ENZYMES

Enzymes - proteins - speed up biochemical reactions

1 molecule is transformed noncatalyzed in comparison to10^6-10^{14} molecules transformed via catalysis by enzyme

The mechanism of action of enzymes consists of reducing the activation energy for the reaction (enable course of metabolic processes at relatively low temperatures (37 ° C) and at pH 6.5 to 7.5 in an aqueous medium)

There are from 1000 to 4000 different types of enzymes in animal cell

- protein part - apoenzyme, nonproteinic – coenzyme
- reactant - substrates, the substances formed –products
- Reaction: The substrate binds to the specific binding site on the enzyme molecule (binding site is wedged into the recess enzyme called. Active center)

The enzymatic reaction-principle

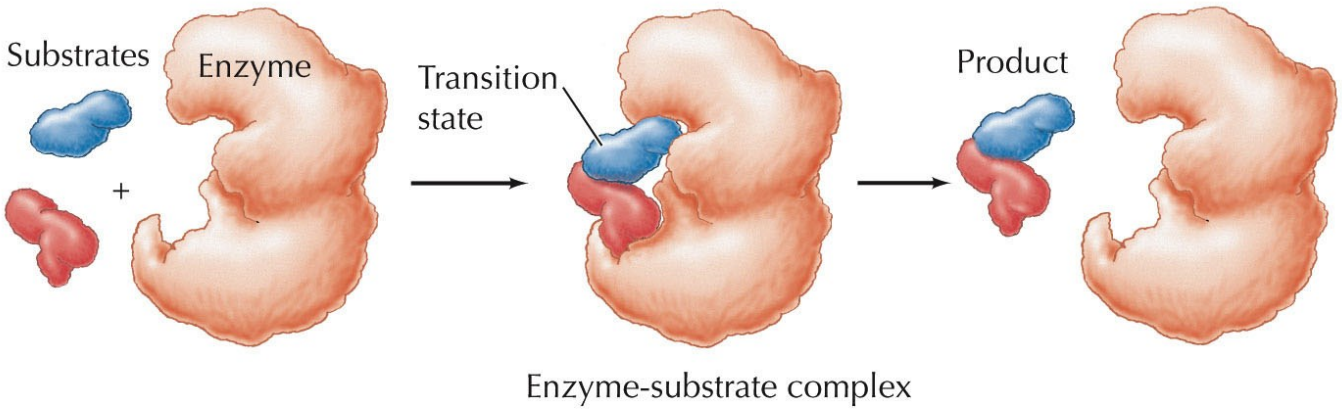
Binding of substrate - enzyme:

- by **non-covalent ionic bond**
- **hydrogen and hydrophobic bridges**
- **van der Waals interactions**

- On the **functional groups of aminoacids residues in active site of enzyme** are linked **coenzymes**, eventually metal atoms (metaloenzymes), participate in the catalyzed reaction

- Functional groups **activate the substrate and reduce** the energy which is required to produce high-energy intermediate stage

Binding of the enzyme-substrate



THE CELL, Fourth Edition, Figure 3.2 © 2006 ASM Press and Sinauer Associates, Inc.

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Pathobiochemistry of regulation enzyme activity

Inhibition of enzyme activity

The effect of **multiple drugs or toxins** resides in its ability to inhibit the enzyme.

Strongest inhibitors - bind **covalently** to functional groups of the active site, eventually substrate analogs - forming enzyme complexes

The rate of enzyme reaction :

a) Concentration of **substrate, product**

b) Concentration of **activators**, concentration od **inhibitors**

The relationship between the rate of the enzyme reaction and the substrate concentration - given by Michaelis-Menten equation

$$E + S \leftarrow \rightarrow ES \rightarrow P + E$$

Products and physiological inhibitors may compete with the substrate for **binding to the active center** of the enzyme and thus slow the rate of reaction

Physiological regulation of metabolic pathways– the ability to alter the rate of progression of metabolic reactions in the pathway via activation of enzymes that catalyze **the slowest part**

Enzymes have so called **allosteric activators or inhibitors** i.e. compounds which bind to a different part of the enzyme molecule than the active site thus affecting the conformation of the enzyme molecule

- multimeric enzymes, CAC enzymes

The regulation also – by modulatory protein or phosphorylation

Isoenzymes: enzymes having a **different amino acid sequence** in the peptide chain, but **catalyzing the same reaction**

INHIBITORS:

The activity of many enzymes can be inhibited by the binding of specific small molecules and ions. This means of inhibiting enzyme activity serves as a major control mechanism in biological systems.

