BIOCHEMISTRY

VEIN BATCH

Lecture : 5

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Enzymes III

Lecture 5

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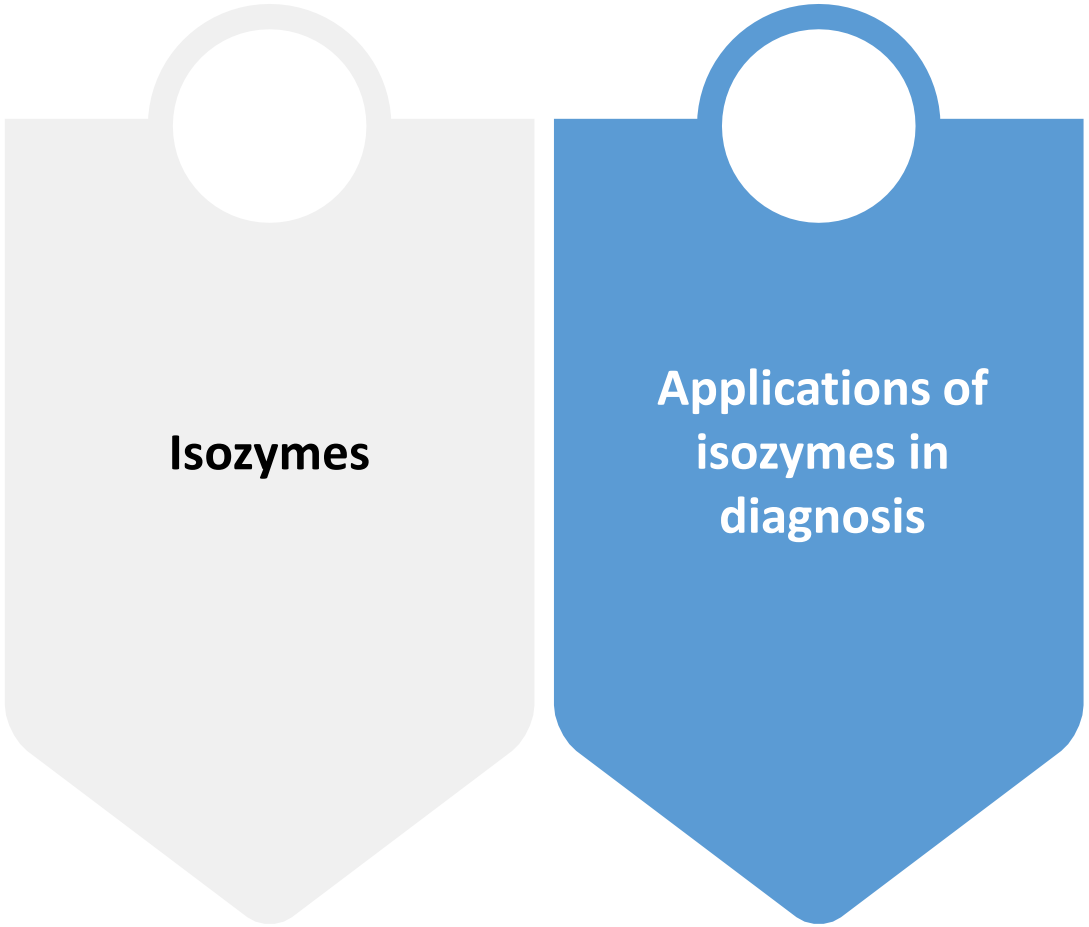
Overview



What are isozymes?

نظائر / شبيهات الenzymes

- **Physically distinct forms of the same enzyme** همه أشكال مميزة (distinct) لنفس الenzyme
  - Multiple molecular forms of an enzyme are described as isozymes or iso-enzymes
  - Different molecular forms of the same enzyme synthesized from various tissues
  - Useful to understand diseases

ف لما يكون الenzyme عنده multiple molecular forms هضول بنسميهم الisoenzymes, وبالأغلب بكونوا مصنعين من tissues مختلفة,
وأهميتهم إنهم بساعدوا بالفهم والdetection ومعرفة الseverity والrisks لبعض الأمراض, ف إلهم أهمية clinically

