Chapter 11
Enzymes and Metabolic Pathways

11.1. Metabolism

Metabolism comes from the Greek metabole, meaning "change". It is an emergent property of life. It includes all the chemical processes needed by **cells** to maintain **homeostasis**. **Respiration**, synthesis of biochemicals, and breakdown of wastes are all carried out by various **metabolic pathways**. The thousands of different reactions that occur in a cell can be thought of as an intricately branched road map -- pathways needed to supply the cell with the molecules it uses for energy, structure and function. Each metabolic pathway, a branch in the road map, is an orderly series of enzyme-mediated chemical reactions resulting in the desired product, each step catalyzed by its own specific enzyme. All of metabolism can be categorized as either **anabolic** or **catabolic**.

In cells **anabolism** is the synthesis of molecules, a building process that requires energy input resulting in a more highly ordered chemical organization and the increase of free energy of the products. Entropy decreases (order increases). A major downhill avenue of catabolism is **cellular respiration**. Glucose is oxidized to water, CO_2 and energy. **Catabolism** produces the breakdown of complex molecules to simpler ones, releasing energy which results in the decrease of chemical organization as well as the supply of free energy. Entropy increases (order decreases). These opposing metabolic pathways are frequently coupled so that energy released by "downhill" exergonic reactions can be used to fuel the "uphill" endergonic ones. Coupling often involves ionic gradients.

Enzymes direct traffic by selectively accelerating those steps which are most necessary to the cell at any given time during its life cycle. Homeostatic mechanisms that regulate enzymes balance metabolic supply and demand - resulting in the most energy efficient system possible. It is evolution and the process of natural selection that has fine tuned the diverse metabolic pathways so that as little energy as possible is wasted.

11.2. Thermodynamics

This is a mathematical treatment of the relationship of heat to various forms of energy. Life needs energy for nearly every step in metabolism, and must conform to the Laws of Thermodynamics. Energy is the capacity to do work - or the ability to move matter against an opposing force. Some types of energy include:

1. **Kinetic energy** - energy of motion. Example: mechanical energy
2. **Potential energy** - stored energy. It is the result of location or arrangement of matter. Example: chemical energy is potential energy stored in molecular structure.
3. **Radiant energy** - light energy in electromagnetic radiation from gamma rays to heat. Example: heat is unavailable energy which is uniformly distributed and at the bottom of the energy hill. Much of the energy released during catabolism is lost as heat.

Energy flows from high to low, and can not be recycled. The energy transformations of life are subject to **two laws of thermodynamics**.

The **first law of thermodynamics**, conservation of energy, states that *energy cannot be created or destroyed.* All types of energy before and after a reaction can be accounted for. This law does allow cells to convert one form of energy into a another form. This

sunlight (radiant energy) may be converted into glucose (potential energy) in chemical bonds between carbon, hydrogen and oxygen)

The **second law of thermodynamics** states that *every time energy changes form, there is an increase in the **entropy*** (S), or disorder, of the universe. Whenever matter becomes more ordered, it does so only as a result of a process that increases the disorder of the surroundings. Another way of stating the second law is: "in a closed system, the order of the system is constantly decreasing"

The only way to overcome the constant increase in entropy is to add energy to the system to increase its organization. Without sunlight life on earth would soon come to a cold and icy end. Living things overcome the tendency to disorder by using up metabolic energy. We live at the expense of free energy. A system's free energy is the amount of energy that can actually be put to work under cellular conditions &emdash; that is, in the absence of temperature gradients. Free energy (ΔG) is directly related to energy locked up in chemical bonds (ΔH) and the **entropy** (ΔS) at a given temperature: ΔG = ΔH - TΔS (Note: if disorder increases ΔS will be negative). Every spontaneous change in a system proceeds with a decrease in free energy (-ΔG).

A spontaneous chemical reaction, one in which the products have less free energy than the reactants, is termed an **exergonic reaction** (-ΔG). **Endergonic** (nonspontaneous) reactions are those that occur only with a supply of energy from the surroundings (+ΔG).

