MINISTRY OF HEALTH OF UKRAINE
ZAPORIZHZHIA STATE MEDICAL UNIVERSITY
Biological chemistry department

ENZYMES

A manual for "Biological chemistry" discipline for teachers

Zaporizhzhia
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and it is recommended for the use in educational process for foreign students.

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This Manual was made in accordance with the program of Biological chemistry for training of students of higher educational institutions of III-IV levels of accreditation for specialty «Medicine».

It is recommended to use for teachers that work with students of International Department (the second year, english medium of study).

Ферменти : навч.-метод. посіб. з дисципліни «Біологічна хімія» для викладачів / уклад. : К. В. Александрова, Н. В. Крісанова, С. В. Левіч. - Запоріжжя : [ЗДМУ], 2017. – 90 с.

Навчально-методичний посібник складено відповідно програми з дисципліни «Біологічна хімія» щодо підготовки студентів медичних ВНЗ ІІІ-ІV рівню акредитації для спеціальності «Лікувальна справа».

Рекомендовано для застосування викладачами в навчанні студентів ІІ-го Міжнародного факультету (2-й рік, англійська мова навчання).

CONTENT

RELEVANCE OF THE THEME

A study of questions about enzymes is the basis for learning of all other trends in Biochemistry. The knowledge about structure and properties of enzymes gives ability to explain in a right way the mechanism of enzyme action, to understand the influence of different factors on enzyme activity, and the enzyme behavior in biological fluids. The study of the principle for enzymes classification is very important, if it is in need to name enzyme according its chemical reaction - this can help any student in study of metabolic pathways later.

The information about the mechanism of enzyme action is the basis for understanding of all the metabolic pathways regulation in human organism, because the regulation of processes is made mainly across the regulation of some enzymes activities. A lot of medicines used for the treatment of somatic diseases are inhibitors of enzymes with different mechanism of influence on enzyme activities in microorganisms and viruses, or they are activators of some processes to enhance the protective properties of human organism thus to help in treatment of diseased person. Main notions about regulation of enzyme activity are useful in understanding of mechanism of medicine action and to choose by the doctor most suitable preparations for the treatment of patients.

The investigation of enzyme activity in biological fluids gives ability for students to understand the importance of enzymatic tests to recognize the situation in diseased person. A lot of clinical enzymology questions are associated with consideration of enzymopathies reasons for the development and ability for their treatment, too.

LESSON 4 OF MODULE 1

THEME:

ENZYMES: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES. CLASSIFICATION AND NOMENCLATURE OF ENZYMES

STUDY GOALS FOR LESSON 4 OF MODULE 1:

1. To learn features of enzyme (simple, conjugated) structure.
2. To investigate properties of enzyme such as salivary amylase: its specificity of action; its behavior under the different temperatures of the medium; its behavior at different pH of the medium
3. To be able to determine the class of enzyme using the the chemical reaction type catalyzed by the enzyme

IT IS IN NEED TO KNOW:

1. The function of enzymes in the organism. Enzyme characteristics in the comparison to non-protein catalysts.
2. Simple and conjugated enzymes structure. Definitions of apoenzyme, cofactor, coenzyme and prosthetic group. A structure of active centres for simple and conjugated enzymes.
3. The role of vitamins in the formation of active centre of enzymes (B1, B2, B3, B5, B6, H).
4. Common properties of enzymes (factors of an influence: pH and temperature of environment, specificity of action).
5. Isozymes: structure and location of their synthesis in tissues (e.g.: Lactate dehydrogenase isozymes).
6. Classification and nomenclature of enzymes: features of reactions catalyzed by each class of enzymes
7. Multienzyme systems of a cell: types of composition and function (e.g.: pyruvate dehydrogenase complex).

IT IS IN NEED TO DO:

1. Determination of salivary amylase specificity.
2. Thermolability investigation for salivary amylase.
3. Investigation of salivary amylase activity at pH region 6.0-8.0.

BASIC LEVEL FOR PREPARATION. DISCIPLINE INTEGRATION

Disciplines	**Obtained skills**
Previous: Organic chemistry	Organic compounds classification. Functional groups in organic compounds. Isomers for organic compounds. Covalent and acid-base catalysis.
Non-organic chemistry	Types of chemical reactions. Catalyst and catalysis
Common chemistry	Energy for activation of reaction. реакції. pH and temperature influencies on enzyme activity. Specificity of action.
Studied in parallel: Normal Physiology	Digestive, metabolic and protective functions of enzymes
Next in study: Clinical biochemistry	Clinical enzymology: principles for enzyme activity determination in biological fluids; the use in diagnostics of diseases ad as agents for methods

CONTENT OF EDUCATIONAL MATERIAL

FOR LESSON 4 OF MODULE 1

1. The function of enzymes in the organism. Enzyme characteristics in the comparison to non-protein catalysts.

2. Simple and conjugated enzymes structure. Definitions of apoenzyme, cofactor, coenzyme and prosthetic group. A structure of active centres for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B1, B2, B3, B5, B6, H).

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4. Isozymes: structure and location of their synthesis in tissues (e.g.: Lactate dehydrogenase isozymes).

5. Classification and nomenclature of enzymes: features of reactions catalyzed by each class of enzymes

6. Multienzyme systems of a cell: types of composition and function (e.g.: Pyruvate dehydrogenase complex).

A PLAN AND ORGANIZATION OF LESSON 4 OF MODULE 1

Stages	**Time/ minutes**	**Materials for lesson**		**Place for dura tion**
		Learning tools/content	**Equipment**	
1.Organizing time	**5**			**Auditory 507,509, 511, 514**
2. A conversation	**20**	**Discussion about catalysts, structure and properties of enzymes**	**1.Textbook on Biochemistry 2. A Manual for submodule 1**	
3. Initial control of	**15**	**Variants for testing**	**Blocks of tests**	

knowledge		**(1-2)**		
4.Independent work for classification of enzymes	**15**	**1.Textbook on Biochemistry 2. Manual for submodule 1**	**Chemical reactions blocks (5 variants)**	
5. Final control for theory	**10**	**Variants for control (1-6)**	**Cards with questions**	
Brake	**5**			
6. Laboratory works	**45**	BIOCHEMISTRY LABORATORY MANUAL Module 1	**Reagents for laboratory works; Test tubes Thermostat**	
7. Final results discussion	**15**	**Protocol N4**		

ALGORITHM FOR LABORATORY WORKS OF LESSON 4 OF MODULE 1

Laboratory work 1. Specificity of salivary amylase

THE PRINCIPLE OF THE METHOD:

Amylase splits starch, glycogen and does not react on sucrose. The specificity of the amylase action is proved by Trommer's test result.

THE COURSE OF THE WORK:

Pour 5 drops of the saliva dissolved in correlation (1:4) into 2 test tubes. Add 10 drops of 1 % starch solution into the 1-st test tube, and 10 drops 1 % of the sucrose solution into the 2-nd one. Put the both test tubes into the thermostat at 38°C for 10 minutes. Carry out the Trommer's test.

Trommer's test:

Pour 3 drops of 5 % copper sulfate (II) solution and a few drops of 10 % sodium hydroxide solution into each test tube until the blue transparent solution appears. Shake up the content of the test tubes. Then cautiously heat up the test tubes and boil for 1 minute. The appearance of red colouring proves the glucose presence.

THE EQUIPMENT AND REAGENTS

Thermostat, measured centrifugal test tube, test tubes, pipettes, support, holder for test tube, the gas burner; 1 % starch solution, 1 % sucrose solution, 5 % copper sulfate (II) solution, 10 % sodium hydroxide solution.

Possible final result: the presence of reddish color sediment after the boiling proves the glucose formation under the action of enzyme, it may be in the test tube with starch.

Laboratory work 2. The thermolability of salivary amylase

THE PRINCIPLE OF THE METHOD:

The influence of temperature on salivary amylase activity is judged at splitting of starch by this enzyme at various temperature conditions. The degree of starch splitting is determined by iodic test, the product formation might be proved by the Trommer's test.

THE COURSE OF THE WORK:

Collect 3 ml of saliva into a test tube. Take away 2 ml of saliva into another tube for to boil 5 minutes, and then cool. Into the third test tube add 1 ml of saliva and dissolve the volume in correlation (1:4). Take the new three test tubes, and pour into each test tube 10 drops of 1% starch solution, after that add 10 drops of the dissolved saliva into the 1-st test tube. Add 10 drops of boiled saliva into the 2-nd test tube. Add 10 drops of water into the 3-rd test tube (control tube). All three test tubes put into the thermostat for 10 minutes at 38^0C. Then divide the content of each test tube into two parts and carry out qualitative reactions for starch and glucose (Trommer's test, see above how to make).

Reaction to starch (iodic test):

Pour 1 drop of the solution of iodine in potassium iodide into all three test tubes. At the starch presence the blue coloured complex appears.

THE EQUIPMENT AND REAGENTS

Thermostat, measured centrifugal test tube, test tubes, pipettes, support, holder for test tube, the gas burner; 1 % starch solution, 5 % copper sulfate (II) solution, 10 % sodium hydroxide solution, solution of 0.1% iodine in potassium iodide.

Possible final result:

Test tube N1 was in investigation for the influence of salivary amylase activity under the $t^oC=38^oC$, iodic test must prove the absence of starch, Trommer`s test must be positive.

Test tube N2 was in investigation for the influence of salivary amylase activity under the $t^oC=100^oC$ (saliva was boiled); iodic test must prove the presence of starch, Trommer`s test must be negative, if complete denaturation was for the enzyme during the boiling of saliva.

Test tube N3 was without the enzyme; iodic test must prove the presence of starch, Trommer`s test must be negative.

Laboratory work 3. The influence of the pH environment on the salivary amylase activity

THE PRINCIPLE OF THE METHOD:

The influence of the pH-environment on amylase activity is judged by the starch splitting in various pH values. The degree of starch splitting is determined by iodic test, the optimum of pH corresponds to a negative iodic test.

THE COURSE OF THE WORK:

The saliva volume is dissolved in correlation (1:100). Take 6 test tubes and pour 2 ml of the phosphatic buffer with various value of pH: 6,0; 6,4; 6,8; 7,2; 7,6; 8,0

into each test tube. Then add 1 ml of 0,5 % starch solution and 1 ml of the dissolved saliva into each one. Mix the con tent of the test tubes and place them into thermostat at 38°C for 10 minutes. Then pour 1 drop of iodine solution into each tube, and mix. You can observe the colouring in each tube and mark the pH optimum.

THE EQUIPMENT AND REAGENTS

Thermostat, measured cylinder, test tubes, pipettes, support, 0.5 % starch solution, phosphatic buffer with various value of pH: 6.0; 6.4; 6.8; 7.2; 7.6; 8.0 ; solution of 0.1% iodine in potassium iodide.

Possible final result:

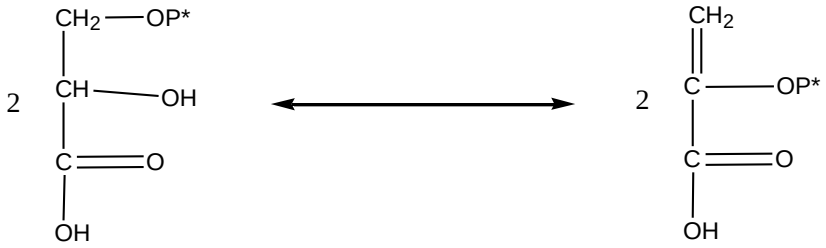
Test tubes with optimum pH after iodic test must prove the absence of starch, it means: they must be most colorless.

INDEPENDENT WORK WITH CHEMICAL REACTIONS BLOCKS TO ESTIMATE CLASS OF ENZYME

Variant 1

Try to classify enzyme according the type of reaction 1-5:

1)



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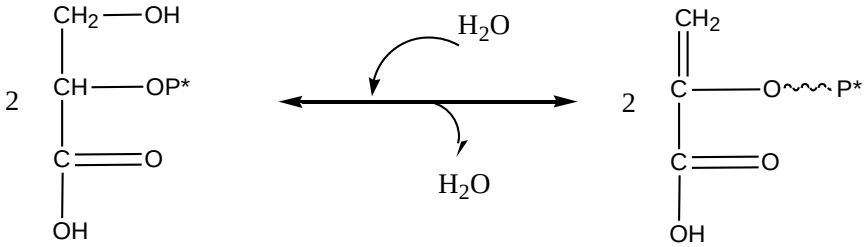

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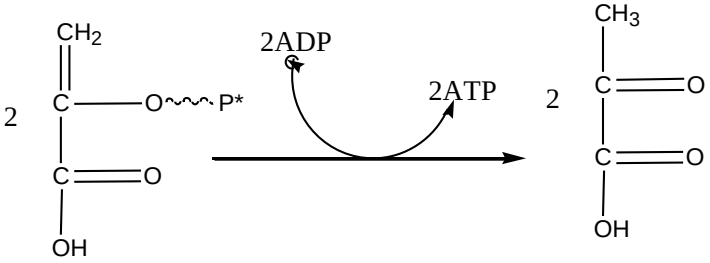

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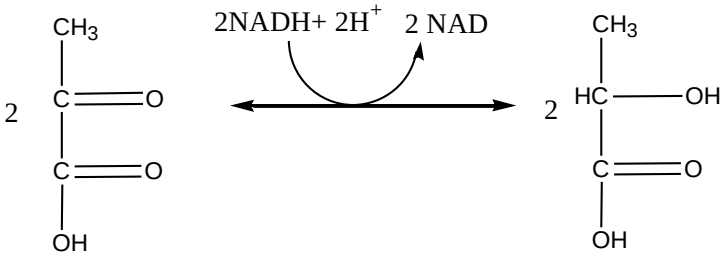

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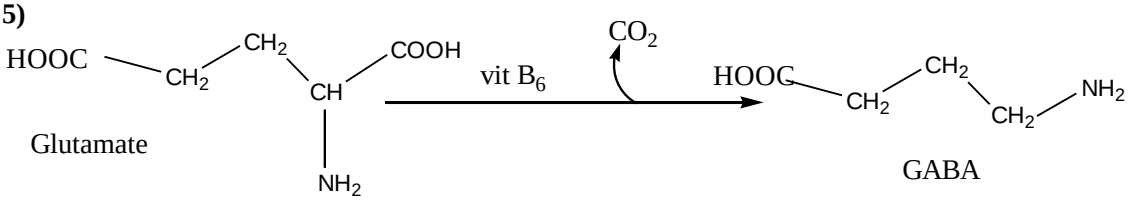


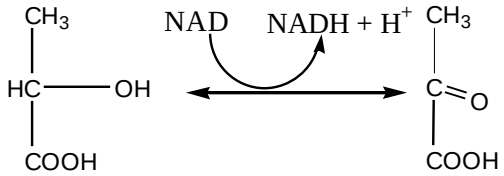
GABA - γ -aminobutyric acid

1-isomerase; 2-lyase; 3-transferase; 4-oxidoreductase; 5-lyase

Variant 2

Try to classify enzyme according the type of reaction 1-5:

1)



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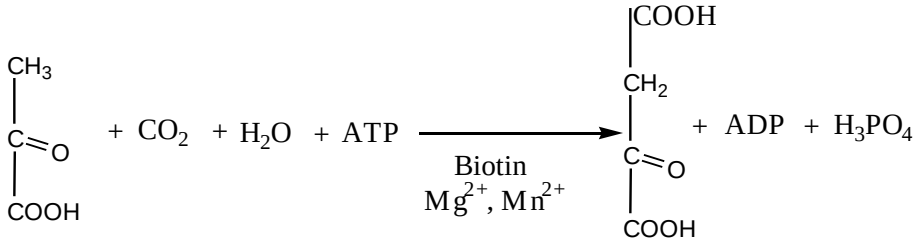

