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SEM-2

PAPER-4: Fundamentals of Biochemistry

Lesson: Enzymes

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Introduction

Video: Enzymes – overview; Lecture Series on BioChemistry I by Prof.S.Dasgupta, Dept of Chemistry, IIT Kharagpur.
Source: http://nptel.ac.in/

Enzymes are biocatalysts, the most amazing specially designed biomolecules having exceptional catalytic potential. They are mainly proteins with the exception of ribozymes or catalytic RNAs. They have very high substrate specificity, accelerate rate of chemical reactions manifold, function under mild temperature and pH conditions in aqueous solutions and can undergo regulation making life feasible. Enzymes play a key role in all biochemical process. Besides this the study of enzymes has prodigious practical significance. Certain genetic diseases and metabolic disorders are caused due to defects in activity of various enzymes. Thus measuring activities of enzymes is a vital tool in diagnosis and prognosis of various diseases and efficacy of drugs. Besides medicine, nowadays enzymes are widely used commercially in chemical industry, agriculture and food processing.

Nomenclature and classification

In nature there exists a plethora of enzymes and the reactions they catalyze. Common names exist for various enzymes that provide little insight about the reactions catalyzed by them, e.g., chymotrypsin is a protease secreted by pancreatic acinar cells. Yet certain other enzymes are named after substrates and reactions catalyzed, with the suffix –*ase;* e.g., collagenase is an enzyme involved

in collagen break down, whereas glycogen synthase is an enzyme involved in glycogen synthesis.

In order to resolve the ambivalence due to inconsistencies in enzyme nomenclature and new ever-increasing discoveries, the International Union of Biochemistry (IUB) set an Enzyme Commission (EC) to establish an explicit and univocal system of enzyme nomenclature. This relies on a numerical system to classify enzymes in groups according to the types of reaction catalysed and systematic naming that deals with the chemical reaction involved. In the system, enzymes are classified into six classes, and further subdivided into subclasses, resulting in a four-digit EC number that would accurately identify all enzymes, and a systematic name, which identifies the reaction catalyzed. For example, the enzyme "hexokinase" is denominated as "ATP: D-hexose 6-phosphotransferase; E.C. 2.7.1.1." This specifies it belongs to class 2 (transferases), subclass 7 (phosphotransferase), 1 specifies phosphotransferase with a hydroxyl group as acceptor and the last 1 suggests glucose is the phosphoryl group acceptor. But still trivial names are widely used for various enzymes—as for the above mentioned E.C.2.7.1.1 it is hexokinase. The following table lists six IUB classes of enzymes and the reactions catalyzed by them :

Table : International Classification of enzymes

S.no	Class	Reaction catalyzed	Examples with trivial name
EC 1	**Oxidoreductases**	Oxidation and reduction reactions (redox); transfer electrons (hydride ions or H atoms) from donor and acceptor molecule	Oxidoreductases, dehydrogenases
EC 2	**Transferases**	Functional group transfer reactions from donor to acceptor	Transaminase, kinase
EC 3	**Hydrolases**	Hydrolysis reactions of substrates leading to formation of two products	Proteases, nucleases
EC 4	**Lyases**	Genesis of double bonds or removal of double bonds	Decarboxylase
EC 5	**Isomerases**	Intramolecular rearrangement, i.e. isomerization reactions	Isomerases, mutases

EC 6	**Ligases**	Joining of two molecules along with hydrolysis of a pyrophosphoryl group of ATP or other nucleoside triphosphates	Synthetases

The Nomenclature Committee of the IUBMB (International Union of Biochemistry and Molecular Biology) provides an entire exhaustive list and characterization of the several enzymes.

In 1897 **Eduard Buchner** discovered that yeast extracts are capable of fermenting sugar to alcohol. **Frederick W. Kühne** called these molecules **Enzymes**. James Sumner isolated and crytallized the enzyme urease in 1926 and found it to be proteinaceous in nature. **J.B.S Haldane** in 1930 wrote a monograph entitled *Enzymes* and suggested that weak interactions between enzyme and substrate are used to promote catalysis.

Cofactors

Cofactors are the small nonprotein molecules and metal ions required by certain enzymes for substrate binding and catalytic activity**.** These cofactors increase the range of catalytic capabilities of various enzymes beyond those provided by amino acyl chains of the protein part of the enzyme. A complete, catalytically active enzyme along with its cofactor is designated as **holoenzyme** and its proteinacoeus part is known as **apoenzyme** or **apoprotein** (Apoenzyme + cofactor = Holoenzyme).

Cofactors can be classified into two groups: **metal ions** or a complex organic and metalloorganic molecule called a **coenzyme.** A **prosthetic group** is a cofactor that is very firmly or even covalently associated with the protein part of the enzyme. Examples of prosthetic groups include biotin, thiamine pyrophosphate (TPP), flavin mononucleotide (FMN), cobalamine, lipoic acid, pyridoxal phosphate (PLP) and various metal ions like Fe^{2+}, Cu^{2+}, Zn^{2+}, etc. Metal ions frequently occur as prosthetic groups in enzymes termed as **metalloenzymes.** Metal ions facilitate plethora of reactions by mediating redox reactions, by the appropriate proximity, placement and orientation of substrates, transient covalent bond formation alongwith reaction intermediates or by increasing the elecrophilic or nucleophilic potential of the substrates. For example, the enzyme carbonic anhydrase, requires Zn^{2+} for its activity. Certain enzymes require a metal ion for activity in a transient, detachable manner are called as **metal-activated**

enzymes. Coenzymes serve as transitory carriers of specific functional or groups atoms and can be designated as group transfer recyclable shuttles. Enzymes usually procure them from vitamins. They may be either loosely or covalently associated with the enzyme. Loosely associated coenzymes are like cosubstrates. For example, Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate. Some examples of enzyme cofactors are listed in Table :

Table: Enzymes Cofactors	
Cofactor	**Enzyme**
Coenzyme	
Thiamine Pyrophosphate	Pyruvate dehydegenase
FAD	Monoamine oxidase
PLP	Aminotransferses
Biotin	Pyruvate carboxylase
Tetrahydrofolate	Thymidylate synthase
Lipoic acid	Pyruvate dehydrogenase
Metal	
Zn^{2+}	Carboxypeptidases A and B, carbonic anhydrase
Mg^{2+}	Hexokinase, pyruvate kinase
Cu^{2+}	Cytochrome oxidase
Mn^{2+}	Arginase
Mo	Dinitrogenase

Activation energy

Enzymes enhance rate of a reaction by lowering the activation energy without affecting the reaction equilibrium. The difference between the transition state of a reaction and the ground state of the reactants is the activation energy ($\Delta G^{\ddagger}$). The part of a reaction at the top of energy hill having the highest free energy and being most unstable is termed as the **transition state**. It is actually an ephemeral molecular moment where bond breakage and formation have proceeded to such a point at which decomposition to either product or substrate is equally possible. A faster rate of a reaction implies lower activation energy and a slower rate means higher activation energy.

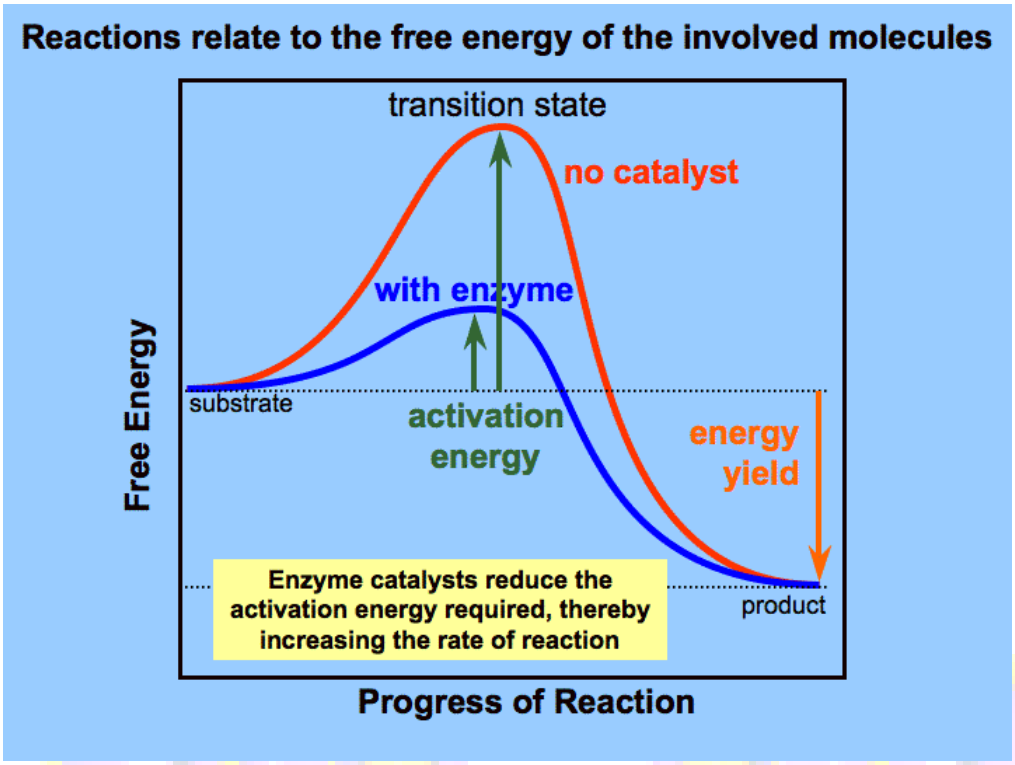


Figure: Enzymes lower activation energy

Source: http://plantphys.info/plant_physiology/images/enzenergy.gif

Active site and substrate specificity

An enzyme-catalyzed reaction always occurs in a pocket like area in the enzyme known as the **active site.** Substrates bind to the active site and are transformed into one or more products. Active site's surface is lined by amino acid residues having functional groups that bind to the substrate and carry catalysis. The active site generally entraps a substrate and sequesters it from solution. There are two proposed hypothesis of substrate specificity of enzymes; the lock and key hypothesis and the induced fit hypothesis.

All enzymes exhibit a very high degree of substrate specificity. Multiple weak interactions like, hydrogen bonding, hydrophobic interactions, vander waals interactions or ionic interactions or may be a combination of all of these lead to the formation of an enzyme-substrate complex. Amino acid residues at the active site serve as proton or other chemical group's donors or acceptors to facilitate catalysis and bring about substrate transformation. Enzymes are absolutely stereospecific in binding chiral substrates and in catalyzing their reactions. This stereospecificity arises as enzymes, because of their inherent chirality (proteins has only L-amino acids), form asymmetric active sites. For example, digestive

protease trypsin hydrolyzes proteins having L-amino acids but not those consisting of D-amino acids. Similarly, the enzymes involved with glucose metabolism are specific for D-glucose residues. Enzymes are also geometrically specific. Some are highly specific for the identity of their substrates, whereas others can bind a wide range of substrates and catalyze a variety of related types of reactions.

Lock and key hypothesis

Emil Fisher first proposed the lock and key hypothesis to explain the mechanism of enzymatic catalysis. The lock represents an enzyme and the key represents a substrate. It is hypothesized that both the enzyme and substrate have fixed complementary conformations that lead to an easy fit. The active site of an enzyme has a specific geometric shape and orientation that only a complementary substrate can fit in perfectly. This model establishes an essential complementarily between the shapes of the enzyme and the substrate. This makes the substrate fit perfectly into enzyme like a lock and a key. A substrate of an enzyme has a predetermined perfectly complementary shape. Thus, the substrate is stabilized on the enzyme.

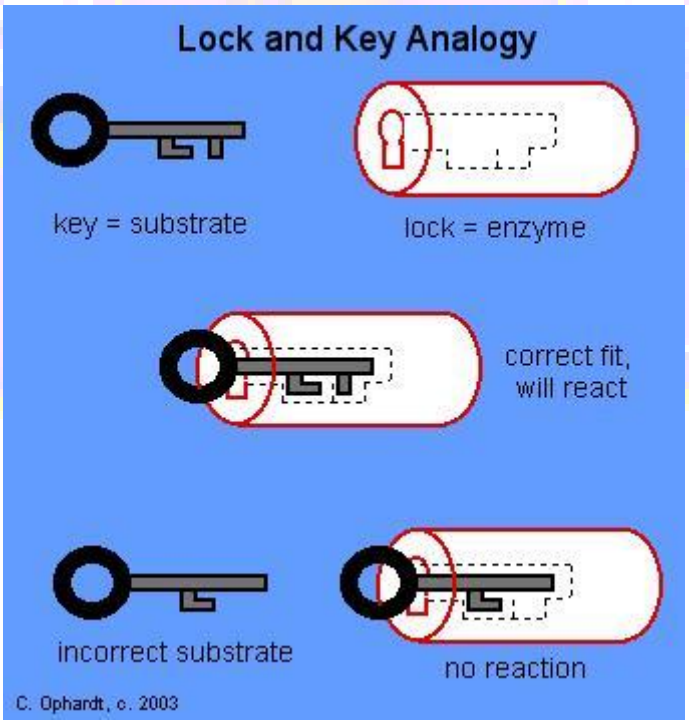


Figure: Lock and key hypothesis

Source:http://upload.wikimedia.org/wikibooks/en/a/af/Lock_and_key2.jpg

Induced fit hypothesis

This model of enzymatic catalysis was first proposed by Daniel Koshland in 1958. Substrate binding leads to a conformational change in the enzyme which is induced by multiple weak interactions with the substrate. Induced fit manages to align precise catalytic functional groups of the enzyme's active site in the correct orientation. This change in conformation also allows optimization of additional weak bonding interactions in the transition state. The new enzyme conformation has increased catalytic capabilities.

