ENZYMES

1. Do not change the **direction** of the reversible reaction.

2. **Do not include in** the composition of **product**.

3. **Are not changed** during the reaction.

The differences of enzymes from inorganic catalysts are:

1. High **specificity**.

2. The **rate** of their catalysis is very high.

3. The rate of their reaction **depends on** the concentration of **enzyme**

4. Their activity is regulated by medium **pH**, **temperature**.

5. They are active under **normal condition**: normal pressure, neutral pH.

Being proteins, enzymes are sort into simple and complex. Simple ones are composed simply of proteins.Complex enzymes are called holoenzymes and have non-protein part. The protein portion of the holoenzyme is called apoenzyme.If non-protein part of holoenzyme is easily dissociated from the protein, it is called a coenzyme, if not separated - prosthetic group.

The cofactor binds to the apoenzyme mainly by ionic and hydrogen bonds, rarely by covalent bonds.

The specificity of the enzyme depends on the apoenzyme.

The active center is the site of the enzyme, which directly contact with the substrate. There are 2 zones in the active center:

• catalytic zone, that performs the substrate convertion to product.

• binding site, or contact zone that provides contact with the substrate. There is also allosteric center in some enzymes, which binds to low molecular particles. Allosteric center connects with compounds called allosteric effector or modifier. So, the allosteric enzyme is an enzyme, whose catalytic center changes under the influence of the allosteric effector.

Vitamin coenzymes

Coenzyme can be of vitamin and non-vitamin nature. FAD, FMN, NAD, NADP are both vitamin and nucleotide cofactors, because they contain vitamin and nucleotide simultaneously. HS-CoA also refers to nucleotide coenzymes.

Being a cofactor of hydrogenases vitamin C converts:

• Vitamin D ⟶ to calciferol

• cholesterol ⟶ to steroid hormones

• procollagen ⟶ to collagen, synthesizing from proline – hydroxyproline.

To non-vitamin coenzymes refer:

1. all triphosphates of purines and pyrimidines: ATP, GTP, TTF, UTP, CTP.

2. phosphates of monosaccharides, such as 2,3-diphosphoglycerate, glucose-1,6-diphosphate.

3. metallo- porphyrins, particularly iron porphyrins in cytochromes, catalase, peroxidase.

4. glutathione in glutathione peroxidase and glutathione reductase.

Metallo enzymes

There are enzymes that have metal ions in their active center. To them refer:

• containing Ca^{2+} α-amylase and lipase

• carboxypeptidase and dipeptidase, which have Zn^{2+}

• xanthine oxidase and aldehyde oxidase, which contain FAD, Mo, Fe.

Xanthine oxidase contains 2FAD, 8 Fe, 2Mo, and -SH group.

In wells where there is a lot of molybdenum, under influence of Mo xanthine oxidase is activated and as a result gout appears. At gout uric acid increases in the blood of the patients.

Proenzymes

Proenzymes are inactive enzymes, which under partial hydrolysis turn to active ones. For instance, inactive pepsinogen, trypsinogen and chymotrypsinogen are proenzymes.

Trypsinogen undergoes partial hydrolysis under the influence of enteropeptidase (enterokinase) turning into trypsin.

Prothrombin under the influence of coagulation factors and Ca^{2+} turns to thrombin.

Plasminogen is converted to plasmin and by this way activated.

Isozymes

Isozymes are formed due to genetic factors. They catalyze the same biochemical reaction. Quaternary structure of isozymes differs from each other in number and features of peptides.

Isozymes differ from each other in:

• electrophoretic mobility

• substrate sensitivity

• optimum pH

• localization in organoids

• specificity to coenzyme

So, LDH has 5 isozymes. Each of them consists of M and H polypeptides. In the fetus and skeletal muscles present mainly LDH_5 and LDH_4. in the heart LDH_1 and LDH_2 are prevalent. An increase in LDH_5 indicates liver and skeletal muscle pathology. At heart damage LDH_1 and LDH_2 increases in the blood.

Polyenzymes

Polyferments are of 3 types:

1. Structural-functional type, when between enzymes is available a structural connection. Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase are examples of structural-functional polyenzymes . Pyruvate dehydrogenase is located on the inner mitochondrial membrane.

2. Functional type - these enzymes can be easily separated from each other. The product of one enzyme is a substrate for another one. Such are enzymes of glycolysis.

