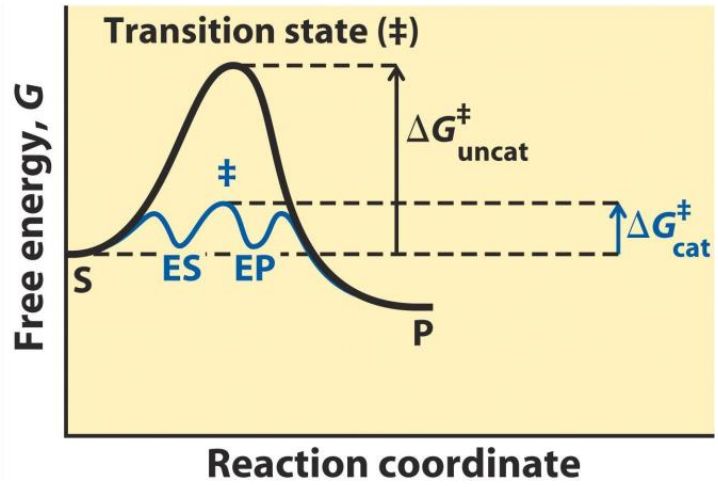
LECTURE NOTE

ENZYMOLOGY (BCH 301)

Enzymes

Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions. Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Most enzymes are proteins, although a few are catalytic RNA molecules. Enzymes are central to every biochemical process acting in organized sequences, they catalyze the hundreds of stepwise reactions that degrade nutrient molecules, conserve and transform chemical energy, and make biological macromolecules from simple precursors. Through the action of regulatory enzymes, metabolic pathways are highly coordinated to yield a harmonious interplay among the many activities necessary to sustain life.

The substance, upon which an enzyme acts, is called as substrate. Enzymes are involved in conversion of substrate into product. Almost all enzymes are globular proteins consisting either of a single polypeptide or of two or more polypeptides held together (in quaternary structure) by non-covalent bonds. Enzymes do nothing but speed up the rates at which the equilibrium positions of reversible reactions are attained. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily at low temperatures - essential for biological systems.



The basic properties of enzymes includes:

- Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.
- Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100^{o}C, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.
- Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.
- Enzymes are highly specific with respect to the substrates on which they act and the products that they form.

- Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.
- They function under strict conditions of temperature and pH in the body.
- Enzymes could be precipitated by protein precipitating agents such as ammonium sulfate and trichloroacetic acid.
- Enzymes are sensitive and labile to heat

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group.

In general, there are four distinct types of specificity:

Absolute specificity – the enzyme will catalyze only one reaction.

Group specificity – the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

Linkage specificity – the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

Stereochemical specificity – the enzyme will act on a particular steric or optical isomer.

Active site of Enzymes

- The active site of an enzyme is the region that binds the substrate and converts it into product.
- It is usually a relatively small part of the whole enzyme molecule and is a three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain.
- The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly nonpolar environment which enhances the binding of the substrate.
- The substrate(s) is bound in the active site by multiple weak forces (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; and in some cases by reversible covalent bonds.

Substrate Specificity of Enzymes

- The properties and spatial arrangement of the amino acid residues forming the active site of an enzyme will determine which molecules can bind and be substrates for that enzyme.
- Substrate specificity is often determined by changes in relatively few amino acids in the active site.
- This is clearly seen in the three digestive enzymes trypsin, chymotrypsin and elastase**.**

Mechanism of Action of Enzymes

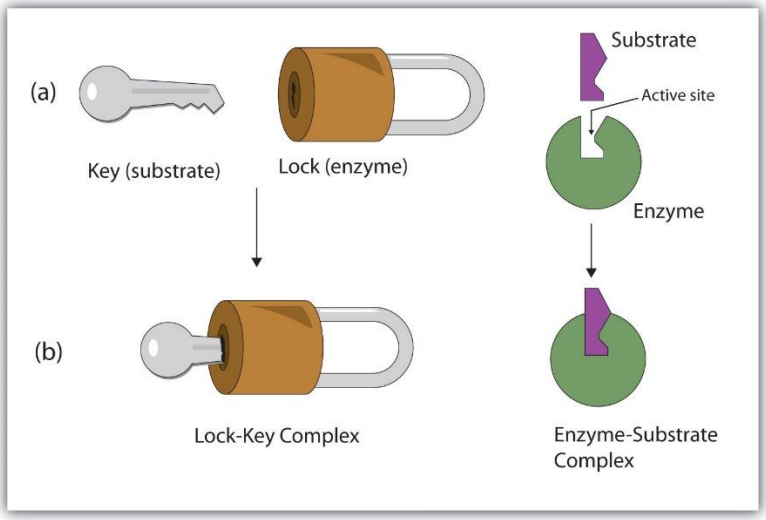
- The substrate(s) is bound in the active site by multiple weak forces which result into the enzyme-substrate complex.
- Once bound active residues within the active site of the enzyme act on the substrate molecule to transform it first into the transition state complex and then into product, which is released.
- The enzyme is now free to bind another molecule of substrate and begin its catalytic cycle again.

The Substrate-Enzyme Binding

Two models were proposed to explain how an enzyme binds its substrate.

The Lock and Key Model

- In the lock-and-key model proposed was proposed by Emil Fischer in 1894.
- According to the model, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.



The Induced Fit Model

- In the induced-fit model was proposed by Daniel E. Koshland, Jr., in 1958.
- It states that the binding of substrate induces a conformational change in the active site of the enzyme.
- In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state.
- For example, the binding of glucose to hexokinase induces a conformational change in the structure of the enzyme such that the active site assumes a shape that is complementary to the substrate (glucose) only after it has bound to the enzyme.

The reality is that different enzymes show features of both models, with some complementarity and some conformational change.

Nomenclature of Enzymes

Many enzymes are named by adding the suffix '-ase' to the name of their substrate.
Example. Urease is the enzyme that catalyzes the hydrolysis of urea, and fructose-1,6-bisphosphatase hydrolyzes fructose-1,6-bisphosphate.

- However, other enzymes, such as trypsin and chymotrypsin, have names that do not denote their substrate.
- Some enzymes have several alternative names.
- To rationalize enzyme names, a system of enzyme nomenclature has been internationally agreed.
- This system places all enzymes into one of six major classes based on the type of reaction catalyzed. Each enzyme is then uniquely identified with a four-digit classification number.

Example: Trypsin has the Enzyme Commission (EC) number 3.4.21.4, where

1. the first number (3) denotes that it is a hydrolase
2. the second number (4) that it is a protease that hydrolyzes peptide bonds
3. the third number (21) that it is a serine protease with a critical serine
4. residue at the active site, and
5. the fourth number (4) indicates that it was the fourth enzyme to be assigned to this class.

Enzyme International Units
Enzyme activity levels are reported in terms of enzyme international units (IU), which defines enzyme activity as the amount of enzyme that will convert a specified amount of substrate to a product within a certain time.
– One standard IU is the quantity of enzyme that catalyzes the conversion of 1 micromole (1 mmol) of substrate per minute under specified conditions.
– Unlike the turnover number, IUs measure how much enzyme is present. (An enzyme preparation having an IU of 40 is forty times more concentrated than the standard solution.

Classification of Enzymes

1. **Oxidoreductases**

- Catalyze oxidation-reduction reactions where electrons are transferred.
- These electrons are usually in the form of hydride ions or hydrogen atoms.
- The most common name used is a dehydrogenase and sometimes reductase is used.
- An oxidase is referred to when the oxygen atom is the acceptor.

2. **Transferases**

- Catalyze group transfer reactions.
- The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor.
- Most of the time, the donor is a cofactor that is charged with the group about to be transferred.
- Example: Hexokinase used in glycolysis.