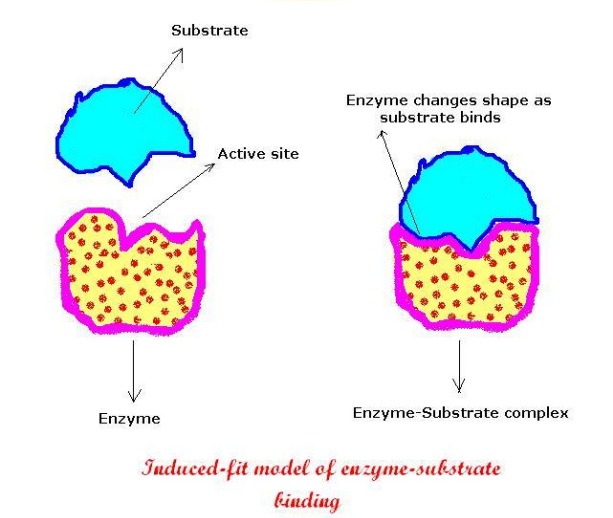
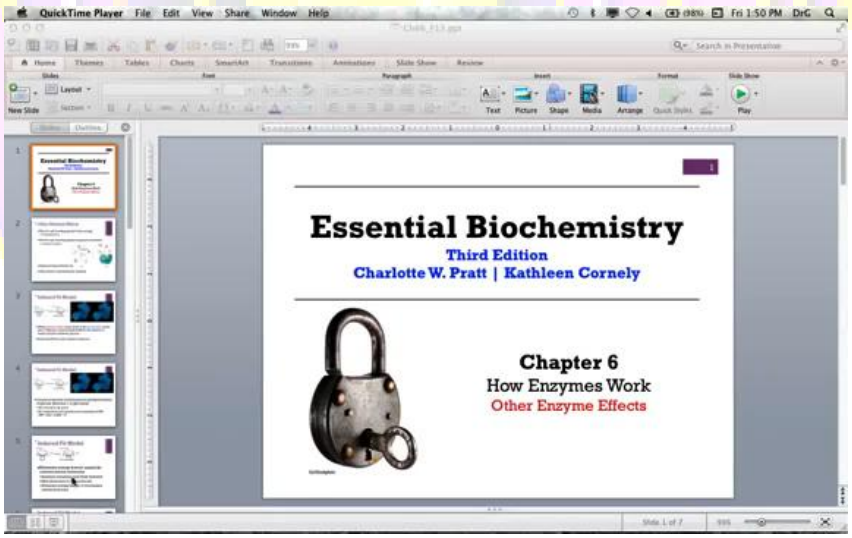


Figure: Induced fit hypothesis

Source: http://upload.wikimedia.org/wikibooks/en/4/4e/Induced_fit.jpg



Video: Mechanism of action of enzymes

Source: https://www.youtube.com/watch?v=QMQG3XQ6s4g

Mechanism of action

Upon substrate binding to an enzyme's active site, appropriately positioned catalytic functional groups facilitate catalysis and aid breakage and formation of various bonds. Enzymes employ various catalytic mechanisms to catalyze various biochemical reactions which are majorly classified into six categories:

1. **Acid-base catalysis**: Various functional groups of aminoacyl side chains and prosthetic groups that can be ionized present at the active site act as acids (proton donors) or bases (proton abstractors). Such acid-base catalysis is of two types either specific or general. Catalysis that uses only the H^+ (H_3O^+) or OH^- ions of water is called specific acid-base catalysis. When such proton transfers are mediated by other molecules except water, such catalysis is referred to as general acid-base catalysis. Various biochemically significant reactions are susceptible to acid and/or base catalysis, including hydrolysis of peptides, tautomerizations, and the reactions of phosphate and carbonyl groups. Mechanism of RNAse action clearly demonstrates acid-base catalysis. RNAse catalyzes the hydrolysis of RNA into smaller components. It has two histidines at the active site, His12 and His119, which act as a general acid and base in a concerted manner. Firstly, His 12 acts a general base and accepts a proton from 2'OH of ribose thereby increasing its nucleophilic character. His 119 acts a general acid, donates its proton and promotes bond breakage and the first product leaves. 2',3' cyclic intermediate is broken when water comes and His12 now acts a general acid and His 119 as a general base to regenerate the active enzyme and the second product of smaller RNA.

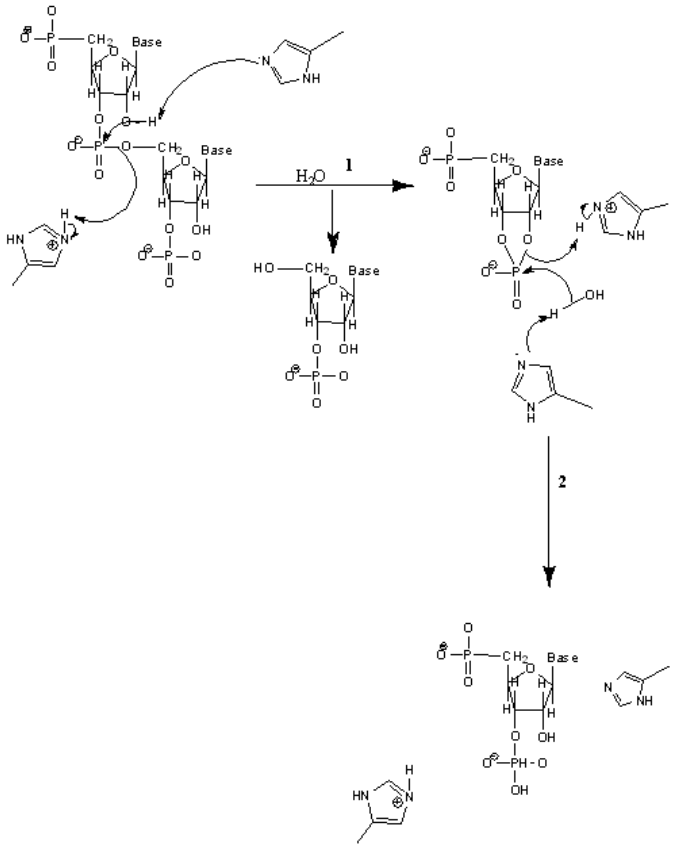


Figure: Mechanism of action of RNAse, an example of acid-base catalysis
Source: http://upload.wikimedia.org/wikipedia/commons/thumb/8/8a/Mechanism_for_RNAse.png/417px-

2. **Covalent catalysis**: In such a mode of catalysis, a transitory covalent bond is established between the enzyme and the substrate. This provides with an alternative enzymatic pathway with lower activation energy. A reactive group, generally a nucleophilic residue is present at the active site of the enzyme which reacts with the substrate through a nucleophilic attack, leading to formation of transient covalent bonds between the enzyme and its substrates. Such fleeting covalent complexes decompose to regenerate the free enzyme and precise products. Covalent catalysis can also be classified as **nucleophilic and electrophilic** substitution reactions. An example of **nucleophilic substitution** reaction is the enzyme acetylcholine esterase (AChE) which inactivates neurotransmitter acetylcholine (ACh) by cleaving it into choline and acetate employs a concerted model for catalysis. From the nerve endings of presynaptic neuron ACh is released into the synaptic cleft at the neuromuscular junction where it binds to ACh receptors present on the post-synaptic

membrane leading to muscle contraction. AChE also found on post-synaptic membrane hydrolyzes ACh thus leading to cessation of the nervous signal. Ach must not reside too long at the synaptic junction as it may lead to uncontrolled muscle contraction (contracture) and death may unused fast. Choline is recycled again by the pre-synaptic nerve by a choline carrier followed by synthesis of ACh by the enzyme choline acetyltransferase. AChE plays a significant role in neurotransmission and is potent target of many neurotoxins and nerve gases. For example, DIPF and sarin are irreversible inhibitors of AChE. The active site of AChE contains a catalytic triad of Ser-His-Glu. The ester bond of ACh is cleaved through a nucleophilic attack by an active Ser cleaving it into choline and acetate. The nucleophilic attack is assisted by a nearby His acts as a base and abstracts a proton from the serine (an example of **concerted catalysis**) thus promoting serine's nucleophilic character. Glu stabilizes the protonation of His. The hydroxyl group of Ser which is electron rich (nucleophilic) attacks the carbonyl carbon of ACh. Electron pair migrates from O of serine to C of carbonyl and now they move to O of carbonyl, then to the double bond of carbonyl and subsequently C-O bond of substrate is broken releasing choline and a transient covalent acyl-enzyme intermediate. Nucleophilic attack by a water molecule, again assisted by His, causes the acyl-enzyme is break into active enzyme and acetate.

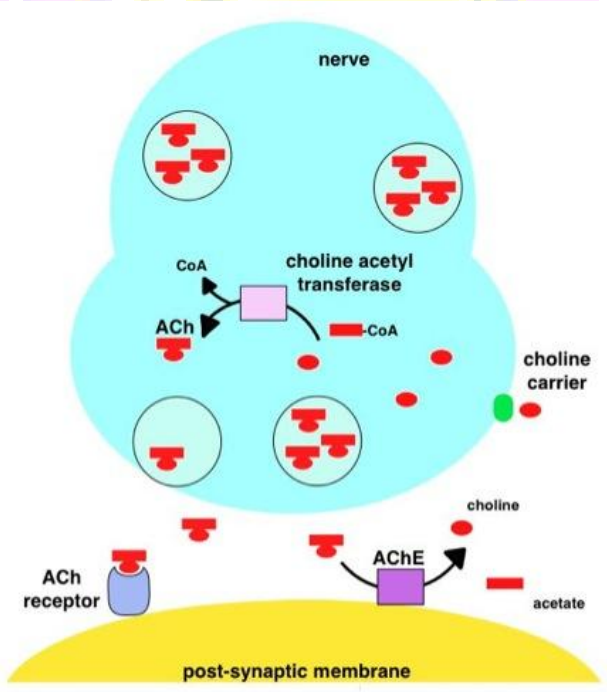


Figure: Acetlycholine esterase hydrolyzes Ach by cleaving it into choline and acetate at the post synaptic nerve

Source:
http://upload.wikimedia.org/wikipedia/commons/8/89/AChe_mechanism_of_action.jpg

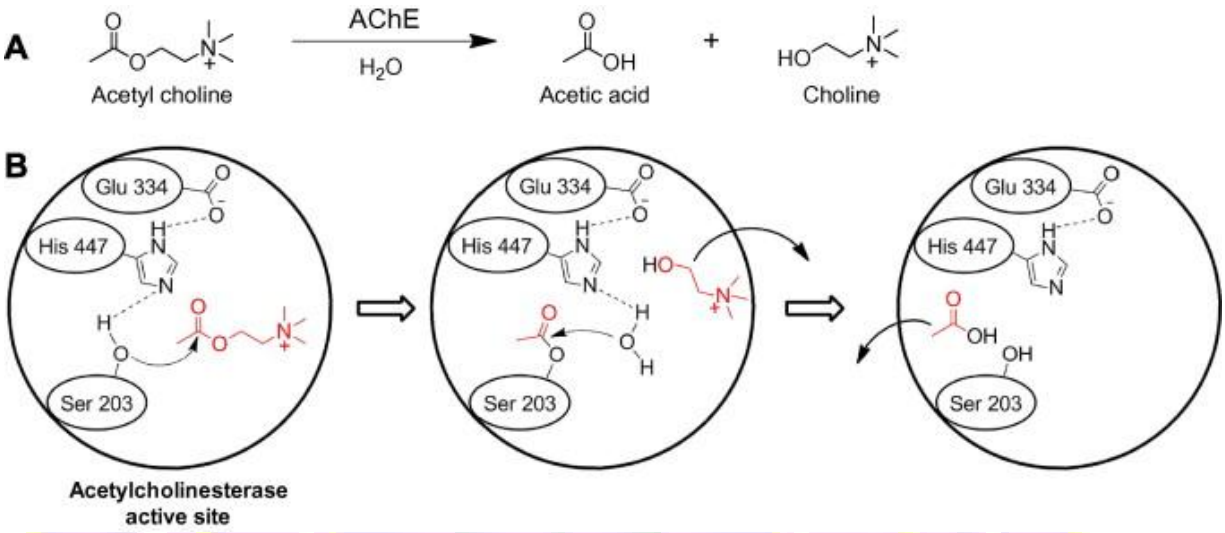


Figure: Mechanism of Acetylcholine esterase. Ser nucleophilically attacks ACh whose nucleophilc nature is enhanced by a nearby His which acts as a base by abstracting its proton and forms a transient covalent intermediate which decomposes to acetate and original enzyme upon nucleophilic attack by a water molecule.

Source:http://amit1b.files.wordpress.com/2012/09/acetylcholine-esterase-mechanism.jpg

3. **Metal ion catalysis**: Nearly about one- third of enzymes we know today need one or more metal ion for catalysis. Generally metal ions facilitate the catalytic process by three ways:
   - **i)** By proper orientation of substrates.
   - **ii)** By mediating redox reactions i.e., oxidation–reduction reactions through variations in the metal ion's oxidation state.
   - **iii)** By electrically stabilizing or shielding negative charges.

4. **Electrostatic catalysis**: The charge distributions about the substrate binding sites of various enzymes are organised so as to preserve the transition state of the enzyme catalyzed reactions and mediate to guide substrates toward their binding sites to bring about rate enhancements. Upon substrate binding water is generally excluded from the active site

resulting in lowering of its dielectric constant and pKa, which further strengthens the electrostatic interactions between the enzyme and polar/charged substrates. These charge distributions probably serve to guide polar substrates toward their binding sites in order to achieve catalytic perfection. An example of electrostatic (also metal ion catalysis) is carboxypeptidase A reaction in which tetrahedral transition state's charge is stablized.