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

- **Isozyme formation:**

كل enzyme بكون فيه subunits, إذا كانت متشابهة بتكون جاية من single gene واسمها Homomultimer, وإذا مختلفة بتكون من different genes واسمها Heteromultimer

1. Products of different genes: **true isozymes** واذا كانوا الsubunits من جينات مختلفة بنسميه true isozyme
2. In some cases all the different forms are present in the same individual e.g. LDH has 5 iso-enzymes وبعض الenzymes ممكن تتواجد كل الforms المختلفة تبعتها داخل الجسم, زي الlactate dehydrogenase (LDH)
3. The same locus of the gene may have different alleles (alternate forms) → alleleic 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 الallozymes بكون الgene نفسه عنده
   أليلات (alleles) مختلفة, زي الG-6-P اللي إله 400 شكل مختلف, ولو تم توزيعهم بالتساوي ف رح يكون إله low frequency of occurrence, لكن في حالة كان
   الfrequency of occurrence < 1% هاض بسميه polymorphism, وهاض بعطينا insight (توقع) لطريقة تأثير drug معين عالمجتمع
4. Molecular heterogeneity of enzymes may also be produced after the protein is synthesized (post-translational modification): **iso-forms** أحيانا ممكن يصير post-translational modification عن طريق الsynthesis
   للprotein وبنتج عنّا iso-forms, واللي بختلف بطريقة تكوينه عن الtrue isozymes اللي بيجي من subunits مختلفة بدون ما يصير للبروتين post-translational modification

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) (ALP= alkaline phosphatase)

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

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

1- عن طريق الelectrophoresis, واللي هي widely used في المختبرات

2- عن طريق الheat, ومعظم الisozymes بصيرلهم denaturation نتيجة التعرض للحرارة

3- عن طريق inhibitors

4- عن طريق الKm value

5- اختلاف الco-factors اللي بستعمله كل isozymes برضه بساعد بالتعرف عليهم

6- كمان معرفة الtissue اللي أنتجه بساعد بالتعرف عليهم

7- برضه في antibodies محددة لبعض الisozymes اللي بساعد بتمييزهم

اللهم إني أسالك التوفيق والنجاح, في الدنيا والآخرة

Applications of isozymes in diagnosis

وبما إنه في عنا 7 طرق to identify isoenzymes ف
التطبيق العملي إلهم بالdiagnosis وبالmedicine واسع جدا

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

ولكن مستواهم بالدم برتفع عند المرض, و بنقدر نستعين بالموضوع هاض عشان نحدد شدة المرض

- 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

ال biomarker هو فحص بالمختبر للكشف عن وجود خلل (dysfunction/disfunction) أو مشكلة في أحد الأعضاء أو اصابته بمرض معين, بالإضافة لدوره في معرفة شدة وتأثير بعض الأمراض

Different markers are used to:

i. **Detect** myocardial ischemia at the earliest

وإلهم كثير استخدامات بال cardiology منها :

- Commonly used biomarkers for **early detection** of acute myocardial infarction are:
  - 1. Cardiac troponins
  - 2. Creatine kinase, CK-MB
    3. Myoglobin

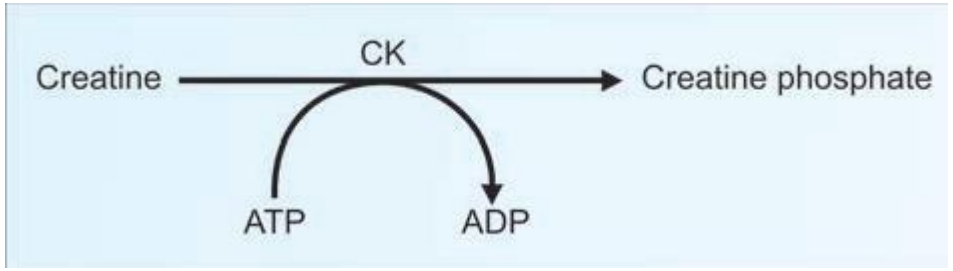
ii. **montoring** the progression of the condition

iii. to **predict** the risk in cardiac dysfunction

CREATINE KINASE (CK)

وكمان مرة.. لما نقول kinase فهاض يعني
إنه بنقل phosphate group

It catalyzes the creatine kinase reaction (was called creatine phosphokinase)



