Biology of Cells and Tissues
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ENZYMES

General

Enzymes are catalysts for chemical reactions in biological systems. As any catalyst, enzymes are neither consumed nor permanently altered during the reaction. Enzymes increase the rate of chemical reactions at least by a factor 10^6.

All enzymes are proteins. The only exception is ribozymes, which are RNAs with intrinsic catalytic activity. Ribozymes are involved in the processing of mRNA to form mature mRNAs. They participate in the removal of introns from the primary transcripts. Ribozymes also participate in the peptidyl transferase activity during protein synthesis.

Nomenclature

Enzymes' systematic names were developed by the International Union of Biochemistry and Molecular Biology (IUBMB). Enzymes are divided into six classes, each with several subgroups. These names are unambiguous and informative about the reaction catalyzed, but are somewhat cumbersome for everyday use. The six categories are: oxido-reductases, transferases, hydrolases, lyases, isomerases, and ligases. According to this systematic nomenclature, enzyme names consist of four numbers following the letters EC (for Enzyme Commission). The first digit is for the class name, the second digit is for subclass, the third digit is for sub-subclass, and the fourth digit is for the specific enzyme.

Oxidoreductases: Oxidation-reduction reactions; most of the time, use coenzymes such as NAD^+, $NADP^+$, FAD, coenzyme Q, or molecular oxygen as the electron acceptors (e.g., dehydrogenases, oxidases, reductases).
Transferases: Transfer of chemical group (amino group, phosphoryl group, glycosyl group, carboxyl group, etc.) from a donor to an acceptor (e.g., transaminases, kinases).
Hydrolases: Hydrolytic cleavage of covalent bonds between carbon and some other atoms (e.g., peptidases, proteases, phosphatases, glycosidases).
Lyases: Non-hydrolytic cleavage of covalent bonds between carbon and other atoms such as carbon, sulfur, and nitrogen (e.g., decarboxylases, dehydratases, aldolase).
Isomerases: Molecular rearrangement within a molecule without any change in molecular formula (e.g., isomerases, epimerases, mutases).
Ligases: Formation of covalent bonds between carbon and other atoms such as sulfur, nitrogen, or oxygen). Frequently, use ATP or GTP as the energy source to carry out the reaction (e.g., carboxylases, thiokinases).

Example: Hexokinase mediates the transfer of phosphate group from ATP to an alcohol group in D-glucose. This enzyme is designated as ATP: D-hexose 6-phosphotransferase EC 2.7.1.1. This identifies the enzyme as a member of class 2 (transferases), subclass 7

(transfer of a phosphoryl group), sub-subclass 1 (alcohol group is the acceptor of the phosphoryl group). The last digit is specific for hexokinase.

Coenzymes and cofactors

Many enzymes contain non-protein components that are essential for the catalytic activity. These non-protein components can be classified into two groups: coenzymes and cofactors.

Coenzymes are those without which the enzymes cannot perform their function. Some coenzymes are bound to enzymes very tightly by covalent or noncovalent interactions. Examples: Heme, Fe, Cu, Se, Zn, biotin, pyridoxal phosphate, FAD, FMN, thiamin pyrophosphate. These coenzymes are also called prosthetic groups. When enzymes are purified, the prosthetic groups stay attached to the enzymes. The enzyme without the prosthetic group is an apoenzyme; enzyme with the prosthetic group is a holoenzyme. When metal ions are prosthetic groups, the enzymes are called metalloenzymes.

Some coenzymes participate in the transfer of functional groups. Examples: tetrahydrofolate (transfer of one-carbon groups), S-adenosylmethionine (transfer of methyl groups), and Coenzyme A (transfer of acyl groups). When enzymes are purified, these non-protein components may or may not stay attached to the enzymes.

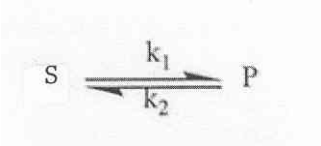
Cofactors are non-protein components that are not absolutely essential for enzyme activity but are needed for maximal activity of the enzymes. Cofactors may interact with either the enzymes or substrates. The most common cofactors are metal ions such as Mg. When metal ions are cofactors, the enzymes are called metal-activated enzymes rather than metalloenzymes.

Many coenzymes are derived from vitamins.

Thiamin – thiamin pyrophosphate
Riboflavin – FAD and FMN
Niacin – NAD^+, $NADP^+$
Pantothenic acid – Coenzyme A

How do enzymes catalyze reactions?

Look at a chemical reaction



k_1 = rate constant for the forward reaction
k_2 = rate constant for the reverse reaction

At equilibrium [S] and [P] are stable (not equal, but stable); the velocity of the forward reaction becomes equal to the velocity of the reverse reaction.

