PROTEINS

LECTURE 6: STRUCTURE AND CATALYSIS OF ENZYMES

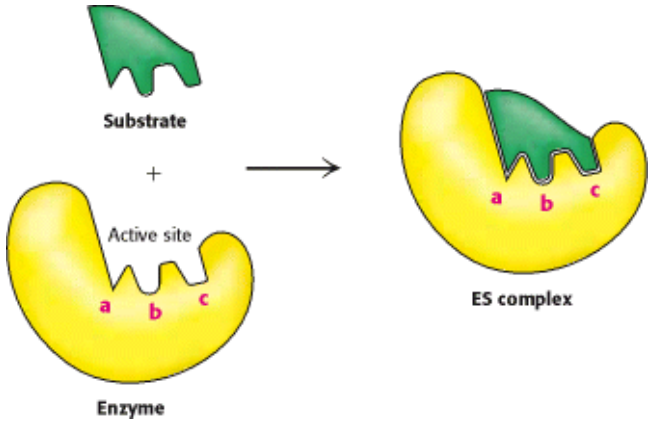
Topic Learning Outcomes: At the end of this topic, you are expected to be able to:

1. Explain what enzymes are, its nomenclature system, structure, properties and general reactions.
2. Discuss how catalysts in general, facilitate chemical reactions
3. Illustrate how enzymes facilitate catalysis in biological systems
4. Calculate Km and Vmax from enzyme kinetic diagrams

THE STRUCTURE AND PROPERTIES OF ENZYMES

Enzymes are biomolecules naturally present in the cell, usually in small amounts, and which speed up the conversion of a substance, called its substrate to another substance(s) called the product. Most enzymes are proteins but there are a few RNA enzymes, called ribozymes. Some protein enzymes also contain metals as part of their structure or are involved in catalysis; these enzymes are called metalloenzymes. For these lectures, we shall only be discussing protein enzymes.

Enzymes are large polypeptides, usually globular with a small hydrophobic pocket. The hydrophobic pocket is usually a cavity within the enzyme molecule where substrates bind, and where reactions take place. This cavity is the enzyme's active or catalytic site. The larger portion of the enzyme forms the scaffold that maintains the structure of the active site. When enzymes bind the substrate, an intermediate called the enzyme-substrate complex (ES) is formed. (Fig. 7.1.)



Source: Stryer, Biochemistry (5th edition)
Fig. 6.1. The ES complex

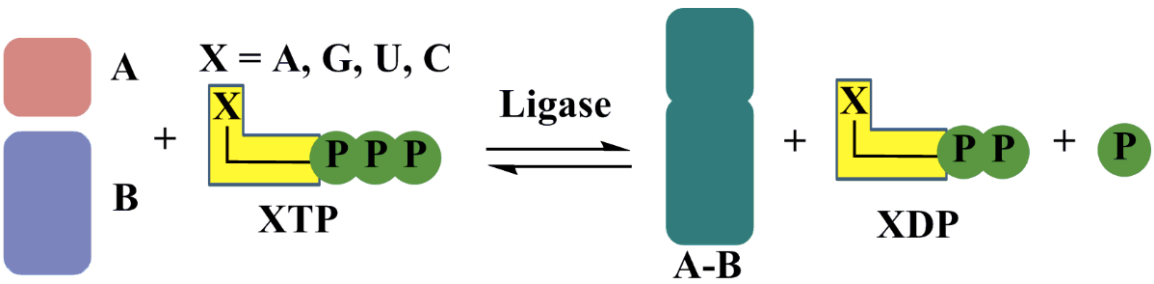
Nomenclature of enzymes

Enzymes are names by simply adding "-ase" as ending or suffix of the substrate they act on. For example, collagenase is an enzyme that acts on collagen; glycosidases are enzymes that act on glycosidic bonds, etc. Another way of naming these is based on the reactions they catalyze, for example, dehydrogenases are

enzymes that catalyzes transfer of H- or oxidation reactions; deaminases are enzymes that transfer amino groups, etc. There are also common names such as trypsin, elastase, etc. which had already been in use before the International Union of Biochemistry and Molecular Biology (IUBMB) came up with a way to systematize enzyme nomenclature. IUBMB created the Enzyme Commission (EC), which grouped together the enzymes into six (6) main groups and used a code number starting with E.C. (for Enzyme Commission).

The main group classification of enzymes is based on similarities in the reactions they catalyzed. These groups are: 1) oxidoreductases; 2) transferases; 3) hydrolases; 4) lyases; 5) isomerases and 6) ligases. The general reactions catalyzed by these groups will be briefly described here.

1. Oxidoreductases. These are enzymes that catalyze redox reactions or oxidation-reduction reactions. Dehydrogenases and reductases are enzymes under this classification.
2. Transferases. These enzymes catalyze the transfer of groups from a donor to a receiver.
3. Hydrolases are enzymes that catalyze the cleavage of bonds (C-O, C-C, C-N, O-P, etc.) with the participation of water. Examples of this group are those that cleave the peptide bond or proteases, those that cleave ATP or ATPase, etc.
4. Lyases are enzymes that cleave bonds (C-O, C-C, C-N, etc.) without water as participant in the reaction and by elimination. The products usually contain double bonds or form cyclic structures. Examples are decarboxylases, dehydratase (an enzyme that removes water), synthase (synthetase is used only if ATP is a participant in the reaction, in which case the enzyme is not a lyase, it falls under the 6th group - ligases), etc. The term synthetase however is no longer used to avoid confusion although there are still authors that use this term.
5. Isomerases catalyze isomerization reactions of compounds such as change in geometry, structure, or position of bonds.
6. Ligases are enzymes that catalyze the joining together of two molecules coupled with the hydrolysis of compounds with high-energy transfer potential such as the hydrolysis of ATP. An example of this enzyme is DNA ligase. The enzyme catalyzes the reaction that joins together short DNA fragments during synthesis and repair of DNA. The reaction is shown in Fig. 7.2



Source: https://www.creative-enzymes.com/resource/ligase-introduction_24.html

Fig. 6.2. The reaction catalyzed by DNA ligase

In general, the system for classifying enzymes and the EC code assigned to them follows this scheme: https://www.qmul.ac.uk/sbcs/iubmb/enzyme/rules.html

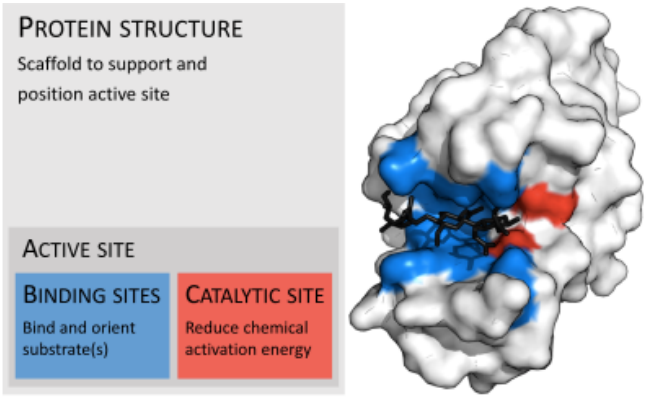
1. The 1st number is the group or the main class number the enzyme belongs to.
2. The 2nd number is the subclass of the enzyme group
3. The 3rd number is the sub-subclass
4. The 4th number is the serial number of the enzyme in the sub-subclass

With many enzymes in nature, there are always exceptions to the general rules. Many common names are still being used today such as trypsin, pepsin, etc. as these are already known to all.