Creatine kinase reaction

بحفز تحويل الcreatine
لcreatine phosphate

Normal serum value for CK is 15-100 U/L for males and 10-80 U/L for females

الNormal value مش مطلوبة, هي فقط مذكورة زيادة العلم

اللهم إنك عفوّ تحب العفو فاعفُ عنا

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 ambiguous
  - A second peak may indicate another ischemic episode

مهم جدا حيث بساهم بالكشف المبكر لحالات الmyocardial infarction, خاصة إنه قراءات الECG بتكون غير واضحة كفاية نعرف منها إنه في مشكلة, وبما إنه بنخفض تركيزه بسرعة ف لو اجيت بعد 4 ايام مثلا من الMI ولقيت إنه تركيزه رجع ارتفع فهاض مؤشر لاحتمال حدوث 2nd ischemic episode

- 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

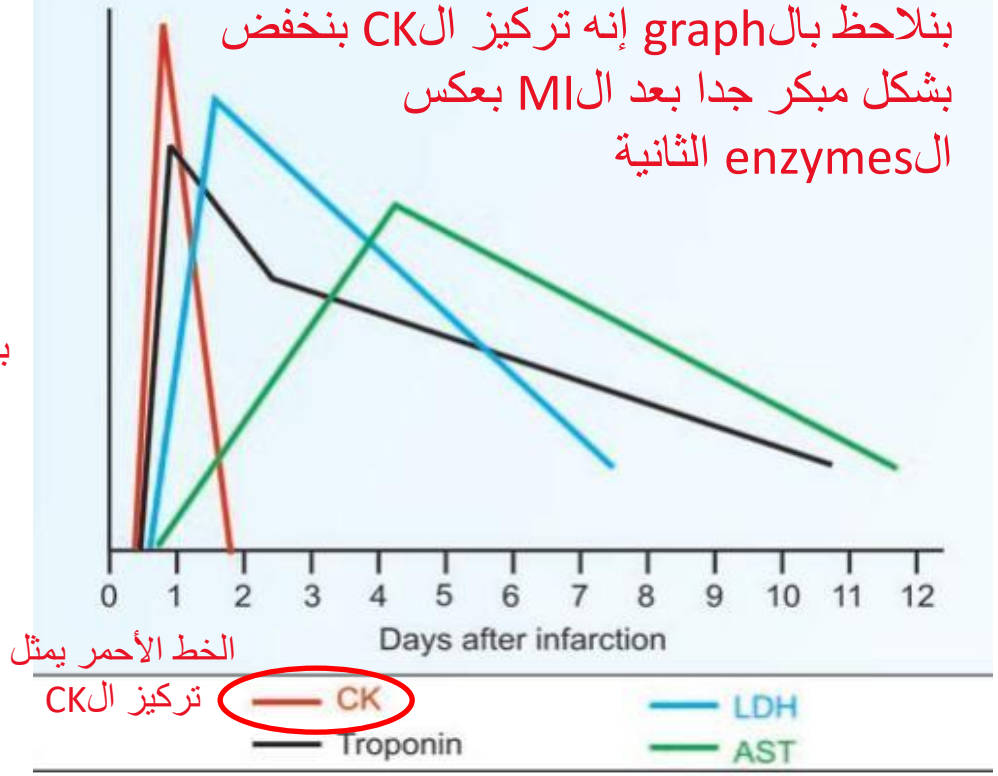
myocardial infraction = MI / نقص التروية الدموية = ischemic episode

* الجدول مش حفظ, لكن لفهم العملية فقط*

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

بنلاحظ بالgraph إنه تركيز الCK بنخفض بشكل مبكر جدا بعد الMI بعكس الenzymes الثانية



الخط الأحمر يمثل تركيز الCK

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 subunits:
  - B (brain)
  - M (muscle)
  - 3 isozymes in circulation (CK-MM, CK-MB and CK-BB)
    (CK2 = CK-MB)

3 isozymes الـsubunits الـpossible همه الـB والـM, واللي رح يكَوْنوا
(CK-BB) B+B الثالث فيه – (CK-MB) M+B الثاني فيه – (CK-MM) M+M الأول فيه -

