Use of enzymes as diagnostic markers

Evaluation of lactate dehydrogenase (LDH) isoenzymes by agarose gel electrophoresis
Lactate dehydrogenase LDH

- tetramer
  - M (gene LDHA, ch.11)
  - H (gene LDHB, ch.12)
- $\text{LDH}_1$ (HHHH) 31-49%
  - heart, liver, erythrocytes
- $\text{LDH}_2$ (HHHM) 38-58%
  - reticuloendothelial system
- $\text{LDH}_3$ (HHMM) 5.5-16.5%
  - lungs
- $\text{LDH}_4$ (HMMM) 0-0.7%
  - kidney
- $\text{LDH}_5$ (MMMM) 0-1.5%
  - skeletal muscle, liver
Lactate dehydrogenase

- LDH 1 and LDH 2 – converts lactate into pyruvate in tissues with aerobic metabolism
- LDH 4 and LDH 5 – converts pyruvate into lactate in tissues with anaerobic glycolysis

![Chemical reaction](image)
Electrophoretic separation of LDH isoenzymes

- agarose gel, TBE buffer
- staining solution
  - lithium lactate
  - NAD$^+$
  - stain nitroblue tetrazolium
  - phenazine methosulphate – carrier of electrons between NADH and the dye
- 5 % acetic acid
Isoenzymes detection

- lactate + NAD$^+$ → pyruvate + NADH + H$^+$
- NADH + H$^+$ + NBT → NAD$^+$ + formazan
Enzymes

- proteins catalyzing chemical reactions (not consumed)
- holoenzyme = apoenzyme + cofactor
- many enzymes rely on cofactor - small molecules required for the catalytic activity of enzymes
  - coenzyme - small organic molecules
  - prosthetic group - tightly bound cofactor

- enzymes decrease the required activation energy
- thus, in the presence of enzymes, reactions proceed at a faster rate
- many enzyme-catalyzed reactions are reversible
Enzyme-catalyzed reaction
Active site of the enzyme

- active site = small portion of enzyme molecule which actually binds the substrate
- active site is the result of precise folding of the polypeptide chain - AA that may have been far apart in the linear sequence can come together to cooperate in the enzyme reaction
Enzyme specificity

- Each enzyme has a unique 3-D shape and recognizes and binds only the specific substrate of a reaction.
- Often – reaction with only one substrate.
- Sometimes – reaction with a group of similar substrates.
- Eg. Aspartase.

\[ \text{Aspartic Acid} \xrightarrow{\text{Aspartase}} \text{Fumaric Acid} \]
Models of enzyme action

- **lock and key model**
  - active site of the enzyme fits the substrate precisely

- **induced fit model**
  - binding of the substrate induces a change in enzyme conformation so that the two fit together better

- enzymes may be found that operate by both mechanisms
Classification of enzymes

1. oxidoreductases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases

- nowadays enzyme names end in „-ase“
- some enzymes were named before this convention was introduced and so have irregular names

LDH  EC 1.1.1.27

Enzymes

Class  | EC 3.-.-. Hydrolases.
Subclass | EC 3.5.-.- Acting on carbon-nitrogen bonds, other than peptide bonds.
Sub-subclass | EC 3.5.2.- In cyclic amides.
Serial number  | EC 3.5.2.6 Beta-lactamase.
Inhibition

Substrate

Enzyme

Competitive inhibitor

Enzyme

Substrate

Uncompetitive inhibitor

Enzyme

Noncompetitive inhibitor

Enzyme

Substrate
Irreversible inhibition

- inhibitor covalently modifies the enzyme (active site)
- eg. the „nerve gas“ sarin interacts with serine residues in the active site of proteins
  - acetylcholine esterase
Factors affecting enzyme kinetics

- enzyme and substrate concentration

More substrate the rate increases.
Factors affecting enzyme kinetics

- **temperature**
  - enzymes have specific temperature ranges. Most denature at high temperatures

- **pH**
  - each enzyme works best at a certain pH
  - altering the pH will denature the enzyme
  - this means that the structure of the enzyme is altered and the "shape" no longer works with its specific substrate
Units of enzyme activity

- enzyme unit (U)
  - the amount of the enzyme that catalyzes the conversion of 1 micro mole of substrate per minute

- katal (International system of units SI)
  - the amount of enzyme that converts 1 mole of substrate per second
Enzyme regulation

- control at the genetic level
- control of activity of an existing protein
  - more rapid cellular response
- the rate depends on
  - level of available substrate
  - how much enzyme protein is present
- negative feedback
Control of enzymes by chemical modification

- shape change is caused by modifying the protein chemically
- chemical group is added and removed later
  - phosphate group, acetyl, methyl, adenyl
Example of control by phosphorylation

- glycogen synthase
  - inactive when phosphorylated
- glycogen phosphorylase
  - active when phosphorylated
Enzyme localization

