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FACTORS AFFECTING ENZYME ACTIVITY

1. Concentration of enzyme
2. Concentration of substrate
3. Effect of temperature
4. Effect of pH
5. Effect of product concentration
6. Effect of activators
7. Effect of time

DIAGNOSTIC IMPORTANCE OF ENZYMES

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical importance. Enzymes in the circulation are divided into two groups – plasma functional and plasma non-functional.

1. Plasma specific or plasma functional enzymes

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, choline esterase, etc.

2. Non-plasma specific or plasma non-functional enzymes

These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as secretory enzymes. All the other plasma enzymes associated with metabolism of the cell are collectively referred to as constitutive enzymes (e.g. lactate dehydrogenase,

transaminases, acid and alkaline phosphatases, creatine phosphokinase). Estimation of the activities of non-plasma specific enzymes is very important for the diagnosis and prognosis of several diseases. The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum enzymes are conveniently used as markers to detect the cellular damage which ultimately helps in the diagnosis of diseases. (Note : The term biomarker refers to any laboratory analyte (enzyme, protein, antigen, antibody, metabolite etc.) that is useful for the diognosis/prognosis of any disease. Biomarker is a vague term, and less frequently used by biochemists.) A summary of the important enzymes useful for the diagnosis of specific diseases is given in Table 3.1, A brief account of selected diagnostic enzymes is discussed

Table 3-1: Important enzymes in the diagnosis of diseases

Serum enzyme (elevated)	*Disease (most important)*
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5′-Nucleotidase	Hepatitis
γ-Glutamyl transpeptidase (GGT)	Alcoholism

Amylase : The activity of serum amylase is increased in acute pancreatitis (reference 80-180 Somogyi units/dl). The peak value is observed within 8-12 hours after the onset of disease which returns to normal by 3rd or 4th day. Elevated activity of amylase is also found in urine of the patients of acute pancreatitis. Serum amylase is also important for the diagnosis of chronic pancreatitis, acute parotitis (mumps) and obstruction of pancreatic duct.

Alanine transaminase (ALT/SGPT) : SGPT is elevated in acute hepatitis of viral or toxic origin, jaundice and cirrhosis of liver (reference 3-40 IU/l). **Aspartate transaminase (AST/SGOT) :** SGOT activity in serum is increased in myocardial infarction and also in liver diseases (reference 4-45 IU/l).

It may be noted that SGPT is more specific for the diagnosis of liver diseases while SGOT is for heart diseases. This is mainly because of their cellular distribution – SGPT is a cytosomal enzyme while SGOT is found in cytosol and mitochondria.

Alkaline phosphatase (ALP) : It is elevated in certain bone and liver diseases (reference 3-13 KA units/dl). ALP is useful for the diagnosis of rickets, hyperparathyroidism, carcinoma of bone, and obstructive jaundice.

Acid phosphatase (ACP) : It is increased in the cancer of prostate gland (reference 0.5-4 KA units/dl).

Gamma-Glutamyl transpeptidase (GGT) : It is a sensitive diagnostic marker for the detection of alcoholism. GGT is also increased in infective hepatitis and obstructive jaundice.

Decreased plasma enzyme activities

Sometimes, the plasma activities of the enzymes may be lower than normal which could be due to decreased enzyme synthesis or congenital deficiency. In Table 3-2, the decreased plasma enzymes in certain disorders are given

Table 3-2: Decrease in plasma (serum) enzymes in certain diseases

Enzyme	*Reference values*	*Disease(s) in which decreased*
Amylase	80–180 Somogyi units/dl	Liver diseases
Pseudocholinesterase (ChE II)	10–20 IU/dl	Viral hepatitis, malnutrition, liver cancer, cirrhosis of liver
Ceruloplasmin	20–50 mg/dl	Wilson's disease (hepatolenticular degeneration)
Glucose 6-phosphate dehydrogenase (G6PD) in RBC	120–260 IU/10^{12} RBC	Congenital deficiency with hemolytic anemia

ISOENZYMES

The multiple forms of an enzyme catalysing the same reaction are isoenzymes or isozymes. They, however, differ in their physical and chemical properties which include the structure and electrophoretic Km and Vmax values, pH optimum.

Many possible reasons are offered to explain the presence of isoenzymes in the living systems.

1. Isoenzymes synthesized from different genes e.g. malate dehydrogenase of cytosol is different from that found in mitochondria.

2. Oligomeric enzymes consisting of more than one type of subunits e.g. lactate dehydro□genase and creatine phosphokinase

Isoenzymes of creatine phosphokinase

Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the inter-conversion of phospho□creatine (or creatine phosphate) to creatine. CPK exists as three isoenzymes. Each isoenzyme is a dimer composed of two subunits—M (muscle) or B (brain) or both.

Isoenzyme	*Subunit*	*Tissue of origin*
CPK_1	BB	Brain
CPK_2	MB	Heart
CPK_3	MM	Skeletal muscle

In healthy individuals, the isoenzyme CPK2 (MB) is almost undetectable in serum with less than 2% of total CPK. After the myocardial infarction (MI), within the first 6-18 hours, CPK2 increases in the serum to as high as 20% (against 2% normal). CPK2 isoenzyme is not elevated in skeletal muscle disorders. Therefore, estimation of the enzyme CPK2 (MB) is the earliest reliable indication of myocardial infarction.