3. Mixed type of polyenzymes – it is a combination of 1st and 2nd types. α-ketoglutarate dehydrogenase of Krebs cycle is structural-functional type of polyenzymes, while the rest enzymes of Krebs cycle are of functional type.

Enzymes localization

*RNA polymerase and DNA polymerase occur in the nucleus.

* Hydrolytic enzymes - in lysosomes.

* Enzymes of glycolysis occur in the cytoplasm.

* enzymes of Krebs cycle and β-oxidation of fatty acids occur in mitochondria.

* The ornithine cycle occurs in the liver.

* Enzymes of glycolysis are prevalent in skeletal muscles.

Enzymes are divided into 6 classes:

1) Oxido reductases:

• if "H" is transferred – enzyme is called dehydrogenase

• if O_2 is an acceptor, enzyme is called oxidase

• if H_2O_2 is an acceptor, enzyme is called peroxidase

• if O_2 is included directly into the substrate, enzyme is called oxygenase

2) Transferases:

• The compound giving a radical is a donor, while the receiving one is an acceptor.

There are methyl-; acyl; glycosyl-; aldehyde-; ketone-; amine transferases and so on.

For example, choline-acetyl-CoA transferase converts choline on acetyl group:

Choline-acetyl-CoA transferase

Choline + AcetylCoA ⟶ Acetyl-Choline

3) Hydrolases cleave intramolecular bonds hydrolytically. They add the water across the bond, hydrolysing it. Lysosomal and digestion enzymes, such as peptidases, phosphatases, DNA-ses belong to hydrolases. These enzymes are simple proteins without coenzymes.

4) Liases cleave C – O, C – H, C – N, C – S, P – O, C – Cl bonds without H_2O or O_2.

Dehydratases are liases that remove H_2O from substrate. Decarboxylases remove CO_2:

Histidine decarboxylase

Histidine ---------- → Histamine + CO_2

Lyases that join to molecule a group, splitting it off from another substrate are called synthases, as for example **citrate synthase.** Unlike synthetases called ligases, they do not use ATP energy at catalising the reaction.

5) Isomerases catalyze intramolecular changes. Depending of isomerisation type, that they catalyze, they are called mutases, tautomerases, racemases, epimerases, cis-trans-isomerases. For example:

Triphosphate isomerase

Glyceraldehyde-phosphate ⟶ Dihydroxyacetone-phosphate

6) Ligases, or synthetases connect 2 substrates at the expense of ATP energy, forming a more complex product. They form C – C, C – O, C – N, C – S, C – P bonds:

Acetyl CoA synthetase

Acetate + HS-CoA + ATP ⟶ acetyl-CoA + ADP

7. Translocases:

EC 7.1 Catalysing the translocation of hydrogens
EC 7.2 Catalysing the translocation of inorganic cations
EC 7.3 Catalysing the translocation of inorganic anions and their chelates
EC 7.4 Catalysing the translocation of amino acids and peptides
EC 7.5 Catalysing the translocation of carbohydrates and their derivatives
EC 7.6 Catalysing the translocation of other compounds

EC 7.1 Catalyse the translocation of hydrogens

EC 7.1.1 Hydrogen translocation or charge separation linked to oxidoreductase reactions

EC 7.1.2 Hydrogen translocation linked to the hydrolysis of a nucleoside triphosphate

EC 7.1.3 Hydrogen translocation or charge separation linked to oxidoreductase reactions etc.

Specificity of enzymes

Urease cleaves only carbamide, arginase - only arginine. This is absolute specificity. Fumarase, oxidase of L- and D- amino acids, lactate dehydrogenase have stereochemical specificity. They act only on one of the stereoisomers of the molecule.

Pepsin, trypsin, chymotrypsin have relative group specificity. They cleave the same type of the bonds – peptide ones. Relative specificity also has cytochrome P_{450}. It catalyzes convertion of nearly all hydrophobic molecules to hydrophylic ones and has the weakest specificity.

Thermolability is the sensitivity of the enzyme to the temperature of the medium.The optimum temperature is that one, at which enzyme is the most active.For most enzymes, t_{opt} = 20-40°C, an average value 37-40°C

But for catalase t_{opt} = 0°C. Adenylate kinase is also an exeption, as it retains its activity at 100°C.

