ENZYMES

What are enzymes?

- All living organisms in the world should possess TWO fundamental properties
- They are:

(1). Must be able to self-replicate

(2). Must be able to catalyze chemical reactions efficiently and selectively

- Enzymes are the molecules which enables each living entity to do these two fundamental activities
- Enzymes are better known as biological catalysts
- Almost all enzymes are highly specialized proteins
- Ribozymes: RNA molecules with catalytic properties
- Abzymes: antibodies with catalytic properties

All known enzymes are proteins. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds. See Figure 1.

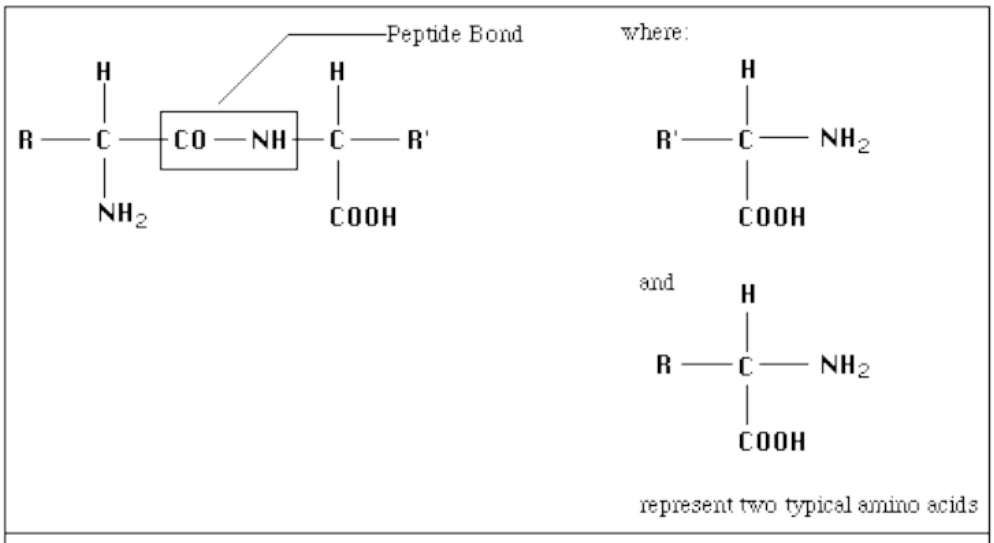


FIGURE 1
Typical protein structure - two amino acids joined by a peptide bond.

Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000.

Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme.

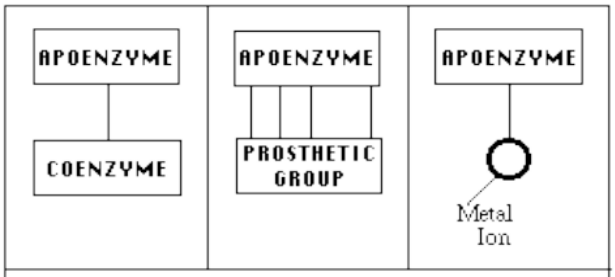


FIGURE 2
Holoenzymes - apoenzymes plus various types of cofactors

Apoenzyme + Cofactor = Holoenzyme

According to Holum, the cofactor may be:

1. A coenzyme - a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.

2. A prosthetic group - an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.

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

Specificity of Enzymes

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

- Absolute specificity - the enzyme will catalyze only one reaction.
- Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names indicate both the substrate acted

upon and the type of reaction catalyzed. Under this system, the enzyme uricase is called urate: O_2 oxidoreductase, while the enzyme *glutamic oxaloacetic* transaminase (GOT*)* is called L-aspartate: 2-oxoglutarate aminotransferase.

Enzymes can be classified by the kind of chemical reaction catalyzed.

1. Addition or removal of water
    A. Hydrolases - these include esterases, carbohydrases, nucleases, deaminases, amidases, and proteases
    B. Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase
2. Transfer of electrons
    A. Oxidases
    B. Dehydrogenases
3. Transfer of a radical
    A. Transglycosidases - of monosaccharides
    B. Transphosphorylases and phosphomutases - of a phosphate group
    C. Transaminases - of amino group
    D. Transmethylases - of a methyl group
    E. Transacetylases - of an acetyl group
4. Splitting or forming a C-C bond
    A. Desmolases
5. Changing geometry or structure of a molecule
    A. Isomerases
6. Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate
    A. Ligases

Enzyme Kinetics: Energy Levels

Chemists have known for almost a century that for most chemical reactions to proceed, some form of energy is needed. They have termed this quantity of energy, "the energy of activation." It is the magnitude of the activation energy which determines just how fast the reaction will proceed. It is believed that enzymes lower the activation energy for the reaction they are catalyzing. Figure 3 illustrates this concept.

