BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

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Unit-3

Enzyme inhibition

Dr. A. Antony Joseph Velanganni
Associate Professor
Department of Biochemistry

Enzyme inhibition occurs when a molecule (inhibitor) binds to an enzyme and decreases its activity. This can be classified into reversible and irreversible inhibition, based on whether the enzyme regains activity after the inhibitor is removed. Here's an overview of the types:

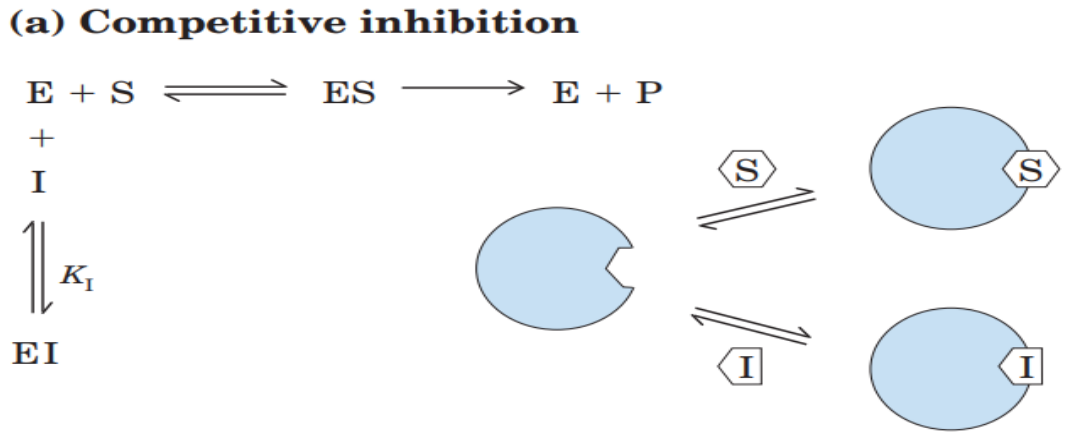
1. Reversible Inhibition

Reversible inhibitors bind to enzymes temporarily and can dissociate to restore enzyme activity. The major types are:

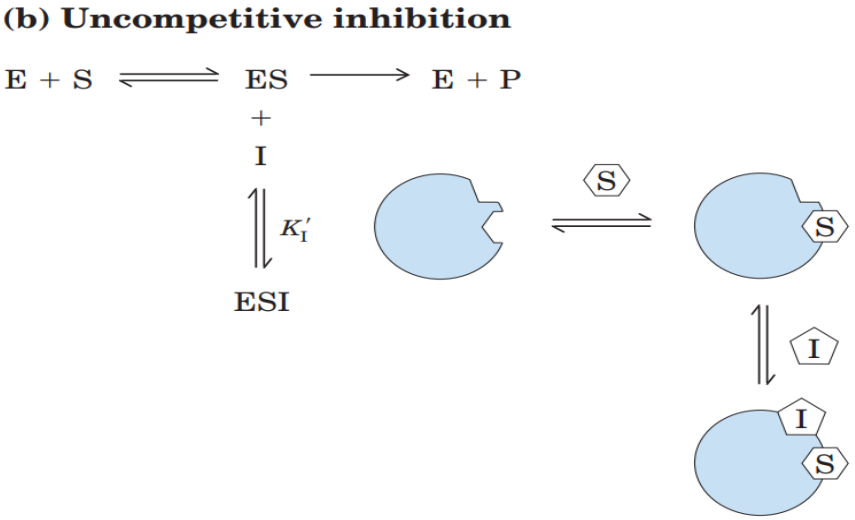
a. Competitive Inhibition

- Mechanism: The inhibitor competes with the substrate for the active site of the enzyme.
- Effect: Reduces the rate of reaction because the substrate cannot bind when the inhibitor occupies the active site.
- Reversal: Can be overcome by increasing substrate concentration.
- Impact on Kinetics:
- VmaxV_{\text{max}}Vmax: Remains unchanged (can be achieved at high substrate concentrations).
- KmK_mKm: Increases (indicating reduced substrate affinity).

Example: Malonate inhibits succinate dehydrogenase by competing with succinate.