3. **Hydrolases**

- Catalyze reactions that involve hydrolysis.
- It usually involves the transfer of functional groups to water.
- When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound
- For example: Chymotrypsin.

4. **Lyases**

- Catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups.
- For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

5. **Isomerases**

- Catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced.
- These enzymes allow for structural or geometric changes within a compound.
- For example: phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate.

6. **Ligases**

- They are involved in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation reactions.
- These reactions are coupled to the cleavage of ATP.

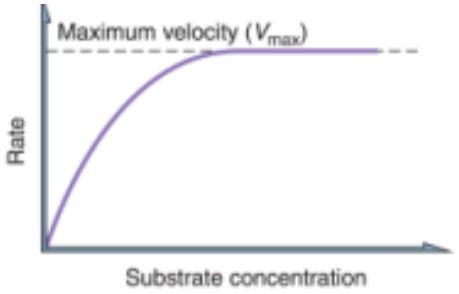
Factors Affecting Enzyme Activity

Velocity or rate of enzymatic reaction is assessed by the rate of change in concentration of substrate or product at a given time duration. Various factors which affect the activity of enzymes include:

1. Substrate concentration
2. Enzyme concentration
3. Product concentration
4. Temperature
5. pH

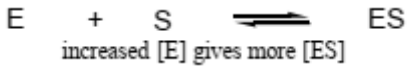
1. Substrate Concentration

• The concentration of substrate, [S], also affects the rate of the reaction.
• Increasing [S] increases the rate of the reaction, but eventually, the rate reaches a maximum (vmax), and remains constant after that.
• The maximum rate is reach when the enzyme is saturated with substrate, and cannot react any faster under those conditions.

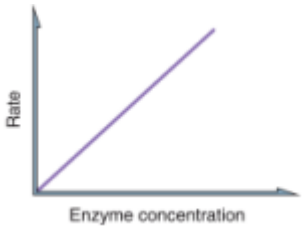


2. Enzyme Concentration

- The concentration of an enzyme, [E], is typically low compared to that of the substrate. Increasing [E] also increases the rate of the reaction:

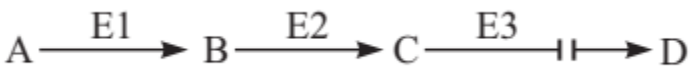


- The rate of the reaction is directly proportional to the concentration of the enzyme (doubling [E] doubles the rate of the reaction), thus, a graph of reaction rate vs. enzyme concentration is a straight line:



3. Product Concentration

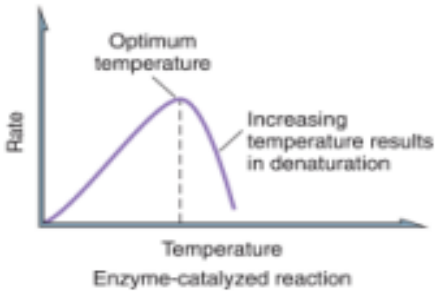
In case of a reversible reaction catalyzed by an enzyme, as per the law of mass action the rate of reaction is slowed down with equilibrium. So, rate of reaction is slowed, stopped or even reversed with increase in product concentration. This phenomena can be better explained by the equation



In the above equation, in case of absence of the enzyme E3, the product C will accumulate. Enzymatic activity of E2 will be inhibited with accumulation of the product C. In such inborn error of one enzyme will block the whole pathway.

4. Temperature

• Like all reactions, the rate of enzyme-catalyzed reactions increases with temperature.
• Because enzymes are proteins, beyond a certain temperature, the enzyme denatures.
• Every enzyme-catalyzed reaction has an optimum temperature at which the enzyme activity is highest, usually from 25º-40ºC; above or below that value, the rate is lower.



5. pH

• Raising or lowering the pH influences the acidic and basic side chains in enzymes. Many enzymes are also denatured by pH extremes. (E.g., pickling in acetic acid [vinegar] preserves food by deactivating bacterial enzymes.)
• Many enzymes have an optimum pH, where activity is highest, near a pH of 7, but some operate better at low pH (e.g., pepsin in the stomach).

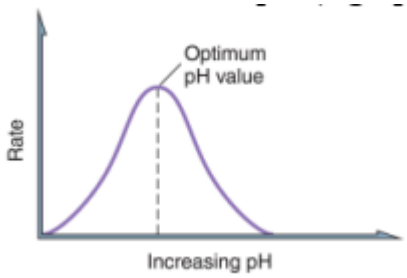


Table 20.4 Examples of Optimum pH for Enzyme Activity

Enzyme	Source	Optimum pH
pepsin	Gastric mucosa	1.5
β-glucosidase	Almond	4.5
sucrase	Intestine	6.2
urease	Soybean	6.8
catalase	Liver	7.3
succinate dehydrogenase	Beef heart	7.6
arginase	Beef liver	9.0
alkaline phosphatase	Bone	9.5

Enzyme	Optimum pH
Lipase (pancreas)	8.0
Lipase (stomach)	4.0 – 5.0
Lipase (castor oil)	4.7
Pepsin	1.5 - 1.6
Trypsin	7.8 - 8.7
Urease	7.0
Invertase	4.5
Maltase	6.1 - 6.8
Amylase (pancreas)	6.7 - 7.0
Amylase (malt)	4.6 - 5.2
Catalase	7.0

Significance of Enzymes

1. In the absence of an enzyme, biochemical reactions hardly proceed at all, whereas in its presence the rate can be increased up to 10^7-fold. Thus, they are crucial for normal metabolism of living systems.
2. Besides in the body, extracted and purified enzymes have many applications.

- Medical applications of enzymes include:

1. To treat enzyme related disorders.
2. To assist in metabolism
3. To assist in drug delivery.
4. To diagnose & detect diseases.
5. In manufacture of medicines.

- Industrial applications of enzymes include:

1. Amylase, lactases, cellulases are enzymes used to break complex sugars into simple sugars.
2. Pectinase like enzymes which act on hard pectin is used in fruit juice manufacture.
3. Lipase enzymes act on lipids to break them in fatty acids and glycerol. Lipases are used to remove stains of grease, oils, butter.
4. Enzymes are used in detergents and washing soaps.
5. Protease enzymes are used to remove stains of protein nature like blood, sweat etc.

Enzyme kinetics, and basic rate determinations

During an enzyme catalyzed reaction, the enzyme (E) first combines with the substrate to generate an enzyme-substrate complex, and then the product is formed and dissociates from the enzyme. The kinetic scheme for an enzyme catalyzed reaction can be written as;

$$E + S \rightarrow ES \rightarrow E + P$$

If the rate limiting step is the breakdown of ES $\rightarrow$ E + P, then the initial velocity (V0) of the reaction will be linearly proportional to the amount of E present, provided S is in great excess and all of the enzyme is combined with S, i.e., the enzyme is "saturated."

In actuality the 2 steps of this reaction are reversible. However, if one measures initial velocity (v0) before P builds up then the equation can be written as;

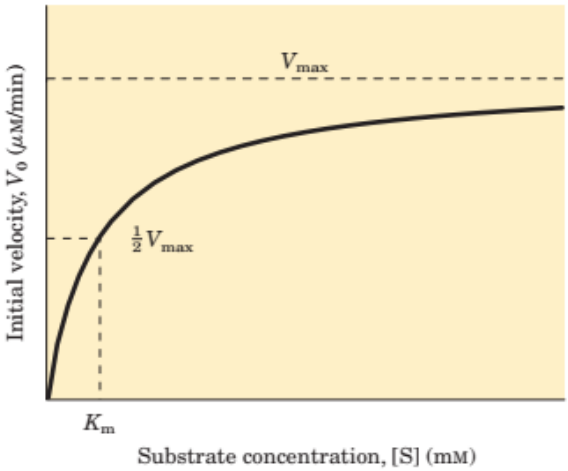
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

In this equation K_1 is the rate constant for the association of E and S, and K_{-1} is the rate constant for the dissociation of the ES complex. K_2 is the rate constant for formation of E and P.