The regulation of allosteric enzymes typifies this type of control.

In addition, many drugs and toxic agents act by inhibiting enzymes. Inhibition by particular chemicals can be a source of insight into the mechanism of enzyme action: specific inhibitors can often be used to identify residues critical for catalysis.

Enzyme inhibition can be either reversible or irreversible.

An ***irreversible inhibitor*** dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme, either covalently or noncovalently.

Some irreversible inhibitors are important drugs.

Mechanisms of regulation of the enzyme activity - INHIBITION

Irreversible inhibition

Reversible inhibition of the active center

The enzyme inhibitor is a compound that reduces the rate of response by **binding to the enzyme**. Reversible inhibitors – there is not a covalent bond, can be detached from the enzyme. Products - are reversible inhibitors for their own reaction.

Competitive inhibition

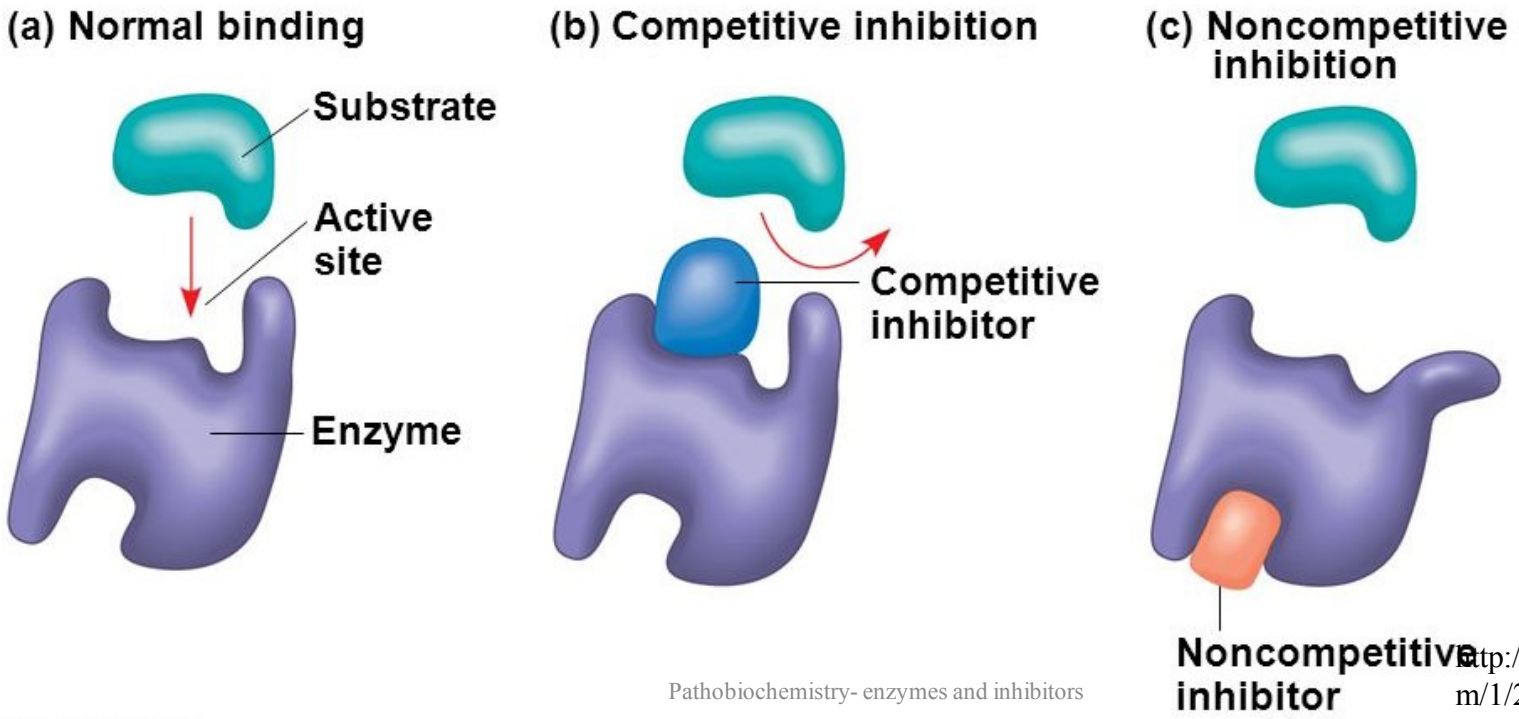
Reversible inhibitor may compete for binding to the active center with the substrate, producing an enzyme complex that may be dissociated into free enzyme and inhibitor.

