3) MM (CK_3) : found in skelatal / Heart muscles
- the heaviest → least electrophoretic mobility
- the highest % in the blood ⇒ 97 - 100 %

- These isoenzymes can be separated by :
1) Electrophoresis 2) Ion exchange chromatography

Note : Biomarkers (Diagnostic markers) should be characterized by :

① High sensitivity : with least duration after occurance of disease the concentration of bio marker should increase four-five times the normal concentration.

② High specificity : Released from one organ

E.g. LH_5 : Highest specificity to diagnose liver diseases
CK-MB : Highest specificity to diagnose MI

* اخر سلايد (enzyme kinetics) ما حكاكنها الدكتور ولا شرحها و هي مش فيها شي ← قراءة فقط

Lecture 2 : Factors affecting enzyme activity

There are 4 factors affecting enzyme activity:

1. Temperature } both of these factors affect the protein structure (tertiary structure)
2. pH
3. Enzyme concentration } both depends on the ratio between enzyme molecules and substrate molecules (نسبة و تناسب)
4. Substrate concentration

Note : when we study one of the above factors we make sure that all other factors are Constant

Note 2 : we study these factors in test tube not under biological media

First : Temperature and pH (Hydrogen ion concentration)

- Every enzyme have an optimum tempreture and an optimum pH in which the enzyme is acting maximally
- Any change in the temp. or pH will reduce the activity of the enzyme thus if the change was much enough the enzyme will denaturate
- Denaturation: breaking the structure (3D struture) of the enzyme (protein) starting from the active site then the whole protein
- Denaturation: affect any protein portion of an enzyme
- From the graphs we can notice that increasing the temp. higher than the optimum destroy the enzyme faster than decreasing the temp. whereas in pH changing the pH in both ways result the same ~~destruction~~ decreasing

Regarding Temperature:

- Q10 or temperature coefficient: the factor by which the rate of biological processes increases for 10°c increase in temp. (in a test tube) تقريبًا بتزيد سرعة التفاعل كل ما أزيد ١٠ س

- Optimum temperature in the body : 37°c (Humans and animals)
  - if the temperature ≤ 36.8°c (Hypothermia)
  - if the temperature > 40°c (Hyperthermia) or (fever) Denaturation
- In higher organisms and plants : 50°c → optimum temp.
  - if the temperature > 55°c → denaturation

Regarding pH:

- For most enzymes ⇒ Optimum pH = 5 to 9 exeptions: pepsin, alkaline phosphatase
  Pepsin optimum pH = 1.5 to 2 // Alkaline phosphatase: 9 pH
- when the pH is near to the optimum value ⇒ amino acids in the active site of the enzyme well be ionized thus enhance the interactions between the substrate and the active site
- when the pH value is a way (lower/Higher) from the optimum pH the interaction between active site and substrate will break

Second: Enzyme concentration & substrate concentration

- In studying the impact of enzyme or substrate concentration on the rate of the catalyzed reaction: we consider that every 1 molecule of enzyme molecules will work only on one molecule of substrate
- Also we consider that all the other factors are constant

* Enzyme concentration:

- We use a test tube with known constant temp. and pH and with a known concentration of substrates and we start adding enzyme. At first the rate of reaction when increase as we add enzymes (first order reaction) then we will notice that there is no increasing in the rate thus we are adding enzymes (zero order reaction).

summery: * when (enzyme concentration) < (substrate concentration) ⇒ adding more enzymes (increasing enzyme concentration) increase the rate of reaction

* when (enzyme concentration) ≥ (substrate concentration) ⇒ adding more enzymes have no affection because we reach the SATURATION POINT: all active sites of enzymes are equiped by substrate.

* Substrate concentration : as same as enzyme concentration but this time the enzyme concentration is constant whereas the substrate concentration is being test

Enzyme concentration and substrate concentration summery :

- The shape of the curve that relates activity to substrate concentratin is hyperbolic
- " " " " " " " " " " Enzyme " " "
- * if enzyme concentration > substrate concentration => adding enzyme result no changment in the rate whereas increasing substrate concentration, increase the rate of reaction
- * if enzyme concentration < substrate concentration => to increase the rate we add enzymes
- * if enzyme concentration = substrate concentration => We have to increase both consentrations to affect the rate of the reaction.

Michaelis - Menten kinetics : { quantitative description of the relationship between the rate of the reaction and the concentration of substrate and two constants Vmax and Km

$$V_i = \frac{V_{max}[S]}{K_m + [S]}$$

نظرية حالة شبه ثابتة : $K_m = (+K_1) + (-K_1) + (+K_2) + (-K_2)$

We have 3 important conditions of the above equation :

1. $[S] <<< K_m$ → we can omit [S] from the denominator → $V_i = \frac{V_{max}[S]}{K_m} = K[S]$
   ∴ $V_i \propto [S]$
2. $[S] >>> K_m$ → we can omit K_m from the denominator → $V_i = \frac{V_{max}[S]}{[S]}$ ∴ $V_i = V_{max}$
3. $[S] = K_m$ → we replace K_m with [S] → $V_i = \frac{V_{max}[S]}{2[S]}$ ∴ $V_i = \frac{1}{2} V_{max}$

∴ K_m = the substrate concentration when $V_i = \frac{1}{2} V_{max}$

Lineweaver - Burk plot : A linear form of the Michaelis - Menten Equation and is used to determine Km & Vmax.

$$\frac{1}{V_i} = \left[\frac{K_m}{V_{max}}\right]\frac{1}{[S]} + \frac{1}{V_{max}}$$

* Lineweaver - Burk plot is also called a double reciprocal

Note : Check the graphs from the slides

Note 2 : We well need the last 2 graphs as we study the affect of inhibitors

Km significance :-

[1] It's very specific to an enzyme (Km لا يعتمد على تركيز الإنزيم)

[2] Can be used for determining the true substrate for a particular enzyme

[3] Determine the affinity of an enzyme for its substrates and the amount of substrate needed to reach the maximum activity (inverse proportional relationship)

∴ ↑ Km → ↓ affinity ∴ ↓ Km → ↑ affinity

↑ Km → ↑ Vmax ↓ Km → ↓ Vmax