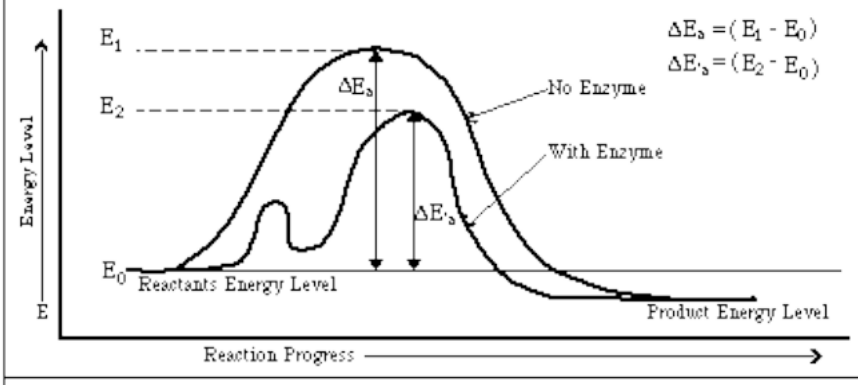


FIGURE 3
Energy of activation ΔE_a is less than $\Delta E'_a$ due to the effect of the enzyme on the substrate.

The enzyme is thought to reduce the "path" of the reaction. This shortened path would require less energy for each molecule of substrate converted to product. Given a total amount of available energy, more molecules of substrate would be converted when the enzyme is present (the shortened "path") than when it is absent. Hence, the reaction is said to go faster in a given period of time.

Enzymes are the catalysts of biological systems and are extremely efficient and specific as catalysts. In fact, typically, an enzyme accelerates the rate of a reaction by factors of at least a million compared to the rate of the same reaction in the absence of the enzyme. Most biological reactions do not occur at perceptible rates in the absence of enzymes.

Enzymes are highly specific. Typically a particular enzyme catalyzes only a single chemical reaction or a set of closely related chemical reactions. As is true of any catalyst, enzymes do not alter the equilibrium point of the reaction. This means that the enzyme accelerates the forward and reverse reaction by precisely the same factor. For example, consider the interconversion of A and B.

$$A \leftrightarrow B \qquad (1)$$

Suppose that in the absence of the enzyme the forward rate constant (kf) is 10-4 s-1 and the reverse rate constant (kr) is 10-6 s-1. The equilibrium constant (Keq) is given by the ratio of the two rate constants.

$$K_{eq} = \frac{[B]}{[A]} = \frac{k_f}{k_r} = \frac{10^{-4}}{10^{-6}} = 100 \qquad (2)$$

The equilibrium concentration of B is 100 times that of A whether or not an enzyme is present. However, in the absence of an enzyme the reaction could take more than an hour to approach this equilibrium, whereas in the presence of an enzyme, equilibrium could be attained within a second. The enzyme lowers the height of the energy barrier to the reaction thereby increasing the rate of the reaction, but since the rate of both the forward and reverse reaction are affected by the same amount, the equilibrium constant is not affected by the presence of the enzyme. the same amount (see Figure 1)

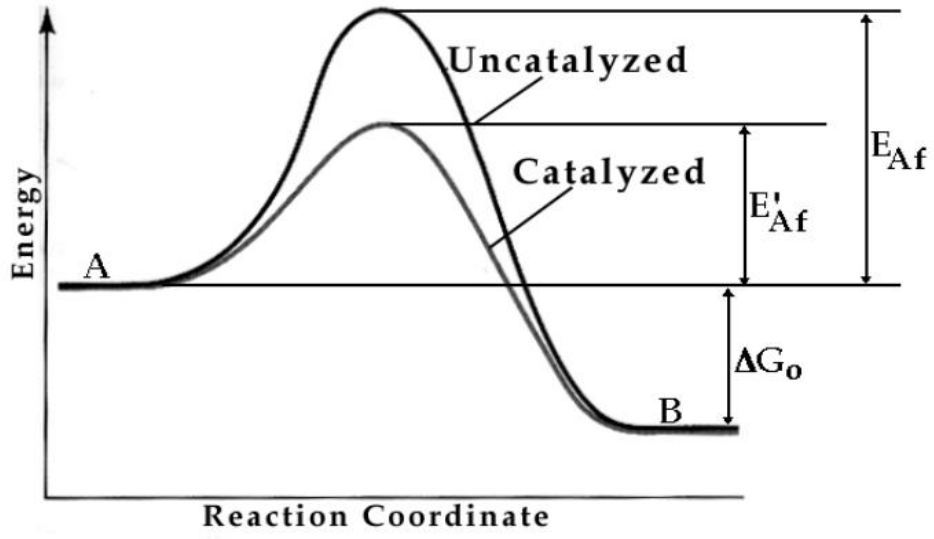


Figure 1

where, E_{Af} is the activation energy for the forward reaction (A□B) in the absence of a catalyst and E'_{Af} is the activation energy for the forward reaction (A□B) in the presence of a catalyst, and ΔG_o is the change in free energy for the reaction.

