is the basic equation of enzyme kinetics and it describes a rectangular hyperbola curve (plot of v_0 against $[S]$).



The Double-Reciprocal Plot

Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting experimental data. One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

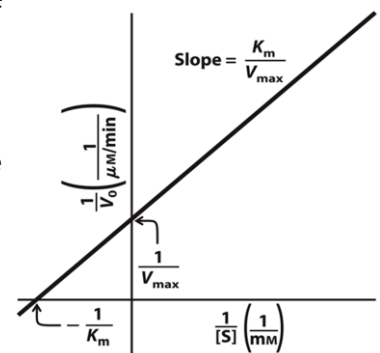
$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{\max} [S]}$$

Separating the components of the numerator on the right side of the equation gives

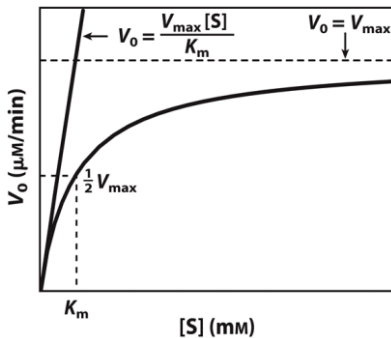
$$\frac{1}{V_0} = \frac{K_m}{V_{\max} [S]} + \frac{[S]}{V_{\max} [S]} \text{ which simplifies to } \frac{1}{V_0} = \frac{K_m}{V_{\max} [S]} + \frac{1}{V_{\max}}$$

This form of the Michaelis-Menten equation is called the **Lineweaver-Burk equation**.

The double-reciprocal presentation, also called a Lineweaver-Burk plot (plot of $\frac{1}{V_0}$ against $\frac{1}{[S]}$), has the great advantage of allowing a more accurate determination of $V_{\max}$, which can only be approximated from a simple plot of V_0 versus $[S]$.



Dependence of initial velocity (V_0) on substrate concentration



- At low $[S]$, $K_m \gg [S]$ and the $[S]$ term in the denominator of the Michaelis-Menten equation becomes insignificant. The equation simplifies to $V_0 = V_{\max}[S]/K_m$ and V_0 exhibits a linear dependence on $[S]$, as observed here.
- At high $[S]$, where $[S] \gg K_m$, the K_m term in the denominator of the Michaelis-Menten equation becomes insignificant and the equation simplifies to $V_0 = V_{\max}$; this is consistent with the plateau observed at high $[S]$.
- An important numerical relationship emerges from the Michaelis-Menten equation in the special case when V_0 is exactly one-half $V_{\max}$.

Then $\frac{V_{\max}}{2} = \frac{V_{\max}[S]}{K_m + [S]}$ on dividing by $V_{\max}$, we obtain $\frac{1}{2} = \frac{[S]}{K_m + [S]}$

$K_m + [S] = 2[S]$, or $K_m = [S]$, when $V_0 = \frac{1}{2} V_{\max}$

K_m is equivalent to the substrate concentration at which V_0 is half $V_{\max}$.

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Definitions of the Michaelis constant (K_m)

- The K_m can vary greatly from enzyme to enzyme, and even for different substrates of the same enzyme. The term is sometimes used as an indicator of the affinity of an enzyme for its substrate. The actual meaning of K_m depends on specific aspects of the reaction mechanism such as the number and relative rates of the individual steps. For reactions with two steps, $K_m = \frac{k_2 + k_{-1}}{k_1}$
- When k_2 is rate-limiting, $k_2 \ll k_{-1}$ and K_m reduces to k_{-1}/k_1 , which is defined as the **dissociation constant (K_d)** of the ES complex. Where these conditions hold, K_m does represent a measure of the affinity of the enzyme for its substrate in the ES complex.
- However, this scenario does not apply for most enzymes. Sometimes $k_2 \gg k_{-1}$, and then $K_m = k_2/k_1$. In other cases, k_2 and k_{-1} are comparable and K_m remains a more complex function of all three rate constants.

