1. History

It is difficult to situate exactly when the concept of enzymes as the only catalysts for chemical reactions occurring in living organisms was discovered.

- ✓ In the 18th century, **L. Spallanzani** reported in **1783** that meat is liquefied by a gastric extract.
- ✓ In the 19th century, **Kirchoff** observed in **1814** that a glutinous component of wheat converts starch into sugar.
- ✓ But it was **A. Payen and J.F. Persoz** who extracted an unpurified solution of diastase (amylase) from malt in **1833**.
- ✓ A year later, **T. Schwan** was able to partially purify the first active agent of animal origin : pepsin.
- ✓ From **1858** to **1871**, the work of **Pasteur** and **Buchener** led to the extraction of a yeast extract responsible for alcoholic fermentation called ferment.
- ✓ At the same time (**1860**), **Berthelot** was able to obtain an active extract that converted sucrose into glucose and fructose called invertase.
- ✓ In **1878**, **Kuhn** introduced the term "enzyme" which means in yeast.
- ✓ At the beginning of the **20th** century, significant work was undertaken to purify enzymes and, above all, to describe their catalytic activity in mathematical terms.
- ✓ In **1905**, **Henri, Michaelis, and Menten**, followed by Briggs and Haldane, developed enzyme kinetics.
- ✓ But due to technical difficulties, it was not until **1926** that **J.B. Sumner** was able to purify and crystallize urease extracted from beans.
- ✓ In **1958**, **Daniel Koshland** proposed the induced adjustment model (the substrate induces a conformational change in the enzyme's active site) ;
- ✓ In **1963**, **Wallace Cleland** proposed a clear and uniform procedure for writing the kinetic equations of multi-substrate enzymatic systems ;
- ✓ In **1965**, **Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux** proposed a kinetic model (MWC model) for allosteric enzymes ;
- ✓ In **1966**, **Daniel Koshland, George Nemethy, and David Filmer** generalized the previous model by including the concept of induced adjustment proposed by Daniel Koshland (KNF model);

- ✓ In **1972**, **Christian Anfinsen** demonstrated the link between the amino acid sequence and the biologically native-active conformation of ribonuclease. While **Stanford Moore and William Stein** demonstrated the link between the chemical structure and the catalytic activity of the active center of ribonuclease ;
- ✓ **Ron Laskey (1978)** and **John Ellis (1987)** discovered the process involving so-called "chaperone" proteins (These proteins help in the folding of other proteins or maintain them in the native conformation when the cell is subjected to certain stresses).

Definition :

Enzymes are catalysts for chemical reactions that occur in living organisms. They are protein macromolecules that accelerate reactions without the formation of unwanted products and they function under average temperature and pH conditions.

Enzymes are the functional units of cellular metabolism, acting in organized sequences. They catalyze the hundreds of reaction steps through which nutrient molecules are broken down, chemical energy is conserved and transformed, and cellular macromolecules are synthesized from simple precursors.

2. Classification of enzymes :

The nomenclature, or classification, of enzymes is very useful ; because knowing the name of an enzyme already tells you the type of reaction catalyzed and the substrate. In fact, there are several rules for enzyme nomenclature :

2. 1. Nomenclature :

2. 1.1. Traditional nomenclature :

At the beginning, enzymes were given traditional names that often evoked the organ in which they were found.

Pepsin : enzyme of gastric juice (stomach).

Zymase : enzyme from yeast extract.

Subsequently, the suffix **"ase"** was used with the name of the substrate.

Peptide : peptidase.

Lipid : lipase.

Oside : osidase.

2. 1. 2. Functional nomenclature :

It is widely used. It takes into account both types of specificity : the substrate and the type of catalyzed reaction. Indeed, to designate an enzyme, we first indicate the name of the substrate, then the type of catalyzed reaction, and we add the suffix "ase."

Glucose-6-phosphate isomerase.

Isocitrate lyase.

Pyruvate carboxylase.

When the enzyme uses two substrates, both are designated by indicating the radical-donating substrate, then the radical-accepting substrate, the name of the exchanged radical, the type of reaction, and adding the suffix "ase."

ATP-glucose phosphotransferase.

UDP glucose-fructose glucosyltransferase.