Properties of Enzymes

1. Efficiency

Enzymes are remarkable molecules in that they are efficient and work with great speeds. That is why they are nature's catalyst, converting substrate to product efficiently and at astonishing rates. Biological reactions are catalyzed at an asymmetric "pocket' called an active site. The active site is a very small fraction of the enzyme's size, and consists of a binding site that contains contact residues responsible for immobilizing the substrate, and a catalytic site that contains residues directly responsible for catalysis. The active site is shown in Fig. 7.3.

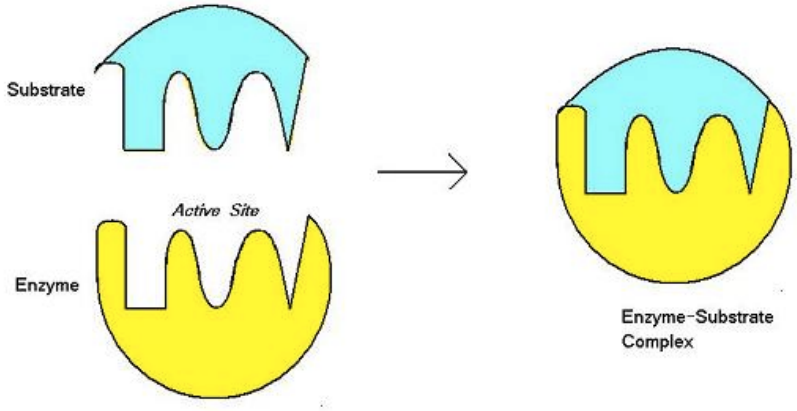


Source: https://en.wikipedia.org/wiki/Active_site

Fig. 6.3. Contact and catalytic residues at an enzyme's active site.

2. Specificity

Enzymes are also very specific. Enzyme proteins are made up of amino acid chains that fold to create a hydrophobic pocket that shows complementarity with its substrate, as a key fits to the lock. This specificity is called the "lock and key" model (Fig. 6.4)



Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

Source: https://biology.stackexchange.com/questions/2489/what-is-the-correct-model-for-enzyme-substrate-complementarity

Fig. 6.4.Lock and key model for enzyme-substrate binding.

On the other hand, the arrangement of molecules in the enzyme can also produce an area called the active site within which the specific substrate(s) will "fit" only after it binds. It is in this site that the substrate is recognized, its bonds reoriented and it is immobilized. This model of specificity is called the “induced-fit” model. A classic example of this is the enzyme glucokinase and hexokinase. Both catalyze the conversion of glucose to glucose-phosphate. The enzyme has an active site that folds onto glucose to enclose it and eliminate water from the reaction. Fig. 7.5 shows the induced-fit model for binding of glucose to the enzyme.

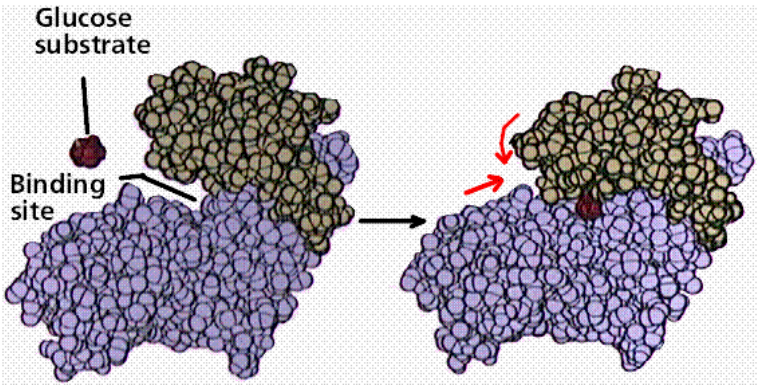


Fig. 6.5. Space filling model of the enzyme (glucokinase or hexokinase) binding its substrate, glucose.

Note the shape change in the enzyme (indicated by the red arrows) after glucose has fit into the binding or active site. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com),

3. Sensitivity to environmental changes

Enzymes, being a biomolecule, are affected by alterations in environmental pH and temperature. These are constraints that biological systems have, i.e. it must maintain a physiological pH of 6.7 to 7.4 and a temperature range of 36-37^{o}C. The

pH range are maintained by buffers; proteins are made up of amino acids that can change its form depending on the pH. A change in the intermolecular forces of attraction between amino acid residues in the enzymes, including the active site can cause denaturation or alter enzyme conformation and function. On the other hand, enzymes may still operate at 39-40^{o}C but prolonged exposure to such temperature can affect cellular efficiency and integrity. This range of temperature is critical in maintaining a kinetic barrier for reactions in a biological system, so that these chemical processes do not occur spontaneously. Thus, the maintenance of pH and T, as well as pressure is essential for conditions compatible with life.

4. Enzymes are true catalysts

Enzymes are true catalysts, in that while they increase reaction rates, they do so without themselves being altered in the process. Enzymes also do not alter the equilibrium constants of reactions they catalyze. While the velocity of the reaction is increased, the equilibrium state is not altered. That is, for a reaction at equilibrium:

$$A \leftrightarrow B$$

Any increase in concentration of B is compensated by an increase in the production of A. An enzyme accelerates the velocities of both forward end backward reaction, but the ratio [B]/[A] is constant, hence Keq is maintained. The equilibrium concentrations will only change, if the reactant or the product is continuously removed from the system.

Many biological pathways are made up of linked series of dependent reactions. Each reaction is catalyzed by enzymes whose product is the substrate of the next enzyme. The products formed in the series, except the end product, are called intermediates. The quality and quantity of the end product however, is dependent on the efficiency of every enzyme in the pathway. To ensure this, enzymes are localized near each other so that intermediates do not accumulate and are immediately removed or acted upon by the next enzyme.

Like other catalysts, these biological catalysts also lower the energy of activation of the reaction it catalyzes. But unlike chemical catalysts, the rate of reactions catalyzed by enzymes is several hundredfold faster than ordinary chemical reactions. Enzymes are also not consumed and are regenerated after the reaction.

5. Allosteric regulation

Most protein enzymes are made up of several polypeptide chains, i.e. they are multimeric. As described before, the quaternary structures of enzymes allows regulation by altering one or a few subunits or by binding of these subunits to ligand causing either the reaction to further speed up of slow down. Thus enzyme activity is subject to regulation in response to concentrations of substrates and other molecules that bind to allosteric sites of the enzyme.

THE MECHANISM OF ENZYME ACTION

In experiments done in the laboratory on chemical reactions, one of the ways by which chemists increase the number of reactants attaining the energy of activation is to increase the kinetic energy of the reacting molecules by increasing temperature. However, given the constraints of pH, temperature and pressure, in biological systems, enzymes must device strategies to ensure that reactions proceed. These strategies, listed below, are key to the efficiency by which enzymes transform substrates to product.