A temperature rise of 10° C doubles the activity of enzymes.

Enzymes of glycoprotein nature are resistant to temperature.
The body enzymes are most active at 37-38 ^{0}C. At 50-55°C and above, the enzymes are denatured and lose their activity.Hibernation is artificial cooling of the body. Hibernation is used in surgical interventions.

The pH of the medium changes the structure of the enzyme, weakens or strengthens the connection between apoenzyme and its cofactor, changes the state of activators and inhibitors, because it affects the degree of their ionization. The optimum pH is such pH of medium,at which the activity of enzyme is maximal. For most enzymes, pH_{opt} = 6.0-8.0. But for pepsin pH_{opt} = 2, for

arginase pH_{opt} = 9.9.

Catal is an enzyme activity that transforms 1 mole of substrate into a product in 1 second. Molecular activity is the amount of enzyme in moles that turns 1 mole of substrate per 1 minute into a product.

Modifiers, or **regulators** of enzymes are divided into 2 groups: activators and inhibitors.

Activators are 2 types:

1. Affecting the active center of the enzyme. These are **substrates and cofactors**. For example, alkaline phosphatase is activated by divalent ions Mg2 +, Mn^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}.

2. Modify a different area, not active center. These activators are represented by **allosteric activators** and compounds, that cleave the fragment from the proenzyme producing **partial hydrolysis**. For example: enterokinase is activator for trypsinogen. This is sample of enzymes activating by partly hydrolysis.

Some activators transform disulfide bonds into sulphhydryl groups, **protecting** the enzyme from oxidation. Such activators are cysteine and glutathione.

The next type of activators **dissociate** the enzyme from its inactive complex with other protein. For example, 3,5-cAMP is such kind activator for protein kinase.

But **inhibitors** weaken the activity of the enzyme. All denaturing factors inactivate enzymes. Salts of heavy metals (for instance Hg-mercury) in small doses are inhibitors of enzymes with SH-groups. In high doses, they denature enzymes.

Inhibitors are divided to reversible and irreversible. After removal of irreversible inhibitor from the medium, enzyme activity is not restored, while after removal of reversible one enzyme's actitity is restored .

Samples of non-competetive irreversible inhibitors are DFP and monoiodo-acetamide. DFP (di-isopropyl-fluorine-phosphate) is an **irreversible** inhibitor of acetylcholine-esterase. It binds to serine in the active center of enzyme. Chlorophos, dichlorvos and DFP zarin and zoman have the same effect. Monoiodo-acetamide is an irreversible inhibitor of enzymes containing the SH- and imidazole group in the active center. Physostigmine, prozerin are reversible inhibitors of acetylcholine esterase.

Reversible inhibitors are competitive and non-competitive. A *competitive* inhibitor competes with the substrate for connection with the active center of the enzyme, because it is similar to substrate. Its reaction is expressed by the formula E + I = EI. This inhibitor never forms a three-component ESI complex. Its inhibitory effect is prevented by increasing the concentration of the substrate in the medium. Malonate and oxaloacetate are competitive inhibitors of SDH (succinate dehydrogenase).

Anti-coenzymes also compete for binding to the active site. Antivitamins are such kind of inhibitors.

A *non-competitive* inhibitors bind to a site, different from active center or to those groups of active site, that do not bind to the substrate. These inhibitors can form a three-component ESI complex, as well as a two-component complex EI. Non-competitive inhibitors can be reversible and irreversible. For example, cyanides are irreversible inhibitors of cytochrome oxidase. They join to most enzymes with sulfhydryl groups. Unithiol, cysteine, citrate binding to metal, for example mercury, that has been inactivating enzyme, serve as antidote and are called reactivators.

Uncompetitive inhibitors do not bind to either free enzyme or free substrate. Binding only to the ES complex, they always form ESI form.

Induction and repression of enzymes

There are *constitutive enzymes*, which do not require an inductor for the availability in the cell. Such enzymes are repressed by the reaction product. But some enzyme need an inductor. For example, lactose induces the synthesis of lactase in intestine, therefore, lactose is called an inductor for lactase. Induction begins 1-2 minutes later after adding the inductor.

Repression of enzyme stops synthesis of enzyme at the gene level.