Glutamate pyruvate aminotransferase.

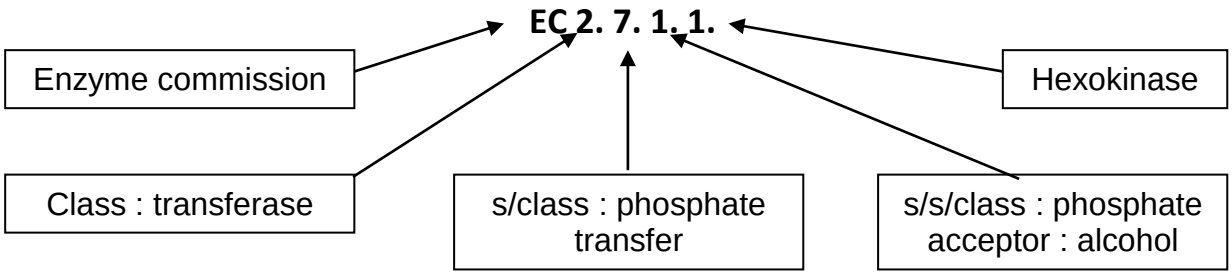
In the case of a reversible balanced reaction, names can be formed from the substrates or products.

2. 1. 3. Official nomenclature :

Starting in 1961, the International Union of Biochemistry (IUB) decided to keep a register of all perfectly characterized enzymes and assign them a systematic classification number consisting of four numbers separated by dots and preceded by EC **(EC x1.x2.x3.x4**.) :

1. The first number, which can range from 1 to 7, designates the enzyme class, which depends on the type of catalyzed reaction.
2. The second number indicates the subclass of the enzyme, which is defined according to its mechanism of action (the nature of the substrate or sometimes the transferred group).
3. The 3rd number indicates the nature of the molecule that serves as the acceptor when it comes to a transfer.
4. The last number is a serial number given to each new enzyme discovery. When an enzyme ends with 99, it means it is incompletely characterized.

Example : **ATP-hexose-6-phosphotransférase** (nom systématique formel).



2. 2. Classification :

2. 2. 1. Oxidoreductases :

Oxidation-reduction reactions are balanced reactions characterised by the flow of electrons. They are either oxygen fixation reactions, hydrogen release reactions, or electron release reactions. They are the most common reactions in biochemistry and their importance is due to their relationship with energy exchanges. These enzymes work with various coenzymes (NAD^+, $NADP^+$, FAD, FMN) that act as intermediate and transient electron acceptors.

Oxidoreductases can be :

Dehydrogenases : enzymes that remove hydrogen atoms or electrons from their substrate.

Oxidases : enzymes whose electron acceptor is molecular oxygen.

Oxygenases : enzymes that use molecular oxygen to modify a substrate by incorporating oxygen (monooxygenases, dioxygenases).

2. 2. 2. Transferases :

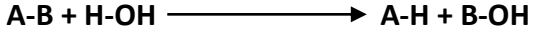
They transfer radicals or groups of atoms from a donor molecule to a recipient molecule. In many cases, the enzyme works with a coenzyme that temporarily binds the radical to be transferred.

Groups that can be transferred:

1. Methyl ($-CH_3$): methyltransferase.
2. Hydroxymethyl ($-CH_2OH$).
3. Carboxyl (-COO-)
4. Carbon group with an aldehyde function (R-CHO).
5. Acyl (R-CO-).
6. Osidyl.
7. Amine ($-NH_2$).
8. Phosphoryl ($-PO_3H_2$): phosphotransferase = kinase.
9. Sulfuryl ($-SO_3H$): sulfotransferase.

2. 2. 3. Hydrolases :

These are degradation enzymes without coenzymes. They cause a molecule to cleavage into two by attaching the elements of a water molecule to the released valences :



The main bonds that can be broken are :

1. Ethers R-CH_2-O-CH_2-R'
2. Acetals or hemiacetals ose-O-alkyl.
3. Carboxylic acid esters R-COO-CH_2-R'
4. Phosphoric esters R-CH_2-O-PO_3H_2
5. Sulfuric esters R-CH_2-O-SO_3H
6. Acid anhydrides R-COO-CO-R'
7. The O-O bonds (peroxides).
8. The C-N bonds of amine, amide, imine functions and especially peptide bonds (-CO-NH-).