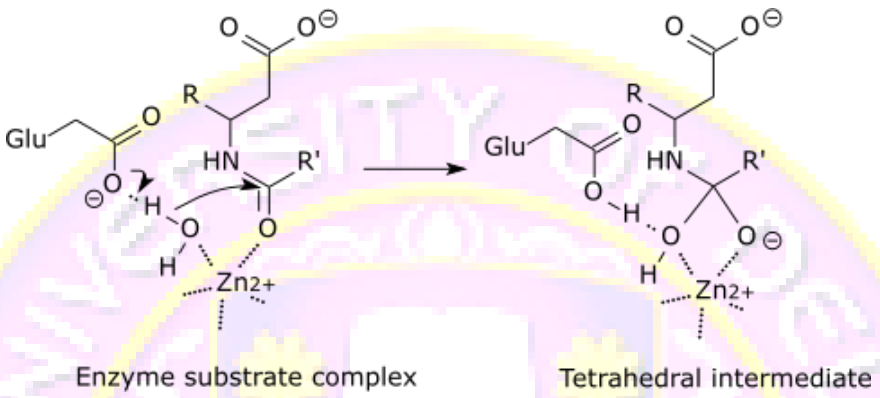


Figure: Carboxypeptidase A catalytic mechanism: an example of electrostatic and metal ion catalysis in which a tetrahedral intermediate is stabilized by a partial ionic bond between the Zn^{2+} ion and the negative charge on the oxygen.
Source:http://upload.wikimedia.org/wikipedia/commons/4/4e/Carboxypeptidase_catalysis.png

5. **Proximity and orientation effects**: Enzymes serve to bring substrates in proper orientation, and close proximity for catalysis, physically at the active site. This aligns the enzyme and substrate and provides an intramolecular nature to the reaction thus resulting in rate enhancements. For example as shown in the figure, massive rate enhancements results if the same reaction is intramolecular because of proper proximity and orientation.

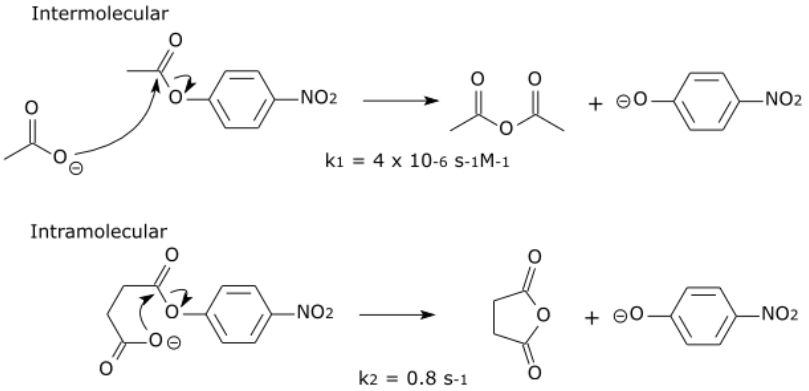


Figure: proximity and orientation effects result in rate enhancements of catalyzed reactions. The effective concentration of acetate in the intramolecular reaction can be estimated as $k_2/k_1 = 2 \times 10^5$ M

Source: http://upload.wikimedia.org/wikipedia/commons/d/d5/Inter_vs_intramolecular_reaction_rates.png

6. **Preferential Binding to Transition State**: Enzymes bind to the transition state of the reaction more strongly than the corresponding substrates or products. Transition state analogs are the compounds that mimic the transition state of an enzyme catalyzed reaction. They are potent inhibitors of enzymes. For example, reaction catalyzed by proline racemase proceeds through a planar transition state, where the tetrahedral α-carbon becomes trigonal as a proton leaves the L-proline. Transition state analog pyrrole 2-carboxylic acid whose α-carbon, like that of the planar transition state is trigonal, binds to racemase 160 times as tightly as L-proline. The inhibitory potential of these analogs underscores the idea of selective binding of the transition state as an important mechanism of catalysis.

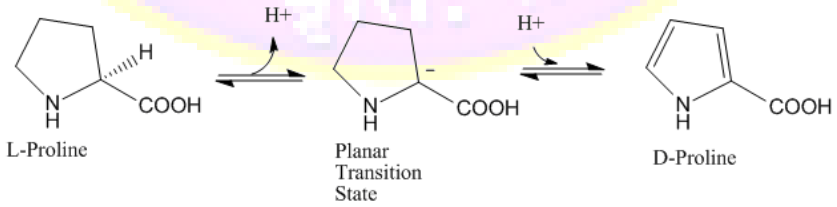


Figure: Isomerization of L-proline to D-proline via proline racemase via a planar transition state

Source: http://upload.wikimedia.org/wikibooks/en/4/49/JLDproline.gif

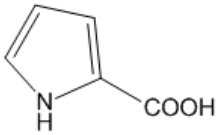
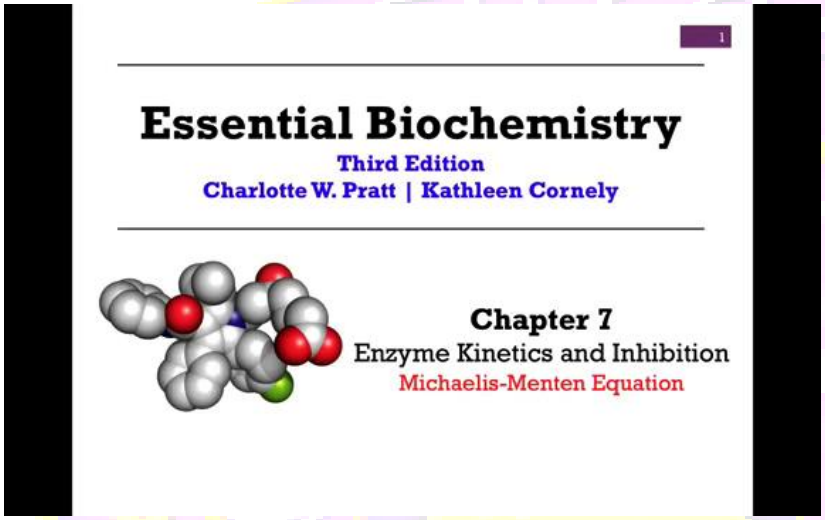


Figure: Transition state analog of proline racemace, pyrrole 2-carboxylic acid

Source: http://upload.wikimedia.org/wikibooks/en/1/12/Jtlog.gif

Enzyme kinetics



In order to understand the mechanism of an enzyme-catalyzed reaction, we study enzyme kinetics, a branch of enzymylogy that determines the *rate* of the reaction and its response to variations in experimental conditions. A major factor that affects the rate of an enzyme catalyzed reaction is the substrate concentration [S]. However, [S] varies during the course of an enzyme catalyzed reaction as substrate gets transformed to product. Therefore, kinetics measures the **initial velocity**, designated $\boldsymbol{V_0}$, when substrate concentration is sufficiently more than the concentration of enzyme, [E]. If only the beginning of the reaction is considered, [S] might be considered as a constant value. The effect on V_0 versus [S] when the enzyme concentration is held constant is depicted in Figure known as the Michaelis-Menten plot:

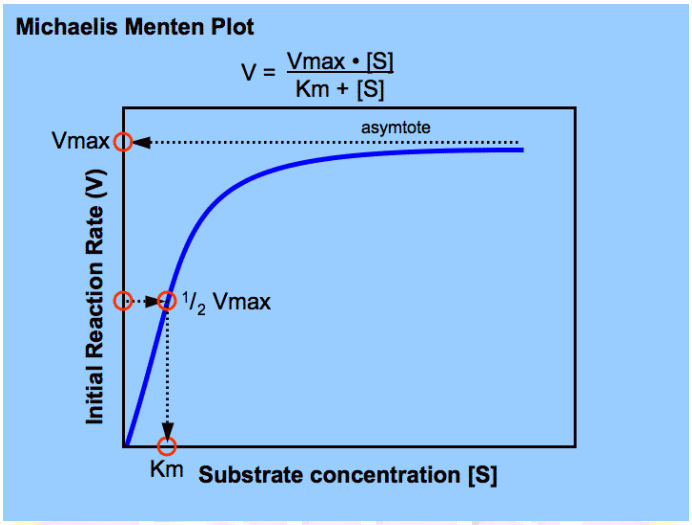


Figure : Michaelis Menten plot

Source: http://plantphys.info/plant_physiology/images/enzmichaelis.gif

In 1913, Leonor Michaelis and Maud Menten, postulated a theory of enzyme action, where enzyme (E) combines with its substrate (S) to form an enzyme-substrate (ES) complex in a fast reversible step. The ES complex then decomposes in a slower second step into free enzyme and its corresponding product P. The general equation for any enzyme catalyzed reaction is

$E + S \leftrightarrow ES \leftrightarrow E + P$

From this equation the rate of the forward reaction from E + S to ES is coined as k_1, and the reverse reaction as k_{-1}. Similarly, for ES complex to E and P, the rate of forward reaction is k_2, and the reverse is k_{-2}.

At the beginning of reaction, when t =0, product formation just starts, therefore rate of backward reaction k_{-2} might be neglected:

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

Rate of catalysis is equal to the product of [ES] and k_2 i.e., $V_0 = k_2[ES]$ ….(1)

Briggs and Haldane gave steady state assumption according to which [ES] remains approximately constant over time, and thus rate equations might be given as:

Rate of formation of ES = $k_1[E][S]$

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

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

On rearrangement,

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

The fraction $(k_{-1} + k_2)/k_1$ is termed as K_m, or the Michaelis constant. It has the units of concentration. K_m is a significant characteristic of enzyme-substrate interactions and is independent of [E] and [S]. Now the above equation becomes:

$[ES]=[E]\,[S]/K_m$ …(2)

Now, since the substrate is often found in much higher concentrations as compared to enzyme so total substrate concentration is approximately equal to the concentration of uncombined sustrate[S]. uncombined enzyme [E] is calculated by deducting concentration of [ES] complex from the total enzyme [E]t present:

$[E]=[E]t-[ES]$

Now, using value of [E] in equation 2 and solving for [ES]:

$$[ES]=\frac{\{[E]t[S]\}[S]}{Km}$$

$$[ES]=\frac{[E]t[S]/Km}{1+[S]/Km}$$

$$[ES]=[E]t\,\frac{[S]}{[S]+Km}$$

Now substituting [ES] in equation 1:

$$V_0=k_2[E]t\frac{[S]}{[S]+Km} \quad \text{…..(3)}$$

Maximum velocity (V_{max}) is achieved when all the active site of enzyme are occupied with the substrate, i.e., [ES] = [E]t, which implies $V_{max}=k_2[E]t$. Now equation 3 beomes,

$$V_0=V_{max}\frac{[S]}{[S]+Km} \quad \text{……(4)}$$

The above equation (4) is known as **Michaelis-Menten** equation. According to Michaelis-Menten's kinetics, at low [S], K_m >>> [S], so the equation becomes V_0 = V_{max} [S]/$K_{m.}$ Since V_{max} and Km are both constants, therefore, V_0 is directly proportional to [S].

At high substrate concentrations, [S] >>> K_m, equation becomes V_0=V_{max}[S]/[S]. The initial velocity approaches V_{max}, and is unaltered by further increase in [S].

When [S] = K_m , Michaelis Menten equation becomes V_0 = V_{max} [S]/K_m+ [S], which implies V_0 = V_{max} [S]/2[S] and therefore V_0 = V_{max}/2. Therefore, K_m is that substrate concentration at which the initial velocity is half-maximal. Km is sometimes used (often inappropriately) as an indicator of the affinity of an enzyme for its substrate. A high K_m indicates weak binding; a low K_m indicates strong binding of ES. The maximal rate, V_{max}, indicates the **turnover number** (k_{cat}) of an enzyme. K_{cat} is maximum number of substrate molecules that an enzyme molecule can transform into product in unit time when the enzyme is fully saturated with its substrate.

Thus, k_{cat}=V_{max}/[E]t

When the concentration of substrates rises at a constant enzyme concentration, the active sites of the enzyme will start getting occupied as the reaction proceeds. When all the active sites get saturated with the substrate, that implies the enzyme is at its maximum capacity and increasing the concentration of substrate will not increase the rate of turnover.

For most enzymes, Km or the Michaelis-Menten constant lies between 10^{-1} and 10^{-7}M. Km varies from one enzyme to another and also from different substrates of the same enzyme. It is also dependent upon on conditions such as temperature, pH and ionic strength. It has two meanings. Firstly, it is that concentration of substrate at which half the active sites are occupied. Thus, it provides a measure of substrate concentration for effective catalysis. Secondly, it is related to rate constants of individual steps in a catalytic scheme as $Km=(k_{-1} + k_2)/k_1$. Now if $k_{-1} >> k_2$, so Km becomes k_{-1}/k_2, also called as dissociation constant of ES complex. So under these conditions Km becomes a measure of strength of ES complex: a high Km indicates weak binding and low indicates strong binding. That means it can be used as a measure of inverse affinity if an enzyme for its substrate (though incorrectly) — a small Km indicates high affinity, i.e., the reaction approaches Vmax quickly. It is also used a kinetic parameter to distinguish between isozymes and is significant for biological function. But if if $k_{-1} << k_2$, Km becomes k_2/k_1 and can no longer be used as a measure of enzyme affinity. Also if individual rate constants are comparable Km becomes a more complex function. Km can also used to measure fES i.e., fraction of active sites occupied. fES=[S]/[S]+Km. *K*cat/Km is a measure of **catalytic efficiency**, where kcat is the turnover number. *K*cat/Km, also called as specificity constant takes into account both catalytic rate with a particular substrate (*k*cat) and strength of ES complex (Km). *k*cat/*K*m is a second-order rate constant having units of $M^{-1}s^{-1}$. It has an upper limit to its value, because of the rate at which E and S can diffuse together in an aqueous environment. This diffusion controlled limit is 10^8 to 10^9 $M^{-1}s^{-1}$, and various enzymes have a *k*cat/*K*m near this range and are said to have attained **catalytic perfection**.

Measurement of V_{max} and Km often requires unrealistic very high concentrations of substrate to reach saturation. Also V_{max} is never attained and only approximated from Michaelis-Menten kinetics. A linear form of the Michaelis-Menten equation overcomes this problem and allows extrapolation of V_{max} and Km from initial velocity data obtained at lower substrate concentration. By taking reciprocal of Michaelis-Menten equation, it can be transformed into a more useful form for plotting experimental data. This is known as **Line weaver Burk equation**:

$$\frac{1}{V} = \left(\frac{K}{V_{\max}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}.$$

Those enzymes that obey the Michaelis-Menten kinetics, a plot of $1/V_0$ versus 1/[S] (the "double reciprocal"plot) yields a straight line, having a slope of Km/V_{max}, an intercept of $1/V_{max}$ on the y-axis, and an intercept of -1/Km on the x-axis. The double-reciprocal plot (Lineweaver-Burk plot), has a greater advantage for more precise measurement of V_{max}, which can only be approximated from Michaelis-Menten plot.