Velocity of the forward reaction: $V_F = k_1 [S]$
Velocity of the reverse reaction: $V_R = k_2 [P]$

Therefore, at equilibrium, $V_F = V_R$

i.e., $k_1 [S] = k_2 [P]$

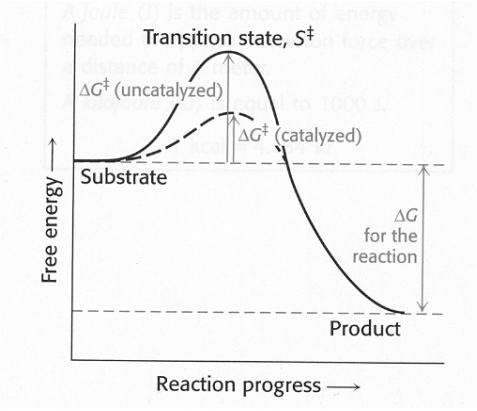
The equilibrium constant (K_{eq}) is expressed as:

$$K = \frac{k_1}{k_2} = \frac{[P]}{[S]}$$

Enzymes activate the chemical reactions by increasing the velocity of forward reaction as well as the velocity of the reverse reaction to an equal extent. Therefore, the concentrations of substrates and products at equilibrium are not altered by the presence of enzymes. Similarly, the equilibrium constant is also not altered by the presence of enzymes. But, the reaction reaches the equilibrium state much faster in the presence of the enzymes.

How does then the enzyme activate the velocity of the chemical reaction?

The free energy change in going from S (substrate) to P (product) is depicted in the figure.



If the free energy of the product (G_p) is lower than that of the substrate (G_s), then the change in free energy for the reaction (ΔG) is negative and the reaction will proceed spontaneously in the direction written. The reaction is exergonic. We normally use the term ΔG^0 to denote the standard free energy change. This is defined as the free energy change for a reaction when the concentrations of the substrates and products are kept at 1 M at the beginning of the reaction.

But what about the path from the substrate to the product?

The reaction from S to P must pass through an energy barrier—a transition state.
The free energy change from S to the transition state is ΔG^*. This is called the "free energy of activation." It is also denoted as E_{act} or $\Delta G^{\ddagger}$

Catalysts (enzymes) act by lowering the free energy of activation (ΔG^*). Enzymes do not affect the free energy of substrates nor the free energy of products. Therefore, enzymes do not alter the change in the free energy of reaction (ΔG^0 or ΔG).

How is the activation barrier lowered by enzymes?

Enzymes bind the transition state in preference to the substrate while forming the enzyme-substrate complex.

The enzyme has a specific site in which the substrate binds = active site. The substrate has a susceptible bond that is attacked by the enzyme. Usually there are other structural requirements for binding to the active site through secondary alignments for proper geometrical relationships.

Features of the active site

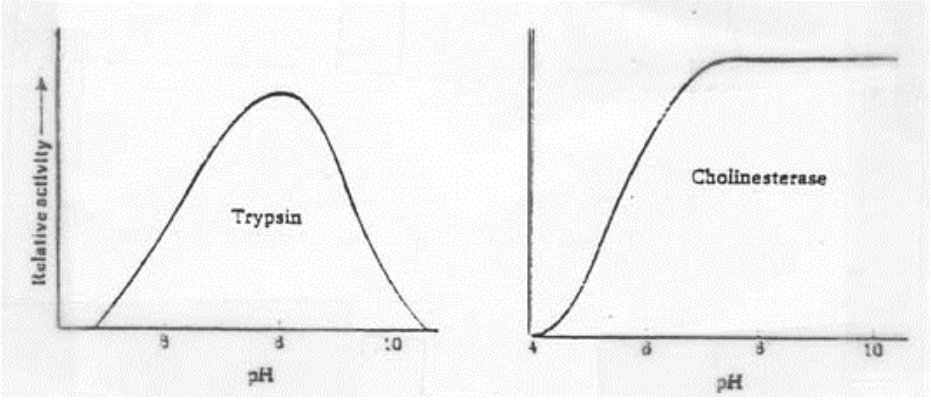
- Occupies a small portion of the total enzyme volume; usually in clefts of the enzyme
- Is 3-dimensional
- Substrates are bound "weakly" to the active site; substrates are bound in geometry resembling the transition state and the enzyme provides groups that enhance the probability that the transition state will occur.
- The amino acid residues that form the active site are not present in sequence in the primary structure of the enzyme. Instead, the active site is formed by amino acid residues located in different parts of the enzyme protein, which come close to each other because of the secondary and tertiary structure of the protein. In multisubunit enzymes, active site may be formed by regions arising from different subunits.
- Substrates bind to the active site mostly via non-covalent interaction with the amino acids lining the active site. This can be electrostatic interaction or hydrophobic interaction.
- Mutations in active site amino acids affect enzyme activity. It can influence the binding of the substrate to the active site and/or catalysis.

Factors affecting reaction velocity

The rate or velocity of a reaction is the number of substrate molecules converted to product per unit time and is usually expressed as moles of product formed per minute per unit quantity of the enzyme.

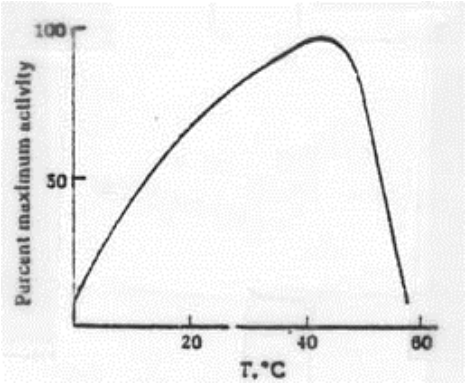
pH

- Enzymes have a pH optimum for catalytic activity; not all exhibit the same pH optimum.
- pH can affect the ionization of the active site if there are chemical groups that are required to be in an ionized or unionized state. pH can also affect the ionization of the substrates.
- pH can affect the denaturation of the enzyme and disrupt the secondary, tertiary, or quaternary structure.