2. 2. 4. Lyases :

Catalyze the displacement of a group from a substrate with the appearance of a double bond on that substrate. There are :

Carboxy-lyases : enzymes that separate or fix the (-COO-) radical. We find carboxylases and decarboxylases.

Acyl-lyases : enzymes that condense acyls from acetyl-coenzyme A (also called synthases).

Lyases that cleave the C-O bond : are enzymes that remove a water molecule, resulting in the formation of a double bond (also called dehydratases).

Lyases that cleave the C-N bond.

2. 2. 5. Isomerases :

They catalyze the transfer of groups within a molecule with the formation of isomers (isomerization). We find :

Epimerases : catalyze the conformational changes of an asymmetric carbon.

Cis-trans isomerases : act on radicals attached to the carbons of a double bond.

Dismutases : are intramolecular oxidoreductases catalyzing the internal transfer of hydrogen atoms.

Intramolecular transferases : move radicals within a molecule.

2. 2. 6. Les ligases (synthetases) :

Are enzymes that form bonds such as : C-C, C-O, C-N, C-P, and C-S during the simultaneous hydrolysis of a pyrophosphate group providing the energy most often contained in an ATP molecule.

1.2.7. Translocases :

This class was added in September 2018. These enzymes catalyze the translocation of protons (H+), inorganic cations and anions, amino acids and peptides, carbohydrates and their derivatives. These enzymes catalyze the movement of molecules or ions across cellular membranes in general.

3. General properties

Enzymes possess all the properties of proteins. Their catalytic activity depends on the integrity of their primary, secondary, and tertiary structure as proteins.

3. 1. Specificity and stereospecificity :

In an enzymatic reaction, the substrate molecule must have two distinct structural characteristics :

1. The specific chemical bond that can be attacked by the enzyme.
2. Another functional group (binding group) that binds to the enzyme and correctly positions the substrate on the site, so that the target bond is precisely located relative to the catalytic group of the enzyme.

3. 1. 1. Specificity of reaction :

It is the most significant property of enzymes. It allows for the regulation of the speed of metabolic processes through a change in catalytic efficiency. This property is only determined by the apoenzyme (enzymatic protein).

3. 1. 2. Substrate specificity :

There are two types of enzymatic specificity towards the substrate:

Close specificity :

In this case, the enzyme has optical specificity (rotatory power of the substrate) and geometric specificity ; therefore, we speak of stereospecificity. Thus, the enzyme recognizes and forms only one type of isomer when a molecule can have several. It precisely recognizes molecular details in space as well as the orientation of substituents on a carbon atom (example : aspartase).

Large specificity :

In this case, there are enzymes with relatively broad specificity that act on many compounds that share a structural characteristic.

Example :

Chymotrypsin catalyzes the hydrolysis of many peptides but only cleaves peptide bonds in which the carbonyl group is supplied by one of the aromatic amino acids (phenylalanine, tyrosine, or tryptophan).

3. 2. Active site :

The active site (or active center) of an enzyme is a small area of the enzyme protein whose geometry is important for specificity. It is located in a hydrophobic (nonpolar) area in the inner part of the structure. It performs two functions :

1. Substrate binding (binding site) ;
2. Substrate transformation (catalytic site).

Structurally, the active site is made up of a small number of amino acids whose side chains are directly involved in electron or radical exchanges with the substrate. The active site consists of a few amino acids that are not necessarily located on the same polypeptide chain (folding due to tertiary structure) and are divided into two groups :

- Spatial recognition (binding) amino acids.
- Chemical transformation (catalytic) amino acids.

Recognition is ensured by complementarity between the surface of the active site and the surface of the substrate, thanks to their proximity to each other, which allows the exchange of non-covalent bonds (hydrogen bonds, Van der Waals forces, hydrophobic bonds).

The active site of monomeric enzymes is often located in an infractuosity (pocket) of the enzyme, while that of multimeric enzymes may be located at the interface between subunits.