Non-competitive inhibition

This inhibitor does not compete for the binding site, but its binding to the enzyme reduces the concentration of active enzyme, i.e. always decreasing Vmax

Scheme of inhibition

In **competitive inhibition** of the binding site for the substrate A competes with structurally very similar other substrate.
In <u>non-competitive inhibition</u> substrate A gets to its binging site, but at the position on the binding site of its partner i.e. substrate B occupies a non-competitive inhibitor (to substrate A), but that is competitive with to B.
In **non-competitive** inhibition the inhibitor binds to the enzyme-substrate complex.



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Examplex of inhibition

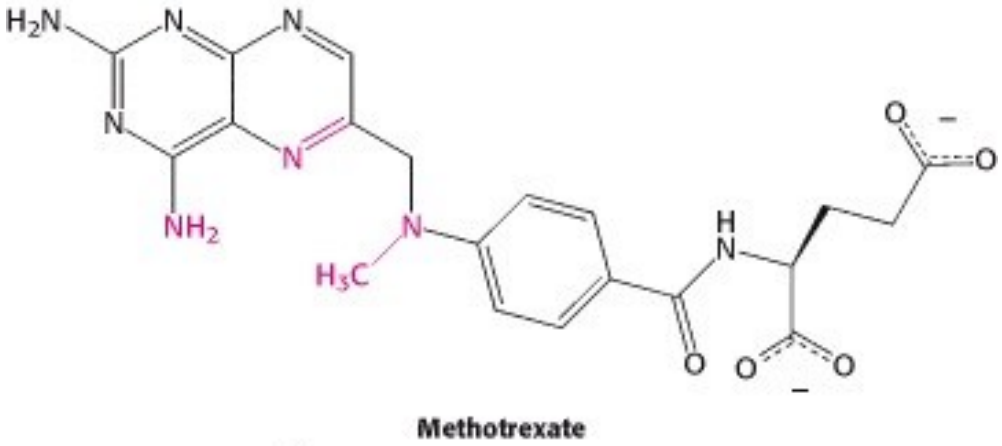
Non-competitive inhibition

- **Non-competitive inhibitor** binds only to the enzyme-substrate complex (when the enzyme binds substrates cotrollably):
- the first substrate molecule induces a change in the conformation of the enzyme molecule, the binding site for either the co-substrate or inhibitor openes. Non-competitive inhibitor reduces Km and Vmax, as well.

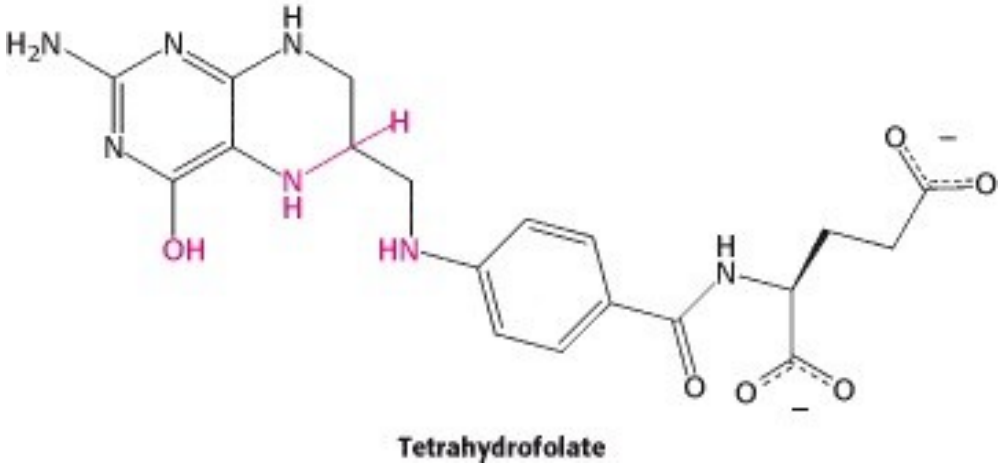
Competitive inhibition

Molecules of inhibitor - structurally similar to the substrate that covalently bind so tightly in the active center that this binding can not be displaced. This way - a common mechanism of action of drugs or antimetabolites

The **competitive inhibitor** resembles the substrate and binds to the active site of the enzyme (Figure 8.15). The substrate is thereby prevented from binding to the same active site. *A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate.* At any given inhibitor concentration, competitive inhibition can be relieved by increasing the substrate concentration. Under these conditions, the substrate "outcompetes" the inhibitor for the active site. **Methotrexate** is a structural analog of **tetrahydrofolate**, a coenzyme for the enzyme **dihydrofolate reductase**, which plays a role in the biosynthesis of purines and pyrimidines (Figure 8.16). It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis. It is used to treat cancer.