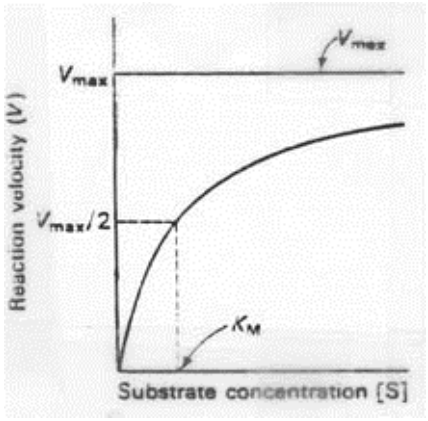
Temperature

- The temperature can increase the velocity by increasing the number of molecules having sufficient energy to reach the activation barrier and form products.
- The temperature can decrease the velocity as a result of denaturation of the enzyme.



Substrate concentration

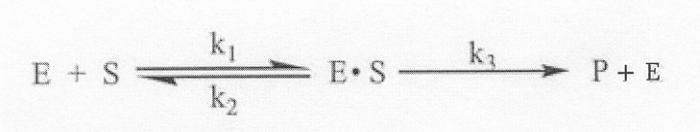
- The rate of the enzyme-catalyzed reaction increases with substrate concentration until a maximum velocity is reached.
- The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme.
- The hyperbolic shape of the plot of velocity vs. [substrate] demonstrates that the enzyme follows Michaelis-Menten kinetics



Kinetics of enzyme-catalyzed reactions

- At low [S], the reaction depends on [S] and the rate is directly proportional to [S]. In other words, it is a first order reaction.
- The reaction shows saturation, meaning that at high [S], the velocity no longer increases with increasing [S]. In other words, it is a zero order reaction when the substrate concentration is very high.
- The reaction velocity at infinite concentration of substrate is known as maximal velocity (V_{max}).
- The substrate concentration that gives a reaction velocity equal to one-half of the maximal velocity is known as the Michaelis constant (K_m).

Michaelis and Menten proposed a simple mathematical model to account for most of the features of enzyme-catalyzed reactions. In this model, the enzyme reversibly combines with its substrate to form an enzyme-substrate (ES) complex that breaks down to product, regenerating the free enzyme. Only initial reaction velocities are used in the analysis of enzyme reactions; i.e., the rate of the reaction is measured as soon as the enzyme and substrate are mixed. At that time, the concentration of product is very small and the rate of the back reaction from P to E·S can be ignored.



E is the concentration of the free enzyme
S is the concentration of the substrate
ES is the concentration of the enzyme-substrate complex
k_1, k_2, and k_3 are the rate constants

Michaelis-Menten Equation

The following equation describes the relationship between the velocity of an enzyme-catalyzed reaction and the substrate concentration.

$$V = \frac{V\ max[S]}{K\ m\ +[S]}$$

V = rate of reaction or velocity of reaction
Vmax = maximal velocity
[S] = substrate concentration
K_m = Michaelis constant

$$Km = \frac{k_2 + k_3}{k_1}$$

Significance of K_m

- Units are moles/liter. Same units as substrate concentration.
- Assume special case where v = 1/2 Vmax

$$\text{i.e, } \frac{1}{2} Vmax = \frac{Vmax\ [S]}{Km + [S]}$$

$$\frac{1}{2} = \frac{[S]}{[Km] + [S]} \qquad [Km] = [S]$$

This is the definition of Km = substrate concentration at which V = ½ Vmax

K_m is a measure of the affinity of the enzyme for its substrate under certain conditions. For most enzymes, $k_2 >>> k_3$. Therefore, K_m becomes equal to k_2/k_1. This means that when the value for K_m is high, $k_2 > k_1$ (i.e., the dissociation of ES to E and S is faster than the association of E and S to form ES). In other words, the affinity of the enzyme for its substrate is low. The opposite is true when the value for K_m is low (i.e., $k_2 < k_1$; the dissociation of ES to E and S is slower than the association of E and S to form ES; the affinity of the enzyme for its substrate is high).

A genetic mutation is aldehyde dehydrogenase (Lysine substitution for glutamate) decreases the affinity of the enzyme for NAD^+. This mutation is common in Orientals and

is believed to be responsible for undesirable side effects of alcohol drinking (flushing, hypotension) and also may protect against alcoholism.

Important points about Michaelis-Menten kinetics

- Km is numerically equal to the substrate concentration at which the reaction velocity is equal to 1/2 Vmax. Km does not vary with the concentration of enzyme. Km is a characteristic of an enzyme and a particular substrate; it reflects the affinity of the enzyme for that substrate.
- Small or low Km reflects a high affinity of the enzyme for substrate because low concentration of substrate is needed to half-saturate the enzyme.
- Large or high Km reflects a low affinity of the enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.
- The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. If enzyme concentration is one third, then the reaction rate is decreased by one third.
- When [S] is much less than Km, the velocity is proportional to the substrate concentration; this is first order with respect to substrate.
- When [S] is much greater than Km, the velocity is constant and equal to Vmax; this is independent of substrate concentration and zero order with respect to substrate concentration.