The equilibrium constant is related to ΔG_o as follows:

$$K_{eq} = e^{-\Delta G_o / RT}$$

Since ΔG_o is the same for the catalyzed and uncatalyzed reaction, K_{eq} is the same for both reactions.

Enzyme Kinetics:

The mechanism of enzyme catalyzed reactions is often studied by making kinetic measurements on enzyme-substrate reaction systems. These studies include measuring rates of the enzyme-catalyzed reactions at different substrate and enzyme concentrations. Here we will look at a simple model for the catalytic behavior of an enzyme and the kinetic model that arises from this model.

For many enzymes, if we were to plot the rate of catalysis, V (also known as the reaction velocity), vs. the substrate concentration, [S] (at a fixed enzyme concentration) we would see a plot as shown in figure 4.

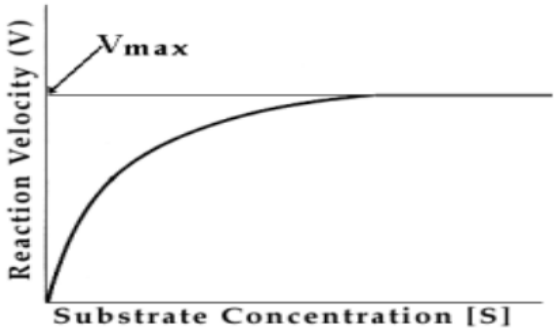


Figure. 4

Looking at this plot, we see that V varies linearly with [S] for small [S]. As [S] increases, V "plateaus" indicating that V becomes independent of [S] at large values of [S]. The simplest model which accounts for this behavior is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P \qquad (3)$$

where E is the enzyme, S the substrate, ES the enzyme-substrate complex, P the product of the enzyme-catalyzed reaction, k_1 the rate constant of the forward reaction of E+S, k_{-1} the rate of the reverse reaction where the enzyme-substrate complex, ES, falls apart to E+S and k_2 the rate constant of the forward reaction of ES forming E+P. In this model, it is assumed that none of the product reacts with the enzyme to form the enzyme-substrate complex, ES (this is true during the initial stages of the reaction when [P] is low, but towards the end of the reaction when [P] is high this may no longer be true).

We need to derive an expression that relates the reaction velocity, V, to the concentrations of the substrate and enzyme and the rates of the individual steps. From equation (3) the reaction velocity, V can be expressed as:

$$V = k_2 [ES] \qquad (4)$$

Since ES is an intermediate and hence its concentration unknown, we have to express [ES] in terms of known values. The rates at which [ES] is formed and falls apart are:

$$\text{Rate of formation of [ES]} = k_1 [E] [S] \qquad (5)$$

$$\text{Rate at which [ES] falls apart} = (k_{-1} + k_2) [ES] \qquad (6)$$

We can use the *steady-state* approximation to express V in terms of known quantities. Under the steady-state approximation, the concentration of the intermediate [ES] stays a constant, while the concentrations of reactants and product change. The steady state occurs when
eqn 5 = eqn 6 i.e.

$$k_1 [E] [S] = (k_{-1} + k_2) [ES] \qquad (7)$$

Rearranging,

$$[ES] = \frac{[E][S]k_1}{k_{-1} + k_2} \qquad (8)$$

Define, K_M, the Michaelis constant, as

$$K_M = \frac{k_{-1} + k_2}{k_1} \qquad (9)$$

Substituting (9) into (8)

$$[ES] = \frac{[E][S]}{K_M} \qquad (10)$$

Since in most situations the enzyme concentration is very small ([E] << [S]), the concentration of the uncombined S is almost equal to the total concentration of S. The concentration of

uncombined E is equal to the total enzyme concentration [E_o] minus the concentration of the complex [ES]

$$[E] = [E_o] - [ES] \quad (11)$$

Substituting (11) into (10)

$$[ES] = \frac{([E_o] - [ES])[S]}{K_M} \quad (12)$$

Solving (12) for [ES],

$$[ES] = [E_o]\frac{[S]/K_M}{1 + S/K_M} \quad (13)$$

or,

$$[ES] = [E_o]\frac{[S]}{[S] + K_M} \quad (14)$$

Substituting (14) into (4)

$$V = k_2[E_o]\frac{[S]}{[S] + K_M} \quad (15)$$

The maximum reaction velocity, V_{max}, is reached when all enzyme sites are saturated with the substrate. This will happen when [S] >>K_M, so that [S]/([S]+K_M) approaches 1. In this limit, we can express V_{max} (from (15)) as:

$$V_{max} = k_2[E_o] \quad (16)$$

Substituting (16) into (15),

$$V = V_{max}\frac{[S]}{[S] + K_M} \quad (17)$$

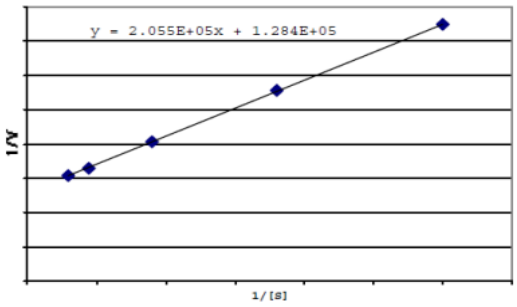
If we were to plot V vs S the resulting plot will have a shape as shown in figure 4. Hence, equation (17) describes the kinetic behavior of an enzyme as modeled by the kinetic scheme in equation (3). Looking at equation (17) at very low [S], when [S] << K_M, V ~ [S]V_{max}/K_M, that is, the rate is proportional to [S] (describes the linear region of the plot in figure 4). At high [S],

when [S]>>K_M, V=V_{max} and hence independent of [S] (the "plateau" region of the plot in figure 4).

Equation (17) can be re-arranged as:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]} \qquad (18)$$

If we were to plot 1/V vs. 1/[S] we would obtain a straight line with a y-intercept = $1/V_{max}$ and a slope =K_M/V_{max} (see figure 5). This plot is called a Lineweaver-Burke plot.



Significance of K_M

From equation 18, when [S] = K_M, then V=$V_{max}/2$. Hence K_M is equal to the substrate concentration at which the reaction rate is half its maximum value. In other words, if an enzyme has a small value of K_M, it achieves its maximum catalytic efficiency at low substrate concentrations. Hence, the smaller the value of K_M, the more efficient is the catalyst. The value of K_M for an enzyme depends on the particular substrate . It also depends on the pH of the solution and the temperature at which the reaction is carried out. For most enzymes K_M lies between 10^{-1} and 10^{-7} M.

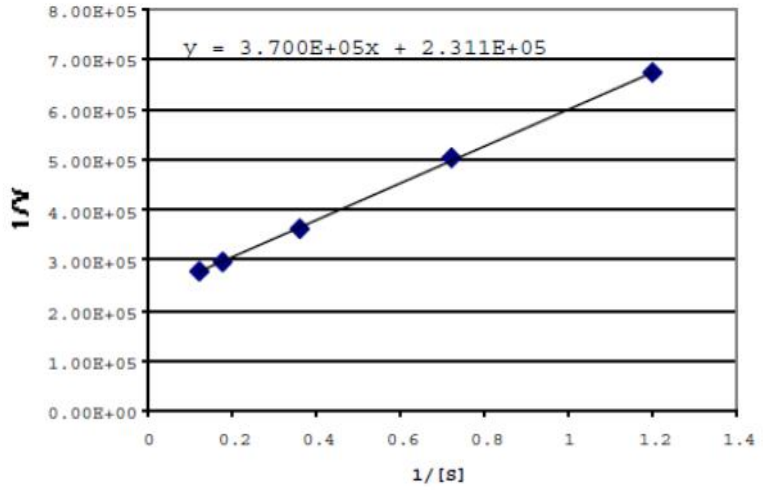
Determining K_M and V_{max} experimentally

To characterize an enzyme-catalyzed reaction K_M and V_{max} need to be determined. The way this is done experimentally is to measure the rate of catalysis (reaction velocity) for different substrate concentrations. In other words, determine V at different values of [S]. Then plotting 1/V vs. 1/[S] we should obtain a straight line described by equation (18). From the y-intercept

and the slope, the values of K_M and V_{max} can be determined. For example, use EXCEL to plot the data shown below. Fit the data to a straight line, and from the equation of the straight line determine the values of K_M and V_{max}.

[S] (mM)	V (mM/sec)	1/[S] (mM-1)	1/V (sec/mM)
8.33	3.62E-06	0.12	2.76E+05
5.55	3.39E-06	0.18	2.95E+05
2.77	2.75E-06	0.36	3.64E+05
1.38	1.99E-06	0.72	5.02E+05
0.83	1.49E-06	1.2	6.73E+05

You should obtain a plot as shown in figure 6 below. From the fit to the data show that $K_M = 1.6$ for this data and V_{max} is 4.32×10^{-6} mM/sec.