11.3. Basic Concepts of Enzymatic Reaction

Enzymes are catalysts. Most are **proteins**. (A few **ribonucleoprotein** enzymes have been discovered and, for some of these, the catalytic activity is in the RNA part rather than the protein part. Enzymes bind temporarily to one or more of the reactants of the reaction they catalyze. In doing so, they lower the amount of **activation energy** needed and thus speed up the reaction.

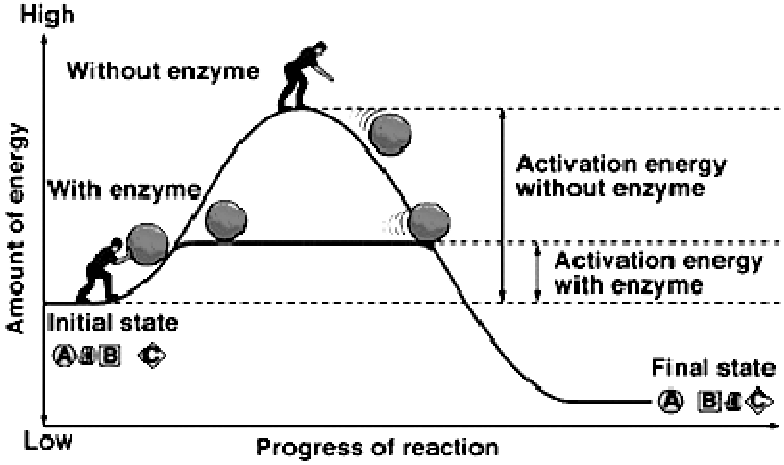

Fig.11.1. Effect of enzymes on the activation energy

Examples:

1. **Catalase.** It catalyzes the decomposition of hydrogen peroxide into water and oxygen. $2H_2O_2 \rightarrow 2H_2O + O_2$. One molecule of catalase can break 40 million molecules of hydrogen peroxide each second.

2. **Carbonic anhydrase.** It is found in red blood cells where it catalyzes the reaction. **$CO_2 + H_2O <-> H_2CO_3$.** It enables red blood cells to transport carbon dioxide from the tissues to the lungs. One molecule of carbonic anhydrase can process one million molecules of CO_2 each second.
3. **Acetylcholinesterase.** It catalyzes the breakdown of the **neurotransmitter acetylcholine** at several types of **synapses** as well as at the **neuromuscular junction** — the specialized synapse that triggers the contraction of skeletal muscle. One molecule of **acetylcholinesterase** breaks down 25,000 molecules of acetylcholine each second. This speed makes possible the rapid "resetting" of the synapse for transmission of another nerve impulse.

Enzyme activity can be analyzed quantitatively. Some of the ways this is done are described in the page **Enzyme Kinetics**. In order to do its work, an enzyme must unite — even if ever so briefly — with at least one of the reactants. In most cases, the forces that hold the enzyme and its substrate are **noncovalent**, an assortment of **hydrogen bonds, ionic interactions** and **hydrophobic interactions**.

Most of these interactions are weak and especially so if the atoms involved are farther than about one **angstrom** from each other. So successful binding of enzyme and substrate requires that the two molecules be able to approach each other closely over a fairly broad surface. Thus the analogy that a substrate molecule binds its enzyme like a **key in a lock**.

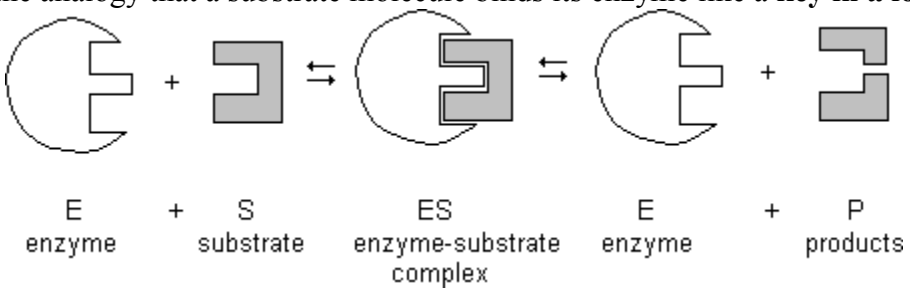

Fig.11.2. Lock-and-Key Hypothesis of Enzyme Action

This requirement for **complementarity** in the configuration of substrate and enzyme explains the remarkable **specificity** of most enzymes. Generally, a given enzyme is able to catalyze only a single chemical reaction or, at most, a few reactions involving substrates sharing the same general structure.

