King Saud University

Foundation Block

Enzyme & Coenzyme

Lecture 7 + 8

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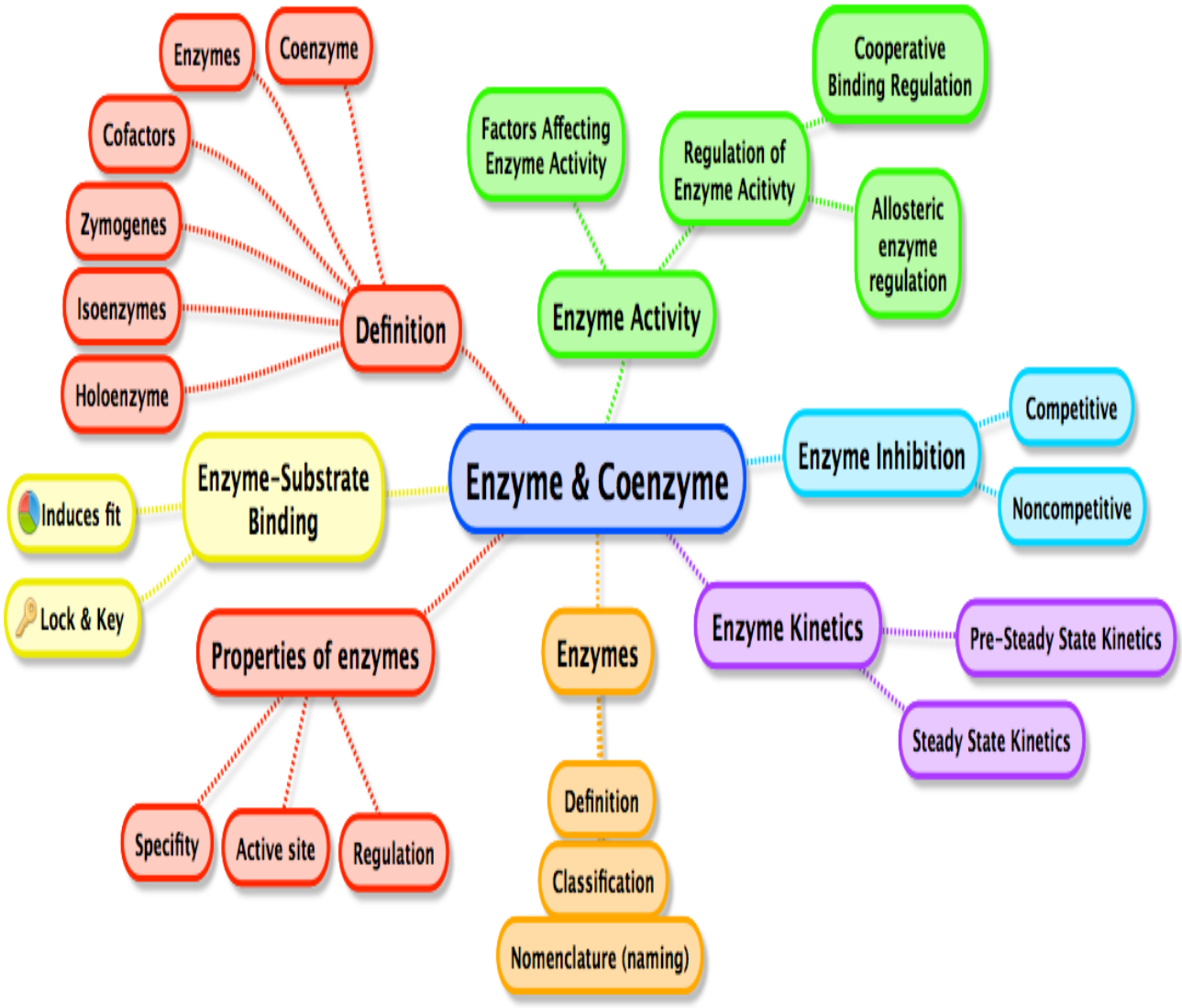
Additional ⇒ Purple