Michaelis-Menten equation

A. Basic assumptions.

For many enzymes, a plot of initial reaction velocity (V_0, measured in units of moles of product per liter per second) vs. substrate concentration [S] gives a hyperbolic curve.



At very low substrate concentration, V_0 increases almost linearly with increasing [S], while at high [S], v0 is independent of [S] and approaches a maximum velocity, Vmax. The French mathematicians Leonor Michaelis and Maud Menten derived a mathematical equation that describes the kinetic behavior of most enzymes. In their derivation, they proposed that; 1) the enzymatic reaction occurs in two stages, and 2) the rate of product formation is determined by the amount of enzyme substrate (ES) complex present, as shown in the equation:

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

They also assumed that the rate of the second stage of the reaction (ES → E + P) is slower than the first. For this reason, the second stage of the reaction sets the rate of the overall reaction, i.e., is "rate limiting". Because 1) the rate of the overall reaction is dependent on [ES], and 2) the second stage is rate-limiting, the velocity of the reaction (V0) is given by the equation:

$$v_0 = k_2[ES]$$

A maximal rate is obtained when all of the enzyme (denoted by [E]total) is in the form of the ES complex, and under these conditions:

$$V_{max} = k_2[E]_{total}$$

Derivation of the Michaelis-Menten equation

Starting with these assumptions it is possible to derive the MM equation. The derivation takes the standpoint that a "steady state", in which the concentration of ES remains relatively constant over time, soon develops after the substrate and enzyme are mixed. The amount of ES formed depends

on the concentration of the substrate and its affinity for the enzyme. When the steady state is achieved, the rate of ES formation equals the rate of ES breakdown, i.e.,

$$\text{Rate of ES formation} = k_1([E]_{total} - [ES])[S]$$

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

and therefore,

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

(Note that the concentration of the free enzyme [E] has been written as [E]total - [ES].) The steady state equation is then rearranged to collect all of the rate constants on one side in the so-called Michaelis constant, K_m:

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

This equation is solved (in several steps) for [ES]:

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

Next, the initial velocity (V_0) term is introduced into the equation after multiplying both sides by the K_2 rate constant and substituting $V_0 = K_2[ES]$ from above:

$$v_0 = k_2[ES] = \frac{k_2[E]_{total}[S]}{K_m + [S]}$$

Finally, the term V_{max} is substituted into the equation for $K_2[E]_{total}$ resulting in the final form of the MM equation:

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

At high substrate concentrations (the extreme right side of the curve), [S]>>Km, we can neglect the term Km in the MM equation. Under these conditions,

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

Thus, at very high substrate concentrations V_0 becomes independent of [S], and the curve becomes flat at the limiting value Vmax. Now, at very low substrate concentrations (the extreme left side of the curve) where [S]<<Km, we can neglect the term [S] in the denominator of the MM equation. Under these conditions,

$$v_0 = \frac{V_{max}[S]}{K_m} = (V_{max}/K_m)[S]$$

This indicates that at very low substrate concentrations the velocity is linearly proportional to [S], i.e., the shape of the curve is approximately linear.

Meaning of Kcat, Km, and Vmax

A. Kcat

In a large percentage of cases, the rate-limiting step is indeed ES → E + P, and the equations $V_0 = K_2[ES]$ and $V_{max} = K_2[E]total$ are directly applicable to the enzyme catalyzed reaction. However, in a significant number of cases, the enzymatic rate may be dependent not only on K_2, but on other rate constants (e.g., K_{-1}) as well. In these cases, it is better to use a generic constant K_{cat} for the rate limiting step. K_{cat} lumps together all of the constants that actually influence the rate of the reaction. Then, the V_0 and V_{max} equations become;

$$v_0 = k_{cat}[ES]$$

$$V_{max} = k_{cat}[E]_{total}.$$

Kcat (like K_2) is a first order rate constant and has the units s^{-1}. When the enzyme is operating at maximal velocity, it is a direct measure of the number of moles of product formed per second in the active site. This is the so-called turnover number for the enzyme, and the higher the turnover number the more efficient the enzyme is in carrying out the reaction. The enzyme carbonic anhydrase which converts dissolved CO_2 into carbonic acid has one of the fastest turnover numbers known and thus is well designed for efficient CO_2 transport to the lungs.

B. K_m

The Km kinetic constant actually has units of moles/liter (M). It can be mathematically proven that the reaction velocity is half-maximal when [S] = Km. To prove this, [S] is substituted for Km in the MM equation:

$$v_0 = \frac{V_{max}[S]}{[S]+[S]} = \frac{V_{max}[S]}{2[S]} = \frac{V_{max}}{2}$$

The Km constant also provides information about the affinity of an enzyme for its substrate. If the breakdown of ES → E + P truly is rate limiting, then K_2 can be neglected from the Km constant and $Km \approx K_{-1}/K1$. Under these conditions, Km is equivalent to the dissociation constant (KES) for the ES complex:

$$ES \xrightarrow[k_1]{k_{-1}} E + S$$

for which

$$K_m = K_{ES} = ([E][S])/[ES] = k_{-1}/k_1.$$

This demonstrates that "the lower the Km, the higher the affinity of the enzyme for its substrate." The Km is an intrinsic property of an enzyme and is independent of the enzyme concentration used in a reaction. The Km of an enzyme often is just slightly higher than the typical intracellular concentration of its substrate. Because the hyperbolic curve is roughly linear in this range of substrate concentration, a slight change in substrate concentration produces a significant change in reaction rate, which is advantageous to metabolism. In contrast, if the Km were much lower than the typical intracellular substrate concentration, then there would be little change in reaction velocity even for substantial changes in S concentration.

C. Vmax

V_{max} is calculated from the equations $V_{max} = K_2[E]total$ or $V_{max} = K_{cat}[E]total$. These equations show that V_{max} is dependent on the concentration of enzyme used to set up a reaction. In other words, the V_{max} measured for a reaction will depend on the amount of enzyme present. This allows one to determine the concentration of a clinically important enzyme in a blood specimen, for example, by determining the V_{max} for the sample and using a calibration curve to calculate the amount of enzyme present. The value of V_{max} also can be used to calculate the turnover number (K_{cat}) for an enzyme.

Determination of Km and Vmax by Lineweaver-Burk plots

It is difficult to accurately determine Km and Vmax values from hyperbolic kinetic curves due to uncertainty as to where the limit Vmax occurs. To better determine these values, data often are graphed in the form of the Lineweaver-Burk plot, which plots $1/V_0$ vs 1/[S]. If one takes the reciprocal of the MM equation, then the LB equation is obtained:

$$1/v_0 = (K_m/V_{max})(1/[S]) + 1/V_{max}$$

This equation is in the form y = mx + c which is the general equation for a straight line. The y intercept of a LB plot is 1/Vmax and the x-intercept is -1/Km. Thus, the values of Vmax and Km are obtained by determining the intercepts of the line with the axes.