11.4. Competitive Inhibition

The necessity for a close, if brief, fit between enzyme and substrate explains the phenomenon of competitive inhibition.

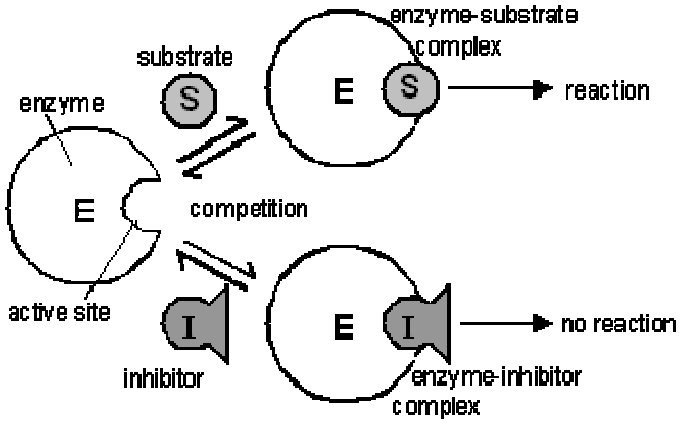

Fig.11.3. Competitive Inhibition

One of the enzymes needed for the release of energy within the cell is **succinic dehydrogenase**. It catalyzes the oxidation (by the removal of two hydrogen atoms) of succinic acid **(a)**. If one adds **malonic acid** to cells, or to a test tube mixture of succinic acid and the enzyme, the action of the enzyme is strongly inhibited. This is because the structure of malonic acid allows it to bind to the same site on the enzyme **(b)**. But there is no oxidation so no speedy release of products. The inhibition is called competitive because if you increase the ratio of succinic to malonic acid in the mixture, you will gradually restore the rate of catalysis. At a 50:1 ratio, the two molecules compete on roughly equal terms for the binding (=catalytic) site on the enzyme.

11.5. Non-Competitive Inhibition

A **non-competitive inhibitor** molecule is quite different in structure from the substrate molecule and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Non-competitive inhibitors therefore simply reduce the amount of active enzyme (just like decreasing the enzyme concentration), so they decrease v_{max}, but have no effect on K_M. Inhibitors that bind fairly weakly and can be washed out are sometimes called **reversible inhibitors**, while those that bind tightly and cannot be washed out are called **irreversible inhibitors**. Poisons like **cyanide**, heavy metal ions and some insecticides are all non-competitive inhibitors.

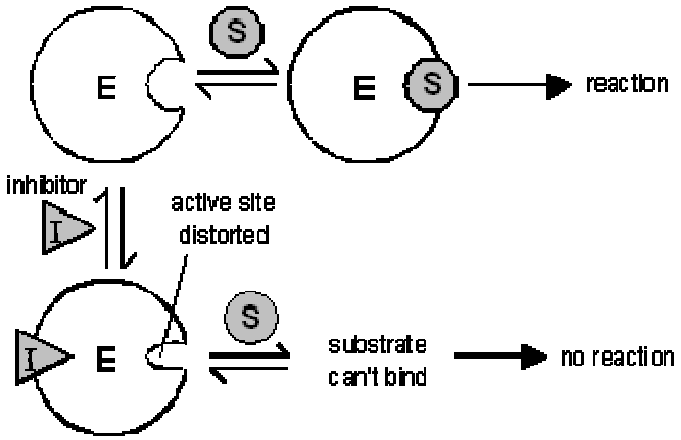

Fig.11.4. Non-Competitive Inhibition

11.6. Enzyme Cofactors

Many enzymes require the presence of an additional, nonprotein, cofactor. Some of these are metal ions such as Zn^{2+} (the cofactor for carbonic anhydrase), Cu^{2+}, Mn^{2+}, K^{+}, and Na^{+}. Some cofactors are small organic molecules called **coenzymes**. The B vitamins **thiamine** (B1), **riboflavin** (B2) and **nicotinamide** are precursors of coenzymes. Coenzymes may be covalently bound to the protein part (called the **apoenzyme**) of enzymes as a **prosthetic group**. Others bind more loosely and, in fact, may bind only transiently to the enzyme as it performs its catalytic act.