Lineweaver-Burk Equation or Double Reciprocal Plot is more useful than Michaelis-Menten equation.

$$V = \frac{Vmax\,[S]}{Km + [S]}$$

$$\text{then } \frac{1}{V} = \frac{Km + [S]}{Vmax\,[S]}$$

or

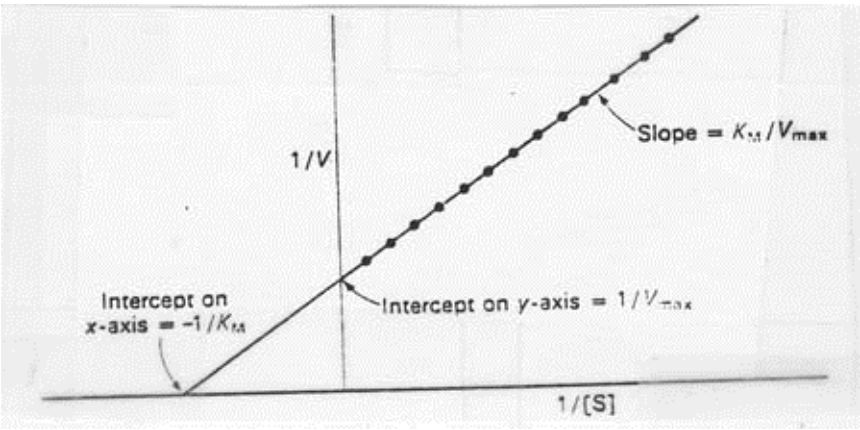
$$\boxed{\frac{1}{V} = \frac{Km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax}}$$

Equation of a straight line is: $y = mx + c$
i.e., when y is plotted against x, we will get a straight line which has a slope of 'm' and a y-intercept of 'c'.

In the Lineweaver-Burk plot,

'y' is 1/V
'x' is 1/S
'm' is K_m/V_{max}

'c' is $1/V_{max}$
Therefore, when 1/V is plotted against 1/S (i.e, a double-reciprocal plot), we will get a straight line, which has a slope of K_m/V_{max} and a 'y-intercept' of $1/V_{max}$.



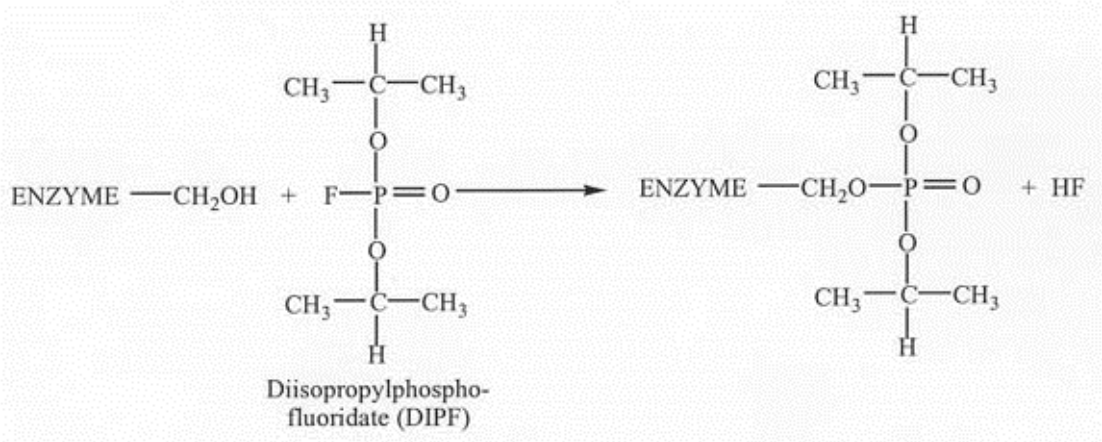
Now, the x-intercept can be calculated. The value for the x-intercept becomes $-1/K_m$.

Influence of enzyme concentration on K_m and V_{max}: The rate of an enzyme-catalyzed reaction is directly proportional to the concentration of the enzyme, provided the concentration of the substrate is sufficient to saturate all the enzymes. The Michaelis constant (K_m) is an inherent property of a given enzyme. It does not vary when the concentration of that given enzyme is varied. In contrast, the maximal velocity (V_{max}) is directly proportional to enzyme concentration.

Enzyme Inhibitors

The rates of enzyme-catalyzed reactions can be decreased by specific inhibitors. Certain inhibitors are poisonous, for example nerve gas.

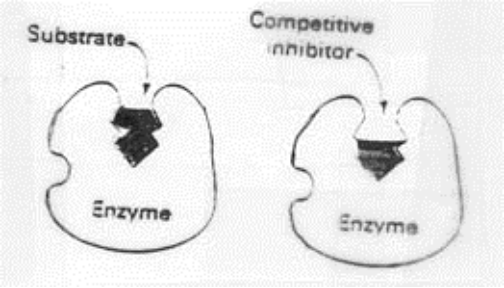
Irreversible enzyme inhibition occurs when an inhibitor covalently binds to the enzyme or binds so tightly that it is not released.