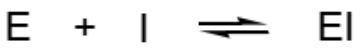
ENZYME INHIBITION

This is the inhibition or decrease of enzymes or some processes related to its production or any other enzyme activity. The study of enzyme inhibition provides information about the structure of active sites, mechanisms of metabolic control, and provides useful information pertinent to the development of pharmaceuticals. Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions. The study of enzyme inhibitors also has provided

valuable information about enzyme mechanisms and has helped define some metabolic pathways. There are two broad classes of enzyme inhibitors: reversible and irreversible.

Reversible inhibitors

Reversible inhibitors bind non-covalently and thus reversibly to their target enzyme. When reversible inhibitors are bound to the enzyme, the enzyme is inactive.



– Once the inhibitor combines with the enzyme, the active site is blocked, and no further catalysis takes place.

– The inhibitor can be removed from the enzyme by shifting the equilibrium.

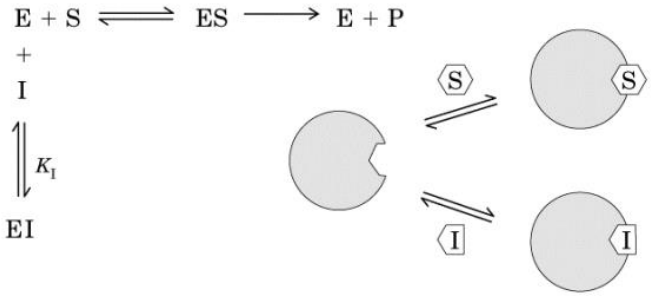
There are different types of reversible inhibitors namely:

Competitive inhibition,

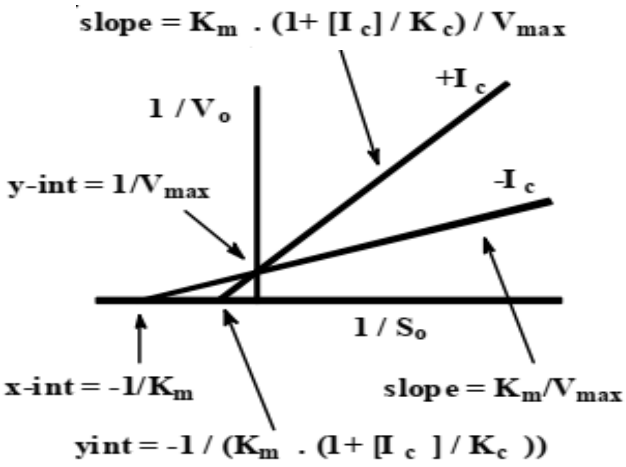
Noncompetitive inhibition,

Uncompetitive inhibition

A) Competitive inhibition



The structural resemblance of the competitor matches that of the substrate and therefore binds to the active site competitively. But when competitive inhibitor's concentration exceeds that of the substrate, competitive inhibitor binds to the active binding site to form enzyme inhibitor complex (EI) and finally no product is formed. It is complete ambiguous information that inhibitors lash out the substrate from the enzyme. Rather, the fact is that the inhibitor would bind to the Enzyme substrate complex and would compel the substrate to dissociate from the enzyme through a thermodynamic principle where the binding between the substrate/inhibitor and the enzyme is governed by the concentration and affinity.



During competitive inhibition, the Vmax remains same in the y intercept at the cost of increasing Km.

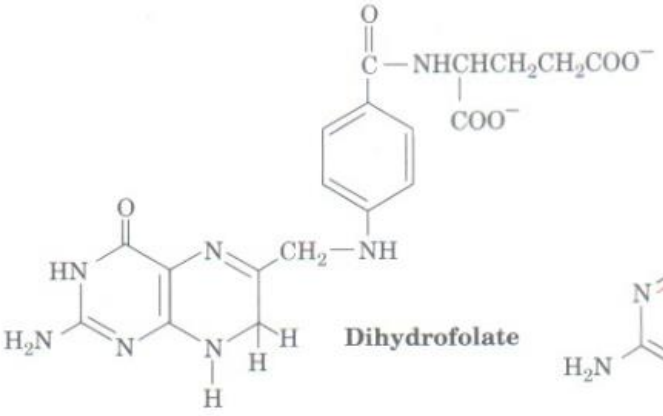
Example of competitive inhibitor used as chemotherapeutic agent

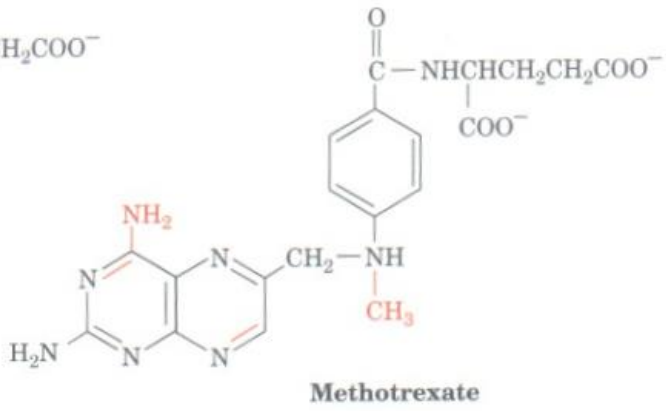
● dihydrofolate reductase catalyzes an essential step in the synthesis of dTMP

● methotrexate binds to and inhibits dihydrofolate reductase preventing dTMP biosynthesis

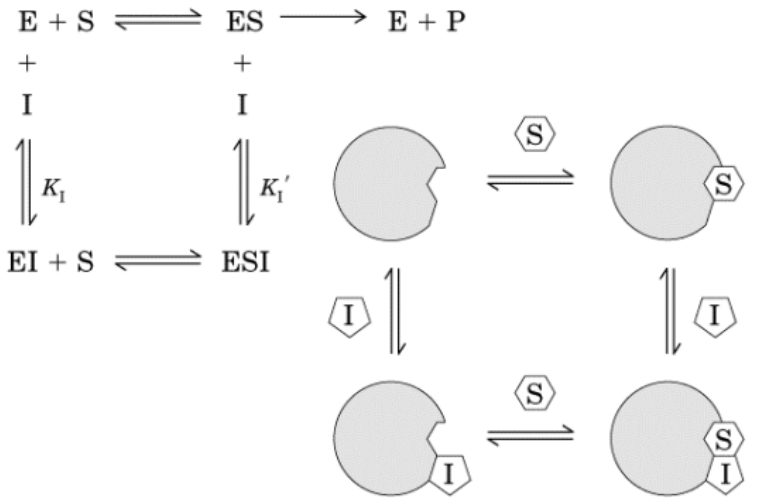
– preferentially kills actively dividing cells (e.g. cancer cells)

– clearly, methotrexate is structurally related to the normal substrate and is known to bind in the enzyme active site in a conformation similar but not identical to the substrate.