Methotrexate



Tetrahydrofolate

Examples of irreversible competitive inhibition

Inhibitor	Enzyme	Effect
Aspirine	cyclooxygenase	Anti inflammatory
Allopurinol	xanthinoxidase	Treatment of gout
5-Fluorouracil	thymidilatesynthase	cancerostatic
Penicilin	transpeptidase	antibiotics
Sarin	cholinesterase	Nerve gas
ß-aminopropionitrile	lysyloxidase	lathyrism

Mechanism of action of irreversible inhibitors

- **Affinity changes of binding site** – the substrate analog has a reactive group that is not the natural substrate and which permanently blocks the active site for substrate (covalent bond with an amino acid residue)

!!! **resistance to antibiotics**

Excessive and prolonged use of antibiotics also in agriculture, veterinary practice… have made some bacterial strains - *Pseudomonas, Streptococcus,Staphylococcus, Mycobacterium tuberculosis*… resistant to some antibiotics

The mechanism of this resistance may be due to induction of the synthesis of enzymes, which modify the antibiotic molecule so that it becomes analog of the transitional stage.

Example of resistance

Induction of ß-lactamases are altered so called ß-lactam antibiotics :

- **penicillins, cephalosporins, carbapenems**

A similar effect may have the induction of:

- **acetyltransferase, fosfotransferase or nukleotidyltransferase on aminoglykosides**
- **acetyltransferase on chloramphenicol**

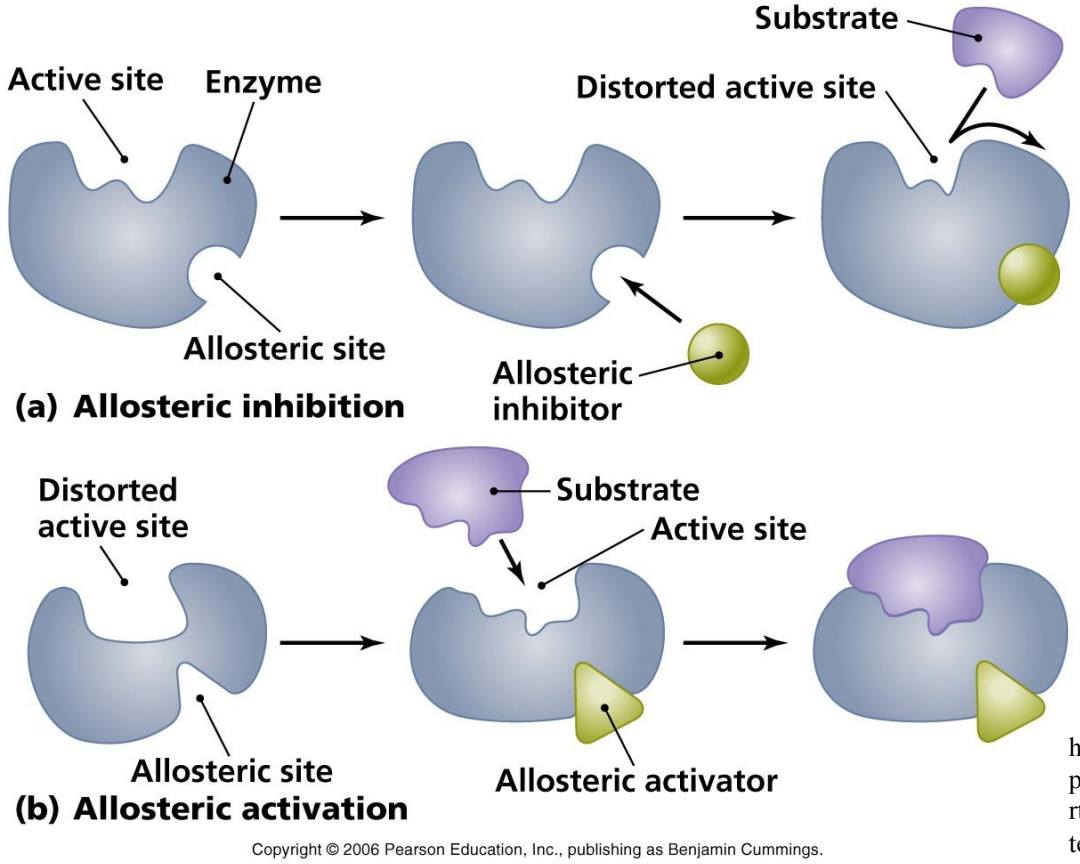
Allosteric regulation
regulation mechanisms of enzyme activity