Interpretation of $V_{\max}$

- At high substrate concentration the overall velocity of the reaction is $V_{\max}$ and the rate is determined by the enzyme concentration. The rate constant observed under these conditions is called the **catalytic constant (k_{cat})** defined as $V_{\max} = k_{cat}[E_t]$ therefore, $k_{cat} = \frac{V_{\max}}{[E_t]}$
- k_{cat} represents the number of moles of substrate converted to product per second per mole of enzyme under saturating conditions. In other words, k_{cat} indicates the maximum number of substrate molecules converted to product each second by each active site. This is often called the **turnover number**.
- The catalytic constant measures how quickly a given enzyme can catalyse a specific reaction; it's a very useful way of describing the effectiveness of an enzyme. The unit for k_{cat} is s^{-1} .
- The reciprocal of k_{cat} is the time required for one catalytic event. In other words, the amount of time required to convert substrate to product is the reciprocal of the turnover rate. $t = \frac{1}{k_{cat}}$ Note that to obtain k_{cat} the enzyme concentration must be known.

Catalytic Efficiencies

- The kinetic parameters k_{cat} and K_m are generally useful for the study and comparison of different enzymes, whether their reaction mechanisms are simple or complex. Each enzyme has values of k_{cat} and K_m that reflect the cellular environment, the concentration of substrate, and the chemistry of the reaction being catalysed. k_{cat} and K_m also allow us to evaluate the kinetic efficiency of enzymes, but either parameter alone is insufficient for this task.
- The best way to compare the catalytic efficiencies of different enzymes is to compare the ratio $\frac{k_{\text{cat}}}{K_m}$ for the two reactions. This parameter, sometimes called the specificity constant, is the rate constant for the conversion of E + S to E + P when $[S] \ll K_m$.
- There is an upper limit to $\frac{k_{\text{cat}}}{K_m}$, imposed by the rate at which E and S can diffuse together in an aqueous solution. This diffusion controlled limit is 10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$, and many enzymes have a $\frac{k_{\text{cat}}}{K_m}$ near this range and such enzymes are said to have achieved catalytic perfection.

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Multi-substrate reactions

- **Multi-substrate reactions** - Most reactions in biological systems usually include two substrates and two products and can be represented by the bi-substrate reaction:

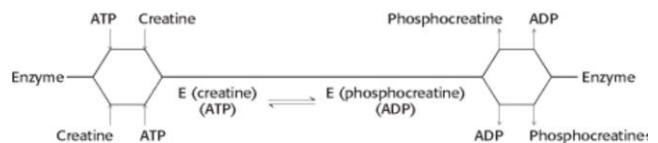
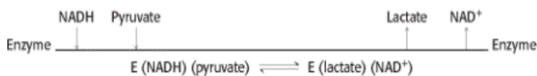


- For example, in the reaction catalysed by hexokinase, ATP and glucose are the substrate molecules, while ADP and glucose-6-phosphate are the products: $\text{ATP} + \text{Glucose} \rightarrow \text{ADP} + \text{Glucose-6-Phosphate}$
- Multiple substrate reactions can be divided into two classes:
 - **Sequential mechanism**
 - **Ping pong mechanism (double displacement)**
- The number of reactants and products in a given reaction are specified, in order by the terms Uni (one), Bi (two), Ter (three), and Quad (four).
- Example a reaction that requires one substrate and yield two products is referred to as Uni-Bi and that requires two substrates and yield three products as Bi-Ter e.t.c.