B) Noncompetitive inhibition

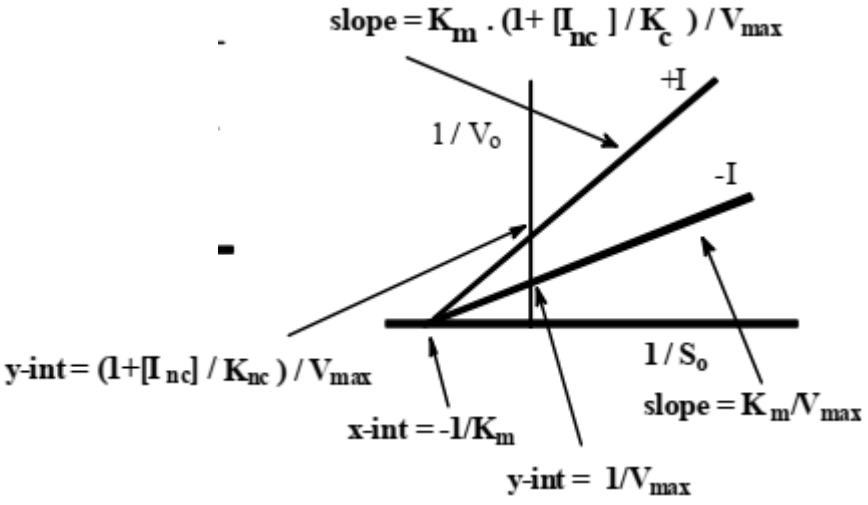


In non-competitive inhibition, there is no similarity between the structure of the substrate and the inhibitor. The inhibitors bind with the enzyme at sites other than the substrate binding site leading to the formation of both enzyme-inhibitor (EI) and enzyme-inhibitor-substrate (EIS) complexes. The inhibitor forms non-covalent bonding with the enzyme and so the enzyme inhibition can be reversed by simply removing the inhibitor. The catalysis is still stopped; the reason behind this may be distortion in the enzyme conformation. The inhibitor binds to some other site located in the same enzyme and changes the total shape of that site for the substrate to fit into as earlier, which ultimately slows down the reaction that is taking place. The reaction gets slowed down but never stopped. Non-competitive inhibition minimizes the turnover rate of enzyme instead of interfering with the quantity of substrate binding to the enzyme.

Enzyme behaviour in presence of non-competitive inhibitor

• Vmax is decreased.

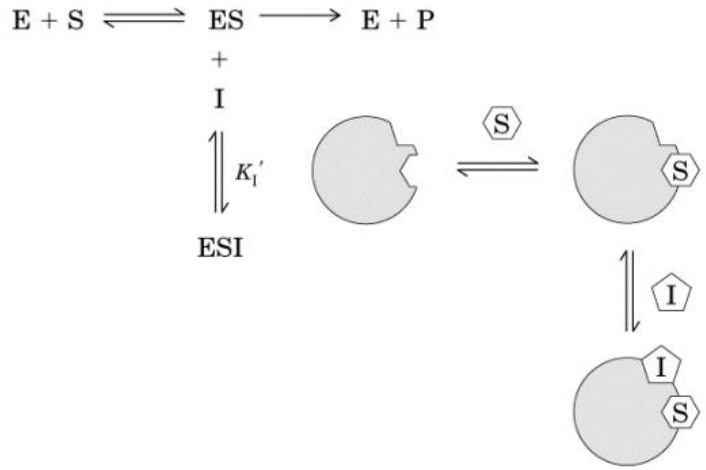
• Michaelis Menten constant (Km) remains unchanged



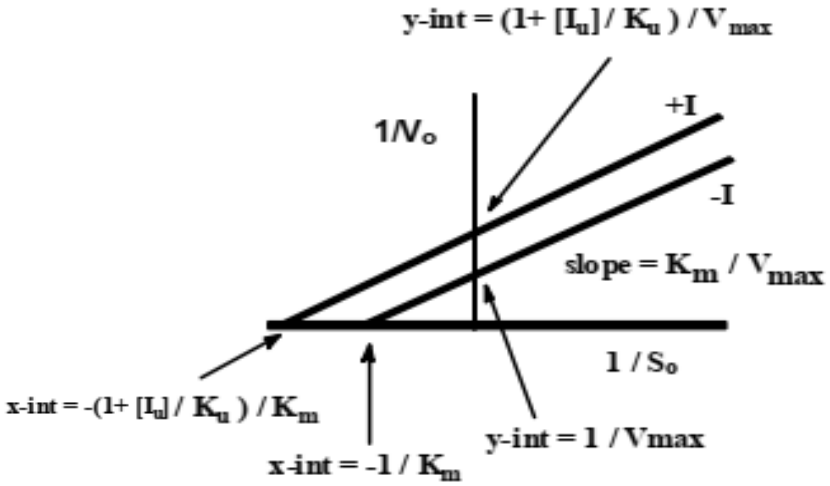
Example of Non-competitive inhibition

Cyanide is a poison which stops the production of ATP by aerobic respiration, ultimately leading to eventual death. It forms boding with allosteric site of cytochrome oxidase which is an important enzyme of the renowned electron transport chain. Cyanide changes the geometry of the active site of cytochrome oxidase because of which electrons can no more pass to the final oxygen (acceptor). As a result, the electron transport chain is not able to function and production of ATP through aerobic respiration is stopped.

C) Uncompetitive Inhibition



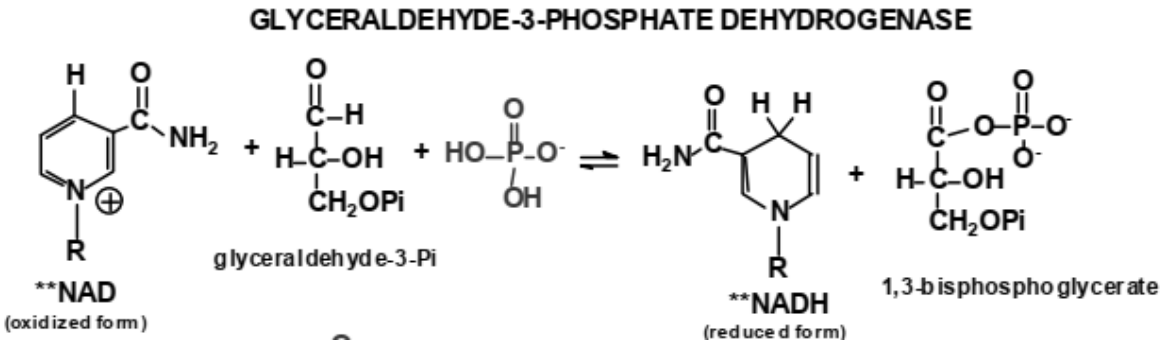
Uncompetitive inhibition occurs when inhibitor binds only to the enzyme-substrate complex and not free enzyme. One can hypothesize that on binding S, a conformational change in the enzyme occurs which presents a binding site for inhibitor. Inhibition occurs since ESI complex cannot form product. It is a dead end complex which has only one fate, to return to ES complex.

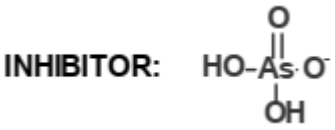


Enzyme behaviour in presence of Uncompetitive inhibitor

The apparent Km and Vm decrease

Example of Uncompetitive inhibition





Irreversible inhibition

Irreversible inhibitors typically react covalently with their target enzymes causing permanent inactivation. Often a key amino acid side-chain in the active site is alkylated or acylated by the inhibitor. A classic example of an irreversible inhibitor is the nerve gas diisopropyl fluorophosphates (DFP). DFP inactivates the enzyme acetylcholinesterase by combining with a serine located within the active site. Acetylcholinesterase is secreted into the synapses of cholinergic nerves, where it serves to degrade acetylcholine molecules once a nerve signal has passed through the synapse. Acetylcholine must be removed from the synapse to allow the neurons to return to their resting state before conduction of another impulse. Other enzymes that contain serines in their active site, such as trypsin and chymotrypsin, also are inactivated by DFP. Another example of an irreversible enzyme inhibitor is aspirin.

A special class of irreversible inhibitors is the suicide inactivators. These compounds are relatively unreactive until they bind to the active site of a specific enzyme. A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted to a very reactive compound that combines irreversibly with the enzyme. These compounds are also called mechanism-based inactivators, because they hijack the normal enzyme reaction mechanism to inactivate the enzyme. Suicide inactivators play a significant role in rational drug design, a modern approach to obtaining new pharmaceutical agents in which chemists synthesize novel substrates based on knowledge of substrates and reaction mechanisms. A well-designed suicide inactivator is specific for a single enzyme and is unreactive until within that enzyme's active site, so drugs based on this approach can offer the important advantage of few side effects.