11.7. Lysozyme: A model of Enzyme Action

A number of lysozymes are found in nature; in human tears and egg white, for examples. The enzyme is antibacterial because it degrades the polysaccharide that is found in the cell walls of many bacteria. It does this by catalyzing the insertion of a water molecule at the position indicated by the red arrow (a **glycosidic bond**). This **hydrolysis** breaks the chain at that point.

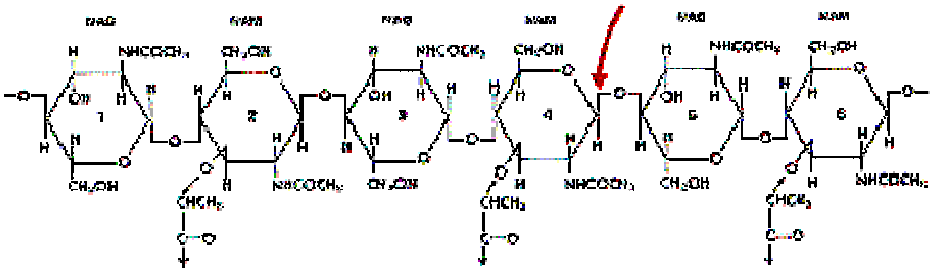

Fig.11.5. A model of Enzyme Action

The bacterial polysaccharide consists of long chains of alternating amino sugars: **N-acetylglucosamine** (**NAG**) and **N-acetylmuramic acid** (**NAM**). These hexose units resemble glucose except for the presence of the side chains containing amino groups. Lysozyme is a **globular protein** with a deep cleft across part of its surface. Six hexoses of the substrate fit into this cleft. With so many oxygen atoms in **sugars**, as many as 14 **hydrogen bonds** form between the six amino sugars and certain amino acid **R groups** such as **Arg-114**, **Asn-37**, **Asn-44**, **Trp-62**, **Trp-63**, and **Asp-101**. Some hydrogen bonds also form with the C=O groups of several **peptide bonds**. In addition, hydrophobic interactions may help hold the substrate in position.

X-ray crystallography has shown that as lysozyme and its substrate unite, each is slightly deformed. The fourth hexose in the chain (ring #4) becomes twisted out of its normal position. This imposes a strain on the C-O bond on the ring-4 side of the oxygen bridge between rings 4 and 5. It is just at this point that the polysaccharide is broken. A molecule of water is inserted between these two hexoses, which breaks the chain. Here, then, is a structural view of what it means to lower activation energy. The energy needed to break this covalent bond is lower now that the atoms connected by the bond have been distorted from their normal position.

As for lysozyme itself, binding of the substrate induces a small (~0.75Å) movement of certain amino acid residues so the cleft closes slightly over its substrate. So the "lock" as well as the "key" changes shape as the two are brought together. (This is sometimes called "induced fit".) The amino acid residues in the vicinity of rings 4 and 5 provide a plausible mechanism for completing the catalytic act. Residue 35, glutamic acid (**Glu-**

35), is about 3Å from the -O- bridge that is to be broken. The free carboxyl group of glutamic acid is a hydrogen ion donor and available to transfer H^+ to the oxygen atom. This would break the already-strained bond between the oxygen atom and the carbon atom of ring 4. Now having lost an electron, the carbon atom acquires a positive charge. Ionized carbon is normally very unstable, but the attraction of the negatively-charged carboxyl ion of **Asp-52** could stabilize it long enough for an -OH ion (from a spontaneously dissociated water molecule) to unite with the carbon. Even at pH 7, water spontaneously dissociates to produce H^+ and OH^- ions. The hydrogen ion (H^+) left over can replace that lost by **Glu-35**. In either case, the chain is broken, the two fragments separate from the enzyme, and the enzyme is free to attach to a new location on the bacterial cell wall and continue its work of digesting it.