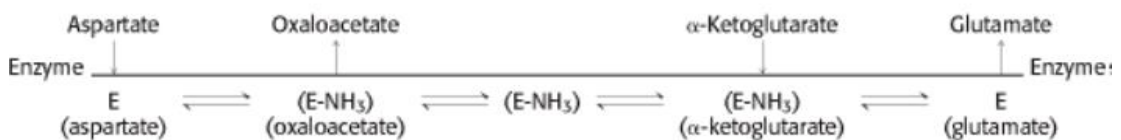
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Sequential mechanism - In the sequential mechanism, all substrates must bind to the enzyme before any product is released. Consequently, in a bi-substrate reaction, a *ternary complex* of the enzyme and both substrates forms. Sequential mechanisms are of two types: **Ordered sequential** and **Random sequential**

- **Ordered sequential**, in which the substrates bind the enzyme in a defined sequence. In the ordered mechanism, the binding of the first substrate is apparently required for the enzyme to form the binding site for the second substrate. Many enzymes that have NAD⁺ or NADH as a substrate exhibit the sequential ordered mechanism.
- **Random sequential**, are those with no preference in the order of substrate addition. In random mechanism, both binding sites are present on the free enzyme. In the random sequential mechanism, the order of addition of substrates and release of products is random. Although the order of certain events is random, the reaction still passes through the ternary complexes including, first, substrates and, then, products.
- The enzyme exists as a ternary complex: first, consisting of the enzyme and substrates and, after catalysis, the enzyme and products.



- **Ping pong (Double displacement)** - In Ping-Pong or double-displacement reactions, one or more products are released before all substrates bind the enzyme. The defining feature of double-displacement reactions is the existence of a [substituted enzyme intermediate](#), in which the enzyme is temporarily modified.
- Reactions that shuttle amino groups between amino acids and α -keto acids are classic examples of double-displacement mechanisms.
- The enzyme aspartate aminotransferase catalyses the transfer of an amino group from aspartate to α -ketoglutarate.
- In the Cleland notation, the substrates appear to bounce on and off the enzyme analogously to a Ping-Pong ball bouncing on a table.

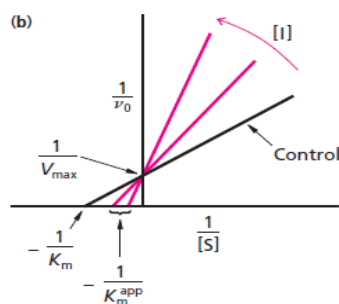
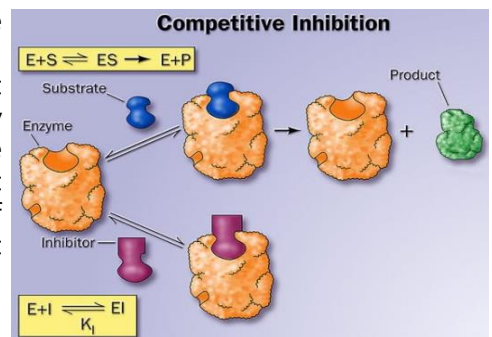


Enzyme Inhibition

- Compounds which convert the enzyme into an inactive form and thus adversely affect the rate of enzyme-catalysed reaction are called enzyme inhibitors. Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions. The study of enzyme inhibition has provided some of the important information about enzyme mechanisms and also helped defined some metabolic pathways. Enzyme inhibition is broadly classified into **reversible** and **irreversible**.
- Reversible inhibition** - In reversible inhibition, the inhibitor dissociates rapidly from the enzyme because it is bound loosely with the enzyme. There are three types of reversible inhibition these include: **Competitive**, **Uncompetitive**, and **Non-competitive (Mixed)**.
- Irreversible inhibition** - In irreversible inhibition, the inhibitor forms a stable covalent bond with an enzyme. Irreversible inhibition occurs typically by alkylation or acylation of the side chain of an active-site amino acid residue. There are many naturally occurring as well as the synthetic irreversible inhibitors.

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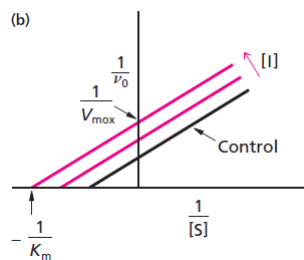
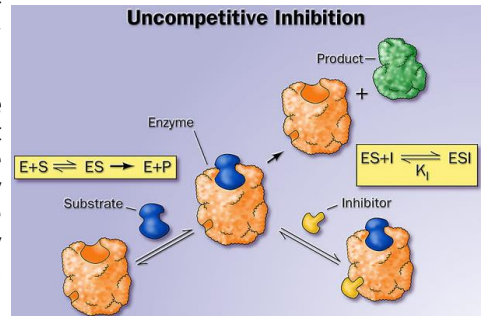
Competitive inhibition – In competitive inhibition, the inhibitor competes with the substrate for the active site of an enzyme. While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme. Many competitive inhibitors are structurally similar to the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis. Even fleeting combinations of this type will reduce the efficiency of the enzyme. The effect of a competitive inhibitor can be reverse by increasing substrate concentration.