(a) **Competitive inhibitors bind to the enzyme's active site.**



(b) **Uncompetitive inhibitors bind at a separate site, but bind only to the ES complex. KI is the equilibrium constant for inhibitor binding to E; KI is the equilibrium constant for inhibitor binding to ES**

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b. Non-Competitive Inhibition

- Mechanism: The inhibitor binds to an allosteric site (not the active site), causing a conformational change that reduces enzyme activity.
- Effect: Inhibitor affects the enzyme regardless of whether the substrate is bound.
- Reversal: Cannot be overcome by increasing substrate concentration.
- Impact on Kinetics:
  - VmaxV_{\text{max}}Vmax: Decreases (less active enzyme is available).
  - KmK_mKm: Remains unchanged (substrate binding is not affected).

Example: Heavy metals like lead inhibit enzymes by binding to sulfhydryl groups.

c. Uncompetitive Inhibition

- Mechanism: The inhibitor binds only to the enzyme-substrate complex, preventing the release of products.
- Effect: Reduces both substrate binding and catalysis.
- Impact on Kinetics:
  - VmaxV_{\text{max}}Vmax: Decreases.
  - KmK_mKm: Decreases (substrate binding appears to improve).

Example: Certain herbicides inhibit enzymes in photosynthesis via uncompetitive inhibition.

d. Mixed Inhibition

- Mechanism: The inhibitor can bind to either the enzyme alone or the enzyme-substrate complex, but with different affinities.
- Effect: A combination of competitive and non-competitive effects.
- Impact on Kinetics:
  - VmaxV_{\text{max}}Vmax: Decreases.
  - KmK_mKm: Can increase or decrease depending on the binding affinity.

Example: Certain allosteric regulators.

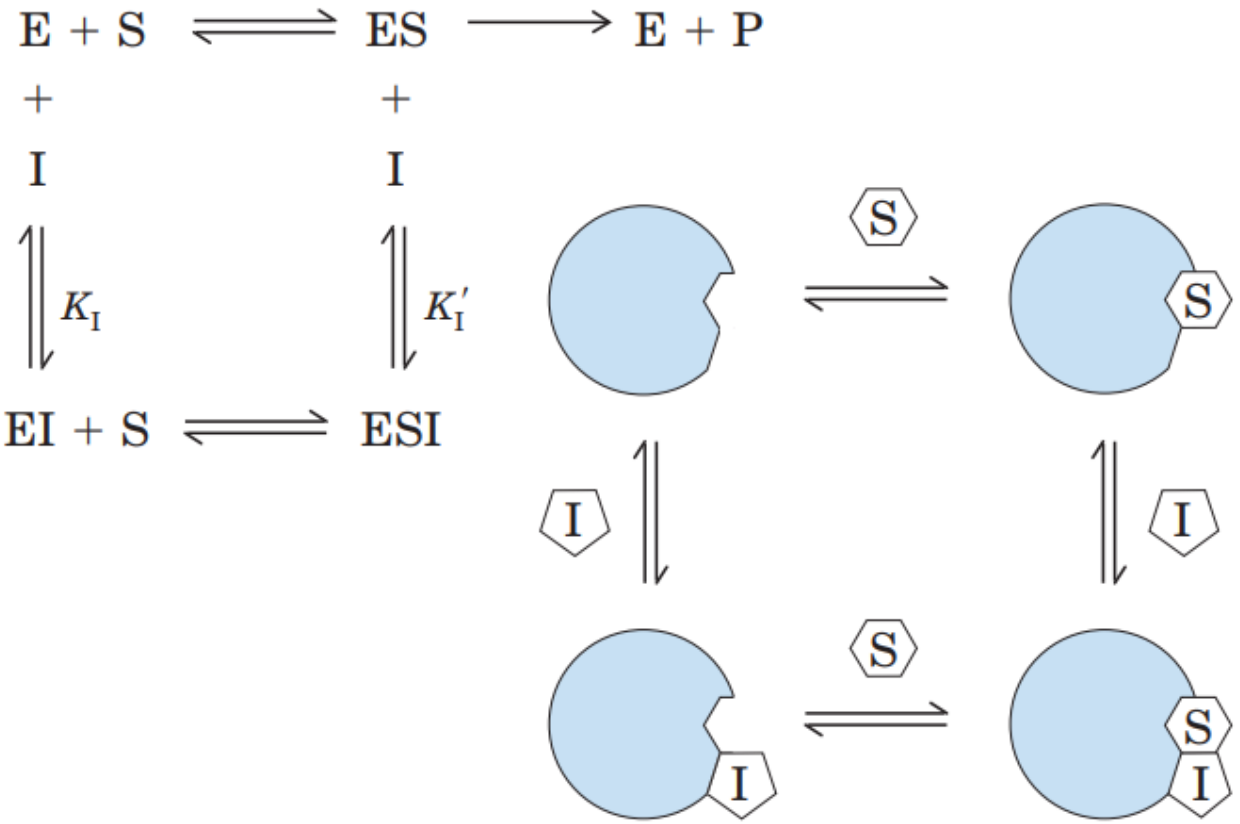
2. Irreversible Inhibition

Irreversible inhibitors bind covalently or tightly to the enzyme, permanently inactivating it. The enzyme cannot regain activity even after the inhibitor is removed.

