University of Baghdad
College of Science – Department of Chemistry

BIOCHEMISTRY (2)

2nd Class - Students of Biology Department – College of Science

2nd Semester

By
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Books

- **Principals of Biochemistry, 4th ed. by Horton *et.al*, 2006.**
- **Biochemistry, 2ed by P. Naik 2007 (JAYPEE).**

Contents:

- Proteins
- Enzymes
- Vitamins

Lecture 1

Proteins

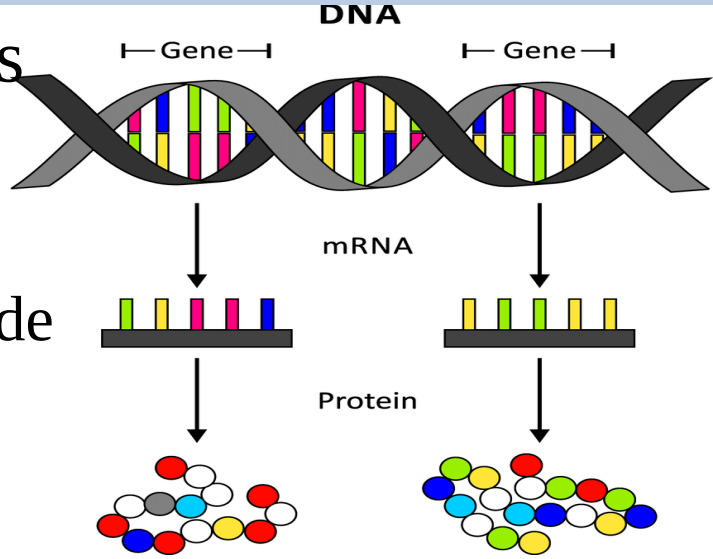
Proteins

Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells.

Proteins also occur in great variety; thousands of different kinds, ranging in size from relatively small peptides to huge polymers with molecular weights in the millions, may be found in a single cell.

Moreover, proteins exhibit enormous diversity of biological function and are the most important final products of the information pathways.

Proteins



Proteins are the molecular instruments through which genetic information is expressed.

Relatively simple monomeric subunits provide the key to the structure of the thousands of different proteins.

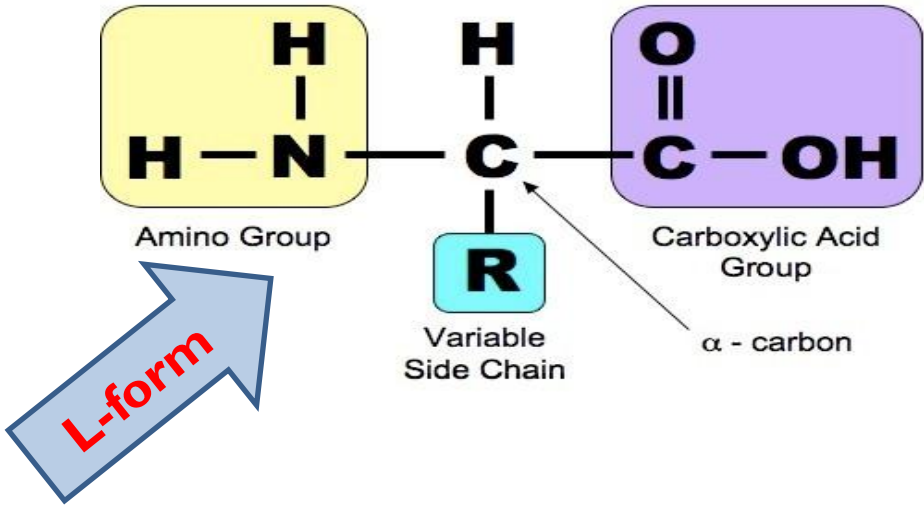
All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same ubiquitous set of **20** amino acids, covalently linked in characteristic linear sequences.

Proteins can be broken down (hydrolyzed) to their constituent amino acids by a variety of methods, and the earliest studies of proteins naturally focused on the free amino acids derived from them.

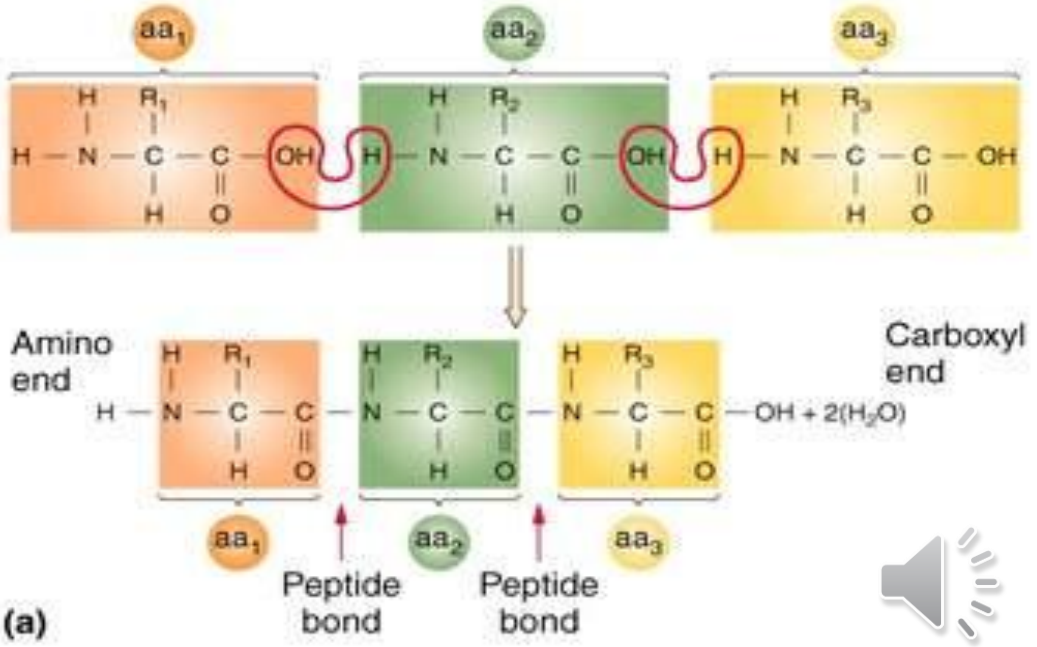
Proteins

Proteins are built from 20 simpler compounds called amino acids.

The amino acids contain a basic amino (NH_2) group at one end and an acid (COOH) group at the other end (L-form)



The amino acids in the protein molecule are linked together through peptide (-CO-NH-) bonds.



Classification of Proteins

Proteins have been classified in several ways

Based on Biological Role (Functions)

1. Catalytic proteins
2. Transport proteins
3. Storage proteins
4. Contractile proteins
5. Structural proteins
6. Defense proteins
7. Regulatory proteins

Based on Molecular shape

1. Fibrous proteins
2. Globular proteins

Based on Physical & Chemical properties

1. Simple proteins
2. Conjugated proteins
3. Derived proteins

Classification of Proteins

A- Classification of proteins Based on Functions

1. Catalytic proteins or enzymes (glucokinase, dehydrogenases, transaminases)
2. Transport proteins (haemoglobin, lipoproteins)
3. Storage proteins (apoferritin, myoglobin)
4. Contractile proteins (actin, myosin)
5. Structural proteins (collagen, elastin, keratin)
6. Defense proteins (immunoglobulins, fibrinogen)
7. Regulatory proteins (insulin, growth hormone)

Classification of Proteins

B- Classification of proteins Based on Molecular shape

On the basis of their shape, proteins may be divided into two classes: Fibrous and Globular.

1. Fibrous Proteins: They are insoluble, high molecular weight fibers (the fibers are long and thin), have axial ratio (length/breadth) greater than 10.

Examples of fibrous proteins: Collagen , Keratin

2. Globular Proteins: These are soluble, relatively low molecular weight. In globular proteins, the polypeptides chain is compactly folded and coiled, have axial ratio (length/breadth) of less than 10.

This group included mainly: Albumin, Globulins, Histones, Actin, Many enzymes.

Lecture 2

Classification of Proteins

C- Classification of proteins Based on Physical & Chemical properties

1. Simple proteins: are defined as those proteins that upon hydrolysis, yield only amino acids or their derivatives. They sub-classified according to their solubility and heat. Such as: Albumins, Globulins, Glutelins, Histones, Protamines.

Classification of Proteins

C- Classification of proteins Based on Physical & Chemical properties

2. Conjugated proteins: are composed of **simple protein** combined with some non-protein substance. The non-protein group is referee to as the ***prosthetic*** (additional) group.

The examples are:

a. Nucleoproteins: are composed of simple basic proteins (histones or protamines) in salt combination with nucleic acids (RNA and DNA) as the prosthetic groups. They are proteins of cell nuclei eg. (nucleohistone and nucleoprotamine).

Classification of Proteins

b. Glycoproteins and Proteoglycans: these consist of simple protein and carbohydrates as a *prosthetic* group.

- When carbohydrates content is less than 4% of protein it is called **glycoproteins** (mucin of saliva, hormones like TSH, FSH, LH).

- When carbohydrates content is more than 4% of protein it is called **proteoglycans** (glycosaminoglycans).

c. Chromoproteins: are composed of simple protein with a colored *prosthetic* group (haemoglobin, cytochromes, catalase).

d. Phosphoprotein: are found by a combination of protein with *prosthetic* group phosphoric acid (Casein of milk, Vitellin of egg yolk).

Classification of Proteins

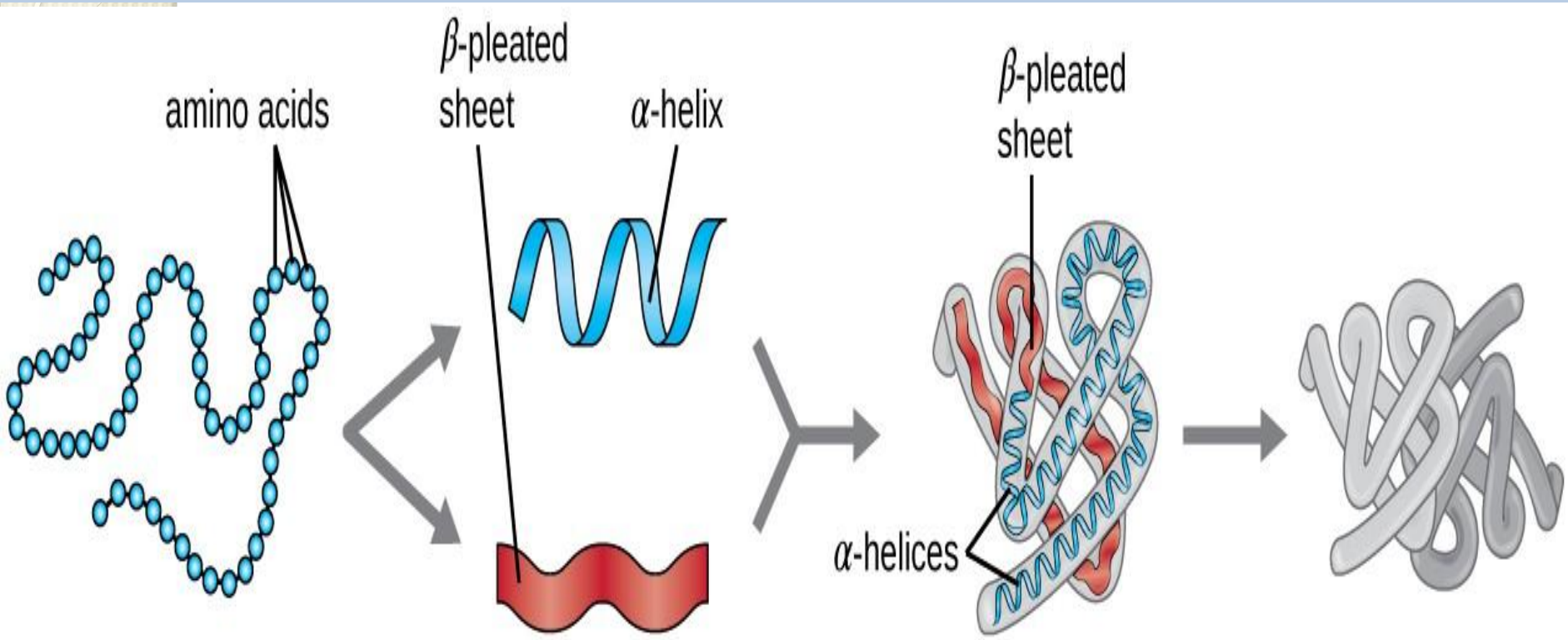
e. Lipoproteins: are formed by a combination of protein with *prosthetic* group Lipid (very low density lipoprotein VLDL, low density lipoprotein LDL, high density lipopretein HDL).

f. Metaloproteins: The *prosthetic* group is metalic elements such as: (Fe, Co, Mn, Zn, Cu). For example: (haemoglobin contain Iron, ceruloplasmin content Copper).

3. Derived proteins: This class of proteins as the name implies, includes those substances formed from simple and conjugated proteins. Derived proteins are subdivided into:

- Primary derived proteins
- Secondary derived proteins

Structure of Protein



Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

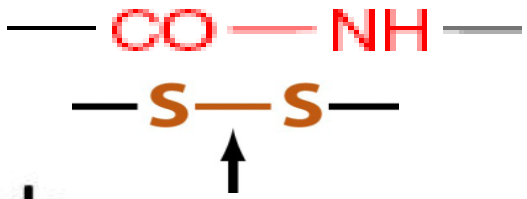
Quaternary Protein Structure
protein consisting of more than one amino acid chain

Bonds responsible for protein structure

Proteins are stablised by **two** types of bonds:-
covalent and non-covalent bonds

Covalent bonds

- Peptide bonds
- Disulphide bonds



Non – covalent bonds

- Hydrogen bonds
- Hydrophobic bonds
- Van der waals forces
- Electrostatic forces

Lecture 3

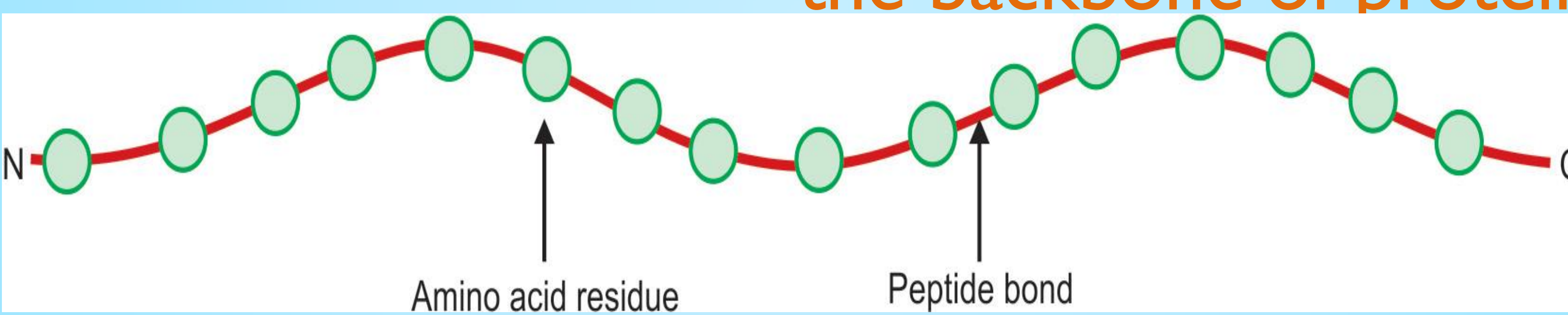
STRUCTURE OF PROTEINS

Proteins are polymers [specifically polypeptides] formed from sequences of amino acids, the monomers of the polymer.

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure.

Primary Structure of Proteins

- The number and sequence of amino acids forming the backbone of proteins.



