Enzymes III

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Overview

- Isozymes - **a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies.**

- Application of isozymes in diagnosis

Isozymes (Isoenzymes)

- **Physically distinct forms of the same enzyme**
  - Multiple molecular forms of an enzyme are described as isozymes or iso-enzymes; enzymes that catalyze the same reaction
  - Different molecular forms of the same enzyme synthesized from various tissues
  - Useful to understand diseases

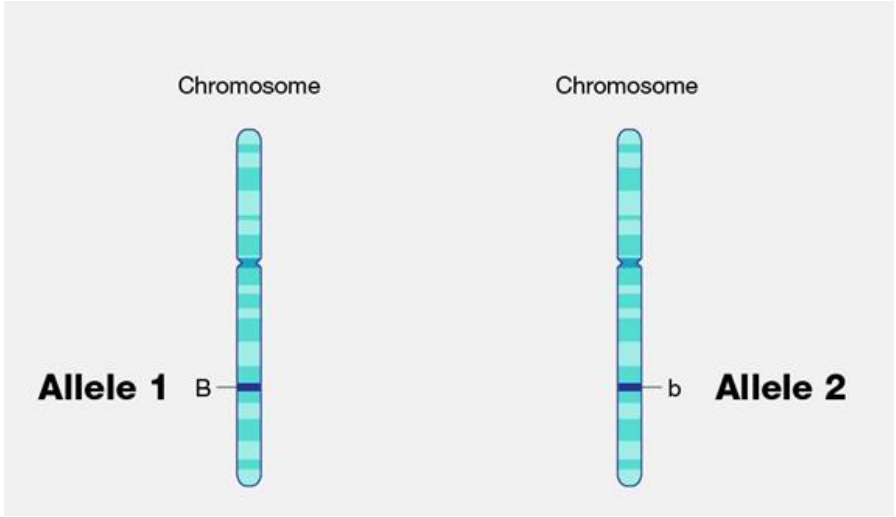
- **Homomultimer** protein: subunits are all the same, represented by a single gene

- **Heteromultimer** protein: subunits are different, produced by different genes

Isozyme formation

- Products of different genes: true isozymes

- The same locus of the gene may have different alleles→ allelic isozymes are called **allozymes** (only one form will be present in the individual)
    - e.g. more than 400 distinct forms of **glucose 6-P dehydrogenase** in population
    - Polymorphism: >1% frequency of variation at a single locus



Isozyme formation

- Molecular heterogeneity of enzymes may also be produced after the protein is synthesized (post-translational modification): **iso-forms**

- In some cases, all the different forms of an enzyme are present in the same individual
    - e.g. **LDH** has 5 iso-enzymes

Identification of isozymes

1. In Agar gel or polyacrylamide gel **electrophoresis**: isozymes have different mobility

   LDH, **CK** and **ALP isozymes** can be separated by electrophoresis

2. **Heat stability**: one of the isozymes may be easily **denatured** by heat

   Bone isozyme of **ALP** (BALP)

