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ENZYME KINETICS

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Learning outcomes

- Kinetics of enzymatic catalysis
- Michaelis- Menten equation and its significance.
- Lineweaver- Burk plot
- Allosteric mechanism
- Isozymes
- Lactate dehydrogenase & Hexokinase

KINETICS OF ENZYMATIC CATALYSIS:

- Reaction is thermodynamically possible, if the free energy of the ground state of product (P) is lower than that of substrate (S).
- For conversion of S $\rightarrow$ P extra energy is needed (represented as energy barrier) that represents the energy required for alignment of reacting groups and bond rearrangements needed for the reaction to occur in either direction.
- The top of the energy barrier is called transition state, where certain events like bond breakage, bond formation etc decide the future course of the reaction.

ACTIVATION ENERGY (E_a)

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- The difference between the energy levels of the ground state and transition state is called **activation energy (E_a)**.

- Activation energy is the minimum amount of energy required for a chemical reaction to occur.

- measured in joules per mole (J/mol) or kilocalories per mole (kcal/mol)

- Enzymes lower the activation energy and increase the rate of reaction.

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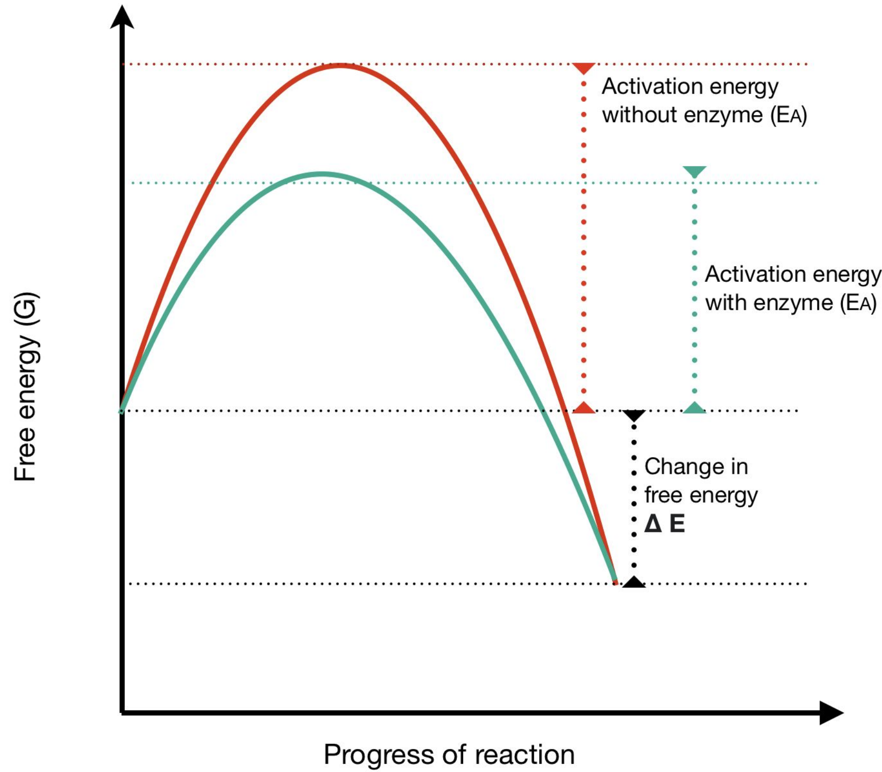
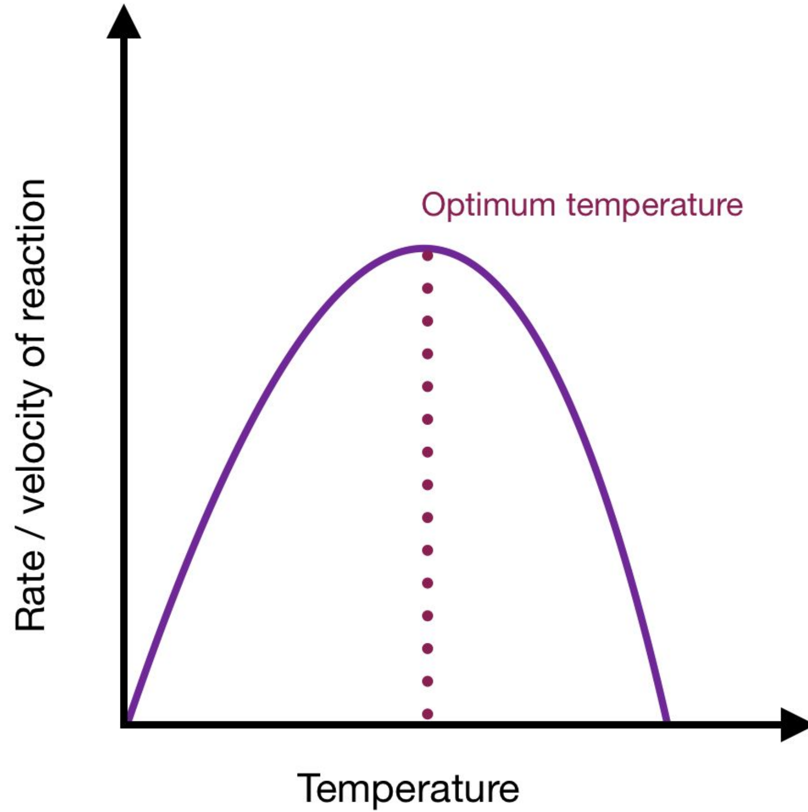