Mechanism:

- Covalent modification or destruction of critical amino acid residues in the enzyme's active site.
- The inhibition is permanent and requires new enzyme synthesis to restore activity

(c) Mixed inhibition



(c) Mixed inhibitors bind at a separate site, but may bind to either E or ES.

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Examples:

1. **Aspirin**: Irreversibly inhibits cyclooxygenase (COX) enzymes by acetylating a serine residue.
2. **Penicillin**: Inhibits bacterial transpeptidase, preventing cell wall synthesis.
3. **Organophosphates**: Irreversibly inhibit acetylcholinesterase, leading to nerve dysfunction.

3. Allosteric Inhibition :

A special type of inhibition where the inhibitor binds to an allosteric site, altering the enzyme's shape and reducing its activity. This can be reversible or irreversible and is common in feedback regulation.Example: ATP acts as an allosteric inhibitor of phosphofructokinase in glycolysis.

Type	Binding Site	Effect on Vmax	Effect on Km
Competitive	Active site	No change	Increases
Non-Competitive	Allosteric site	Decreases	No change
Uncompetitive	Enzyme-substrate complex	Decreases	Decreases
Irreversible	Active or allosteric site	Decreases	No change or irrelevant

The proximity and orientation effect is a critical concept in enzymatic catalysis, ensuring that substrates are brought into an optimal position and orientation for a reaction to occur. Enzymes utilize several factors to achieve this, enhancing reaction rates significantly. Here are the key factors contributing to proximity and orientation in enzymes:

1. Active Site Architecture

- The enzyme's **active site** is specifically shaped to bind the substrate in a way that positions reactive groups close to one another.
- The three-dimensional structure ensures the substrate is held in the correct alignment for effective catalysis.

2. Substrate Binding Specificity

- Enzymes exhibit **specificity** for their substrates due to precise interactions such as hydrogen bonding, ionic bonds, and hydrophobic interactions.
- This ensures the substrate is bound in the correct orientation relative to the active site.

3. Catalytic Groups in the Active Site

• Specific amino acid residues in the active site contribute to proximity and orientation by:

- **Polarizing bonds**: Weakening specific bonds in the substrate to favor the reaction.
- **Positioning functional groups**: Aligning reactive groups like -OH, -NH_2, or -COOH for optimal interaction.
- 4**. Substrate-Induced FitIn** some enzymes, binding of the substrate induces a conformational change in the enzyme (induced fit), improving substrate alignment and proximity for catalysis.
- 5. **Multisubstrate Reactions**For reactions involving two or more substrates, enzymes often arrange them in the correct spatial orientation to facilitate interaction:Example: DNA polymerase aligns nucleotides with the template strand during DNA synthesis.
- 6**. Conformational FlexibilityEnzymes** are not rigid; their flexible regions allow minor adjustments to align substrates better and stabilize the transition state.
- 7**. Covalent Intermediates**Some enzymes form temporary covalent bonds with substrates, holding them in the correct position for reaction.
- 8. **Electrostatic Interactions**Charged residues in the active site create an electrostatic environment that:Attracts substrates.Holds them in a precise orientation conducive to reaction.

9. Enzyme-Substrate Complex Stability

- Non-covalent interactions (hydrogen bonds, Van der Waals forces) stabilize the enzyme-substrate complex, ensuring proper alignment for catalysis.

10. Macroenvironmental Factors

- The **pH and ionic strength** around the enzyme can influence its conformation, helping maintain the correct orientation of active site residues and substrates.

Proximity and Orientation Benefits:

- **Reduced Activation Energy**: Correct substrate positioning minimizes the energy required to reach the transition state.
- **Enhanced Reaction Rate**: By holding substrates close and in the right orientation, the enzyme facilitates faster bond formation or cleavage.
- **Specificity**: Ensures the enzyme catalyzes the desired reaction without side reactions.

