Enzymes

Teaching aims

- Gaining new knowledge about properties of enzymes and their importance for appropriate metabolism as well as clinical diagnostics
- Gaining new knowledge about vitamin coenzymes and their importance for appropriate activity of enzymes

Learning effects

- Ability to use enzymologic knowledge for laboratory diagnostics – the interpretation of obtained results
- Understanding of metabolic consequences of alterations in enzyme activities
- Understanding of meaning of vitmain coenzymes for metabolism

Definition

Specific protein biocatalisator which is able to decrease activation energy leading to the increase of velocity or making possibe at all the reaction.

Classification

- in accordance to structure:

  - simple
  - conjugated – with prosthetic group
    - with coenzyme

Classification

- in accordance to function:

- OXIDOREDUCTASES – catalyse redox reactions
- TRANSFERASES – catalyse the transfer of groups of atoms between molecules
- HYDROLASES – catalyse the degradation of bonds with the use of water
- LYASES – catalyse the degradation of bonds without the use of water
- ISOMERASES – catalyse the reactions of isomerisation
- LIGASES (SYNTHETASES) – catalyse the synthesis of bonds between atoms in molecules of substrates

Classification

- in accordance to function:

- 7 classes

Class 1

Oxidoreductases catalyze redox reactions. They transport electrons and protons between the oxidant and the reductant.

Class 2

Transferases catalyze the transfer of specific functional groups (-SH, NH_2, $-CH_3$ between individual compounds

Class 3

Hydrolases catalyze the breakdown of various bonds involving a water molecule.

Class 4

Lyases catalyze the detachment of groups from the substrate without the participation of water.

Class 5

Isomerases catalyze isomerization reactions – reconstruction within the molecule

Class 6

Ligases (SYNTHETASES) – catalyze the formation of bonds between atoms in substrate molecules

Class 7

TRANSLOCASES – proteins that help transport ions or molecules across the cell membrane or within it (ATP synthase)

Nomenclature

- 4 digit number
- systematic names defined in accordance to catalysed reaction

  oxidoreductase alcohol:NAD
- working names (commonly used)

  alcohol dehydrogenase
- historical names (pepsin)

number

Specificity of enzymes

- In accordance to catalysed reaction
- In accordance to substrate – low, high, absolute

Mechanism of enzymatic catalysis

1. Contact between molecules of substrate and the surface of enzyme molecules
2. Electrostatic interaction between substrate and enzyme leading to the formation of active form of **complex enzyme-substrate**
3. Enzymatic catalysis – complex enzyme-substrate is converted into complex **enzyme-product**
4. Product and free enzyme are released

Complex enzyme - substrate

The structure of active center (catalytic)

- Area for binding the substrate – contact groups
- Area for enzymatic catalysis – catalytic groups

Models of active center

- in accordance to Fischer – „key and lock”
- in accordance to Koshland – „hand and glove”
- current

Factors that influence **the velocity** of enzymatic reactions

- temperature
- the concentration of hydrogen ions
- redox potential
- modulators (activators/inhibitors)

Enzymatic kinetics

Deals with topics related to mechanisms of reactions and their velocity

The reaction rate **v** is measured by the change in the concentration **c** of reacting substances (substrates or products) per unit of time **t**.

Reaction rate constant (proportionality constant k) – depends on the type of chemical reaction, the conditions of its course and is characteristic for a given temperature. It is numerically equal to the rate of such a reaction in which the concentrations of all reacting substances are equal to 1 mol/l. It is determined experimentally.

The reaction is expressed by the stoichiometric equation:

$$aA + bB \leftrightarrow cC + dD$$

and kinetic equation:

$$v = -\frac{dc}{dt} = kc_A^a c_B^b$$

Kinetic classification of chemical reactions

Reaction order – the sum of exponents from the concentration of reactants. These exponents are selected experimentally, they are not related to stoichiometric coefficients. The reaction order does not define the reaction mechanism, but is helpful in considering the reaction mechanism.

Reaction molecularity – indicates the number of molecules participating in a given process, should not be identified with the reaction order.