1. Entropic effects
2. Immobilization
3. Strain effects
4. Catalysis
    a. Acid-base
    b. Nucleophilic catalysis
    c. Metal catalysis

Entropic effects

Majority of enzyme reactions occurs in aqueous medium. The first step is the formation of the enzyme-substrate complex, called ES. Both enzyme and substrate are dissolved in water. The substrate, surrounded by water must bind to the enzyme's active site, which is a hydrophobic pocket within the polypeptide. Binding necessitate several steps: 1) exclusion of water which involves disruption of H-bonding interactions called dissolvation; 2) transfer of the substrate to the hydrophobic environment; 3) rearrangement of molecules in the substrate and the active site to effect interactions between substrate and enzyme, whether it is H-bonding, electrostatic or hydrophobic interactions; 4) replacement of the H-bonding interactions with water, with interactions with the enzyme. Dissolvation is possible only if the interactions of the substrate with water can be replaced by a similar to stronger interactions with contact residues of the active site. This will require rearrangement of or reorientation of molecules to maximize bonding with contact residues and bring the substrate closer to the catalytic residues (Fig. 7.6). The disruption of H-bonds with water and the replacement with new bonds with contact residues at the active site involves a change in entropy of water and the reacting species. A correct orientation will mean stronger binding and therefore less entropy for the ES complex as disorder is minimized; however the ensuing order is at the expense of disorder in the surrounding water molecules.

Orientation results in movements of the molecules in the substrate or the enzymes' active site in some cases, causing the translocation of these molecules to a position that will allow for maximum interactions. As long as enzyme and substrate cannot interact or "effectively collide", the ES complex can still break up to yield the enzyme and the unchanged substrate.

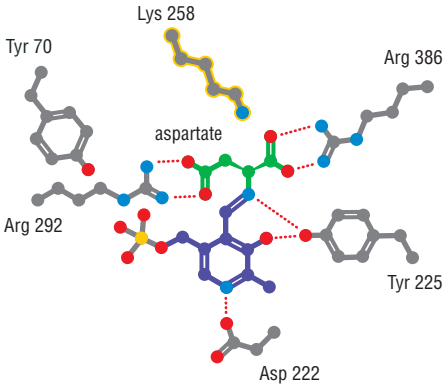


Fig. 6.6. Active site of E. coli aspartate aminotransferase showing the binding of the substrate with binding and catalytic residues at the enyzme's active site.

Immobilization

An effective orientation that allows maximum interaction between substrate and active site will result in immobilization of substrate. Immobilization coupled by proximal localization with the catalytic subunit will then allow catalysis to proceed. Immobilization may be likened to the strategy of entrapment of prey by spiders.

Fig. 6.7. Spider attacking its prey after immobilizing them.

Source: https://www.foxnews.com/science/double-sided-tape-inspired-by-spiders-webs-could-replace-surgical-sutures

Entropic effects and immobilization ensure that the reactants will effectively be acted upon by molecules at the active site of the enzyme involved in catalysis, forming product. It is ensuring the occurrence of "effective collision" between the reacting species and transform one to the product.

Strain Effects

When breaking of bonds are involved, it is oftentimes necessary to put a strain in these bonds to facilitate cleavage. Strain can be induced on a molecule by increasing electrophilicity of reactive site or substrate, or increasing nucleophilicity also of substrate at reactive site. It can also be induced by twisting of bonds or compression of bond angles particularly in a ring, such as when an sp2 hybridized carbon is changed to sp3 or when a chair conformation is changed to a twist boat conformation.

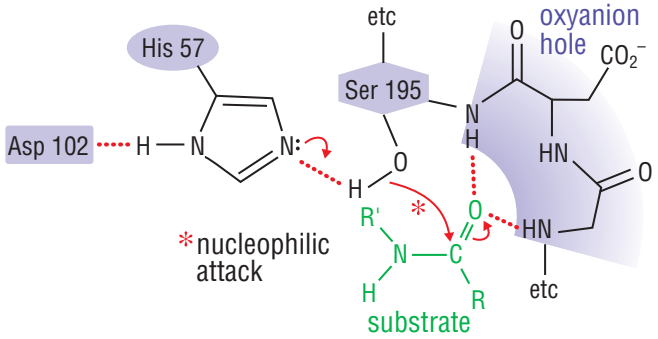


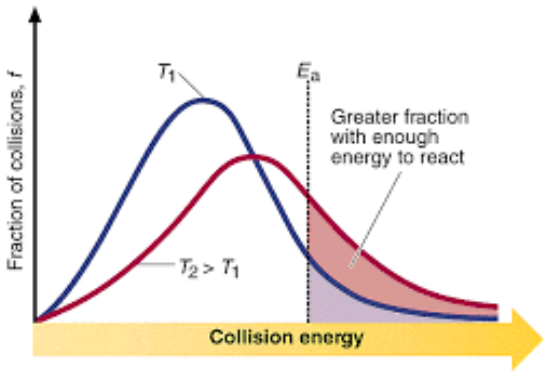
Fig. 6.8. An example of how strain can be produced by H-bonding interactions to draw electron density from the carbonyl carbon towards oxygen. This increases the d-positivity of the carbonyl C or its electrophilicity such that a weak nucleophile such as the O- from serine can easily break the bond. This is the mechanism of the catalytic triad of serine proteases.

Catalysis

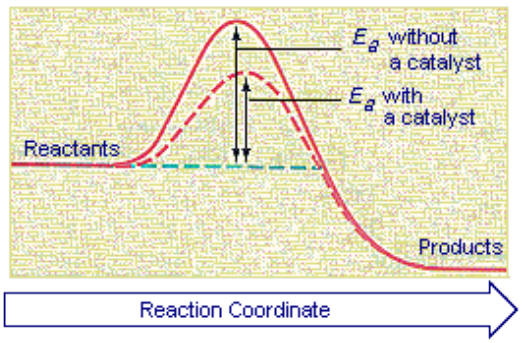
For reactions to occur, the free energy of the reactants must be raised to energy equivalent to the transition state energy, or the reactants must become less stable. Many organic reactions cannot proceed under ordinary conditions. Thus, catalysts are employed to decrease the transition state energy of organic reactions, or to achieve the experimental activation energy faster. Catalysts are substances that facilitate chemical reactions without themselves being modified nor consumed.

Lowering the experimental activation energy can be achieved in three (3) ways:

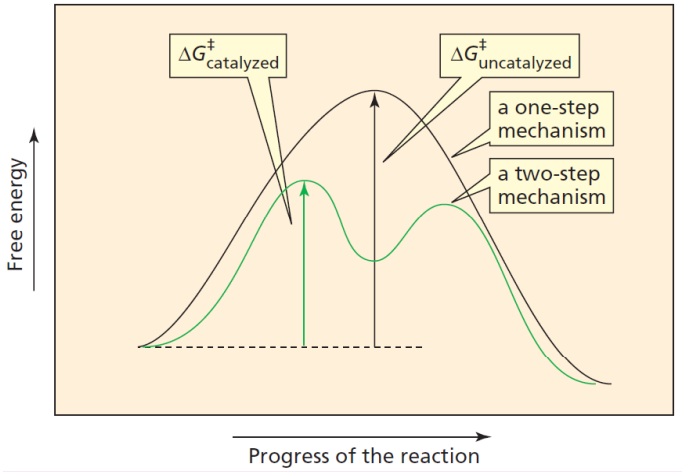
1. The reaction undergoes a similar mechanism as uncatalyzed reaction but with the catalyst converting the reactants to species that are less stable.
2. The reaction undergoes a similar mechanism as uncatalyzed reaction with the catalyst providing ways to make the transition state more stable.
3. The reaction proceeds with a completely changed mechanism.