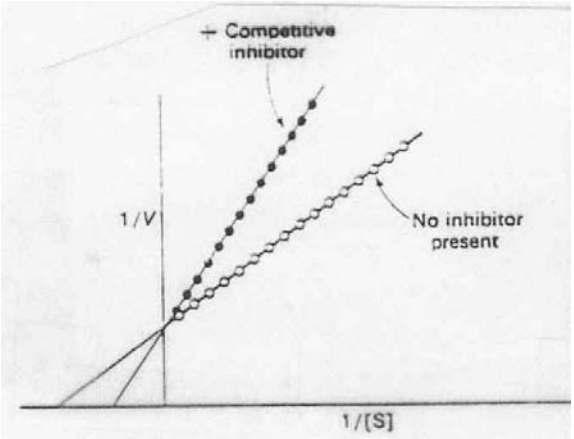
- Diisopropylphosphofluoridate (DFP or nerve gas) binds to acetylcholinesterase and increases the concentration of acetylcholine at the motor endplate. This results in continued depolarization of the muscle and thus paralysis. Paralysis of respiratory muscles leads to death.
- Some of the alkylating agents used in cancer chemotherapy are irreversible inhibitors.
- Some irreversible inhibitors are suicide substrates; i.e., substrate analogs which bind to the active site and get covalently attached to the enzyme.

Reversible Enzyme Inhibitors bind to enzymes through non-covalent bonds; dilution of the enzyme-inhibitor complex results in dissociation of the reversibly-bound inhibitor and recovery of enzyme activity. The two most common types of reversible inhibitors are competitive and non-competitive.

Competitive Inhibitors



- Competitive inhibitors resemble the substrate and bind to the active site of the enzyme; thus it competes with the substrate for that site.
- The inhibition can be overcome by increasing the substrate concentration.
- At sufficiently high substrate concentration, the velocity reaches the Vmax observed in the absence of inhibitor. Thus, Vmax does not change.
- A competitive inhibitor increases the Km for a given substrate. This means that in the presence of the inhibitor, more substrate is needed to achieve 1/2 Vmax.
- On a Lineweaver-Burk plot, the lines in the presence and absence of inhibitor intersect on the y-axis at 1/Vmax. Both lines show different x-axis intercepts.



Double reciprocal plot - fixed [I]; vary [S]

a) Vmax does not change
b) Km changes
c) Velocity changes.

Slope and Km are increased by a factor of

$$Km\ (app) = Km \times \left[1 + \frac{[I]}{K_I}\right]$$

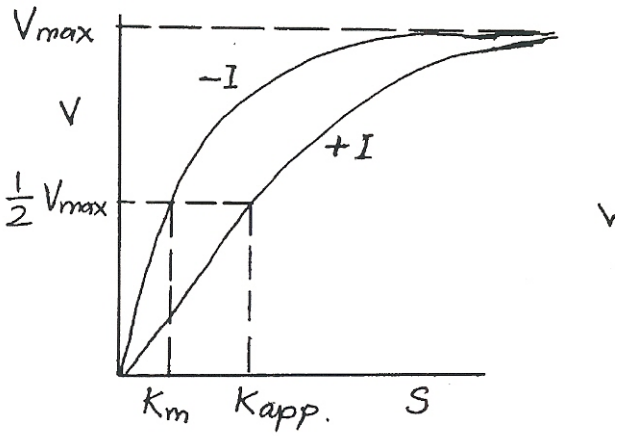
Where Km(app) is the Michaelis constant in the presence of the inhibitor, [I] is the concentration of the inhibitor, and K_I is the inhibition constant (an equivalent of Km for the interaction of the inhibitor with the enzyme; i.e., lower the K_I, higher is the affinity of the inhibitor for the enzyme and vice versa).

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

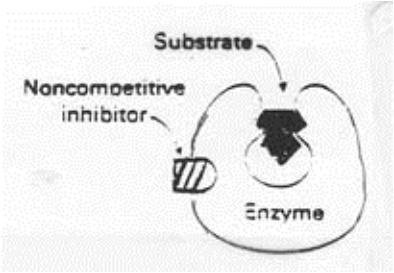
$$E + I \rightleftharpoons EI$$

Since S and I compete for the same site (active site), formation of ESI complex is not possible.

Below is the V versus S plot for an enzyme reaction in the absence (− I) and presence (+ I) of a competitive inhibitor.



Non-competitive inhibitors



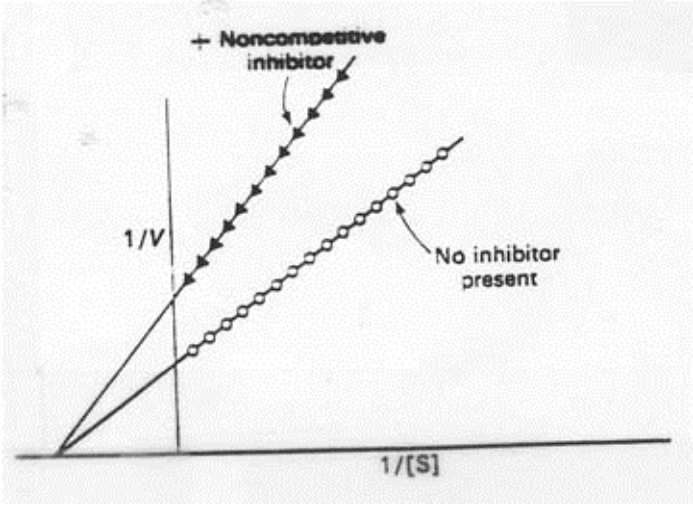
- These inhibitors do not bind to the active site and thus they do not compete with the substrate for the active site.
- The non-competitive inhibitor can bind either free enzyme or the ES complex preventing the reaction from occurring.
- This type of inhibitor may prevent a necessary conformational change in the enzyme.
- This type of inhibition is recognized by its characteristic effect on Vmax
- Vmax is decreased even at high substrate concentrations; non-competitive inhibition cannot be overcome by increasing the substrate concentration
- Km is unchanged