Regulation of enzyme activity

Regulation of enzyme activity is important to coordinate the different metabolic processes.
It is also important for homeostasis i.e. to maintain the internal environment of the organism constant.
Regulation of enzyme activity can be achieved by two general mechanisms:

1- **Control of enzyme quantity**
Enzyme quantity is affected by:
A- Altering the rate of enzyme synthesis and degradation,
B- Induction
C- Repression

2- **Altering the catalytic efficiency of the enzyme by**
Catalytic efficiency of enzymes is affected by:
A- Allosteric regulation
B- Feedback inhibition
C- Proenzyme (zymogen)
D- Covalent modification
E- Protein – Protein interaction

1- Control of enzyme quantity

A- Control of the rates of enzyme synthesis and degradation.

As enzymes are protein in nature, they are synthesized from amino acids under gene control and degraded again to amino acids after doing its work.
Enzyme quantity depends on the rate of enzyme synthesis and the rate of its degradation.

- **Increased enzyme quantity** may be due to an increase in the rate of synthesis, a decrease in the rate of degradation or both.
- **Decreased enzyme quantity** may be due to a decrease in the rate of synthesis, an increase in the rate of degradation or both.
- For example, the quantity of liver arginase enzyme increases after protein rich meal due to an increase in the rate of its synthesis; also it increases in starved animals due to a decrease in the rate of its degradation.

B- Induction

- Induction means an increase in the rate of enzyme synthesis by substances called inducers
- According to the response to inducers, enzymes are classified into:
  i- Constitutive enzymes, the concentration of these enzymes does not depend on inducers.
  ii- Inducible enzymes, the concentration of these enzymes depends on the presence of inducers
- For example, induction of lactase enzyme in bacteria grown on glucose media.

C- Repression

- Repression means a decrease in the rate of enzyme synthesis by substances called repressors.
- Repressors are low molecular weight substances that decrease the rate of enzyme synthesis at the level of gene expression.
- Repressors are usually end products of biosynthetic reaction, so repression is sometimes called feedback regulation.
- For example, dietary cholesterol decreases the rate of synthesis of HMG CoA reductase (β-hydroxy β-methyl glutaryl CoA reductase), which is a key enzyme in cholesterol biosynthesis.

D- Derepression

Following removal of the repressor or its exhaustion, enzyme synthesis retains its normal rate.

E- Concentration of substrates, coenzymes and metal ion activator

The susceptibility of the enzyme to degradation depends on its conformation. Presence of substrate, coenzyme or metal ion activator causes changes in the enzyme conformation decreasing its rate of degradation.

2- Control of catalytic efficiency of enzymes

A- Allosteric Regulation

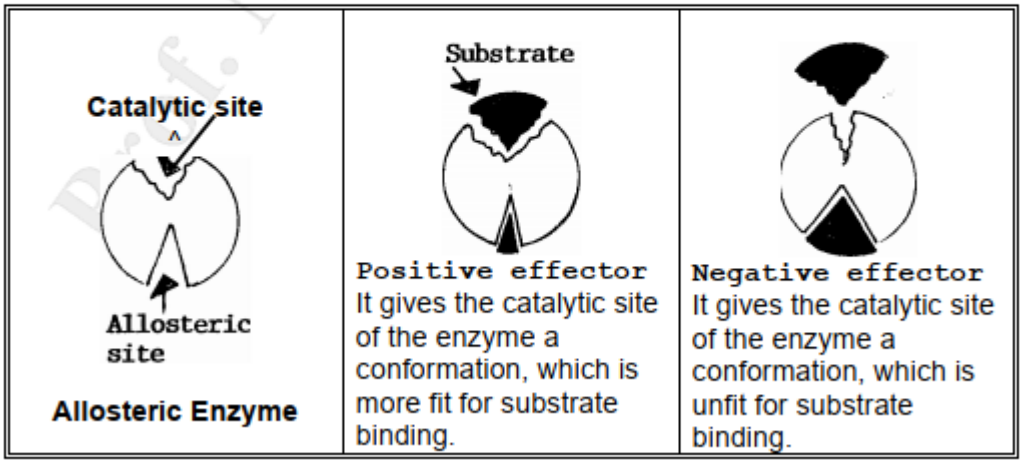
Allosteric enzyme is formed of more than one protein subunit. It has two sites; a catalytic site for substrate binding and another site (allosteric site), that is the regulatory site, to which an effector binds.
Allosteric means another site
If binding of the effector to the enzyme increases it activity, it is called positive effector or allosteric activator e.g. ADP is allosteric activator for phosphofructokinase enzyme.

If binding of the effector to the enzyme causes a decrease in its activity, it is called negative effector or allosteric inhibitor e.g.

- ATP and citrate are allosteric inhibitors for phosphofructokinase enzyme.
- Glucose-6-phosphate is allosteric inhibitor for hexokinase enzyme.

Mechanism of allosteric regulation

Binding of the allosteric effector to the regulatory site causes conformational changes in the catalytic site, which becomes more fit for substrate binding in positive effector (allosteric activator), and becomes unfit for substrate binding in negative effector (allosteric inhibitor) as shown in the following diagram.



A representative diagram for the mechanisms of allosteric regulation

Allosteric Enzyme are those enzymes possess additional sites, known as allosteric sites besides the active site. The allosteric sites are unique places on the enzyme molecules; allosteric enzymes have one or more allosteric site.

The term allosteric has been introduced by the two Noble laureates, MONOD AND JACOB, to denote an enzyme site, different from the active site, which non competitively binds molecule other than the substrate and may influence the enzyme activity.

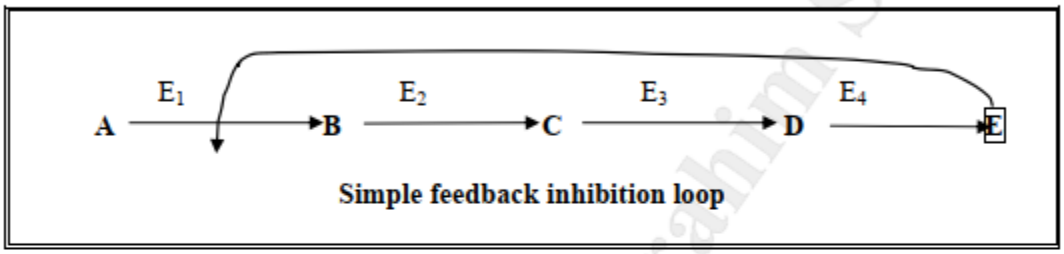
PROPERTIES OF ALLOSTERIC ENZYME

I. Allosteric enzyme have one or more allosteric sites
II. Allosteric sites are binding sites distinct from an enzyme active site or substrate binding site
III. Molecule that bind to allosteric sites are called effector or modulator
IV. Effector may be positive or negative, this effector regulate the enzyme activity. The enzyme activity is increased when a positive allosteric effector binds at the allosteric site known as activator site. On the other hand, negative allosteric effector bind at the allosteric site called inhibitor site and inhibit the enzyme activity.
V. Binding to allosteric sites alter the activity of the enzyme, this is called cooperative binding. Allosteric enzymes display sigmoidal plot of V_0 vs [S].

B- Feedback Inhibition

In biosynthetic pathways, an end product may directly inhibit an enzyme early in the pathway. Such enzyme catalyzes the early functionally irreversible step specific to a particular biosynthetic pathway.