1. Less stable species



2. More stable transition state



3. Changed mechanism

Sources: https://260h.pbworks.com/w/page/70917374/Rate%20Laws
http://chemistry.tutorvista.com/physical-chemistry/reaction-rates.html

Fig. 6.9. Ways by which catalysts facilitate reactions

Catalysts provide a favorable pathway for the reaction by the following mechanism:

1. Catalysts increase susceptibility of electrophile to nucleophilic attack
2. Catalysts increase reactivity of nucleophile
3. Catalysts increase the leaving ability of groups by converting these to weaker bases

Types of catalysis:

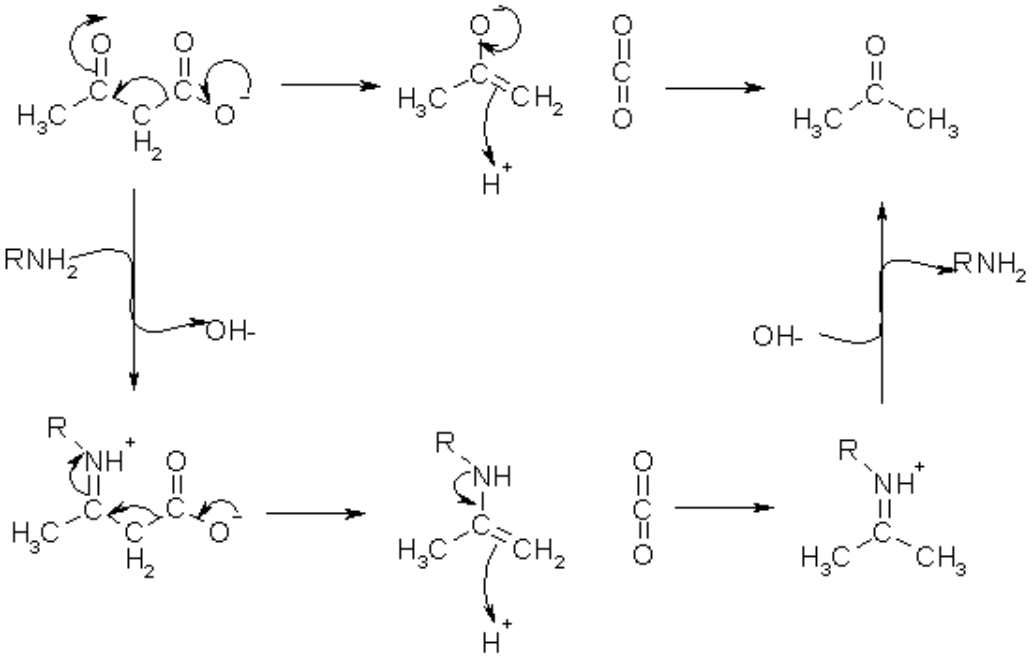
1. Nucleophilic catalysis is facilitated by substances or nucleophiles that form covalent bonds with reactants. These substances are also called covalent catalysts. These catalysts act as nucleophiles and forms intermediates.

Nucleophilic or Covalent catalysis

Alcoholic and basic amino acids are also involved in covalent catalysis. These amino acids are shown in the table below:

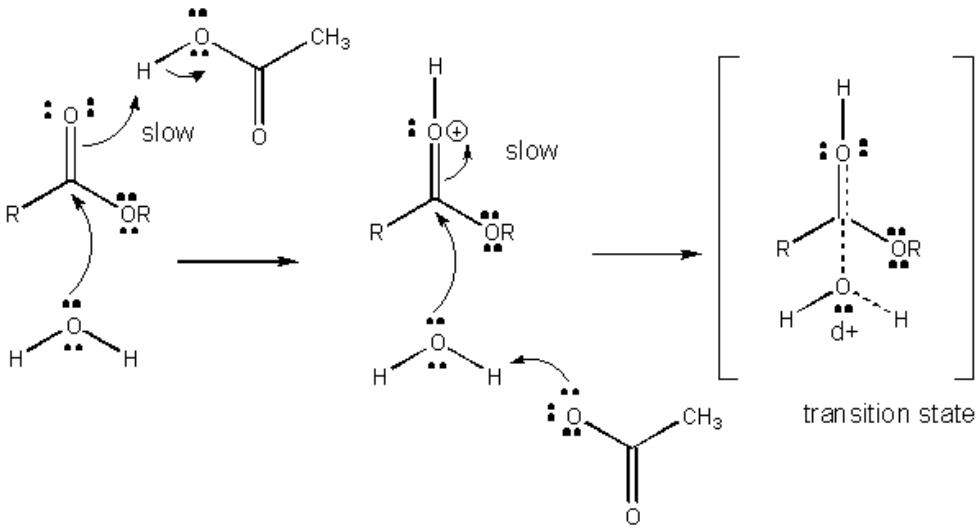
Amino acid	**Group**
Threonine	-OH
Serine	-OH
Tyrosine	-OH
Cysteine	-SH
Histidine	-N=
Lysine	$-NH_2$
Arginine	$-CH(NH_2)_2$

Covalent catalysis in the decarboxylation of acetoacetate



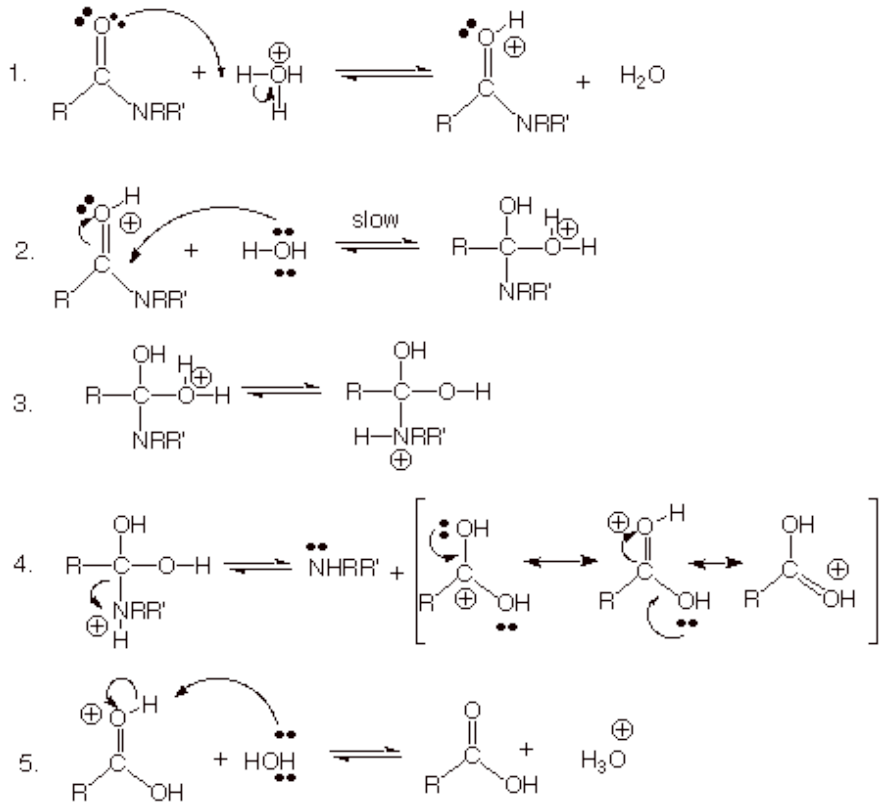
2. Acid catalysis – increases rate of slow step by:
    a. Specific catalysis – proton is completely transferred before the slow step
    b. General catalysis – partial transfer of protons simultaneous with nucleophilic attack (slow step)

