

Biochemistry II - Seminars

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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:
LD₁ (H₄), LD₂ (H₃M), LD₃ (H₂M₂), LD₄ (HM₃), LD₅ (M₄)
- Widely distributed in body
- Total activity determination – nonspecific finding
- LD₁ + LD₂ 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- Today is LD assay considered out-of-date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 | ? | ? | ? |

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Q. 6

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

A. 6

Low activities of intracellular enzymes
in extracellular 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 $\mu\text{kat/l}$ |
| AST | 0.1 - 0.7 $\mu\text{kat/l}$ |
| LD | up to 7.5 $\mu\text{kat/l}$ |
| CK | up to 4 $\mu\text{kat/l}$ |

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

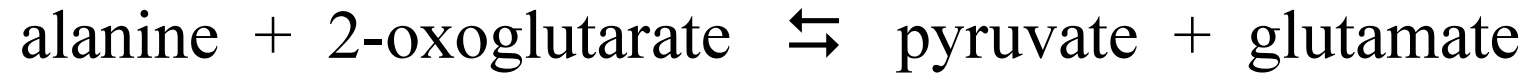
LD

ALT Reaction

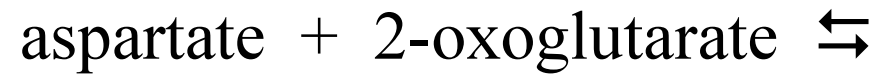
alanine + 2-oxoglutarate \rightleftharpoons

plné názvy enzymů !!

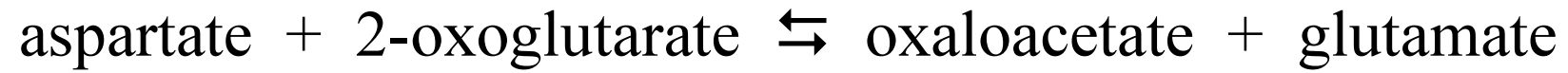
ALT Reaction



AST reaction



AST reaction



LD reaction



LD reaction



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 cardiac form | 2 |
| CK-MB | 13 |
| AST | 17 |
| LD ₁₂ | 110 |