- Normally CK2 (heart isozyme) is only 5% of the total activity

(When we measure the activity of CK2 in blood we will find out that it is 5%, which represent a relatively low activity of the total CK activity)

- Even doubling of the value of CK2 (MB) isozyme may not be detected, if total value of CK alone is estimated

- **MBisoenzyme** estimation is the best diagnostic marker in MI

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

هسا لما نيجي بدنا نعمل فحص عشان نشوف خطورة التعرض لـMI مثلا فإحنا ما رح نستفيد غير من الـCK2 (لإنه الوحيد اللي اله علاقة بالقلب زي ما الجدول موضح) و الـCK2 ما بطلع لحاله مباشرة, اللي بدنا نعمله بالأول عملية الـidentification عن طريق الـelectrophoresis عشان نفصلهم بعدين نشوف شو بطلع معنا

CK isozyme characteristics *الجدول حفظ*

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%

السلايد هاض زيادة علم برضه (بس ما فيه اشي يعني, مش غلط تقرأوه)

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

سبحان الله وبحمده, سبحان الله العظيم

LACTATE DEHYDROGENASE (LDH) (LD)

- LDH convert pyruvate to lactate

(ال normal value للعلم فقط, مش مطلوبة)

- **Normal value** of LDH in serum is 100-200 U/L

- Values in the upper range are generally seen in children

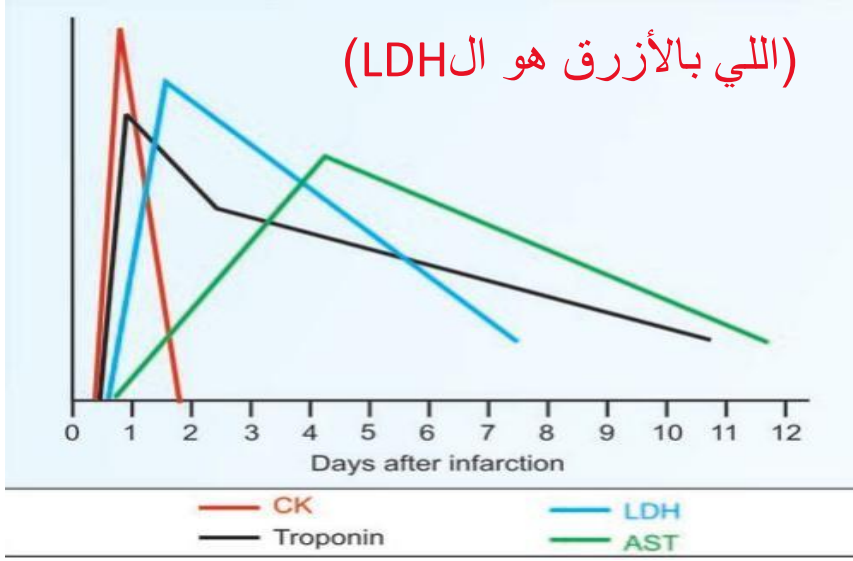
- Strenuous exercise will slightly increase the value التمرين وبذل الجهد بزيد من ال value لل LDH

- LDH level is 100 times more inside the RBC than in plasma **هاي النقطة مهمة**

- Minor amount of **hemolysis** will result in a false positive test

وأهميتها تتجلى أثناء عملية فحص ال LDH بالدم, حيث اذا صار hemolysis وتكسرت ال RBCs هاض رح يأدي
لزيادة نسبة ال LDH بال plasma وبالتالي يعطينا قراءة خاطئة

LDH and Heart Attack



- In MI, total LDH activity is increased
- **H4 isozyme** is increased 5-10 times more
- The magnitude of the peak value and area under the graph will be roughly proportional to the size of the myocardial infarct

info from the clinical standpoint is not that important..