Mind Map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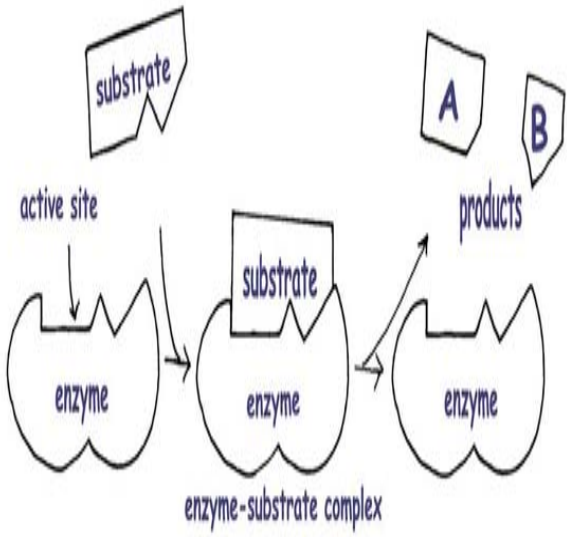
Enzymes

- They are biological catalysts محفز that speed up the rate of a reaction without being consumed by the reaction.

- All enzymes are proteins, but not all proteins are enzymes.

Substrate is the substance that enzymes act on, and convert it into products.



Classification of Enzymes

Enzymes are classified into 6 classes according to the reaction catalyzed

	Classification	Type of Reaction Catalyzed
1	Oxidoreductases	Oxidation-Reduction reactions
2	Transferases	Transfer of functional groups
3	Hydrolases	Hydrolysis reaction
4	Lyases	Group elimination to form double bonds
5	Isomerases	Isomerization
6	Ligases	Bond formation + ATP hydrolysis

Enzyme Nomenclature (Naming)

Enzyme Nomenclature is based on the rules given by IUBMB (International Union of Biochemistry and Molecular Biology.

EC 3.4.17.1 (Carboxypeptidase)

EC = Enzyme Commission

Class.subclass.subsubclass.Enzyme number

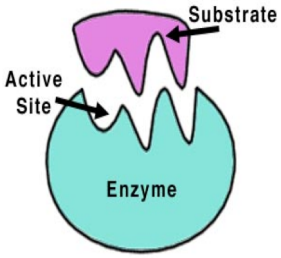
Properties of Enzymes

Active Site

- The region of enzyme that binds with substrate

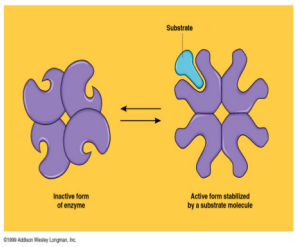
- Where Catalysis occur

- All enzymes have 1 or more active sites



Regulation

- Enzymes can be activated or inhibited so that the rate of product formation responds to the need of the cell.



Specificity

- Enzymes bind to their specific substrates in the active site to convert them to products

- Enzymes are highly specific

- They interact with only one or few substrates

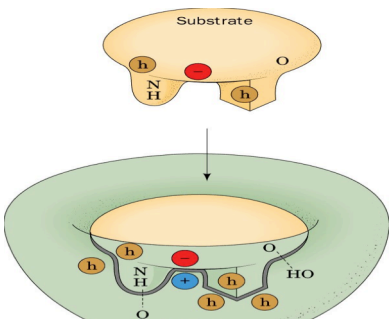
- Catalyze only one reaction

Enzyme-Substrate Binding

Lock and Key Binding

The enzyme has an active site that fits the exact dimensions of the substrate.

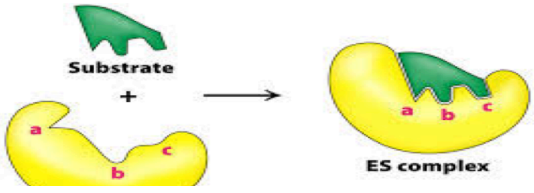
From the beginning the active site is complementary to the shape of substrate.



Induces Fit Binding

In this case, after binding to the substrate, the enzyme changes its shape to fit more perfectly with substrate.

The active is not complementary to the substrate, but when binding happens the active site changes its confirmation according to the substrate.



- Some enzymes require non-protein groups to become active.
-The inactive form of the enzyme "without its non-protein part" is called Apoenzyme.
-The active form of the enzyme "with its non-protein part" is called Holoenzyme.