3. 3. Activation energy:

3. 3. 1. Genralities :

The kinetic theory or collision theory of chemical kinetics involves two key concepts :

1. Only molecules that collide can react. These are molecules separated by a distance that allows bonds to form.
2. For each reaction, there is an energy barrier that must be overcome for it to occur.

In fact, for a collision to cause a reaction, the reacting molecules must have enough energy to overcome this energy barrier.

The transformation of a system (substrate) involves a transition state represented by two partial reactions, each involving a characteristic change in energy :

1. Formation of the transition state.
2. Decline of this transition state.

3. 3. 2. Definition :

Activation energy (Ea) : this is the energy that the reactant molecules must absorb in order to react ; it is therefore the potential barrier that the reactant must overcome for the reaction to take place.

3. 3. 3. Enzymes and activation energy :

In general, a catalyst only accelerates thermodynamically possible reactions (those that result in a decrease in energy).

Thus, the enzyme increases the speed of a reaction by decreasing the activation energy (Ea).

It lowers the energy barrier (ΔG*) that the substrate must overcome, thereby facilitating the transition to the transition state (activated complex). In fact, in the presence of enzymes, a large proportion of the molecules in a system will be able to react, even though these molecules are not all in the same energy state.

Therefore, the enzyme offers the reaction a different and more accessible path than the one taken in its absence.

3. 3. 4. Mechanism :

- The architecture of the enzyme makes catalytic transformation possible.
- The tertiary structure of the enzyme allows electrons or protons to flow through channels formed by charged amino acid residues, surrounded and electrically isolated by nonpolar regions.
- The enzyme-substrate combination creates a new reaction pathway whose transition state energy is lower than that of the pathway the reaction would take if it occurred in the absence of the enzyme.
- The amino acids of the enzyme located around the substrate participate in catalysis by modifying its electronic distribution, which allows for all possible types of reactions.

3. 4. Enzymatic effectors :

The speed of an enzymatic reaction depends on both :

1. The enzyme concentration.
2. The substrate concentration present in the reaction medium.
3. The presence or absence of effectors.

The latter play a key role *in vivo*, as they act on and adapt the functioning of enzymes to their biological environment. They are also effective in the experimental study of enzymes outside living organisms.

3. 4. 1. Temperature :

An increase in temperature increases the speed of an enzymatic reaction, but only within a very limited range, due to :

1. An increase in the number of molecules that are in an activated state.

2. An increase in the probability of combination between the enzyme and the substrate.

The factor that leads to an increase in the speed of a biological process following a temperature increase of around 10°C is called Q10 (temperature coefficient).

3. 4. 2. pH :

pH affects the ionization state of the enzyme and that of the substrate. Consequently, the rates of enzymatic reactions are sensitive to pH variations in the range [2,11]. pH modifies:

1. The hydrogen bonds, ionic bonds, or Van der Waals forces that bind the substrate to the active site.
2. The forces binding the monomers of oligomeric enzymes.
3. The conditions for electron or proton transport within the enzyme.

Enzyme behavior towards pH :

1. Some enzymes are not greatly affected by pH variations, such as salivary amylase.

2. Some enzymes act within a narrow pH range, such as pepsin and trypsin.

3. Others, which catalyze reversible reactions, have a different optimum pH when acting in one direction or the other.

3. 4. 3. Inhibitors :

The inhibition of enzyme activity by small molecules or ions acts as an essential control mechanism in biological systems (regulation of metabolic pathways by retro-inhibition).

Experimentally, enzyme inhibition can provide information on the mechanism of action of enzymes and their substrate specificity: residues essential for catalysis can be identified using specific inhibitors.

3. 4. 4. Allosteric effectors :

- The functioning of allosteric enzymes is controlled by effectors : activators or inhibitors. These can be small molecules that are different from the substrate. They can be organic molecules or mineral ions (Ca^{++}, Zn^{++}, Ni^{++}, Mg^{++}, Mn^{++}, Co^{++}, Fe^{++} or Fe^{+++}, Mo^{4+}).
- They have a distinct binding site.
- They cause a successive change in the conformation of the subunits, producing an increase in enzyme activity (in the case of activators).
- In some cases, the substrate binds to the enzyme, forming a common complex with the activator.