Cofactors -Coenzymes

==We have seen that most enzymes are simple globuar proteins. Some others are conjugated proteins which have non-protein fraction called prosthetic group.== A prosthetic group is essential co-factor attached to protein part of a conjugated enzyme. Some enzymes need the presence of co-factors. Co-factors are non-protein substances that take part in enzymatic reactions and are regenerated for further reaction. Metal ions frequently play such role. They are one of two important classes of cofactors. The other important class is that of coenzyme. ==Coenzymes are organic non-protein substances which are not bonded to enzyme molecules like other prosthetic groups.==

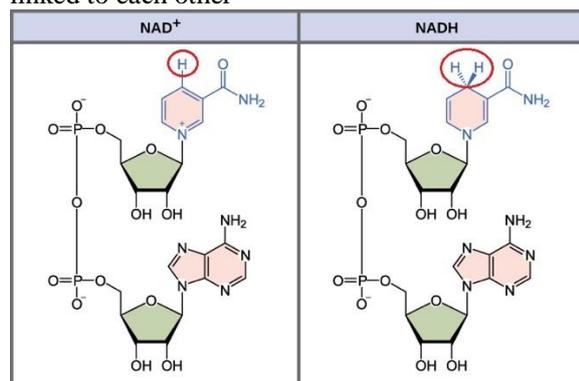
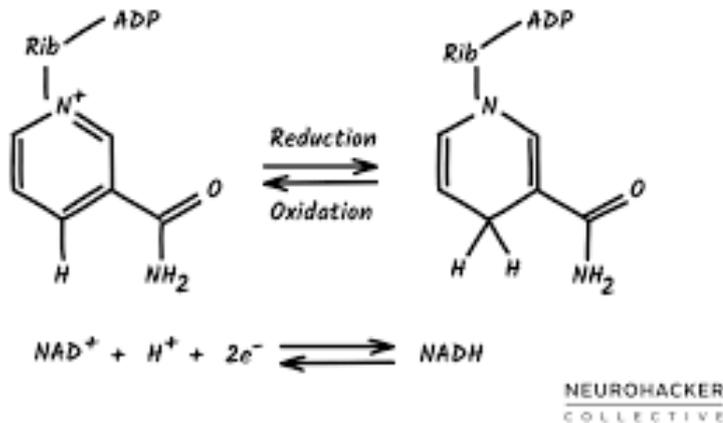
A prosthetic group is an essential co-factor attached to the protein part of a conjugated enzyme. If the prosthetic group is removed the enzymes fails to function. Mineral ions must be mixed with reactants before some enzymes will work. It is partly for those reasons that minerals are essential to living organisms.

Metal ions are Lewis acids i.e., electron acceptors. Therefore they can act as Lewis acid base catalysts. They can form coordination compounds by behaving as Lewis acids. these coordination compounds are an important part of metal ions in biological systems. These coordination compound formed by metal ions tend to have specific geometries, which help in positioning the groups involved in the reaction for optimum catalysis. zinc, iron and magnesium act as enzyme activator in this way. e==.g., Zn(II) in carboxypeptidase and Fe(III) in haemoglobin.==

Several important coenzymes are vitamins and their derivatives. Nicotinamide adenine dinucleotide (NAD) and its phosphate ester (NADP) are derived from nicotinamide, a B-group vitamin(B_3-Niacin). Riboflavin(Vitamin B_2) forms part of flavin-adenine dinucleotide, (FAD). Many of these coenzymes are involved in oxidation-reduction reactions which provide energy for the organism. Other serve as group transfer agents in metabolic processes.

Some Coenzymes with their Reactions and Vitamins Precursors

Coenzyme	Reaction Type	Vitamin Precursor
Biotin	Carboxylation	Biotin
Coenzyme A	Acyl transfer	Pantothenic acid
Flavin Coenzymes	Oxidation-reduction	Riboflavin(B_2)
Lipoic acid	Acyl transfer	
Nicotinamide-adenine coenzymes	Oxidation -reduction	Niacin
Pyridoxal phosphate	Transamination	Pyridoxine(B_6)
Thiamine pyrophosphate	Aldehyde transfer	Thiamine (B_1)

Nicotinamide adenine dinucleotide(NAD^+) is a coenzyme which takes part in many oxidation-reduction reactions. Its structure has three parts- a nicotinamide ring, an adenine ring and two phosphate group linked to each other

The phosphorylated forms of the B_6 vitamins, pyridoxal, pyridoxamine and pyridoxine are important coenzymes. They are involved in the transfer of amino groups from one molecule to another, an important step in the biosynthesis of amino acids.