General catalysis in the hydrolysis of an ester



Source:
http://biowiki.ucdavis.edu/Core/Biochemistry/Catalysis/METHODS_OF_CATALYSIS/General_Acid%2F%2FBase_Catalysis

Specific catalysis in the hydrolysis of an amide



Source: http://research.cm.utexas.edu/nbauld/teach/ch610bnotes/ch18.htm

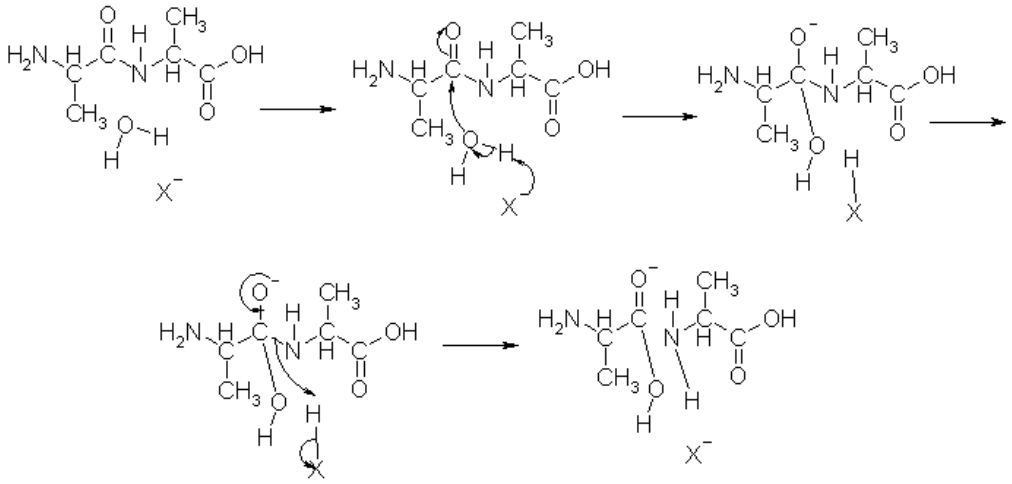
3. Base catalysis
   a. Specific – proton is removed first, then the slow step of the reaction occurs; base must be strong to be able to remove proton prior to the slow step of the reaction.

   b. General – proton is removed during the slow step of the reaction and occurs simultaneously with formation of transition state.

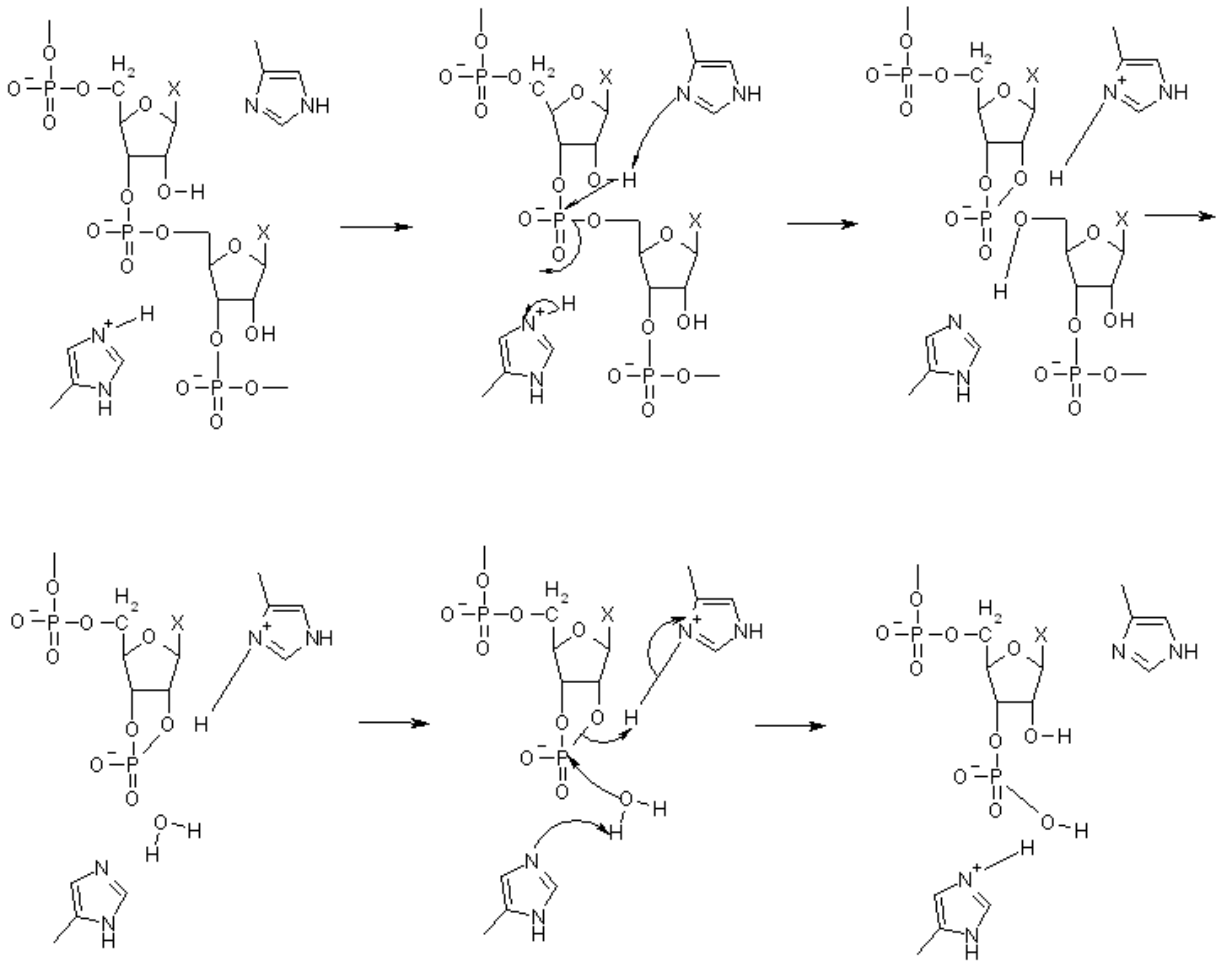
Acid-base catalysis

Acid-base catalysis is provided by certain amino acid residues in the enzyme. The table shows the amino acids that take part in acid-base catalysis:

Amino acid	**Group**	**pK_a**	**Functions**
Aspartate	-COOH	3.9	Bind H^+ and other cations
Glutamate	-COOH	4.5	
Serine	-OH	13.0	Covalent bonding of acyl groups
Cysteine	-SH	8.5	
Tyrosine	-OH	10.1	H-bond to substrates
Histidine	$-NH_2^+-$	6.0	Bind H^+
Lysine	$-NH_3^+$	10.5	Bind anions
Arginine	$-CH(NH_2)^{2+}$	12.5	