11.8. Factors Affecting Enzyme Action

The activity of enzymes is strongly affected by changes in **pH** and **temperature**. Each enzyme works best at a certain pH (left graph) and temperature (right graph), its activity decreasing at values above and below that point. This is not surprising considering the importance of **tertiary structure** (i.e. shape) in enzyme function and noncovalent forces, e.g., ionic interactions and hydrogen bonds, in determining that shape.

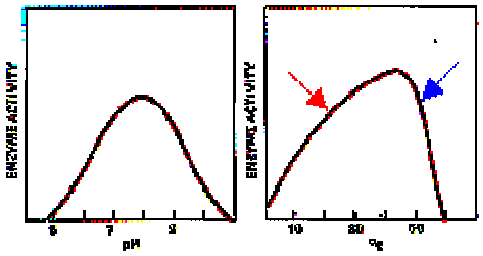

Fig.11.6a. Effect of pH and Temperature on Enzyme Activity

Examples: The protease **pepsin** works best as a pH of 1–2 (found in the stomach) while the protease **trypsin** is inactive at such a low pH but very active at a pH of 8 (found in the small intestine as the bicarbonate of the pancreatic fluid neutralizes the arriving stomach contents). Changes in pH alter the state of ionization of charged amino acids (e.g., Asp, Lys) that may play a crucial role in substrate binding and/or the catalytic action itself. Without the unionized -COOH group of **Glu-35** and the ionized $-COO^-$ of **Asp-52**, the catalytic action of lysozyme would cease.

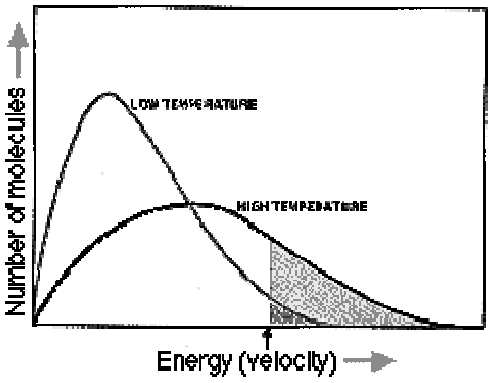

Fig.11.6b. Lysozyme Activity

Hydrogen bonds are easily disrupted by increasing temperature. This, in turn, may disrupt the shape of the enzyme so that its affinity for its substrate diminishes. The ascending portion of the temperature curve reflects the **general effect of increasing temperature** on the rate of chemical reactions (graph at left). The descending portion of the curve above (blue arrow) reflects the loss of catalytic activity as the enzyme molecules become **denatured** at high temperatures.

11.9. Regulation of Enzyme Activity

Several mechanisms work to make enzyme activity within the cell efficient and well-coordinated.

(a) Anchoring Enzymes in Membranes

Many enzymes are inserted into cell membranes, for examples, the **plasma membrane**, the membranes of **mitochondria** and **chloroplasts**, the **endoplasmic reticulum** and the **nuclear envelope**. These are locked into spatial relationships that enable them to interact efficiently.

(b) Inactive Precursors

Enzymes, such as proteases, that can attack the cell itself are inhibited while within the cell that synthesizes them. For example, pepsin is synthesized within the **chief cells** (in gastric glands) as an inactive precursor, **pepsinogen**. Only when exposed to the low pH outside the cell is the inhibiting portion of the molecule removed and active pepsin produced.

(c) Feedback Inhibition

If the product of a series of enzymatic reactions, e.g., an amino acid, begins to accumulate within the cell, it may specifically inhibit the action of the first enzyme involved in its synthesis. Thus further production of the enzyme is halted.

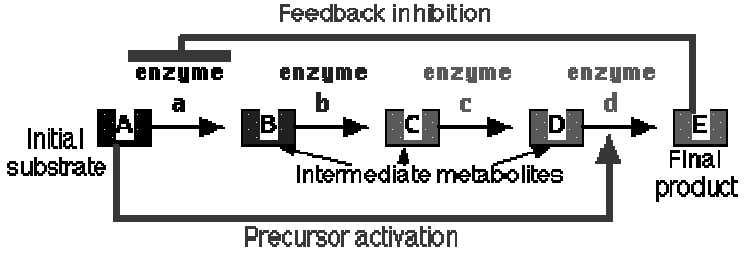

Fig.11.7a. Precursor Activation and Feedback Inhibition

(d) Precursor Activation

The accumulation of a substance within a cell may specifically activate (blue arrow) an enzyme that sets in motion a sequence of reactions for which that substance is the initial substrate. This reduces the concentration of the initial substrate.