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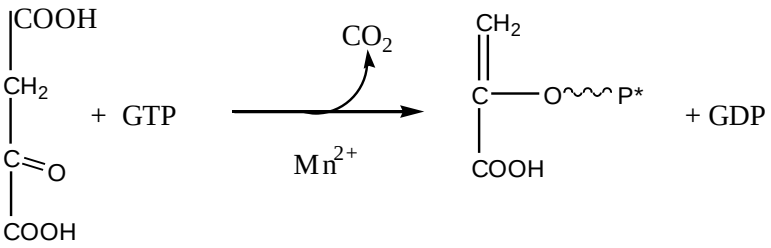

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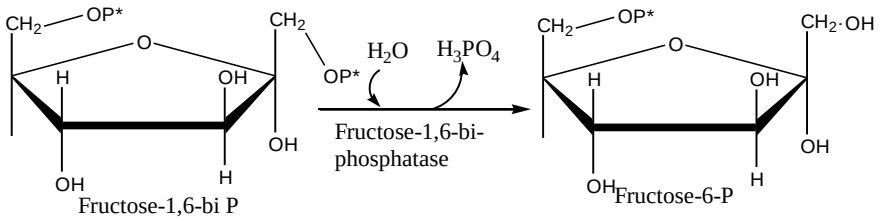

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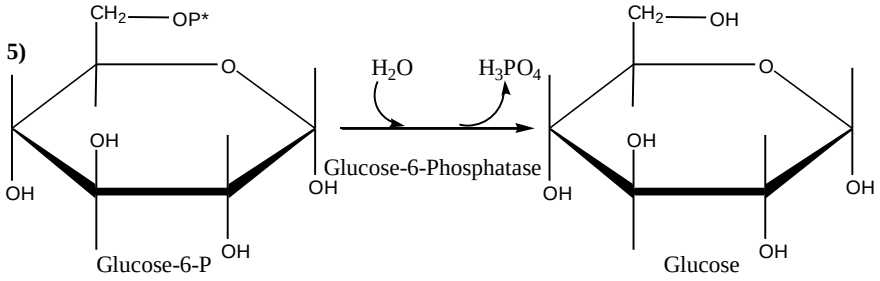


1- oxidoreductase; 2-ligase; 3- mixed transferase; 4-hydrolase; 5- hydrolase

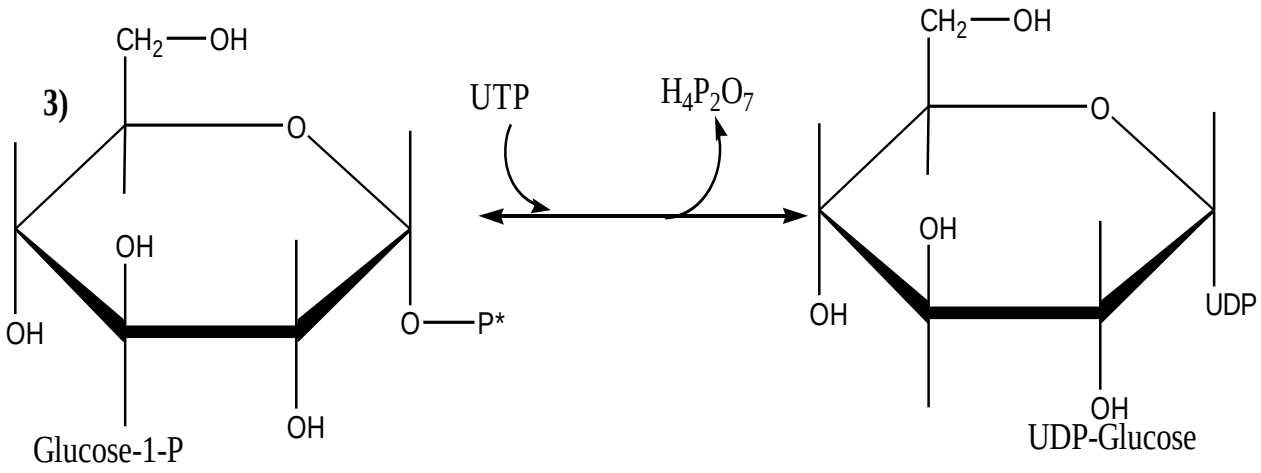
Variant 3

Try to classify enzyme according the type of reaction 1-5:

1) Glucose $\xrightarrow[Mg^{2+}]{ATP \rightarrow ADP}$ Glucose-6-P

2) Glucose-6-P $\longleftrightarrow$ Glucose-1-P

3)



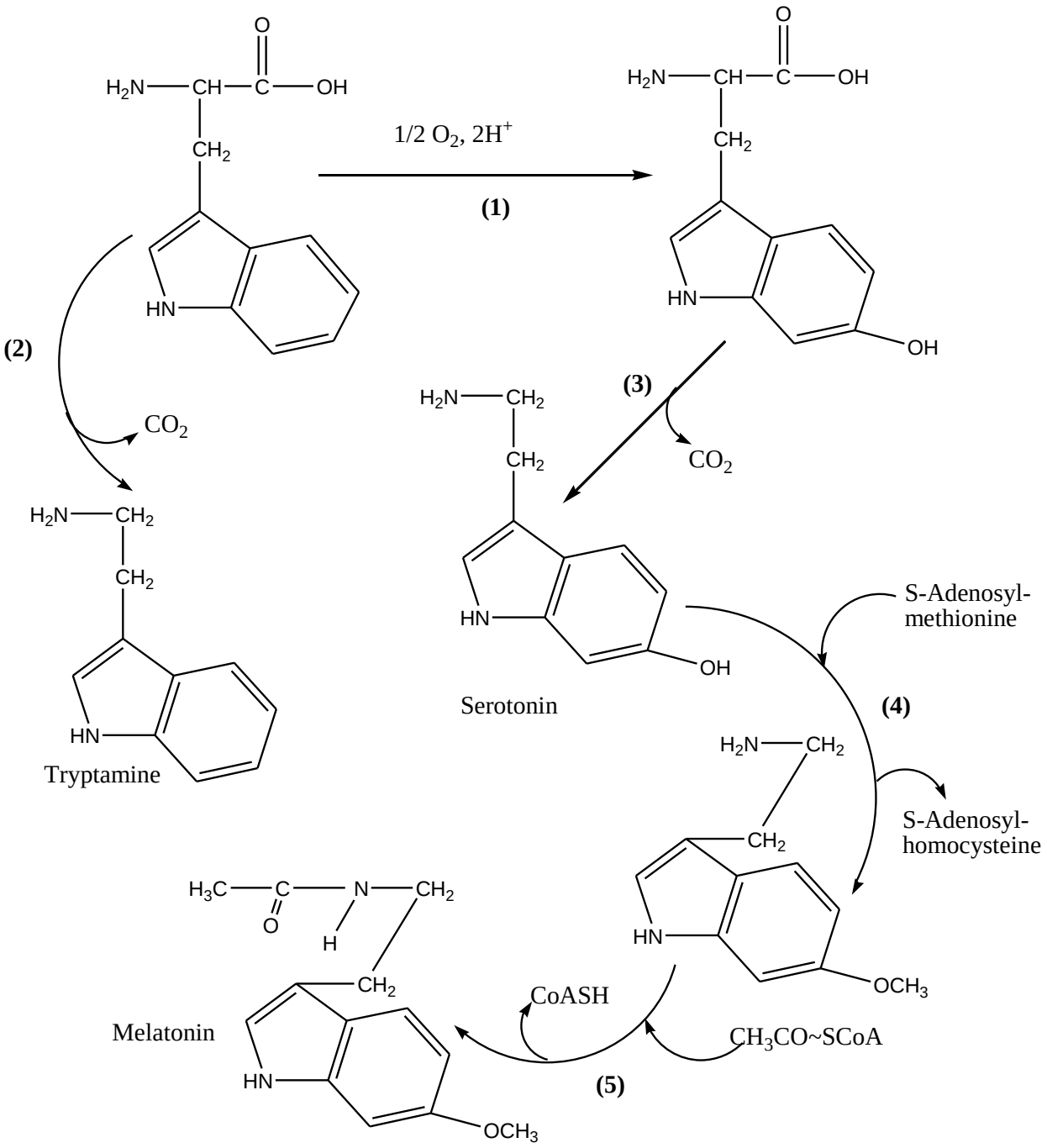
4) $H_4P_2O_7 \xrightarrow{+ H_2O} 2\ H_3PO_4$

5) UDP-Glucose + $(C_6H_{10}O_5)n \longrightarrow (C_6H_{10}O_5)n+1$ + UDP

1-transferase; 2- isomerase; 3- transferase; 4- hydrolase; 5- transferase

Variant 4

Try to classify enzyme according the type of reaction 1-5:



1- oxidoreductase; 2- lyase; 3- lyase; 4- transferase; 5transferase

FINAL CONTROL OF KNOWLEDGE FOR LESSON 4 OF MODULE 1

Variant-1

1. Salivary amylase can cleave the alpha-1.4-glycosidic bond in any polysaccharide structure. Determine the type of specificity for this enzyme. Explain your choice, please.

2. The Trommer's test is in need for salivary amylase specificity investigation in the course of the laboratory work. Explain, please, the principle of Trommer's reaction and its observation after its use in the laboratory work.

Variant-2

1. The optimum pH for pepsin action in gastric juice is in the region 1.5-2.5. What amino acid residues are in the active centre promoted the acidic properties of this enzyme? Explain your answer, please.

2. What is the color of iodic test on the starch in the experimental test tube containing the starch solution and boiled saliva? Explain your answer, please.

Variant 3

1. Salivary amylase can cleave the alpha-1,4-glycosidic bonds in any polysaccharide structure at pH in the region 6.8-7.6 mainly. Try to show the graph curve for the influence o pH on enzyme activity.

2. What is the color of Trommer's reaction in the test tube whish contained dissolved saliva, starch solution, and was placed before the reaction into the thermostat at 38ºC? Explain, please, your answer.

Variant 4

1. The enzyme contains 4 polypeptide chains and coenzyme NAD^+. Name the type of chemical reaction catalyzed by this enzyme. Name the class of this enzyme according the type of the reaction which you will choose.

2. Explain the principle of the iodic test on the starch, and its use in the investigation of temperature influence on salivary amylase activity.

Variant 5

ATP molecule may be used for phosphorylation of a substrate as the donor of phosphate group (case *a*), or as the energy source for the formation of a new bond between two structural fragments of two substances (case *b*). Name the class of enzymes for both cases (*a*, *b*), and the products from ATP in each case.

1. Explain, please, the principle of the qualitative test on glucose (Trommer's test), and its use in the laboratory work for the investigation salivary amylase specificity.

Variant 6

1. The cleavage of the fragment –C-C- in the molecule of a substrate may be due to hydrolase class, or due to lyase class. What is the principal difference between hydrolase and lyase action on this structural fragment?

2. The phosphate buffer solutions with different pH (6 values) are in need to investigate the influence of the pH medium on salivary amylase activity. Explain, please, the use of iodic test for the search of pH optimum for this enzyme.

TESTS FOR FINAL CONTROL

Variant 1

1. Conjugated enzymes contain cofactors in their structure. Point out the location of vitamin derivative cofactor in the structure of enzyme:

A. Active centre
B. Allosteric centre
C. Hydrophobic fragment of structure
D. Hydrophilic fragment of structure
E. Near the metal-ion-cofactor in the structure

2. Only one factor can influence on the charge of amino acid radicals in the enzyme active centre. Name it:

A. Temperature
B. Pressure

C. pH medium

D. The presence of a competitive inhibitor

E. The surplus of a reaction product

3. Find out the method for separation of isozymes mixture to determine each isozyme fraction in the blood serum of patient:

A. Dialysis

B. Electrophoresis

C. Spectrophotometry

D. Gel chromatography

E. Salting-out

4. Ribonuclease cleaves phosphodiester bonds in the structure of any type of RNA. Point out, please, its type of specificity:

A. Absolute

B. Absolute group

C. Absolute relative

D. Relative group

E. Stereochemical

5. Pyruvate dehydrogenase complex is multienzyme system because it contains:

A. Two enzymes and one coenzyme

B. Two enzymes and five coenzymes

C. Three enzymes and three coenzymes

D. Three enzymes and five coenzymes

E. Five enzymes and five coenzymes

6. One of the important properties of enzymes is their specificity of action. Check up a type of specificity for salivary amylase:

A. Absolute

B. Absolute group

C. Absolute relative

D. Relative group

E. Stereochemical

7. Some terms are used for the description of non-protein part of an enzyme. Point out the term for non-protein part that easily dissociates from polypeptide chain:

A. Apoenzyme

B. Coenzyme

C. Prosthetic group

D. Cofactor

E. Metall ions

8. The change of the temperature of environment from 0°C to 38°C can cause this effect:

A. The probability of enzyme-substrate complex formation is increased

B. A denaturation of enzymes occurs

C. The enzyme molecular charge changes

D. The substrate molecular charge changes

E. Enzyme action specificity varies

9. The optimum pH for cytoplasm enzymes activity varies from 7.2 to 7.6. Point out all possible changes in active centre structure of such enzyme at pH=7. 0:

A. Changes are not presented

B. Radicals of amino acids get negative charge

C. Neutralization of negatively charged radicals

D. Formation of ester bonds between radicals

E. Destruction of the active centre

10. There are some factors influencing enzyme activity. Point out one of them resulting in complete loss of enzymatic activity:

A. Vitamin H

B. Carbon dioxide

C. T = 100° C

D. P =101325 Pa

E. Sodium chloride solution

Variant 2

1. There are some characteristic sites in the enzyme structure. Choose the most important site for enzyme function:

   A. Allosteric centre

   B. Active centre

   C. Cofactor

   D. Apoenzyme

   E. Catalytic site

2. Lactate dehydrogenase (LDH) isozymes catalyze the transformation of pyruvate to lactic acid in different types of tissues. Point out the structural distinctive peculiarity of each LDH isozyme:

   A. Different native protein structure

   B. Different level of structural organization in native molecule

   C. Different by the type of coenzyme in native molecule

   D. Different by the quantity of subunits

   E. Different by the combination of subunits, forming a native molecule

3. Point out the factor that can cause the damage of enzyme function in a cell:

   A. Temperature 37°C

   B. The presence of activator of enzyme

   C. pH medium about 7.2

   D. The presence of a product of enzymatic reaction

   E. The surplus of protons in a cell

4. Enzymes are the catalysts of protein nature. Name the property of enzymes which is not represented at the inorganic catalysts:

   A. Ability to the denaturation

   B. Wide specificity

C. Inert to chemical substrates

D. Big half-life

E. Ability to lowering the energy to activate the reaction

5. The oxidation of a substrate may be catalyzed by enzyme - flavoprotein that contains FAD as prosthetic group. Name, please, the vitamin used for this non-protein part of enzyme formation:

A. Ascorbic acid

B. Nicotinamide

C. Riboflavin

D. Biotin

E. Adenosine triphosphate

6. Many important catabolic processes are located in the matrix of mitochondria. Name the matrix multienzyme system (MS):

A. Citrate synthetase

B. Pyruvate dehydrogenase

C. Isocitrate dehydrogenase

D. Pyruvate carboxylase

E. Glucokinase

7. The enzyme hexokinase can catalyze the conversion of glucose or fructose in tissues. Find out the type of this enzyme specificity:

A. Absolute

B. Absolute group

C. Absolute relative

D. Relative group

E. Stereochemical

8. Vitamin B_1 is the precursor for thiamine pyrophosphate (TPP) synthesis that is used as the non-protein part of some enzymes: Find out one of them:

A. Chymotrypsin

B. Hexokinase

C. Ribonuclease

D. Pyruve dehydrogenase

E. Alanine transaminase

9. The catalytic site of active center of enzyme is used for:

A. Conversion of a substrate in the reaction

B. Binding with the substrate

C. Binding with activator

D. Binding with inhibitor

E. Removal of a product of the reaction

10. There are different cofactors in the structure of conjugated enzymes but only one is used for transfer of amine group from amino acid to ketoacid. Name it:

A. Carboxybiotin

B. Pyridoxal phosphate

C. Thiamine pyrophosphate

D. FAD

E. NAD^+

Keys for tests:

Variant 1: 1-A 2-C 3—B 4 -D 5-D 6- D 7-B 8-A 9-C 10-C

Variant 2: 1- B 2- E 3—E 4 - A 5- C 6- B 7- D 8- D 9- A 10- B

INFORMATIONAL MATERIAL FOR THE LESSON 4 OF MODULE 1

ENZYMES ARE CATALYSTS OF PROTEIN NATURE.