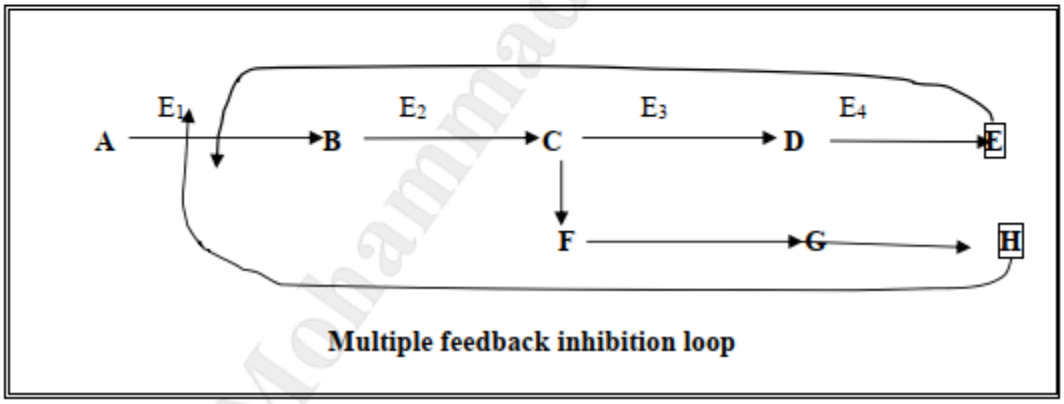
Feedback inhibition may occur by simple feedback loop.as in the following diagram



Simple feedback inhibition loop

Where A is the substrate, E is the end product, B, C, D are intermediate metabolites, E_1, E_2, E_3 and E_4 are enzymes in biosynthetic pathway.

Feedback inhibition can occur by multiple feedback inhibition loops as occurs in branched biosynthetic pathways.



Multiple feedback inhibition loop

N.B.: Feedback regulation is different from feedback inhibition.

Feedback regulation:

- It means that an end product in the reaction decreases the rate of enzyme synthesis at the level of gene expression.
- It does not affect the enzyme activity.
- It decreases the enzyme quantity through the action on the gene that encodes the enzyme.
- It is a complicated process that takes hours to days.
- For example, inhibition of HMG CoA reductase enzyme by dietary cholesterol.

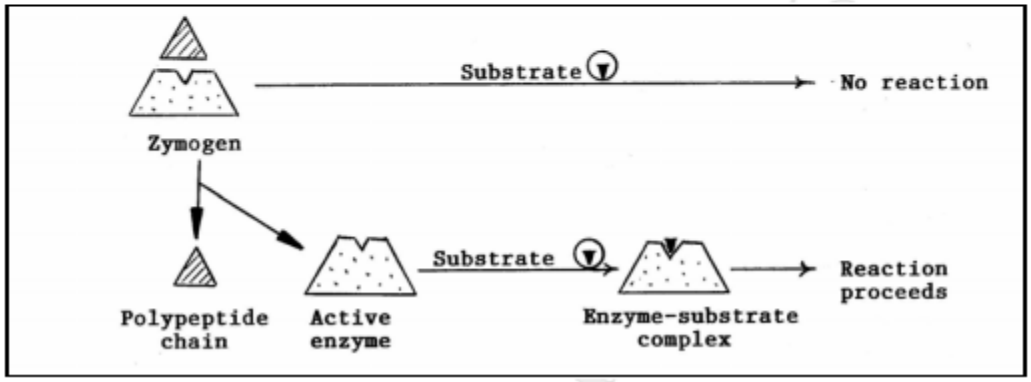
Feedback inhibition

- It means that an end product directly inhibits an enzyme early in biosynthetic pathways.
- It does not affect enzyme quantity
- It decreases enzyme activity.
- It is a direct and rapid process that occurs in seconds to minutes.

- For example, CTP inhibits aspartate- transcarbamylase enzyme in pyrimidine synthesis

C- Proenzymes (Zymogens)

Some enzymes are secreted in inactive forms called proenzymes or zymogens.
Examples for zymogens include pepsinogen, trypsinogen, chymotrypsinogen, prothrombin and clotting factors.
Zymogen is inactive because it contains an additional polypeptide chain that masks (blocks) the active site of the enzyme
Activation of zymogen occurs by removal of the polypeptide chain that masks the active site as shown in the following figure.



Activation of zymogens can occur by one of the following methods:

1- **Activation by HCl**

$$\text{Pepsinogen} \xrightarrow{\text{HCl}} \text{Pepsin}$$

2- **Activation by other enzymes**

$$\text{Trypsinogen} \xrightarrow{\text{Enterokinase}} \text{Trypsin}$$

$$\text{Prothrombin} \xrightarrow{\text{Thrombokinase} + Ca^{++}} \text{Thrombin}$$

3- **Auto activation** i.e. the enzyme activates itself.

$$\text{Pepsinogen} \xrightarrow{\text{Pepsin}} \text{Pepsin}$$

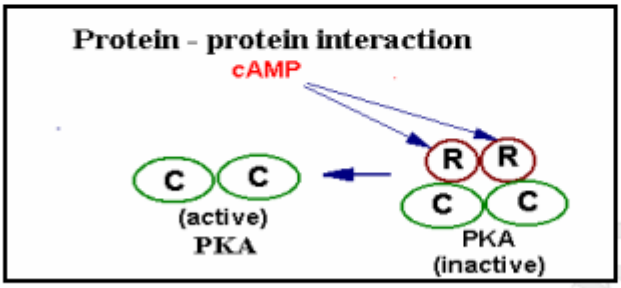
Biological importance of zymogens

1. Some enzymes are secreted in zymogen form to protect the tissues of origin from auto digestion.
2. Another biological importance of zymogens is to insure rapid mobilization of enzyme activity at the time of needs in response to physiological demands.

D- Protein-protein interaction

In enzymes that are formed from of many protein subunits, the enzyme may be present in an inactive form through interaction between its protein subunits.

The whole enzyme, formed of regulatory and catalytic subunits, is inactive. Activation of the enzyme occurs by separation of the catalytic subunits from the regulatory subunits.
Protein kinase A enzyme is an example for regulation of enzyme activity through protein interaction.
This enzyme is formed of 4 subunits, 2 regulatory (2R) and 2 catalytic (2C) subunits. The whole enzyme (2R2C) is inactive. cAMP (cyclic adenosine monophosphate) activates the enzyme by binding to the 2 regulatory (2R) subunits releasing the 2 catalytic (2C) subunits and hence activating the enzyme.



Where PKA is protein kinase A enzyme, C is catalytic subunit and R is regulatory subunit.

E- Covalent modification

It means modification of enzyme activity of many enzymes through formation of covalent bonds e.g.

1. Methylation (addition of methyl group).
2. Hydroxylation (addition of hydroxyl group).
3. Adenylation (addition of adenylic acid).
4. Phosphorylation (addition of phosphate group)

Phosphorylation is the most covalent modification used to regulate enzyme activity.
Phosphorylation of the enzyme occurs by addition of phosphate group to the enzyme at the hydroxyl group of serine, threonine or tyrosine. This occurs by protein kinase enzyme.
Dephosphorylation of the enzyme occurs by removal of phosphate group from the hydroxyl group of serine, threonine or tyrosine. This occurs by phosphatase enzyme.
The phosphorylated form is the active form in some enzymes, while the dephosphorylated form is the active form in other enzymes.
Examples of enzymes activated by phosphorylation. These are usually enzymes of degradative (breakdown) reactions e.g.

1. Glycogen phosphorylase that breaks down glycogen into glucose.
2. Citrate lyase, which breaks down citrate.
3. Lipase that hydrolyzes triglyceride into glycerol and 3 fatty acids.