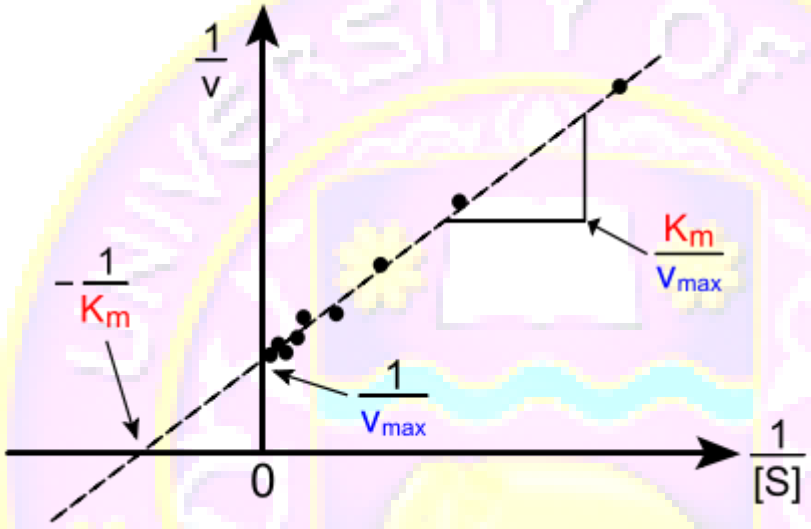
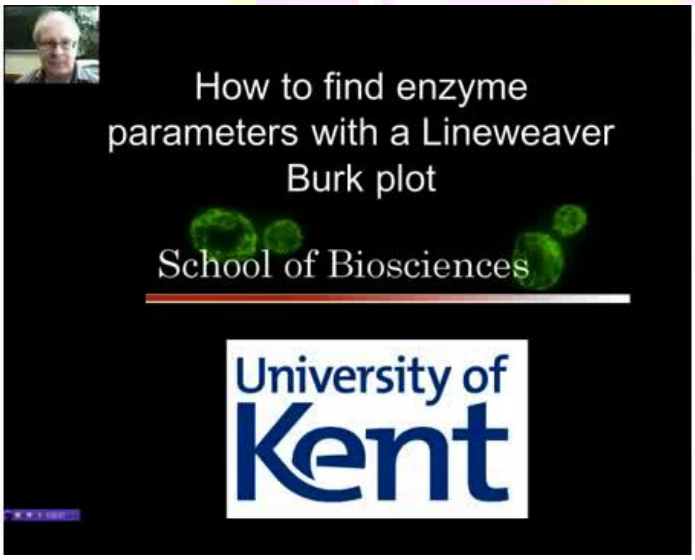


Figure: Line weaver burk plot or double reciprocal plot

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/7/70/Lineweaver-Burke_plot.svg/420px-Lineweaver-Burke_plot.svg.png



Enzyme inhibition

Enzymes are subject to inhibition by various molecules called inhibitors, which bind to them and decreases or halt their enzymatic activity. These act as both pharmaceutical agents and as research tools for study of the mechanism various enzymes. These inhibitors are majorly classified into two broad categories: reversible and irreversible.

Reversible inhibition

Reversible inhibitors combine with enzymes via non-covalent multiple weak interactions to produce strong and specific binding. They do not undergo chemical reactions when bound to the enzyme unlike substrates and irreversible inhibitors and thus can be removed using dialysis. They can be categorized into three major categories:

I) Competitive inhibiton: In such a inhibition there is a competition between the substrate and the inhibitor for the active site of an enzyme. The inhibitor (I) prevents binding of the substrate to the enzyme as it sits at the substrate binding site; the substrate and inhibitor *compete* for access to the enzyme's active site. Many of such inhibitors are compounds that bear resemblance to the substrate and combine with the enzyme to form an EI complex, thus abolishing catalysis. These inhibitors are also termed as substrate analogs. Their effects can be circumvented by increasing the substrate concentration, as it overcompetes the inhibitor for the active site.

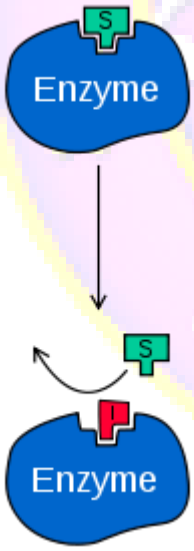


Figure: Competitive inhibition

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/5/50/Competitive_inhibitor.svg/134px

An example of competitive inhibitor is malonate which is inhibitor of the enzyme succinate dehydrogenase that catalyzes the dehydrgenation of succinate to fumarate. Both succinate and malonate are capable of binding to the active site of

enzyme, forming an ES or an EI complex, respectively. However, since malonate is not the actual substrate as it has only one methylene carbon, it cannot undergo dehydrogenation and thus enzyme is reversibly inactivated and no catalysis occur.

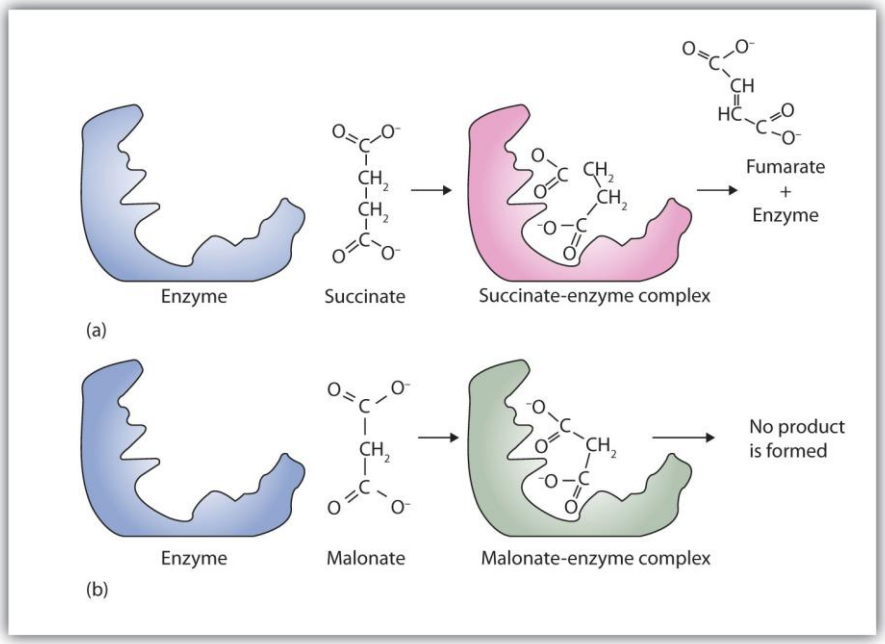


Figure : a) Succinate binds to the enzyme succinate dehydrogenase. A dehydrogenation reaction occurs, and the product—fumarate—is released from the enzyme. (b) Malonate also binds to the active site of succinate dehydrogenase. In this case, however, no subsequent reaction occurs while malonate remains bound to the enzyme

Source: http://2012books.lardbucket.org/books/introduction-to-chemistry-general-organic-and-biological/section_21/c4856898d875b151ce6627716c8a6dc3.jpg (CC)

Another example of competitive inhibitor is a chemotherapeutic drug Methotrexate. It bears structural resemblance to tetrahydrofolate, a coenzyme of dihydrofolate reductase, that plays a central role in the nucleotide biosynthesis. It competitively binds to dihydrofolate reductase and thus inhibits nucleotide biosynthesis and thus being used in cancer therapy.

Kinetically, K_m increases in presence of a competitive inhibitor as it interferes with substrate binding, but does not alter $V_{max.}$ The Michaelis-Menten equation thus becomes $V_o = V_{max}[S]/ aKm + [S]$ Where $a = 1 + [I]/Ki$ and $Ki = [E][I]/[EI]$

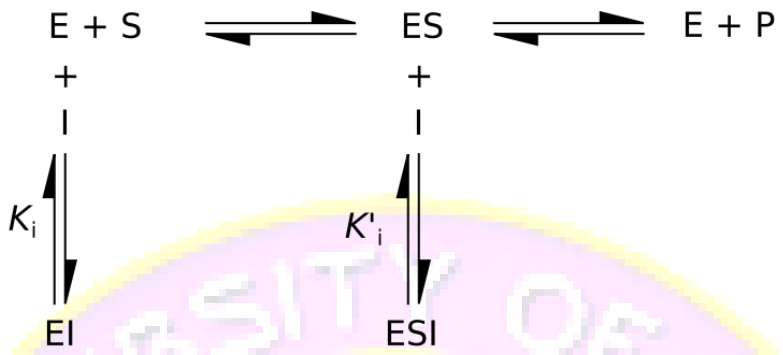


Figure: Kinetic scheme for reversible enzyme inhibitors
Source:http://upload.wikimedia.org/wikipedia/commons/thumb/f/f3/Reversible_inhibition.svg/761px

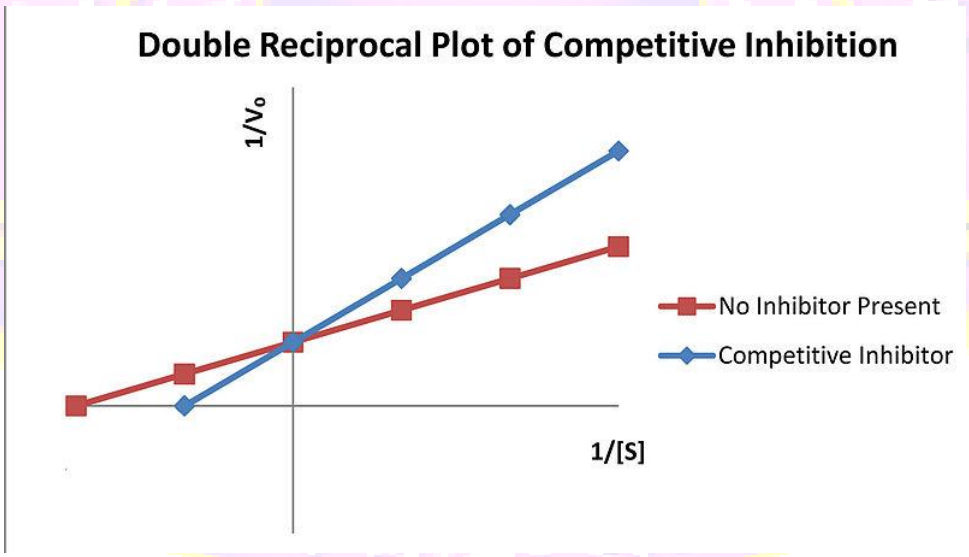


Figure: Lineweaver–Burk plots of competitive enzyme inhibitors
Source:http://upload.wikimedia.org/wikibooks/en/thumb/9/9d/Competitive_Image.jpg/800px-

II) Uncompetitive Inhibition: It is also known as **anticompetitive inhibition.** Such an inhibitor binds at a site other than the active site of the enzyme and can only bind to the enzyme- substrate complex. It has little or no affinity for free enzyme. Such an inhibition cannot be overcome by Increasing the concentration of substrate and affinity of these inhibitors is highest at the saturating levels of

substrate. An example is lithium, which is used as a drug in bipolar disorders and schizophrenia. It inhibit the enzyme inositol monophosphatase[IMPase] and prevent recycling of inositol for signal transduction pathway. Lithium functions to trap the cleaved product at the active site and thus uncompetitively inhibits the enzyme.

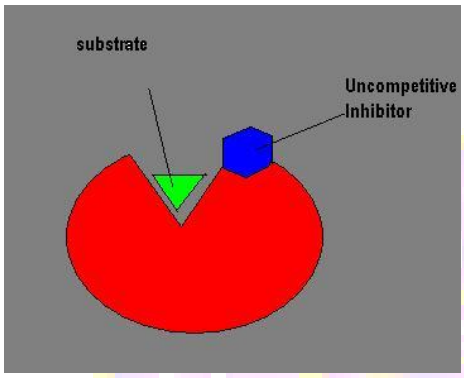


Figure : Uncompetitive inhibitor

Source:http://upload.wikimedia.org/wikibooks/en/9/94/Uncompetitiveinhibitor.jpg

Kinetically, uncompetitive inhibitors alter both Km and V_{max} (decrease them) as they interfere with substrate binding and affect catalysis in the ES complex. The Michaelis-Menten equation becomes $V_o = V_{max}[S]/ Km + a'[S]$ Where $a' = 1 + [I]/Ki'$ and $Ki' = [ES][I]/[ESI]$

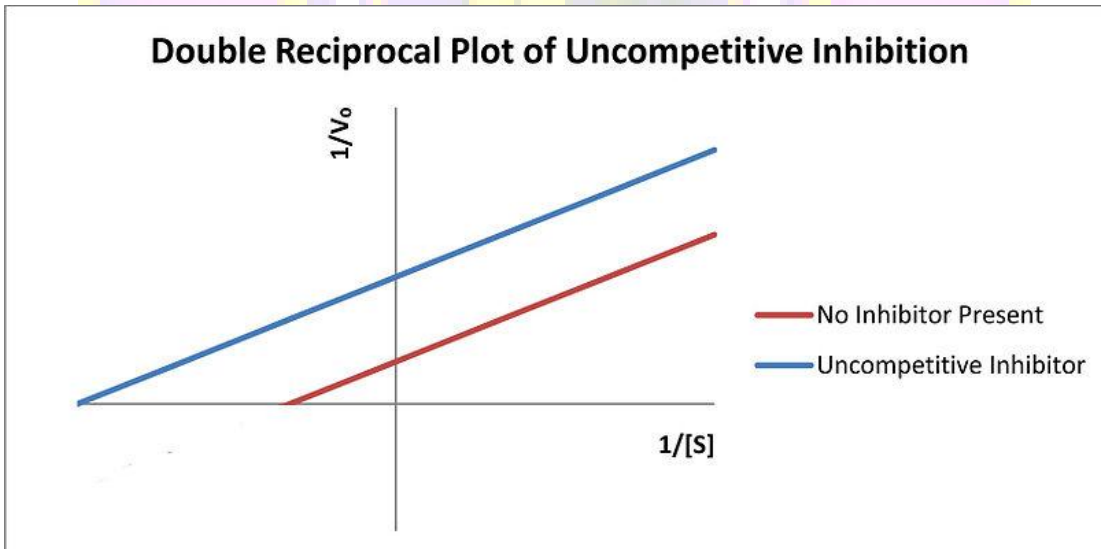


Figure: Lineweaver–Burk plots of uncompetitive enzyme inhibitors

Source:http://upload.wikimedia.org/wikibooks/en/thumb/c/c3/Uncompetitive_Image.jpg/800px-

III) **Mixed inhibition(Noncompetetive inhibition)**: A mixed inhibitor binds to a site other than the active site of an enzyme but it can either bind to free enzyme or to the enzyme substrate complex. In this both Km and V_{max} are altered. In certain cases of mixed inhibition known as **non competitive inhibition** V_{max} is decreased whereas Km remains unaltered. Noncompetitive inhibition cannot be prevailed over by raising the substrate concentration. The Michaelis-Menten equation for mixed inhibition becomes $V_o = V_{max}[S]/ aKm + a'[S]$ where a and a' are same as above. When a=a', noncompetitive inhibition occurs. Examples of non-competitive inhibitors of many enzymes include metal ions, like Cu, Hg and Ag, that reversibly bind to the –SH groups of cysteine of many proteins, thus disrupting their structure by breaking disulphide bridges and non competitively inhibiting the enzyme.