Allosteric regulation

Allosteric effector reversibily binds to another than binding site of the enzyme. This bond induces: conformational change of the active site, so it may **activate** or

inhibit the enzyme

Scheme of allosteric activation and inhibition



http://academic.pgcc.edu/~kroberts/Lecture/Chapter%205/05-11_AllostericControl_L.jpg

Other regulation mechanisms of enzyme activity

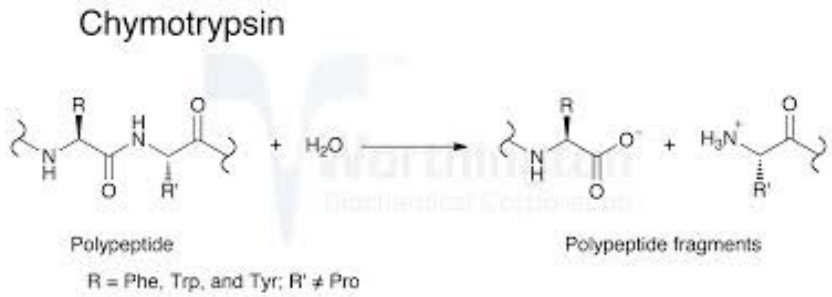
Covalent modifications – phosphorylation of hydroxyl group by phosphorylasakinase. Phosphorylation of enzyme **activates the active site** of enzyme → activ conformatin, dephosphorylatin → inaktivation.

Limited proteolysis - aktivation induced by cleaving the short polypeptide chain from the peptide, e.i. proenzyme or enzymogene

✓leads to change in the conformation of the active site that can bing the substrate in this form.

Examples: chymotrypsinogen ® chymotrypsin

prothrombin ® thrombin



Note: - proteolytic pancreatic enzymes, which are formed in the pancreas as prodrugs, are activated in the gut lumen. Otherwise, they would digeste own pancreatic tissue.

http://www.worthington-biochem.com/chy/images/reaction.jpg

Other regulation mechanisms of enzyme activity

➢Induction of enzyme synthesis

the amount of enzyme in the cell depends on the rate of its synthesis or degradation (lasts several hours or days, previous methods of control activities last only)

➢Suppression synthesis of the enzyme

➢**Example: by this mechanism acts e.g. omeprazole – suppresses protein synthesis e.i. proton pump in the gastric mucosa, thus preventing the production of HCl. It is used to reduce the acidity of gastric secretion, thereby limiting its aggressive action (treatment of peptic ulcer disease).**

➢feedback regulation

final product regulates the speed of its own synthesis, the final product of a metabolic pathway may inhibit or related metabolites may activate regulatory key) enzyme

final product may regulate the speed of its own synthesis - action on gene transcription key enzyme in the metabolic pathway – much more slower process than regulation by allosteric mechanism.

Classification and nomenclature of enzymes

Classes of enzymes:

- 1 Oxidoreductases
- 2 Transferases
- 3 Hydrolases
- 4 Lyases
- 5 Isomerases
- 6 Ligases
- 7 Translocases

The ending of enzyme names: **substrate + -ase**

e.g. lactate dehydrogenase, amylase, alcohol dehydrogenase, aspartate transaminase…

Oxidoreductases catalyze oxidation-reduction reactions where electrons are transferred. These electrons are usually in the form of hydride ions or hydrogen atoms. When a substrate is being oxidized it is the hydrogen donor. The most common name used is a **dehydrogenase** and sometimes reductase will be used. An oxidase is referred to when the oxygen atom is the acceptor.

Transferases catalyze group transfer reactions. The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor. Most of the time, the donor is a cofactor that is charged with the group about to be transferred. Example: **Hexokinase used in glycolysis.**

Hydrolases catalyze reactions that involve hydrolysis. This cases usually involves the transfer of functional groups to water. When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound. These enzymes could also be classified under transferases since hydrolysis can be viewed as a transfer of a functional group to water as an acceptor. However, as the acceptor's reaction with water was discovered very early, it's considered the main function of the enzyme which allows it to fall under this classification. **For example: Chymotrypsin.**

Lyases catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups. For example: **Fructose bisphosphate aldolase** used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

Isomerases catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced. These enzymes allow for structural or geometric changes within a compound. Sometime the interconversion is carried out by an intramolecular oxidoreduction. In this case, one molecule is both the hydrogen acceptor and donor, so there's no oxidized product. The lack of a oxidized product is the reason this enzyme falls under this classification. The subclasses are created under this category by the type of isomerism. For example: **phosphoglucose isomerase** for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate.