- extracellular
- intracellular
  - membrane bound
  - cytosolic
  - in organelles
## Classification of Enzymes in Blood

<table>
<thead>
<tr>
<th>Classification</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma-Specific enzymes</strong></td>
<td>Serine protease procoagulants: thrombin, factor XII (Hageman factor), factor X (Stuart-Prower factor), and others</td>
</tr>
<tr>
<td></td>
<td>Fibrinolytic enzymes or precursors: plasminogen, plasminogen proactivator</td>
</tr>
<tr>
<td><strong>Secreted enzymes</strong></td>
<td>Lipase (from salivary glands, gastric oxyntic glands, and pancreas), ( \alpha )-amylase (from salivary glands and pancreas), trypsinogen, cholinesterase, prostatic acid phosphatase, prostate-specific antigen</td>
</tr>
<tr>
<td><strong>Cellular enzymes</strong></td>
<td>Lactate dehydrogenase, aminotransferases, alkaline phosphatase, and others</td>
</tr>
</tbody>
</table>
Factors affecting plasmatic enzyme level

- enzyme activity in the cell
- localization of enzyme in the cell
- cytoplasmic membrane permeability
- the extent of cell damage
- the amount of affected cells
- the rate of enzyme elimination
Průběh aktivit AST a ALT při virové hepatitidě
(Průběh aktivit AST a ALT při infarktu myokardu)
Different forms of enzymes

- proenzymes (zymogens)
- isoenzymes
  - primary
  - secondary
Diagram of the origin of isoenzymes, assuming the existence of two distinct gene loci

- Structural genes
- mRNA
- Polypeptides
- Subunits
- Possible dimers
- Possible tetramers

Diagram:

- a: Structural genes → mRNA → Polypeptides → A
- b: Structural genes → mRNA → Polypeptides → B

Possible dimers and tetramers are shown with different connections.
Evaluation of isoenzymes

- physical-chemical
  - electrophoresis
  - chromatography
- imunochemical
- chemical
  - determination of reaction speed in different conditions – pH, temperature, substrate concentration
Biochemical examination of liver function

- indicators of hepatocyte damage
  - ALT, AST, LDH
- indicator of bile ducts obstruction
  - ALP, GMT
- indicators of synthetic liver function
  - albumin, CHE, LCAT, PT
- tests of conjugation and liver transport of organic anions
  - bilirubin, urobilinogen
Hepatocyte damage

- **Alanin aminotransferase (ALT)**
  - L-alanin+2-oxoglutarate $\leftrightarrow$ pyruvate+L-glutamate
  - reaction is reversible, it proceeds in the synthesis, degradation and transformation of aminoacids
- cytoplasmatic enzyme
- the most abundant in hepatocytes, plasmatic level elevated as early as in the disorder of membrane permeability

- **Aspartate aminotransferase (AST)**
  - L-aspartate+2-oxoglutarate $\leftrightarrow$ oxalacetate+L-glutamate
  - reaction is reversible, it proceeds in the synthesis, degradation and transformation of aminoacids
- cytoplasmic and mitochondrial isoenzymes
- occurs in liver, myocard, skeletal muscle, kidney and pancreas
- plasmatic level of cytoplasmic isoenzyme elevated as early as in the disorder of membrane permeability, releasing of mitochondrial isoenzyme accompanies hepatocellular necrosis
Interpretation of ALT/AST elevation

- increased activity of both ALT and AST in many liver diseases
  - extremely high values (10-100x) in toxic and acute viral hepatitis and shock conditions
- plasmatic aminotransferase activity does not tell us anything about excretoric or metabolic function of hepatocytes
- correlation between level of amino transferases and the extent of liver lesions is not the rule
- De Rittis index = AST/ALT
  - less than 0.7...good prognosis
  - 1 and more...bad prognosis (necrosis)
- physiologically and in majority of liver diseases ALT > AST
- exception - AST/ALT > 2
  - alcoholic damage
  - postnecrotic cirrhosis
Bile ducts obstruction

- **Alcaline phosphatese (ALP)**
  - membrane bound enzyme catalyzes hydrolysis of phosphate esters at alkalic pH
    - tetramer, into the circulation released as dimer
  - widespread - occurs primarily in liver, gut and bones (different isoenzymes)
  - plasmatic ALP level – diagnosis of bone and hepatobiliar disorders
  - considerable part of liver ALP is localized membranes of cells covering bile ducts
    - membranes are disturbed in cholestasis and ALP is released
  - elevated also in other conditions (liver tumors, cirrhosis)

- **γ-glutamyl transferase (GMT)**
  - membrane bound enzyme found in liver, kidney, pancreas, gut and prostate
  - catalyzes transfer of γ-glutamyl from glutathione on aminoacid and enables the aminoacid transport through membrane
  - serum GMT activity determination is used for evaluation of hepatobiliar diseases
Synthetic liver function