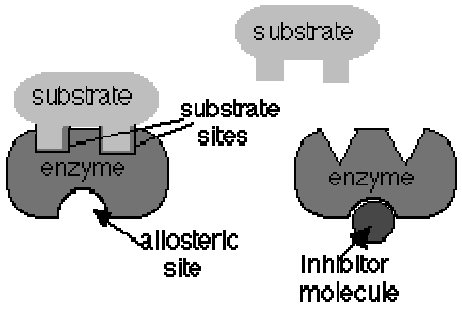

Fig.11.7b. Allosteric effect of enzyme Action

In the case if **feedback inhibition** and **precursor activation**, the activity of the enzyme is being regulated by a molecule which is **not** its substrate. In these cases, the regulator molecule binds to the enzyme at a different site than the one to which the substrate binds. When the regulator binds to its site, it alters the shape of the enzyme so that its activity is changed. This is called an **allosteric** effect. In feedback inhibition, the **allosteric effect** lowers the affinity of the enzyme for its substrate. In **precursor activation**, the regulator molecule increases the affinity of the enzyme in the series for its substrate.

11.10. Regulation of Enzyme Synthesis

The four mechanisms described above regulate the activity of enzymes already present within the cell. What about enzymes that are not needed or are needed but not present? Here, too, control mechanisms are at work that regulate the rate at which new enzymes are synthesized. Most of these controls work by turning on — or off — the **transcription of genes**. If, for example, ample quantities of an amino acid are already available to the cell from its **extracellular fluid**, synthesis of the enzymes that would enable the cell to produce that amino acid for itself is shut down. Conversely, if a new substrate is made available to the cell, it may induce the synthesis of the enzymes needed to cope with it. Yeast cells, for example, do not ordinarily metabolize lactose and no **lactase** can be detected in them. However, if grown in a medium containing lactose, they soon begin synthesizing lactase — by transcribing and translating the necessary gene(s) — and so can begin to metabolize the sugar. *E. coli* also has a mechanism which regulates enzyme synthesis by controlling **translation** of a needed messenger RNA.

11.11. Enzyme Kinetics

Enzymes are **protein catalysts** that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process. They achieve their effect by temporarily binding to the **substrate** and, in doing so, lowering the **activation energy** needed to convert it to a product. The rate at which an enzyme works is influenced by several factors, e.g.,

1. The **concentration** of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated **[S]** and is expressed in unit of molarity.
2. The t**emperature**. As the temperature rises, molecular motion - and hence collisions between enzyme and substrate - speed up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes **denatured** and ineffective.
3. The presence of **inhibitors**.

a. **Competitive inhibitors** are molecules that bind to the same site as the substrate - preventing the substrate from binding as they do so - but are not changed by the enzyme.
b. **Noncompetitive inhibitors** are molecules that bind to some other site on the enzyme reducing its catalytic power.

4. **pH**. The conformation of a protein is influenced by pH and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected.

The study of the rate at which an enzyme works is called **enzyme kinetics**. Let us examine enzyme kinetics as a function of the **concentration of substrate** available to the enzyme.

- We set up a series of tubes containing graded concentrations of substrate, **[S]**.
- At time zero, we add a fixed amount of the enzyme preparation.
- Over the next few minutes, we measure the concentration of product formed. If the product absorbs light, we can easily do this in a spectrophotometer.
- Early in the run, when the amount of substrate is in substantial excess to the amount of enzyme, the rate we observe is the initial velocity of $\mathbf{V_i}$.