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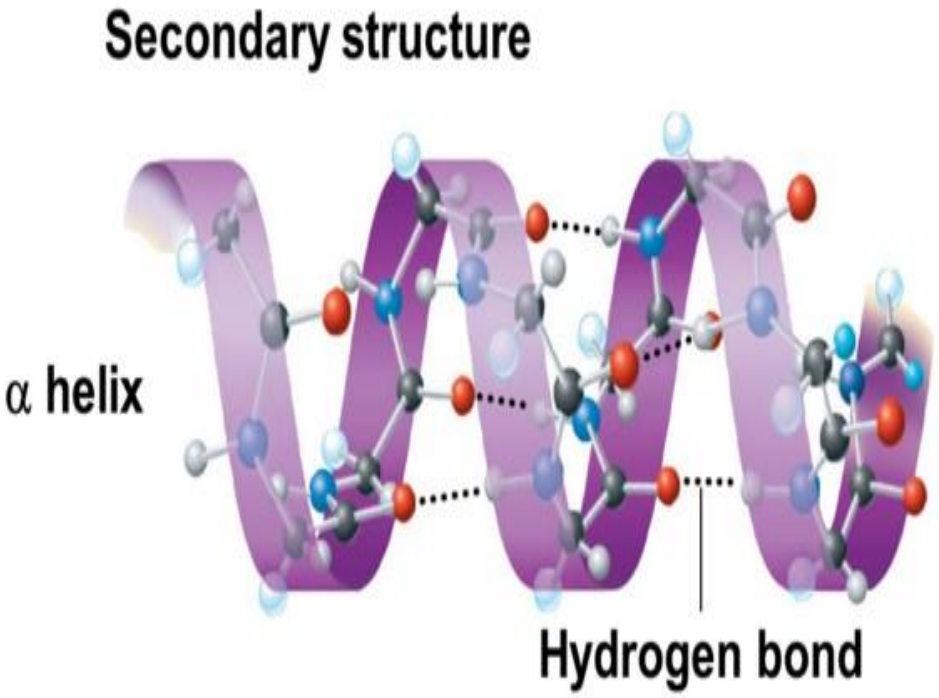
Secondary Structure of Proteins

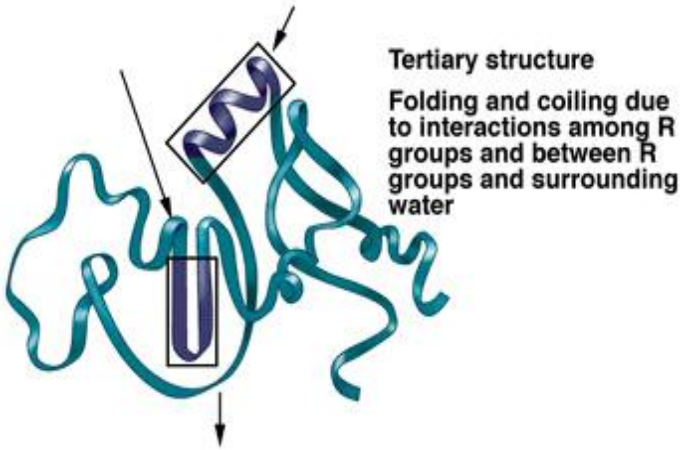
• Regular folding and twisting of the polypeptide chain brought about by **hydrogen bonding**(between the hydrogen of NH (amide) and oxygen of C=O (carbonyl) groups within the polypeptide chain).

 # kinds of secondary stru

– α-Helix
– β-Pleated sheet
– β-bends or β-turn
– loop regions
– Disordered regions.
- Triple helix



Tertiary Structure of Proteins



- The peptide chain, with its secondary structure, may be further folded and twisted about itself forming three-dimensional arrangement.

• Amino acid residues which are very distant from one another in the sequence can be brought very near due to the folding and thus form regions essential for the functioning of the protein, like active site of enzymes.

• Thus, the three dimensional folded compact and **biologically active conformation of a protein** **is refer to its tertiary structure**.

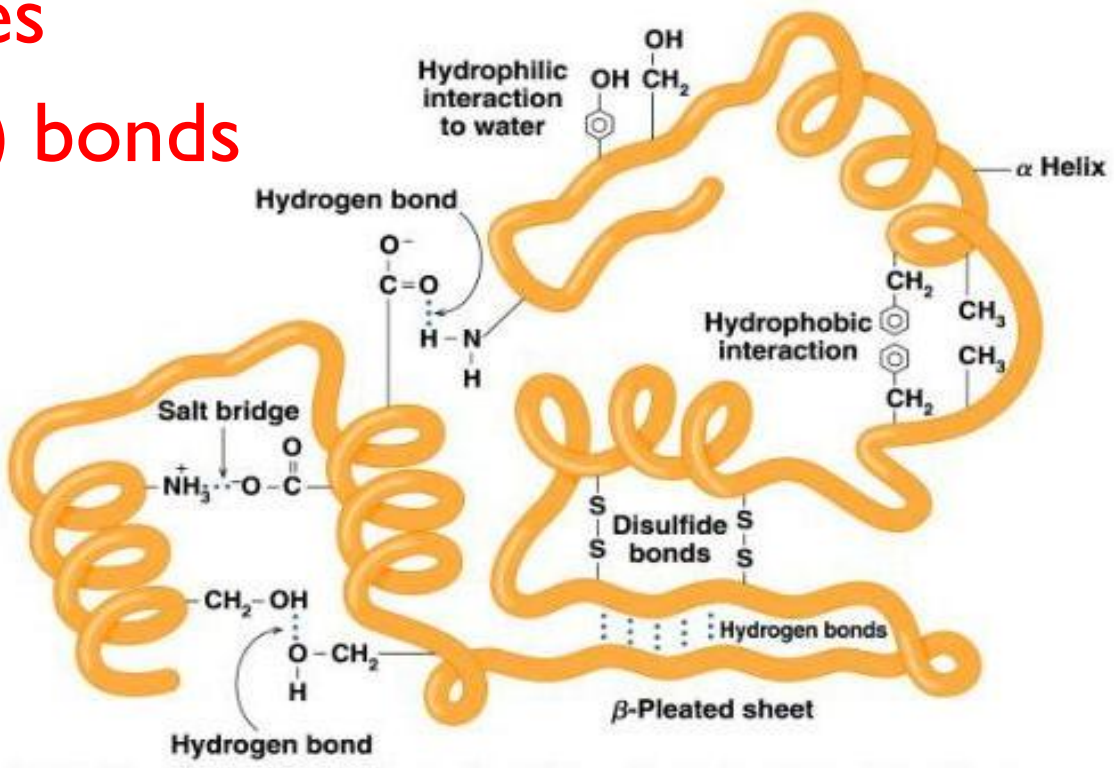
- Every protein has a unique three-dimensional structure that reflects its function.

- ***The three dimensional folded compact and biologically active conformation of protein it referred to its Tertiary structure***

Tertiary Structure Stabilizing Forces

- Disulfide bond
- Hydrogen bonds
- Hydrophobic interactions
- Van der Waals forces
- Ionic (electrostatic) bonds

or salt bridges.

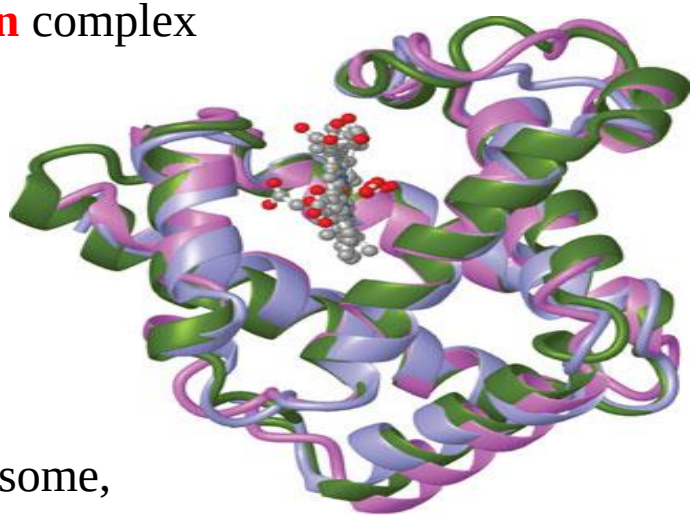


Lecture 4

Protein folding & Stability

Protein folding is the physical process by which a protein chain acquires its native 3D structure, a conformation that is usually biologically functional.

New polypeptides are synthesized in the cell by a **translation** complex that includes ribosomes, mRNA, and various factors.



Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids.

As the newly synthesized polypeptide emerges from the ribosome, it **folds** into its characteristic three-dimensional shape.

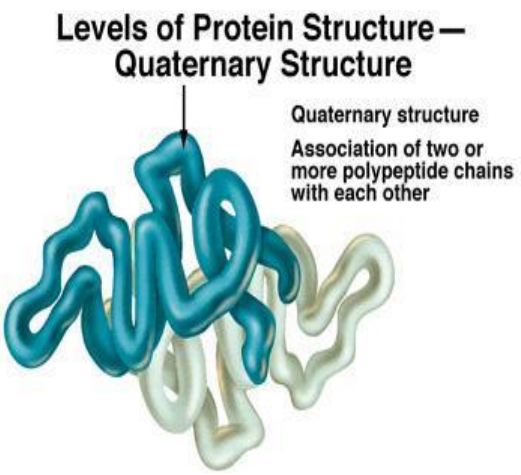
Folded proteins to produce a well-defined three-dimensional structure, known as the **native state or native structure**

Every protein in its **native state** has a unique three dimensional structure which is referred to as its **conformation** and made up of only 20 different amino acids. The number and sequence of these amino acids are different in different proteins.

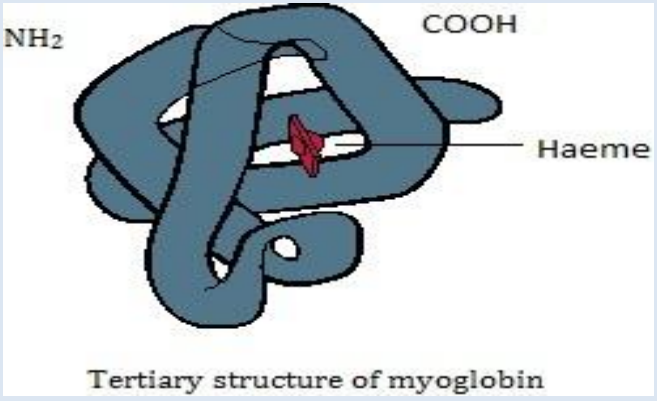
Quaternary Structure of Protein

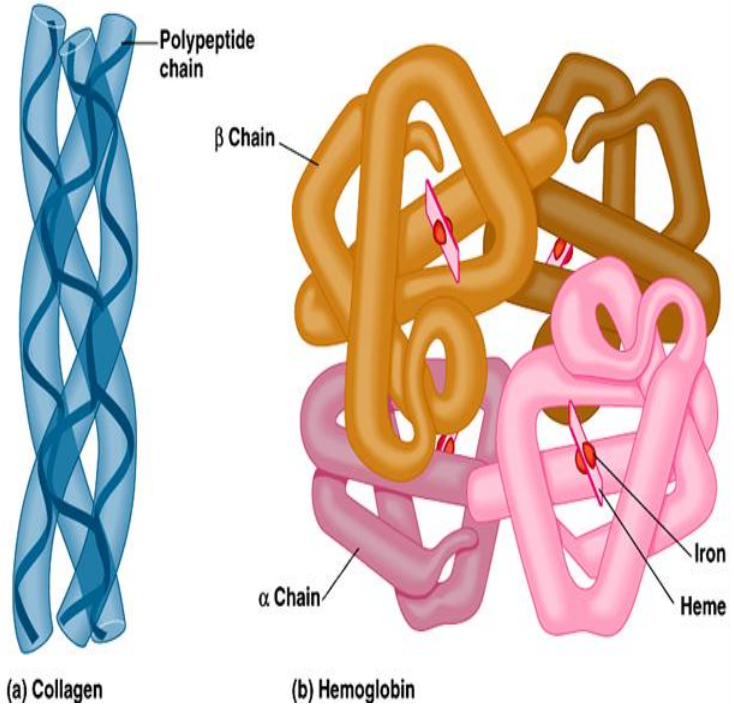
- Only proteins that have **more than one polypeptide chain** (**polymeric**) have a quaternary structure.
- The arrangement of these polymeric polypeptide subunits in three-dimensional complexes is called the quaternary structure of the protein.

• Examples: Collagen and Hemoglobin.



- Many proteins consist of a **single polypeptide chain and are called monomeric** proteins, e.g. myoglobin.



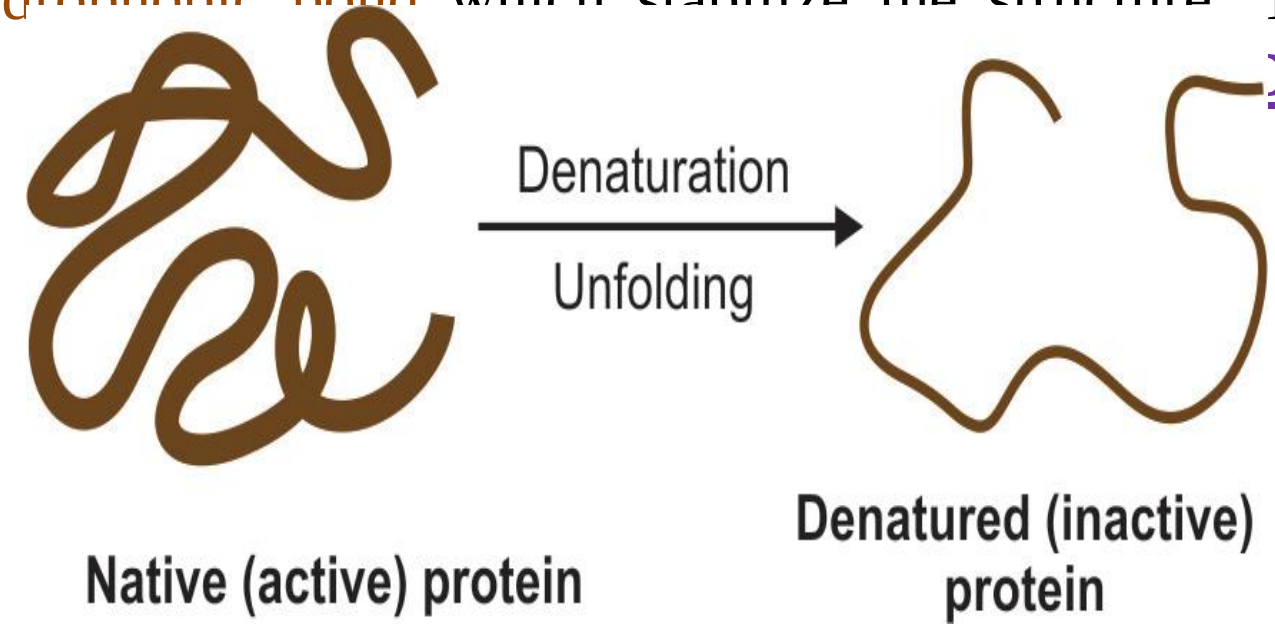


 # Quaternary Structure Stabilizing Forces (noncovalent interactions):

- Hydrophobic interactions
- Hydrogen bond
- Ionic bonds

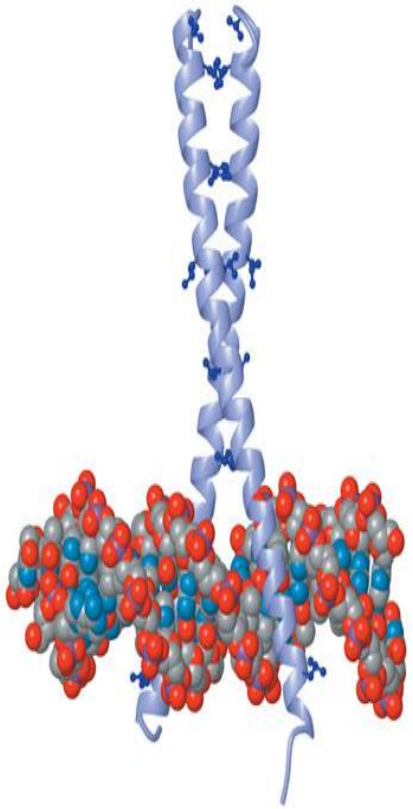
DENATURATION OF PROTEINS

The three-dimensional conformation, the **primary, secondary, tertiary** and even in some cases **quaternary** structure is characteristic of a native protein. **Hydrogen bond, ionic bond** and **hydrophobic bond** stabilize the structure to maintain its conformation in space. This conformation can upset and disorganized **without breakage of any peptide linkage,** only by the rupture of ionic bond, hydrogen bonds and hydrophobic bond which stabilize the structure. This is called **Denaturation.**



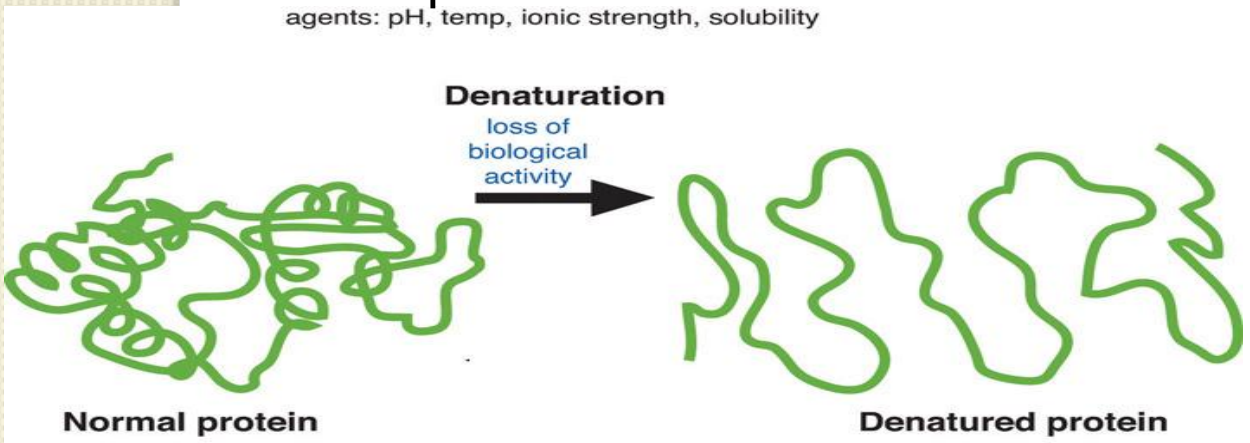
Denaturation of proteins leads to:

- Unfolding of natural coils of native protein.
- Decrease in solubility and increase in precipitability.
- Loss of biological activities, (e.g. enzyme activity) and antigenic properties.
- Increased digestibility.