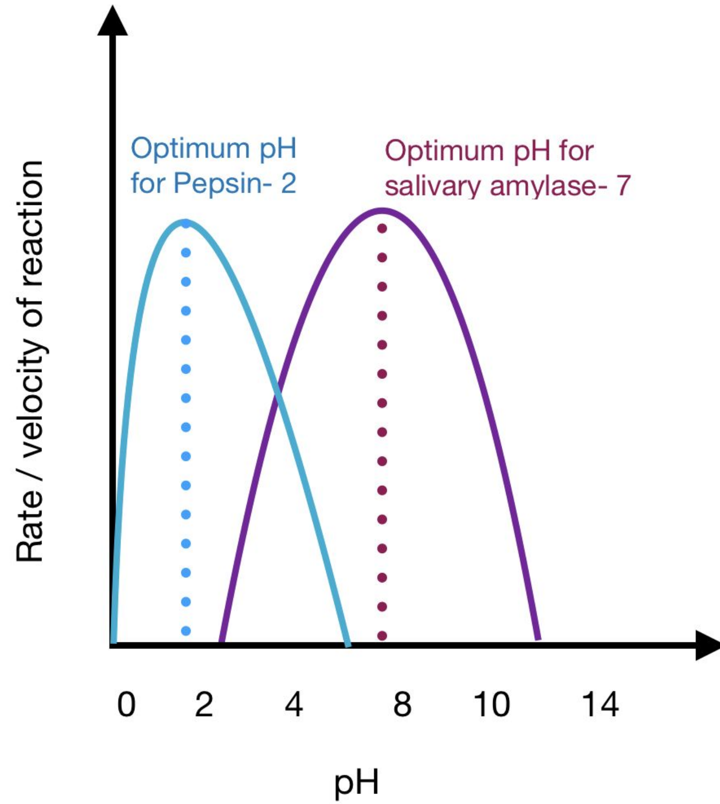
Free energy change during a chemical reaction

FACTORS AFFECTING ENZYME ACTIVITY

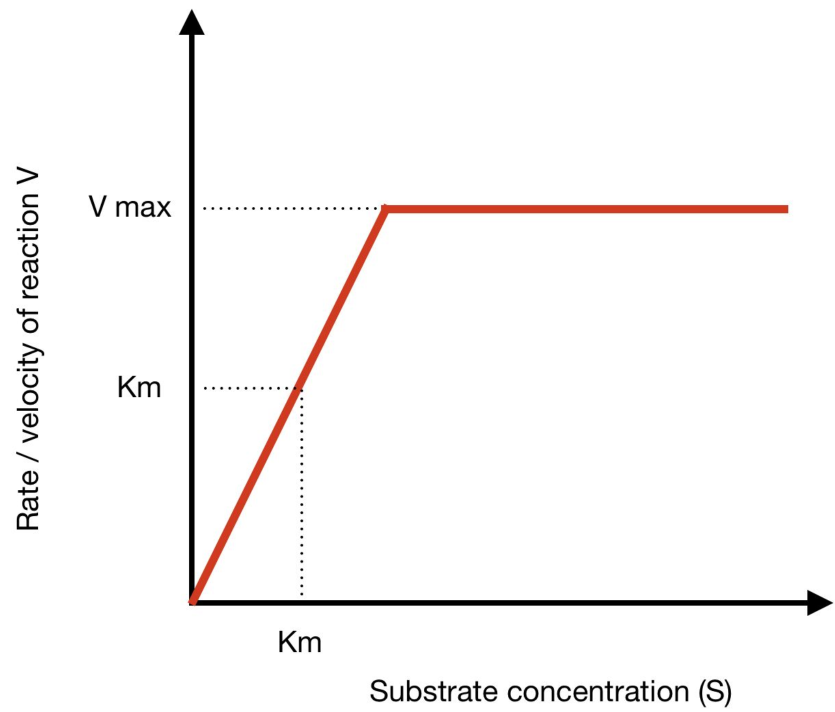
- Temperature
- pH
- Concentration of substrate molecule [S]
- Activators/ Inhibitors