Ligases are used in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation reactions. These reactions are couple to the cleavage of ATP.

Translocase are enzymes that catalyze the movement of ions or molecules across membranes or their separation within membranes. It is a general term for a protein that assists in moving another molecule, usually across a cell membrane. The reaction is designated as a transfer from "side 1" to "side 2" because the designations "in" and "out", which had previously been used, can be ambiguous. Translocases are the most common secretion system in Gram positive bacteria.

The causes of increased activity, enzyme concentration in plasma

1. **Patological ↑ conc. of enzymes in plasma** – the result of the increased permeability of the cell membrane - damaged by chemicals, anoxia, hypoxia, viruses, inflammation. May lead to cell degradation.

Cell degradation→ Increased phospholipase activity, the degradation of cytoplasmic membrane phospholipids → perforation→ release of contents + enzymes into extracellular space → plasma

2. **Incerased synthesis E** – is not pathological, but is associated with a condition in organism :

e.g. bone growth ↑ osteoblasts activity↑ **alkaline phosphatase** in blood

In children ALP 3x-7x higher than in adults

3. **Drugs, alcohol ↑ enzyme activity** (liver) – **ALP, GMT etc.**

The causes of increased enzyme concentration in plasma

3. **Release from cells not associated with cell death or increased synthesis** –

E.g. ethanol releases expression of mitochondrial AST in hepatocytes, its transport to the surface hepatocytes → release into blood.

E.g. Food intake→ intestinal ALP →into lymph, ↑ in blood.

E.g. Liver enzymes are bound to the surface of hepatocytes ….

4. **Some cases of increased concentration** – inadequate removal from circulation.

E.g. Small enzymes– amylase, lipase – removed from circulation by glomerular filtration.

Renal impairment, **renal failure** ↑ their concentration in the blood.

Formation of complexes E-Ab, macroenzyme, half-life Ig-3 weaks

Time course of the increase and decrease of enzyme activity in plasma

Time course – influenced by many factors:

- **During apoptosis the cell membrane defects deepen with time.** Result – from cells are first released small molecules of enzymes and later large enzymes.

E.g. myocardial infarction (IM) – **at first in plasma AST and CK** (small molecules), **later LD** (bigger). In IM – concentration of CK in plasma depends on the size of the bearing affected by IM.

- When the **cause of cell damage disappear** enzyme **concentration persists for some time, then decreases**.

E.g. **acute hepatitis** can be distinguished from **toxic** liver damage. At virus hepatitis – imunological cell damage, longer persistance of increased enzyme concentration, toxic damage – faster return to normal levels of the enzyme (GGT,AST, ALT).

The activity of enzymes in plasma

- Enzyme concentration gradient between the cell and plasma– inside hepatocytes in cytoplasm = **higher concentration of AST** than ALT , LD minimum there.
- E.g. Hepatocyte damage- fastest-growing AST, but LD at least.
- E.g. Myocardium – hogh concentration of CK, low of LD, damage → hogh increase of CK in plasma

Enzyme concentration in plasma is determined by **the rate of its removal.**

- **low molecular weight -glomerul. filtration**
- **others (the majority) - inactivated in plasma, removed by cells of the RES via receptor, endocytosis**

Definition - the half-life of enzyme

The period during which the enzyme is increased in plasma is determined by its own half-life.