3. **Inhibitors**: one of the isozymes may be sensitive to one inhibitor

   Tartrate labile ACP

4. **Km** value or **substrate specificity** may be different for isozymes

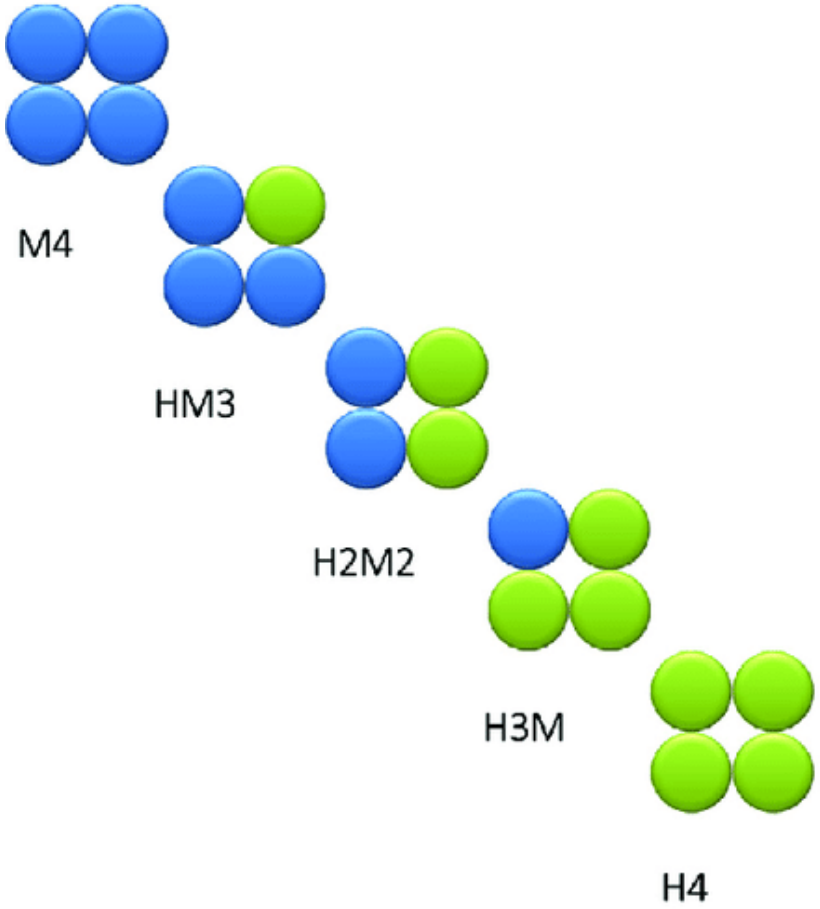
   Glucokinase has high Km and hexokinase has low Km for glucose

Identification of isozymes

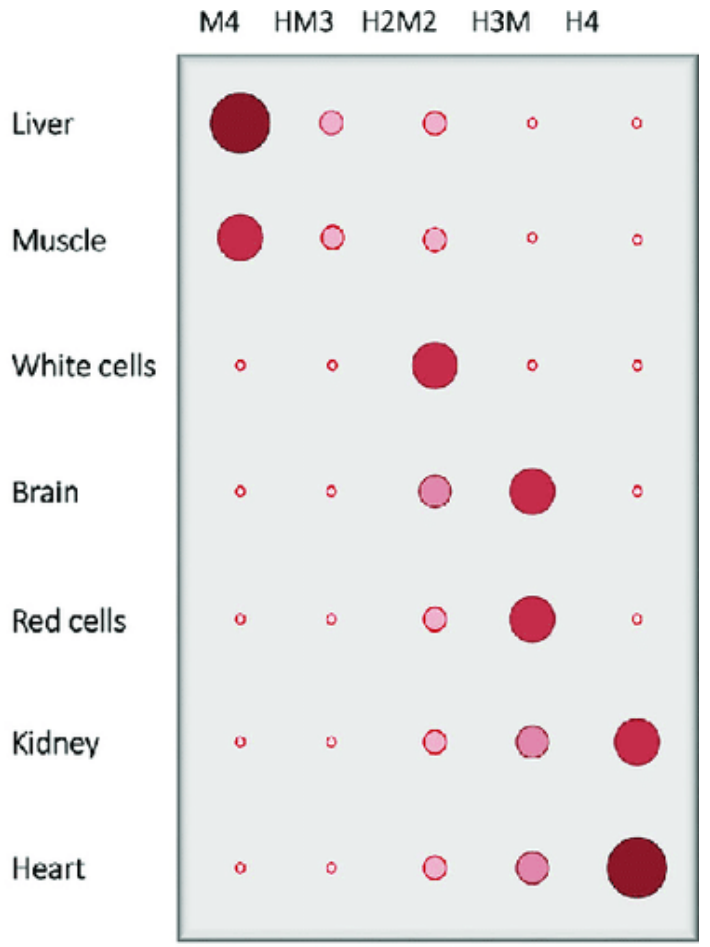
5. **Cofactor** requirements may be different for isozymes
   - Mitochondrial isocitrate dehydrogenase is NAD^+ dependent
   - Cytoplasmic isozyme is $NADP^+$ dependent

6. Tissue **localization** may be different for isozymes
   - H4 form of LDH is present in heart
   - M4 variety is seen in skeletal muscle

7. Specific antibodies may identify different types of isozymes
   - CK iso-enzymes are separated by antibodies



LDH isomere subunit composition



Prevalence of the five isotype in different organs and tissues

Applications of isozymes in diagnosis

Clinical Enzymology

- Plasma contains many functional enzymes, which are **actively secreted** into plasma.