Denaturing Agents

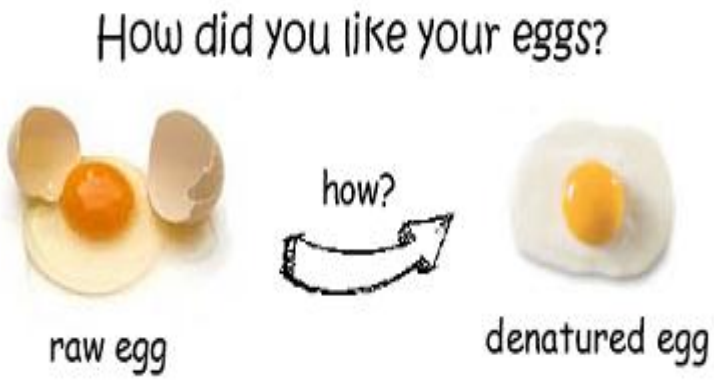
- **Physical agents :** Heat, Ultraviolet rays and ionizing radiations
- **Chemical agents :** Acids, alkalies and certain acid solutions of heavy metals, e.g. mercury, lead, detergents; organic solvents like alcohol, acetone, etc.
- **Mechanical means :** Vigorous shaking or grinding leads to denaturation of the protein.



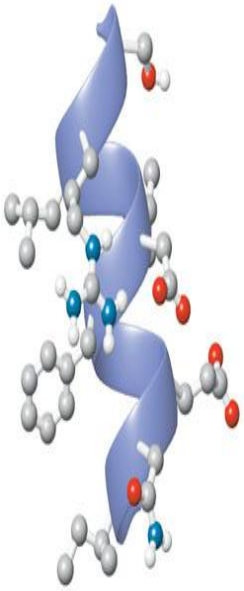
▲ Christian B. Anfinsen (1916–1995). Anfinsen was awarded the Nobel Prize in Chemistry in 1972 for his work on the refolding of proteins.

❖ **Examples of Denatured Protein**

Cooked meat , boiled egg, etc.



Significance of Denaturation



1. Digestibility of native protein is increased on denaturation

by gastric HCl or by heat on cooking.

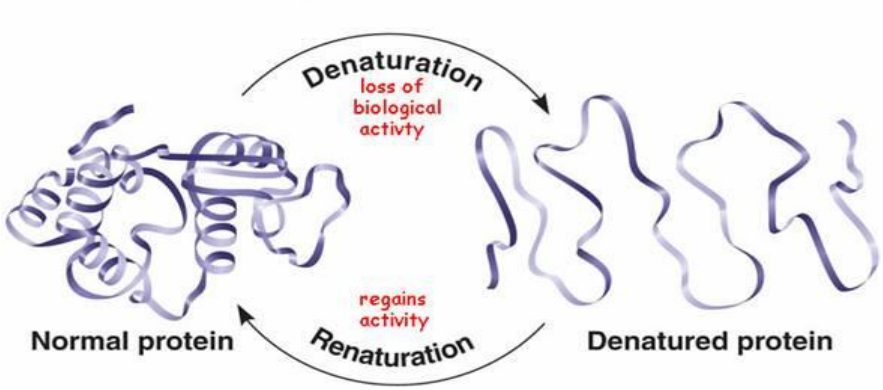
2. Denaturation causes unfolding of native polypeptide coil so that hidden peptide bonds are exposed to the action of proteolytic enzyme in the gut. It also increases reactivity of certain groups.

3. Denaturation property of a protein is used in blood analysis to eliminate the proteins of the blood (deproteinization of blood).

Lecture 5

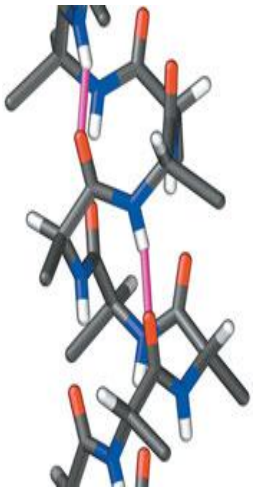
Coagulation

- Denaturation may, in rare cases be **reversible**, in which case the protein refolds into its original native structure, when the denaturing agent is removed.



- However, most proteins, once denatured, remain permanently disordered and are called **irreversible denaturations** or **coagulation**, e.g. coagulated egg white of boiled egg.

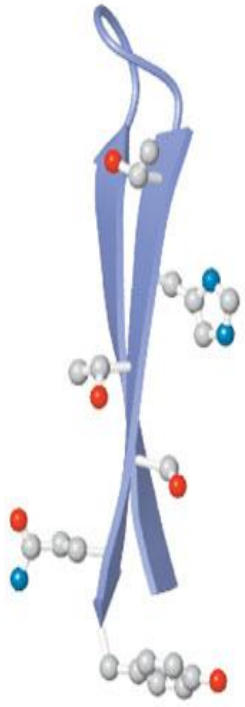
Protein Separation and Purification



Protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms.

Proteins are separated and purified on the basis of differences in their properties, such as:

- solubility,
- molecular size,
- molecular charge,
- specific binding of the protein to a specific substances.

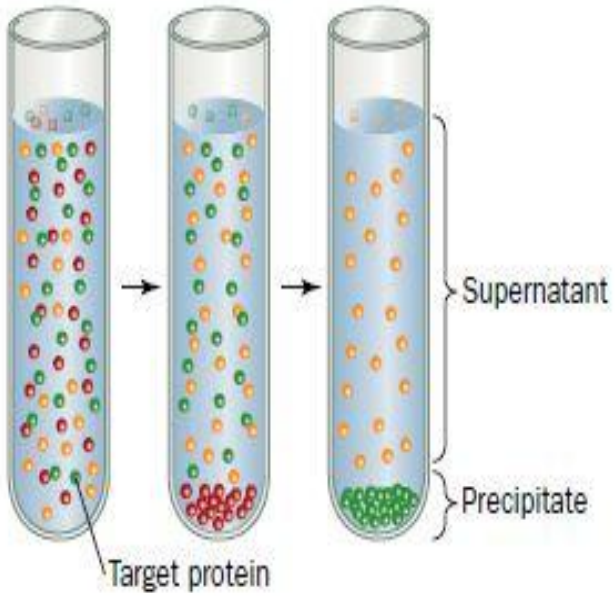


1. Separation of proteins based on solubility:

The solubility of protein is dependent on the salt concentration of the solution.

- **Salting in**: the solubility of a protein increased by addition of salt at low concentration.
- **Salting out**: the solubility of a protein decreased by addition of salt at high concentration,

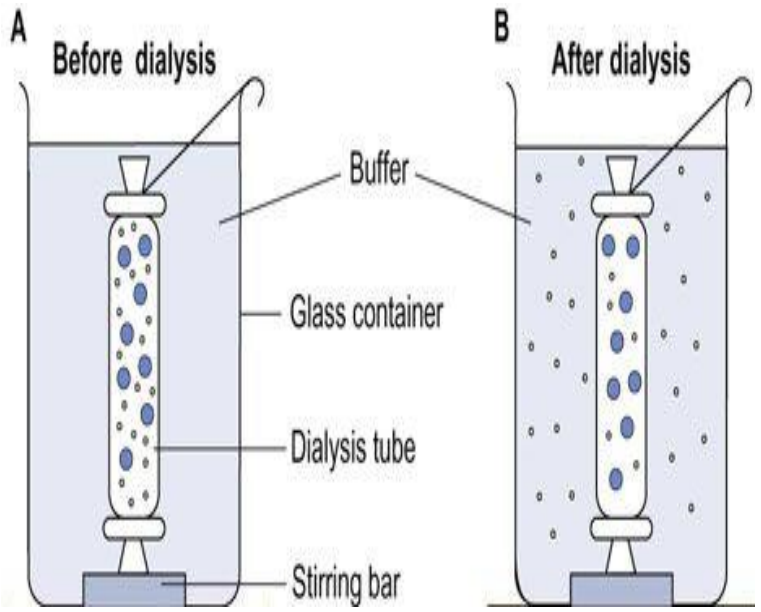
such as, ammonium sulfate or sodium sulfate are added to a protein solution, the addition may precipitate a protein from its solution.



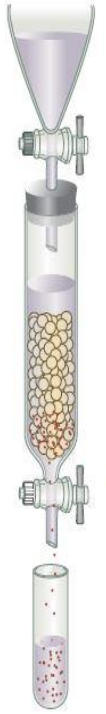
2. Separation of proteins based on molecular size:

- **Dialysis** is a procedure that separates proteins from small solutes by taking advantage of the proteins' larger size.

The extract is placed in a bag or tube made of a semipermeable membrane. When this is suspended in a much larger volume of buffered solution, the membrane allows the exchange of salt and buffer but not proteins. Thus dialysis retains large proteins within the membranous bag or tube, while allowing the concentration of other solutes in the protein preparation to change until they come into equilibrium with the solution outside the membrane.

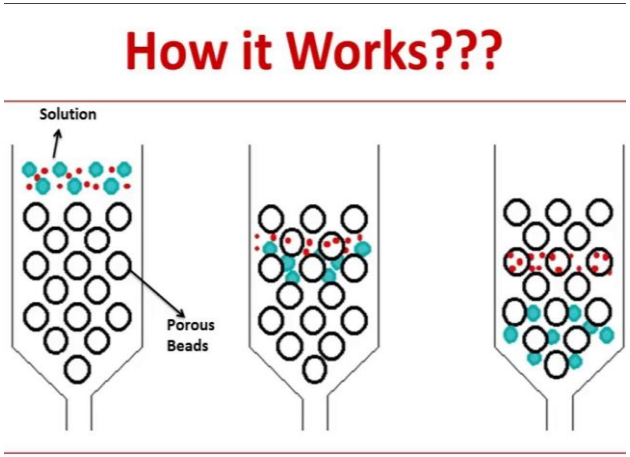


2. Separation of proteins based on molecular size:

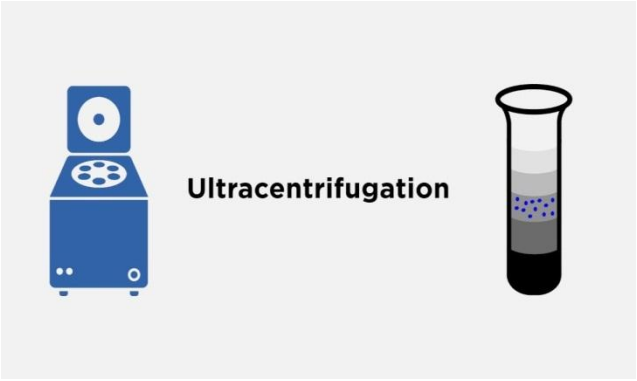


Chromatography, the separation of soluble substances by their rate of movement through an insoluble matrix, is a technique for purifying molecules by charge (ion exchange chromatography), hydrophobicity (hydrophobic interaction chromatography), size (gel filtration chromatography),and binding specificity (affinity chromatography).

- **Gel filtration: Size-exclusion chromatography** In this method, large proteins emerge from the column sooner than small. The solid phase consists of cross-linked polymer beads with engineered pores or cavities of a particular size.



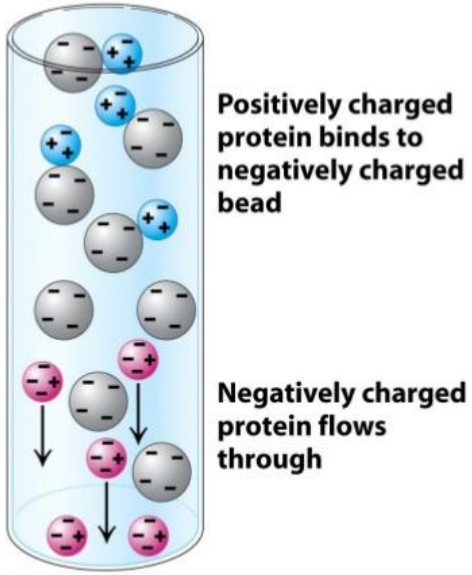
- **Ultracentrifugation:** is a high speed centrifuge able to separate mixtures of particles of varying masses or densities suspended in a liquid.



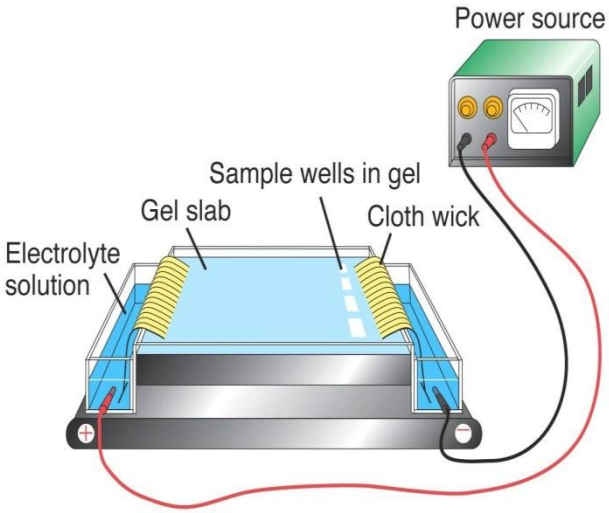
3. Separation of proteins based on molecular charge:

- **Ion Exchange Chromatography:** is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger.

Separates Anions and Cations charged molecules bind to oppositely charged groups that are chemically linked to a matrix such as: cellulose or agarose.



- **Electrophoresis:** another important technique for the separation of proteins is based on the migration of charged proteins ir an electric field. Electrophoresis of proteins is generally carried out in gels made up of the cross-linked polymer Polyacrylamide (PAGE: Polyacrylamide gel electrophoresis).



Enzymes

Lecture 6

Enzymes

Enzymes, the catalysts of biological systems, are remarkable molecular devices that determine the patterns of chemical transformations.

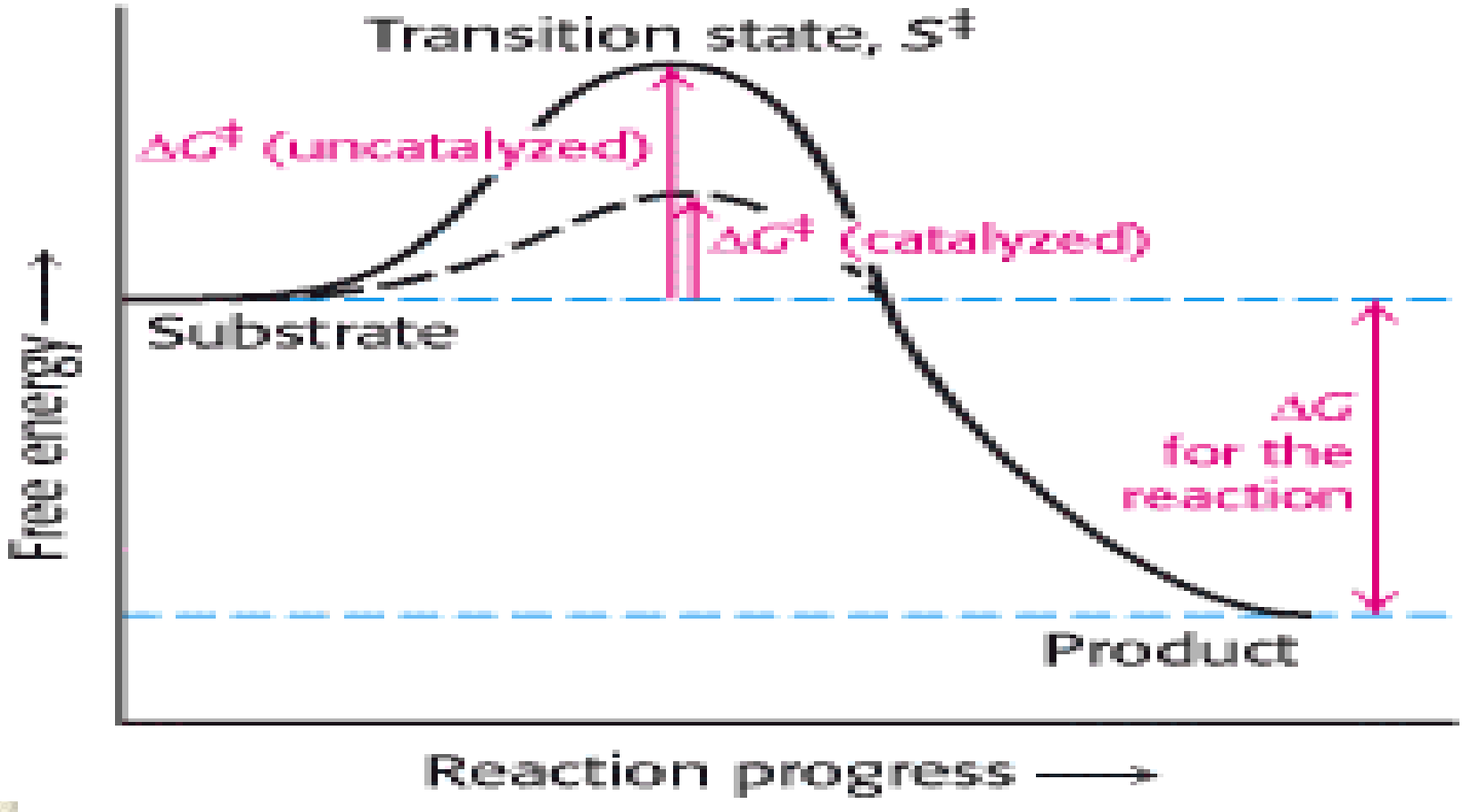
They also mediate the transformation of one form of energy into another.

