

T.D. n°1 : Enzyme structure

1. Protein structure :

Proteins are polymers of amino acids linked by a peptide bond.

1. 1. Primary structure :

This is the description of the polypeptide chain that forms the protein. It is determined by:

- Number of polypeptide chains.
- Number and nature of the constituent amino acids.
- Sequence of amino acids in each chain, starting from the N-terminal amino acid to the C-terminal.
- Location of inter- and intra-chain disulfide bridges.

Example :

Bovine insulin consists of two polypeptide chains of 21 and 30 amino acids. It contains three disulfide bridges: one intra-chain bridge linking amino acids 6 and 11, and two inter-chain bridges between amino acids A7–B7 and A20–B19.

1. 2. Secondary structure :

It is a regular form adopted by part of the protein (β -sheet, α -helix, turn). In other words, it is the conformation of the peptide bonds.

The α -helix structure is characterized by:

- ✓ The cohesion of the structure is due to hydrogen bonds between the C=O and N-H atoms of two superimposed peptide bonds. Van der Waals interactions, established by the tightly packed atoms at the core of the helix, give it additional stability.
- ✓ The helix turn contains 3.6 amino acids.
- ✓ The side chains of the amino acids (radicals) are located outside the helix.

The β -sheet structure is characterized by:

- ✓ The cohesion of this structure is due to hydrogen bonds between C=O and N-H atoms between two elongated polypeptide chains or between two regions of the same chain.
- ✓ Formation of parallel sheets and other antiparallel sheets.
- ✓ The side chains of amino acids (radicals) are located on either side of the sheet.

1. 3. Tertiary structure :

This is the final form of the protein. The assembled protein folds into a precise three-dimensional structure:

Globular proteins: most proteins have a compact form (like a small cloud).

Fibrous proteins: these are long and thread-like. They are formed from a single α -helix and can bind together to form strong fibers.

This structure is stabilized by hydrogen bonds, hydrophobic interactions, salt interactions, disulfide bridges, and Van der Waals forces.

2. Monomeric enzymes :

Some enzymes consist of a single subunit or a single polypeptide chain. These are most often secreted enzymes such as chymotrypsin and pancreatic ribonuclease (proteases).

3. Oligomeric enzymes :

Other enzymes, which are more numerous, consist of several polypeptide chains or subunits that are either identical (homo-oligomer) or different (hetero-oligomer) and linked by non-covalent bonds. These are referred to as protomers or monomers forming an oligomer.

Oligomeric protein :

Has a globular form and comprises few subunits (2-8) (Oligo: few).

Multimeric (polymeric) protein:

Is often fibrillar and contains several subunits; more than 12.

3. 1. Quaternary structure :

The quaternary structure describes the association of several protein subunits by non-covalent bonds (hydrogen, ionic, and very often hydrophobic) to form the final protein.

The assembly of subunits occurs spontaneously and is highly specific. It is more stable than each isolated subunit.

The association between subunits is achieved through contact zones between protomers or monomers. These zones must be mobile in order for the oligomer to perform its functions.

3. 2. Molecular formula :

The composition of polypeptide chains and monomers is symbolized by the molecular formula, which indicates:

- The nature of the subunits

Designated by capital letters (A, B, C, etc.), Greek letters (α , β , γ , etc.) or letters evoking the property of each monomer.

- The number of subunits per complete molecule.

Example :

Hemoglobin = $2\alpha 2\beta$.

Insulin = (AB)₂ in the presence of Zn^{++} ions.

Lactate dehydrogenase (LDH) = H_4, H_3M, H_2M_2, HM_3 , or M_4 .

4. Isoenzymes (isozymes):

Isoenzymes are enzyme proteins that are very similar in their properties, synthesized by the same organism, and catalyze the same reaction. They are physically distinct forms with the same catalytic activity and may be present in different tissues of the same organ, in different cell types, or in different subcellular compartments.

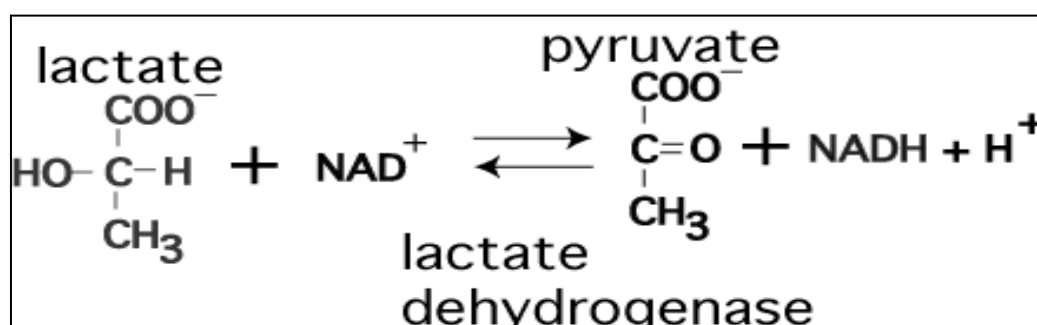
4. 1. Differences between isoenzymes :

- Structurally, the enzyme may have a single subunit whose polypeptide sequence varies to some extent from one organ to another, or several subunits arranged differently.
- This is because the synthesis of subunits is controlled by genetically distinct loci, expressed differently in various tissues, cells, or organelles, which result from physiological adaptation.
- In addition, isoenzymes appear to differ from each other in terms of specific properties, particularly their kinetic coefficients (K_m and V_m) and their specificity.