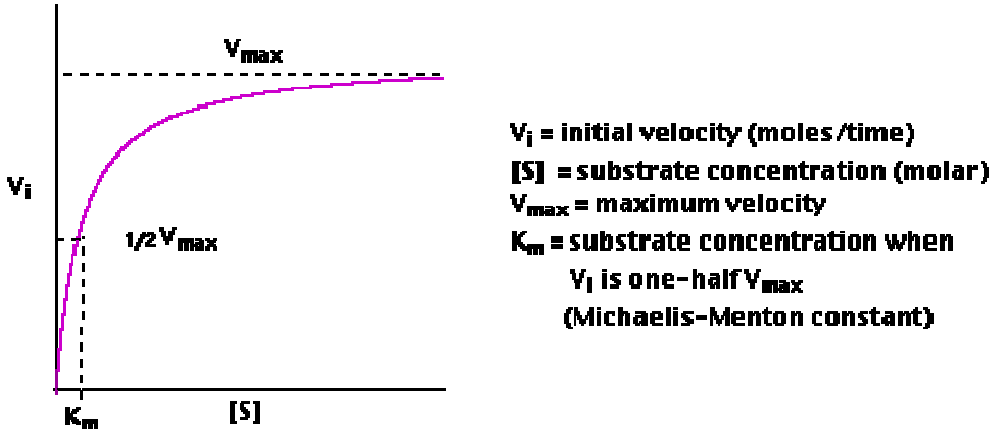

Fig.11.8a. Effect of Concentration on enzyme Kinetics

Plotting $\mathbf{V_i}$ as a function of **[S]**, we find that At low values of **[S]**, the initial velocity,$\mathbf{V_i}$, rises almost linearly with increasing **[S]**. But as **[S]** increases, the gains in $\mathbf{V_i}$ level off (forming a rectangular hyperbola). The asymptote represents the maximum velocity of the reaction, designated $\mathbf{V_{max}}$. The substrate concentration that produces a $\mathbf{V_i}$ that is one-half of $\mathbf{V_{max}}$ is designated the Michaelis-Menten constant, $\mathbf{K_m}$(named after the scientists who developed the study of enzyme kinetics). $\mathbf{K_m}$ is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the $\mathbf{K_m}$, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

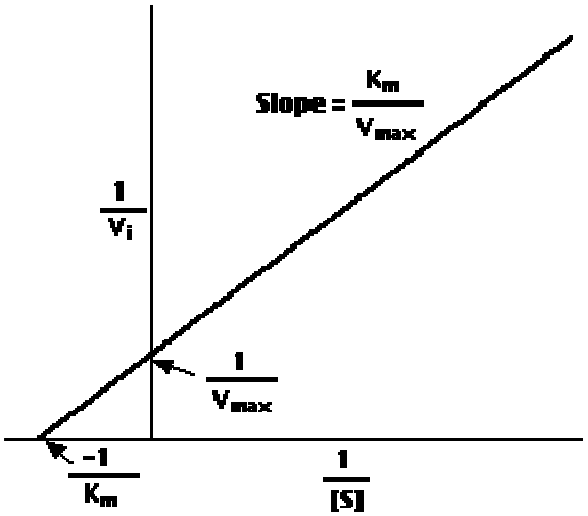

Fig.11.8b. Michaelis-Menten constant

Plotting the reciprocals of the **same data points** yields a "double-reciprocal" or Lineweaver-Burk plot. This provides a more precise way to determine $\mathbf{V_{max}}$ and $\mathbf{K_m}$. $\mathbf{V_{max}}$ is determined by the point where the line crosses the $1/\mathbf{V_i} = 0$ axis (so the **[S]** is infinite). Note that the magnitude represented by the data points in this plot **decrease** from lower left to upper right. $\mathbf{K_m}$ equals $\mathbf{V_{max}}$ times the slope of line. This is easily determined from the intercept on the X axis.

(a) The Effects of Enzyme Inhibitors

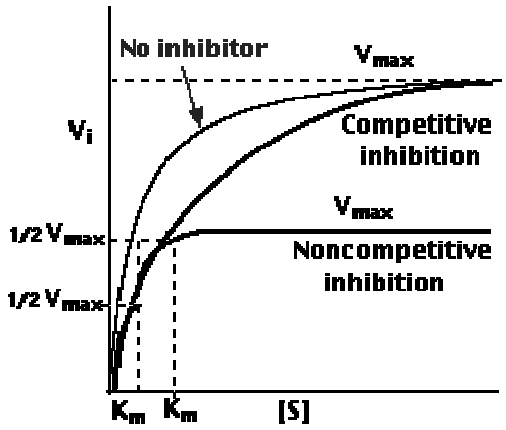

Fig.11.9a. Effects of Enzyme Inhibitors

Enzymes can be inhibited **competitively**, when the substrate and inhibitor compete for binding to the same active site or **noncompetitively**, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency. The distinction can be determined by plotting enzyme activity with and without the inhibitor present.