The most striking characteristics of enzymes are their **catalytic power** and **specificity**.

Catalysis takes place at a particular site on the enzyme called the **active site**.

Enzymes

Nearly all known enzymes are **proteins**. However, proteins do not have an absolute monopoly on catalysis; the discovery of catalytically active RNA molecules provides compelling evidence that RNA was an early biocatalyst.



Enzymes

The catalytic activity of many enzymes depends on the presence of small molecules termed ***Cofactors (nonprotein component)*** , *although the* precise role varies with the cofactor and the enzyme.

Such an enzyme without its cofactor is referred to as an ***Apoenzyme; (protein component)*** the complete, catalytically active enzyme is called a ***Holoenzyme***.

Apoenzyme + cofactor = holoenzyme

Enzymes

Cofactors can be subdivided into two groups:

1- metals (it is called a **cofactor or activators**)

For example, the enzyme **carbonic anhydrase**, requires **Zn^{2+}** and **Superoxide dismutase** requires **Mn^{2+}** , for its activity .

2- small organic molecules (it is termed a **coenzyme** *often derived from vitamins*).

Glycogen phosphorylase , which mobilizes glycogen for energy, requires the small organic molecule pyridoxal phosphate (PLP).

Monoamine oxidase requires [Flavin adenine dinucleotide (FAD)]

Lactate dehydrogenase requires [Nicotinamide adenine dinucleotide (NAD)]

Acetyl CoA carboxylase requires [Coenzyme A (CoA)]

Enzymes

Cofactors that are small organic molecules are called ***coenzymes***.

Coenzymes can be either **tightly** or **loosely** bound to the enzyme.

If tightly bound, they are called ***prosthetic groups***.

Loosely associated coenzymes are more like **cosubstrates** because they bind to and are released from the enzyme just as substrates and products are.

Flavin adenine dinucleotide (FAD) contains **Riboflavin (vitamin B2)**

Nicotinamide adenine dinucleotide (NAD) contains **Niacin (vitamin B3)**

Coenzyme A (CoA) contains **pantothenic acid (vitamin B5)**

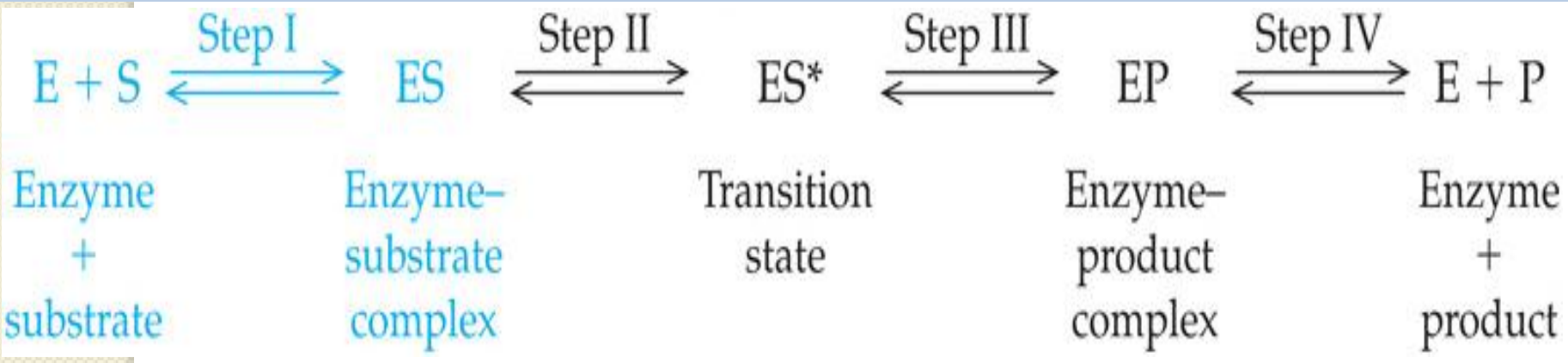
Enzymes

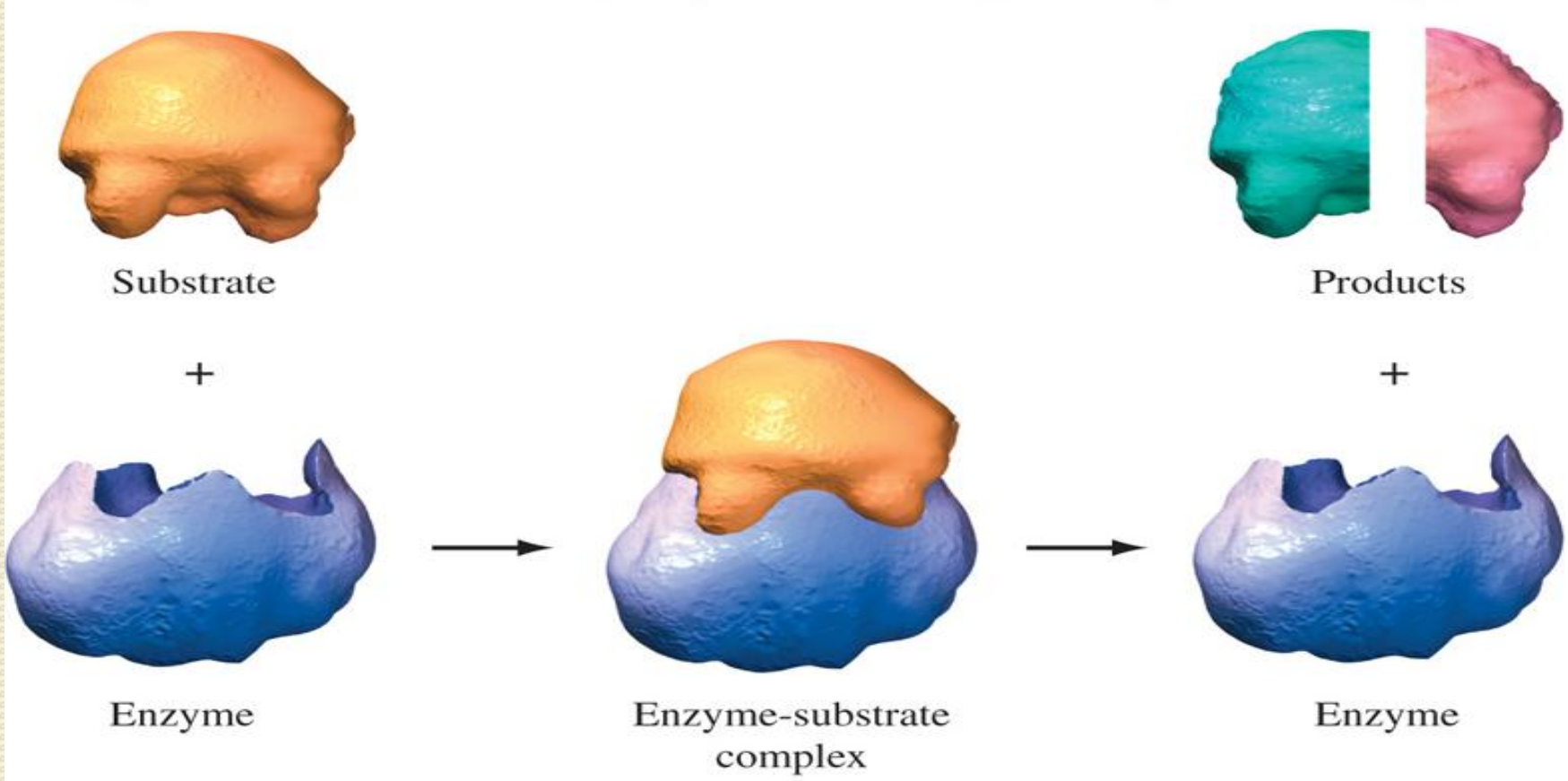
Much of the catalytic power of enzymes comes from their bringing substrates together in favorable orientations to promote the formation of the transition states in ***enzyme-substrate (ES) complexes***.

The substrates are bound to a specific region of the enzyme called the ***active site***.

Most enzymes are highly selective in the substrates that they bind. Indeed, the catalytic specificity of enzymes depends in part on the specificity of binding

Enzyme Catalysis





Lecture 7

Models of Enzyme action (mechanism)

The specificity of binding depends on the precisely defined arrangement of atoms in an active site. Because the enzyme and the substrate interact by means of short-range forces that require close contact, a substrate must have a matching shape to fit into the site.

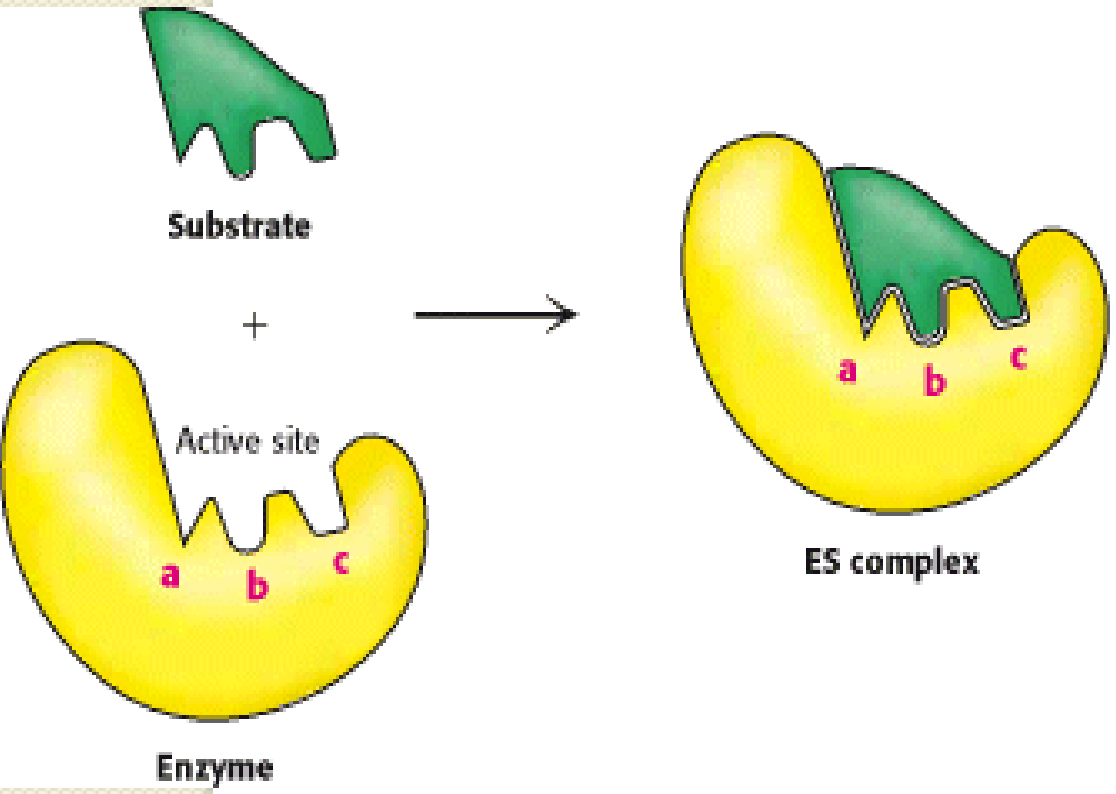
The models of Enzyme action (mechanism)

1- Lock and Key model (rigid templet model)

2- Induced fit model (hand in glove model)

1- Lock and Key model (rigid templet model)

Emil Fischer's analogy of the lock and key, expressed in 1894, has proved to be highly stimulating and fruitful. However, we now know that enzymes are flexible and that the shapes of the active sites can be markedly modified by the binding of substrate.



Lock-and-Key Model of Enzyme-Substrate Binding

A. Substrate (key) fits into a perfectly shaped space in the enzyme (lock)

B. As we've said, there is lots of similarity between the shape of the enzyme and the shape of the substrate

C. Highly stereospecific

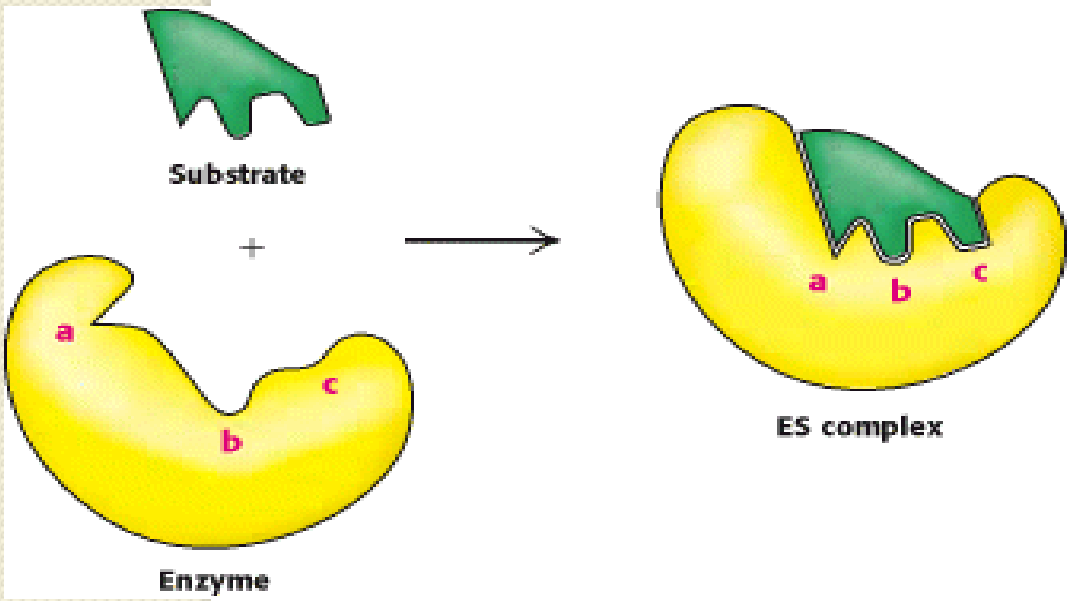
D. Implies a very RIGID inflexible active site

E. Site is preformed and rigid

2- Induced fit model (hand in glove model)

It was postulated by Daniel E. Koshland, Jr., in 1958, suggested a modification to the lock and key model.

The active sites of some enzymes assume a shape that is complementary to that of the transition state only after the substrate is bound. This process of dynamic recognition is called induced fit.



Induced-Fit Model of Enzyme-Substrate Binding

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

A. Takes into account the flexibility of proteins

B. A substrate fits into a general **shape** in the enzyme, causing the enzyme to change shape (conformation); close but not perfect fit of E + S

C. Change in protein configuration leads to a near perfect fit of substrate with enzyme

Specificity of Enzymes

Specificity:

Specificity of an enzyme depends on its own shape, charge and hydrophobic/hydrophilic characteristics of the enzyme. Enzymes are usually impressively specific in their action. The specificity toward substrate is sometimes almost absolute.

Types of specificity

1- Absolute Specificity: the enzyme will catalyze only one reaction

2-Group Specificity: the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

3- Reaction Specificity: the enzyme will act on a specific reaction.

4- Stereo Specificity: the enzyme will act on a particular steric or optical isomer

Lecture 8

Enzymes : Nomenclature

A system of enzyme nomenclature that is comprehensive, consistent, and at the same time easy to use has proved elusive.

The common names for most enzymes derive from their most distinctive characteristic: their ability to catalyze a specific chemical reaction.

In general, an enzyme's name consists of a term that identifies the type of reaction catalyzed followed by the suffix -***ase***.

For example, dehydrogenases remove hydrogen atoms, *proteases* hydrolyze proteins,

and isomerases catalyze rearrangements in configuration.

Enzymes : Nomenclature

The International Union of Biochemists (IUB) developed a complex but unambiguous system of enzyme nomenclature. In the IUB system, each enzyme has a unique name and code number that reflect the type of reaction catalyzed and the substrates involved.

Enzyme Commission number - EC number [a.b.c.d] consist of four digits

Example:

- Hexokinase [EC 2.7.1.1]

-Transferase (EC2) that adds a phosphate group (EC 2.7) to a hexose sugar, a molecule containing an alcohol group (EC 2.7.1.1)

Enzymes Classification

Enzymes are grouped into six classes, each with several subclasses.

1- Oxidoreductases: catalyze oxidation-reduction reaction. (EC 1)

(يحفز تفاعلات الاكسدة والاختزال ينقل H او O او الكترونات من مادة الى اخرى)

2- Transferases: catalyze transfer of groups C-, N- or P- such as: (EC 2)

methyl or glycosyl groups from a donor molecule to an acceptor molecule.