Examples of enzymes inactivated by phosphorylation. These are usually enzymes of biosynthetic reactions e.g.

1. Glycogen Synthetase, which catalyzes biosynthesis of glycogen.
2. Acetyl CoA carboxylase, an enzyme in fatty acid biosynthesis.
3. HMG CoA reductase, an enzyme in cholesterol biosynthesis.

Isoenzymes

- Isoenzymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors.
- Different isoenzyme forms of a given enzyme are usually derived from different genes and often occur in different tissues of the body.
- An example of an enzyme which has different isoenzyme forms is lactate dehydrogenase (LDH) which catalyzes the reversible conversion of pyruvate into lactate in the presence of the coenzyme NADH.
- LDH is a tetramer of two different types of subunits, called H and M, which have small differences in amino acid sequence. The two subunits can combine randomly with each other, forming five isoenzymes that have the compositions H_4, H_3M, H_2M_2, HM_3 and M_4. The five isoenzymes can be resolved electrophoretically.

Selected Blood Enzyme Assays Used in Diagnostic Medicine

Enzyme	Condition Indicated by Abnormal Level
lactate dehydrogenase (LDH)	heart disease, liver disease
creatine phosphokinase (CPK)	heart disease
aspartate transaminase (AST)	heart disease, liver disease, muscle damage
alanine transaminase (ALT)	heart disease, liver disease, muscle damage
gamma-glutamyl transpeptidase (GGTP)	heart disease, liver disease
alkaline phosphatase (ALP)	bone disease, liver disease

Stoker 2014, Table 21-3 p768

Cofactors, Coenzymes, prosthetic groups, **Holoenzymes and Apoenzymes**

Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Many enzymes require the presence of small, non-protein units or cofactors to carry out their particular reaction. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component.

The cofactor may be:

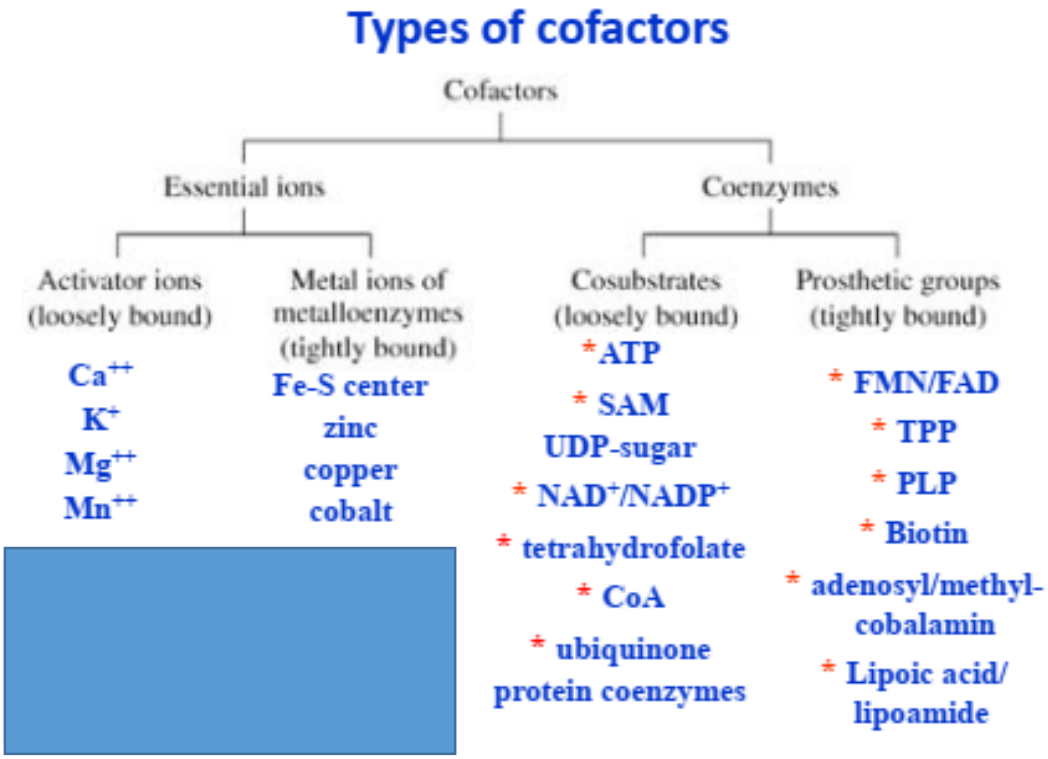
1. A coenzyme – a complex organic or metallo-organic molecule **loosely attached to the enzyme.** Some coenzymes, such as NAD+, are bound and released by the enzyme during its catalytic cycle and in effect function as co-substrates. Many coenzymes are derived from vitamin precursors.

2. A prosthetic group – A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein (e.g. heme in hemoglobin).

3. A metal-ion-activator – these include K^+, Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Mo^{3+}.

A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **Holoenzyme**. The protein part of such an enzyme is called the **Apoenzyme** or **Apoprotein**.

Enzyme Cofactors



Cofactor	Enzyme*
Coenzyme†	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine nucleotide (FAD)	Monoamine oxidase
Nicotinamide adenine dinucleotide (NAD^+)	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
6′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Mg^{2+}	*Eco*RV
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase
K^+	Acetoacetyl CoA thiolase

Some activated carriers in metabolism

Carrier molecule in activated form	Group carried	Vitamin precursor
ATP *	Phosphoryl	
NADH and NADPH 2 electrons	Electrons	Nicotinate (niacin)
$FADH_2$ 1 or 2 electrons	Electrons	Riboflavin (vitamin B_2)
$FMNH_2$ 1 or 2 electrons	Electrons	Riboflavin (vitamin B_2)
Coenzyme A	Acyl	Pantothenate
Lipoamide	Acyl	
Thiamine pyrophosphate 2 carbon groups containing carbonyl	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2 ATP-dependent carboxylation	Biotin
Tetrahydrofolate	One-carbon units	Folate
S-Adenosylmethionine	Methyl	
Uridine diphosphate glucose	Glucose	
Cytidine diphosphate diacylglycerol	Phosphatidate	
Nucleoside triphosphates	Nucleotides	

Selected Important Coenzymes in Which B Vitamins Are Present

B Vitamin	Coenzymes	Groups Transferred
thiamin	thiamin pyrophosphate (TPP)	aldehydes
riboflavin	flavin mononucleotide (FMN) flavin adenine dinucleotide (FAD)	hydrogen atoms
niacin	nicotinamide adenine dinucleotide (NAD^+) nicotinamide adenine dinucleotide phosphate ($NADP^+$)	hydrogen atoms
pantothenic acid	coenzyme A (CoA)	acyl groups
vitamin B_6	pyridoxal-5-phosphate (PLP) pyridoxine-5′-phophate (PNP) pyridoxamine-5′-phosphate (PMP)	amino groups
biotin	biotin	carbon dioxide (carboxyl group)
folate	tetrahydrofolate (THF)	one-carbon groups other than CO_2
vitamin B_{12}	methylcobalamin	methyl groups, hydrogen atoms

Stoker 2014, Table 21-7 p780

Vitamins, nutritional deficiency diseases

Vitamin	Disease
Ascorbate (C)	Scurvy
Nicotinic acid (B_3)	Pellagra
Riboflavin (B_2)	Growth retardation
Pantothenate (B_5)	Dermatitis in chickens
Thiamine (B_1)	Beriberi
Pyridoxal (B_6)	Dermatitis in rats
Biotin	Dermatitis in humans
Folate	Anemia, spina bifida
Cobalamin (B_{12})	Pernicious anemia

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