Enzymes' activity regulation

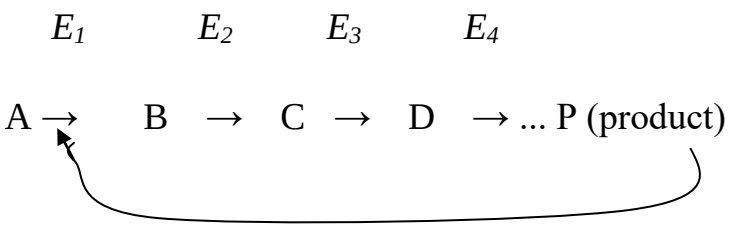
As enzymes are walled up with organell membranes, they work in limited spase. This phenomenon is referred to the *compartmentalization.* Thus, the compartmentalization is the regulation of enzymes by limitation of their localisation via membrane of organoids. In this case, the metabolism is regulated by "shuttle mechanism" - the substance passes through the membrane after it transformation into the appropriate molecule.

Enzyme activity is also regulated by:

1. The masses law of action:

$$\text{Alanine} + \alpha\text{KG} \xleftrightarrow{ALAT} \text{PVC} + \text{glutamate};$$

2. The principle of feedback:



3. Transformation of proenzyme into active enzyme. Sample is proteinkinase activation via cAMP.

4. Chemical modification of enzyme, which means phosphorylation and dephosphorylation of enzyme. So, adrenaline phosphorylates glycogen phosphorylase “b”, turning it into the active form “a”. Glycogen synthetase after phosphorylation is inactivated.

5. Allosteric regulation via effectors. Effector can be positive and negative. Positive one increases the activity of the enzyme, while negative - reduces.

Enzymology

Enzymology has 3 directions:

• Enzymopathology

• enzymodiagnostics

• enzymotherapy

Enzymopathy can be hereditary and acquired. Acquired is a result of wrong nutrition (alimentary), toxic factors, impaired neurohumoral regulation. Alimentary enzymopathy occurs due to the lack of vitamins, proteins, microelements.

Toxic enzymopathy is a result of poisons action. Thus, arsen salts and mercury are bound to the thiol —SH groups of the enzyme. Floridzin is also a poison;

this glycoside inactivates hexokinase. As a result, the reabsorption of glucose in the kidneys is disturbed, and glycosuria occurs.

Enzyme diagnostics. Some enzymes are used as indicators of diseases, or for determination of compounds in the biological fluids.For example, at myocardial infarction in the blood increases CK (creatine-phosphokinase), aspartate aminotransferase and LDH (lactate dehydrogenase). Especially characteristic at heart attac is increase of MB isozyme of CK and LDH_1 isoenzyme of LDH. At liver disease in the blood rises ALAT (alanine aminotransferase). At inflammation of the pancreas in the blood increases trypsin, chymotrypsin, amylase, lipase. In acute pancreatitis, α-amylase is determined in the blood. Glucose oxidase is a reagent for glucose determination in the blood, urease – for carbamide.

Enzyme therapy. Nucleases, such as ribonucleases and deoxyribonucleases are used in the treatment of purulent wounds. Ribonuclease stops the growth of RNA viruses. Lysozyme and hyaluronidase increase the permeability of cell membranes. Hyaluronidase also softens scars.

Vitamin	Coenzyme	Enzyme
PP-nicotinamide	NAD, NADP	OXIDOREDUCTASES
B_2-riboflavin	FAD, FMN	
KoQ,		
lipoic acid		

B_6-pyridoxal	PALP, PAMP	TRANSFERASES
B_3 - pantothenic acid	CoA, dephosphon-CoA, 4-phospho-pantothenate	
B_9-folic acid	THFA	
B_{12}-Cobalamin carnitine	Methyl-Cobalamin	

UDF, CDF, PAPS, ATP, S-adenosyl-methionine are non-vitamin coenzymes of Transferases.

Do not have coenzymes - HYDROLASES

B_6	PALP	LIASES
B_3	HS-KoA	
B_{12}	DOAC (deoxyadenosyl-cobalamin)	
B_1	TPP (in ketoacid decarboxylases)	

B_6 PALP,

B_{12} DOAC

} ISOMERASES

Glucose 1,6-diphosphate; 2,3BFG (diphosphoglycerate) are non-vitamin coenzymes of Isomerases.

THFA } LIGASES

UDP, CDP are non-vitamin coenzymes of Ligases.