(ينقل مجموعة وظيفية من مادة الى اخرى مثل مجموعة مثيل او فوسفات او امينو)

Enzymes Classification

3. Hydrolases: catalyze the hydrolytic cleavage of (EC 3)

C-C, C-O, C-N, P-O, and certain other bonds, including acid anhydride bonds.

(يحفز تفاعلات التحلل المائي للعديد من الروابط)

4. Lyases: catalyze cleavage of C-C, C-S, C-N, (EC 4)

and other bonds by elimination, leaving double bonds, and also add groups to double bonds.

(فك روابط بوسائل غير التحلل المائي والاكسدة مثل C-C, C-S, C-N)

Enzymes Classification

5. Isomerases: catalyze geometric or structural (EC 5)

changes within a single molecule.

(تحفيز التغيرات الهندسية أو الهيكلية داخل جزيء واحد)

6. Ligases: catalyze the joining together of two molecules, (EC 6)

coupled to the hydrolysis of a pyrophosphoryl group in ATP or a similar nucleoside triphosphate.

تحفيز ربط جزيئتين بواسطة الروابط التساهمية مثل C-C, C-S, C-N

Enzyme Kinetics

Enzyme kinetics is the study of the **chemical reactions** that are catalyzed by enzymes.

In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme

Enzyme Kinetics

-The ***VELOCITY*** **(reaction rate) (product formation of disappearance of substrate/time)** of an enzyme catalyzed reaction is dependent upon the substrate concentration [S].

The velocity (V) of an enzyme-catalyzed reaction is dependent upon the substrate concentration [S]

Michaelis-Menten plot

The **Michaelis-Menten equation describes the kinetic behavior of many enzymes**

$$E + S \underset{k-1}{\overset{k1}{\leftrightarrow}} ES \xrightarrow{k2} E + P$$

Michaelis-Menten Equation (equation for a hyperbola)

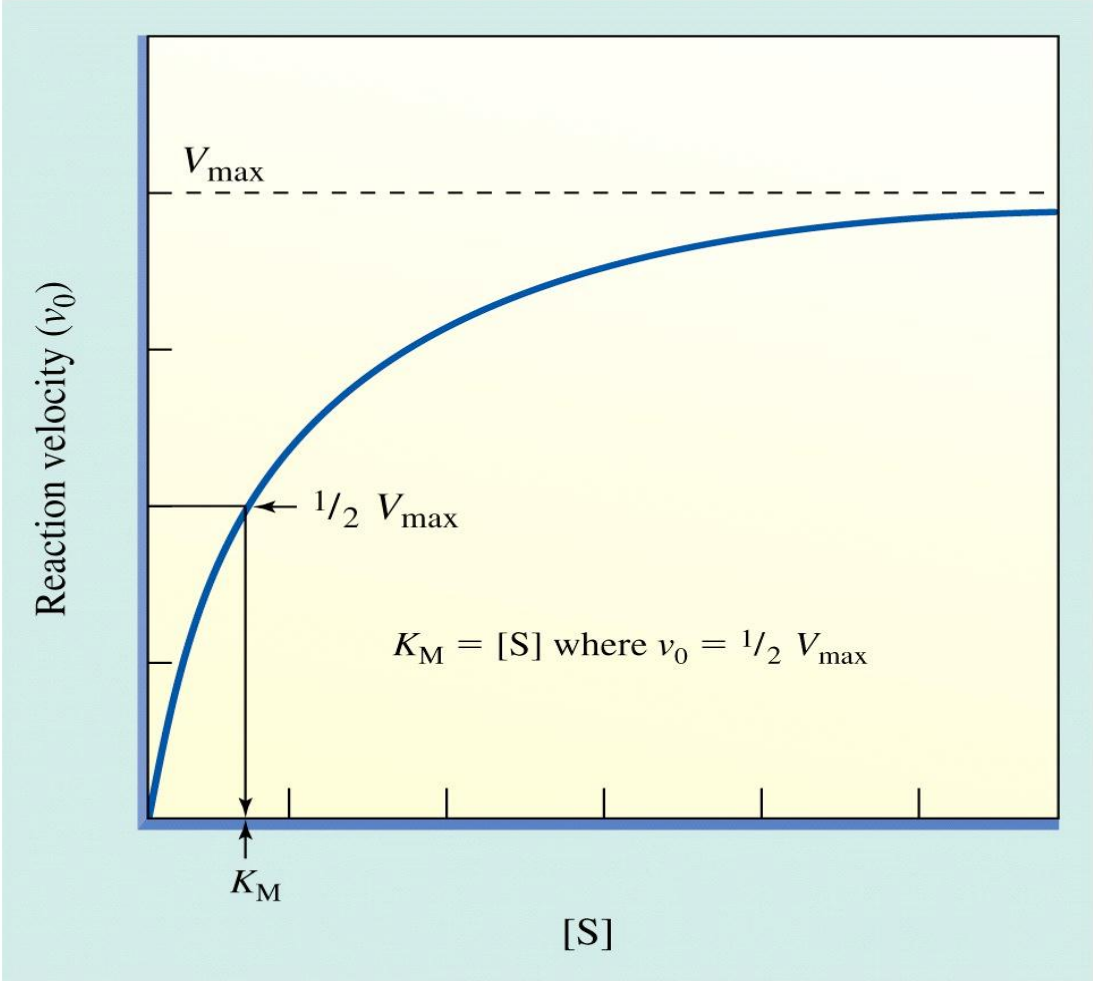
$$V = \frac{Vmax\,[S]}{K_M + [S]}$$

V: is the reaction rate (velocity)

[S]: substrate concentration

Vmax: is the maximum velocity

K_M: is the Michaelis constant = [S] when v =0.5 V max



Enzyme Kinetics

For certain enzymes under certain conditions, K_M can also be a measure of affinity between E and S – approximates the dissociation constant of the ES complex

-If K_M is LOW (small number) = Substrate is held tightly

(HIGH affinity)

1. Reaches Vmax at a lower [S]

2. Small number means less than 10^{-3}M

- If K_M is HIGH (large number) =

Substrate is held weakly (LOW affinity)

1. Reaches Vmax at a higher [S]

2. Large number means 10^{-1} – 10^{-3}M

Lineweaver-Burk Plot

Michaelis-Menten plot is not useful for estimating K_M and Vmax

• it is better to transform the Michaelis-Menten equation to a linear form (Lineweaver-Burk equation obtained by taking the reciprocal of the Michaelis-Menten equation)

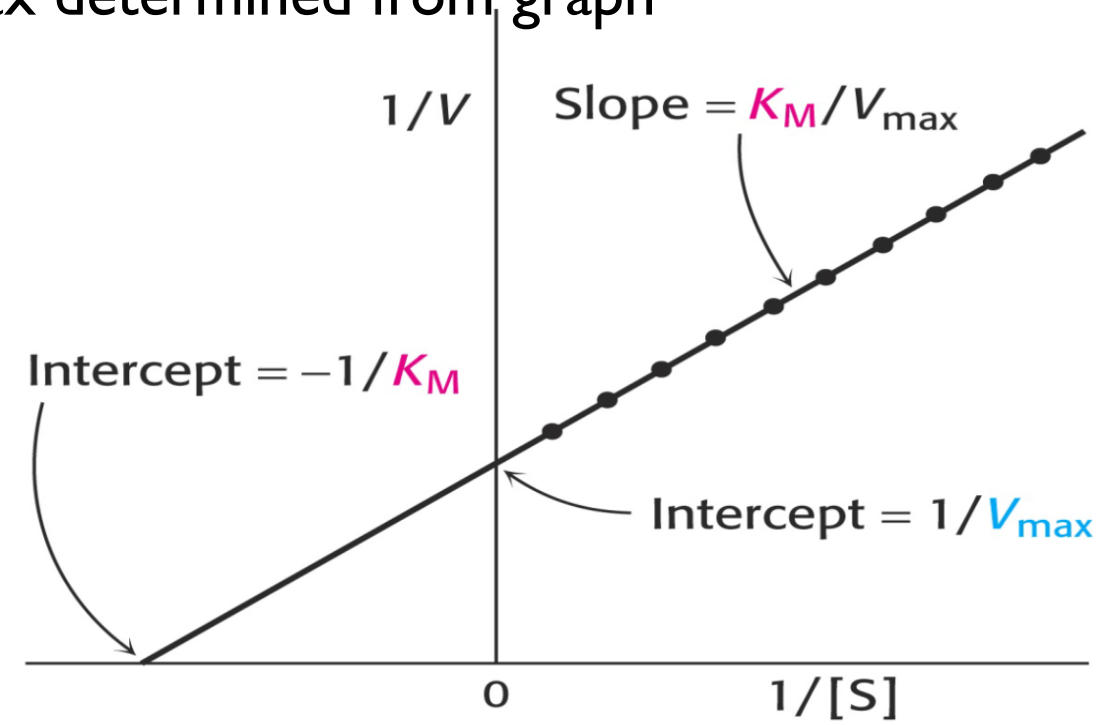
– actual values for K_M and Vmax determined from graph

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

$$\frac{1}{V} = \frac{KM}{Vmax\,[S]} + \frac{[S]}{Vmax\,[S]}$$

Lineweaver-Burk equation

$$\frac{1}{V0} = \frac{Km}{Vmax\,[S]} + \frac{1}{Vmax}$$



same form as y = mx + b
plot is y vs x
y is 1/V x is 1/[S]

K_M/V_{max} is slope
y intercept is $1/V_{max}$
x intercept is $-1/K_M$

Factors affecting Enzyme Activity

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 :

1- Substrate concentration

2- Temperature

3- pH

4- Enzyme concentration

5- Time

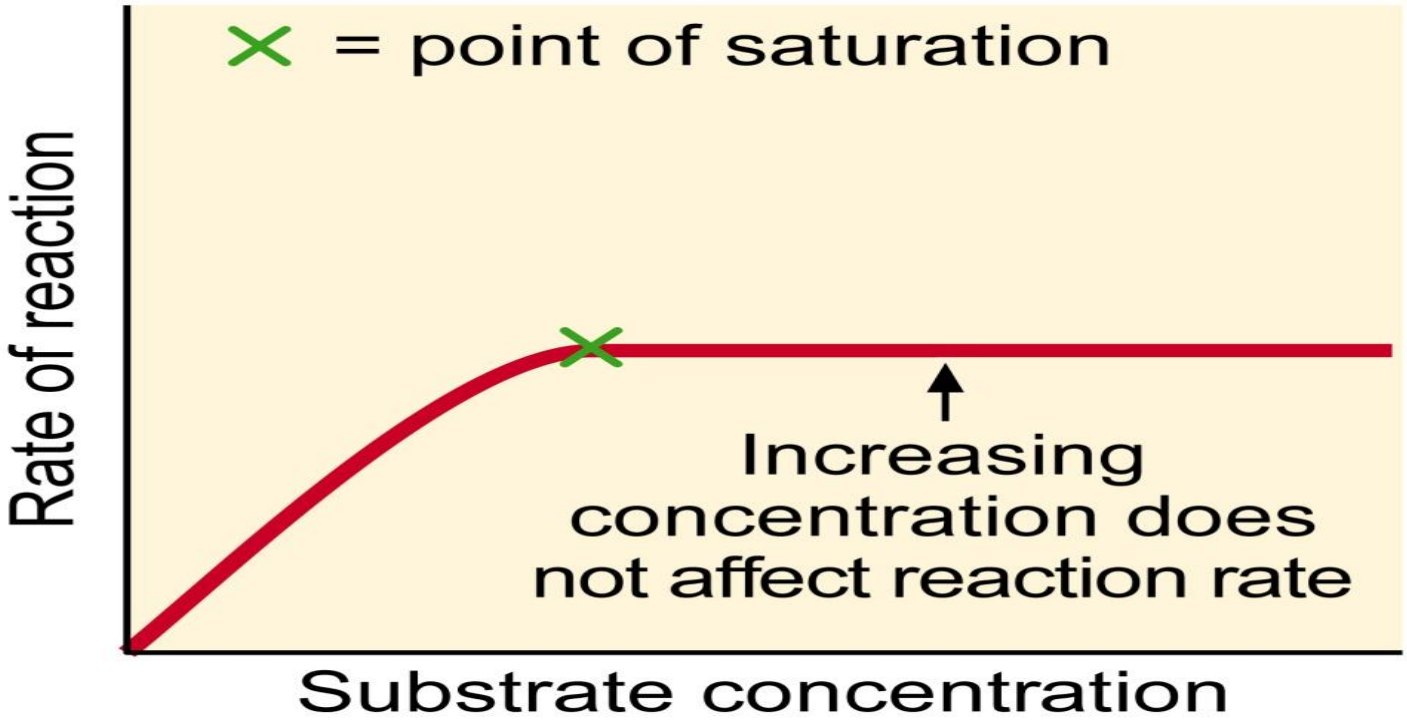
6- The presence of any inhibitors or activators.

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.

The **Michaelis-Menten**



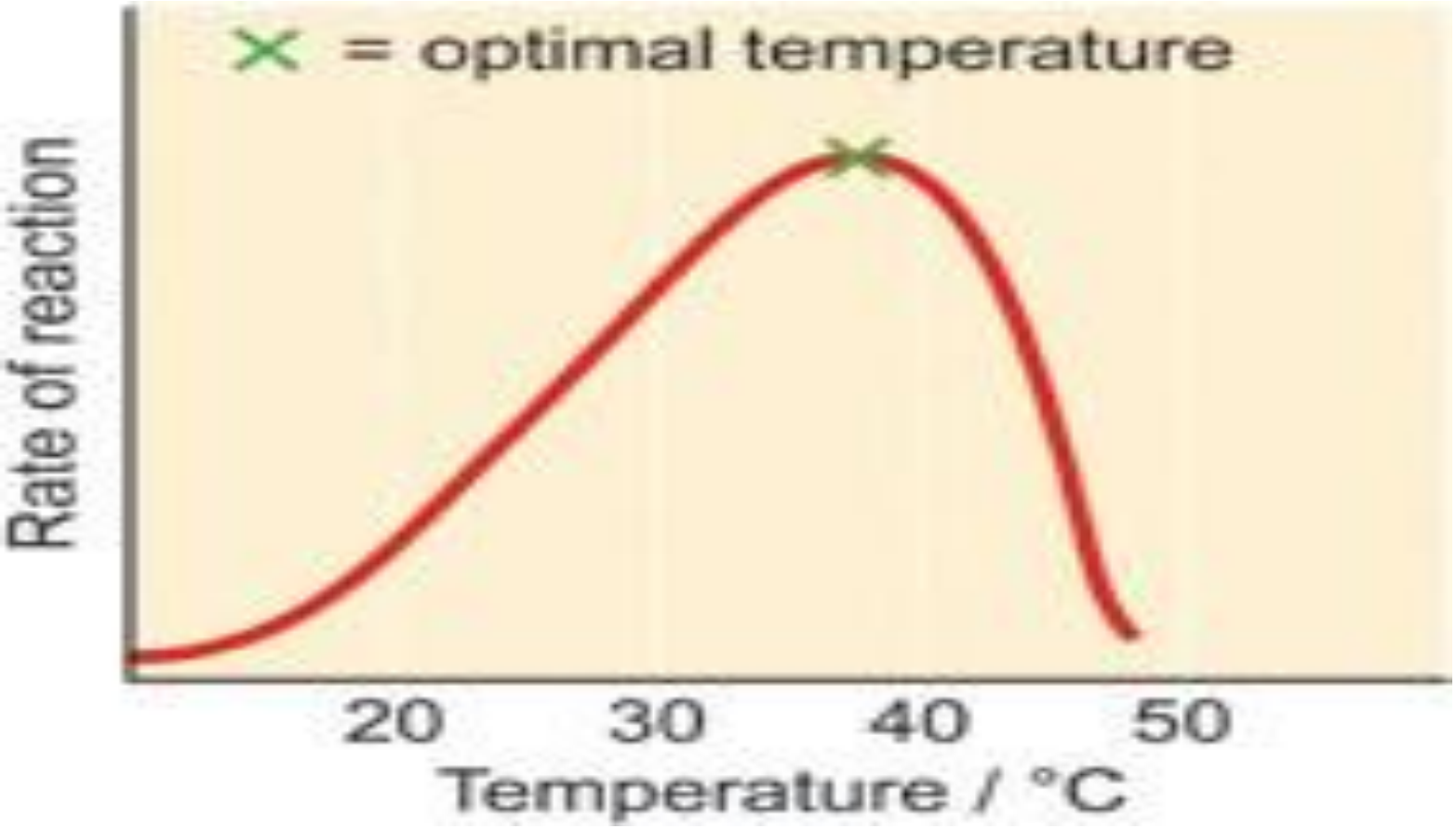
Temperature

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised.

The reaction velocity is increased until a peak velocity is reached.