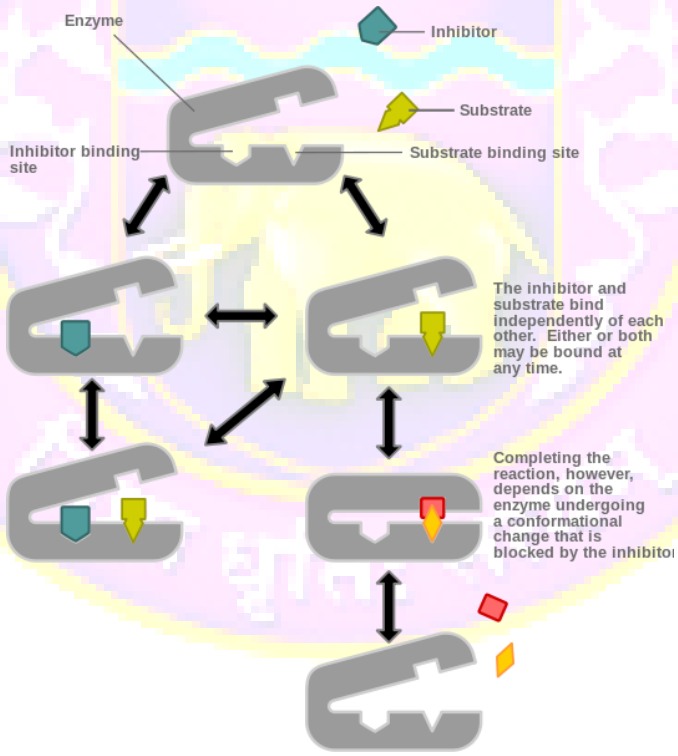


Figure: Non-competitive inhibitors

Source: http://upload.wikimedia.org/wikipedia/commons/thumb/5/55/Non-competitive_inhibition.svg/536px-Non-

Table: Effect of reversible inhibitors on Vmax, Km and Vmax/Km			
Inhibition type	Vmax	Km	Vmax/Km
Competitive	No change	Increased	Decreased
Uncompetitive	Decreased	Decreased	No change
Mixed	Decreased	No change	Decreased

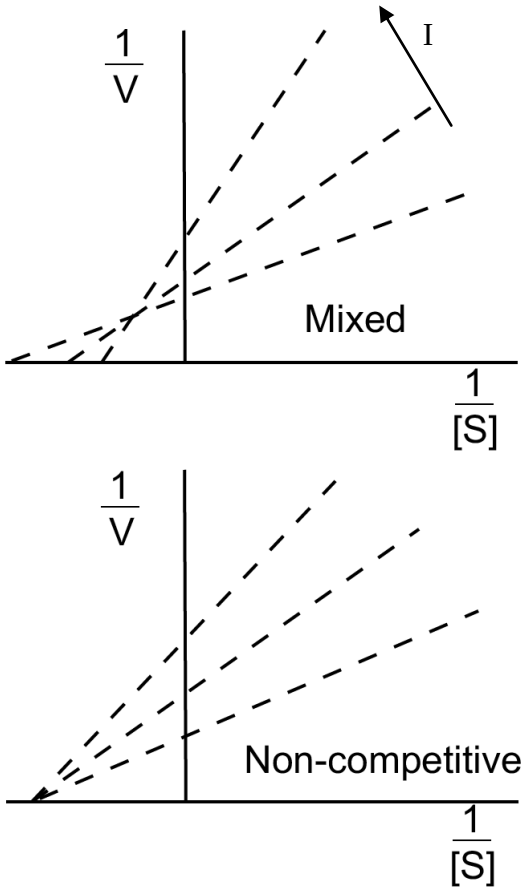


Figure: Lineweaver–Burk plots of mixed and non-competitive enzyme inhibitors. The arrow shows the effect of increasing concentrations of inhibitor
Source:http://upload.wikimedia.org/wikipedia/commons/a/a3/Inhibition_diagrams.png

Feedback inhibition is a mechanism to regulate enzymatic processes using noncompetitive inhibitors *in vivo*. One of the end products of a metabolic pathway inhibits the enzyme usually catalyzing the first committed step of the pathway. Such a feedback mechanism is used to regulate many important biochemical pathways. For example, certain bacteria employ a series of five enzyme catalyzed reactions for the conversion of threonine to isoleucine. As the concentration of end product, isoleucine rises, some of it binds as a noncompetitive inhibitor to the first enzyme of the series (threonine deaminase), thus decreasing the amount of isoleucine being synthesized by the bacterium.

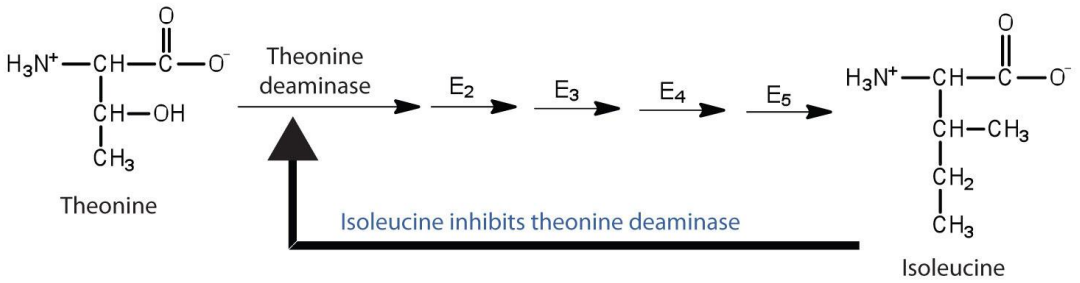


Figure: Feedback inhibition by Threonine deaminase

Source: http://2012books.lardbucket.org/books/introduction-to-chemistry-general-organic-and

Irreversible inhibition

Irreversible inhibitors are those that associate stably with the enzyme, either covalently or non-covalently. They dissociate very slowly from the enzyme because of their tight association. They serve as vital tools for investigating mechanism of enzyme catalyzed reactions. Amino acids with major catalytic functions in the active site can sometimes be identified by determining which residue is covalently linked to an inhibitor after the enzyme is irreversibly inactivated. There are three broad classes of irreversible inhibitors: group-specific reagents, reactive substrate analogs also termed as affinity labels and suicide inhibitors.

Group-specific reagents react with specific amino acyl side chains present at the active site. For example diisopropylphosphofluoridate (DIPF) reacts with serine 195 of chymotrypsin which is present at the active site. DIPF and similar compounds act as potent nerve poisons as they inactivate acetylcholinesterase involved in nerve transmission. This also indicated that there is an active serine present in its active site.

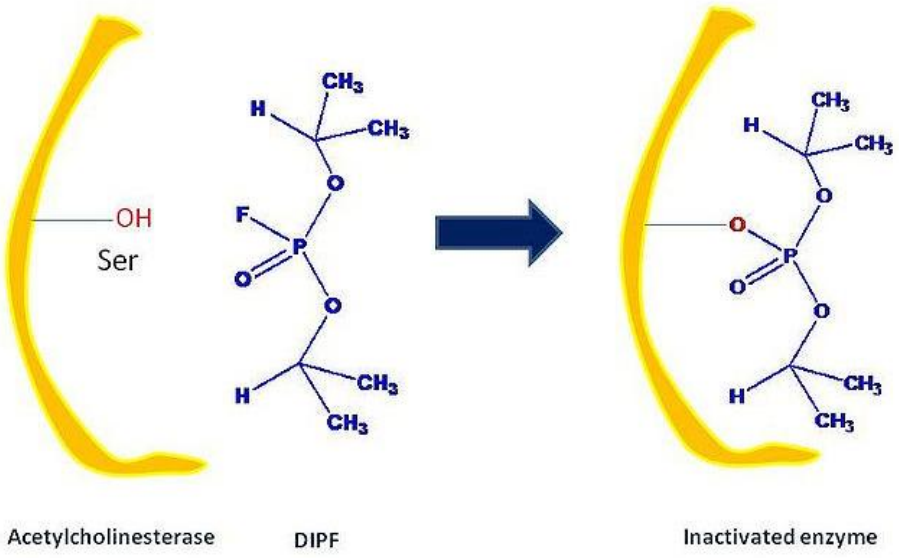


Figure: Group specific reagent DIPF reacts with a reactive serine present at the active site of acetylcholinesterase

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/2/21/Group_specific_reagent.jpg/800px-Group_specific_reagent.jpg

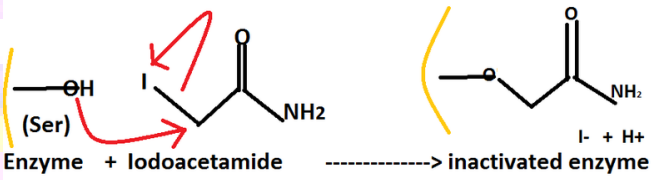
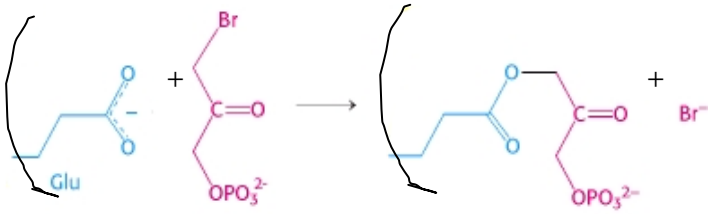


Figure: Another group specific reagent Iodoacetamide reacts with a critical serine present at the active site of enzymes

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/5/57/Irreversible_inhibitors.png/220px-

Affinity labels, also known as reactive substrate analogs are molecules that structurally resemble the substrate and covalently modify enzyme's active site residues and thus are very specific the above group-specific reagents. An example is Tosyl-l-phenylalanine chloromethyl ketone (TPCK) which is a substrate analog for chymotrypsin and binds irreversibly with a critical histidine at the active site. Another example is 3-bromoacetol which acts as an affinity label for

triose phosphate isomerase (TIM) as it mimics its substrate, dihydroxyacetone phosphate. It binds at the active site, covalently modifies the enzyme and irreversibly inhibits it.



TIM + Bromoacetol phosphate Inactivated enzyme

Figure: Suicide inactivator Bromoacetol phosphate
Source: Author

Suicide inhibitors are actually modified substrates that the most specifically modify an enzyme's active site. The inhibitor is initially processed by the normal catalytic mechanism which then generates a very reactive chemical reactive intermediate that irreversibly inactivates the enzyme covalently. Thus, they are also known as **mechanism-based inactivators, as** they hijack the normal enzyme reaction mechanism to inactivate the enzyme itself. An example of a suicide inhibitor is N,N dimethylpropargylamine, which inhibits the enzyme, monoamine oxidase (MAO). MAO breaks various neurotransmitters such as dopamine and serotonin, reducing their concentration in brain. Reduced levels neurotransmitters is the major cause of neurodegerative disorders like Parkinson and depression. Thus, to raise the neurotransmitter levels, N,N dimethylpropargylamine can be used as a suicide inhibitor to inhibit MAO from breaking down more neurotransmitters. A flavin prosthetic group of MAO is involved in oxidization of the *N*,*N*-dimethylpropargylamine, which in turn inactivates the enzyme by alkylating N-5 of the flavin prosthetic group. The drug (-)deprenyl, is a suicide inhibitor of monoamine oxidase and is used in the treatment of Parkinson disease and depression,.

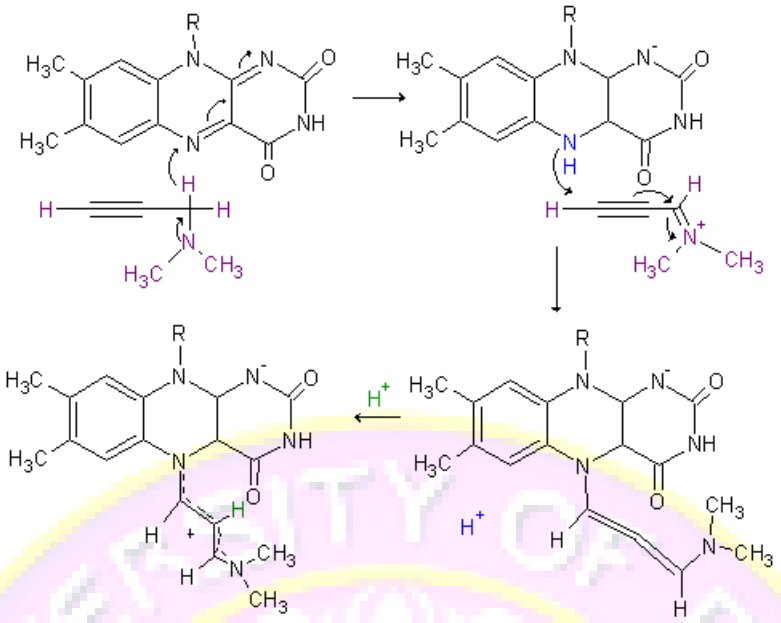


Figure: N,N dimethylpropargylamine (shown in purple) acts as a suicide inhibitor on the flavin prosthetic group of the enzyme monoamine oxidase.