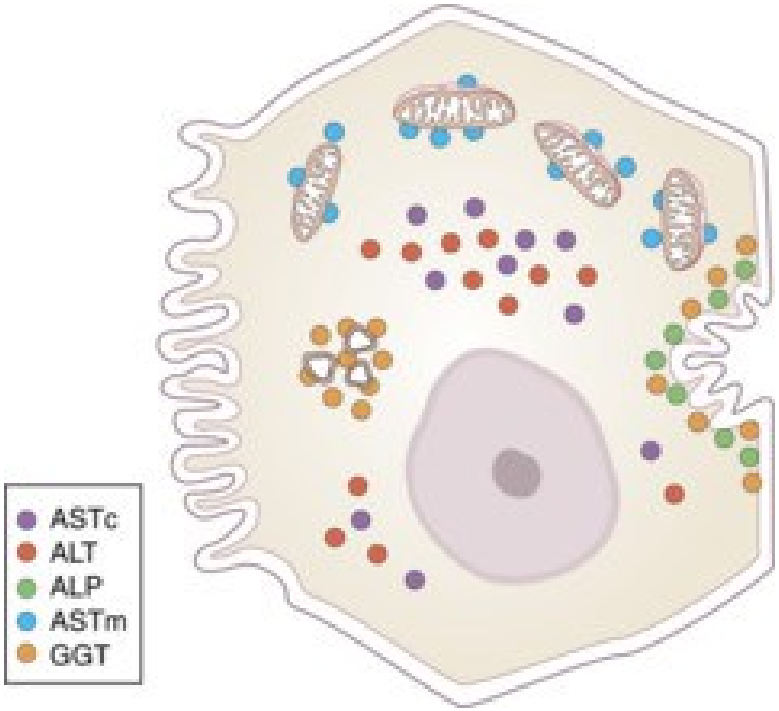
The biological half-life of enzyme – the period during which the amount of enzyme decreases half, unless added from tissues.

Enzyme	Half-life
ALP	3-7 days
AMS	9-18 hours
ALT	2 days
AST	12-14 hours
CHS	10 days
CK	10 hours
GMT	3-4 days
LDH1(HHHH)	4-5 days
LDH5(MMMM)	10 hours

The use of enzymes in clinical diagnosis

- Detection of tissue damage
- Identification of the beginning of tissue damage
- Determining the extent of damage
- Estimation of the severity of cell damage
- Diagnosis of basic disease
- Specification diagnosis within the damaged organ

- The main diagnostic hepatocellular enzymes are localized in different areas of hepatocyte. ALT and cytoplazmatic isoenzyme AST are placed in cytoplasm.



http://o.quizlet.com/i/eHnZCzz2IZU1GLwWW5FTwg_m.jpg

- When is the membrane damaged (e.g. viral or chemical), these enzymes are released and entered the sinusoid. The result is an increase in plasma.
- Mitochondrial AST is primarily released during mitochondrial damage, egg. when exposed to alcohol.
- ALP and GGT are located on the canalicular surface of hepatocytes and released in cholestasis, particularly due to the effect of bile acids on the membrane.
- GGT is also located in the microsomes, where is induced by certain drugs. The administration of these drugs increases the plasma levels of GGT

Specification of diagnosis

- Information suitable for precise diagnosis is obtained :
- - from values of the **catalytic enzyme concentration** in a body fluid (a direct correlation between the **degree of organ damage** and **increased activity of enzymes** in blood).
- - **spectrum of enzymes** present in the blood (e.g. in severe liver damage accompanied by cell necrosis is increased activity of enzymes in the blood subsequently : LD> AST> ALT).
- - calculating the ratio of the enzyme activities (e.g. according to the ratio of AST / ALT in serum can be distinguished initial obstructive jaundice (AST / ALT <1) from chronic active hepatitis (AST / ALT> 1). For acute disease can be from the ratio of enzyme activities with short and long biological half-life to determine the stage of disease or predict the course of the disease, e.g. in acute hepatitis decrease in

  the ratio of AST (half-life 12 h) / ALT (half-life of 2 days) helps to qualify the type of hepatitis.
- - monitoring the enzyme activity (mechanism of enzyme release into the blood from damaged tissues and their clearance is characteristic curves of typical kinetic activity of which can be derived during the period of time during which the disease may be present or to determine the stage of disease)
- - determination of isoenzymes.

Macroenzymes

- Macroenzymes are complexes formed by enzyme linked immunoglobulin, a lipoprotein, protein, or cell membrane fragments.
- Generally **macroenzymes** have higher molecular weight and longer half-life in blood. Macroenzyme´s presence in serum may affect its analytical determination or cause misinterpretations of results.
- **Macroamylasemia** is a well-known example when the amylase forms a complex with other macromolecules and therefore is not filterable into urine. As a result, accumulates in the blood. The level of amylase in the blood is then increased without causing its increased release from damaged tissue (about 1-3% of patients with elevated serum amylase).
- Because **macroamylase** does not penetrate into the urine, urine amylase level is normal or reduced in this case. Unlike the situation during e.g. pancreatitis when elevated levels of serum amylase is accompanied by an increase of amylase in urine.