Enzymes share some properties with chemical catalysts.

Shared properties:

1. Enzymes are neither consumed nor produced during the course of a reaction.
2. Enzymes do not cause reactions to take place; they expedite reactions that would ordinarily proceed, but at a much slower rate, in their absence. They don't alter the equilibrium constants of reactions that they catalyze.

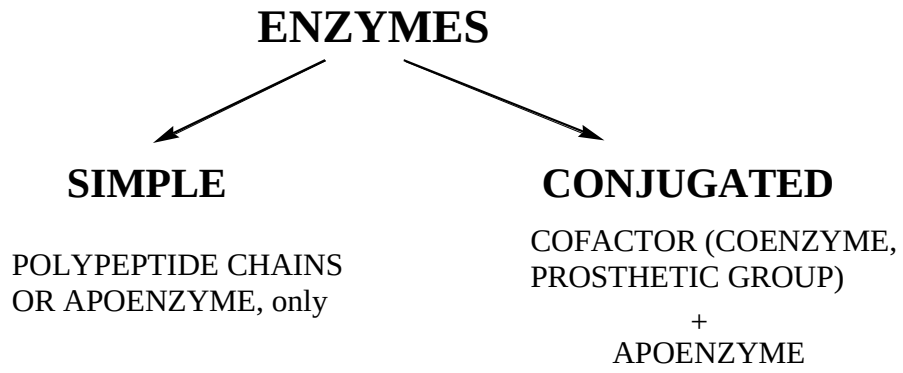
Differences between enzymes and chemical catalysts:

1. Enzymes are invariably proteins
2. Enzymes are highly specific for the reactions they catalyze and produce only the expected products from the given reactants (or substrates)
3. Enzymes often show a high specificity toward one substrate, although some enzymes have a broader specificity, using more then one substrate.
4. Enzymes function within a moderate pH and temperature range.

A majority of enzymes are globular proteins. So, all specified properties of globular proteins are introduced in enzymes.

COMPOSITION AND STRUCTURE OF ENZYMES

Enzymes may be simple or conjugated, it depends upon the presence of the non-protein part (see below):



Cofactor (is the common term for non-protein part)

Prosthetic group

If the non-protein part is linked to polypeptide chains by covalent bonds, can`t dissociate

Coenzyme

If the non-protein part is linked to polypeptide chains weak bonds and can dissociate

Cofactors may be non-organic or organic compounds.

Non-organic cofactors:

1. Metal ions: Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, K^{+}, Na^{+}, Cu^{2+}, selenium for glutathione peroxidase, etc.

2. Phosphoric acid residues: $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}.

Organic compounds-cofactors:

1. Nucleotides: ATP, AMP, ADP, etc.

2. Carbohydrates: glucose, galactose, mannose, etc.

3. Vitamins and their derivatives (look figure N1)

4. Heme and its deravatives:

1) Cytochromes *b*, c_1, *c*, aa_3, P_{450}; 2) Catalase, Peroxidase

5. Short peptides: Glutathione (GSH/GS-SG), etc.

Figure N1. The use of some vitamins in the structure of enzymes catalyzed special type of the reaction

Vitamin	Coenzyme or prosthetic group	Type of the reaction catalyzed by the enzyme
Thiamine	TPP (thiamine pyrophosphate)	Oxidative decarboxylation of keto acids; Transketolase reactions
Riboflavin	FMN (Flavin MonoNucleotide), FAD (Flavin Adenine Dinucleotide)	Oxidation-Reduction

Pantothenic acid	Coenzyme A 4´-phosphopantetheine	Activation of free acids Fatty acid synthase complex (Acyl carrier protein)
Nicotinic acid or nicotin amide	NAD^+, $NADP^+$ (Nicotinamide Adenine Dinucleotide, Nicotinamide Adenine Dinucleotide Phosphate) and their reduced forms	Oxidation-Reduction Hydroxylation (NADPH mainly)
Pyridoxine	Pyridoxal phosphate, Pyridoxamine	Alpha-decarboxylation, Transamination of amino acids
Lipoic acid	Lipoic acid	Oxidative decarboxylation of keto acids
Biotin	Carboxybiotin	Carboxylation of some acids

It should be noted that some medicines may be found in the formation of so named pseudo-coenzymes thus they can block activity of enzymes, for example: izoniazide is precursor for pseudo-coenzyme similar in structure to NAD+.

Specific sites of enzyme

The most important part of any enzyme is ***the active centre. It is a structural fragment of enzyme which attaches a substrate (one or more), and there is a conversion of substrates to the products of enzymatic reaction*** in this centre. There are two parts in each active centre of enzymes: a *catalytic site* and *binding site* for substrates.

Active centre of simple enzymes is composed from amino acid residues, only. The most frequently used amino acid residues in active centre of many enzymes are: Serine, Aspartic acid, Histidine, Lysine, Glutamic acid, Cysteine.

Active centre of conjugated enzymes usually keeps the non-protein part, for example:

a) Alcohol Dehydrogenase has NAD^+;

b) Cytochrome oxidase has heme-containing Fe^{2+}/Fe^{3+} and Cu^+/Cu^{2+}.

As a rule vitamin derivatives are in the active centre of conjugated enzymes. There are some amino acid residues in the active centre of conjugated enzymes, too. A conformation of active centre is formed only when a three-dimensional structure of enzymes is formed.

A majority of enzymes are synthesized as precursors of enzymes (*inactive form, proenzyme*). ***There are some ways of activation of inactive enzymes to form active molecule:***

1. ***Non-complete proteolysis of precursor*:** a part of polypeptide chain of precursor is eliminated by some another enzyme (protease). For example: 1) Enteropeptidase action on trypsinogen: N-terminal hexopeptide is eliminated from precursor to form active enzyme trypsin; 2) Trypsin produces chymotrypsin from its precursor chymotrypsinogen. The subtype of limited proteolysis is ***Autocatalysis: ability of active form of enzyme to produce itself from proenzyme.*** This way is discussed for pepsin, trypsin and chymotrypsin formation.

2. ***Allosteric*** activation of inactive molecule. As a rule the key enzymes of process have allosteric centers. Allosteric centre is a site in the enzyme molecule structure which is able to adopt some organic or non-organic compounds. They are named effectors. The effector changes the conformation of enzyme (or proenzyme) after its linkage:

1) to form the active centre in the structure of proenzyme. In this case it is named allosteric activator;

2) to destroy the active centre of enzyme. In this case it is named allosteric inhibitor.

Phosphorylation–Dephosphorylation is this type of enzyme activation (or inhibition). As example, look in your textbook at two key enzymes regulation in glycogen metabolism: ***glycogen phosphorylase and glycogen synthase:***

Glycogen synthase kinase

ATP→ ADP

Simple protein →→→→→→→→→→→→→→→→→→→→ Phosphoprotein

Glycogen synthase ←←←←← Phosphatase ←←←←← Glycogen synthase

Active form (- H_3PO_4) Inactive form

Glycogen phosphorylase kinase

ATP→ ADP

Simple protein →→→→→→→→→→→→→→→→→→→→ Phosphoprotein

Glycogen phosphorylase ←←← Phosphatase←←← Glycogen phosphorylase

Inactive form (- H_3PO_4) Active form

Specificity of enzyme

Absolute specificity. This is specificity of enzyme action that is determined by its ability to act with only one substrate. For example: enzyme *urease* can destroy the urea, only, and can't react with any other substrate.

Relative group specificity. Many enzymes in nature have more then one substrate. This type of specificity may be named as *relative group* one. Term "relative" is used for the enzyme catalyzing the conversion of the same fragment in the structure of its substrate molecules. For example: A salivary amylase has the relative group specificity. It can destroy the ά–1.4–glycosidic bond in the structure of polysaccharides such as starch, glycogen and their non-complete digestion products. But this enzyme can't react with disaccharides such as sucrose or maltose and monosaccharides as substrates. Second enzyme from saliva named lysozyme

(or muramidase) can destroy proteoglycans in bacterial wall and has the same type of specificity.

Stereochemical specificity. For example: There are two types of alanine oxidase in the liver: L–oxidase and D–oxidase. L–oxidase can react with L–alanine, only. D–oxidase can react with D– alanine, only.

Any type of specificity of enzyme is determined by:

1) The functional groups of the substrate (or product);
2) The functional groups in the active centre of enzyme and its cofactors (coenzymes);
3) The physical proximity of these various functional groups during the duration of the reaction.

Thermolability of enzymes

High temperature of environment (more then 60^{o}C) should be considered as a factor for denaturation of human enzymes. Optimal temperature for enzymatic action in human organism is about 38-40^{o}C. The low enzymatic activity is keeping at low temperature in region -8^{o}C – 0^{o}C. So the curve of enzymatic activity (A) dependence on temperature is like this one:

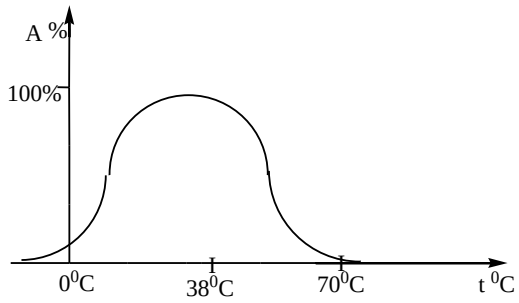


Figure 2. A temperature of environment influences the enzyme activity.

Effect of pH medium on enzymatic activity

Each enzyme-catalyzed reaction has its pH optimum. For majority tissue enzymes in humans pH optimum is about 7.2–7.4. Pepsin of gastric juice has very low pH optimum 1.5–2.5 at healthy adults. Enzymes of small intestine have the pH optimum about 8.0–8.4. So, the pH optimum of enzymes is very individual

characteristic for them. The curve of the enzymatic activity (A) dependence on pH environment may be shown for tissue enzymes like this graph curve:

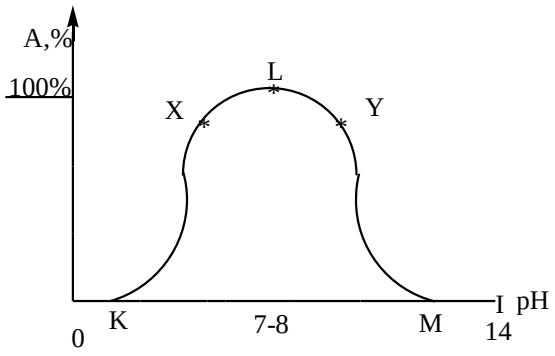


Figure 3. The influence of pH medium on enzyme activity.

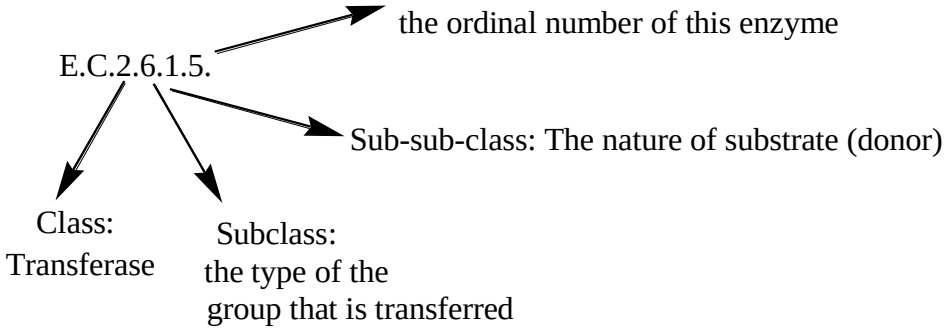
There is the denaturation of tissue enzymes at points K and M because of strong acidic (point K) or strong alkalic (point M) medium around enzyme is found as denaturation factor. There is the lower enzyme activity in point X and Y in comparison with the point L because the charge of amino acid residues in active centre of enzyme is changed at pH values related to points X and Y. This change influences the rate of enzymatic reaction, and it is decreased.

Classification and nomenclature of enzymes

The International Union of Biochemistry recommended to introduce a decimal system of enzymes based on the nature of the catalyzed reaction. In 1972, the Commission for Biochemical Nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) published a new addition of enzyme nomenclature. Before this time the substrate name was usually taken and the suffix “ase” was attached. In other cases, the suffix was attached to the name of the catalyzed reaction. Some of the yearly described enzymes have special names, such as trypsin, pepsin, catalase. Each enzyme receives a four-part-number code and is also given a systematic name and recommended trivial name. For example: membrane carrier proteins that facilitate diffusion are named permeases, because it is difficult to estimate type of the reaction catalyzed by them.

Example of enzyme class code:

Tyrosine amino transferase

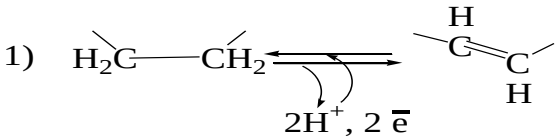


Today according to the classification there are six classes of enzymes.

Classes of enzymes:

1. ***Oxidoreductases*** are involved in oxidation and reduction. The trivial names: dehydrogenases, oxidases, oxygenases, cytochromes. All the enzymes of this class are conjugated proteins. The cofactors of this class: FAD/$FADH_2$, FMN/$FMNH_2$, NAD^+/NADH, $NADP^+$/NADPH, heme (Fe^{2+}/Fe^{3+}), Cu^+/Cu^{2+}

Scheme of reactions, related to oxidation / reduction are shown below:



2) $A + 2\bar{e} \longleftrightarrow B$

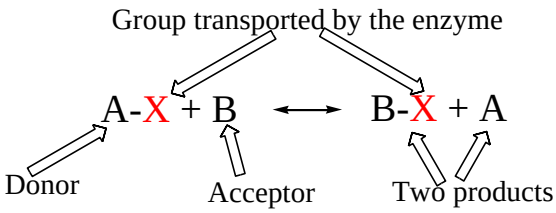
3) $A + O_2 \longrightarrow AO_2$

4) $SH + O_2 \longrightarrow S\text{-}OH + H_2O$

$2H^+, 2e$

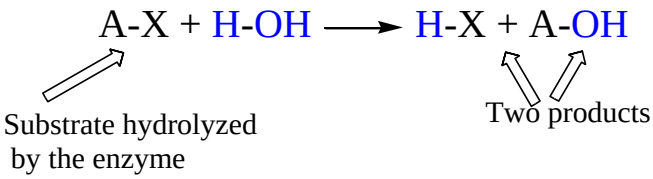
2. ***Transferases*** transfer structural fragment from one substrate (donor) to another one (acceptor)

Scheme of this type of reaction:



Transferases catalyze usually reversible reactions. Fragments that may be transported: Amino – NH_2; Methyl – CH_3; Acetyl – CH_3CO-; Phosphate – OPO_3H_2 and many others.

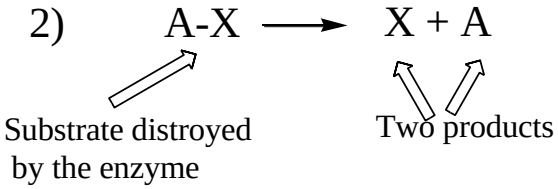
3. ***Hydrolases*** catalyze the hydrolysis of a substrate. The structural fragment (or bond) of a substrate is digested, water molecule is used in the formation of products. A scheme of this type of reaction:



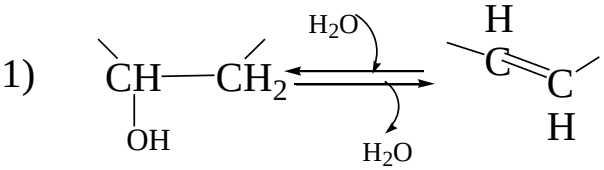
The digestion of proteins, polysaccharides, some lipids is carried out by this class of enzymes. Invasive properties of phytopathogenic microorganisms are due to this enzyme class.