First order reactions

Those whose rate, determined experimentally, changes in proportion to the concentration of one of the reacting substances

$$v = -\frac{dc}{dt} = k_I c$$

$$k_I = \frac{2{,}3}{t} \log \frac{c_0}{c}$$

Half-life – is the time after which the concentration of the substrate as a result of the reaction has decreased by half of its initial concentration. It does not depend on the initial concentration of the substance.

$$t_{1/2} = \frac{0{,}693}{k_I}$$

Second order reactions

Those whose rate, determined experimentally, is proportional to the product of the concentrations of the two reacting substances or the square of the concentration of one substrate.

$$v = -\frac{dc}{dt} = k_{II} c_1 c_2 = k_{II} c^2$$

$$k_{II} = \frac{2{,}3}{t(c_{01} - c_{02})} \log \frac{c_{02} c}{c_{01} c}$$

The half-life is inversely proportional to the initial substrate concentration.

$$t_{1/2} = \frac{1}{k_{II} c_0}$$

Third order reactions

They are relatively rare and include those reactions whose rate, determined experimentally, is proportional to the product of the three reacting substances or to the cube of the concentration of one substrate.

$$v = -\frac{dc}{dt} = k_{III} c_1 c_2 c_3 = k_{III} c^3$$

$$k_{III} = \frac{c_0^{\,2} - c^2}{t 2 c_0^{\,2} c^2}$$

The half-life is inversely proportional to the square of the initial concentration

$$t_{1/2} = \frac{3}{2 k_{III} c_0^{\,2}}$$

Zero order reactions

They proceed at a rate independent of the concentration of the reacting substances. They depend on the enzyme concentration.

$$v = -\frac{dc}{dt} = k_0$$

Half-life time:

$$t_{1/2} = \frac{c_0}{2k_0}$$

Example

A drug was produced in January 1992. If its decomposition follows first-order kinetics, how long will it take for half of the drug to disintegrate? The rate constant k for this reaction is $7.9 \times 10^{-5}\ h^{-1}$

$$t_{1/2} = \frac{0,693}{k}$$

$$t_{1/2} = \frac{0,693}{7,9x10^{-5}} = 8772,15godz$$

$$\frac{8772,15godz}{24godz} = 365,5dni$$

Michaelis Constant

This is the substrate concentration at which the reaction rate is half the maximum rate. It is a characteristic value for each enzyme under appropriate pH and temperature conditions. It determines the affinity of substrate for the enzyme.

Michaelis constant

According to the Michaelis–Menten model, the enzyme-catalyzed reaction proceeds according to the scheme:

$$S + E \underset{k_1}{\overset{k_2}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

The rate of the actual enzymatic reaction, i.e. the rate of product formation and release, depends on the concentration of the ES complex and is characterized by the reaction rate constant k_3

$$v = k_3[ES]$$

In describing the kinetics of such an enzyme, Michaelis and Menten based their description on the following assumptions:

1. The concentration of the ES complex is constant over time. The concentration of the substrate and product can change, but not the ES. If the rate of formation of the ES complex is equal to the rate of its disintegration, then:

$$k_1[E][S] = (k_2 + k_3)[ES]$$

And by introducing constant K_m

$$K_m = \frac{k_2 + k_3}{k_1}$$

We receive:

$$K_m = \frac{[E][S]}{[ES]}$$

2. If all of the enzyme is in the ES form, then for a given enzyme concentration the rate reaches a maximum value:

$$v_{\max} = k_3[ES] = k_3[E]$$

Hence, the final equation for the rate of the enzymatic reaction is:

$$v = \frac{v_{\max}[S]}{[S] + K_m}$$

Inhibitions

- competitive inhibition

Inhibitions

- non competitive inhibition

Inhibitions

- allosteric inhibition

Medical meaning

- Toxicity – many compounds naturally occuring or synthesised by companies are irreversible inhibitors of some enzymes eg sarin (poison gas) inhibits acetylcholinesterase.
- Therapeutic use – natural and synthetic coumpunds are used for the inhibition of selected enzymes for therapeutic reasons:

Lowastatin inhibits HMG-CoA reductase and decreases the concentration of cholesterol

Penicylin inhibits transpeptidase and has antibacterial activity

Pargylin inhibits monoaminooxidase and has hypotensic activity

Anticoagulants

Heparin – binds to antithrombin III and increases its inhibitory activity

Hirudin – natural inhibitor of thrombin

Kumarins – inhibit vitamin K dependent γ-carboxylase of Gla residues in coagulation factors