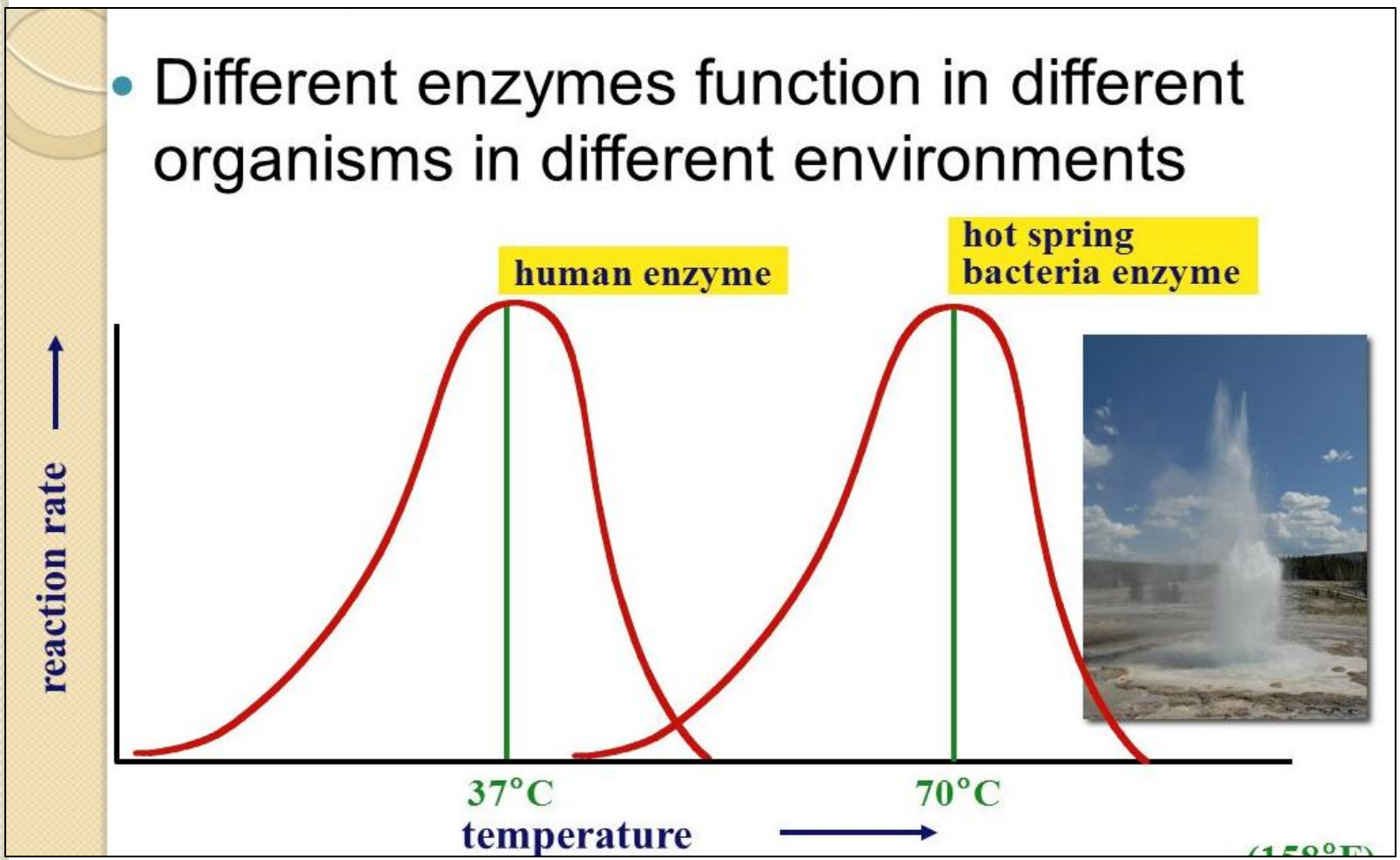
There is a decrease of velocity with higher temperature because the high temperature results in **denaturation** of the enzyme. Because most enzymes rapidly become denatured at temperatures **above 40 C.**

35 ^{0}C – 40^{0}C is the optimum temperature required for human enzymes



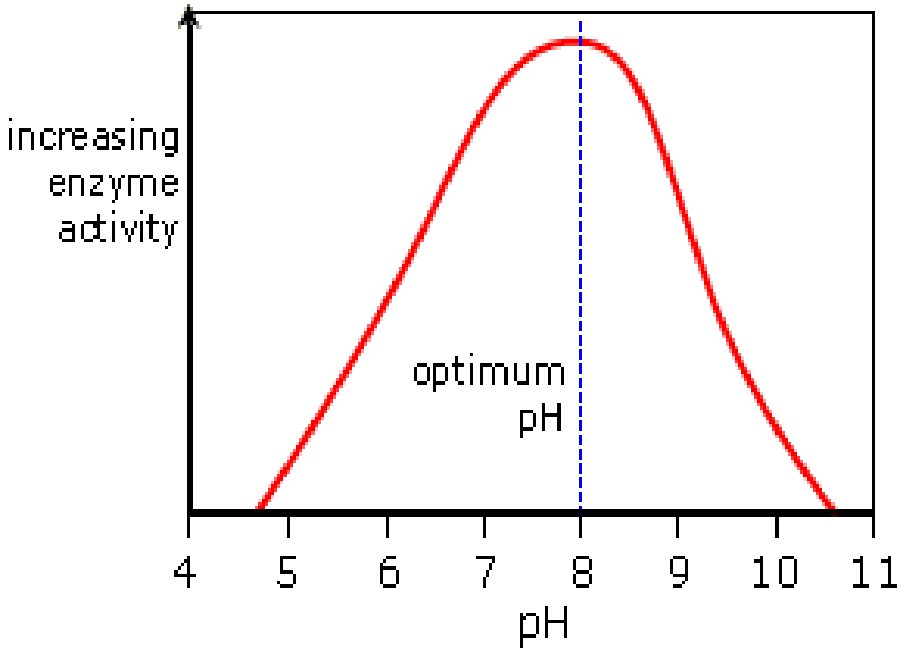
Thermophilic bacteria found in the hot springs have optimum temperature of **70 ^{0}C**

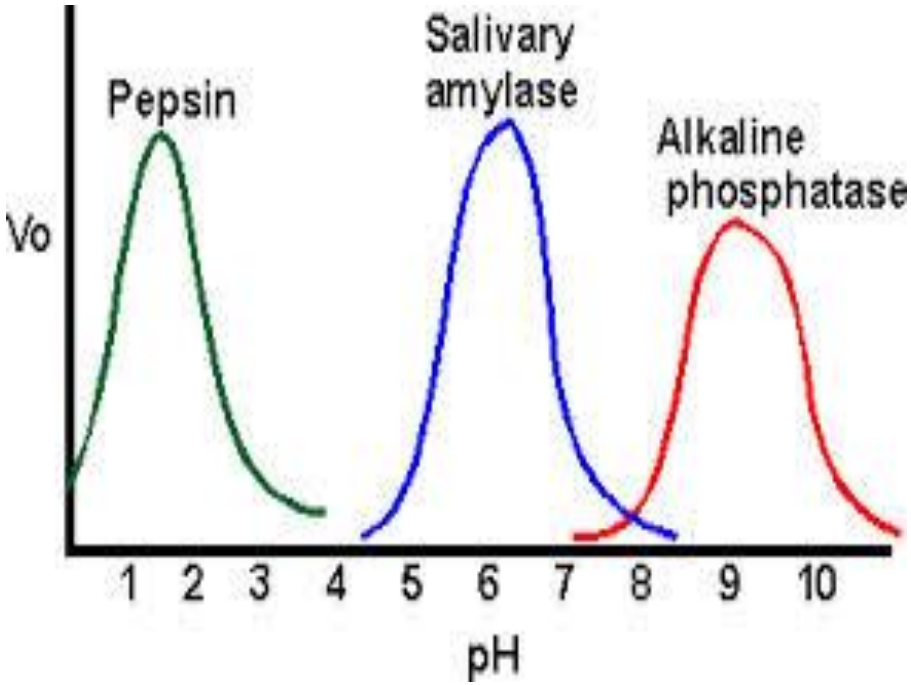
- Different enzymes function in different organisms in different environments



pH

Changes in pH have influence on enzymes. The most favorable pH value is known as the ***optimum pH***. This is the point that the enzyme is most active. Extremely high or low pH values generally result in complete loss of activity for most enzymes.





1- Effect of pH on the ionization of the active site.

The conc. of H^+ affects reaction velocity in several ways. The catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un ionized state to order to interact.

2- Effect of pH on enzyme denaturation.

Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

Lecture 9

Inhibition of Enzyme Activity

- Interfere with the action of an enzyme تتداخل مع عمل انزيم
- Decrease the rates of their catalysis تخفض معدلات التحفيز
- Inhibitors are a great focus of many drug companies (want to develop compounds to prevent/control certain diseases due to an enzymatic activity)

المثبطات: العديد من شركات الأدوية لها تركيز كبير على دراسة المثبطات وترغب في تطوير المركبات لمنع / السيطرة على بعض الأمراض بسبب النشاط الأنزيمي

Inhibitors can be **REVERSIBLE or IRREVERSIBLE**

Irreversible Inhibitors

No effect on K_m , V_{max} decreased

An irreversible inhibitor (I) will bind to an enzyme so that no other enzyme-substrate complexes can form. It will bind to the enzyme using a ***covalent bond*** at the active site which therefore makes the **enzyme denatured**

Many Es are inhibited irreversibly by **heavy metal ions** such as **Hg^{+2}, Cu^{+2} or Ag^{+1}**

Many drugs that are in clinical use work by irreversibly inhibiting specific enzymes. Although those irreversible inhibitors that react with specific groups in the enzyme protein generally inhibit more than one enzyme, those that initially form a noncovalent complex with the enzyme, with subsequent reaction within that complex leading to the formation of a covalent bond, can show a high degree of selectivity.

§**Aspirin: Acetylates Ser in active site of cyclooxygenase (COX) enzyme**

Reversible Inhibitors

- Bind to enzyme and are subsequently released
- Leave enzyme in original condition

- **Three subclasses**:

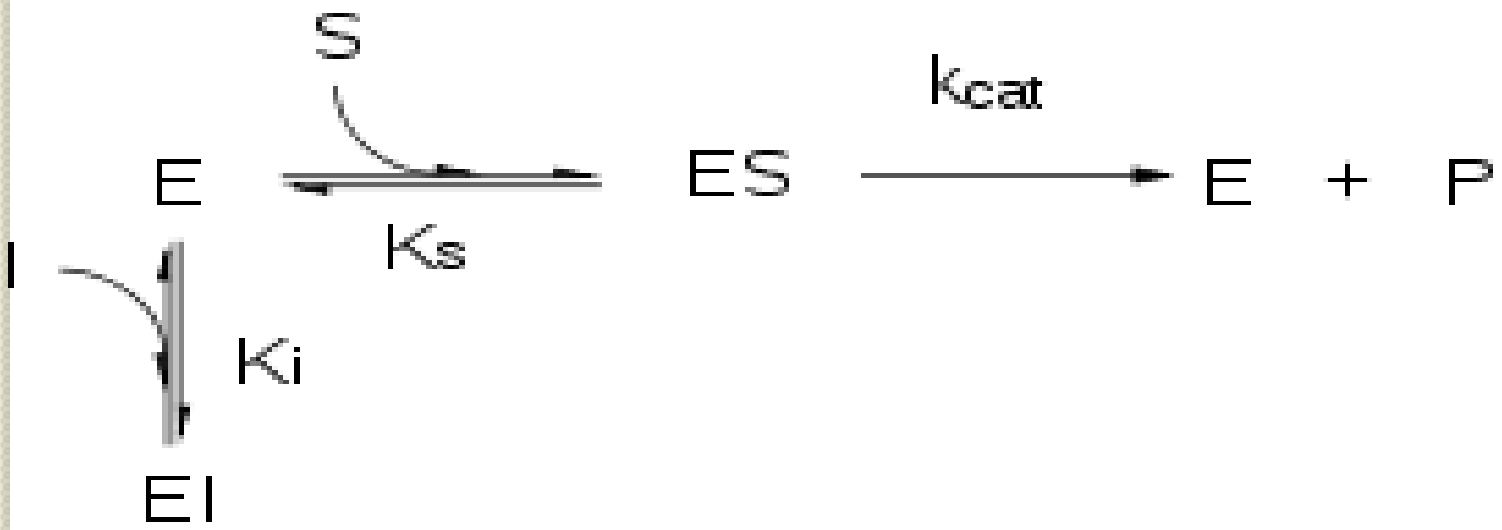
§ **Competitive Inhibitors** مثبطات تنافسية

§ **Non-competitive Inhibitors** مثبطات غير تنافسية

§ **Uncompetitive Inhibitors** مثبطات لا تنافسية

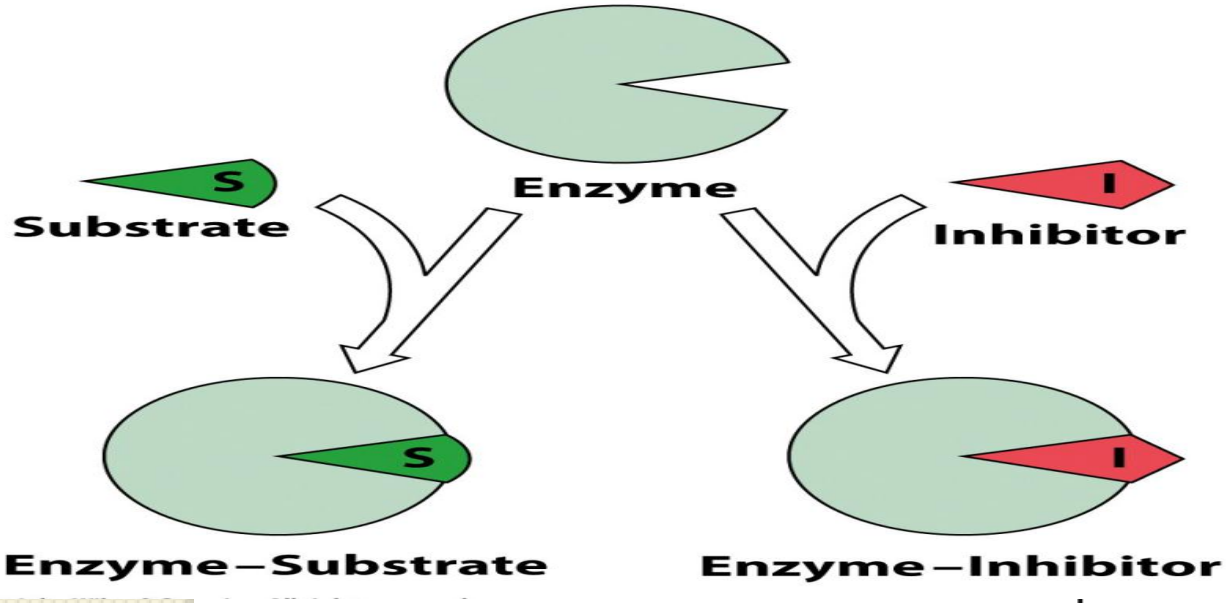
Competitive Inhibitors

In **competitive inhibition**, the substrate and inhibitor cannot bind to the enzyme at the same time. This usually results from when inhibitor **(I)** binds reversibly to the free enzyme **(E)** [to the same site that the substrate would normally occupy and therefore competes with the substrate for the site]. Competitive inhibitors are often similar in structure to the real substrate.