4. ***Lyases*** add (or remove) the elements of water, ammonia, or carbon dioxide (CO_2) to (or from) double bonds. They can destroy the bond without water molecule utilization.

1) alpha-decarboxylation of amino acid

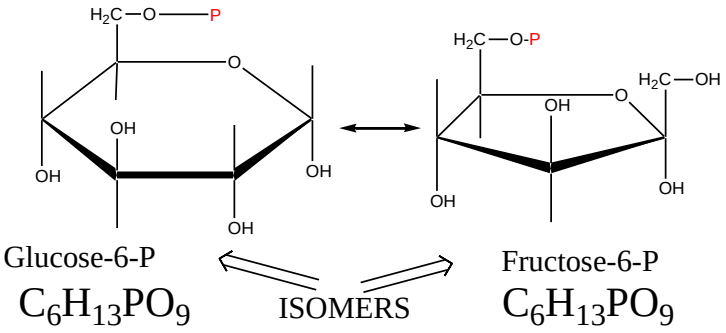


2) dehydration of beta-hydroxyacyl-CoA fragment:



5. ***Isomerases*** catalyze changes within one molecule; they include *racemases* and *mutases*, as well as *epimerases*. Isomers are different in structures, but quantitative composition is the same for both substances.

For example, reaction catalyzed by glucose-6-phosphate isomerase:

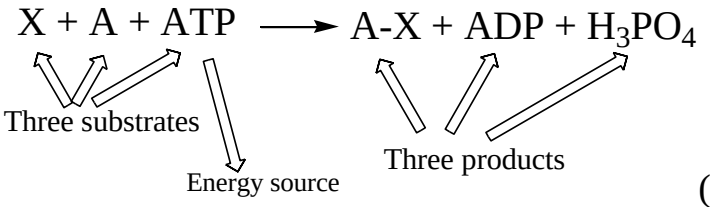


$C_6H_{13}PO_9$ $C_6H_{13}PO_9$

A sign for this type of reaction: the reaction is obligatory reversible!

6. ***Ligases*** (trivial name: synthetases) join two or more molecules (substrates) together at the expense of energy released after degradation of high-energy bond of nucleoside triphosphate (ATP, GTP, UTP and others).

The schemes of this type of reaction are:



(1)

or:

$A + B + ATP \rightarrow A - B + AMP + H_4P_2O_7$ (2)

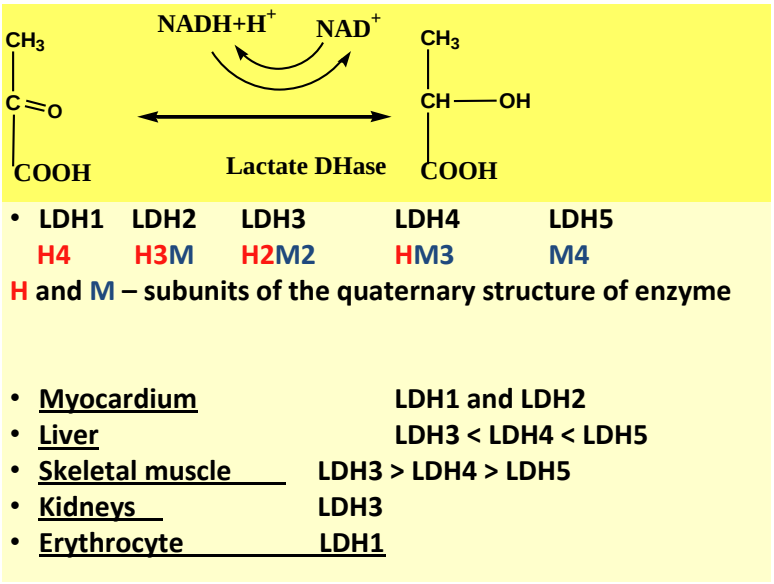
ATP may be used as the agent for phosphorylation (as a donor of phosphate group) catalyzed by phosphotransferase:

$S + ATP \rightarrow S\text{-}OPO_3H_2 + ADP$ (3)

Compare equation (1) and (3) and care for the transformation of ATP molecule in both reactions to differ them.

Isozymes: the definition and properties

The genetic information about the same enzyme may be represented in different tissues of human organism by variation of genes. In this case genetic forms of this enzyme may be differ partially in variation of subunits which are in creation of the native molecule of the enzyme. As example, let us consider those genetic forms for lactate dehydrogenase (LDH):



These genetic forms are named ***isozymes***. The active site in isozymes structure is the same, and any isozyme of LDH catalyzes the same reaction. But they are different in quaternary structures, physicochemical properties and location in tissues, that is because the determination of activity and concentration of each isozyme in the blood serum may be used in clinical diagnostics of diseases.

Multienzyme complexes

This is a complex of enzymes that are located together and carry out the same reaction or process.

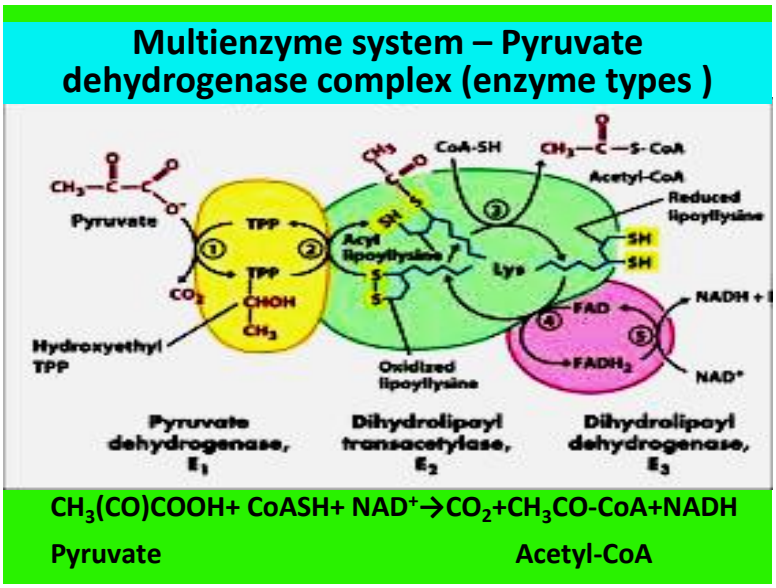


Figure 4. The composition of pyruvate dehydrogenase complex

For example: Pyruvate dehydrogenase complex is composed from three enzymes :

1) Pyruvate Dehydrogenase: E_1-TPP

2) Dihydrolipoyl transacetylase: E_2 (Lipoic Acid in two forms, CoA~SH)

3) Dihydrolipoyl dehydrogenase: E_3 (FAD, NAD^+)

The inhibition of any one enzyme from this complex causes the inactivation of the whole system. There are many Multienzyme complexes (MC) in cells: MC for High Fatty Acids Synthesis; MC for Oxidative decarboxylation of alpha–ketoglutarate; MC for β–oxidation of HFA; a respiratory chain in the inner membrane of mitochondria, etc.

LESSON 5 OF MODULE 1

THEME:

THE MECHANISM OF ACTION AND KINETIC PROPERTIES OF ENZYMES. THE REGULATION OF ENZYMATIC ACTIVITY

STUDY GOALS FOR LESSON 5 OF MODULE 1:

1. To study basic notions about mechanism of enzyme action
2. To learn main ways to control the rate of enzymatic reaction
3. To recognize the influence of activators and inhibitors on enzyme activity

IT IS IN NEED TO KNOW:

1. Modern notions about the mechanism of enzymatic catalysis: the definition of energy activation for enzymatic reaction; the stages of the formation of an enzyme-substrate complex; the mechanisms for products formation (covalent and acidic catalysis). A significance of scientific works written by D. Keilin, B. Chance, D. Koshland, L. Michaelis and M. Menten.

2. Enzymes kinetics: the determination of kinetic indexes (Km and Vmax) using the Michaelis-Menten equation curve and Lineweaver-Burk equation curve. A significance of Michaelis constant determination for enzymes with relative group specificity.

3. The factors for enzyme activity regulation: concentration of substrate; concentration of product; concentration of enzyme; pH and temperature of environment.

4. Common notions about inhibitors. Inhibition Types: reversible - competitive, uncompetitive, noncompetitive; irreversible - suicide inhibition, affinity labels (examples). The change of kinetic indexes for enzyme under the influence of competitive, non-competitive inhibitors (the determination of inhibitor type using Lineweaver-Burk equation curves).

5. Allosteric centre of enzyme: its location, structure and function in enzymatic catalysis. The common notion about Allosteric type of enzyme activity regulation. Feed-back type of inhibition.

IT IS IN NEED TO DO:

1. Investigation of inhibitor and activator influences the activity of salivary amylase.
2. Investigation of enzyme concentration influence the rate of reaction catalyzed by salivary amylase.

BASIC LEVEL FOR PREPARATION. DISCIPLINE INTEGRATION

Disciplines	**Obtained skills**
Previous: Organic chemistry	Covalent and acid-base catalysis. Types of reactions.
Non-organic chemistry	Catalyst and catalysis. Reversible and irreversible reactions. Factors to influence the rate of chemical reaction.
Common chemistry	Energy for activation of reaction. pH and temperature influencies on enzyme activity. Specificity of action.
Next in study: Clinical biochemistry Pharmacology	Clinical enzymology: principles for enzyme activity determination in biological fluids; the use in diagnostics of diseases ad as agents for methods. Medicines as enzyme inhibitors.

CONTENT OF EDUCATIONAL MATERIAL

IN LESSON 5 OF MODULE 1

1. Modern notions about the mechanism of enzymatic catalysis: the definition of energy activation for enzymatic reaction; the stages of the formation of an enzyme-substrate complex; the mechanisms for products formation (covalent and acidic catalysis). A significance of scientific works written by D. Keilin, B. Chance, D. Koshland, L. Michaelis and M. Menten.

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5. Allosteric centre of enzyme: its location, structure and function in enzymatic catalysis. The common notion about allosteric type of enzyme activity regulation. Feed-back type of inhibition.

A PLAN AND ORGANIZATION OF LESSON 5 OF MODULE 1

Stages	Time/ minutes	Materials for lesson		Place for Duration Of lesson
		Learning tools	Equipment	
1.Organizing time	**5**			**Auditory 507,509, 511, 514**
2. A conversation	**20**	**1.Textbook on Biochemistry 2. A Manual for submodule 1**		
3. Initial control	**15**	**Variants for testing (1-2)**	**Blocks of tests**	
4.Independent work for recognition of inhibition type	**15**	**1.Textbook on Biochemistry 2. A Manual for submodule 1**	**Graph curves (3 variants)**	
5. Terminal control for theory	**10**	**Variants for control (1-2)**	**Blocks of tests**	
Brake	**5**			
6. Laboratory works	**45**	BIOCHEMISTRY LABORATORY MANUAL Module 1	**Reagents for laboratory works; Test tubes Thermostat**	
7. Final results discussion	**15**	**Protocol N5**		

ALGORITHM FOR LABORATORY WORKS

OF LESSON 5 OF MODULE 1

Laboratory work 1. The influence of activators and inhibitors on the salivary amylase activity

THE PRINCIPLE OF THE METHOD:

The activator of salivary amylase is sodium chloride, and the inhibitor of one is copper sulfate. The influence of these substances on the amylase activity is judged by the degree of starch hydrolysis under the enzyme influence at the presence of sodium chloride and copper sulfate.

THE COURSE OF THE WORK:

The saliva is dissolved in correlation (1:200). Take 3 test tubes. Pour on 2 drops of 1 % sodium chloride solution into the 1-st one, and 2 drops of 1 % copper sulfate solution into the 2-nd one, and 2 drops of water into the 3-rd one. Add 1 ml of the dissolved saliva and 5 drops of 1% starch solution into each test tube. Mix the content and keep it at a room temperature for 2 minutes. Pour 1 drop of iodine solution into each tube, mix and observe the colouring.

THE EQUIPMENT AND REAGENTS

Thermostat, measured centrifugal test tube, measured cylinder, pipettes, support, 1 % starch solution, 1 % sodium chloride solution, 1 % copper sulfate (II) solution, distal water, solution of 0.1% iodine in potassium iodide.

Possible final result: most colorless solution after iodic test will be in the test tube with activator; copper sulfate must completely block activity of enzyme - the color final will be blue. Color of 3-d test tube mixture may be with some violet shade, because the enzyme will be in work, but not so active as at the presence of actvator.

Laboratory work 2. The investigation of the rate of enzymatic reaction dependence on the enzyme concentration

THE PRINCIPLE OF THE METHOD:

The method is based on test of amylase concentration influenced the velocity of starch hydrolysis (the remainder of starch is determined after amylase action by reaction with iodine solution).

THE COURSE OF THE WORK:

Number 4 test tubes and 1 ml of the saliva dissolved accordingly in 5, 10, 20 and 40 times put into each test tube. 5 ml of 1 % starch solution add in each test tube. Mix the contents of test tubes quickly, place the test tubes into thermostat at 38^0C and note the time of beginning of reaction. Using a glass stick take out 1-2 drops of the reactionary solution on the clock glass every 2 minutes and add 1 drop of 0,1 % of iodine solution. First tests give dark blue colouring, then - violet, then - red-violet and at the end - red colouring.

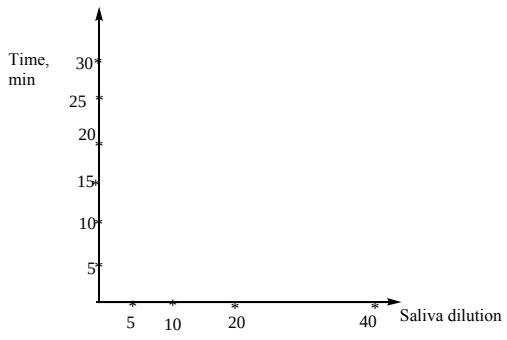
Splitting of starch gives products - erythrodextrins, their formation is proved by red colouring of iodic test. Fix the time of red colouring occurrence (by iodic test) from the beginning of reaction for each test tube. Fill in results in table below and show the results on graph, marking relative amylase concentration (dilution) on X-line and time (min) of erythrodextrin formation on Y-line, and make the curve.

THE EQUIPMENT AND REAGENTS

Thermostat, measured centrifugal test tube, test tubes, pipettes, support, 1 % starch solution, distal water, solution of 0.1% iodine in potassium iodide, glass clock, timer.