Apoenzyme (Inactive) + **Non-protein part** = **Holoenzyme (active)**

This non-protein part can either be

Coenzyme

If the non-protein part is a small organic molecule its called Coenzyme.

and they are also divided into 2 types

1-Prosthetic Groups

They are coenzymes hat are **permanently** attached with an enzyme e.g. FAD

2-Cosubstrates

They are coenzymes that only **temporarily** associate with an enzyme e.g. NAD

or

Cofactor

If the non-protein part is a metal ion such as Cu+2, Fe+3, Zn+2 its called Cofactor

Permanently = All the time

Temporarily = Not all the time

Riboenzymes

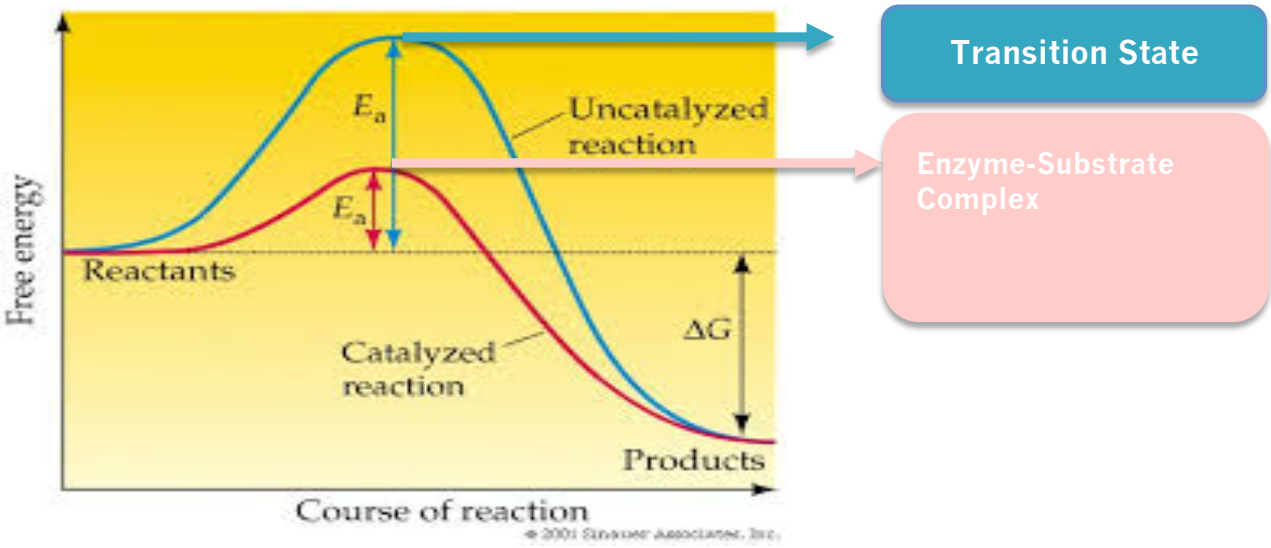
RNAs with enzyme activity

Isoenzymes

Enzymes that catalyze the same reaction, but they have slightly different structure.

Zymogens

They are inactive enzyme precursors that require a biochemical change to become active. Usually, the biochemical change is removing a peptide chain from the active site, so the zymogen becomes active.



Transition State: it's a state that reactants pass through, in every chemical reaction, which has greater energy than reactant and products alone. "without enzymes"

Activation Energy (Ea): The deference in energy between the reactants and the transition state + It's the energy required to

- If the activation energy is available, then the reaction can be proceed forming production.

Free Energy GΔ: The difference in energy between the reactants and the products. ADDITIONAL BUT IMPORTANT TO KNOW.

Enzymes decrease the activation energy, but they don't affect free energy GΔ. "Check the graph"

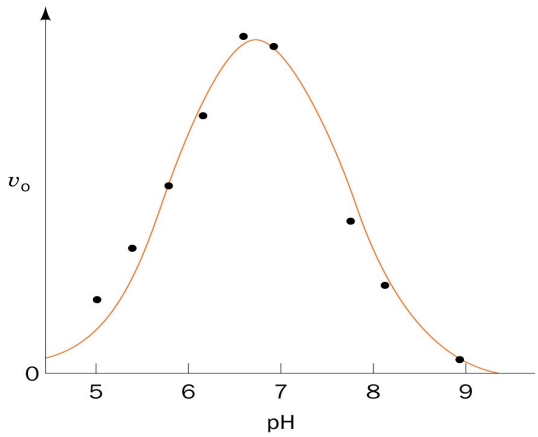
- Enzymes provide alternative transition state with lower activation energy called Enzyme Substrate Complex, thus it speeds up the reaction.

Enzyme Activity or Velocity

It is the rate of a reaction catalyzed by an enzyme.

Measured in millimole of product formed/Minute/mg of enzyme.