اللي بهمنا فعليا هو الisozymes إله

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 **هاي النقطة مهمة**

- Subunit may be either H (heart) or M (muscle) polypeptide chains

- Both of them have the same molecular weight (32 kD), there are minor amino acid variation → 5 combinations of H and M chains are possible
  - H4 (**in heart**), H3M, H2M2, M3H and M4 (in skeletal muscles) varieties → 5 isozymes
  - All these 5 forms are seen in all persons
  - The isozymes are usually separated by **electrophoresis**

هاي النقطة مهمة جدا واكيد جاية بالامتحان

ف في عنا 5 isozymes, **أول واحد** (ال LDH-1 / H4) كل ال polypeptide chains فيه مكونة من H (من ال heart subunit), وبنلاقيه بال cardiac muscle وال RBCs, **الثاني** (ال LDH-2 / H3M) برضه موجود بال cardiac muscle وال RBCs, الآن **الثالث** (ال LDH-3 / H2M2) موجود بال lungs, بينما **الرابع** (ال LDH-4 / M3H) موجود بال liver وال skeletal muscles, **الخامس** (ال LDH-5 / M4) موجود in the other tissues

- Normally LDH-2 (H3M1) concentration in blood is > LDH-1 (H4)

تركيز ال H3M في الدم بالعادة بكون أعلى من ال H4

- This pattern is reversed in MI; this is called **flipped pattern**

- LDH has only limited diagnostic value because its non-specific

من الآخر السلايد كله مهم

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)

لما يصير مشكلة بالliver ف إحنا بنتوجه لدراسة هاي الenzymes, لإنه تركيزها رح يرتفع في حال حدوث أي خلل بالliver

اللهم إني أسألك الهدى والتقى والعفاف والغنى

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

زي اللي قبله, الnormal range للعلم

- 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

تركيزه برتفع بشكل كبير عند حدوث أي ضرر للعضلات

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

Enzyme Patterns (Enzyme profiles) in Diseases

I. Hepatic diseases

1. Alanine amino transferase (ALT)
   Marked increase in parenchymal liver diseases
2. Aspartate amino transferase (AST)
   Elevated in parenchymal liver disease
3. Alkaline phosphatase (ALP)
   Marked increase in obstructive liver disease
4. Gamma glutamyl transferase (GGT)
   Increase in obstructive and alcoholic liver

II. Myocardial infarction

1. Creatine kinase (CK-MB)
   First enzyme to rise following infarction
   CK-MB isoenzyme is specific
2. Aspartate amino transferase (AST)
   Rises after the rise in CK and returns to normal in 4-5 days
3. Lactate dehydrogenase (LDH)
   LDH-1 becomes more than 2 (flipped pattern)

III. Muscle diseases

1. Creatine kinase (CK-MM)
   Marked increase in muscle diseases.
   CK-MM fraction is elevated
2. Aspartate amino transferase (AST)
   Increase in muscle disease; not specific
3. Aldolase (ALD)
   Earliest enzyme to rise, but not specific

IV. Bone diseases

1. Alkaline phosphatase (ALP)
   Marked elevation in rickets and Paget's disease
   Heat labile bone isoenzyme is elevated (BAP).

V. Prostate cancer

1. Prostate specific antigen (PSA)
   Marker for prostate cancer.
   Mild increase in benign prostate enlargement
2. Acid phosphatase (ACP)
   Marker for prostate cancer. Metastatic bone disease especially from a primary from prostate.
   Inhibited by L tartrate.

اللي بهمنا أكثر اشي بالجدول هو اللي ركزنا عليه من isozymes بالسلايدات اللي قبل, وبالأخص الCK-MB والLDH, أما اللي ما تم ذكرهم أو التركيز عليهم ف ما تركزوا عليهم كثير

Enzymes in other body fluids

Enzyme	Clinical significance
Adenosine deaminase in pleural fluid	Elevated in tuberculous pleural effusion, but not in malignant effusion.
Lactate dehydro-genase in CSF, pleural fluid, ascitic fluid	Elevated levels indicate the presence of a malignant tumor. But not diagnostic, as the enzyme is not tissue specific.