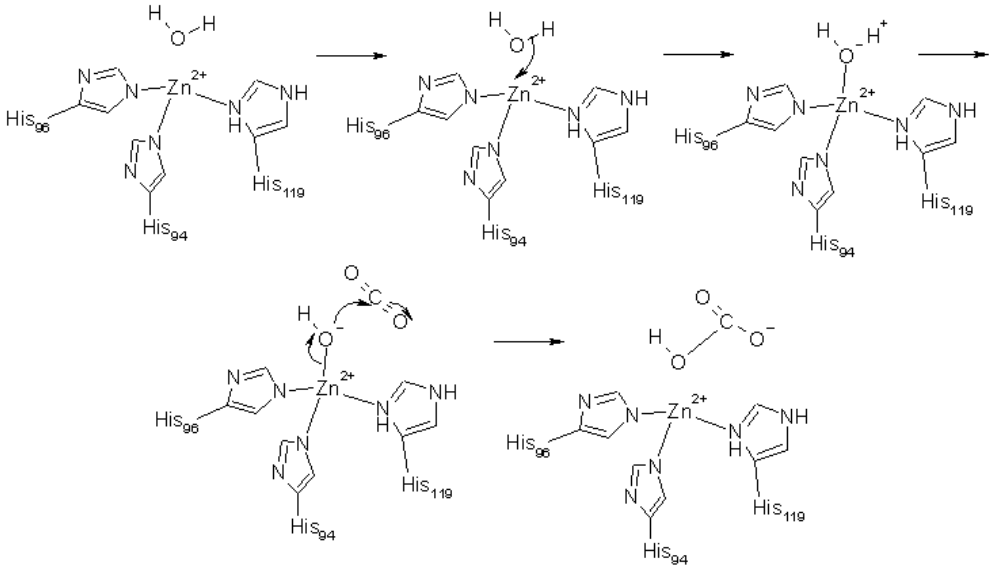
Acid-base catalysis in the action of ribonuclease (RNAse)



4. Metal catalysis
   Mode of action:
   a. Makes reactants more susceptible to nucleophilic attack by receiving electrons
   b. Converts leaving group to a weaker base
   c. Increase nucleophilicity of weak nucleophiles, e.g. water

Metal-ion catalysis

Many enzymes have metals as prosthetic groups. An example is carboxypeptidase that utilizes zinc in catalysis:



Metals facilitate enzyme reactions by a) acting as an electron sink b) immobilizing substrate and c) increasing nucleophilicity and electrophilicity of reacting groups.

5. Intramolecular catalysis – groups within the molecule increases the reaction rate by either increasing the electrophilicity of certain sites in the molecule or by providing an arrangement or conformation that makes a site susceptible to nucleophilic attack.

Watch this video: https://www.youtube.com/watch?v=yk14dOOvwMk

Catalysis in Biological Systems

Enzymes can act rapidly. Some enzymes can speed up reactions up to 1000 times. The factors that contribute to the ease and efficiency by which enzymes react include the presence of intermediary molecules called coenzymes, effect of cofactors, and specificity of substrate.

Factors affecting enzyme reactions:

1. **Temperature**

Generally, when temperature increases, the kinetic energy of molecules, and hence the probability of effective collision to form product also increases. The temperature versus velocity of enzyme reaction follows a bell shape curve such that after the optimum temperature is achieved, a higher temperature can decrease enzyme activity because of protein denaturation. On the other hand, at

lower temperatures, kinetic energy of the molecules is also low and therefore the probability of effective collision decreases. The optimum temperature varies from enzyme to enzyme. Some, such as Taq polymerase can work at very high temperatures, while others such as those found in arctic animals have very low optimum temperatures.

2. pH

The plot of the pH versus the activity of the enzyme has also a bell-shaped curve. Below and above the optimum, enzyme denaturation occurs because of changes in the ionic forms of the amino acid residues affecting intermolecular forces of attraction of these residues within the protein scaffold. The optimum pH also varies from enzyme to enzyme. Figure 6.10 shows that all the enzymes in the diagram exhibit a pH optimum, i.e. the pH at which the enzyme works most efficiently.

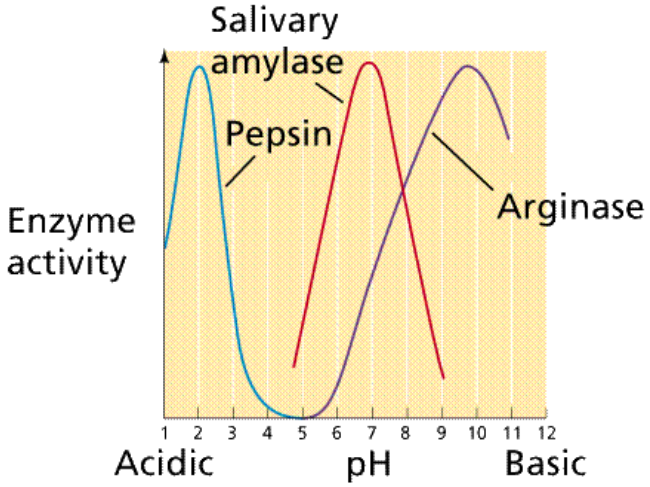


Fig. 6.10. Plot of enzyme activity as a function of pH for several enzymes.

Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).

3. Concentration of substrate and product

The rates of enzyme reactions are determined by the concentrations of both substrates and products. The affinity constant or Km is also the minimum concentration required of a substrate for the reaction to occur. On the other hand an increase in the concentration of substrates or products can also cause a feedback mechanism that can slow down the enzyme reaction. Concentration of substrate is determined by the rate of absorption of the substrate, activity of the enzyme producing the substrate in a series, and gene regulation.

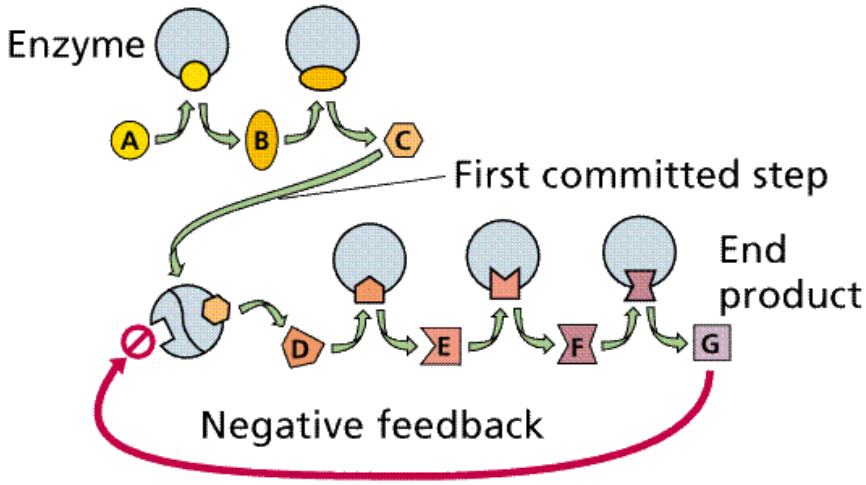


Fig. 6.11. Negative feedback in a metabolic pathway.

The synthesis of the end product (G) in sufficient quantity to fill the square feedback slot in the enzyme will turn off this pathway between step C and D. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).

4. Concentration of co-factors

Cofactors are of two types: 1) small organic biomolecules usually derived from the Vitamin B complex and 2) metals, also called micronutrients. Small organic molecules called coenzymes are enzyme "helpers" that received hydrides during redox reactions or groups that are to be transferred from one substrate to another. Some coenzymes bind to enzyme during the reaction while others are covalently bound to enzymes as one of the reactive groups at the enzymes' active site. Metal cofactors are ligands that form coordination complexes with enzymes and are important in enhancing electrophilicity of reactive residues at enzymes' active sites.