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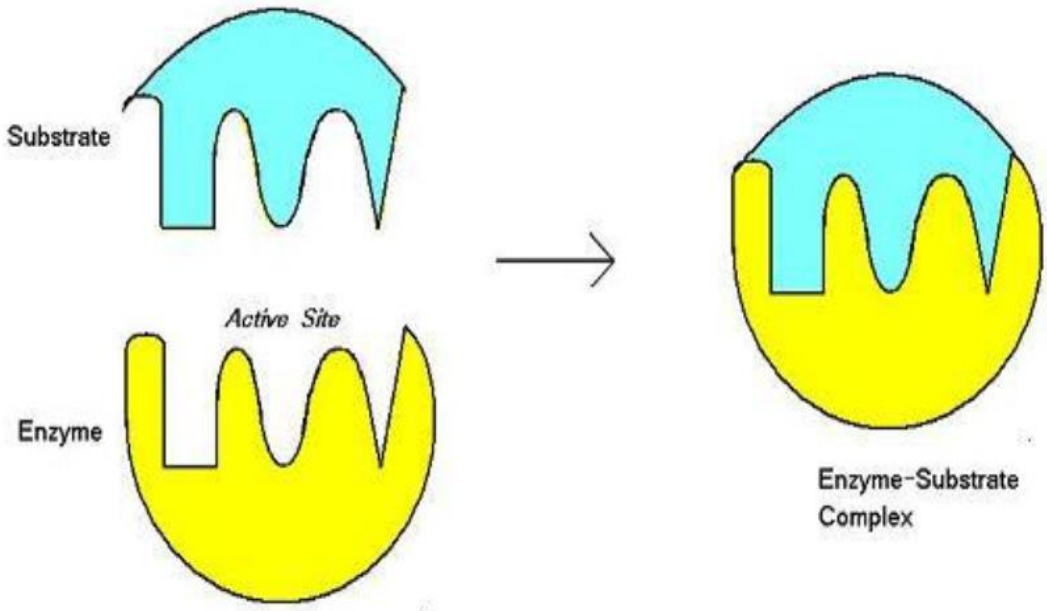
The Lock-and-key Hypothesis

- The Lock-and-key Hypothesis is a model of how Enzymes catalyseSubstrate reactions. It states that the shape of the Active Sites of Enzymes are exactly Complementary to the shape of the Substrate.

- When a substrate molecule collides with an enzyme whose Active Site shape is complementary, the substrate will fit into the Active Site and anEnzyme-Substrate Complex will form.

- The enzyme will catalyse the reaction, and the products, together with the enzyme, will form an Enzyme-Product Complex. According to this model, it is possible for an enzyme to catalyse a reverse reaction.

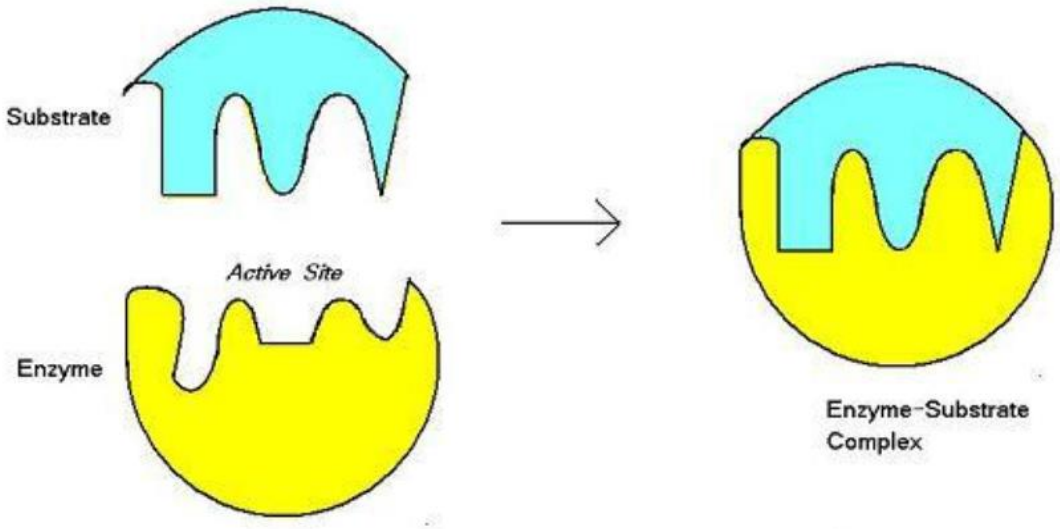


Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

The Induced-Fit Hypothesis

- A more recent model, which is backed up by evidence ,and is widely accepted as describing the way enzymes work, is the Induced-Fit Hypothesis. It states that the shape of Active Sites are not exactly Complementary, but change shape in the presence of a specific substrate to become Complementary.

- When a substrate molecule collides with an enzyme, if its composition isspecifically correct, the shape of the enzyme's Active Site will change so that the substrate fits into it and an Enzyme-Substrate Complex can form. The reaction is then catalysed and an Enzyme-Product Complexforms.



Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.

Factors affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

- The activity of an Enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which areadapted to function well in extreme conditions where they live.

Temperature

- Increasing temperature increases the Kinetic Energy that moleculespossess. In a fluid, this means that there are more random collisionsbetween molecules per unit time.

- Since enzymes catalyse reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.