- There are a few **nonfunctional enzymes in plasma**, which are coming out from cells of various tissues due to normal wear and tear
    - Their normal levels in blood are very low; but are **drastically increased** during necrosis or disease

- Assays of these enzymes are very useful in diagnosis of diseases

Enzymes as (cardiac) Biomarkers

- A **biomarker** is a clinical laboratory test which is useful in detecting dysfunction of an organ

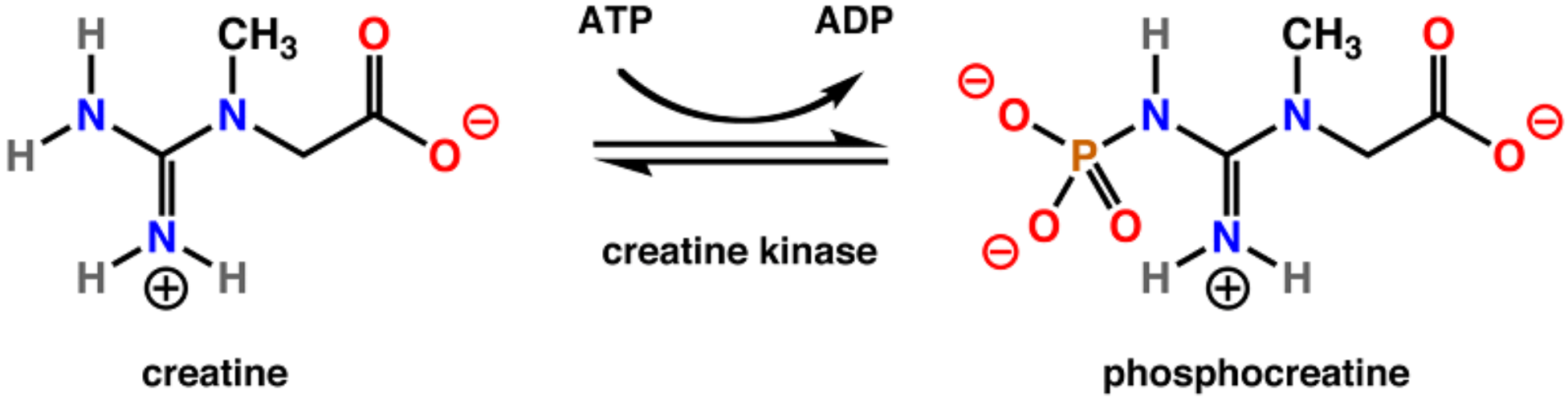
Different markers are used to:

1. **Detect** myocardial ischemia at the earliest
   - ❖Commonly used biomarkers for **early detection** of acute myocardial infarction are:
     - Cardiac troponins
     - Creatine kinase, CK-MB
     - Myoglobin

2. **Monitoring** the progression of the condition
3. **Predict** the risk in cardiac dysfunction

CREATINE KINASE (CK)

It catalyzes the creatinine kinase reaction



CK and Heart Attack

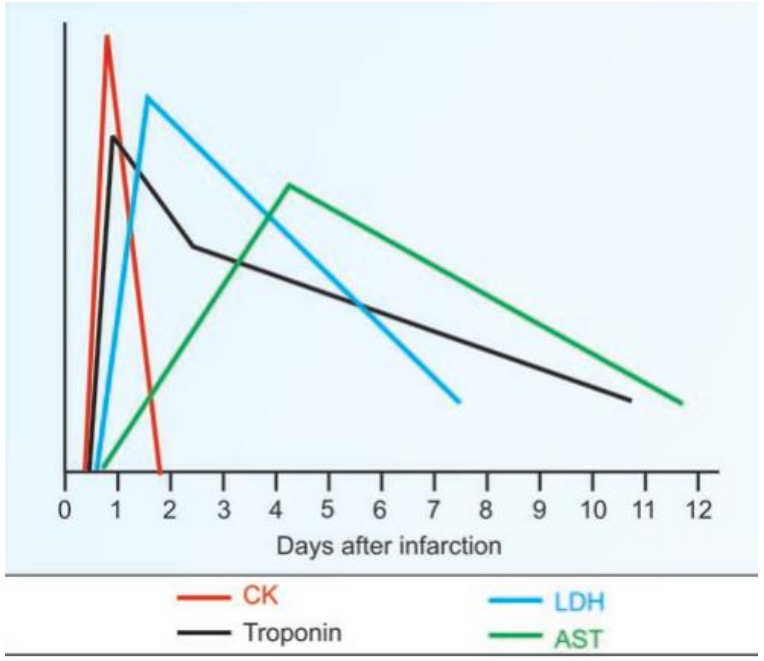
- CK value in serum is **increased** in **myocardial infarction**
  - The CK level starts to rise **within 3-6 hours** of infarction
- CK estimation is very useful to **detect early cases**, where ECG changes may be ambiguou
  - A second peak may indicate another ischemic episode