Fibrinolysins

Streptokinase – enzyme of β-hemolytic streptococci which dissolves clot

Ways of expressing enzyme activity

Enzymatic units

- S**tandard Unit** (U) – amount of enzyme that catalyses the conversion of 1 micromol substrate during 1minute in optimal conditions
- C**atal** (cat) – activity of enzyme that converts 1mol of substrate during 1 second in optimal conditions
- **Specific activity** – amount of U per 1 mg of protein (in SI system – cat/kg protein) – defines the degree of purity of enzymatic preparates

Regulation of enzyme **activity**

- Changes in direct amount of enzymatic protein
- Changes in concentrations of reagents
- Changes in catalytic capacity of enzyme

Changes in direct amount of enzymatic protein

Direct amount of enzymatic protein is the result of balance between its synthesis and degradation – processes that occur independently and are independently regulated

- inductors of synthesis of enzymatic protein
- represors of synthesis (eg feedback via the product of reaction)
- metabolic turnover
- environmental influence – hormones, diet
- synthesis of inactive precursors

Changes in concentrations of reagents

Activation or inhibition of enzyme activity via end products or intermediates (enzymatic induction – adaptive enzymes)

Changes in catalytic capacity of enzyme

The change of enzyme activity without the change in the concentration of enzymatic protein

- compartmentation
- multimolecule complexes
- coenzymes
- covalent modifications – phosphorylation /dephosphorylation → kinases/phosphatases
- allosteric efectors

Covalent modifications

- reversible phosphorylation of serine, tyrosine, threonine residues
- reversible nucleotidation
- proteolytic clevage – proteolytic enzymes, blood clothing

Allosteric effectors

- activators and inhibitors change the velocity of enzymatic reactions and influence the regulation of metabolic pathways
- feedback inhibition – inhibition via end product of pathway that prevents unnecesssary formation of the excess of end product

Isoenzymes

- Physically different forms with the same catalytic activity.
- May appear in different tissues of the same organism, in different cells or subcellular compartments.
- May differ from molecular weight and electrophoretic mobility.
- The determination of isoenzymes possess diagnostic meaning – they can be organ specific.

Isoenzymes

Diagnostic meaning of the determination of enzyme activity

- secretory enzymes – secreted directly to vascular bed (cholinesterase, proteases of coagulation and fibrynolysis)
- excretory enzymes – secreted to eg digestive tract (amylase, lipase)
- indicative enzymes – intracellular – their activity increases during the damage of cells (AST, ALT, LDH, CK)

Plasma – is obtained after the centrifugation of full blood taken into tube with anticoagulant

Serum – is obtaine after the centrifugation of blood taken into dry tube

Some typical causes of changes in enzyme activities in blood

- increased proliferation of cells and the induction of enzymes - changes in elimination of enzyme
- changes in premeability of cell membranes – release of cytoplasmic indicative enzymes
- difficulties in flowing off the secretes of gland containing secretory enzymes (eg. cholestasis)
- the degradation of cells due to pathologic process (release of indicative enzymes)
- defect of synthesis (the decrease in enzyme activity mainly of secretory enzymes due to the damage of tissue by disease)

Alanine Aminotransferase (ALT)

Glutamate + pyruvate $\leftrightarrow$ α keto glutarate + alanine

The increase in actvity indicates on diffuse damage to cells but not the disturbances in the function of organ. May appear during the course of:

- liver cancers, inflammation of pancreas, haemolysis in vitro and in vivo
- liver cholestasis, cirrhosis, treatment with high doses of salicylates
- viral inflammation of liver, toxic damage to liver, insuficiency of circulation

Asparagine Aminotransferase (AST)

glutamate + oxalacetate $\leftrightarrow$ α keto glutarate + aspartate

The increase in activity appears during the course of:

- cirrhosis, inflammation of pancreas, haemolysis in vitro and in vivo
- diseases of skeletal muscles, chronic inflammation of liver, surgery, parasites, insuficiency of Se and vit E
- infarct, viral inflammation of liver, toxic damage to liver, cancers of liver, intensive effort in sport horses

Isoforms of aminotransferases

Amylase

The enzyme hydrolyses the breakdown of α 1,4-glycans containing at least 3 residues of glucose. The best substrates are, however, polysaccharides belonging to α-glycans such as amyloses, amylopectins and glycogens, which are metabolised to dextrins $\rightarrow$ maltotrioses $\rightarrow$ maltoses and small amounts of glucose.

The enzyme is present in pancreas, salivary glands, liver and muscles. The activity can be determined in the course of diseases of pancreas.