4. 2. Origin :

Isoenzymes are observed in the form of multiple bands after separation, most often by electrophoresis. They are often observed during the purification of enzymes involved in glycolysis and intermediate metabolism (hexokinase, enolase, aldolase, xanthine oxidase, isocitrate dehydrogenase, and malate dehydrogenase).

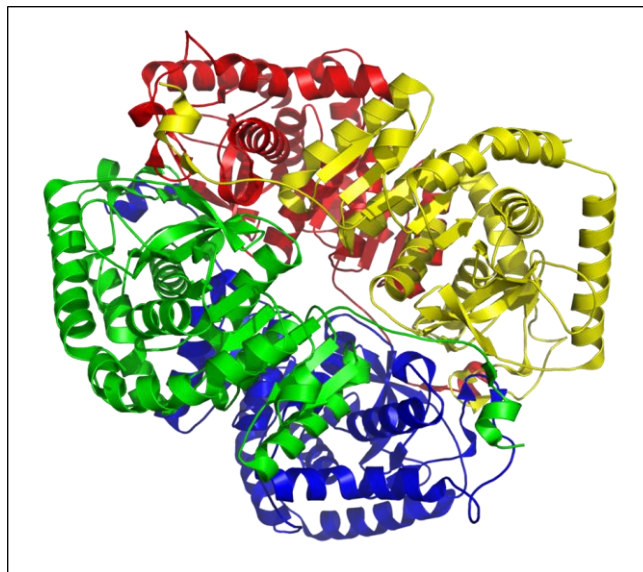
It can be assumed that the body contains isoenzymes in order to optimize the functioning of a particular reaction according to the specific physiological needs of different tissues.

4. 3. Example (case of lactate dehydrogenase LDH):

In mammals and humans, this enzyme consists of four polypeptide chains linked together by non-covalent bonds. There are two types of chains: H (Heart) and M (Muscle).

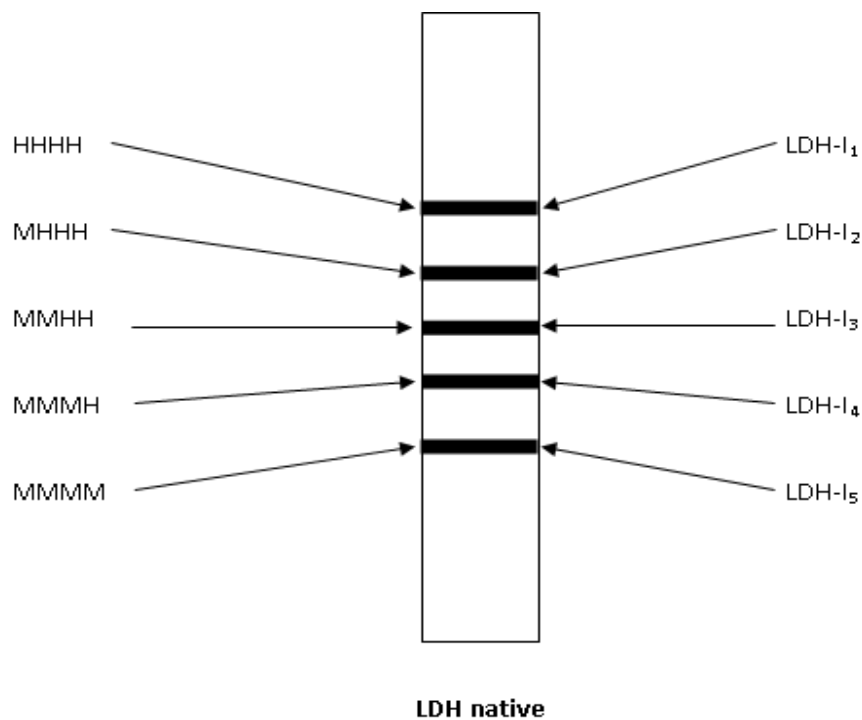
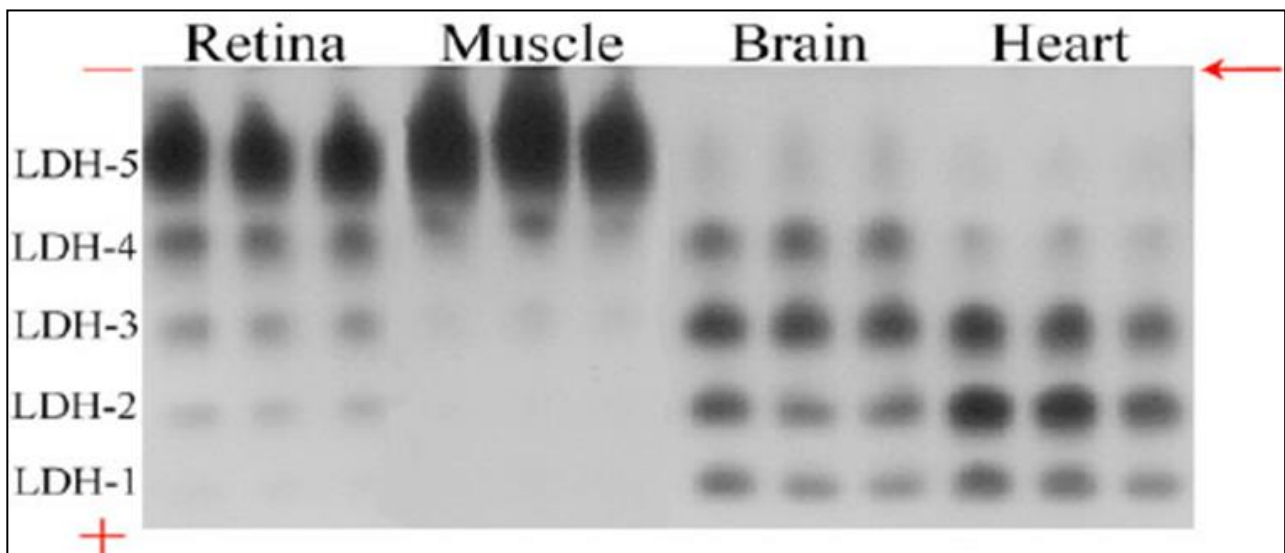
This enzyme is found in all organs, but the composition of polypeptide chains varies depending on the organ:

- In cardiac muscle, LDH has four H subunits (H_4 or HHHH).
- In other muscles and the liver, LDH has four M subunits (M_4 or MMMM).
- In other organs, there are intermediate combinations: H_3M (HHHM), H_2M_2 (HHMM) or HM_3 (HMMM).



In humans, the two genes M and H are carried by different chromosomes. Compared to isoenzyme M_4 , isoenzyme H_4 has:

1. A higher Michaelis constant (K_m) for pyruvate and a lower maximum velocity (V_m) for its reduction.
2. Less marked inhibition by pyruvate.
3. Lower specificity; H_4 can reduce 2-oxobutyrates instead of pyruvate and accept an NAD^+ analog.



5. Multi-enzymatic system :

Enzymes that catalyze a series of reactions in the same metabolic pathway can combine within a multienzyme complex whose molecular mass can reach several thousand units. These systems generally include a key enzyme that can be activated or inhibited by specific effectors (negative feedback, feedback control).

6. Allosteric enzymes :

6. 1. Generalities :

This is a special category of enzymes that play a fundamental role in regulating metabolic pathways in all living organisms.

Example :

In the metabolic pathway: $A \longrightarrow B \longrightarrow C \longrightarrow \dots\dots \longrightarrow Z$

The enzyme that catalyzes the first reaction is inhibited by product Z (feedback inhibition).

In fact, the enzyme reversibly binds Z at a site other than the catalytic site. In this case, Z is called an effector or allosteric ligand of the enzyme.

6. 2. Allostery :

The term allostery (allos: Greek for “other,” “different”) comes from the fact that these enzymes are inhibited or activated by effectors, inhibitors, or activators that are sterically different from the substrate(s).

These effectors bind to sites distinct from the active site, called allosteric sites, through non-covalent bonds. Thus, the enzyme-effector complex is dissociable (reversible).

Effectors are therefore regulators of allosteric enzyme activity, since their binding results in a decrease or increase in the initial speed.

6. 3. Properties of allosteric enzymes:

- They have a quaternary structure.
- They exhibit kinetic behavior that does not conform to the Michaelis function.
- They are subject to conformational changes without loss of biological activity. These changes are detectable by various physical or kinetic measurements.
- They generally have a regulatory function in metabolism, particularly in synthesis pathways.

6. 4. Cooperativity :

Allosteric interactions between subunits of the same enzyme are cooperative interactions caused by the binding of a ligand (effector or modulator) to a specific site, modifying the binding of a second ligand to another site on the enzyme protein. In other words, the binding of a substrate molecule to one subunit may or may not promote the binding of other molecules to the remaining subunits.

This cooperativity is reflected in the sigmoidal curve that links the initial velocity to the ligand concentration.