Effect of temperature on enzyme catalyzed reaction



Effect of pH on enzyme catalyzed reaction



Effect of substrate concentration on enzyme catalyzed reactions

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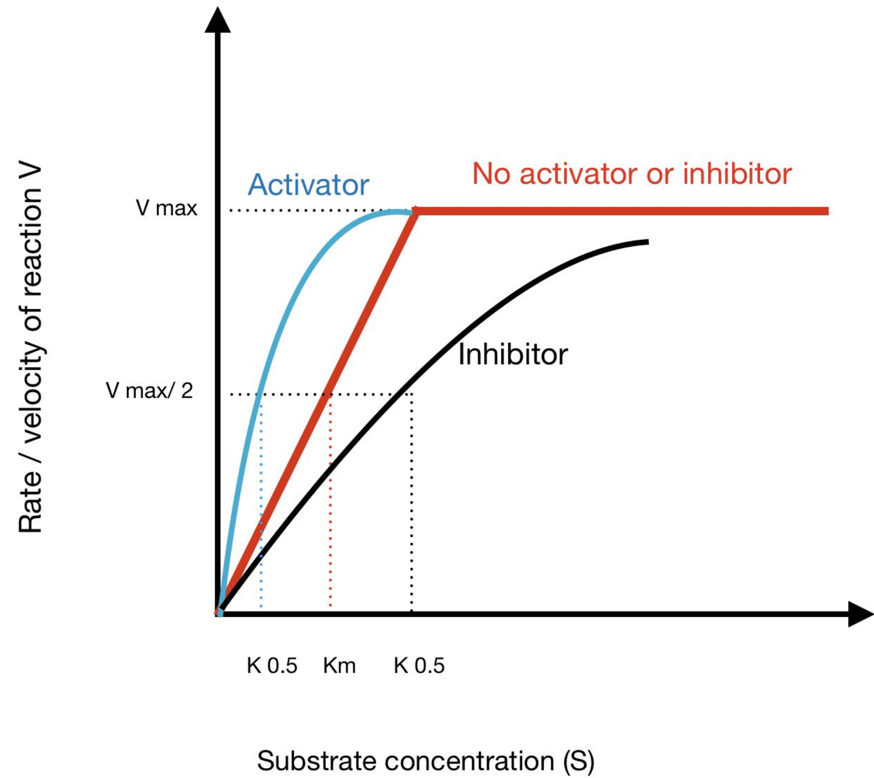

Effect of activator and inhibitor on enzyme catalysed reaction

MICHAELIS- MENTEN EQUATION AND ITS SIGNIFICANCE:

- Rate of reaction increase with the increase in substrate concentration.
- Finally, a point is reached when the addition of substrate has no effect on the rate of enzyme catalysed reaction.
- This is called maximum rate (Vmax) at which the enzyme is saturated with the substrate .
- This saturation effect is exhibited by nearly all enzymes.

MICHAELIS- MENTEN EQUATION

- If a graph is plotted between rate of reaction versus substrate concentration V/ [S], a rectangular hyperbolic curve is obtained.
- The mathematical expression of this curve was developed by **Leonor Michaelis and Maud Menten** (1912-1913).
- The curve flattens at the maximum velocity (V_M), when all the active sites of the enzyme are filled with substrate.