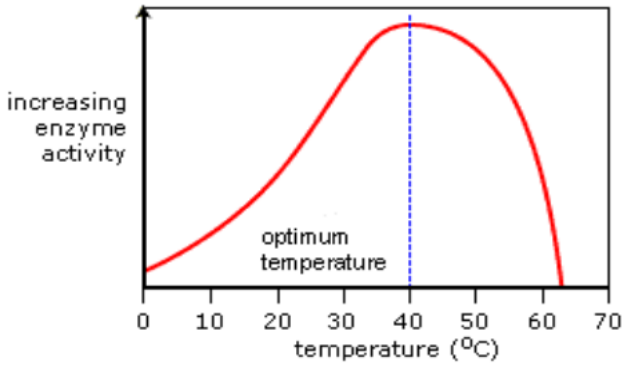
- However, increasing temperature also increases the Vibrational Energythat molecules have, specifically in this case enzyme molecules, which putsstrain on the bonds that hold them together.

- As temperature increases, more bonds, especially the weaker Hydrogenand Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.

- This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become Denatured and will no longer function.

- As temperature increases, more enzymes' molecules' Active Sites' shapes will be less Complementary to the shape of their Substrate, andmore enzymes will be Denatured. This will decrease the rate of reaction.

- In summary, as temperature increases, initially the rate of reaction willincrease, because of increased Kinetic Energy. However, the effect ofbond breaking will become greater and greater, and the rate of reaction will begin to decrease.



- The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. *Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.*

pH - Acidity and Basicity

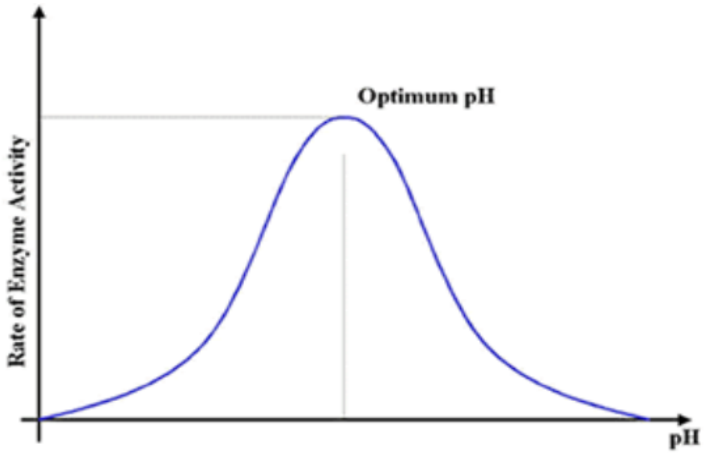
- pH measures the Acidity and Basicity of a solution. It is a measure of theHydrogen Ion (H^+) concentration, and therefore a good indicator of theHydroxide Ion (OH^-) concentration. It ranges from pH1 to pH14. Lower pHvalues mean higher H^+ concentrations and lower OH^- concentrations.

- Acid solutions have pH values below 7, and Basic solutions (alkalis are bases) have pH values above 7. Deionised water is pH7, which is termed 'neutral'.

- H^+ and OH^- Ions are charged and therefore interfere with Hydrogen andIonic bonds that hold together an enzyme, since they will be attracted orrepelled by the charges created by the bonds. This interference causes achange in shape of the enzyme, and importantly, its Active Site.

- Different enzymes have different Optimum pH values. This is the pH value at which the bonds within them are influenced by H^+ and OH^- Ions in such a way that the shape of their Active Site is the most Complementaryto the shape of their Substrate. At the Optimum pH, the rate of reaction is at an optimum.

- Any change in pH above or below the Optimum will quickly cause adecrease in the rate of reaction, since more of the enzyme molecules will have Active Sites whose shapes are not (or at least are less)Complementary to the shape of their Substrate.



- Small changes in pH above or below the Optimum do not cause apermanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to Denature andpermanently lose their function.

- Enzymes in different locations have different Optimum pH values since their environmental conditions may be different. *For example, the enzyme Pepsin functions best at around pH2 and is found in the stomach, which contains Hydrochloric Acid (pH2).*

Concentration

- Changing the **Enzyme** and **Substrate concentrations** affect the **rate** of reaction of an enzyme-catalysed reaction. **Controlling** these factors in a**cell** is one way that an organism **regulates** its **enzyme activity** and so its**Metabolism**.

- Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate.

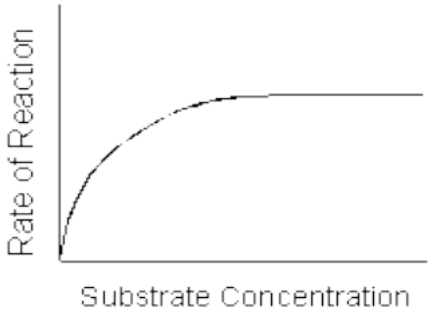
- If it is the limiting factor, increasing concentration will increase the rateof reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will no longer be the limiting factor andanother factor will be limiting the maximum rate of reaction.