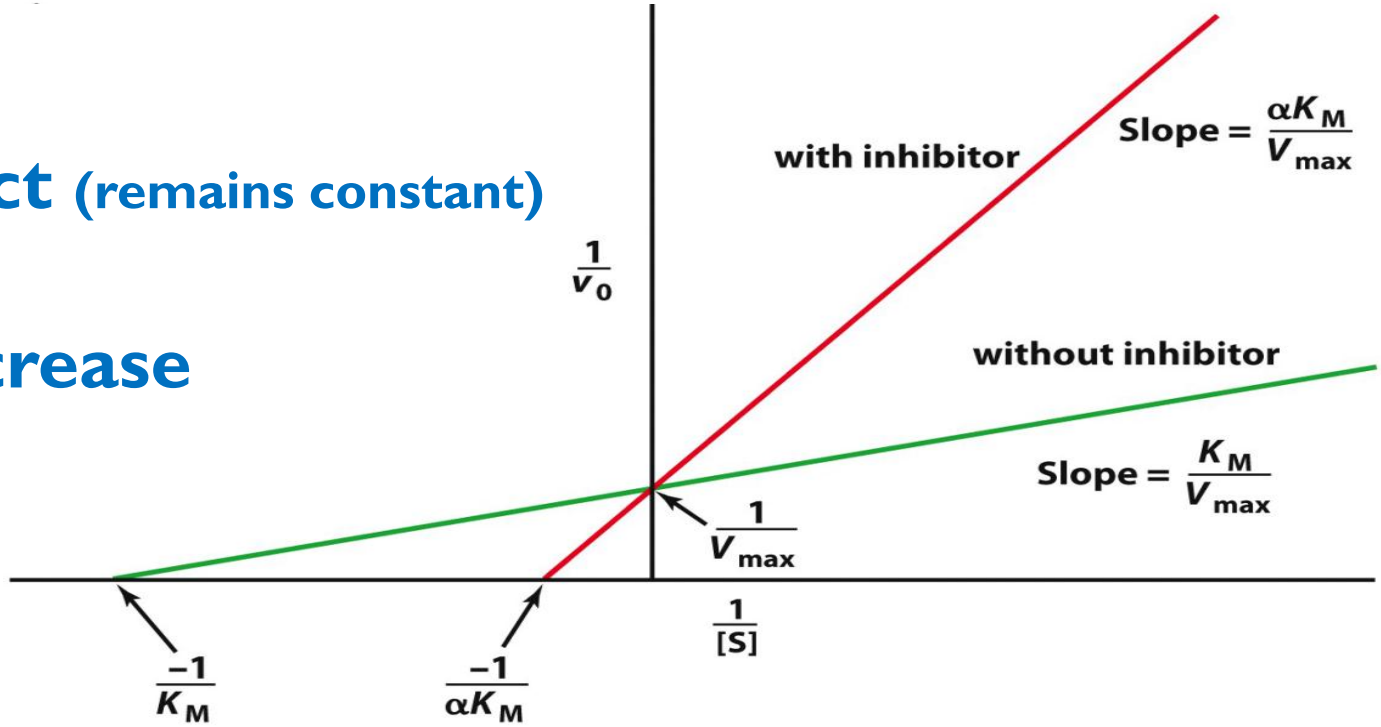
Reversible Inhibitors

Competitive Inhibitors



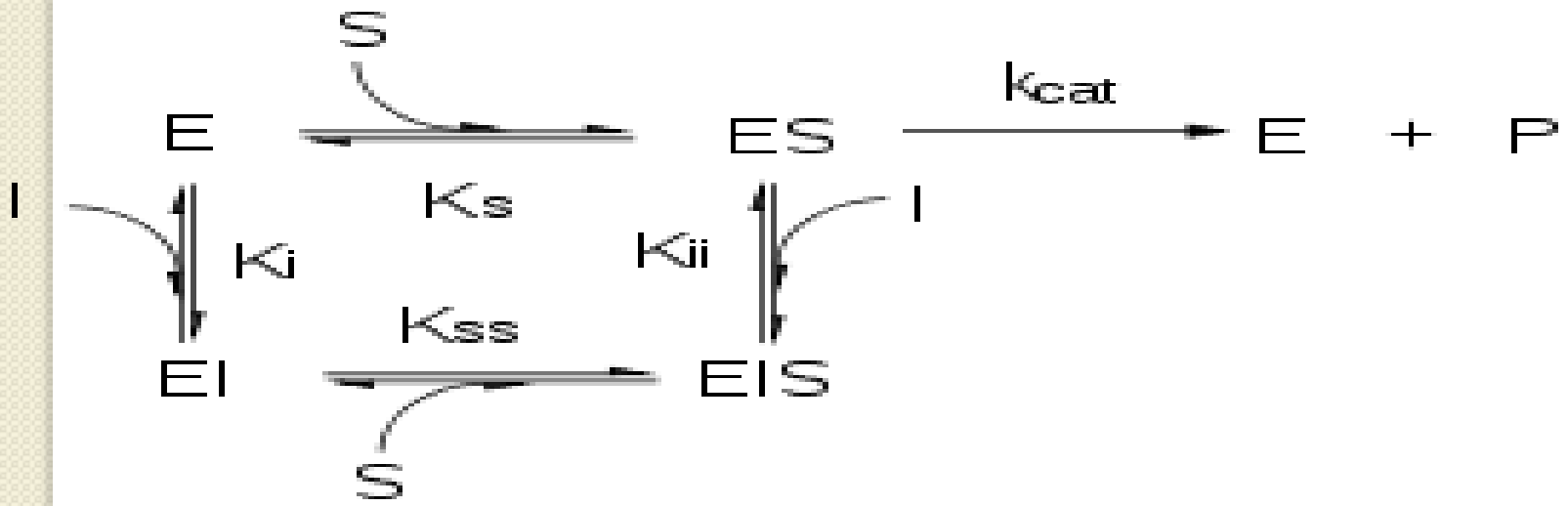
V_{max} **no effect (remains constant)**

K_m **will increase**



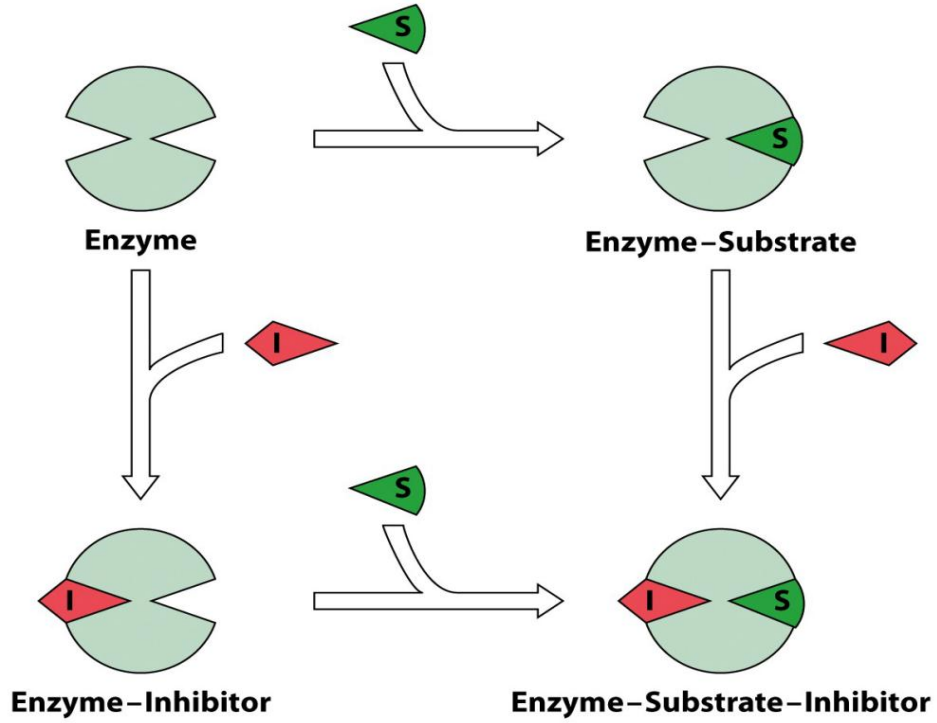
Non-Competitive Inhibitors

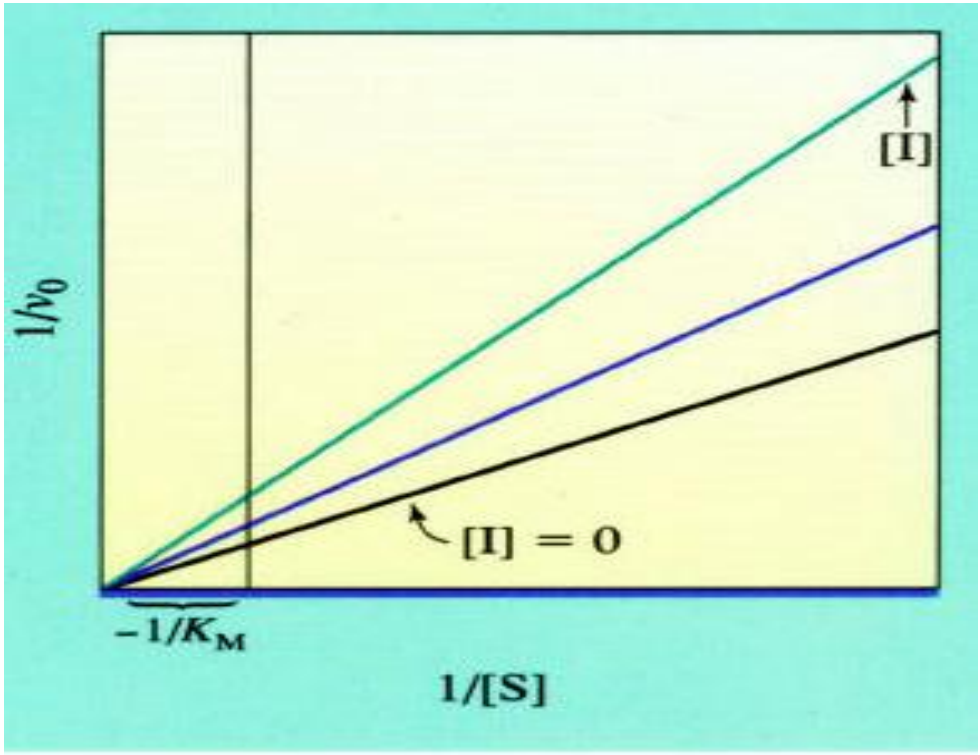
In **non-competitive inhibition**, occur when the inhibitor **(I)** binds either free enzyme **(E)** or the **ES** complex, thereby preventing the reaction from occurring [the inhibitor and substrate bind at different sites on the enzyme].



Reversible Inhibitors

Non-Competitive Inhibitors

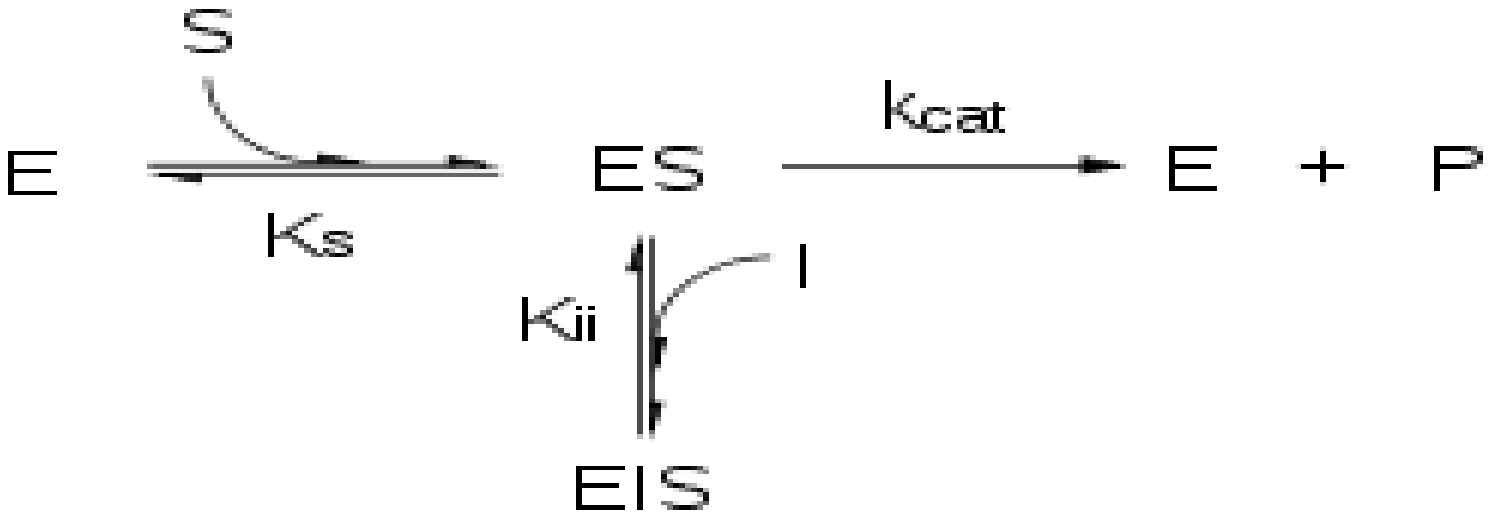




V_{max} will decrease

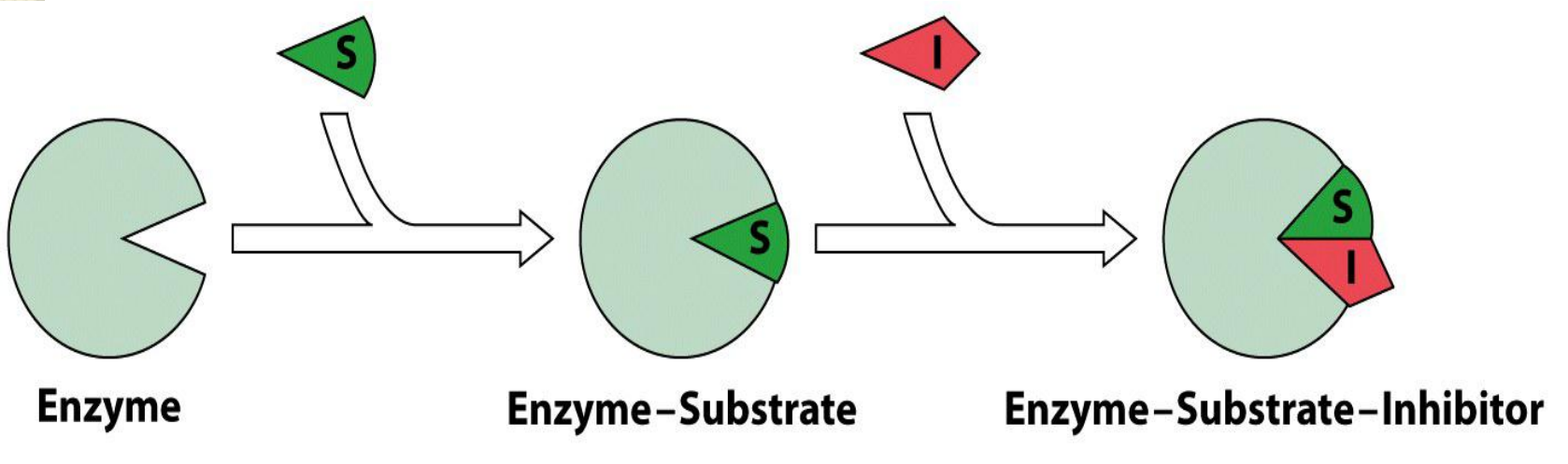
K_m no effect (remains constant)

In **uncompetitive inhibition**, the inhibitor **(I)** binds only to the enzyme-substrate- complex not to the free enzyme. The **EIS** complex is catalytically inactive].