MICHAELIS- MENTEN EQUATION

At half the maximum velocity, the substrate concentration in moles per litre (*M*) is equal to the **Michaelis- Menten constant Km**, which is a rough measure of the affinity of the substrate molecule for the enzyme.

- lower Km- higher binding affinity
- higher Km- lower binding affinity

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

V0 = Initial velocity (moles/times)

[S] = substrate concentration (molar)

V_{max}= maximum velocity

K_m = substrate concentration at half V_{max}

- The two variables in this equation are the reaction velocity V and the concentration of the substrate [S]
- Vmax and Km are the two constants
- By **Michaelis – Menten equation** we can calculate reaction rate of an enzyme at any given concentration of its substrate

LINEWEAVER BURK PLOT

- It is impossible to obtain a definitive value of Vmax and Km from a typical Michaelis-Menten plot.

Plotting the reciprocal of V/ [S] yields a "double-reciprocal" or Lineweaver-Burk plot (Hans Lineweaver and Dean Burk in 1934) . This provides a more precise way to determine $\mathbf{V_{max}}$ and $\mathbf{K_m}$.

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Michaelis-Menten equation

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

invert

$$\frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]}$$

factor

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

and simplify

Double-Reciprocal or Lineweaver-Burk equation

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

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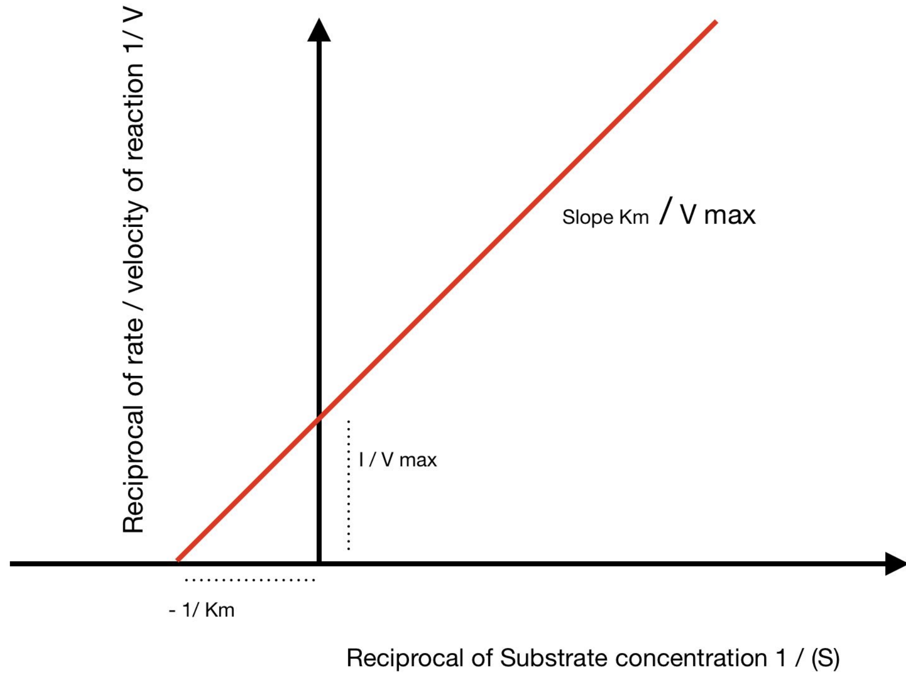