5. Activation

Zymogens are enzymes that have to be post-translationally modified before activation. Many enzymes produced by the pancreas are released as zymogens or pro-enzyme forms. This protects the cell from degradation by ensuring that the enzyme acts on its substrate in predetermined sites. Upon the release of the zymogens, the molecules undergo specific cleavage to remove some amino acid residues or a short peptide and activate the enzymes.

6. Allosteric Interactions

Allosteric sites are sites to which another substance other than the substrate may bind. These sites are different from the active site and may influence the activity of the enzyme. Binding of an allosteric effector changes the shape of the enzyme. As long as the effector is bound, the enzyme is either inhibited or activated. Allosteric effectors can either inhibit the transformation of substrate and therefore the activity of the enzyme or it can act as enhancer and facilitate

transformation of substrate to product. By acting as inhibitors or enhancers, these allosteric effectors play very important roles in regulating enzyme activities.

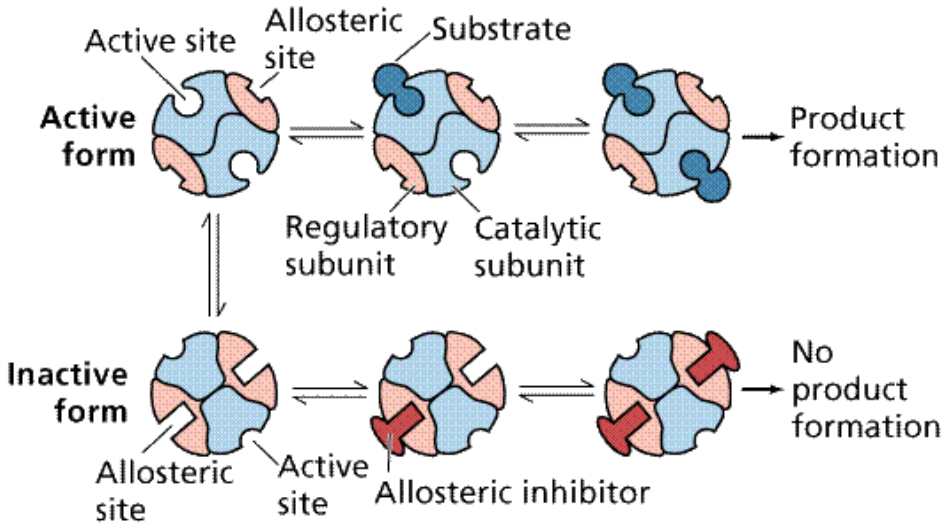


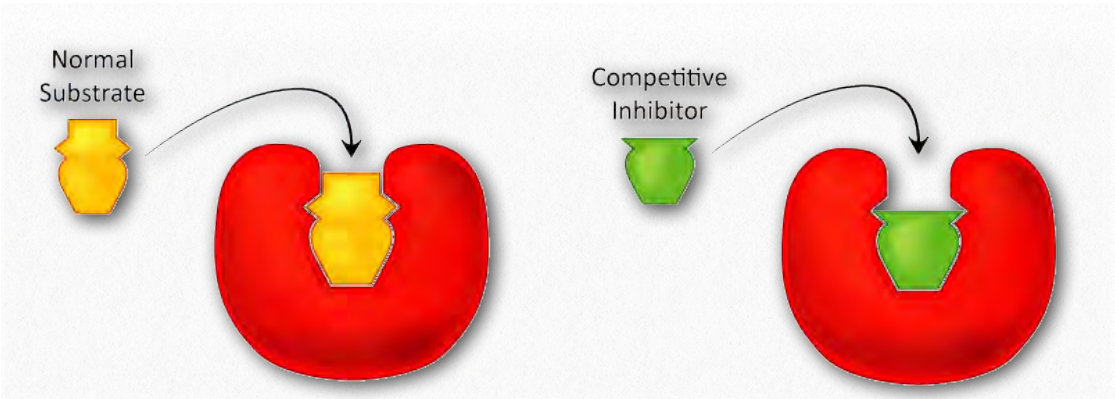
Fig. 6.12. Action of an allosteric inhibitor as a negative control on the action of an enzyme.

Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).

a. Inhibition

There are two general types of inhibition: irreversible and reversible inhibition. The discussion here will focus on reversible inhibition, which are of three (3) types:

1). **Competitive Inhibition** works by the competition of inhibitor and the substrate for the binding site. Both substrate and inhibitor bind at the active site. This type of inhibition is sensitive to the concentration and binding constant (Km for substrate and Ki for inhibitor) of both substrate and inhibitor. Competitive inhibitors are usually structural analogues of substrates.



Source: https://biochem.oregonstate.edu/content/biochemistry-free-and-easy

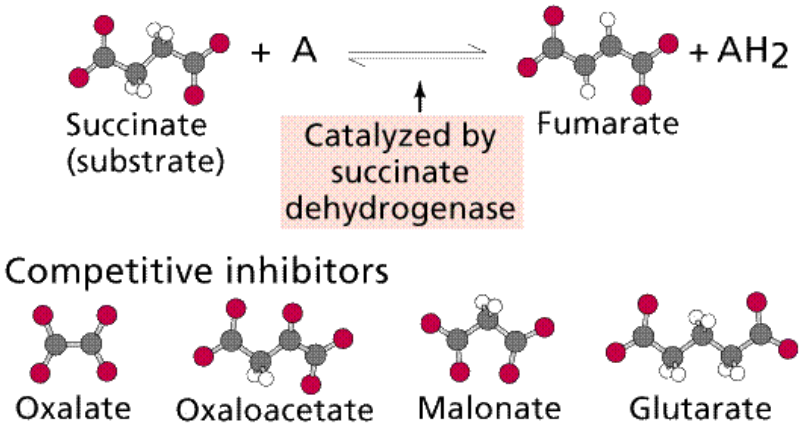


Fig. 6.13. Top: general diagram showing competitive inhibitor in the active site normally occupied by the natural substrate; Bottom: specific case of succinate dehydrogenase and its natural substrate (succinate) and the structure of some competitive inhibitors, oxalate, oxaloacetate, malonate and glutarate.

Images from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).

2). **Noncompetitive Inhibition** occurs when inhibitor binds to sites other than the active site (called allosteric site). The inhibitor is also an allosteric effector. The shape, size and structure of the inhibitor are usually different from the substrate. Purely noncompetitive inhibition is not common; most inhibitors under this group exhibit mixed inhibition.

3). **Uncompetitive inhibition** occurs when the inhibitor binds to the enzyme-substrate complex preventing the formation of product.

b. Enhancers are also molecules that bind to allosteric sites of enzymes. In contrast to inhibitors, enhancers or activators increase the velocity of enzyme reactions. Activators can either be ions, small organic molecules or even peptides, some proteins and lipids.

KINETIC MECHANISMS

The Michaelis-Menten assumptions

Leonor Michaelis and Maud Leonora Menten studied enzyme reactions in the test tube or *in vitro*. The kinetics data they plotted was based on the assumption that for every one enzyme, there is one active site and therefore one substrate. The results of their kinetics studies are applicable not just for one-substrate enzyme but also for multi-substrate enzyme where each reaction is treated as a one-substrate reaction.