Enzymes as Therapeutic Agents

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can lyse intravascular clots and are therefore used in myocardial infarction

كان يُستخدَم قديما مع مرضى الMI لإذابة الجلطات (الclots),
لكن حاليا تم استبداله بالPCI (Percutaneous coronary intervention)

- **Pepsin** and **trypsin** are given to patients with defective digestion

- **Asparaginase** is used as an anticancer drug

الجدول حفظ

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

اللهم إني أستودعك أموري كلها, فوفّقني لما تحبه وترضاه

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)

Immobilized Enzymes

(بما إنه الenzymes من protein,
فهاض يعني إنهم soluble in water)

- Enzymes have been fixed or rendered insoluble by attaching them to insoluble matrix such as plastic beads or cellulose strips

لكن اذا صارلهم attachment مع مواد insoluble رح يصيروا الenzymes برضه insoluble

- These strips are used for detection of abnormal substances in blood or urine

وممكن نستفيد من هاض الاشي بكثير diagnostic tests

- Immobilized urease, hexokinase, amylase etc. are also used for diagnostic purpose

Coenzymes

هسا مبدأيا موضوع الheat stable هو للinorganic Co-factors , هسا الCo-
enzymes بنقدر نعتبرهم زي proteins ف رح يكون في قابلية إنه يصيرلهم
denaturation زي الenzymes اذا تعرضوا لextreme temperature ,
فالheat stable بالسلايد هون هي للCo-factors

- Are heat stable, low molecular weight non-protein compounds
- Strictly required by some enzymes for their actions
- **Actions of coenzymes:** function as group transfer agents
- **Important:** co-factor is used as a collective term to include co-enzymes and metal ions. Co-enzyme is an organic co-factor.

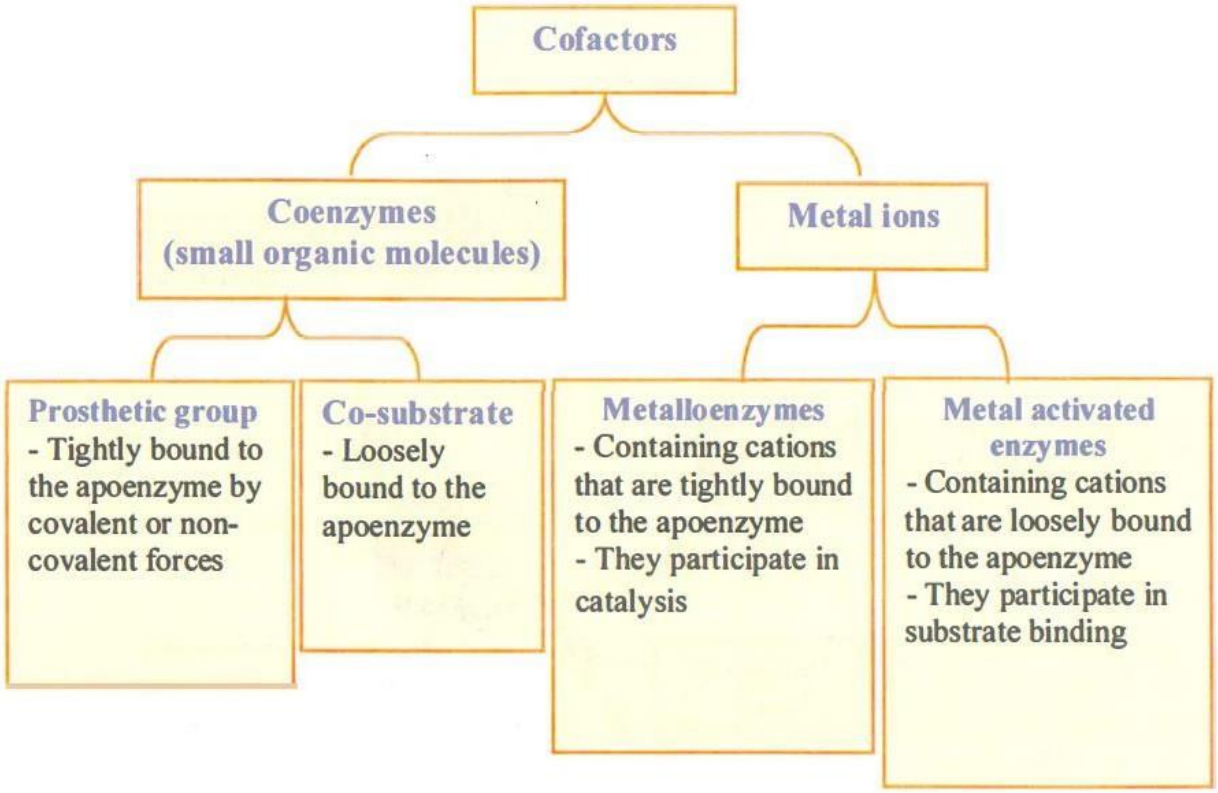
note : أي inorganic cofactor فهو لازم يكون heat stable