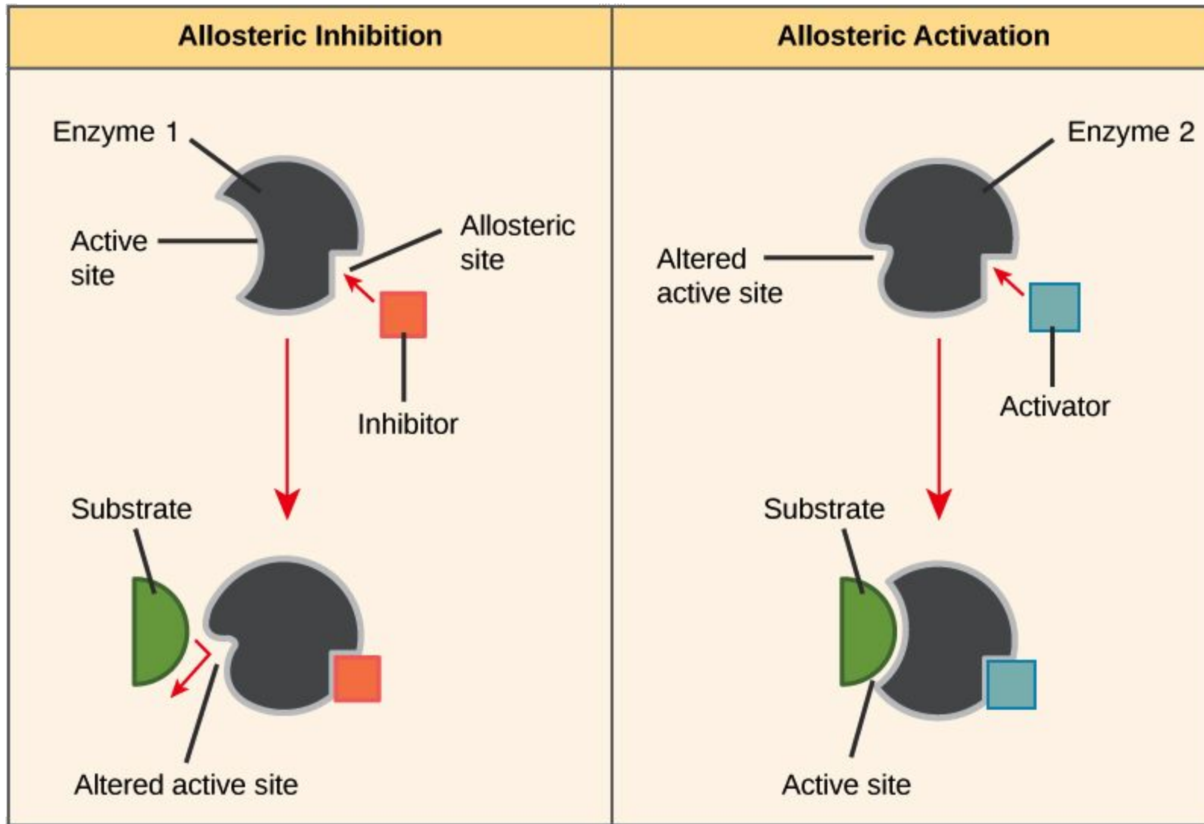
A double-reciprocal plot of enzyme kinetics is generated by plotting $1/V_0$ versus 1/[S]. The slope is the K_M/V_{max}, the intercept on the vertical axis is $1/V_{max}$, and the intercept on the horizontal axis is $-1/K_M$.

ALLOSTERIC MECHANISM:

- **Allosteric enzymes** are structurally different from simple non- regulatory enzymes.
- Besides the presence of an active or catalytic site, the allosteric enzyme have one or more regulatory or allosteric site (allos = other, stereos = space/ site) for the binding of modulator/ effector.
- A modulator is a metabolite which when bound to the allosteric site of an enzyme, alters its kinetic characteristics.
- The modulator may be either an inhibitor or an activator. A stimulator is often the substrate itself.

Allosteric enzymes

- The regulatory enzymes for which substrate and modulator are identical are called homo-tropic.
- When the modulator has a structure different than the substrate, the enzyme is called heterotropic.
- In most of the case , the allosteric inhibitors are the end products of the metabolic pathway in which that particular enzyme is participating. This kind of enzyme inhibition is also called feedback/ end product/retro inhibition.
- The effect of allosteric modulators is reversible, when they are withdrawn, the enzyme resumes the original activity.
- Binding of modulator changes the physical configuration of the enzyme molecule.