***Possible final result**:* the graph curve must show the first-order dependence .

Saliva dilution	Erythrodextrins formation time
5	
10	
20	
40	



INITIAL CONTROL OF KNOWLEDGE FOR LESSON 5 OF MODULE 1

Variant 1

1. E. Fisher`s theory explains the mechanism of enzyme action with the fixed type of specificity, only. Name it:

A. Absolute

B. Absolute group

C. Absolute relative

D. Relative group

E. Stereochemical

2. There are some factors influencing enzyme activity. Point out one of them resulting in complete loss of enzymatic activity:

A. Vitamin H

B. Oxygen

C. t^0 C = 100^0 C

D. P =101325 Pa

E. Sodium chloride solution

3. There are some characteristic sites in the enzyme structure. Choose the most important site for enzyme function:

A. Allosteric centre

B. Active centre

C. Cofactor

D. Apoenzyme

E. Catalytic site, only

4. Choose the factor that changes the cytoplasmic enzyme conformation mainly:

A. Suicide inhibitor

B. Environmental pH value about 7.4

C. Environmental temperature value about 25^0 C

D. Allosteric inhibitor

E. Water

5. Point out the way of proenzyme transformation to the active enzyme:

A. Limited proteolysis

B. Dehydration

C. Decarboxylation

D. Inhibitor action

E. Vitamin non-protein part dissociation from enzyme

6. Competitive inhibitor always interacts with enzyme active centre. Find out the explanation of this phenomenon:

A. Inhibitor causes the denaturation of active centre

B. Inhibitor is similar to a substrate structure

C. Inhibitor is an exact copy of a substrate structure

D. Inhibitor is similar to the product's structure

E. Inhibitor forms a covalent type of bonds with amino acid residues of active centre

7. Covalent modification of inactive form of enzyme may be catalyzed by special enzyme in a cell. Name it:

A. Esterase

B. Ligase

C. Protein kinase

D. Hydroxylase

E. Oxygenase

8. Find out the irreversible type of enzyme inhibition:

A. Competitive

B. Noncompetitive

C. Uncompetitive

D. Allosteric

E. Suicide

9. Find out the mathematic sense of Michaelis constant (Km):

A. It is a time for complete degradation of a substrate

B. It is a 1/2 of a substrate concentration for obtaining of Vmax

C. It is a substrate concentration for obtaining of 1/2 Vmax

D. It is a constant for ES-complex dissociation

E. It is a product concentration formed after enzymatic reaction

10. The active centre of the enzyme contains amino acid residues of Aspartic acid. The substrate for this enzyme is cyclic organic alcohol. Point out the type of bond that may be formed between this substrate molecule and active centre of this enzyme:

A. Glycosidic bond only

B. Hydrogen bond mainly

C. Peptide bond

D. Ester bond mainly

E. Disulfide bond

Variant 2

1. Phosphorylation may be named as Covalent modification of inactive form of the enzyme, and it is catalyzed by special enzyme in a cell. Name it:

A. Esterase

B. Peptidase

C. Protein kinase

D. Hydroxylase

E. Oxygenase

2. Find out the irreversible type of enzyme inhibition:

A. Competitive

B. Noncompetitive

C. Uncompetitive

D. Allosteric

E. Suicide

3. Point out the activator used for the determination of amylase activity:

A. $CuSO_4$

B. NaCl

C. H_3PO_4

D. ATP

E. Ca^{2+}

4. The majority of key enzymes contain the allosteric centre. Specify a role of this centre:

A. It attaches the substrate

B. It attaches the regulatory factor

C. It changes the structure of the substrate

D. It promotes the dissociation of a coenzyme

E. It blocks the active centre

5. Glycogen phosphorylase *b* is transformed to the active form *a* by the action of special kinase with the use of ATP as donor of phosphate group. Find out, please, the type of enzyme activation:

A. Limited proteolysis

B. Covalent modification

C. Activation by Ca^{2+}

D. The change of pH medium

E. Competitive inhibition

6. Sodium chloride is considered as activator of one enzyme from gastro-intestinal tract. Find out it:

A. Lipase

B. Salivary amylase

C. Pepsin

D. Trypsin

E. Cholesterol esterase

7. Sulfanilamides are used as drugs to protect our organism from some bacteria. Enzyme in bacterial cell producing folic acid from para-aminobenzoate is inhibited by this group of drugs. Choose the type of inhibition for sulfanilamides:

A. Competitive

B. Noncompetitive

C. Uncompetitive

D. Allosteric

E. Suicide

8. Name, please, the inhibitor for salivary amylase:

A. Copper sulfate

B. Sodium chloride

C. Potassium cyanide

D. Alanine

E. Hydrogen peroxide

9. Name the kinetic index that is changed under the influence of competitive inhibitor on enzyme:

A. Michaelis constant

B. The initial velocity of enzymatic reaction

C. The maximal velocity of enzymatic reaction

D. The dissociation constant of enzyme-substrate complex (ES)

E. The rate constant for the formation of ES

10. Point out the way of pro-enzyme transformation to the active enzyme:

A. Limited proteolysis

B. Dehydration

C. Decarboxylation

D. Inhibitor action

E. Non-protein part dissociation from enzyme

Keys for tests:

Variant 1: 1-A 2-E 3-B 4-B 5-B 6-B 7-A 8-A 9-A 10-A

Variant 2

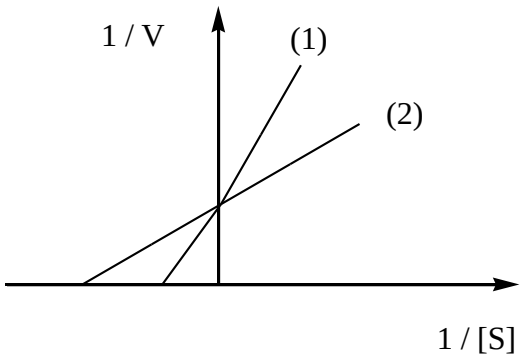
1C 2- E 3—B 4 - B 5- B 6- B 7- A 8- A 9- A 10- A

INDEPENDENT WORK

FOR RECOGNITION OF INHIBITION TYPE

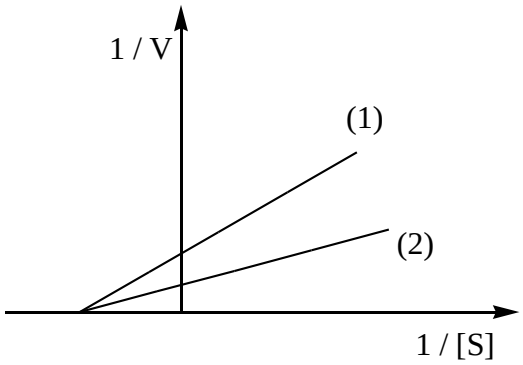
Variant 1

You can see two curves for Lineweaver-Burk method: one curve for inhibitor influence the velocity of enzymatic reaction, the other one without inhibitor. Name, please, the type of inhibition and the number of curve for inhibitor influence the velocity of enzymatic reaction. Explain your choice.



Variant 2

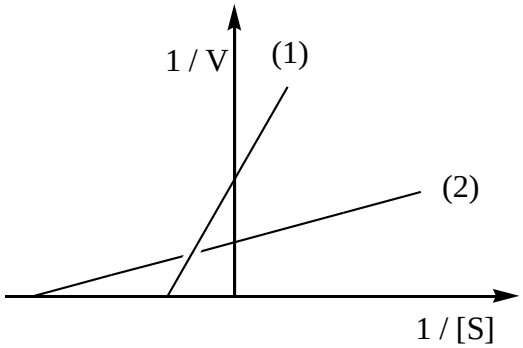
You can see two curves for Lineweaver-Burk method: one curve for inhibitor influence the velocity of enzymatic reaction, the other one without inhibitor. Name, please, the type of inhibition and the number of curve for inhibitor influence the velocity of enzymatic reaction. Explain your choice.



Variant 3

You can see two curves for Lineweaver-Burk method: one curve for inhibitor influence the velocity of enzymatic reaction, the other one without

inhibitor. Name, please, the type of inhibition and the number of curve for inhibitor influence the velocity of enzymatic reaction. Explain your choice.



FINAL CONTROL OF KNOWLEDGE FOR LESSON 5 OF MODULE 2

Variant 1

1. Point out the type of bonds that are formed between Ser, Tyr and Cys residues in active centre of the enzyme and the molecule of substrate containing functional group: --COOH:

A. Disulfide bond and hydrophobic interaction

B. Disulfide and ester bonds

C. Ester and hydrogen bonds

D. Ester bond and hydrophobic interaction

E. Hydrophobic interactions, only

2. The formation of ES complex is due to various types of bonds between E and S. Specify the type of bond, which is usually formed between charged functional groups in this case:

A. Peptide bond

B. Hydrophobic interaction

C. Hydrogen bond

D. Donor-acceptor bond

E. Electrostatic interaction

3. Find out the mathematic significance of Michaelis constant (Km):

A. It is a time for complete degradation of a substrate

B. It is a substrate concentration needed to promote the Vmax

C. It is a substrate concentration needed to promote the 1/2 Vmax

D. It is a constant for ES-complex dissociation

E. It is a product concentration formed after enzymatic reaction

4. Point out the characteristic index that is taken into account during the election of the substrate for the determination of the activity of enzyme with relative group specificity:

A. Km for this substrate conversion

B. Total activity of enzyme for this substrate conversion

C. Specific activity of enzyme for this substrate conversion

D. Ks for this substrate conversion

E. V_{max} for this substrate conversion

5. The common enzymatic reaction may be represented so:

E + S ↔ ES ↔ ES*↔EP ↔ E + P. Name using this equation all the factors that can influence the rate of this reaction:

A. The concentration of a substrate, only

B. The concentration of enzyme, only

C. The concentration of a substrate, enzyme and product, only

D. The concentration of enzyme-substrate complex, only

E. The concentration of a substrate, enzyme, product and stability of ES-complex

6. Acid-base catalysis is considered as mechanism of enzyme catalysis for enzymes that contain in active centre donors of protons or acceptors of protons. Choose the amino acid whose residue may be the acceptor of protons at pH=7.0:

A. Alanine

B. Aspartic acid

C. Glutamic acid

D. Cysteine

E. Arginine

7. Heme synthesis starts from glycine and succinyl-SCoA interaction with δ-aminolevulinate synthetase help. It is inhibited by the terminal metabolic product - heme. Name the inhibition type:

A. Competitive Inhibition

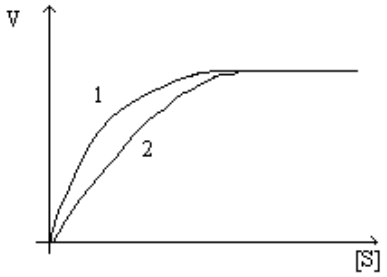
B. Uncompetitive Inhibition

C. Non-competitive Inhibition

D. Limited proteolysis

E. Feedback Inhibition

8. The inhibitor influence on the enzymatic reaction rate is investigated. The graph dependences V - [S] without the inhibitor (1) and at the presence of the inhibitor (2) are constructed.



Name the type of the inhibitor:

A. Competitive

B. Non-competitive

C. Uncompetitive

D. Allosteric

E. Complete

9. The regulation of the enzymatic activity is carried out by different ways. Point out the way that is used more often in the regulation of key enzymes:

A. Limited proteolysis

B. Allosteric regulation

C. Activation by Ca^{2+}

D. The change of pH medium

E. Competitive inhibition

10. Choose the factor that changes the enzyme conformation:

A. Suicide inhibitor

B. pH=7.2

C. Temperature 37° C

D. Allosteric inhibitor

E. The product of reversible reaction

Variant 2

1. Fisher`s theory explains the mechanism of enzyme action with the fixed type of specificity, only. Name it:

A. Absolute

B. Absolute group

C. Absolute relative

D. Relative group

E. Stereochemical

2. The determination of Michaelis constant (Km) is of great importance for enzymes with the relative group specificity. Explain the necessity of its determination:

A. It helps to determine the activity of enzyme in katals

B. It helps to differentiate the influence of enzyme on its substrates

C. It helps to calculate the Vmax

D. It helps to determine the structure of the apoenzyme

E. It helps to determine products` influence on an enzyme

3. There is the moment of conformational conversions both in the molecule of enzyme and in the molecule of a substrate to make ES-complex due to various types of bonds between functional groups. Find out the surname of the author of this notion:

A. Michaelis L.

B. Menten M.

C. Henri V.

D. Koshland D.

E. Keilin D.

4. The rate of an enzymatic reaction may be calculated according to the Briggs-Haldane equation. Find out it:

A. $Vmax = Km \cdot [S] / V + [S]$

B. $V_{max} = V \cdot [S] / K_m + [S]$

C. $V = V_{max} \cdot [S] / K_m + [S]$

D. $V = V_{max} \cdot [S] / K_s + [S]$

E. $V_{max} = V \cdot [S] / K_s + [S]$

5. The active centre of the enzyme contains amino acid residues of Aspartic acid. The substrate for this enzyme is organic alcohol. Point out the type of bond that may be formed between this substrate molecule and active centre of this enzyme:

A. Glycoside bond

B. Hydrogen bond

C. Peptide bond

D. Ester bond

E. Disulfide bond

6. Covalent catalysis as mechanism of enzyme catalysis was studied in experimental works with one proteolytic enzyme. Find out it:

A. Pepsin

B. Aldolase

C. Chymotrypsin

D. Decarboxylase

E. Glucoisomerase

7. Succinate dehydrogenase catalyzes the dehydrogenation of succinate HOOC-CH_2-CH_2-COOH. Malonic acid HOOC-CH_2-COOH can interrupt the action of this enzyme. Name the inhibition type:

A. Allosteric

B. Competitive

C. Non-competitive

D. Limited proteolysis

E. Dephosphorylation

8. Suicide type of inhibition is considered when:

A. The inhibitor structure is similar to substrate one

B. The product of reaction is the allosteric inhibitor for enzyme

C. There is the intermediate metabolite formation from the inhibitor which tightly binds to the active centre of enzyme to block it

D. The end-product of reaction binds to the structure of the substrate to give non-soluble complex

E. The end-product of reaction is not removed from the environment

9. Choose the method that is more often used for the determination of inhibitor type:

A. Nuclear magnetic resonance method

B. Michaelis-Menten graphical method

C. Briggs-Haldane graphical method

D. Lineweaver-Burk graphical method

E. Eadie-Hofstee graphical method

10. Choose the inhibition type that is mainly considered for key enzymes of processes:

A. Competitive Inhibition

B. Uncompetitive Inhibition

C. Non-competitive Inhibition

D. Suicide Inhibition

E. Allosteric Inhibition

Keys for tests:

Variant 1: 1-C 2-E 3—C 4 -A 5-E 6- E 7-E 8-A 9-B 10-D

Variant 2: 1-A 2-B 3—D 4 -C 5-D 6- C 7-B 8-C 9-D 10-E

INFORMATIONAL MATERIAL FOR THE LESSON 5 OF MODULE 1

Modern notions about the mechanism of enzymatic catalysis

Enzymes decrease the energy of activation. A chemical reaction occurs when a certain proportion of the substrate molecules are sufficiently energized to reach a transition state in which there is high probability that a chemical bond will be made or to form the product. The effect of enzymes is to decrease the energy of activation (fig. 1).

$$E_{activation} = E_{transition\ state} - E_{initial\ state}$$

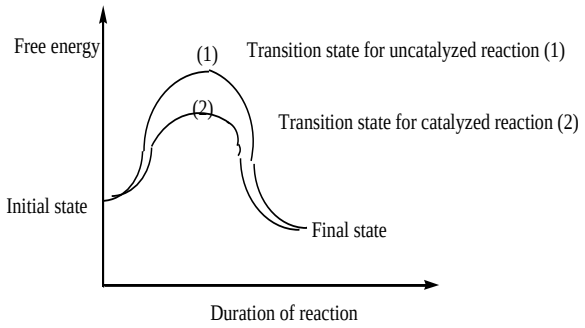


Figure 1. Free energy of chemical reaction for uncatalyzed reaction and catalyzed by enzyme. ***Energy activation for enzymatic reaction is lower!***

In 1913, L. Michaelis and M. Menten noted that an enzyme – substrate complex ES is formed which undergoes a chemical reaction and is broken down to free enzyme E and the product P.

So, the common equation of reversible enzymatic reaction must be:

$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$ (1)

$E + S \rightleftharpoons ES \rightarrow EP \rightarrow E + P$ (2),

where case (1) – equation for reversible reaction; case (2) - equation for irreversible reaction.

The rate of both reactions is depended on the substrate, enzyme concentration, and the rate to reach transition state is promoted by ES complex concentration. Product concentration influences the rate of reaction (2), only.

***The types of bonds for ES complex formation*:** Hydrogen bonds; Electrostatic interactions; Covalent bonds; Magnetic attractions.