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

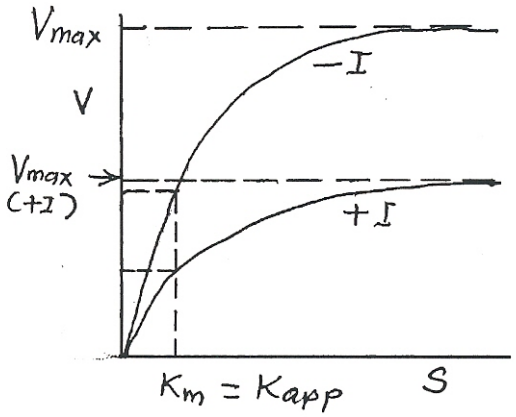
$E + S \rightleftharpoons ES;\ ES + I \rightarrow ESI$

$E + I \rightleftharpoons EI$

$E + I \rightleftharpoons EI;\ EI + S \rightleftharpoons EIS$

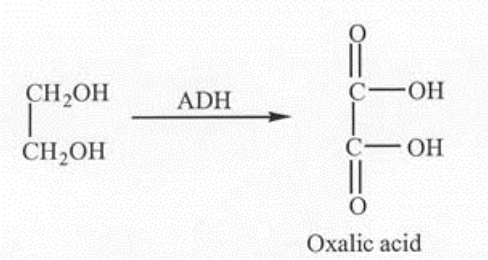


Below is the V versus S plot of an enzyme reaction in the absence (− I) and presence (+ I) of a noncompetitive inhibitor.



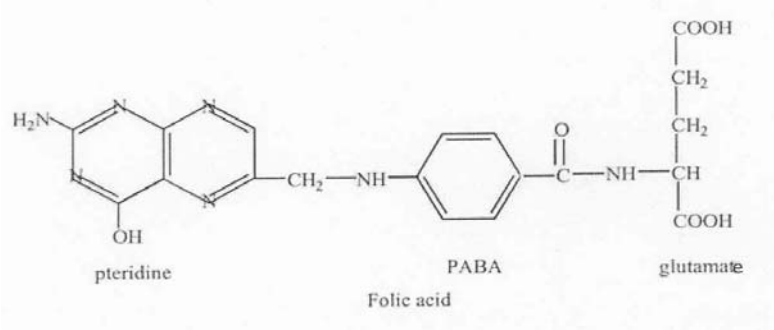
Examples of enzyme inhibition with clinical relevance

Ethylene glycol ingestion: Antifreeze contains ethylene glycol. Some alcoholics who cannot afford to buy alcohol consume antifreeze to support their drinking habit. But, consumption of ethylene glycol is toxic. Ethylene glycol poisoning is associated with confusion, convulsions, metabolic acidosis, tachypnea, and coma.

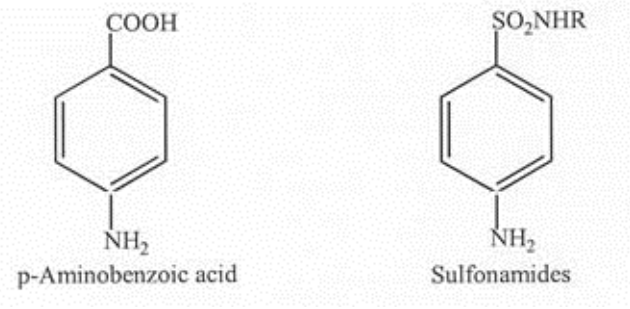


- Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) sequentially oxidize ethylene glycol to oxalic acid (HOOC-COOH). Both of these metabolites are toxic.
- Ethanol is administered to individuals who consume ethylene glycol. Ethanol competes for the active site on ADH and thus prevents the metabolism of ethylene glycol and the generation of the toxic metabolites glycolic acid and oxalic acid.
- Ethanol is oxidized to acetic acid and ethylene glycol is excreted unchanged.
- The same is true for those who consume wood alcohol (methanol). Methanol is converted to formic acid via ADH. Formic acid is toxic. Administration of ethanol to these individuals prevents the formation of formic acid.

Sulfa drugs



- Para-aminobenzoic acid (PABA) is one of the components of folic acid. Humans must ingest folic acid, but bacteria can synthesize it from the component parts. Bacteria require PABA to synthesize folic acid, which is used in many reactions in bacterial metabolism.



- Sulfa drugs are structurally similar to PABA and are competitive inhibitors of the enzyme incorporating PABA into the folic acid.

Many commonly prescribed drugs are enzyme inhibitors. Penicillin, amoxicillin, enalapril, captopril, prilosec, etc.

ACE
Angiotensin I $\rightarrow$ Angiotensin II (vasoconstrictor)
ACE = angiotensin converting enzyme

This enzyme is inhibited by captopril with a K_i (inhibition constant) of 0.2 nM.

If the Km for the enzyme is 2 μM, what would the apparent Km be in the presence of 0.2 μM (i.e, 200 nM) captopril?

$K_{app} = K_m (1 + [I]/K_i)$
$= 2 (1 + 200/0.2) = 2 \times 1001 = 2002$ μM.