The increase in amylase activity in blood may result in its release with urine via not damaged renal glomeruli – it is possible to monitor the diseases of pancreas in urine.

Amylase

The increase of activity is observed in:

- acute inflammation of pancreas, intestinal occlusion, ketone acidosis in diabetes, renal insufficiency, hyperadrenocorticism, salivary gland occlusion

The decrease of activity:

- pancreatic necrosis, diffuse combustio, intoxication with heavy metals

Lipase

The enzyme catalyses the breakdown of esters of glycerol and fatty acids. It may confirm the presence of pathological processes in pancreas.

The increase of activity is observed in:

- acute inflammation of pancreas, cancers of pancreas, diseases of kidneys, intestinal occlusion,

Haemolysis of examined plasma may result in the decrease of results due to the inhibition of lipase activity by haemoglobin

Lactic dehydrogenase (LDH)

Cytoplasmatic enzyme present in every cell in brain, erythrocytes, heart muscle (H), leukocytes, kidneys, liver, muscles (M), lungs. It consists from 4 chains – M type for organs with lower oxygen needs and H type for organs with intensive oxygen metabolism. 5 tissue specific isoenzymes are known: M4; HM3; H2M2; **H3M**; H4.

Lactate + NAD $\leftrightarrow$ pyruvate + $NADH_2$

The increase in activity is observed during:

- diseases of liver, haemolytic anemia, leukemia, diseases of skeletal muscles, lung inflammation, infarct, longlasting stress

Isoenzymes of LDH

γ Glutamyl-transpeptidase (GGT)

It catalyses the transportation of γ-glutamyl group from donor to appropriate acceptor. Glutathione or γ- glutamyl peptides can be donors while glycil-glycine, α aminoacids or γ-glutamyl substrates can be acceptors

$$\gamma\text{-glu A} + \text{A`} \rightarrow \gamma\text{-glu A`} + \text{A}$$

It is present in kidneys, liver, cells of bile tract, pancreas, intestines. It belongs to inductive enzymes – for example by barbiturates, estrogens, alcohol

The increase of activity is observed during:

- intrahepatic and extrachepatic cholestasis, acute and chronic inflammations of pancreas, acute inflammation of liver, colonic ulcer, after treatment with corticosteroids in dogs

Alkaline phosphatase

It catalyses the hydrolysis of ortophosphate monoesters:

$R\text{-}O\text{-}PO_3H_2 + H_2O \rightarrow R\text{-}OH + H_3PO_4$

Isoforms are present in liver, bones, intestines, placenta, kidneys, spleen

The increase in activity is observed during:

- jaundice congestive, viral and toxic inflammation of liver, cirrhosis, leukemia, bone cancers, osteomalacia, rickets, after treatment with corticoids, hyperadrenocorticism in dogs, moderate increase in bone fractures

Acid phosphatase

Lysosomal enzyme – optimum pH 5.
Isoforms are present in **prostate**, liver, kidneys, erythrocytes, spleen, osteoclasts

The increase in activity is observed during:

- prostate cancer, malignant bone cancers, haemolysis in vitro and in vivo, damaged blood platelets, primary hyperfunction of parathyroid glands,

Haemolysis interrupts in appropriate determination

Creatine kinase (CK)

Cytoplasmatic and mitochondrial enzyme. The highest activity is in **muscles (M), brain (B), heart (MB)**, intestines. 3 tissue specific isoenzymes are defined – CK-MM; CK-BB; CK-MB.

It catalyses the transportation of phosphate group from ATP to creatine with the formation of phosphocreatine and ADP

Creatine + ATP $\leftrightarrow$ phosphocreatine + ADP

The increase in activity is observed during:

- damage to muscle tissue, infarct, progresive degeneration of muscles, intoxications with strychnine or carbon oxide

Cholinesterase (pseudocholinesterase)

Enzymes that catalyse the hydrolysis of choline esters to choline and appropriate fatty acids. The most important are:

- acetylocholinesterase from nervous system and erythrocytes which decomposes acetylcholine
- pseudocholinesterase produced in liver and liberated to circulation.

Activity is decreased in persons exposed to constant contact with pesticides, which block the enzyme

Haemolysis interrupts in appropriate determination

Glutamate dehydrogenase

Mitochondrial enzyme present mainly in hepatocytes. The activity is higher in men as in women.

It belongs to „liver profile” of diagnostic research.