- As a reaction proceeds, the rate of reaction will decrease, since theSubstrate will get used up. The highest rate of reaction, known as theInitial Reaction Rate is the maximum reaction rate for an enzyme in anexperimental situation.

Substrate Concentration

- Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.

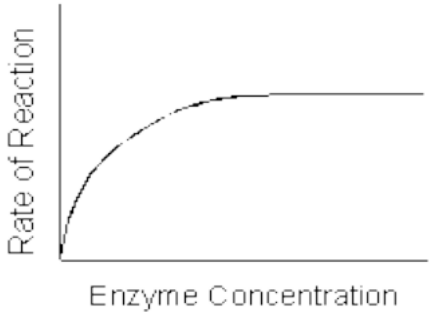
- However, after a certain concentration, any increase will have no effecton the rate of reaction, since Substrate Concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.



Enzyme Concentration

- Increasing Enzyme Concentration will increase the rate of reaction, asmore enzymes will be colliding with substrate molecules.

- However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.



Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity (delta A/delta T). This is represented graphically in Figure 8.

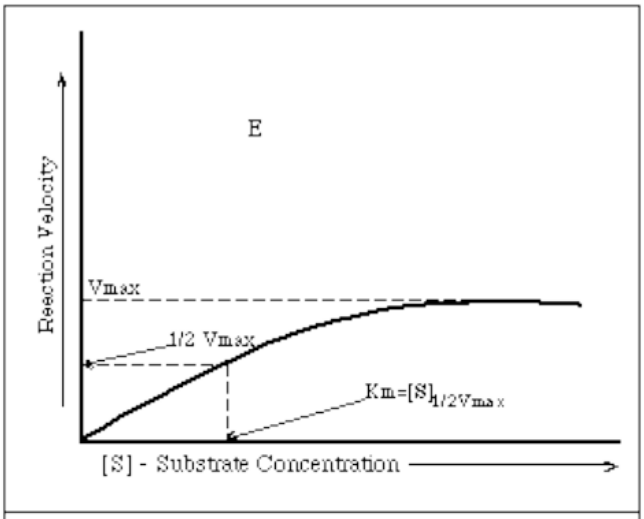


FIGURE 8
Effect of substrate concentration

It is theorized that when this maximum velocity had been reached, all of the available enzyme has been converted to ES, the enzyme substrate complex. This point on the graph is designated Vmax. Using this maximum velocity and equation (7), Michaelis developed a set of mathematical expressions to calculate enzyme activity in terms of reaction speed from measurable laboratory data.

Effects of Inhibitors on Enzyme Activity

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition.

Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. As mentioned earlier, the existence of temporary ES structures has been verified in the laboratory.

Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

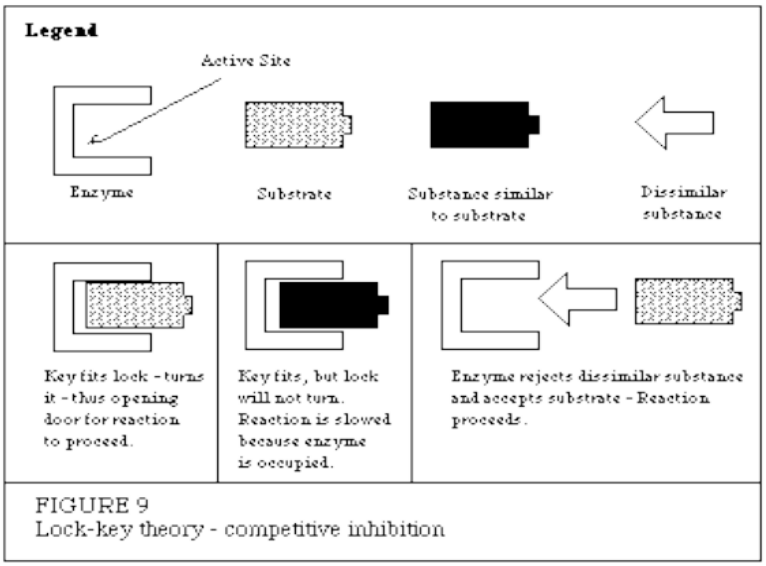


FIGURE 9
Lock-key theory - competitive inhibition

The lock and key theory utilizes the concept of an "active site." The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favorable. If we consider the enzyme as the lock and the substrate the key (Figure 9) - the key is inserted in the lock, is turned, and the door is opened and the reaction proceeds. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the enzyme lock. When the inhibitor wins, it gains the lock position but is unable to open the lock. Hence, the observed reaction is slowed down because some of the available enzyme sites are occupied by the inhibitor. If a dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.

Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate. Figure 10.