(i) Competitive Inhibition

In the presence of a competitive inhibitor, it takes a higher substrate concentration to achieve the same velocities that were reached in its absence. So while $\mathbf{V_{max}}$ can still be reached if sufficient substrate is available, one-half $\mathbf{V_{max}}$ requires a higher **[S]** than before and thus $\mathbf{K_m}$ is larger.

(ii) Noncompetitive Inhibition

With noncompetitive inhibition, enzyme molecules that have been bound by the inhibitor are taken out of the game so enzyme rate (velocity) is reduced for all values of **[S]**, including $\mathbf{V_{max}}$ and one-half $\mathbf{V_{max}}$ but $\mathbf{K_m}$ remains unchanged because the active site of those enzyme molecules that have not been inhibited is unchanged.

This Lineweaver-Burk plot displays these results.

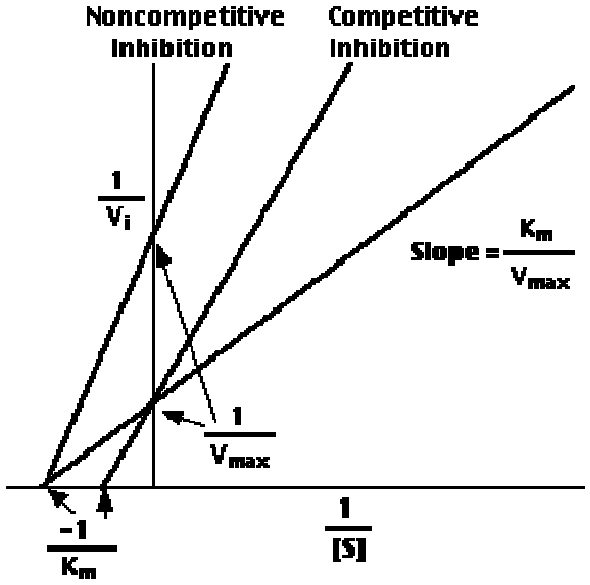

Fig.11.9b. Lineweaver-Burk Plot

An Example: When a slice of apple is exposed to air, it quickly turns brown. This is because the enzyme **o-diphenol oxidase** catalyzes the oxidation of phenols in the apple to dark-coloured products. (A similar enzyme, tyrosinase, converts **tyrosine** to **melanin**.)

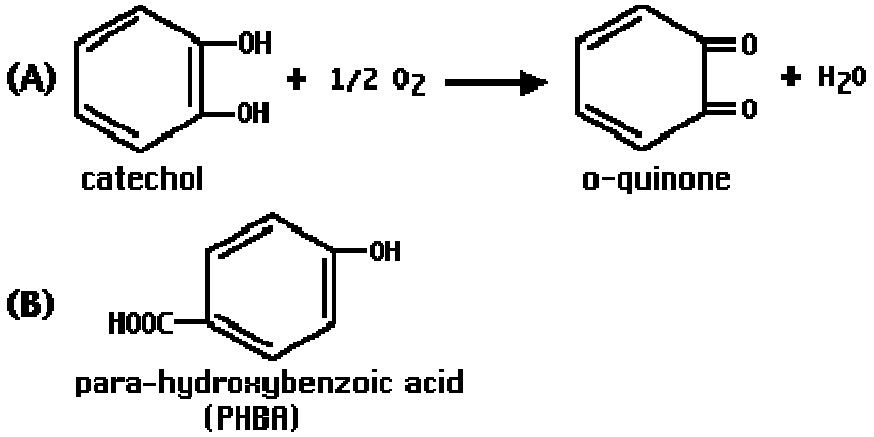

Fig.11.10. Enzymatic oxidation reaction in apple