Markers of myocardial infarction

Marker	Onset	Peak	Duration
CK-MB	3-6 hr	18-24 hr	36-72 hr
Troponins	4-10 hr	18-24 hr	8-14 days
LDH	6-12 hr	24-48 hr	6-8 days
AST	24-36 hr	4-5 d	10-12 days
Myoglobin	1-4 hr	6-7 hr	24 hr

CK and Heart Attack

- The CK level is not increased in **hemolysis** or in **congestive cardiac failure**
  - CK has an advantage over LDH
  - The area under the peak and slope of initial rise are proportional to the size of infarct



Time course of CK, LDH, Troponin and AST in blood of MI patients

Iso-enzymes of CK

- CK is a **dimer**; each subunit has a molecular weight of 40 kD
- The **3 isozymes of CK**:
  - BB (brain)
  - MM (muscle)
  - MB (heart)
- Normally CK2 (heart isozyme) is only 5% of the total activity.
- Even doubling of the value of CK2 (MB) isozyme may not be detected, if total value of CK alone is estimated
- **MB isoenzyme** estimation is the best diagnostic marker in MI

CK isozyme characteristics

Iso-enzyme	Electrophoretic mobility	Tissue of origin	Mean percentage in blood
MM (CK3)	Least	Skeletal muscle	80%
MB (CK2)	Intermediate	Heart	5%
BB (CK1)	Maximum	Brain	1%

Estimation of **total CK** is employed in **muscular dystrophies** and MB isozyme is estimated in MI

CARDIAC TROPONINS (CTI/CTT)

- They are not enzymes
- Troponins are now accepted as reliable markers for **MI**
- Measurement of cardiac troponins are among the main tests in early detection of an ischemic episode and in monitoring

LACTATE DEHYDROGENASE (LDH)

- **LDH convert pyruvate to lactate** (Normal value of LDH in serum is 100-200 U/L)
- Values in the upper range are generally seen in children
- Strenuous exercise will slightly increase the value
- LDH level is **100 times** more inside the **RBC** than in plasma
- Minor amount of **hemolysis** will result in a false positive test

LDH and Heart Attack

- In MI, total LDH activity is increased

- **LDH1 (H4) isozyme** is increased 5-10 times more

Differential Diagnosis

- Increase in total LDH level is seen in hemolytic anemias, hepatocellular damage, muscular dystrophy, carcinomas, leukemias, and any condition which causes necrosis of body cells
- Total LDH is ↑ in many conditions → LDH isozyme study is important

LDH isozymes

LDH enzyme is a tetramer with 4 subunits (subunit may be either H (heart) or M (muscle) polypeptide chains).

- ❖H subunit and M subunit have the same molecular weight with minor amino acid variation
- ❖5 (Isozymes) combinations of H and M chains are possible
  - LDH1, LDH2, LDH3, LDH4, LDH5
  - All these 5 forms are seen in all persons
  - The isozymes are usually separated by **electrophoresis**

- Normally LDH2 concentration in blood is > LDH1
- LDH has only limited diagnostic value because its non-specific

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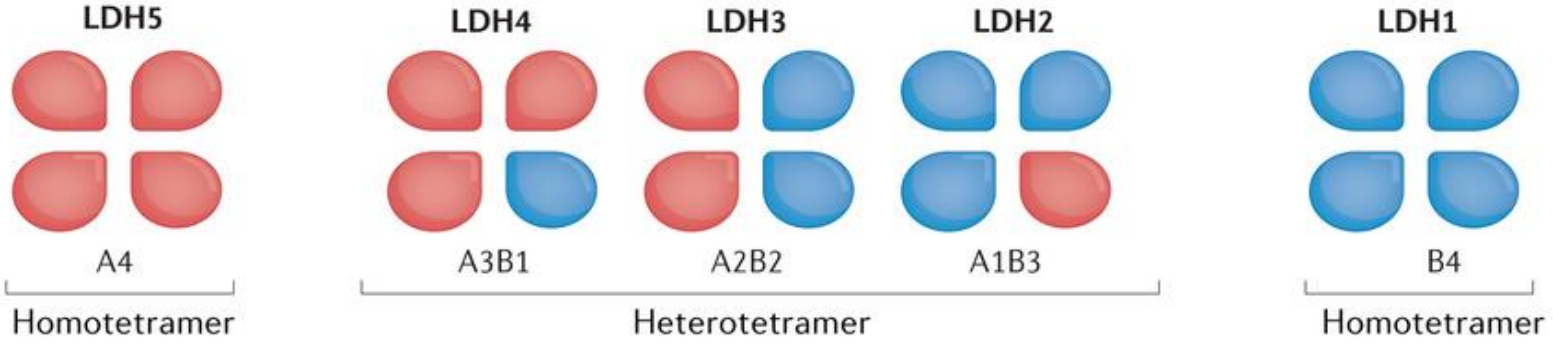