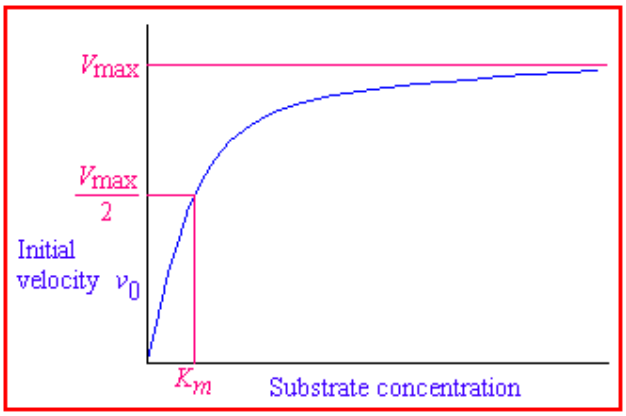


Fig. 6.14. The Michaelis-Menten plot of substrate vs velocity of enzyme-catalyzed reactions.

The first step in any enzyme reaction is the formation of the enzyme-substrate complex as follows:

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

Where E = Enzyme
S = Substrate
P = Products

The rate of formation of this enzyme-substrate complex (ES) is given by:

$$\frac{d\,ES}{dt} = k_1(E)(S) \quad \text{(Eq. 1)}$$

While the rate of disappearance of ES is given by:

$$-\frac{d\,ES}{dt} = k_{-1}(ES) + k_2(ES) \quad \text{(Eq. 2)}$$

When steady-state conditions are reached, and *d*ES/*dt* = -*d*ES/*dt*:

$$k_1(E)(S) = k_{-1}(ES) + k_2(ES) = (k_{-1} + k_2)(ES) \quad \text{(Eq. 3)}$$

The free enzyme concentration (E) cannot be measured experimentally but is related to the total enzyme concentration (E_0):

$$(E) = (E_0) - (ES) \quad \text{(Eq. 4)}$$

Substitution of Eq. 4 into Eq. 3 gives:

$$k_1[(E_0) - (ES)](S) = k_1(E_0)(S) - k_1(ES)(S) = (k_{-1} + k_2)(ES) \quad \text{(Eq. 5)}$$

Rearrangement gives:

$$k_1(E_0)(S) = (k_{-1} + k_2)(ES) + k_1(ES)(S)$$
$$= (ES)[k_{-1} + k_2 + k_1(S)] \quad \text{(Eq. 6)}$$

Solving for (ES) gives:

$$(ES) = \frac{k_1(E_0)(S)}{k_{-1} + k_2 + k_1(S)} \quad \text{(Eq. 7)}$$

Dividing the numerator and denominator by k_1 gives:

$$(ES) = \frac{(E_0)(S)}{(k_{-1} + k_2)/k_1 + (S)} \quad \text{(Eq. 8)}$$

The individual rate constants can be grouped together:

$$K_m = \frac{(k_{-1} + k_2)}{k_1} \quad \text{(Eq. 9)}$$

K_m is the *Michaelis constant*. Substituion of Eq. 9 into Eq. 8 gives:

$$(ES) = \frac{(E_0)(S)}{K_m + (S)} \quad \text{(Eq. 10)}$$

(ES) cannot usually be measured experimentally but we can substitute the rate of product formation as follows:

$$\frac{dP}{dt} = k_2(ES) = v_0 \quad \text{(Eq. 11)}$$

where v_0 is the initial velocity. Combining Eq. 10 and Eq. 11 gives us:

$$v_0 = \frac{k_2(E_0)(S)}{K_m + (S)} \quad \text{(Eq. 12)}$$

The maximum velocity, V_{max} is achieved when the enzyme is saturated with substrate. At saturation, (E_0) = (ES), hence:

$$k_2(ES) = k_2(E_0) = V_{max} \quad \text{(Eq. 13)}$$

Therefore:

$$v_0 = \frac{V_{max}(S)}{K_m + (S)} \quad \text{(Eq. 14)}$$

This is the *Michaelis-Menten* equation used for kinetic studies of enzymes.

Taken from R.A. Rastall (1997). http://www.food.rdg.ac.uk/online/fs916/lect9/lect9.htm

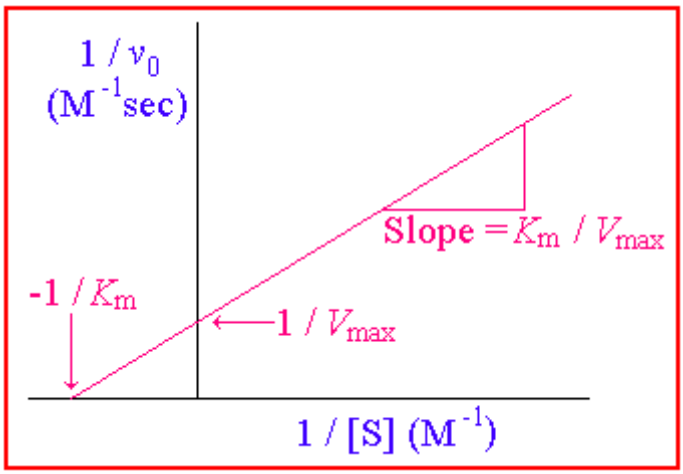
Km is the measure of the affinity of the enzyme for the substrate and is similar to the dissociation constant, Kd for divalent substances or acids. Km is also the minimum concentration to effect catalysis in enzyme reactions. Vmax is the rate when all the enzymes' active sites are filled up such that no amount of additional substrate will change the rate of the reaction.

The Lineweaver-Burk plot and measurement of Km and Vmax

The Lineweaver-Burk plot is obtained from taking the reciprocal of the Michaelis-Menten equation. When 1/S is plotted against $1/v_o$, the result is a linear equation of the form:

$$\frac{1}{v_0} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}$$

The Lineweaver-Burk plot is shown below:



The reciprocal of the y-intercept is 1/Vmax. The x-intercept is -1/Km.

Summary

1. Enzymes are nature's biological catalyst that functions to speed up chemical reactions necessary to maintain and support life.
2. Enzymes are true catalysts in that they are present in small amounts and are regenerated after the reaction.
3. Enzymes are large molecules with a small hydrophobic pocket called the active site. It is in the active site that substrate binds to. The active site contains both contact and catalytic residues to immobilize substrate and transform substrate to product. The active site is also very specific for the substrate it will bind with and act on.
4. Enzymes ensure that the energy of activation to effect transformation is

decreased using entropic strategies, immobilization, strain and catalysis.

5. Protein enzymes are also sensitive to changes in its environment like all other proteins thus the rate and efficiency by which they catalyze enzyme reactions are affected by the factors such as temperature, pH, concentrations of substrates, products, and co-factors, activation requirements, allosteric interactions including inhibition.

Additional References

1. Bruice, Paula Y. (2004). Chap. 3. "Alkenes: Thermodynamics and Kinetics" in Organic Chemistry 4th Edition. NJ: Pearson Education, Inc. pp. 126-134.
2. Bruice, Paula Y. (2004). Chap. 24. "Catalysis" in Organic Chemistry 4th Edition. NJ: Pearson Education, Inc. pp. 999-1029.
3. https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Kaiser)/Unit_7%3A_Microbial_Genetics_and_Microbial_Metabolism/19%3A_Review_of_Molecular_Genetics/19.2%3A_Enzymes

4. Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).
5. R.A. Rastall (1997). http://www.food.rdg.ac.uk/online/fs916/lect9/lect9.htm

Fun video:

https://www.youtube.com/watch?v=XTUm-75-PL4