Factors Affecting Enzyme Activity

Temperature	pH	Concentration of Enzyme [E] and Concentration of Substrate [S]
- Every enzyme has an optimal''perfect'' temperature for catalyzing a reaction. - At first, the enzyme reaction increase with increasing temperature - If the temperature is very HIGH, enzymes are denatured and become inactive. - Most human enzymes have an optimal energy of 37° C.	- any effect of pH on the ionizable groups in the active site or in the substrate can affect catalysis. - Every enzyme has an optimal pH for catalyzing - Most enzymes have highest activity between 6 pH and 8 pH - Pepsin has highest activity at pH 2 because it works in the stomach which is an acidic medium. The bell-shaped curve  	-Reaction y ,initially, increases with increasing substrate concentration [S] but when the enzyme reaches saturation, the addition of substrate will not affect enzyme velocity - At low substrate concentration [S], the reaction velocity is low. - If the substrate concentration is higher than enzyme, the rate of an enzyme reaction will be directly proportional to the concentration of enzyme.

Enzyme Kinetics

Michaelis and Menten first proposed the model of enzyme kinetics ‘‘Movement

E = Enzyme

S = Substrate

P = Product

Pre-steady state kinetics

When an enzyme is mixed with high [S], there is an initial short of period of time (few hundred microseconds) during which intermediates* build up, that leads to the formation of product

Steady State Kinetics

· After initial state, the reaction rate and the concentration of intermediates change **slowly** with time called steady state reaction.

· An intermediate is called steady state **when** its rate of synthesis is equal to its rate of degradation

The Michaelis Menten Equation:

It describes the relationship of the initial rate of an enzyme to the concentration substrate [S]. It measures the initial velocity (v_o) of an enzyme reaction.

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

V_{max} = maximum velocity

K_m = Michaelis constant

K_m (Michaelis Constant)

is the substrate concentration[S] at which the initial rate is one-half of the maximum rate (½ V_{max})

It is the [S] required to saturate half of all of the active sites of an enzyme

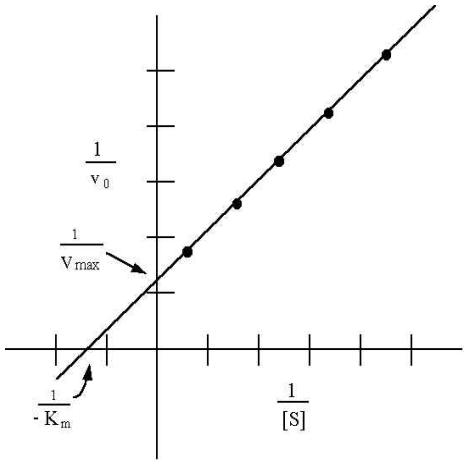
K_m value of a substrate depends on its affinity with the enzyme