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	LDH5	LDH4	LDH3	LDH2	LDH1
Expressed in	• Liver • Skeletal muscle • Kidney (medulla)	• Liver • Skeletal muscle • Kidney (medulla)	• Lymphoid tissue • Lung • Platelets • Brain	• Heart • Red blood cells • Kidney (cortex)	• Heart • Red blood cells • Germ cells
Non-malignant serum (% of activity)	8–20%	9–15%	18–25%	27–37%	17–27%
Related diseases	• Liver diseases • Skeletal muscle diseases • Lung cancer • Advanced-stage CRC	• Breast cancer • Advanced-stage melanoma • Advanced-stage CRC	• Leukaemia • Breast cancer • Advanced-stage melanoma	• Haemolytic diseases • Non-Hodgkin lymphoma • Lymphocytic leukaemia • Myeloproliferative syndrome	• Myocardial infarction • Haemolytic anaemia • Ovarian cancer • Testicular cancer

Isoenzymes of lactate dehydrogenase	Highest levels found in the following:	Isoenzymes of lactate dehydrogenase	Highest levels found in the following:
		 H_2M_2 (LDH_3)	Brain, lung, white blood cells
 H_4 (LDH_1)	Heart, kidneys	 HM_3 (LDH_4)	Lung, skeletal muscle
 H_3M (LDH_2)	Red blood cells, heart, kidney, brain	 M_4 (LDH_5)	Skeletal muscle, liver

Enzyme Profiles in Liver Diseases

Enzymes commonly studied for diagnosis of liver diseases are:

- Alanine amino transferase (ALT)
- Alkaline phosphatase (ALP)
- Nucleotide phosphatase (NTP)
- Gamma glutamyl transferase (GGT)

ENOLASE

- A glycolytic enzyme
- Neuron-specific enolase (NSE) is an isozyme seen in neural tissues and **Apudomas**
- NSE is a **tumor marker** for cancers associated with neuroendocrine origin, small cell lung cancer, neuroblastoma, pheochro-mocytoma, medullary carcinoma of thyroid

Aldolase (ALD)

- A tetrameric enzyme with A and B subunits → 5 isozymes
- A **glycolytic enzyme**

- Normal range of serum is 1.5-7 U/L

- Drastically ↑ in **muscle damages** such as progressive muscular dystrophy, poliomyelitis, myasthenia gravis and multiple sclerosis

- It is a **very sensitive early index in muscle wasting diseases**

Enzymes as Therapeutic Agents

Enzyme	Therapeutic application
1. Asparaginase	Acute lymphoblastic leukemia
2. Streptokinase	To lyse intravascular clot
3. Urokinase	do
4. Streptodornase	DNAse; applied locally
5. Pancreatin (trypsin and lipase)	Pancreatic insufficiency; oral administration
6. Papain	Anti-inflammatory
7. Alpha-1-antitrypsin	AAT deficiency; emphysema

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can **lyse intravascular clots** and are therefore used in myocardial infarction
- **Pepsin** and **trypsin** are given to patients with **defective digestion**
- **Asparaginase** is used as an anticancer drug

Enzymes Used for Diagnosis

Enzyme	Used for testing
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Peroxidase	Glucose; Cholesterol
Hexokinase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Horse radish peroxidase	ELISA
Alkaline phosphatase	ELISA
Restriction endonuclease	Southern blot; RFLP
Reverse transcriptase	Polymerase chain reaction (RT=PCR)

Required

- Read on **enzyme regulation** (allosteric enzymes) the difference between **homotropic and heterotropic effectors**.