***Two theories have been proposed to explain specificity of enzyme action*:**

a) The lock and the key theory (Fisher E., 1940th)

The active centre of the enzyme (the lock) is complementary in conformation to the substrate (the key), so that enzyme and substrate "recognize" one other.

b) The induced-fit theory (D.E. Koshland, 1950th)

The enzyme changes shape upon binding the substrate, so that the conformations of a substrate and enzyme protein are only complementary after binding reaction. The "enduced-fit" hypothesis presumes the existence between the enzyme and the substrate of not only spatial ore geometrical complanarity, but also electrostatic charge complementary: it means interactions of oppositely charged groups of the substrate and the active centre of the enzyme.

Today a majority of scientists agree with the second theory, because it can explain any type of specificity of enzymes, and the least level of energy activation for enzymatic reaction. Step by step whole mechanism of enzymatic reaction may be explained so:

- there is a moment of orientation and approach of enzyme and substrate relatively (may be at the expense of high-energy bond digestion) one to another in space;
- then, it is a moment of an enzyme contact with the substrate – as the result ES complex is formed, and there is the induced fit of enzyme to substrate at this moment too. The attachment of a substrate provokes the spatial changes in the enzyme conformation. There is some strain in the conformation of active centre, and there is some deformation in substrate structure attached to the active centre. All these changes promote quickly the reaching of the transition state of the reaction.

Enzymes catalyze reactions by utilizing the same general reactions as studied in organic chemistry:

- ***Covalent catalysis***
- ***Metal ion catalysis***

– ***Catalysis by alignment (approximation)***

– ***Acid-base catalysis***

- Additional free energy is obtained through the "Binding Energy" (binding of the substrate to the enzyme);
- Binding energy often helps stabilize the transition state, lowering energy for activation of enzyme.

Acid-base catalysis. There are some specific amino acid residues in active centre of enzymes that can be donors or acceptors of protons during the catalysis. Such as:

Donors	Acceptors
- COOH	- COO^-
- NH_3^+	- NH_2
- SH	- S^-

These groups take part in catalysis of many organic reactions in water phase.

Covalent catalysis (*fig.2)*. In some cases enzyme (E-OH) can replace the functional group in a substrate RCO-X to form the covalent complex E-OCOR and first product HX (step A: acylation). This complex is not stable and is quickly hydrolyzed due to water use (step B: deacylation).

The hydroxylic group –OH in the enzyme active site may be from amino acid residues such as Serine or Threonine. This mechanism of enzymatic action is discussed for chymotrypsin and is named as covalent catalysis.

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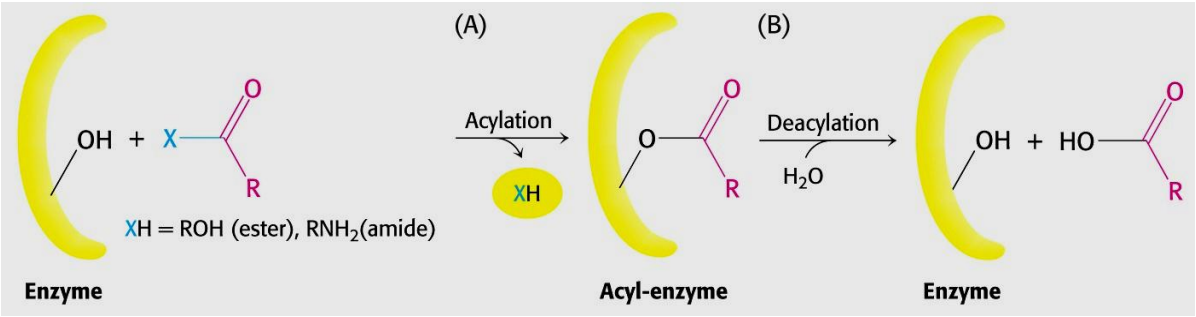


Figure 2. Covalent catalysis mechanism in steps (A, B) for chymptrypsin.

Enzymes kinetics: the determination of kinetic indexes (Km and Vmax) using the Michaelis-Menten equation curve and Lineweaver-Burk equation curve

Kinetic is the trend of enzymology that is concerned with study of all the factors which can influence the rate of enzymatic reaction. The determination of special indexes for each enzyme (Km and V) at normal condition (or in a case of some factors influence the rate of enzymatic reaction) is made. These indexes can help us to estimate the behavior of enzyme in living system.

Substrate concentration influences the rate of enzymatic reaction

Common equation of reversible enzymatic reaction is:

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \rightleftharpoons EP \underset{K_{-2}}{\overset{K_{+2}}{\rightleftharpoons}} E + P \qquad (1)$$

K_{+1} – the rate constant for the formation of ES

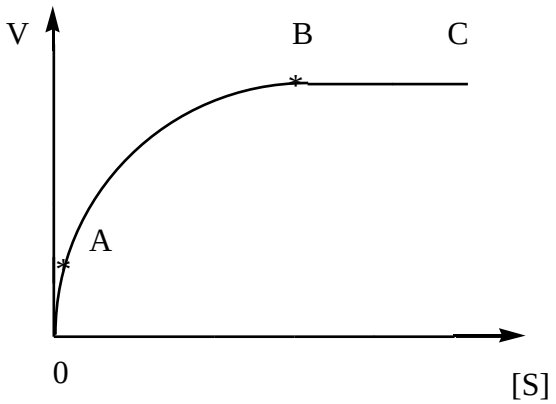
K_{-1} – the rate constant for dissociation of ES

K_{+2} – the rate constant for dissociation of ES to E plus P.

K_{-2} – the rate constant of ES formation from E and P.

If the substrate concentration [S] equals zero, the rate of enzymatic reaction equals zero, too. The rate of enzymatic reaction depends upon the rate of saturation of active centers of enzyme by substrate molecules. The curve of reaction velocity (V) dependence on the substrate concentration [S] is this one (fig.3):

Figure 3. The curve of reaction velocity (V) dependence on the substrate concentration [S]



1) when the [S] is low, the reaction is first-order with respect to substrate: V ~ [S] → intercept 0A
2) in the middle of the curve (part AB) the reaction is mixed-order.

3) the part BC is discussed as a complete saturation of active centers of enzyme by substrate molecules. The velocity is maximal V = V_{max}. The [S] corresponding to the point B is named as the substrate concentration for saturation of active centers.

This curve may be described by mathematic equation (Michaelis-Menten equation):

$$V = Vmax \bullet [S]/Ks+[S] \qquad (2),$$

where V_{max} - maximal reaction velocity; K_s - dissociation constant of enzyme-substrate complex ES.

Briggs and Haldeine later decided to replace the constant K_s by a new one → K_m (Michaelis constant), that may be calculated as:

$$K_m = K_s + \frac{K_{+2}}{K_{-1}}; \text{ and the new equation is } V = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad (3)$$

Physical sense of K_m:

K_m equals to the substrate concentration at which the velocity is half-maximal, that is because it may found using the curve (fig.4)

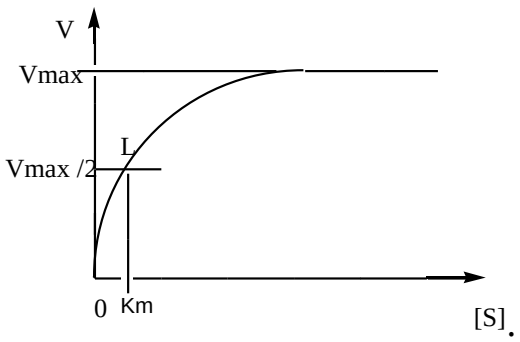


Figure 4. An example of Km determination for enzyme using the graph.

The affinity of an enzyme for its substrate is estimated by K_m:

The lower the value of K_m the greater the affinity of the enzyme for its substrate

V_{max} and K_m are very important characteristics which are placed in special reference books for each enzyme.

Because it is difficult to estimate V_{max} from the position of an asymptote, as in the plot of a rectangular hyperbola (Michaelis-Menthen curve), linear transforms of the Michaelis-Menten equation are often used. The equation (3) is transformed into (4) and (5).

The reverse value to V are produced from equation (3):

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max} \cdot [S]} \quad (4); \qquad \frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}} \quad (5)$$

This method is named as *Lineweaver-Burk method*. It shows the straight-line graph obtained by plotting of 1/V opposite 1/[S] (fig.5) , where the y-intercept equals $1/V_{max}$, and the x–intercept equals $-1/K_m$, and the slop equals K_m/V_{max}. This method is often used at the research of inhibitors` influence on the rate of enzymatic reaction.

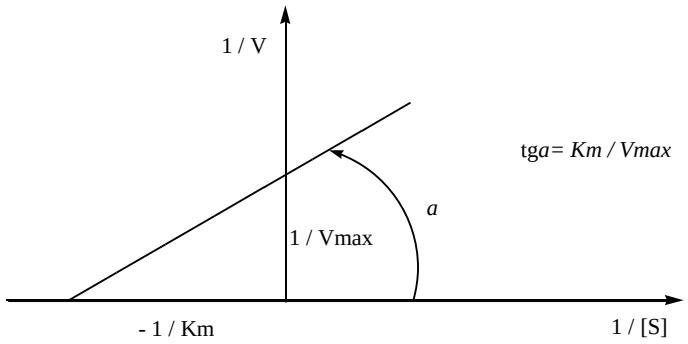


Figure 5. Lineweaver-Burk method graph.

Enzyme concentration

Enzyme activity is regulated by Enzyme concentration (fig.6). This dependence is considered only if:

$$pH,\ t^0C - optimal,$$

$$[S] >> [S]_{saturation}$$

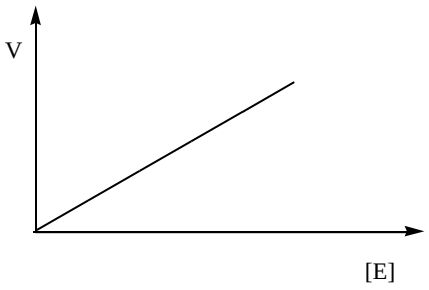


Figure 6. First-order dependence of V from [E].

Common notions about inhibitors. Inhibition Types: reversible - competitive, uncompetitive, noncompetitive; irreversible - suicide inhibition, affinity labels (examples).

Inhibitor is a substance which can decrease the rate of enzymatic reaction after its linkage with enzyme. The inhibition may be partial or complete (in latter case inhibitor may be named *stunner*).

Reversible inhibition of enzyme activity

Different types of reversible inhibition are possible, and they may be easily distinguished by analysis of Lineweaver-Burk plots.

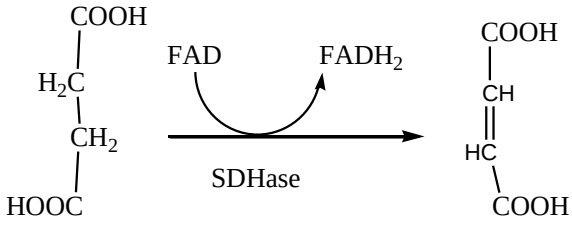
Competitive inhibition features:

a) Inhibitor (I) is similar in a structure to substrate (S).

b) I makes linkage only with active centre of enzyme (E). The inhibition is observed if [I] > [S].

c) If [I] << [S], I is displaced by substrate molecule from active centre of E.

Example 1: the inhibition of succinate dehydrogenase (SDHase) by malonic acid. Equation is represented below for this reaction:



Succinate (Amber acid) Fumarate

Malonic acid HOOC – CH_2 – COOH is the competitive I for succinate dehydrogenase reaction in Krebs cycle. Thanks to two carboxylic groups in structure Malonic acid blocks active centre of E. It increases the K_m, but V_{max} is not changed in value. Lineweaver-Burk plots are as shown in fig.7.

Example 2: Proserin preparation influences the acetylcholine esterase activity: proserin competes with acetylcholine to attach active site of this enzyme, thus it decreases activity of acetylcholine esterase in treatment of myasthenia.

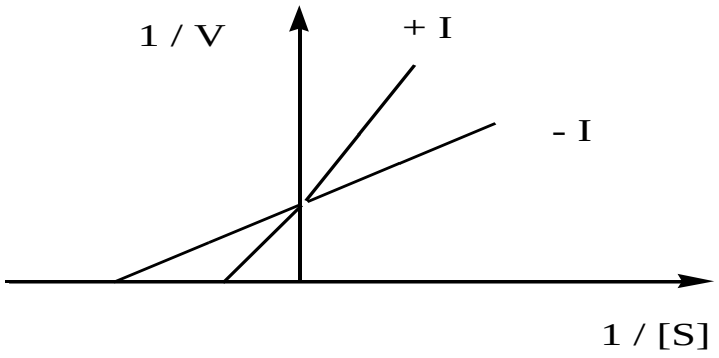


Figure 7. Lineweaver-Burk plots for competitive inhibition investigation and succinate dehydrogenase reaction.

Example 3: Antimicrobial effect of Sulfonamide preparations is associated with the damage of folic acid (vitamin B_9) synthesis from para-amino benzoic acid, and sulfonamide competes with para-amino benzoic acid to be linked to active site of the enzyme involved in production of this very important vitamin.

Example 4: Ethanol is used for treatment of patients with methanol poisoning (per os or intravenously) in a quantity that can cause separately toxicity for healthy person. The effect of ethanol use as drug in this case is explained so: affinity of ethanol to active site of alcohol dehydrogenase is much higher then for methanol, and it can replace methanol by itself under condition of ethanol excess intake.

Non-competitive inhibition features

a) I has another structure in a comparison with *S*

b) I may be attached not only with active centre of *E*.

c) The complex EIS is formed due to weak or covalent bonds.

d) I changes the V_{max} value, but K_m is not changed.

Lineweaver-Burk plots for this type of inhibition are shown in fig.8.

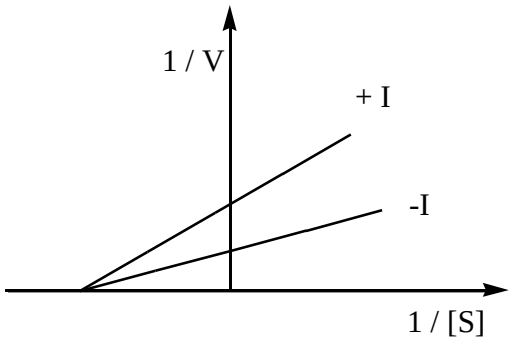


Figure 8. Lineweaver-Burk plots for non-competitive inhibition investigation. For example: E – cytochrome C oxidase (heme-containing); I – cyanide ions CN^-

Heavy metal ions (lead, mercury), arsenic ions toxicity is explained from their influence on enzymes as non-competitive inhibitors to block SH-groups in active site of enzymes. Reversibility for this type of inhibition may be due to decrease of their concentration in the reaction medium due to dilution of solution where reaction occurs.

Uncompetitive inhibition features:

a) I can bind only to the ES at a site distinct from the active centre.
b) I has another structure in comparison with S
c) EIS is formed due to weak or covalent bonds
d) I changes as V_{max}, as K_m

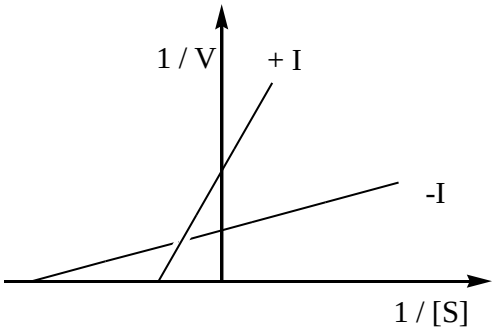


Figure 9. Lineweaver-Burk plots uncompetitive inhibition investigation. For example: E – xanthine oxidase (heme containing); I – cyanide ions CN^-

Irreversible inhibition of enzyme activity

This type of inhibitors binds covalently or so tightly to the active centre of enzymes that they are inactivated irreversibly. There are those subtypes:

Affinity labels. There are substrate analogs that possess a highly reactive group that is not present on the natural substrate. The reactive group of ***I*** permanently blocks the active centre of the ***E*** from the ***S*** because the group reacts covalently with amino acid residue. The residue that is modified is not necessarily involved in catalysis.