ENZYME PATTERN IN DISEASES

For the right diagnosis of a particular disease, it is always better to estimate a few (three or more) serum enzymes, instead of a single enzyme. Examples of enzyme patterns in important diseases are given here.

II	**Hepatic disease**	
	Alanine transaminase (ALT)	Marketdly elevated in viral hepatitis
	Aspartate transaminse (AST)	Increased in liver diseases. Significantly elevated in obstructive jaundice (gall stones).
	γ-Glutamyl transpeptidase (GGT)	Marketdly increased in alcoholic liver diseases.
	5′-Nucleotidase	Elevated in hapatic cholestasis.
III	**Muscle disease**	
	Creatine kinase (CK)	Markedly increased in muscle disease (CK-MM more sensitive).
	Aldolase (ALD)	Early marker (not specific)
	Aspartate transaminase (AST)	Significantly increased, although not specific.
IV	**Bone disease**	
	Alkaline phosphatase (ALP)	Increased in rickets and Paget's disease.
V	**Pancreatic disease**	
	Amylase	Significantly elevated in acute pancreatitis.
	Lipase	Markedly increased in acute pancreatitis.
VI	**Prostate cancer**	
	Acid phosphatase (ACP)	Marker enzyme for prostate cancer.
	Prostate specific antigen (PSA)	Significantly elvated in prostate cancer (not an enzyme).

Enzymes in myocardial infarction

The enzymes – namely creatine phosphokinase (CPK), aspartate transaminase (AST) and lactate dehydrogenase (LDH)—are important for the diagnosis of myocardial infarction (MI). The elevation of these enzymes in serum in relation to hours/days of MI is given in the Fig above

Creatine phosphokinase (precisely isoenzyme MB) is the first enzyme to be released into circulation within 6-18 hours after the infarction. Therefore, CPK estimation is highly useful for the early diagnosis of MI. This enzyme reaches a peak value within 24-30 hours, and returns to normal level by the 2nd or 3rd day.

Aspartate transaminase (AST or SGOT) rises sharply after CPK, and reaches a peak within 48 hours of the myocardial infarction. AST takes 4-5 days to return to normal level.

Lactate dehydrogenase (LDH1) generally rises from the second day after infarction, attains a peak by the 3rd or 4th day and takes about 10-15 days to reach normal level. Thus, LDH is the last enzyme to rise and also the last enzyme to return to normal level in MI.

Cardiac troponins (CT) : Although not enzymes, the proteins cardiac troponins are highly useful for the early diagnosis of MI. Among these, troponin I (inhibitory element of actomysin ATPase) and troponin T (tropomysin binding element) are important. Cardiac troponin I (CTI) is released into circulation within four hours after the onset of MI, reaches a peak value by 12–24 hours, and remains elevated for about a week.

The protein myoglobin is also an early marker for the diagnosis of MI. However, it is not specific to cardiac diseases. High serum concentration of brain natriuretic peptide is a marker for congestive cardiac failure. In the Table 3.3, a summary of the diagnostic markers used in MI is given. Table 6.13 gives enzyme patterns in various diseases.

Table 3-3 : Summary of diagnostic markers used for the evaluation of acute myocardial infarction

Diagnostic marker	*Time of peak elevation*	*Time of return to normal level*	*Diagnostic importance*
Myoglobin	4-6 hrs	20–25 hrs	Earliest marker, however not cardiac specific.
Cardiac troponin I	12-24 hrs	5-9 days	Early marker and cardiac specific.
Cardiac troponin T	18-36 hrs	5-14 days	Relatively early marker and cardiac specific. However, elevated in other degenerative diseases.
Creatine phosphokinase (MB)	20-30 hrs	24-48 hrs	Cardiac specific and early marker.
Lactate dehydrogenase (LDH I)	48-72 hrs	10-15 days	Relatively late marker and cardiac specific.
Aspartate transaminase	30-48 hrs	4-6 days	Not cardiac specific.

Enzymes in liver diseases

The following enzymes—when elevated in serum – are useful for the diagnosis of liver dysfunction due to viral hepatitis (jaundice), toxic hepatitis, cirrhosis and hepatic necrosis 1. Alanine transaminase 2. Aspartate transaminase 3. Lactate dehydrogenase. The enzymes that markedly increase in intrahepatic and extrahepatic cholestasis are : (1)

Alkaline phosphatase, (2) 5'-Nucleotidase Serum-J-glutamyl transpeptidase is useful in the diagnosis of alcoholic liver diseases.

Enzymes in muscle diseases

In the muscular dystrophies, serum levels of certain muscle enzymes are increased. These include creatine phosphokinase, aldolase and aspartate transaminase clinical significant of enzyme assay

Enzymes in cancers

Increase in the serum **acid phosphatase(ACP)** is specific for the detection of prostatic carcinoma. [Note : Prostate specific antigen (PSA; mol wt. 32 KD), though not an enzyme, is a more reliable marker for the detection of prostate cancer. Normal serum concentration of PSA is 1-4 ng/ml].**Neuron–specific enolase** serves as a marker for lung cance