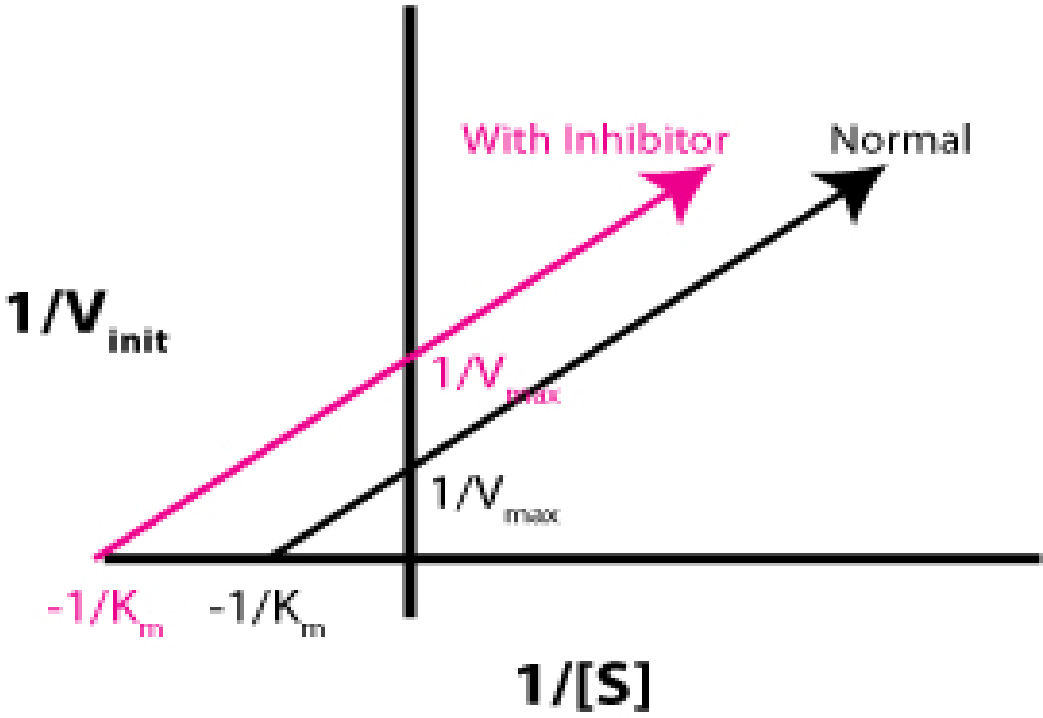
Reversible Inhibitors

UnCompetitive Inhibitors



V_{max} will decrease

K_m will decrease



Location of Enzyme

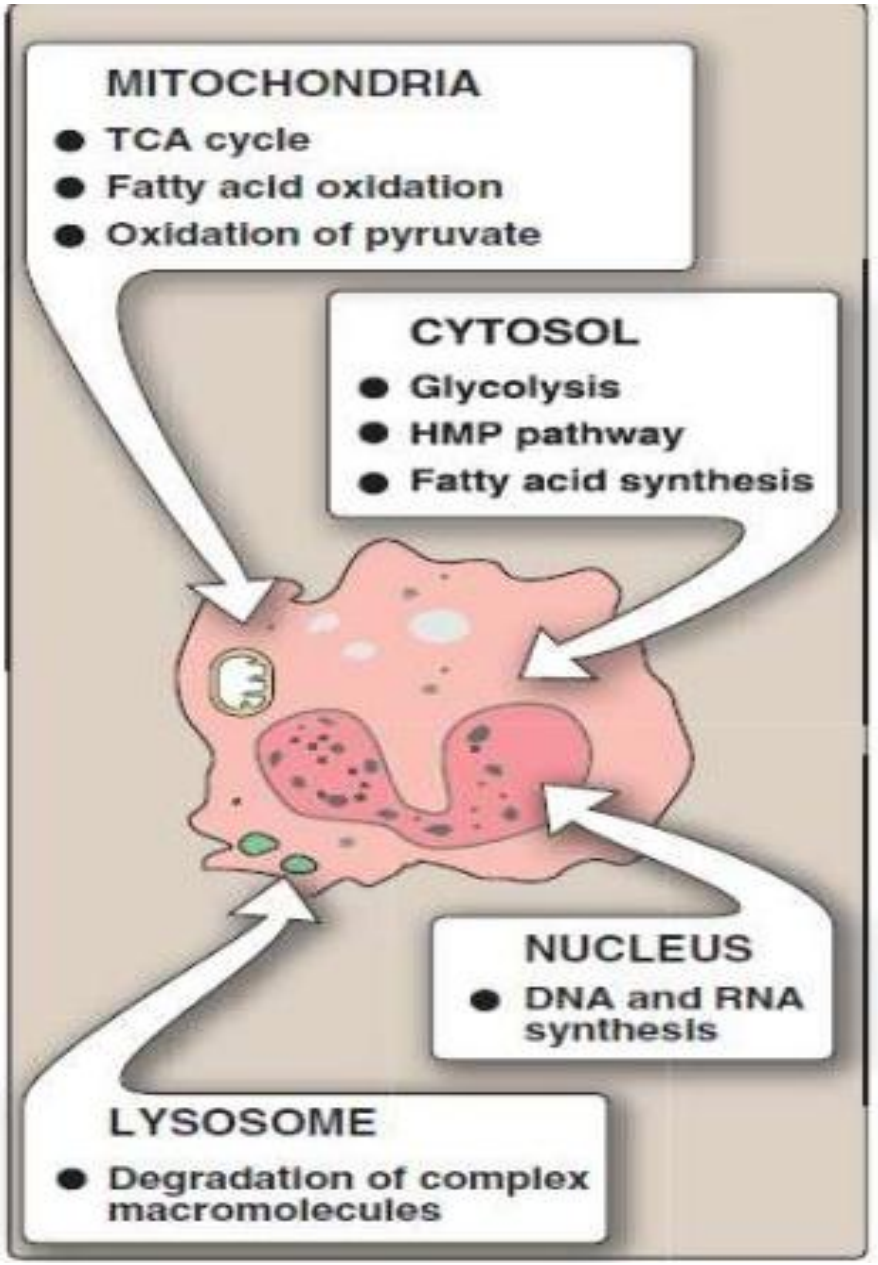
Intracellular enzyme

Enzymes that act inside cells are responsible for catalysing the millions of reactions that occur in metabolic pathways such as **glycolysis** in the mitochondria and in the **photosynthetic pathway** in the chloroplast.

Extracellular enzyme

The are secreted out from the cell and function outside the cell origin. The enzymes that function in our digestive systems are manufactured in cells - but work extracellularly .

Example: α-amylase secreted by salivary gland



Enzymes in Clinical Diagnosis

Measurements of the activity of enzymes in plasma are of value in the diagnosis and management of a wide variety of diseases.

***One* international unit (U)** is the amount of enzyme that will convert one micromole (μmol) of substrate to product per minute under optimal condition of measurement.

First, different tissues may contain two or more enzymes in different proportions; thus alanine and aspartate aminotransferases are both present in cardiac and skeletal muscle and hepatocytes, but there is only a very little alanine aminotransferase in either type of muscle

Enzymes in Clinical Diagnosis

Table 7–2. Principal serum enzymes used in clinical diagnosis. Many of the enzymes are not specific for the disease listed.

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

Second, some enzymes exist in different forms (**isoforms**), colloquially termed **(Isoenzymes)**. Individual isoforms are often characteristic of a particular tissue: although they may have similar catalytic activities, they often differ in some other measurable property, such as heat stability or sensitivity to inhibitors.

Isoenzyme (Isozyme)

Are multiple forms (isomers) of the same enzyme that catalyze the same biological reaction at different places in the specie **but they differ in structure**.

However, they do not necessarily have the same physical properties like kinetic properties and electrophoretic mobility.

Creatine kinase (CK) isoenzymes

Isoenzyme name	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases brain tumors
CK-2	MB	Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle	Skeletal muscle diseases

Industrial uses of Enzymes

Enzymes are used in the food, agricultural, cosmetic, and pharmaceutical industries to control and speed up reactions in order to quickly and accurately **obtain a valuable final product**.

Enzymes are also increasingly being used in the production of biofuels and biopolymers. The enzymes can be harvested from microbial sources or can be made synthetically.

Yeast and *E. coli* are commonly engineered to overexpress an enzyme of interest. This type of enzyme engineering is a powerful way to obtain large amounts of enzyme for biocatalysis in order to replace traditional .

Industrial uses of Enzymes

Enzyme	Industry	Application
Palatase	Food	Enhance cheese flavor
Lipozyme TL IM	Food	Interesterification of vegetable oil
Lipase AK Amano	Pharmaceutical	Synthesis of chiral compounds
Lipopan F	Food	Emulsifier
Cellulase	Biofuel	Class of enzymes that degrade cellulose to glucose monomers
Amylase	Food/biofuel	Class of enzymes that degrade starch to glucose monomers
Xylose isomerase	Food	High fructose corn syrup production
Resinase	Paper	Pitch control in paper processing
Penicillin amidase	Pharmaceutical	Synthetic antibiotic production
Amidase	Chemical	Class of enzymes used for non-proteinogenic entiomericically pure amino acid production

Vitamins

Lecture 10

VITAMINS

- A **vitamin** is an organic compound and an essential nutrient that an organism requires in limited amounts. An organic chemical compound is called a vitamin when the organism cannot make the compound in sufficient quantities, and it must be obtained through the diet; thus, the term *vitamin* is conditional upon the circumstances and the particular organism.

- *For example*, vitamin C is a vitamin for humans, but not most other animals which make enough internally. Vitamin D is essential only for people who do not have adequate skin exposure to sunlight, because the ultraviolet light in sunlight normally promotes synthesis of vitamin D. While vitamin supplements are important for the treatment of certain health problems, otherwise healthy people generally receive no benefit from using vitamin supplements.

Classification of vitamins

- **Water soluble**

  - Vitamin B1 or thiamine
  - Vitamin B2 or riboflavin
  - Vitamin B3 or niacin
  - Vitamin B5 or pantothenic acid
  - Vitamin B6 or pyridoxine
  - Vitamin B7/H or biotin
  - Vitamin B9 or folic acid
  - Vitamin B12 or cobalamin
  - Vitamin C or ascorbic acid

  Cannot be stored and is readily extracted from the body

- **Fat soluble**

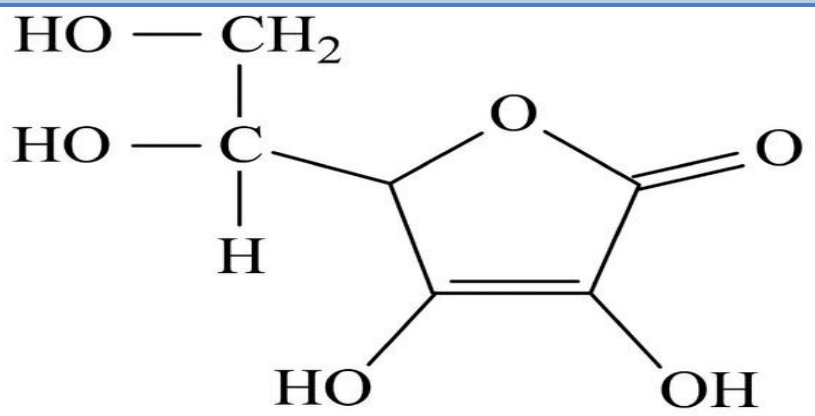
  - Vitamin A or retinol
  - Vitamin D or cholecalciferol
  - Vitamin E or tocopherol
  - Vitamin K

  Can be stored in fat cells when excess is present and easily absorbed through the intestinal tract

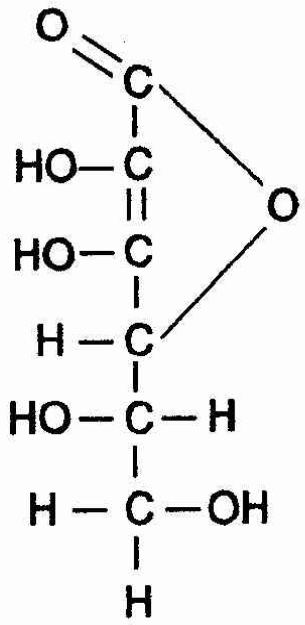
Difference between fat soluble and water soluble Vitamins

Water Soluble	Fat Soluble
Function as precursor for coenzymes and antioxidants	Function as coenzymes , hormones and antioxidants
Are usually non-toxic (since excess amounts are excreted in the urine)	Toxic and even lethal when taken in excessive quantities
Not stored extensively except vitamin B_{12}, so their intake has to be more frequent	Stored
Examples : Vitamin B complex and Vitamin C	*Examples:* Vitamins A, E, D and K

VITAMIN C (Ascorbic Acid)



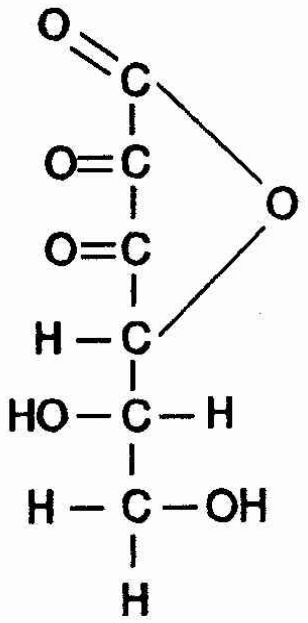
Ascorbic acid
(Vitamin C)



ascorbic acid

oxidation (- 2 H)

reduction (+ 2 H)



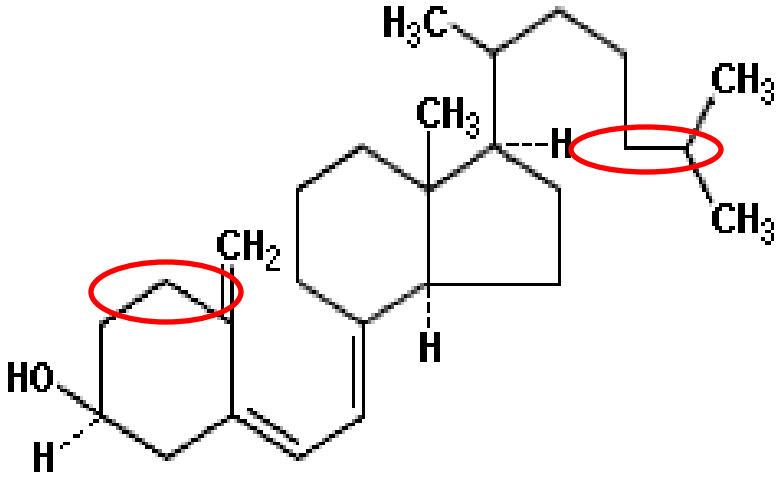
dehydroascorbic acid

VITAMIN D (Cholecalciferol)

- **Vitamin D** is also known as:
- 1- **Calciferol:** for its role in calcium metabolism
- 2- **Antirachitic factor**: because it prevents rickets

Vitamin D could be thought of as a hormone rather than a vitamin:

- As it can be synthesized in the body
- It is released in the circulation
- Has district target organs



- **Vitamin D forms**

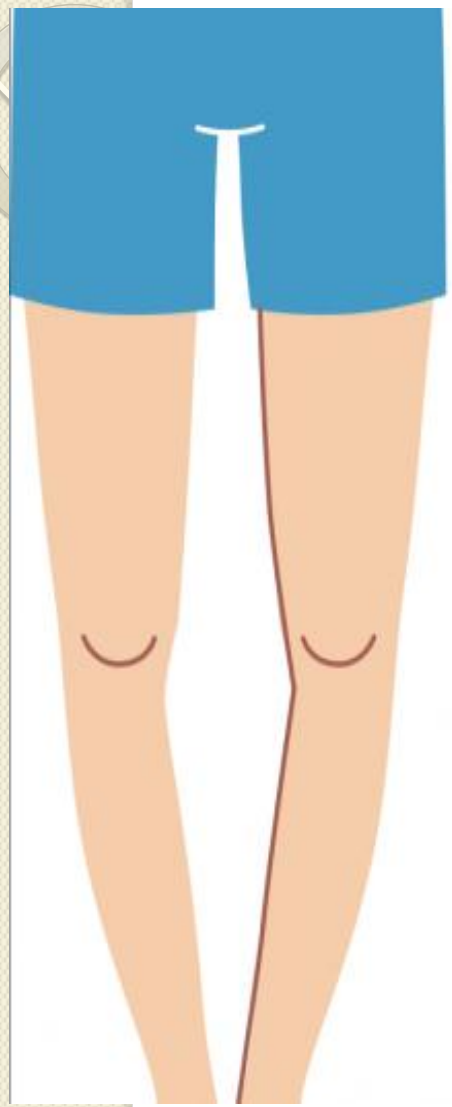
1- The naturally produced D3 (Cholecalciferol)

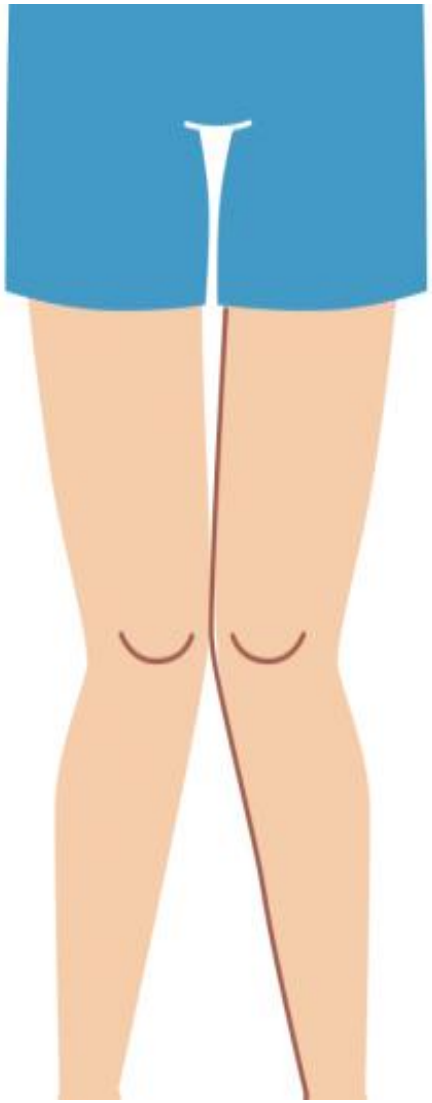
2- The artificially produced D2 (Ergocalciferol)

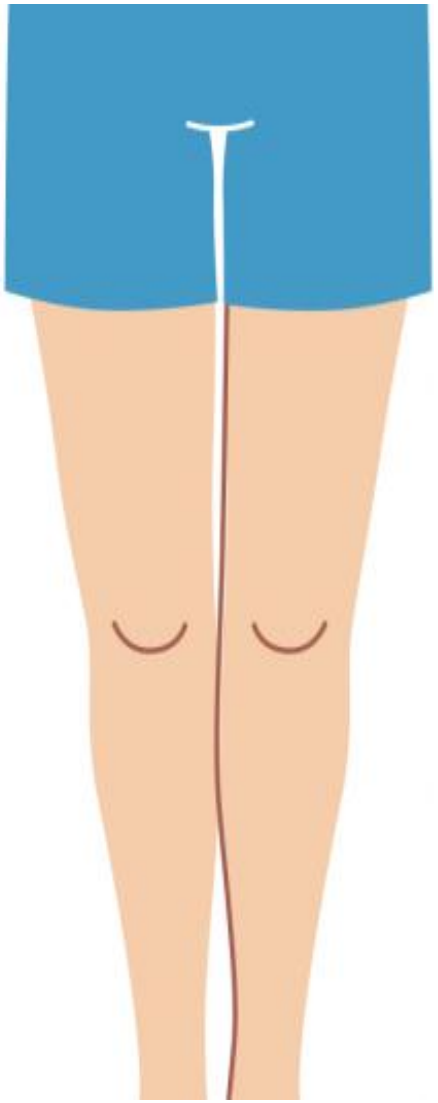
VITAMIN D (Cholecalciferol)

- A diet deficient in vitamin D in conjunction with inadequate sun exposure causes **osteomalacia** (or rickets when it occurs in children), which is a softening of the bones. In the developed world, this is a rare disease. However, vitamin D deficiency has become a worldwide problem in the elderly and remains common in children and adults. Low blood calcifediol (25-hydroxy-vitamin D) can result from avoiding the sun. Deficiency results in impaired bone mineralization and bone damage which leads to bone-softening diseases, including rickets and osteomalacia.

Rickets







مع تمنياتي بالموفقية والنجاح

د. صبا زهير حسين