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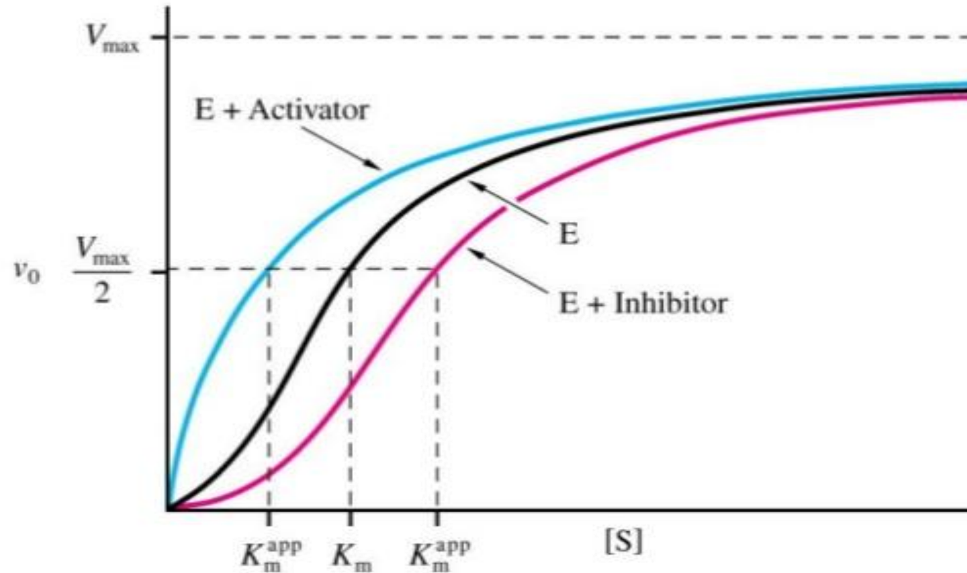
Kinetic properties of allosteric enzymes

- The allosteric enzymes often display sigmoidal saturation curve of the reaction velocity versus substrate concentration V/ [S] .

- Although value of [S] at half maximum velocity can be found out on sigmoid saturation curve, designation Km is not used because the enzyme does not follow the hyperbolic Michaelis- Menten relationship.

- Instead the symbol $[S]_{0.5}$ or $K_{0.5}$ is often used to represent the substrate concentration giving half maximum velocity of the reaction.



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Reaction velocity (V) versus substrate concentration [S]

ISOZYMES

- Isozymes are the different molecular forms of the same enzyme.
- Isozymes are the product of different genes and are encoded by different genetic loci which usually arise through gene duplication and divergence.

ISOZYMES

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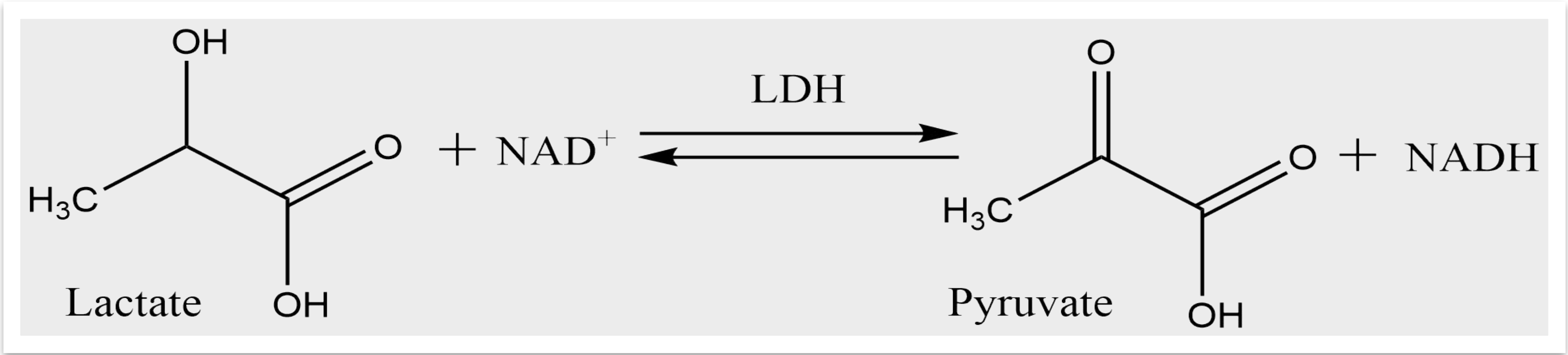
- Isozymes differ in few amino acid sequences, yet catalyze the same reaction. They
- differ in their physic-chemical properties
- differ in their electrophoretic mobility,
- isoelectric pH,
- kinetic parameters such as Km,
- binding with some specific ligands and
- regulatory properties.



Example of isozymes – **lactate dehydrogenase (LDH)**

LDH catalyzes the oxidation of lactic acid to pyruvic acid with the help of NAD^+.