- **albumin**
  - synthesized in liver, plasmatic level determination
  - long half-life – does not fall in acute disorders
  - exclusion of another causes of decline (malabsorption, reduced intake of proteins, kidney disease) → liver disease
  - significant decline in alcoholic cirrhosis

- **cholinesterase**
  - enzyme generated in hepatocytes and released into blood (secretory enzyme)
  - catalyzes hydrolysis of cholin esters in plasma
  - enzyme production (thereby plasmatic activity) is decreased when liver parenchyme is damaged or in malnutrition
  - irreversibly inhibited by organophosphates
Synthetic liver function

- **coagulation factors**
  - produced in liver, short half-life – quick changes
  - Quick test – extrinsic coagulation system
  - values are changed in disorders of liver parenchyma accompanied by proteosynthesis failure or in obstructive icterus with disorder of lipid and lipid soluble vitamins uptake
Cardiac markers

- the ideal cardiac marker
  - high sensitivity
    - high concentration in myocardium
    - rapid release for early diagnosis
    - long half-life in blood for late diagnosis
  - high specificity
    - absent in non-myocardial tissue
  - analytical characteristics
    - measurable by cost-effective and simple method
  - clinical characteristics
    - ability to influence therapy and to improve patients outcome

- the ideal cardiac marker does not yet exist
Cardiac markers

- **Creatine kinase (CK)**
  - cytoplasmic and mitochondrial enzyme
  - catalyzes reversible transfer of phosphate from ATP onto creatine
  - ATP + creatine $\rightarrow$ ADP + creatine phosphate
  - dimeric – M (muscle) and B (brain)
  - 3 isoform
    - CK-BB – smooth muscle, brain, prostate
    - CK-MB – myocardium (also in skeletal muscle)
    - CK-MM – skeletal muscle, myocardium
  - CK-MB – diagnosis of acute myocardial infarction and monitoring of reperfusion in the course of trombolytic treatment of AMI

- **Myoglobin**
  - intracellular protein found in cardiac and skeletal muscle cells concerned in aerobic metabolism
  - released quickly from damaged cells into circulation (small size, 0.5 – 2 hours)
  - the smallest cardiac marker – quick propagation and degradation
  - non-specific marker (present also in skeletal muscle)
Cardiac markers

- **troponins**
  - troponin complex – part of the structural proteins, which participates on muscle contraction
    - heterotrimer consisting of troponins I, T and C
  - tightly connected with contractile apparatus – low levels of cardiac troponins in the circulation
    - TnI level is undetectable if the heart is not injured (even in the presence of skeletal muscle damage)
- cardiac isoform troponin I (TnI) differs from skeletal muscle isoform - specific determination
Troponin I determination

- **arguments for**
  - absolute cardiospecificity
  - long period of liberation – monitoring of course
  - sensitivity – detection of smaller injury
  - not affected by chronic renal insufficiency

- **arguments against**
  - slower onset than myoglobin (nonspecific)

<table>
<thead>
<tr>
<th></th>
<th>Myoglobin</th>
<th>TnI</th>
<th>CK-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>increased after</td>
<td>0.5 - 2 h</td>
<td>3 - 6 h</td>
<td>3 – 8h</td>
</tr>
<tr>
<td>peaks between</td>
<td>5 - 12 h</td>
<td>14 - 20 h</td>
<td>9-30 h</td>
</tr>
<tr>
<td>remains elevated</td>
<td>18 – 30 h</td>
<td>5 - 7 days</td>
<td>48-72 h</td>
</tr>
</tbody>
</table>
# Cardiac markers

<table>
<thead>
<tr>
<th>enzyme</th>
<th>beginning of rise</th>
<th>maximum</th>
<th>normalization</th>
<th>fold in maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>4-8 hours</td>
<td>16-48</td>
<td>3-6 days</td>
<td>up to 25</td>
</tr>
<tr>
<td>CK</td>
<td>3-6 h</td>
<td>16-36</td>
<td>3-5 days</td>
<td>up to 25</td>
</tr>
<tr>
<td>LD</td>
<td>6-12 h</td>
<td>24-60</td>
<td>7-15 days</td>
<td>up to 8</td>
</tr>
<tr>
<td>myoglobin</td>
<td>0,5-2 h</td>
<td>6-12</td>
<td>0,5-1 days</td>
<td>up to 20</td>
</tr>
<tr>
<td>troponin I</td>
<td>3,5-10 h</td>
<td>12-18</td>
<td>7-20 days</td>
<td>Up to 300</td>
</tr>
</tbody>
</table>
Figure 1. Release of cardiac biomarkers into blood following AMI. Time zero is defined as the moment of onset of symptoms. Marker concentrations are expressed in a common scale—as multiples of the upper reference limit for that marker.

\[\text{cTnT} = \text{cardiac troponin T}\]

\[\text{cTnI} = \text{cardiac troponin I}\]