Mechanisms of enzyme reactions

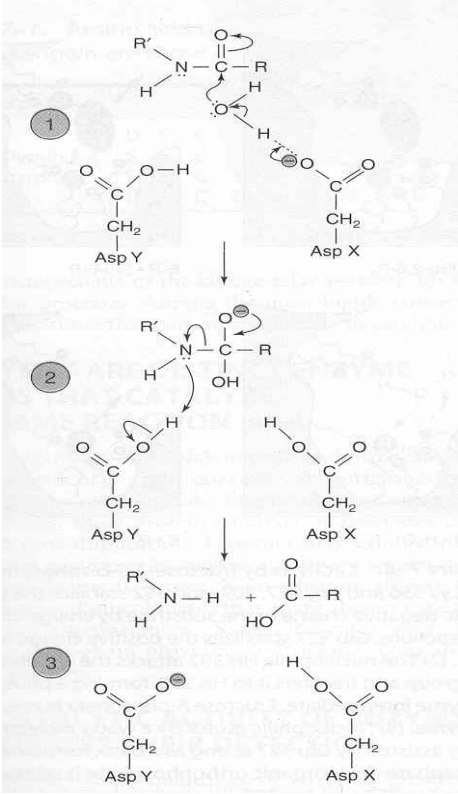
Enzymes employ various combinations of four general mechanisms to facilitate catalysis. The active site plays an obligatory role irrespective of the mechanism. The active site of the enzyme contains chemical groups, which can attract substrates, hold them closely, and transform them into products (*Circe effect*).

Catalysis by proximity: The active site assembles the substrates in close proximity within bond-forming distance of one another. The higher concentration of the substrates, the greater is the chance that the substrates will occupy the active site in close proximity to enhance catalysis.

Catalysis by strain: Enzymes that catalyze lytic reactions breaking a covalent bond bind its substrate at the active site in a strained position so that the conformation of the substrate at the active site places the targeted covalent bond in a thermodynamically unfavorable position so that it will undergo cleavage.

Acid-Base catalysis: The ionizable functional groups at the active site contribute to catalysis by serving as acids or bases. An example of acid-base catalysis is the reaction mediated by pepsin, an aspartic protease. This enzyme hydrolyzes peptide bonds at the carboxyl end of large hydrophobic amino acids in proteins using water. Pepsin has two aspartic acid residues at its active site, which serve as acids (proton donors: Asp-COOH) and bases (proton acceptors: Asp-COO^-).

R'-**NH-CO**-R + $H_2O \rightarrow$ R'-NH_2 + HOOC-R



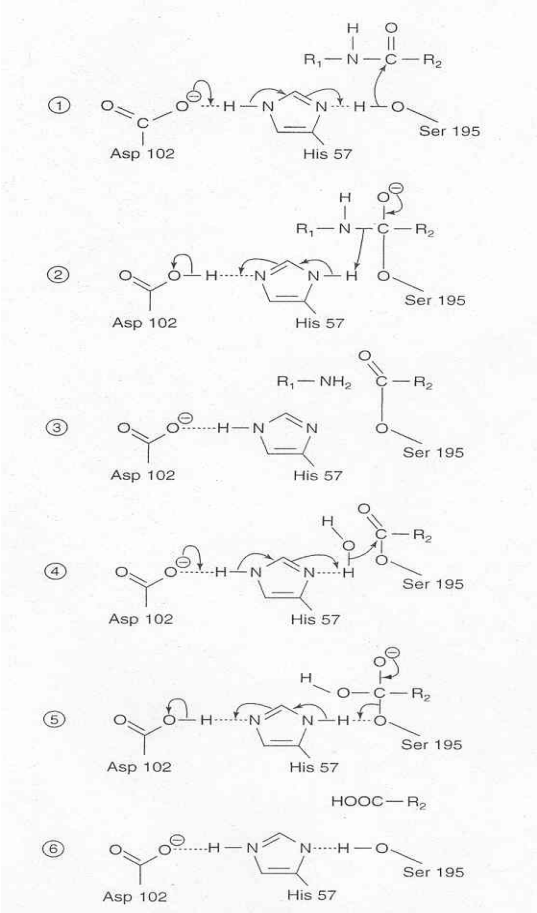
Covalent catalysis: This involves formation of a transient covalent bond between an active site residue of the enzyme and one or more substrates. On completion of the reaction, the covalent intermediate disappears, regenerating the original active site residue. Covalent catalysis often follows a ping-pong mechanism. Chymotrypsin is an example for covalent catalysis. It is a serine protease, meaning that serine is one of the residues at the active site, playing an obligatory role in the catalysis. The active site contains three residues: Asp-102, His-57, and Ser-195. These three residues form a charge-relay network, involving acid-base catalysis. At the same time, Ser-195 forms a transient covalent intermediate with the substrate (covalent catalysis). This enzyme hydrolyzes peptide bonds at the carboxyl end of aromatic amino acids in proteins using water. The overall reaction is the same as that catalyzed by pepsin.

$$R_1\text{-}\underline{\mathbf{NH\text{-}CO}}\text{-}R_2 + H_2O \rightarrow R_1\text{-}NH_2 + HOOC\text{-}R_2$$

But, chymotrypsin uses a mechanism that is different from that of pepsin. The reaction catalyzed by chymotrypsin involves formation of a transient covalent intermediate between the enzyme (active site Ser-195) and the substrate.

$$R_1\text{-}NH\text{-}CO\text{-}R_2 + \text{E-Ser-OH} \rightarrow R_1NH_2 + \text{E-Ser-OOC-}R_2$$
$$\text{E-Ser-OOC-}R_2 + H_2O \rightarrow \text{E-Ser-OH} + HOOC\text{-}R_2$$

Asp-102 and His-57 help the formation of the covalent intermediate by serving as acids/bases.