Lactate dehydrogenase (LDH)

- LDH enzyme is a tetramer consisting of two distinct types of subunits called H type and M type.
- Depending upon the relative number of H and M type subunits, there are five types of isozymes of LDH- **LDH1 (H4), LDH2 (H3M1), LDH3 (H2M2), LDH4 (H1M3),LDH5 (M4)**
- Both H and M subunit have two polypeptide chains, and each share 75% of the amino acid sequence for the chains.
- The H4 type (LDH1) functions better in aerobic environments such as the heart.
- The M4 type (LDH5) functions better in anaerobic environments such as the muscle.

Use of LDH isozyme in clinical diagnosis

- When heart muscle cells are damaged during a heart attack or myocardial infarction they release LDH1 .
- If the LDH1 (H4) isozymes appear in increased levels in blood samples, then there is a possibility that the heart cells are damaged.

Hexokinase isozymes

- In mammals there are four **hexokinase isozymes** with different tissue distributions.

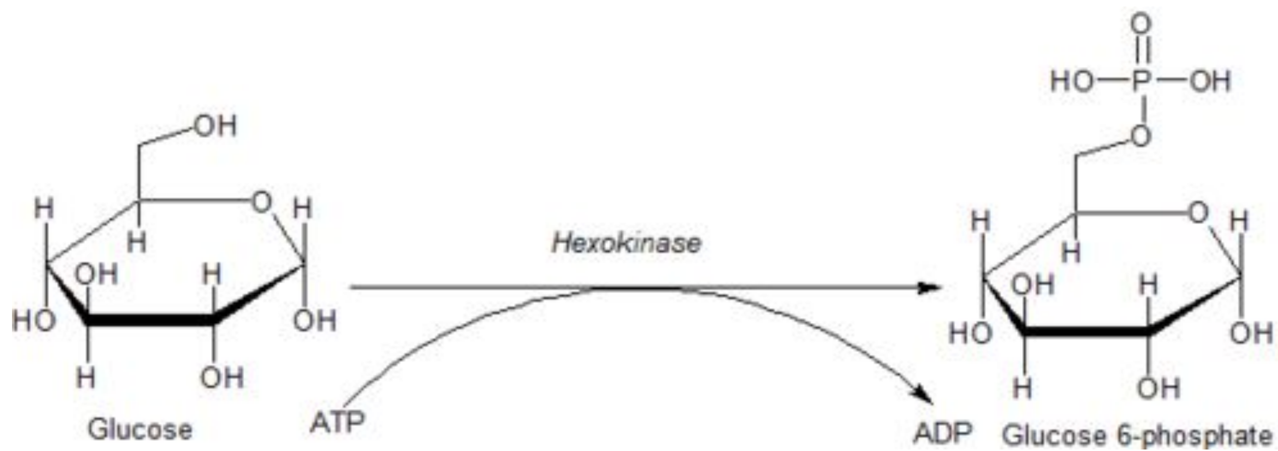
- They are designated hexokinases **I, II, III, and IV or hexokinases A, B, C, and D**.

- **Hexokinases I, II, and III** are referred to as "low-K_m" isozymes because of a high affinity for glucose (below 1 mM).

Hexokinase isozymes

- **Hexokinase IV**, also referred to as **glucokinase** only phosphorylate glucose if the concentration of glucose is high enough; its Km for glucose is 100 times higher than that of hexokinases I, II, and III.

- **Hexokinase IV** (Glucokinase **)** serves as a glucose sensor in beta cells of the pancreatic islets to control insulin release, and similarly controls glucagon release in the alpha cells.


OH
H
O
H
H
OH
H
HO
OH
H
OH
Glucose
Hexokinase
ATP
ADP
O
HO
P
OH
O
H
O
H
H
OH
H
HO
OH
H
OH
Glucose 6-phosphate

Let’s revise

Q.1 Define activation energy.

Q.2 What are the different factors that affect rate of enzyme catalysed reaction.

Q.3 What is the effect of substrate concentration on enzyme catalysed reaction?

Q.4 Write Michaelis-Menten equation. What is its significance?

Q.5 Define Km

Let's revise

Q.6 Describe Lineweaver-Burk plot.

Q.7 Describe the kinetic properties of allosteric enzymes.

Q.8 What are isozymes? Write a note on different isozymes of lactate dehydrogenase.

Q.9 Write a note on different isozymes of Hexokinase and compare their properties.