Source: http://upload.wikimedia.org/wikibooks/en/2/22/Suicideinhibition.png

Regulation of enzyme activity

Precise and sensitive metabolic controls over enzyme activity are required to balance an organism's cellular needs and varying conditions. Enzyme activity is regulated in five principal ways:

1. Allosteric control:

Allosteric enzymes are those enzymes that reversibly and non-covalently bind to modulators at sites other than the active site leading to changes in the catalytic activity. These modulators lead to subtle conformational changes in the active site usually transmitted between various subunits of enzymes. These modulators of enzymatic catalysis are known as allosteric modulators or effectors.

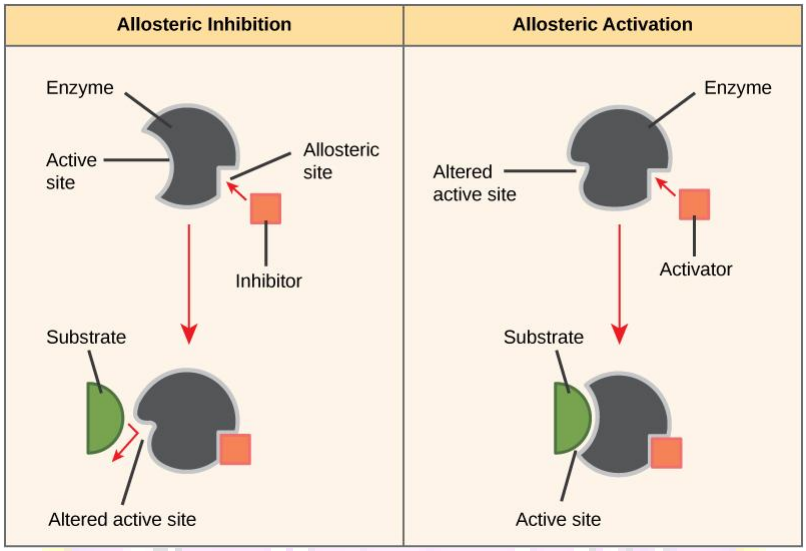


Figure: Allosteric inhibitors modify the active site so as to reduce or prevent substrate binding as compared to allosteric activators that enhance the substrate binding affinity

Source: http://cnx.org/content/m48610/latest/Figure_06_05_05.jpg

Allosteric enzymes differ quite significantly from other enzymes. Firstly, in addition to substrate binding site they have sites for binding for allosteric effectors. They may have one or more such sites which are specific for each modulator. Secondly these enzymes are larger and more complicated than other enzymes having two or more subunits. Thirdly these enzymes do not obey Michaelis Menten kinetics but undergo sigmoidal kinetics. Sigmoid kinetic behaviour indicates the property of cooperativity, where changes in the structure of one subunit are communicated into structural changes in adjacent subunits, mediated by non covalent interactions between various subunits. Also these enzymes have two states, namely R state or the relaxed state which is high substrate affinity state and the T state or the tense state which is low substrate affinity state.

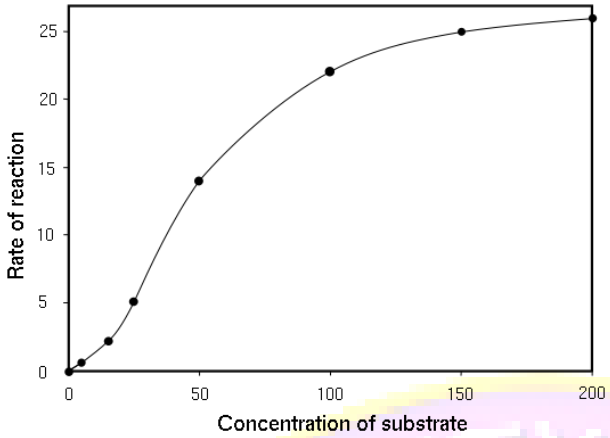


Figure: Sigmoidal kinetics

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/4/44/Allosteric_v_by_S_curve.svg/565px-

A classic example of allosteric enzyme is Aspartate transcarbamoylase (ATCase) from *E.coli* that catalyzes the first committed step in pyrimidine biosynthesis. It catalyzes the condensation of aspartate and carbamoyl phosphate to form carbamoyl aspartate.

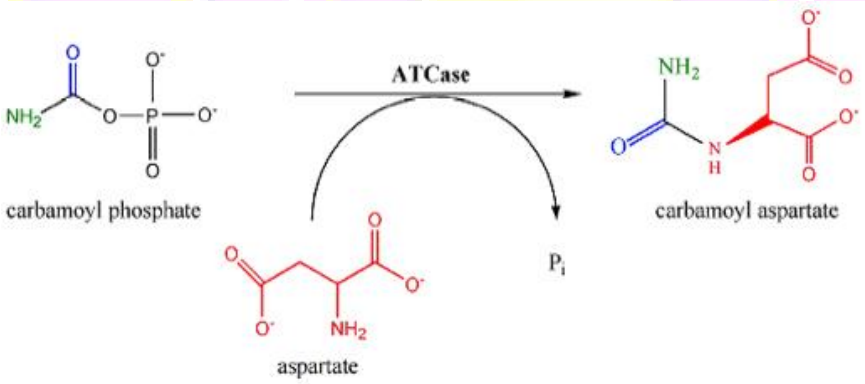


Figure: ATCase reaction

Source:http://upload.wikimedia.org/wikipedia/commons/f/f1/ATCase_reaction.jpg

It has 12 polypeptide subunits organized in six catalytic and six regulatory subunits (c_6r_6). They are further organised in the form of two catalytic trimers and three regulatory dimers. Isolated catalytic trimers have their catalytic activity, and show hyperbolic i.e., Michaelis-Menten kinetics and are unperturbed by the presence of allosteric modulators; whereas regulatory dimers bind allosteric modulators but do not exhibit catalytic ability.

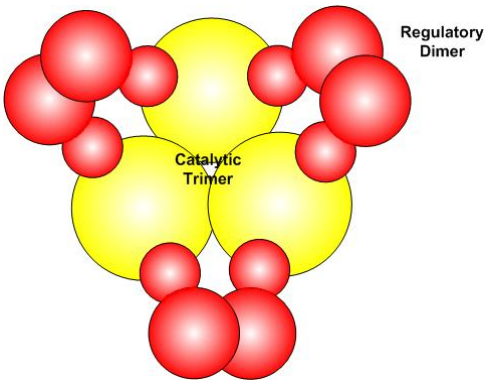


Figure: ATCase

Source:http://upload.wikimedia.org/wikibooks/en/thumb/b/b7/ATCaseTop.png/220px-ATCaseTop.png

This enzyme displays positive homotropic cooperativity on binding of its substrates. Homotropic enzymes have the same active and allosteric sites. Also **CTP,** a pyrimidine nucleotide acts as a negative modulator of enzyme activity, and **ATP,** a purine nucleotide, acts a positive modulator of enzyme activity. This is an example of **feedback inhibition**, where the end product of a pathway controls the rate of its synthesis by regulating a key enzyme in the pathway according to the cells need.

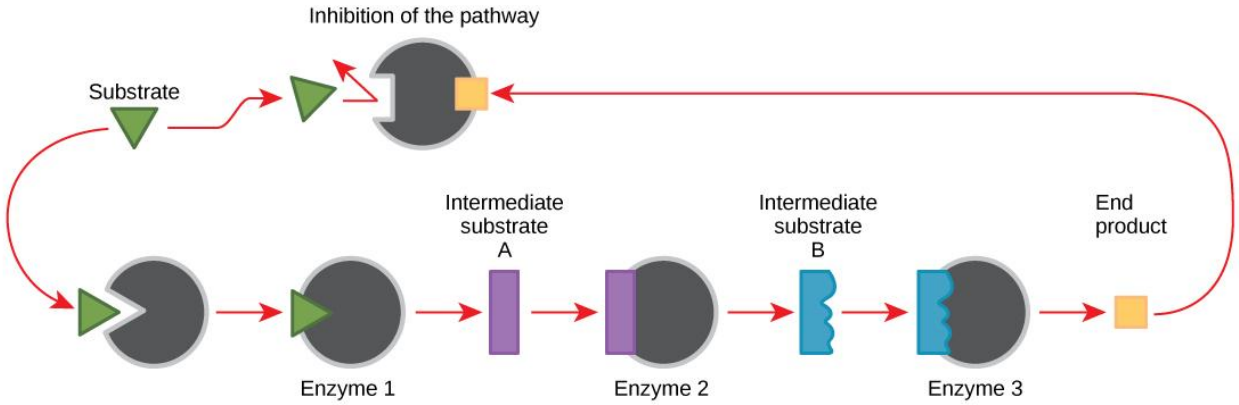


Figure: Series of reactions occur in metabolic pathways catalyzed by multiple enzymes. Feedback inhibition by the end product is an important regulatory mechanism

Source: http://cnx.org/content/m48610/latest/Figure_06_05_07.jpg

ATP activates ATCase in order to balance the pools of purines and pyrimidines for nucleotide biosynthesis. CTP binding favours the T state and ATP binding favours the R state.

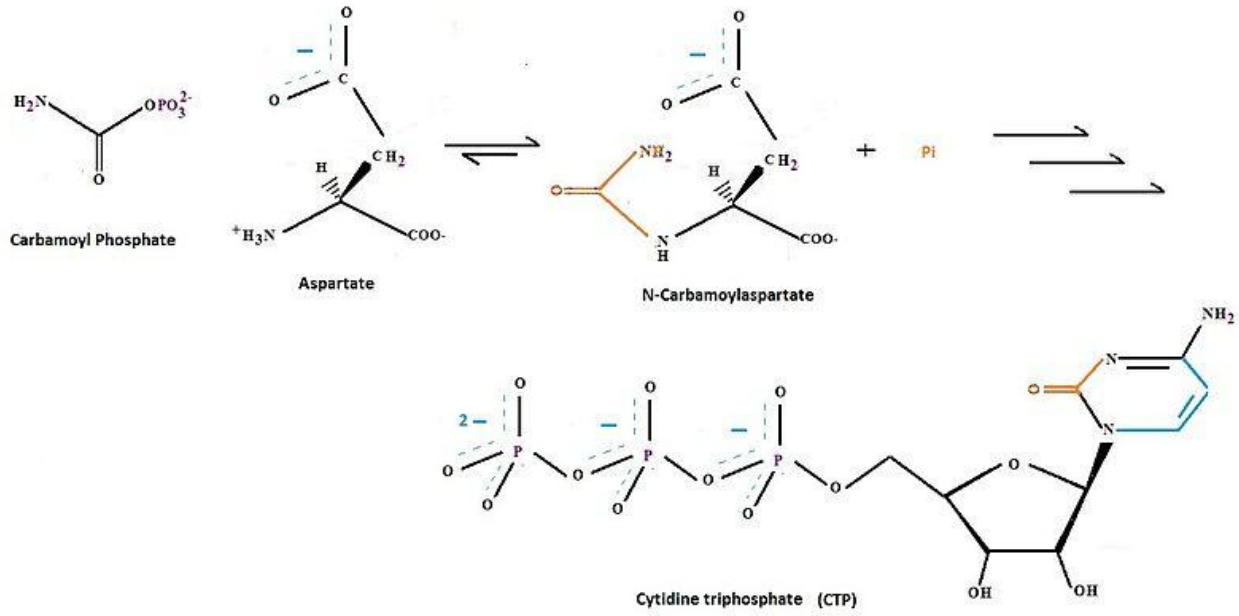


Figure: Feedback inhibition

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/6/61/ATCase_Reaction.jpg/800px-

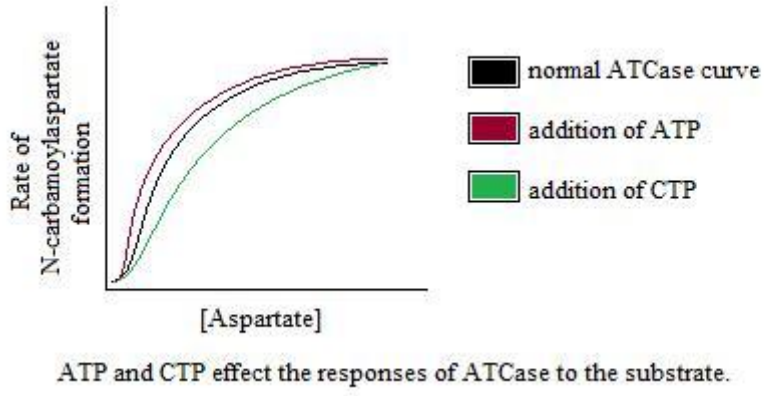


Figure: CTP acts as negative modulator and ATP acts as a positive modulator of ATCase.

Source: http://upload.wikimedia.org/wikibooks/en/8/8c/ATP_CTP_ATCase.jpg

2. **Isozymes or isoenzymes** are enzymes catalyzing the same reaction but having different amino acid sequence, kinetic parametres (Km and Vmax), regulatory properties and subcellular distribution (soluble or membrane bound). They are also encoded by different genes. They allow adjustment and regulation of metabolism according to tissue or particular developmental stage. For example, the enzyme Lactate Dehydrogenase (LDH) plays an essential role in

glucose metabolism and is a tetramer made up two types of subunits- H (overexpressed in heart) and M (overexpressed in skeletal muscles) having nearly 75% similar primary sequence. LDH exists in five different isozymes (LDH-1 to LDH-5) in humans depending on the subunit composition and have different electrophoretic mobilities, tissue distribution and properties. Levels of LDH-1 are highest in heart and LDH-4 highest in liver. Serum has the highest concentration of LDH-2. Study of isozymes is important as their levels change in several disease and might be used for clinical diagnosis. Shortly after a heart attack serum levels of LDH-1 rise due to damage to the heart tissues. After 24h LDH-1/LDH-2 ratio rises indicating a myocardial infarction.