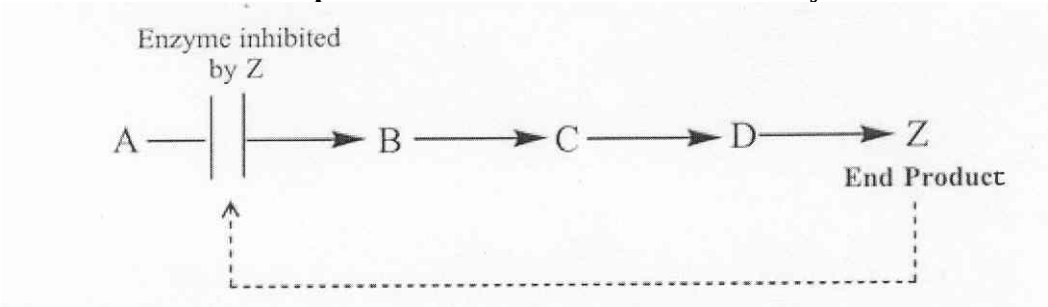
Allosteric Enzymes

- The kinetics of many single substrate enzyme reactions do not fit the Michaelis-Menten model
- On a velocity vs. [substrate] plot, some enzymes will give a sigmoidal curve rather than hyperbolic; the sigmoidal shape is indicative of allosteric enzymes.
- Most allosteric enzymes have quaternary structure. They consist of multiple subunits. They also have multiple active sites (i.e., substrate binding sites).
- One active site in the enzyme can effect another active site in the same enzyme.
- The allosteric site of an enzyme is not the same as the active site.
- K-class effectors; alters Km but not Vmax; binds at an allosteric site which then affects the affinity of substrate binding.
- V-class effectors; alters Vmax but not Km.
- The equation that describes the sigmoidal behavior of the velocity (V) versus substrate concentration (S) plot is a modification of the Michaelis-Menten equation.

$$V = V_{max}\ S^n/(K_m^n + S^n)$$

where n is called Hill coefficient. This number denotes the number of substrate binding sites present on the enzyme. The value for Hill coefficient is greater than 1 for allosteric enzymes whereas the value is 1 for normal enzymes.

Feedback inhibition or end product inhibition and allosteric enzymes



- A classical example of allosteric enzyme is aspartate transcarbamoylase. This enzyme is involved in the biosynthesis of pyrimidine nucleotides. CTP is one of the end products of the biosynthetic pathway. Thus, this enzyme is allosterically inhibited by CTP.
- This enzyme is also regulated by ATP even though ATP is not the end product of the biosynthetic pathway involving the enzyme. ATP is a positive modulator and thus stimulates the enzyme activity.
- Both of them are K-class effectors. They alter the substrate affinity but not the maximal velocity.

Covalent modification and enzyme regulation

Enzymes can be regulated by covalent modification. Examples of covalent modification include methylation, adenylylation, ADPRibosylation, and phosphorylation.

The most frequent utilization of covalent modification is through phosphorylation and dephosphorylation of amino acid residues, serine, threonine, and/or tyrosine. Phosphorylation may activate or inhibit enzymatic activity.

Enzyme synthesis and degradation as a means of regulation

Cells also regulate enzyme activity through altering the rate of synthesis or modulating degradation of the protein.

Isozymes

- Some enzymes occur in more than one molecular form in the same species.
- Catalyze the same reaction.
- May have different kinetic properties for Km and/or Vmax.
- Irrespective of whether a given reaction is catalyzed by one isoenzyme or the other, equilibrium constant, ΔG and ΔG° remain unaltered for the reaction. But, $\Delta G^{\ddagger}$ (i.e., activation energy) will be different for each isoenzyme.

- May have different effector binding sites.
- Are different gene products (arising from different chromosomes) regulated differentially in various tissues. Thus, isozymes have different physico-chemical properties such as molecular mass, electrophoretic mobility, isoelectric pH, subunit composition, etc.

Lactate dehydrogenase (LDH) exists in five different isoforms, each as a tetramer. Two genes code for the two different subunits: LDH-H and LDH-M

LDH1 = H_4; LDH2 = H_3M; LDH3 = H_2M_2; LDH4 = HM_3; LDH5 = M_4

LDH1 is predominant in heart whereas LDH5 is predominant in erythrocytes, skeletal muscle and liver.

Isoenzymes can be used for differential diagnosis. When tissues get destroyed, the intracellular enzymes are released into blood. Therefore, serum levels of these enzymes can be used to diagnose destruction of specific tissues because these enzymes are expressed in a tissue-specific manner. For example, LDH1 is expressed predominantly in the heart whereas LDH5 is expressed predominantly in erythrocytes, skeletal muscle and liver. Therefore, an increase in LDH1 isoenzyme in serum could be an indicator of heart damage (heart attack or myocardial infarction) whereas an increase in LDH5 isoenzyme in serum could be an indicator of hemolytic anemia or myolysis or liver disease.