Cofactors

Cofactors: organic or inorganic molecules that are required for the activity of certain enzymes

Haloenzyme: refers to the active enzyme with its non-protein component (cofactor)

Apoenzyme: enzyme without its cofactor and is inactive



Coenzymes

- Are regarded sometimes as second substrate:
  - Chemical changes in co-enzymes are opposite the substrate (if substrate is oxidised coenzyme is reduced)
  - Reaction in coenzyme is sometimes of greater physiological importance than substrate

- Coenzymes are required by:
  - Oxido-reductases
  - Transferases
  - Isomerase
  - Ligase

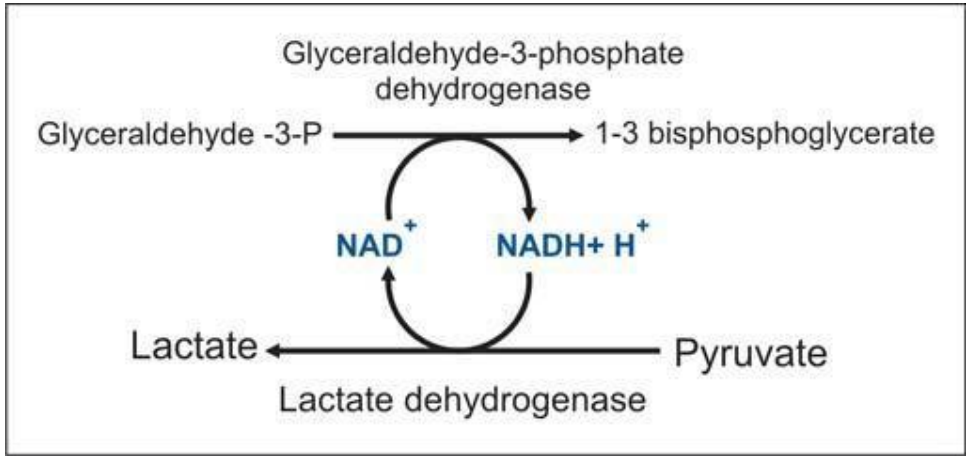
- Coenzymes are **not** required by:
  - Hydrolases
  - Lyases

سبحان الله وبحمده, عدد خلقه, ورضى نفسه, و زِنة عرشه, ومداد كلماته

السلايد كله مهم جدا للامتحان, والنقاط حفظ كاملة

Coenzymes are classified into

One co-enzyme molecule can work with different enzymes



- Involved in hydrogen or electron transfer
  - Nicotinamide nucleotides (NAD, NADP)
  - Flavin nucleotides (FMN, FAD)
  - Glutathione
  - Coenzyme Q

- Involved in transfer of other groups
  - Thiamine pyrophosphate (carries alpha keto acids and glycoladehyde)
  - Pyridoxal phosphate (carries amino acids and amino groups)
  - Coenzyme A (carries carboxylic acid)
  - Biotin (carries carbon dioxide) (أي reaction فيه carbon dioxide رح نشوف فيه biotin)
  - Tetrahydrofolic acid (carries one carbon unit)
  - Adenosine triphosphate (ATP) (carries phosphate)

Metallo-enzymes: These are enzymes which require certain metal ions for their activity

الجدول حفظ ومهم

Table 5.2. Metallo-enzymes

Metal	Enzyme containing the metal
Zinc	Carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase
Magnesium	Hexokinase, phospho fructo kinase, enolase, glucose-6-phosphatase
Manganese	Phospho gluco mutase, hexokinase, enolase, glycosyl transferases
Copper	Tyrosinase, cytochrome oxidase, lysyl oxidase, superoxide dismutase
Iron	Cytochrome oxidase, catalase, peroxidase, xanthine oxidase
Calcium	Lecithinase, lipase
Molybdenum	Xanthine oxidase

اللهم إني أستودعك ما درست وقرأت وحفظت وفهمت.. فرُدَّه لي عند حاجتي إليه