Table: LDH isozymes

S.no	Isozyme	Subunit composition	Location	% 0f total serum LDH
1.	LDH-1	H_4	Heart and erythrocytes	15-25
2.	LDH-2	H_3M	Heart and erythrocytes	30-40
3.	LDH-3	H_2M_2	Brain and kidney	20-26
4.	LDH-4	HM_3	Skeletal and kidney	8-16
5.	LDH-5	M_4	Skeletal and kidney	6-15

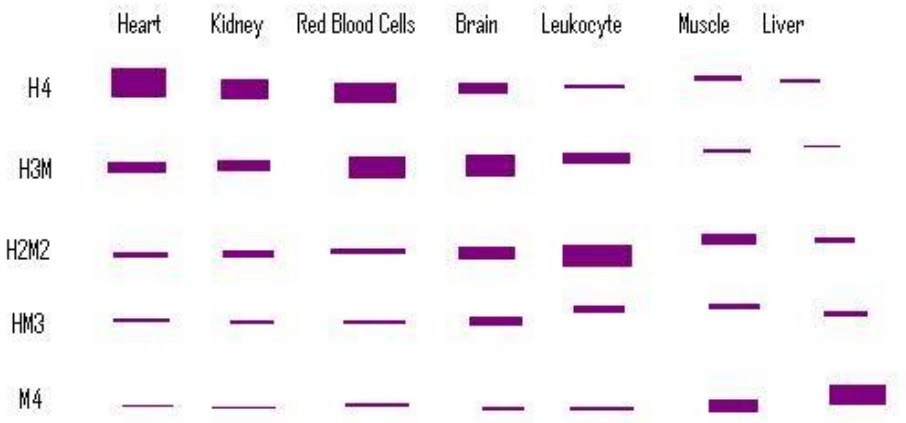


Figure: Different tissues expressing different forms of isozymes

Source: http://upload.wikimedia.org/wikibooks/en/9/9b/Figure_B.JPG

3**. Reversible Covalent modification**: Certain enzymes are regulated by reversible covalent modifications which markedly alter its catalytic properties.

These modifying groups include phosphoryl, adenylyl, uridylyl, methyl, acetyl and ADP ribosyl groups which are usually added to or removed to various enzymes by separate enzymes. An example of enzyme regulated by adenylylation is Glutamine Synthetase(GS). Adenylyl transferase catalyzes the adenylylation reaction and is regulated by two regulatory proteins P_A and P_D, which add or remove AMP group to GS. Unadenylated GS is more active than the adenylylated form.

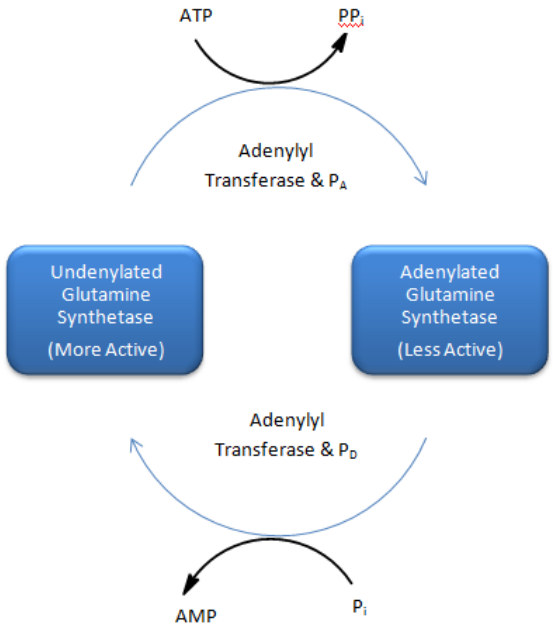


Figure: Covalent modification of glutamine synthetase
Source:http://upload.wikimedia.org/wikipedia/commons/3/38/Adenylylated_Unadenylylated_GS.PNG

Reversible phosphorylation is the most common form of regulation. Nearly about one-third of enzymes are regualted by it. Protein kinases catalyze the addition of phosphoryl group whereas phosphatase catalyzes its removal. For example, glycogen phosphorylase of liver and muscles is regulated by phosphorylation. It exists in two forms: phosphorylase a or the more active and phosphorylase b or the less active form. Phosphorylase a has two subunits each having a Ser14 residue that can be phosphorylated. Phosphorylase a is converted to b form, by enzymatic removal of phosphoryl groups by phosphorylase a phosphatase or phosphoprotein phosphatase 1, PP1. phosphorylase b kinase calalyzes the interconvertion of Phosphorylase b to more active phosphorylase a.

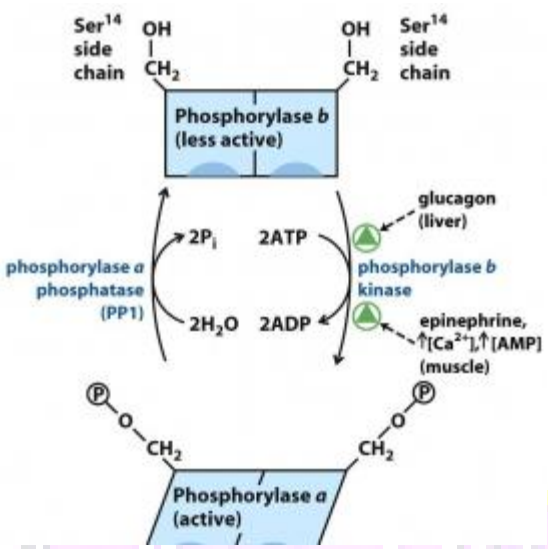


Figure: Covalent modification of glycogen phosphorylase

Source: http://www.studydroid.com/imageCards/0m/n1/card-23823604-front.jpg

4. **Proteolytic activation**: Certain enzymes cycle between inactive and active forms irreversibly. These enzymes are synthesized as inactive precursors known as **zymogens** or **proproteins,** and are proteolytically cleaved into active forms. This regulatory mechanism generates many of the digestive enzymes of stomach and pancreas. Blood clotting also occurs as a result of zymogen activation cascade. Apoptosis or programmed cell death also occurs as a result of proteolytic activation of certain proteins known as caspases which are synthesized as procaspases. Also, certain protein hormones like insulin are synthesized as the inactive precursor form as proinsulin which activated by proteolytic cleavage. Another example is of procollagen which gets activated to collagen, a fibrous protein. Active forms of these enzymes are switched off by irreversible binding to certain inhibitory proteins.

As already mentioned, certain digestive enzymes are activated by proteolysis. For example, Chymotrypsin is synthesized as an inactive precursor, chymotrypsinogen and it hydrolyzes proteins in the small interstine. Chymotrypsinogen and other zymogens are synthesized in the pancreatic acinar cells. And stored in membrane bound granules. When the cells are stimulated by hormal or a nervous signal, the granules contents are released into a duct which leads to duodeneum. Chymotrypsinogen is a single polypeptide of 245 amino acid residues and is inactive. It is converted to active form π-chymotrypsin, by action of trypsin which catalyzes the hydrolyses of peptide bond between Arg15 and

Iso16. The resulting π-chymotrypsin autocatalyzes removal of two dipeptides yielding α-chymotrypsin. Three chains in α-chymotrypsin are joined by disulphide linkages. Proteolytic cleavage of the inactive form leads to the formation of a active substrate binding site in chymotrypsin.

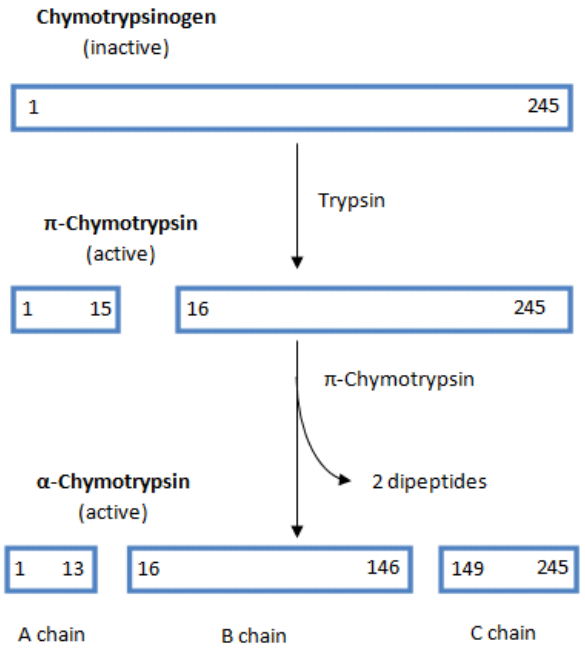


Figure: Activation of chymotrypsinogen

Source:http://upload.wikimedia.org/wikibooks/en/d/da/ChymotrypsinActivation.png

The generation of trypsin from trypsinogen results in activation of other zymogens and thus concurrent digestion of all types proteins in the small interstine. Enteropeptidase secreted by cells of duodenum hydrolyze a unique Lys-Ile bond in the trypsinogen and activates it. This is considered as the master activation step. Trypsin thus formed activates all pancreatic zymogens- trypsinogen, chymotrypsinogen, prolipase, proelastase and procarboxypeptidase. As this is irreversible activation, specific protease inhibitors are present eg, pancreatic trypsin inhibitor which binds to the active site of trypsin. This inhibition prevents inappropriate activation of the cascade and thus severe damage to tissues, in whose absence severe pancreatitis can occur.

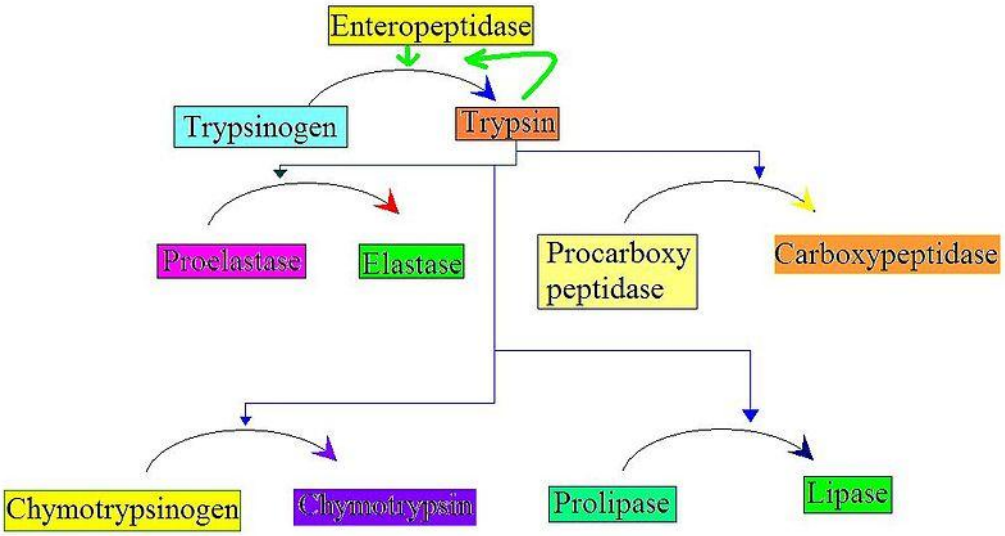


Figure: Zymogen activation by proteolytic cleavage

Source:http://upload.wikimedia.org/wikibooks/en/thumb/f/f7/Tinozymogen.jpg/800px-Tinozymogen.jpg

5. **Genetic control:** By controlling the amount of enzyme present according to cell's needs is known as enzyme induction and it usually occurs at the level of transcription. For example in presence of glucose no other sugar is utilized by E.coli as it is the preferred source of energy. But in absence of glucose, the bacteria looks for other sources of energy. If lactose is present in the medium, lac operon is switched on and lactose is metabolized by the enzyme β-galactosidase. The details of lac operon are beyond the scope of the chapter.