Examples

1.Chymotrypsin:

Positions peptide bonds near a serine residue to facilitate cleavage.

2.Carbonic Anhydrase:

Holds water and carbon dioxide in the correct orientation for hydration to bicarbonate.

3.ATP Synthase:

Precisely aligns ADP and inorganic phosphate for ATP synthesis.

Proximity and orientation effects underline how enzymes efficiently overcome kinetic barriers to catalysis, exemplifying the elegance of biological systems.

Mechanism of Action: Lysozyme

Lysozyme is an enzyme that catalyzes the hydrolysis of the β-(1→4) glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) in the peptidoglycan layer of bacterial cell walls. This action compromises bacterial integrity, leading to cell lysis.

Steps of Lysozyme Mechanism:

1.Substrate Binding:

1. The active site of lysozyme has subsites (A-F) that bind six sugar residues of the substrate.
2. Residues at position **D** are distorted into a half-chair conformation, making the glycosidic bond more susceptible to cleavage.

2.Catalysis:

1. Lysozyme operates via **acid-base catalysis** involving two key amino acids: **Glu35** (acid catalyst) and **Asp52** (nucleophile).
2. **Step 1: Protonation**:
    1. Glu35 donates a proton to the glycosidic oxygen, destabilizing the bond and creating a positively charged oxocarbenium ion transition state.

3. **Step 2: Nucleophilic Attack**:
    1. Asp52 stabilizes the oxocarbenium ion by forming a covalent intermediate with the C1 carbon of the sugar at site D.
4. **Step 3: Hydrolysis**:
    1. A water molecule, activated by Glu35, attacks the covalent intermediate, regenerating the enzyme and releasing the cleaved products.

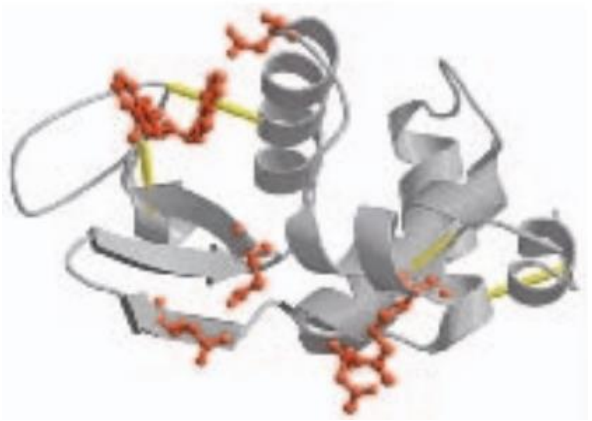
3.Product Release:

1. The cleaved polysaccharides (NAG and NAM fragments) are released, and lysozyme is free to catalyze another reaction.

Key Features:

• Lysozyme lowers the activation energy by stabilizing the distorted substrate and the transition state.

• This enzymatic action is critical for the immune defense system in humans (e.g., in tears, saliva, and mucus).



3D structure of lysozyme

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RIBONUCLEASE A

Mechanism of Action: Ribonuclease (RNase A)

Ribonuclease (RNase A) catalyzes the hydrolysis of RNA, specifically cleaving the phosphodiester bonds between nucleotides.

Steps of RNase A Mechanism:

1.Substrate Binding:

RNase A binds single-stranded RNA in its active site, which contains two key histidine residues: His12 and His119.

RNA is positioned so that the phosphate group of the target bond is between these residues.

2.Catalysis:

RNase A operates via a two-step acid-base catalysis mechanism.

Step 1: Cleavage of Phosphodiester Bond:

His12 acts as a base, abstracting a proton from the 2'-OH group of the ribose, generating a 2'-oxyanion.

The 2'-oxyanion performs a nucleophilic attack on the phosphate group, breaking the phosphodiester bond.

His119 donates a proton to the 5'-OH group of the leaving ribose, completing the first step.

Step 2: Hydrolysis of the Cyclic Intermediate:

A cyclic 2’,3’-phosphate intermediate is formed.

His119 activates a water molecule, which hydrolyzes the cyclic intermediate into a 2’-phosphate nucleotide.

3.Product Release:

The hydrolyzed RNA fragments are released, and RNase A is regenerated for further catalysis.

Key Features:

- RNase A is highly specific for RNA due to its ability to bind ribose sugars selectively.
- It is critical in cellular RNA turnover and defense mechanisms, such as in antiviral responses

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