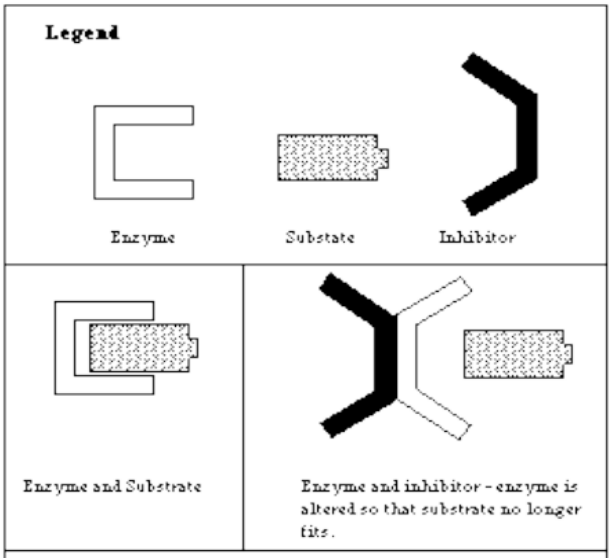


FIGURE 10
Non-competitive inhibition

Substrate inhibition will sometimes occur when excessive amounts of substrate are present. Figure 11 shows the reaction velocity decreasing after the maximum velocity has been reached.

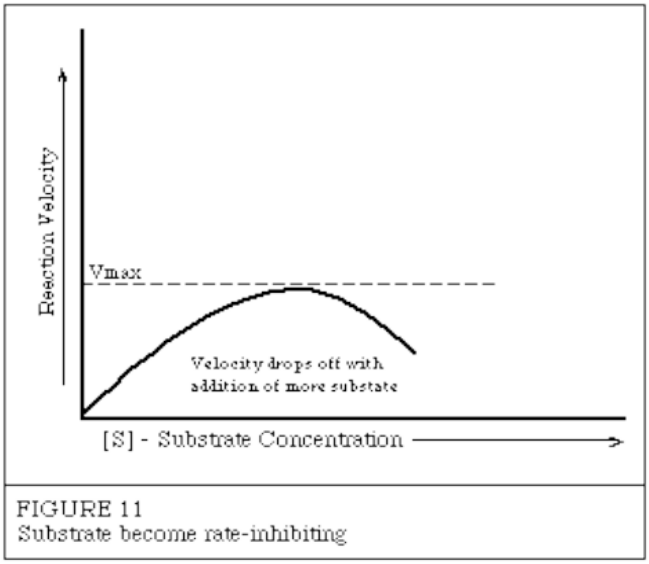


FIGURE 11
Substrate become rate-inhibiting

Additional amounts of substrate added to the reaction mixture after this point actually decrease the reaction rate. This is thought to be due to the fact that there are so many substrate molecules competing for the active sites on the enzyme surfaces that they block the sites (Figure 12) and prevent any other substrate molecules from occupying them.

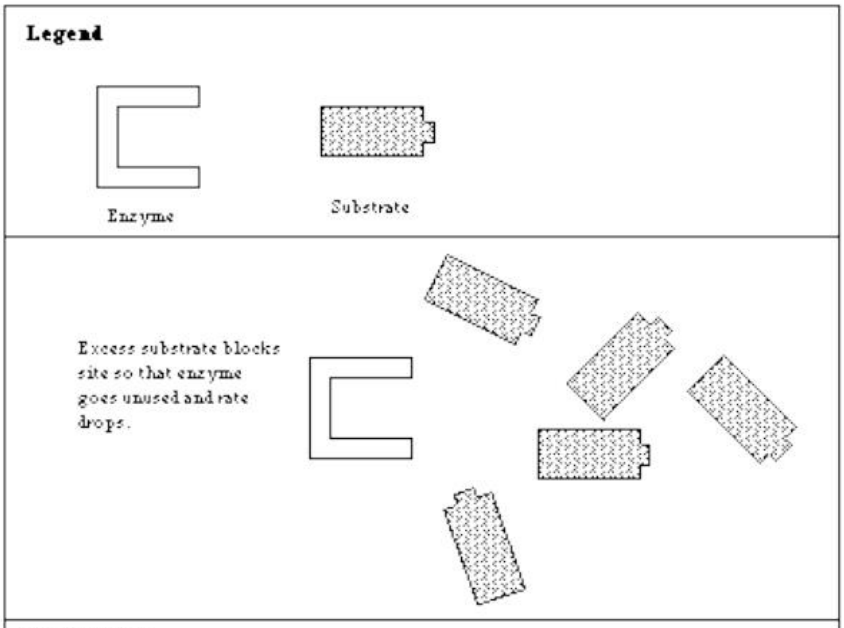


FIGURE 12
Substrate inhibition

This causes the reaction rate to drop since all of the enzyme present is not being used.