Double-reciprocal (Lineweaver-Burk) plot In competitive inhibition, $V_{\max}$ remains unchanged and K_m increases. The black line labelled "Control" is the result in the absence of inhibitor. The red lines are the results in the presence of inhibitor, with the arrow showing the direction of increasing $[I]$.

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Uncompetitive Inhibition - The uncompetitive inhibitor binds at a site distinct from the substrate active site (allosteric site) and, unlike a competitive inhibitor, it binds only to the ES complex and not the free enzyme. Uncompetitive inhibitor brings about a decrease in $V_{\max}$ ($1/v_{\max}$ is increased) by the conversion of some molecule of E to an inactive form ESI. As it is the ES complex that binds I, increase in the concentration of substrate will not reverse the decrease in $V_{\max}$. K_m value is also affected (decrease) by uncompetitive inhibitor, this is because the equilibria for the formation of both ES and ESI are shifted toward the complexes by the binding of I. This type of inhibition usually occurs only with multi-substrate reactions.

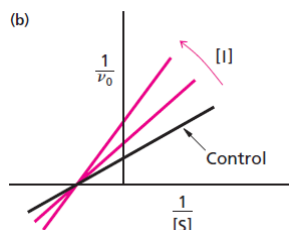
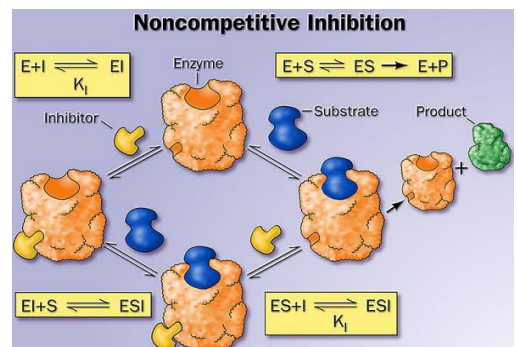


Double reciprocal (Lineweaver-Burk) plot

In uncompetitive inhibition, both $V_{\max}$ and K_m decrease (i.e., the absolute values of both $1/V_{\max}$ and $1/K_m$ obtained from the y and x intercepts, respectively, increase). The ratio $K_m/V_{\max}$, the slope of the lines, remains unchanged.

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Non-competitive (Mixed) Inhibition - Also has a structure that is different than that of the substrate. It binds at a site distinct from the substrate active site (allosteric site), but it binds to either E or ES. It distorts the shape of the enzyme, which alters the shape of the active site and prevents the binding of the substrate. The effect can not be reversed by adding more substrate.



Double-reciprocal (Lineweaver-Burk) plot

$V_{\max}$ decreases, but K_m remains the same.

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Uses of Enzyme Inhibition

- Reversible enzyme inhibition provides a powerful tool for probing enzyme activity and for altering it in the treatment of disease.
- The pharmaceutical industry uses enzyme inhibition studies to design clinically useful drugs. In many cases, a naturally occurring enzyme inhibitor is used as the starting point for drug design.
- The advances made in drug synthesis are exemplified by the design of a series of inhibitors of the enzyme purine nucleoside phosphorylase. This enzyme catalyses a degradative reaction between phosphate and the nucleoside guanosine.
- Some organophosphorus inhibitors are used in agriculture as insecticides; others, are useful reagents for enzyme research.
- Amino acids with key catalytic functions in the active site can sometimes be identified by determining which residue is covalently linked to an inhibitor after the enzyme is inactivated.