Clinically important enzymes

Aminotransferases

- provide the conversion of amino acids and keto acids a-amino transfer, the donor and acceptor amino group is a 2-oxoglutarate / L-glutamate
- **Alanine aminotransferase, ALT** - donor -NH2 Ala to form pyruvate, marker- liver (viral hepatitis, alcohol, hepatopathy, ...)
- **Aspartate aminotransferase, AST** - donor – NH_2 Asp to form oxaloacetate, a marker of damage - liver, heart, myocardial infarction, muscular damage

Clinically important enzymes

α-amylase, AMS

- synthesized in the pancreas, cleaves α-1,4-glycosidic linkage in starch and glycogen to produce maltose and maltotriose, endoglycosidase, marker - acute pancreatitis

- alkaline phosphatase, ALP

- hydrolysis of monoesters of phosphoric acid with alcohols, phenols, Glycine - nonspecific - cleaves POC, POP, PS, PN, has several isoforms according to the synthesis of tissue - bone, liver, placenta, intestinal, optimum in the alkaline environment, marker - bone damage, liver

- acid phosphatase, ACP

- properties ALP, optimum in the acidic region, marker-prostate

Other clinically important enzymes

creatine kinase , CK

- catalyzes the reversible phosphorylation of creatine to phosphocreatine for ATP consumption, a marker of muscle damage, heart

lactate dehydrogenase, LDH

- isoforms (tissue) of the heart, liver, catalyzes the reaction:

  Lactate + NAD + ↔ pyruvate + NADH + H + (reversible) is non-specific tissue damage according isoforms - electrophoretically (LDH3 pulmonary embolism, myocardial infarction LDH1,2, hepatopathy + disease koster.svalstva LD4,5).

Clinically significant enzymes

TEST

- **Causes of increased activity in serum**
- **AST** *aspartate aminotransferase* myocardial infarction; hepatopathia; blood diseases; muscle damage
- **ALT** *alanine aminotransferase* hepatic dysfunction, heart disease, AST / ALT ratio> 1 alcoholic liver disease, myocardial infarction, AST / ALT <1 viral hepatitis
- **LD** *lactate dehydrogenase* LD1,2 - myocardial infarction, hemolytic anemia; LD3 - pulmonary embolism; LD4,5 - hepatopathy diseases of skeletal muscle
- HBD *hydroxybutyrátdehydrogenase* activity subunit H (LD1,2), myocardial infarction
- **GGT** *gamma-glutamyltransferase* hepatopathia (inflammation, alcohol, drugs); test of chronic alcohol consumption; cholestasis
- **ALP** *isoenzyme of alkaline liver phosphatase* - diseases of the biliary tract, bone isoenzyme - bone disease (Paget's disease, rachitis, tumors), *physiologically increased during growth*
- **ACP** *acid phosphatase prostatic isoenzyme* - prostate tumors, bone isoenzyme - tumor metastasis to bone, osteoporosis marker
- **CK** *creatine kinase*, CK-MB - particularly myocardial infarction, but also in the regeneration of skeletal muscles, chronic muscular diseases and acute renal failure
- CK-MM - diseases of skeletal muscle, intramuscular injections, physical activity
- **AMS** *amylase* (Mr ~ 50,000) pancreatic isoenzyme - acute pancreatitis, salivary isoenzyme – parotiditis
- **LPS** lipase of *acute pancreatitis*, acute reversal of chronic pancreatitis
- **PSA** *prostate specific antigen* in prostate cancer
- **Causes of decreased activity in serum**
- **CHE** *cholinesterase* chronic hepatopathy, alcoholic-toxic hepatitis (organophosphate intoxication); indicator of hepatic protein synthesis

Tissue distribution of diagnostically important enzymes

- Tissue damage can be diagnostically prooved indirectly either by determining the activity of tissue-specific enzymes or by isoenzymes in the blood.
- Tissue-specific enzymes are found preferentially in a particular tissue or have high activity in that tissue. Exemples of tissue-specific enzymes are listed in the following table.
- Expressionof isoenzymes is mostly determined genetically for each tissue . Therefore, determination of isoenzymes in the blood enables to identify damaged tissue which they come from (e.g. pancreatic lipase, CK-MB, LD1).

Organ	AST	ALT	LD	LD_1	CK	GGT	ALP	ACP	AMS	LPS	CHS
Liver	x	xx	x			xxx	x				xxx
Myocardium	x	x	x	xx	xx						
Muscle	x	x	x		xx						
Bile duct							xx				
Kidneys	x		x	x		x	x				
Bones							xx	x			
Erythrocytes	x		x	x				xx			
Prostate								xxx			
Pancreas	x					x			xx	xxx	
Parotid gland									xx		