# Biochemistry II - Seminars

Doc. RNDr. Jiří Dostál, CSc.

Department of Biochemistry, Fac. Med., MU Brno

**jrdostal@med.muni.cz**

# Literature

- J. Tomandl, E. Táborská:

  Biochemistry – Seminars II, MU Brno, 1996

  **selected chapters,  see the syllabus**

# Enzymes in Clinical Biochemistry

Seminar No. 1

- Chapter 4 -

# You are supposed to know …

- Enzymes – main features, properties
- Coenzymes – structures, functions
- Enzyme kinetics
- Enzyme activity

# Isoenzymes – General Features

- Genetically determined differences in primary structure
- Catalyze the same reaction
- May have **different subcellular distribution**
  (cytoplasm × mitochondria)
- May have **different tissue distribution**
- May be combined from more subunits (quarternary structure)
- May differ in kinetic properties ($K_M$)
- Usually are determined by electrophoresis

# Q. 2 (p. 27)

Explain the terms: proenzyme, isoenzyme, isoform

# A. 2

- **Proenzyme** (zymogen) – inactive form of enzyme that becomes active after partial proteolysis

  example: pepsinogen → pepsin
- **Isoenzyme** – see previous page
- **Isoform** – more general term, includes true isoenzymes and pseudoisoenzymes (posttranslational variations)

# Lactate dehydrogenase (LD)

- **Tetramer**
- Two different chains (H - heart, M - muscle)
- Five isoenzymes:

  **$LD_1$** ($H_4$), **$LD_2$** ($H_3M$), **$LD_3$** ($H_2M_2$), **$LD_4$** ($HM_3$), **$LD_5$** ($M_4$)
- Widely distributed in body
- Total activity determination – nonspecific finding
- $LD_1 + LD_2$ ….. marker of myocardial infarction (MI)
- Today is LD assay considered out-of-date

# Creatine kinase (CK)

- **Dimer**
- Two different chains (M – muscle, B – brain)
- Three isoenzymes: **MM** (muscle), **MB** (heart), **BB** (brain)
- Major isoenzyme in blood is MM (95 %)
- MB form in blood: 0 – 6 %
- BB in blood: traces (BB cannot pass across blood-brain barrier)
- MB isoenzyme …. excellent marker of myocardial infarction

# Enzymes in Blood

| Feature | Plasmatic enzymes | Secretory enzymes | Intracellular enzymes |
|---|---|---|---|
| Example | coag. factors | amylase, lipase | AST, … |
| Source organ | liver | pancreas | various |
| Function in | blood | GIT | cells |
| Enzyme activity in blood after source organ damage | **?** | **?** | **?** |

# Enzymes in Blood

| Feature | Plasmatic enzymes | Secretory enzymes | Intracellular enzymes |
|---|---|---|---|
| Example | coag. factors | amylase, lipase | AST, … |
| Source organ | liver | pancreas | various |
| Function in | blood | GIT | cells |
| Enzyme activity in blood after source organ damage | ↓ | ↑ | ↑ |

# Q. 6

Why are low activities of cellular enzymes detected even in serum of healthy people?

# A. 6

Low activities of **intracelular** enzymes

in **extracelular** fluid (blood plasma, serum)

are the consequence

of physiological cell disintegration.

# Main Tissue Distribution of Enzymes

| | |
|---|---|
| **AST** | liver, myocard |
| **ALT** | **liver** |
| **LD** | not specific |
| **CK** | **myocard, muscles** |
| **GMT** | **liver** |
| **ALP** | biliary tract, bones |
| **ACP** | **prostate** |
| **AMS** | **pancreas** |
| **LPS** | **pancreas** |
| **CHS** | **liver** |

# Intracellular Location of Enzymes

| Intracellular Location | Enzymes |
|---|---|
| Cytoplasm | LD, **ALT**, **30 % AST** |
| Mitochondria | **70 % AST** |
| Golgi complex, ER | CHS, AMS |
| Lysosome | ACP |
| Membrane | **GMT, ALP** |

# Consider the AST/ALT ratio

- AST/ALT > 1 ……. severe liver damage

- AST/ALT < 1 ……. mild liver damage

# Enzymes of Clinical Significance

| Enzyme | Source of blood elevation |
|---|---|
| ALT | hepatopathy |
| AST | MI, hepatopathy |
| GMT | hepatopathy (alcohol, drugs) |
| ALP | biliary tract diseases, bone diseases |
| ACP | prostatic cancer |
| CK | MI (CK-MB), muscle diseases |
| AMS | pancreatitis |
| LPS | pancreatitis |
| CHS | hepatopathy (alcohol, drugs) – decreased |

# Catalytic concentration of some enzymes

| Enzyme | Reference values (serum) |
|---|---|
| ALT | 0.1 - 0.9 μkat/l |
| AST | 0.1 - 0.7 μkat/l |
| LD | up to 7.5 μkat/l |
| CK | up to 4 μkat/l |

see also the lab manual

# Q. 7

What enzymes might appear in blood

a) In mild hepatocellular damage

b) In serious hepatocellular damage

# A. 7

a) Mild hepatocellular damage:

enzymes from cytoplasm and/or membrane are released into ECF – ALT, GMT, ALP

b) Severe hepatocellular damage:

enzymes from mitochondria are released into ECF – AST

# Q. 8

Write equations of reactions catalyzed by:

ALT

AST

LD

# ALT Reaction

alanine + 2-oxoglutarate ⇆

plné názvy enzymů !!

# ALT Reaction

alanine + 2-oxoglutarate ⇆ pyruvate + glutamate

# AST reaction

aspartate + 2-oxoglutarate ⇆

# AST reaction

aspartate + 2-oxoglutarate ⇆ oxaloacetate + glutamate

# LD reaction

lactate + $NAD^+$ ⇆

# LD reaction

$$\text{lactate} + NAD^+ \leftrightarrows \text{pyruvate} + NADH + H^+$$

# Q. 9

The levels of most blood enzymes are increased in newborns and infants. What enzyme persists elevated till puberty?

# A. 9

ALP – the bone isoenzyme activity persists till puberty

# Biochemical Diagnostic of MI

| Enzyme / Protein | Half-time (hrs) |
|---|---|
| Myoglobin | 0,25 |
| Troponine T cardial form | 2 |
| CK-MB | 13 |
| AST | 17 |
| $LD_{12}$ | 110 |