***Mechanism-based or suicide inhibitors*.** These are substrate analogs that are transformed by the catalytic action of the enzyme. Their structures are such that the product of this reaction is highly reactive and subsequently combines covalently with an amino acid residue in the active centre, thus inactivating the enzyme.

Transition – state analogs. There are substrate-analogs which do not covalently modify the enzyme but bind the active centre so tightly that they irreversible inactivate the E.

Many highly toxic, naturally occurring and man-made compounds are irreversible enzyme inhibitors. Some organic compounds are poisons for humans (diisopropyl fluorophosphate, organophosphorus insecticides are among them). Phosphor-containing organic compounds inhibit acetyl choline transferase across blockage of OH-groups of serine residues in active sites of enzyme to cause CNS paralysis

Natural compounds used as drugs can also inhibit enzymes. For example:

1) Penicillin, which is a transition-state analog that inhibits the reaction with transpeptidase that is important in the development of bacterial membranes, thus destroying normal growth of the bacteria.

2) Allopurinol is the suicide inhibitor of xanthine oxidase and is used in the treatment of gout.

Allosteric centre of enzyme: its location, structure and function in enzymatic catalysis. The common notion about Allosteric type of enzyme activity regulation. Feed-back type of inhibition.

Allosteric inhibition features

It is usually the reversible inhibition. That is because ***the I makes linkage with allosteric centre by non-covalent bonds to change conformation of enzyme molecule*** *(fig.10).*

Allosterically regulated enzymes are key enzymes for metabolic processes. So, allosteric activation and inhibition are the most important regulative processes in promotion of homeostasis in a cell. ***Feed-back inhibition is discussed as the case of allosteric inhibition.*** Sometimes a product of enzymatic reaction (or terminal product of a process) may be as allosteric inhibitor at condition of its accumulation in a cell.

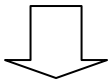
Example 1: NADH is produced due to isocitrate dehydrogenase reaction, under condition of its accumulation the enzyme activity is blocked. The terminal product of a process may be the feed-back inhibitor , too.

Example 2: Cholesterol synthesis from acetyl-SCoA is controlled so: the key enzyme – β hydroxy-β—methyl-glutaryl~SCoA-reductase is inactivated by cholesterol if its concentration is increased in a cell.

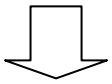
Example 3: Acetyl-CoA-carboxylase (the key enzyme in fatty acid synthesis) is regulated by feed-back influence of end-product – Palmityl-CoA.

Example 4: δ-Aminolevulinate synthase (the key enzyme of the heme synthesis placed in the matrix of mitochondria) is inhibited under accumulation of heme in a cell.

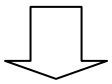
Allosteric inhibitor connects with allosteric centre



An enzyme conformation is changed at this moment



The conformation of active centre is changed, too (or there is the destruction of active centre)



Result: it is impossible to create the ES

Figure 10. All the steps for the influence of allosteric inhibitor on enzyme.

LESSON 6 OF MODULE 1

THEME:

PRINCIPLES OF ENZYME ACTIVITY DETERMINATION. GENETIC DEFICIENCY OF ENZYMES. MEDICAL ENZYMOLOGY

STUDY GOALS FOR LESSON 6 OF MODULE 1:

1. To study main principles of enzyme activity determination in biological fluids.
2. To learn units of total activity of enzymes.
3. To study basic notions about the use of enzymes as preparations, methodical reagents or in diagnostics of diseases.

IT IS IN NEED TO KNOW:

1. The principles of enzyme activity determination. Total and specific enzyme activity. The units of enzyme activity. Turnover number of enzyme.
2. Common notions about enzymatic pathologies; the reasons of their development (examples).
3. General trends in the development of medical enzymology:

1) the elaboration of diagnostic methods using enzymes as reagents;
2) enzymatic tests for diagnosis of diseases (examples);
3) the use of enzymes and their inhibitors as drugs (examples).

IT IS IN NEED TO DO:

1. Calculation of total activity of enzyme in katals and IU.
2. Determination of amylase activity in the urine by Volgemut's method.
3. Determination of cholinesterase activity in the blood serum.

BASIC LEVEL FOR PREPARATION.

DISCIPLINE INTEGRATION

Disciplines	**Obtained skills**
Previous: Organic chemistry	Covalent and acid-base catalysis. Types of reactions.
Non-organic chemistry	Catalyst and catalysis. Reversible and irreversible reactions. Factors to influence the rate of chemical reaction.
Common chemistry	pH and temperature influencies on enzyme activity. Specificity of action.
Next in study: Clinical biochemistry Pharmacology Pathological physiology	Clinical enzymology: principles for enzyme activity determination in biological fluids; the use in diagnostics of diseases ad as agents for methods. Enzymes, enzyme inhibitors as Medicines Recognition of term “normal state” and “pathological state”. Clinical indexes change for enzyme activity under acute pancreatitis, liver disorders. Enzymopathies as genetic disorders.

CONTENT OF EDUCATIONAL MATERIAL

IN LESSON 6 OF MODULE 1

1. The principles of enzyme activity determination. Total and specific enzyme activity. The units of enzyme activity. Turnover number of enzyme.
2. Common notions about enzymatic pathologies; the reasons of their development (examples).
3. General trends in the development of medical enzymology:

1) the elaboration of diagnostic methods using enzymes as reagents;

2) enzymatic tests for diagnosis of diseases (examples);

3) the use of enzymes and their inhibitors as drugs (examples).

A **PLAN AND ORGANIZATION OF LESSON 6 OF MODULE 1**

Stages	**Time/ minutes**	**Materials for lesson**		**Place for Duration Of lesson**
		Learning tools\content	**Equipment**	
1.Organizing time	**5**			**Auditory 507,509, 511, 514**
2. A conversation	**30**	**Consideration of main principles to determine enzyme activity, its units and example for its calculation**	**1.Textbook on Biochemistry 2. A Manual for submodule 1**	
3.Laboratory works beginning	**20**	**The group of students is divided in two subgroups with aim to start laboratory works from the beginning up to the operation : to place test tubes with reagents in the thermostat for 30 minutes**		
4. Initial control	**20**	**Variants for calculation of total activity of enzymes (1-4)**	**Cards : each with two questions**	
Brake	**10**			
5. Laboratory works continuation	**25**	BIOCHEMISTRY LABORATORY MANUAL Module 1	**Reagents for laboratory works; Test tubes Thermostat**	

6. Terminal control for theory	**10**	**Variants for control (1-2)**	**Blocks of tests**	
7. Final results discussion	**15**	**Protocol N6**		

ALGORITHM FOR LABORATORY WORKS OF LESSON 6 OF MODULE 1

Laboratory work 1. Determination of amylase activity in the urine (Volgemut`s method)

THE PRINCIPLE OF THE METHOD:

The Volgemut's method is based on the minimal quantity of the enzyme determination, which is capable to split completely 2ml of 0,1% starch solution. This quantity of enzyme is accepted for a unit of the amylase activity.

THE COURSE OF THE WORK:

Pour 1 ml of 0, 85 % sodium chloride solution into each test tube (8 test tubes). Add 1 ml of patient's urine into the 1-st test tube and mix thoroughly. Then transfer 1 ml of the mixture into the 2-nd test tube and repeat all the operations with the test tubes: from the 2-nd one into the 3rd one, etc. Pour 1 ml of liquid out of the 8-th test tube. Add 2 ml of 0, 1 % starch solution into each test tube, mix and put them into the thermostat at 38^0C for 30 minutes. At the end of the incubation take the test tubes out, cool them and add 2 drops of the iodine solution into each one. Mix the content of the tubes and mark the latest test tube with no coloured solution (where there was full starch splitting).

The calculation is made according to the formula:

X (units)= 1 • 2 • dilution;
1 - urine volume (1ml); 2 - volume of 0,1 % starch solution in ml;
X - salivary amylase activity in standard units.

Dilution is in each test tube (respectively): N1 - 2; N2 - 4; N3 -8; N4 - 16; N5 - 32; N6 - 64; N7 - 128; N8 - 256.

THE EQUIPMENT AND REAGENTS

Thermostat, test tubes, pipettes, 0, 85 % sodium chloride solution; 0, 1 % starch solution; 0, 1 % iodine solution

THE CLINICAL SIGNIFICANCE OF THE TEST:

Normal values of the amylase activity in the urine (by Volgemut) are 16 - 64 units. At sharp pancreatitis the activity of amylase in the urine and the blood serum arises 10 - 30 times.

Laboratory work 2. Cholinesterase activity determination in the blood serum

THE PRINCIPLE OF THE METHOD:

Cholinesterase (CE) hydrolyzes acetylcholine to obtain an acetic acid and choline. The acetic acid decreases the pH value of solution that is because an indicator changes its colour: from crimson colour to yellow one.

THE COURSE OF THE WORK:

Keep all the reagents for 10 minutes at 37ºC in the thermostat. Take three test tubes and make all the operations according to scheme:

Add, ml	N1 (test sample)	N2 (control sample)	N3 (empty sample)
Indicator solution	2, 5	-	2,5
Blood serum	0, 05	0, 05	-
0, 9% NaCl sol-n	-	2, 7	0,05
Acetylcholine sol-n	0, 1	-	0,1
Take all test tubes into the thermostat (37°C) and keep there for 30 minutes. Then add:			
Stop-reagent	0,1	-	0,1

You have to determine an optical density of each sample against distalled water at 540 nm (green colour filter) in cuvettes (5 mm). Calculate the E according the formula:

E = E(empty) +E(control) – E(test)

Use this E value to find out on a graph the CE activity.

THE EQUIPMENT AND REAGENTS

Thermostat, photocolorimeter, cuvettes (5 mm), test tubes, dozators for 0.05ml, 0.1ml; pipette 5ml; 0, 85 % sodium chloride solution; acetyl choline solution, indicator solution, stop-reagent.

THE CLINICAL SIGNIFICANCE OF THE CHOLINESTERASE (CE) DETERMINATION IN THE BLOOD SERUM:

The normal value of CE activity is 45-95 μmol/sec•lit

The distinct decrease of the CE activity in blood serum takes place at the diseases of the liver, hypothyroidism, the bronchial asthma, articulate rheumatism, heart attacks of the myocardium, burns, traumatic shocks, in postoperative conditions. In severe forms of Botkin's disease the CE activity is decreased. In a case of the aggravation of the disease the decrease of the cholinesterase activity outstrips the bilirubin peak, playing a role of a harbinger of the aggravation. The dynamics of CE activity changes plays a valuable prognostic role at the patient's treatment.

INITIAL CONTROL OF KNOWLEDGE FOR LESSON 6 OF MODULE 1

Variant 1

1. There was the decrease of the concentration of the substrate from the value 5 mmol / l to the value 1 mmol / l during 5 min of the enzymatic reaction duration. Determine the total activity of the enzyme in the international units of activity.
2. What is the purpose to determine the activity of amylase in the urine of patients? Propose the normal value for the activity of this enzyme in the urine.

Variant 2

1. There was the increase of the concentration of the product from the value 0 mmol / l to the value 6 mmol / l during 1 min of the enzymatic reaction duration. Determine the total activity of the enzyme in kat/l.

2. There is the choline esterase activity 50 micromol/sec•l in the blood plasma of patient. Is it the normal value for the activity of this enzyme or you can propose the probable diagnosis for patient? Transform the value for the enzyme activity in units kat/l.

Variant 3

1. There was the decrease of the concentration of the substrate from the value 12 mmol / l to the value 6 mmol / l during 1 min of the enzymatic reaction duration. Determine the total activity of the enzyme in katals/l.

3. There is the decrease of choline esterase activity to the value 25 micromol/sec•l in the blood plasma of patient. Propose the probable diagnosis for patient, and transform the value for the enzyme activity in kat/l.

Variant 4

1. There was the decrease of the concentration of the substrate from the value 6 mmol / l to the value 0 mmol / l during 5 min of the enzymatic reaction duration in the blood plasma. Determine the total activity of the enzyme in kat/l.

2. Why the activity of amylase in the urine is determined in the relative units of activity?

Answers for positions 1, 2 in cards:

Variant 1: 1) 800 micromol / min x L; 2) Acute pancreatitis; parotitis: testing for the damage of pancreas by viral infection.

Variant 2: 1) 0,1 kat/l; 2) $50x10^{-6}$ kat/L; normal value.

Variant 3: 1) 0,1 kat/l; 2) $25\ x10^{-6}$ kat/L; viral hepatitis; liver cirrhosis.

Variant 4: 1) $0,02x10^{-3}$kat/L; 2) the substrate for amylase is starch – polysaccharide, it is impossible to determine 1 mole of starch.

FINAL CONTROL OF KNOWLEDGE FOR LESSON 6 OF MODULE 1

Variant 1

1. There is the treatment of patients with achlorhydria (the complete lack of hydrochloric acid in the gastric juice of patient) by an enzyme as a drug. Name it:

A. Casein

B. Pyruvate

C. Pepsin

D. Trypsin

E. Chymotrypsin

2. Choose the enzyme used as reagent for glucose content determination in the blood:

A. Glucose-6-phosphatase

B. Pyruvate kinase

C. Maltase

D. Glucose oxidase

E. Amylase

3. A lot of factors must be taken into account to promote methodic requirements for the determination of the enzyme activity in biological fluids. Choose, please, the most important from them:

A. pH of the environment

B. Temperature of the environment

C. Substrate concentration

D. Enzyme concentration

E. All the positions placed above

4 The determination of Lactate dehydrogenase (LDH) isozymes content showed the increase of LDH_4 and LDH_5 fractions in the patient's blood plasma. Point out the presumable diagnosis:

A. Viral hepatitis

B. Skeletal muscle dystrophy

C. Diabetes mellitus

D. Myocardial infarction

E. Acute pancreatitis

5. Find out the parameters that are in need to calculate specific activity of enzyme:

A. The protein content in the test sample and total activity

B. The volume of the test sample and total activity

C. Turnover number and total activity

D. The pH value of the environment and turnover number

E. The product concentration in the test sample and total activity

6. The cytoplasm fraction of tissue homogenate has been obtained for some enzyme activity determination. Point out the enzyme whose activity may be determined in this fraction:

A. Citrate synthase

B. Urease

C. Maltase

D. Glucokinase

E. Pepsin

7. Name, please, the reagent that is added to urine of patient to increase the activity of amylase:

A. Sodium phosphate

B. Sodium chloride

C. Copper sulfate

D. Glucose

E. Choline

8. Name the unit of the enzyme activity, if the reaction is carried out by the quantity of the enzyme at a rate of 1 mol of the substrate conversion per second:

A. Standard international unit

B. Katal

C. Specific activity

D. Turnover number

E. Conditional unit of activity

9. Patient's amylase activity in the urine equals 16 units. Point out the possible state for this patient:

A. There is viral hepatitis in patient

B. There is diabetes mellitus in patient

C. There is the sharp pancreatitis in patient

D. The patient is apparently healthy

E. There is Angina in patient

10. Find out the term for unit of enzyme activity that is estimated as the number of molecules of a substrate acted upon in a period 1 second by a single enzyme molecule:

A. Total activity

B. Special activity

C. Turnover number

D. Katal

E. Unit of an enzyme activity

Variant 2

1. Find out the activator used for amylase activity determination in the urine of patient:

A. Copper sulfate

B. Pyruvate

C. Sodium chloride

D. Ammonia sulfate

E. Starch

2. Pancreatine is proposed as the drug to promote the normal digestion of proteins in the small intestine of patients with chronic pancreatitis. Choose the enzyme that is the component of this drug and is possible to destroy protein structure:

A. Amylase

B. Lipase

C. Pepsin

D. Trypsin

E. Maltase

3. Competitive inhibitors of enzymes may be used as drugs. Try to find out the medicine that is used to decrease the rate of folic acid synthesis from para-aminobenzoic acid in microorganisms which can cause the inflammation state of tissues in humans:

A. Antimycin A

B. Pancreatine

C. Phenobarbital

D. Sulfonamide

E. Phosphogluconate

4. Enzyme deficiency in patient usually is discussed as severe form of pathology because:

A. It is genetic disorder that is difficult to treat

B. It is secondary reason of pathology

C. It is difficult to determine it

D. It cannot be prevented in the prenatal period of organism development

E. It can be discovered in adults, only

5. We cannot use the enzymes as medicines for oral administration because oral dose of enzyme:

A. Causes the allergic reactions in human organism

B. Stimulates the production of albumins by the liver

C. Can lead to the cleavage of blood plasma proteins

D. Is digested in gastrointestinal tract

E. Changes the acid-alkaline balance in the blood

6. The decrease of Choline esterase activity in the blood serum of patient is the signal to care for the function of one organ mainly. Name it:

A. Liver

B. Spleen

C. Brain

D. Kidney

E. Pancreas gland

7. The principle of the method for Choline esterase activity determination is based on the ability of the product for the reaction catalyzed by this enzyme to change the pH of the incubation phase. Try to give the name of this product:

A. Choline

B. Serum Albumin

C. Acetyl choline

D. Phosphatidyl choline

E. Acetic acid

8. The activity of choline esterase in the blood serum of patient equals 95 μmole/sec• lit. Propose, please, the way for the transformation of this value into units [mmole/lit●min]:

A. Divide this result into 1000, only

B. Divide this result into 1000 times, and multiple on 60

C. Multiple this result into 1000 times, only

D. Multiple this result into 60 times, only

E. Divide this result into 60, only

9. Find out the parameter that changes in the incubation phase of experimental test tube for the determination of choline esterase activity in the blood plasma:

A. The pH

B. The acetylcholine concentration

C. The acetic acid content

D. The volume of the blood plasma

E. Positions A, B, C are right

10. Genetic disorder associated with enzymatic pathology may be caused by the:

A. Deficiency of the non-protein part of enzyme, only

B. Disorder in the regulation of the transcription of mRNA for enzyme synthesis

C. The damage of the feed-back mechanism of enzyme regulation

D. Super-activation of inducer synthesis that is used to stimulate transcription of mRNA for enzyme

E. All the reasons that are described above

Keys for tests:

Variant 1: 1-C 2-D 3—E 4 -A 5-A 6- D 7-B 8-B 9-D 10-C

Variant 2: 1- C 2- D 3—D 4 - A 5- D 6- A 7- E 8- B 9- E 10- E

INFORMATIONAL MATERIAL FOR THE LESSON 6 OF MODULE 1

Principles of enzyme activity determination. Total and specific enzyme activity. The Units of enzyme activity. Turnover number of enzyme.

The determination of enzyme activity is of great importance for scientists. The enzyme activity is determined for tissue enzymes in homogenates of tissues or cellular fractions at research works. It is determined in whole blood, plasma or serum, in saliva, in gastric juice, in the urine, in cerebrospinal fluid for disease diagnostics in patients.

There are some methodic requirements for the enzyme activity determination in biological fluids:

- The substrate concentration must be more then substrate concentration for saturation of active centers of enzyme molecules found in investigated sample;
- The pH and temperature of the environment must be optimal;
- The activator for the enzyme in some cases must be added.

Total activity (T.A.) units:

- ***An International Unit (IU)*** is the amount of enzyme that catalyzes the transformation of 1 μmole of a substrate per minute under optimal conditions of measurement.
- ***Katal*** is the amount of enzyme that catalyzes the transformation of 1 mole of a substrate per second under optimal conditions of measurement.

These units are used for Total enzyme Activity (T.A.) determination.

Specific activity (S.A.) is the number of units of total activity per milligram of total protein [C] present in a sample: S.A. = $\frac{T.A}{[C]}$. This type of activity is used in researching works in biochemistry.

Turnover number (N) is the number of substrate molecules metabolized per one enzyme molecule per unit of time. For example, Carbonic unhydrase has turnover number 36000000/min.

Common notions about enzymatic pathologies: the reasons oftheir development

A determination of enzyme activity in the blood plasma is of great importance for medicine. It helps to make diagnosis for some diseases, to find out the tissue damage, to differentiate the type of infringements for the same organ when other indexes of the blood plasma can't help.

Example 1*: Aspartate aminotransferase (AsAT) activity* in the blood plasma increases 10-100 times more then normal value at myocardium infarction in the first 3-4 hours of the damage development when cardiogram may be normal for patient.

Example 2 : The knowledge about tissue distribution of *isozymes of Lactate Dehydrogenase (LDH):*

Myocardium	LDH_1 and LDH_2
Liver	$LDH_3 < LDH_4 < LDH_5$
Skeletal muscular tissue	$LDH_3 > LDH_4 > LDH_5$
Kidneys	LDH_3

It helps to find out the damage of some tissues if special type of isozymes is allocated from damaged tissue cell into the blood plasma. The determination of isozymes activity in the blood plasma is very important for diagnostic of heart, liver disease and many others (Fig. 1).

Example 3*:* Bilirubin indexes (conjugated and unconjugated) may be high in blood serum at various types of jaundice. The hepatic jaundice is accompanied with parenchyma damage of the liver. The liver parenchyma damage may be proved by the determination of *alanine aminotransferase (AlAT) activity* and *choline esterase activity* in the blood plasma of patients. Beside this the dynamic of choline esterase activity plays a valuable prognostic role at the treatment of patient: the decrease of the cholinesterase activity plays a role of a harbinger of the aggravation.

Example 4*: Amylase activity in blood plasma and in urine* may be increased in 10-60 times or more at sharp pancreatitis in patients. This test is used also to check up the pancreatic gland function after treatment of patient with parotitis (mumps).

Genetic disorders of enzyme synthesis

A lot of genetic disorders are associated with the damage of enzymes synthesis or with the infringements of their regulation in tissues. These disorders are the most difficult in treatment, and the diagnosis is made at newborns or at prenatal state using the determination of some substrates or products concentration for enzymes that are in deficiency. DNA probes are available for prenatal diagnosis using amniotic liquid.

Example 1: Defects in the Phenylalanine 4-monooxygenase (hyperphenylalaninemia type I) or classic phenylketonuria. There is no transformation of phenylalanine into tyrosine in patient. Phenylalanine is accumulated in tissues and in the blood; the transformation of it may be to phenyl pyruvate, only. Phenyl pyruvate levels are also high in tissues and all the liquids. The major consequence of untreated type I hyperphenylalaninemia is mental retardation. Additional clinical signs include seizures, psychoses, eczema and a

mould odour of urine. Screening of newborn infants for phenylketonuria now is compulsory (in a few days after born, 1-6 days).

	Normal Plasma / Urine	Phenylketonuric patient Plasma / Urine
Phenylalanine, mg/DL	1 - 2 / 30	15 - 63 / 300 - 1000
Phenylpyruvate, mg/DL	- / -	0,3 - 1,8 / 300 - 2000

Example 2: Alkaptonuria is caused by a defect in Homogentisate oxidase used for transformation of homogentesic acid to 4-maleylacetoacetate (tyrosine conversions). Homogentisate is accumulated in the blood and is excreted in large amounts in the urine causing the urine to darken after being exposed to air. Later in life, patients may develop pigmentation of connective tissue and suffer from arthritis.

General trends in the development of medical enzymology:

1) the elaboration of diagnostic methods using enzymes as reagents;
2) enzymatic tests for diagnosis of diseases (examples);
3) the use of enzymes and their inhibitors as drugs (examples).

Enzymes with their respective substrates and inhibitors: the use in medicine

Competitive inhibitors			
Enzyme	**Substrate**	**Inhibitor**	**Significance of inhibitor**
Monoamine oxidase	Epinephrine norepinephrine	Ephidrene, amphetamine	Useful for elevating catecholamine levels
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients

Enzyme	Substrate	Inhibitor	Significance of inhibitor
Dihydropteroate synthase	Para-Amino Benzoic Acid	Sulfonamide	Prevents bacterial synthesis of folic acid
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant
Betta-Hydroxy-betta-Methyl-Glutaryl-CoA (HMG CoA)-reductase	HMG CoA	Lovastatin, compactin	Inhibits cholesterol biosynthesis
Irreversible inhibitors			
Enzyme	**Substrate**	**Inhibitor**	**Significance of inhibitor**
Aldehyde Dehydrogenase	Acetaldehyde	Disulfiram (antabuse)	Used in the treatment of alcoholism
Xanthine oxidase	Xanthine hypoxanthine	Allopurinol (suicide inhibitor)	Used in the control of gout to reduce excess production of uric acid from hypoxanthine
Cyclooxygenase	Arachidonic acid	Acetyl salicylic acid (aspirin) Phenyl butazone Indomethacin Ibuprofen	Anti-inflammatory drug : antipyretic (fever-reducing) and analgesic (pain relieving)
Aldehyde dehydrogenase	Acetyc aldehyde produced from ethanol	Teturam	Accumulation of acetic aldehyde in the blood of alcoholics will be, and it causes the aversion to alcohol under its use
Trypsin	Proteins of blood plasma	Trasilol and other protease inhibitors	To prevent spread proteolysis of proteins in pancreas and in the blood
Kallikrein	Proteins-regulators of blood vessels tonicity	Contrical	To control blood pressure in norm

Enzymes Use in Medicine

Basic sections	***Enzyme name***	***Examples of use***
Diagnostics a) serum enzyme The increased level	Lactate dehydrogenase [isozyme LDH_1] [isozyme LDH_5]	Heart attack Liver diseases
	Aspartate aminotransferase [SGOT], AsAT	Heart attack (myocardial infarction)
	Alanine aminotransferase [SGPT], AlAT	Viral hepatitis, liver damage
	Creatine phosphokinase [CPK]: Isozyme MM (CPK_3) Isozyme MB (CPK_2)	Muscle disorders Heart attack
	Acid phosphatase [ACP]	Prostate cancer
	Alkaline phosphatase [ALP]	Liver diseases, bone disorders
b) serum enzyme The lowered level	Choline esterase [ChE]	Liver parenchyma damage, hypothyroidism, nephritic syndrome, myocardial infarction
	γ-Glutamyl transpeptidase [GGT]	Alcoholism
	α-Amylase	Acute pancreatitis
	Lipase	Acute pancreatitis
	Aldolase	Muscular dystrophy
	5'-Nucleotidase	Hepatitis
	Glucose 6-phosphate dehydrogenase [G6PD]	Congenital deficiency with hemolytic anemia
	Ceruloplasmin	Wilson's disease
Treatment	Pepsin	Disordered digestion of proteins in stomach, deranged synthesis or secretion of pepsin
	Trypsin, chymotrypsin (immobilized forms)	Treatment of purulent wounds
	Streptokinase, urocaninase Its preparation form as "Streptodekase" (immobilized enzyme)	Prevention of clots formation at transplantation of organs and other operations To promote fibrinolysis at patients

	Enzyme name	***Examples of use***
Treatment	Hyaluronidase	Resorption of a scar tissue, keloids due to the degradation of substrate - hyaluronic acid
	Asparaginase	Treatment of some malignant neoplasms, leucosis to prevent the accumulation of tumor growth factor -asparagine
	Nucleases (DNAase)	Viral conjunctivitis, rhinitis, purulent bronchitis
	Urease	Removal of urea from an organism in artificial kidney apparatus
Use of enzymes as analytical reagents	Glucose oxidase	The determination of glucose content in blood
	Cholesterol oxidase	The determination of cholesterol content in blood
	Lipase	The determination of triacylglycerols content in blood
	Urease	The determination of urea content in blood

COLLECTION OF TESTS ABOUT ENZYMES FOR PREPARATION TO LICENSING EXAMINATION "KROK-1"

1. Succinate dehydrogenase catalyses the dehydrogenation of succinate. Malonic acid HOOC-CH_2-COOH is used to interrupt the action of this enzyme. Choose the inhibition type:

A. Allosteric

B. Competitive*

C. Non-competitive

D. Dephosphorylation

E. Limited proteolysis

2. Only one factor can influence the charge of amino acid radicals in the active centre of enzyme. Name this factor:

A. The presence of a competitive inhibitor

B. Temperature

C. Pressure

D. pH medium*

E. The surplus of a product

3. Researchers isolated five isozymes of Lactate dehydrogenase from human blood serum and studied their properties. What property of these isozymes indicates that they are genetic forms of the same enzyme?

A. They have the same molecular weight

B. They catalyze the same reaction*

C. The same tissue localization

D. The same electrophoretic mobility

E. The same net charge of the molecule

4. In case of enterobiasis acrihine - the structural analogue of vitamin B2 - is administered. The synthesis disorder of which enzymes does this medicine cause in microorganisms?

A. FAD-dependent dehydrogenases *

B. Cytochromeoxidases

C. Peptidases

D. NAD-dependet dehydrogenases

E. Aminotransferases

5. In clinical practice tuberculosis is treated with izoniazid preparation – that is an anti-vitamin able to penetrate into the tuberculosis bacillus. Tuberculostatic effect is induced by the interference with replication processes and oxidation-reduction reactions due to the buildup of pseudo-coenzyme:

A. FMN

B. NAD+ *

C. CoQ

D. FAD

E. TDP

6. Profuse foam appeared when dentist put hydrogen peroxide on the mucous of the oral cavity. What enzyme caused such activity?

A. Catalase

B. Cholinesterase

C. Acetyltransferase

D. Glucose-6-phosphatdehydrogenase

E. Methemoglobinreductase

7. Twelve hours after an accute attack of retrosternal pain in patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?

A. Myocardium infarction *

B. Viral hepatitis

C. Collagenosis

D. Diabetes mellitus

E. Diabetes insipidus

8. Marked increase of activity of MB-forms of CPK (creatine phosphokinase) and LDH-1 were revealed on the examination of the patient's blood. What is the most likely pathology?

A. Miocardial infarction*

B. Hepatitis

C. Rheumatism

D. Pancreatitis

E. Cholecystitis

9. A patient presents high activity of LDH1, LDH2, aspartate aminotransferase, creatine phosphokinase. In what organ (organs) the development of pathological process is the most probable?

A. In the heart muscle (initial stage of myocardium infarction)*

B. In skeletal muscles (dystrophy, atrophy)

C. In kidneys and adrenals

D. In connective tissue

E. In liver and kidneys

10. During metabolic process active forms of the oxygen including superoxide anion radical are formed in the human body. With help of what enzyme is this anion inactivated?

A. Superoxide dismutase*

B. Catalase

C. Peroxidase

D. Glutathione peroxidase

E. Glutathione reductase

11. 6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isozyme should be expected in this case?

A. LDH_4

B. LDH_1*

C. LDH_5

D. LDH_3

E. LDH_2

12. Those organisms which in the process of evolution failed the protection from H_2O_2 can exist only in anaerobic conditions. Which of the following enzymes can break hydrogen peroxide down?

A. Oxygenase and hydroxylase

B. Oxygenase and catalase

C. Cytochrome oxidase, cytochrome b5

D. Flavin-dependent oxidase

E. *Peroxidase and catalase

13. Cyanide is a poison that causes instant death of the organism. What enzymes found in mitochondria are affected by cyanide?

A. Flavin enzymes

B. Cytochrome oxidase (*aa*3) *

C. Cytochrome B5

D. NAD+- dependent dehydrogenase

E. Cytochrome P-450

14. A patient is diagnosed with cardiac infarction. Blood test for cardio-specific enzymes activity was performed. Which of enzymes has three isoforms?

A. Creatine kinase*

B. Aspartate transaminase

C. Alanine transaminase

D. Lactate dehydrogenase

E. Pyruvate kinase

15. A 50-year-old woman diagnosed with cardiac infarction has been delivered into intensive care ward. What enzyme will be the most active in the blood plasma during the first two days?

A. LDH4

B. Alanine aminotranferase

C. LDH5

D. Alanine transpeptidase

E. Aspartate aminotransferase*

16. When investigating human saliva it is necessary to assess its hydrolytic properties. What substance should be used as a substrate in the process?

A. Starch*

B. Fiber

C. Fats

D. Proteins

E. Amino acids

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