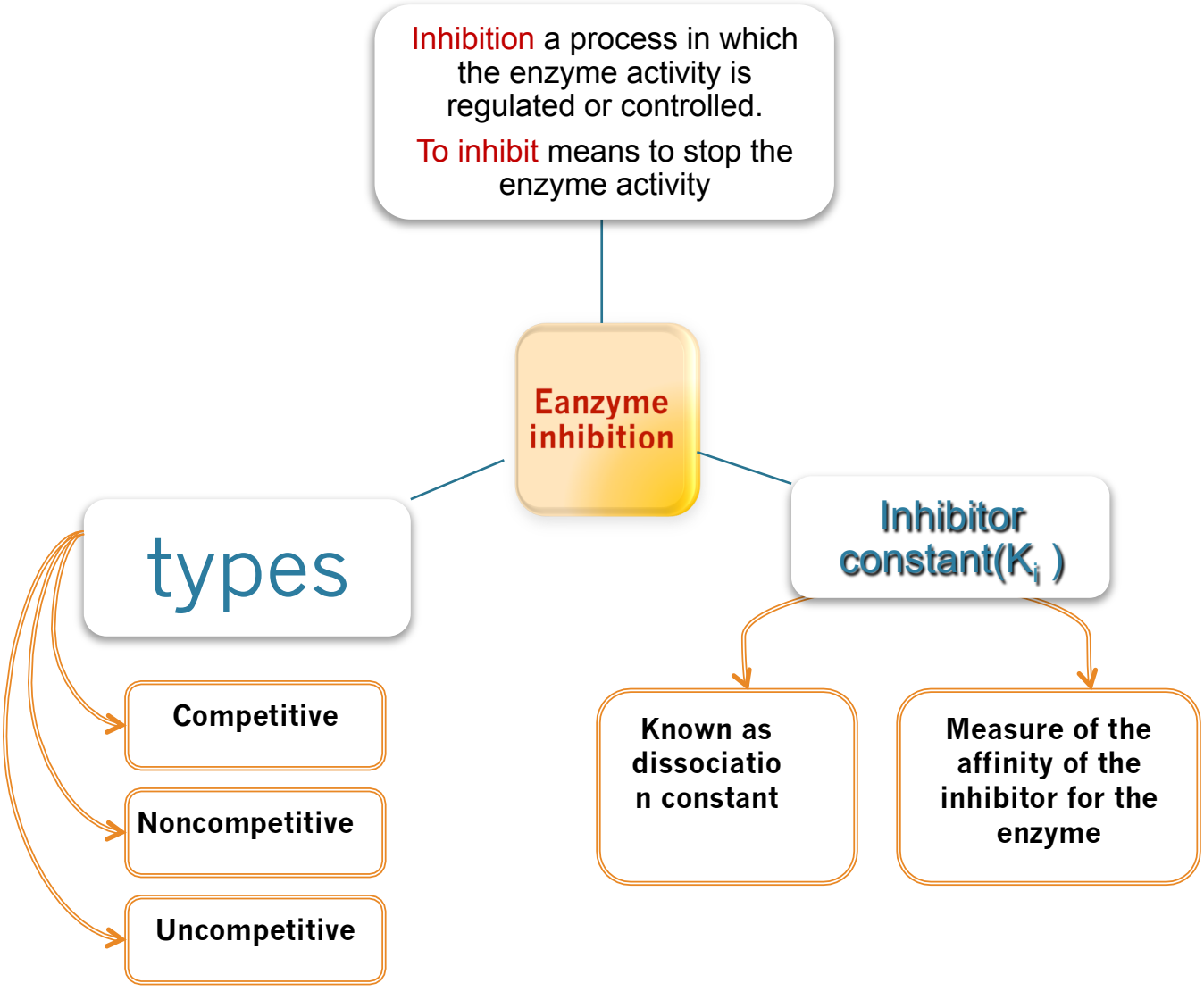
High K_m means low affinity with enzyme (more substrate needed to saturate the enzyme)

Low K_m means high affinity with enzyme (less substrate needed to saturate the enzyme)