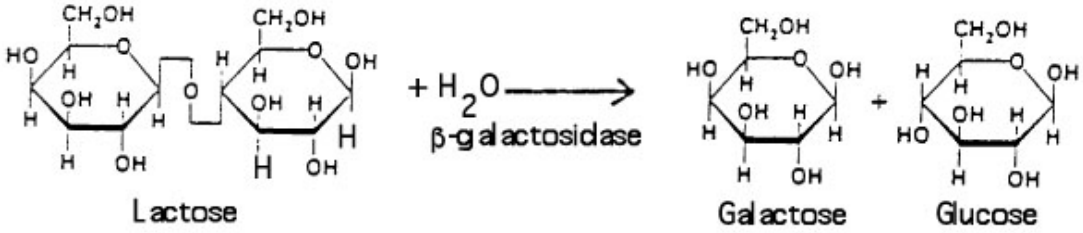


Figure: Catalysis by β-galactosidase

Source: https://encrypted-bn1.gstatic.com/images?q=tbn:ANd9GcRxY6B32TeGZ4t71HLNqZx-

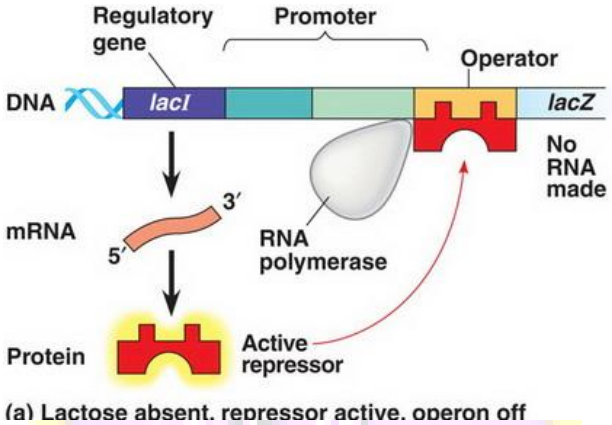


Figure: Lac operon is repressed when glucose is available and lactose is absent

Source:http://m.blog.hu/bi/biokemia/image/pic%20for%20posts/c8_18x4a_lac_operon_resize.jpg

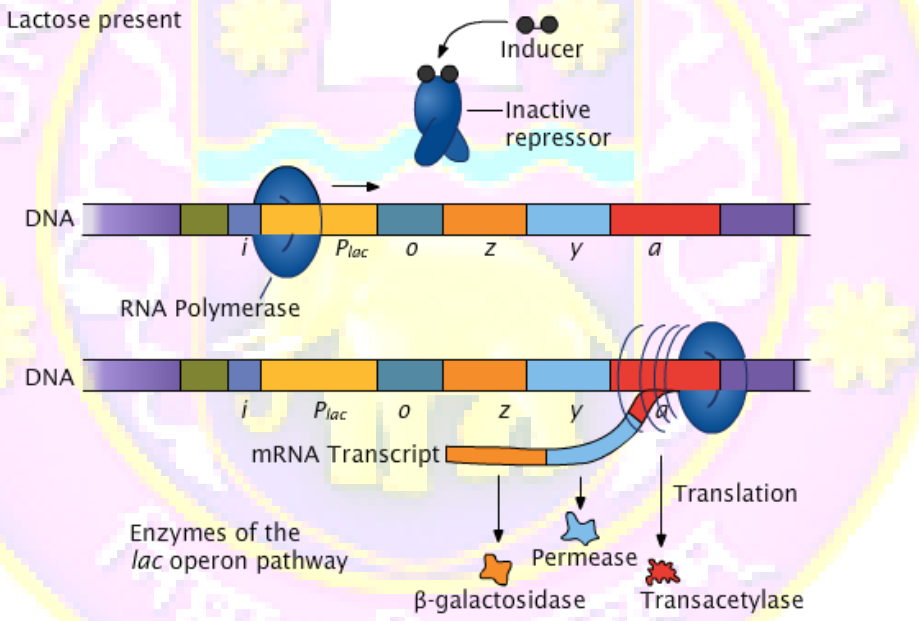


Figure: Lac operon is switched on as lactose is available and glucose is present

Source: Author

Factors affecting enzyme activity

Various factors affect the rate of enzyme catalyzed reactions like temperature, pH and substrate concentration.

Temperature

All enzymes work within a specific range of temperature specific to a particular organism. Increasing temperature increases the rate of enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reactants. However, further rise in temperature increases the kinetic energy of the enzyme and disrupts the enzyme's three-dimensional structure by breaking the noncovalent interactions that maintain it. Thus the enzyme begins to denature and loss its catalytic activity. The temperature range over which an enzyme maintains its native, catalytically active conformation depends on and only moderately exceeds the normal temperature of the organism where it is found. The "optimum" temperature for human enzymes is generally between 35 and 40 °C above which they begin to denature. By contrast, enzymes from the thermophilic bacteria may be stable up to or above 100 °C. The **Q_{10} value or the temperature coefficient** is a measure of the rate of change of a biological process as a result of increasing the temperature by 10 °C. The rate of enzymatic reactions typically doubles for every 10 °C rise in temperature (Q_{10} = 2) at temperatures at which enzymes are stable. In short, as temperature increases, initially the rate of enzyme catalyzed reaction will rise, because of increase in Kinetic Energy. However, due to loss of non covalent weak interactions the enzyme will begin to denature and the rate of enzyme catalyzed reaction will begin to decline.

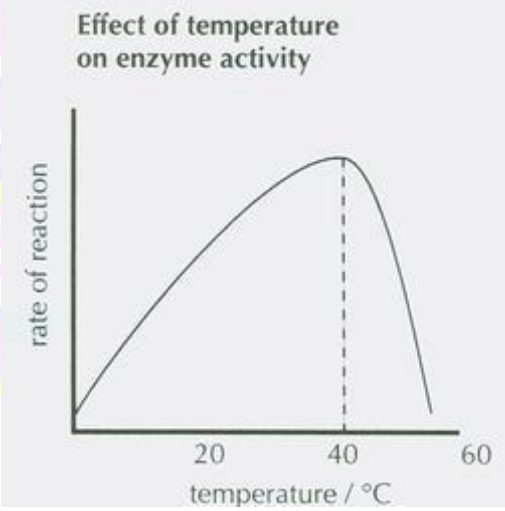


Figure: Effect of temperature on enzyme activity

Source:http://ibhumanbiochemistry.wikispaces.com/file/view/Effect_of_Temperature,pH%

Hydrogen ion concentration or pH

Generally all enzymes have an optimum pH at which they exhibit maximum activity; at higher or lower pH, activity declines. Most of the intracellular enzymes show maximum activity within a narrow pH range of 5-9. pH exerts its effect by a combination of factors. At the active site of enzymes amino acyl side chains act as weak acids and bases with important mechanistic roles which depends on their state of ionization, especially those involving acid-base catalysis. The amino acid residues at the active site must be appropriately protonated or unprotonated. pH also effects the ionization of substrates and the binding of substrate to the enzyme. Also ionization state of various amino acids in the enzyme helps to maintain is three dimensional native structures (at extremes of pH). The graph of pH against the reaction rate is a bell shaped curve and reflects the balance between enzyme denaturation at high or low pH and ionization state of the substrates, enzyme, or both of them.

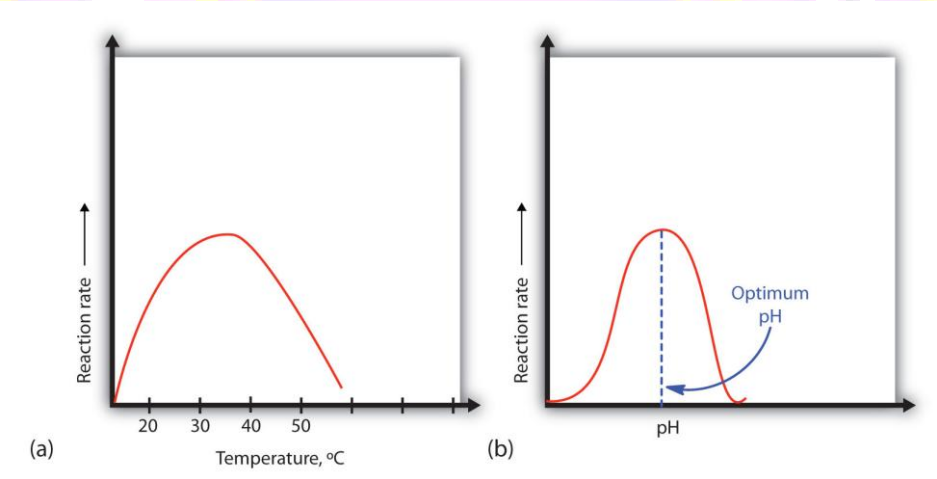


Figure: a) This graph depicts the effect of temperature on the rate of a reaction that is catalyzed by a fixed amount of enzyme. (b) This graph depicts the effect of pH on the rate of a reaction that is catalyzed by a fixed amount of enzyme.
Source:http://2012books.lardbucket.org/books/introduction-to-chemistry-general-organic-and-biological/section_21/9de13b61d3f051cfd2e49c1abea2ab46.jpg

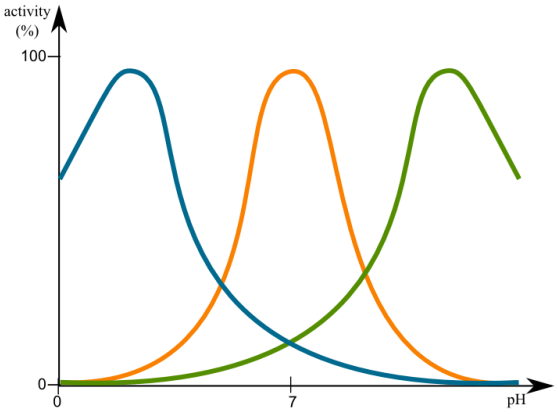


Figure: graph of enzyme activity in against pH. green- high pH enzyme; blue- low pH enzyme; orange- neutral pH enzyme

Source: http://upload.wikimedia.org/wikipedia/commons/a/ac/Enzyme-ph.png

Ribozymes or catalytic RNAs are RNA molecules that act as catalyst for a biochemical reaction. Thomas Cech and Sidney Altman recieved the Nobel Prize for their discovery of catalytic properties of RNA. Thomas Cech, worked on the self splicing introns of ciliated protozoan *Tetrahymena thermophila* which spliced RNA accurately without any protein enzymes (removed itself from the transcript). S. Altman worked on the bacterial RNase P complex involved in the maturation of pre-tRNAs. Other examples of ribozymes are hammerhead ribozyme, the VS ribozyme and the hairpin ribozyme. Ribozymes clearly demonstrate that RNA molecules can act both as a genetic material and as biocatalysts. They clearly point towards the significance of RNA in the evolution of life.

Source: http://en.wikipedia.org/wiki/Ribozyme - cite_note-7#cite_note-7

Summary

Enzymes are generally a protein molecule (exception ribozymes) which acts as biocatalysts to bring about a specific biochemical reaction. Enzymes are classified as EC classification number which is indicates of the type of reaction catalyzed by the enzyme. Certain enzymes require small nonprotein molecules and metal ions for substrate binding and catalytic activity, designated as cofactors. They lower the activation energy of a reaction for catalysis but do not affect the reaction equilibrium. Transition state theory assumes that the rate of a reaction depends

on the free energy of formation of its activated complex, which occurs at the free energy maximum of the reaction coordinate, is poised between reactants and products and is therefore also termed the transition state. Enzyme kinetics is the study of the enzyme catalysed chemical reactions, in which, the reaction rate is measured and the effects of varying the conditions of the reaction is studied. The enzyme and substrate reversibly combine to form an enzyme–substrate complex which decays into corresponding products. Enzymes are subject to inhibition by various molecules called inhibitors, which bind to them and decreases or halt their enzymatic activity. These act as both pharmaceutical agents and as research tools for study of the mechanism various enzymes. These inhibitors are majorly classified into two broad categories: reversible and irreversible. Also enzymes can be regulated according to cellular needs.

Exercises

Q1: State True (T) or False (F) and justify your answer:

i) EC classifies the enzymes into six major classes based on the reaction catalyzed

ii) All enzymes are proteins

iii) Coenzymes are not required for catalysis

iv) The state of ionization of amino acid residues in the active site of an enzyme is pH dependent

v) Increasing temperature increases the rate of enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reactants upto a certain point

vi) Enzymes alter the reaction equilibrium

vii) Enzymes exhibit stereo specificity

viii) Lock and key model assumes enzyme undergoes a conformational change on substrate binding

ix) Km is the substrate concentration at which the velocity is maximum

x) A Competitive inhibitor cannot competes with the substrate for the active site of an enzyme

Ans i) T ii) F iii) F iv) T v) T vi) F vii) T viii) F ix) F x) F

Q.2: Fill in the blanks:

i) A catalytically active enzyme–cofactor complex is called a_____.

ii) A Michaelis-Menten curve resembles a _____.

iii) Enzymes lower the _____ of a reaction to enhance rate of a reaction

iv) Lock and key hypothesis was proposed by _____

v) The graph of pH against the reaction rate is generally a _____ curve

Ans i) Holoenzyme ii) Hyperbola iii) Activation energy iv) Emil Fisher v) bell shaped

Q.3: Identify the following:

i) An enzyme lacking its cofactor

ii) Number of substrate molecules that can be converted to product in a given unit of time on a single enzyme when the enzyme is saturated with the substrate

iii) A metal ion or an organic compound that is covalently attached to a protein

iv) Molecules that bind to enzymes and decrease or abolish their activity

v) Inhibitors that hijack the normal catalytic mechanism to inactivate the enzymes

Ans i) Apoenzyme ii) turnover number iii) Prosthetic group iv) Inhibitors v) suicide inhibitors

Q.4: Write short notes on:

i) Competitive inhibition

ii) Suicide inhibition

iii) Mechanism of catalysis

iv) Regulation of enzymes

Q.5: What is the ratio of [S] to Km when velocity of enzyme catalysed reaction is 80% of Vmax?

Ans: 4:1

Q.6: The reaction is half maximal when the enzyme is one half with substrate. Show that this is true algebraically.

Glossary

Enzyme: Generally a protein molecule (exception ribozymes) which acts as a catalyst to bring about a specific biochemical reaction.

Active site: The part of an enzyme at which catalysis of the substrate occurs.

Transition State: The state corresponding to the highest energy state along a reaction coordinate.

Cofactors: Certain enzymes require small nonprotein molecules and metal ions for substrate binding and catalytic activity

Prosthetic group: A cofactor that is very tightly or even covalently associated with the enzyme protein

Coenzymes: A nonproteinaceous organic substance usually a vitamin or mineral that combines with a specific protein, the apoenzyme, to form an active enzyme system.

Holoenzyme: A catalytically active enzyme along with its cofactor

Inhibitors: Molecules that bind to enzymes and decreases or halt their enzymatic activity.

Feedback inhibition: A cellular control mechanism in which an enzyme that catalyzes the transformation of a particular substrate is inhibited when the product has accumulated to a certain level, thereby balancing the amount provided with the amount required.

Temperature coefficient: A measure of the rate of change of a biological process as a result of increasing the temperature by 10 °C.

Isozymes: Enzymes that catalyze the same reaction but differ in their amino acid composition.

Allosteric enzymes: Enzymes that bind to modulators at sites other than the active site leading to changes in the catalytic activity.

Induced fit: Dynamic fit between the enzyme and its substrate, leading to subtle conformational changes in their structures to allow ideal binding

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Web links

http://nptel.ac.in/courses/103105054/9

http://www.elmhurst.edu/~chm/vchembook/570enzymes.html

http://cnx.org/content/m44429/latest/?collection=col11448/latest