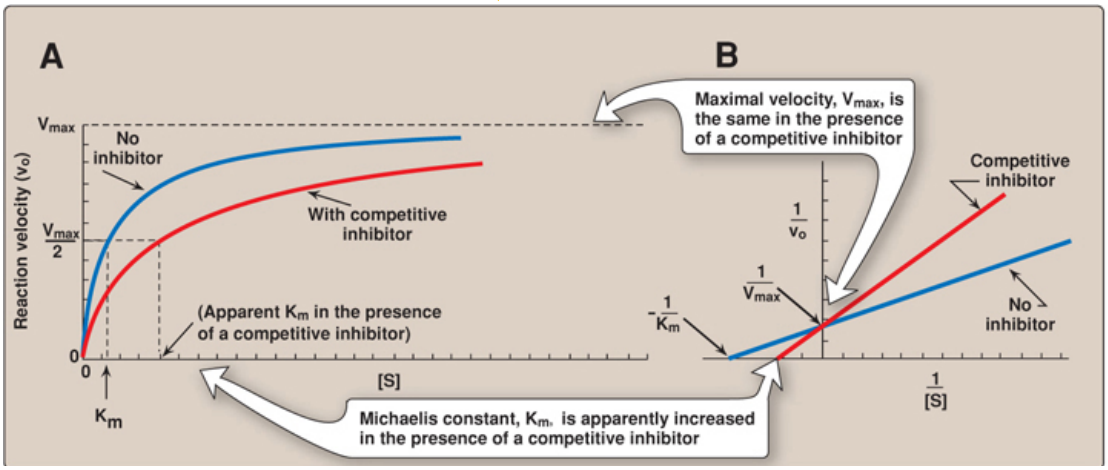
Lineweaver-Burk plot

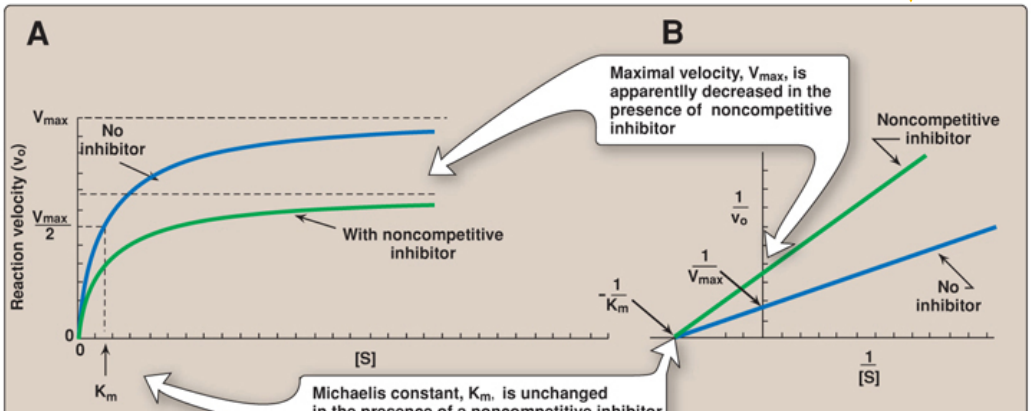
- **Also called the double-reciprocal plot، obtained by taking reciprocals of the Michaelis Menten equation**
- **It is plotted to calculate the Km and Vmax values and to determine the mechanism of action of enzyme inhibitors**




Inhibition a process in which the enzyme activity is regulated or controlled.
To inhibit means to stop the enzyme activity
Eanzyme inhibition
types
Competitive
Noncompetitive
Uncompetitive
Inhibitor constant(K_i)
Known as dissociatio n constant
Measure of the affinity of the inhibitor for the enzyme

	Competitive inhibition	Noncompetitive inhibition
Inhibitor shape	**Inhibitor & substrate are Structural analogue**	**Inhibitor does not have structural similarity to the substrate**
Competition with substrate	**<u>Competes with</u> the substrate for binding <u>at the active</u> site of enzyme**	**Inhibitor binds to the enzyme at a site <u>away from</u> the substrate binding site (No competition exists between the inhibitor and the substrate).**
Possible equilibria	• **E + S ⇔ ES → E + P (active)** • **E + I ⇔ EI (inactive)**	• **ES + I ⇔ ESI (inactive)** • **E + I ⇔ EI (inactive)**
Value of V_{max}	**<u>Unchanged</u> in the presence and the absence of inhibitor**	**Decreased by the inhibitor**
Value of K_m	**Increased (because substrate and inhibitor compete for binding at the same site)**	**K_m is unchanged (because the affinity of S for E is unchanged)**
	Higher concentration of substrate is required to achieve half-maximal velocity	





Regulation of enzyme activity

Characteristi

- **Regulatory enzymes usually catalyze the first or an early reaction in a metabolic pathway**
- **They catalyze a rate limiting reaction that controls the overall pathway**
- **They may also catalyze a reaction unique to that pathway known as committed step**

FEEDBACK INHIBITION

When the end product of a metabolic pathway exceeds its concentration limit, it inhibits the regulatory enzyme to normalize the pathway

FEED POSITIVE ACTIVATION:

When the end product of a metabolic pathway is below its concentration limit, it activates the regulatory enzyme to normalize the pathway

Types of regulation

Know that:

- The **term "allosteric" came from Greek word "allos" meaning "other"**
- **E= Enzyme**
- **S= Substrate**

Allosteric enzyme regulation	**Cooperative binding**
Compounds **that bind to enzyme other than the catalytic site.** **Binding of an allosteric modulator causes a change in the conformation of the enzyme** **Change in the binding affinity of enzyme for the substrate**	**Process by which binding of a ligand to a regulatory site affects binding of the same or of another ligand to the enzyme**

Modulator may be

- Positive: increased E, S affinity(activation)
- Negative decreased E, S affinity(inhibition)

The subunits are known as protomers

Most allosteric enzymes are oligomers (two or more polypeptide chains or subunits)

Allosteric enzyme regulation

Types of interactions occur in allosteric enzymes

Homotropic: Effect of one ligand on the binding of the same ligand (a regulatory enzyme modulated by its own substrate)

Heterotropic: Effect of one ligand on the binding of a different ligand

Enzymatic diagnosis and prognosis of diseases

Enzymes are used clinically in three ways:

- As indicators of enzyme activity or conc. in body fluids (serum, urine) in the diagnosis/prognosis of diseases
- As analytical reagents in measuring activity of other enzymes or compounds in body fluids
- As therapeutic agents

Serum markers in the diagnosis of diseases

- Heart disease
- Pancreatic diseases
- Liver

The most commonly used body fluids for measuring enzyme activity are

- **Serum**
- **Plasma**

There are

- **Plasma-specific enzymes**
- **Nonplasma-specific enzymes**