Pyridoxal-5-phosphate is a versatile coenzyme which takes part in variety of reactions required for the synthesis and catabolism of alpha amino acids.

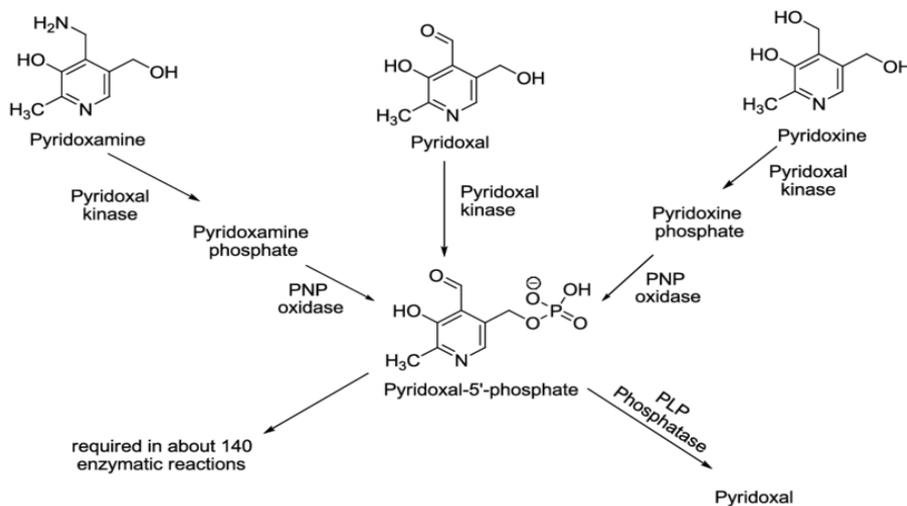



Figure 3: Metabolism of B_6 vitamers (Salvage pathway)

CoFactor

A **cofactor** is a non-protein chemical compound or metallic ion that is required for an enzyme's activity as a catalyst (a catalyst is a substance that increases the rate of a chemical reaction). **Cofactors** can be considered "helper molecules" that assist in biochemical transformations.

Cofactor, a component, other than the protein portion, of many enzymes. If the cofactor is removed from a complete enzyme (holoenzyme), the protein component (apoenzyme) no longer has catalytic activity. A cofactor that is firmly bound to the apoenzyme and cannot be removed without denaturing the latter is termed a prosthetic group; most such groups contain an atom of metal such as copper or iron. A cofactor that is bound loosely to the apoenzyme and can be readily separated from it is called a coenzyme. Coenzymes take part in the catalysed reaction, are modified during the reaction, and may require another enzyme-catalysed reaction for restoration to their original state.

A **coenzyme** is an organic non-protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often broadly called cofactors, but they are chemically different. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme.

Holoenzymes

- Some enzymes require molecules other than proteins for enzymatic activity.
- The term **holoenzyme** refers to the **active** enzyme with its **nonprotein** component.
- The term **apoenzyme** is **inactive** enzyme without its **nonprotein** part.
- If the **nonprotein part** is a **metal ion** such as Zn 2+ or Fe2+, it is called a **cofactor**.
- If it is a **small organic molecule**, it is termed a **coenzyme.**

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ENZYME INHIBITORS

An inhibitor, as the name implies is a substance that interferes with the action of an enzyme and slows the rate of a reaction. many substances inhibit the activity of enzymes. enzymes are sensitive to a variety of chemical reagent that react with groups at or near the surface of the protein, producing partial or complete inhibition of chemical activity. Inhibitors fall into categories reversible and non-reversible.

A.Reversible inhibitors

Reversible inhibitors are substances which prevent enzyme from combining with the substrates. activity of the enzyme is restored when inhibitor is removed. A reversible inhibitor can bind to the enzyme and subsequently be released, leaving the enzyme in its original condition .
there are 2 major classes or reversible inhibitors-

1.Competitive inhibitors :

These compete with the normal substrate molecule for combining with the active site of the enzyme. they can bind to the active site and block the substrate from binding to the enzyme. Thus, they affect enzyme action by becoming attached to the active centres stop in the substrate from binding to the enzyme. Such inhibitors are very similar in structure to the substrate.

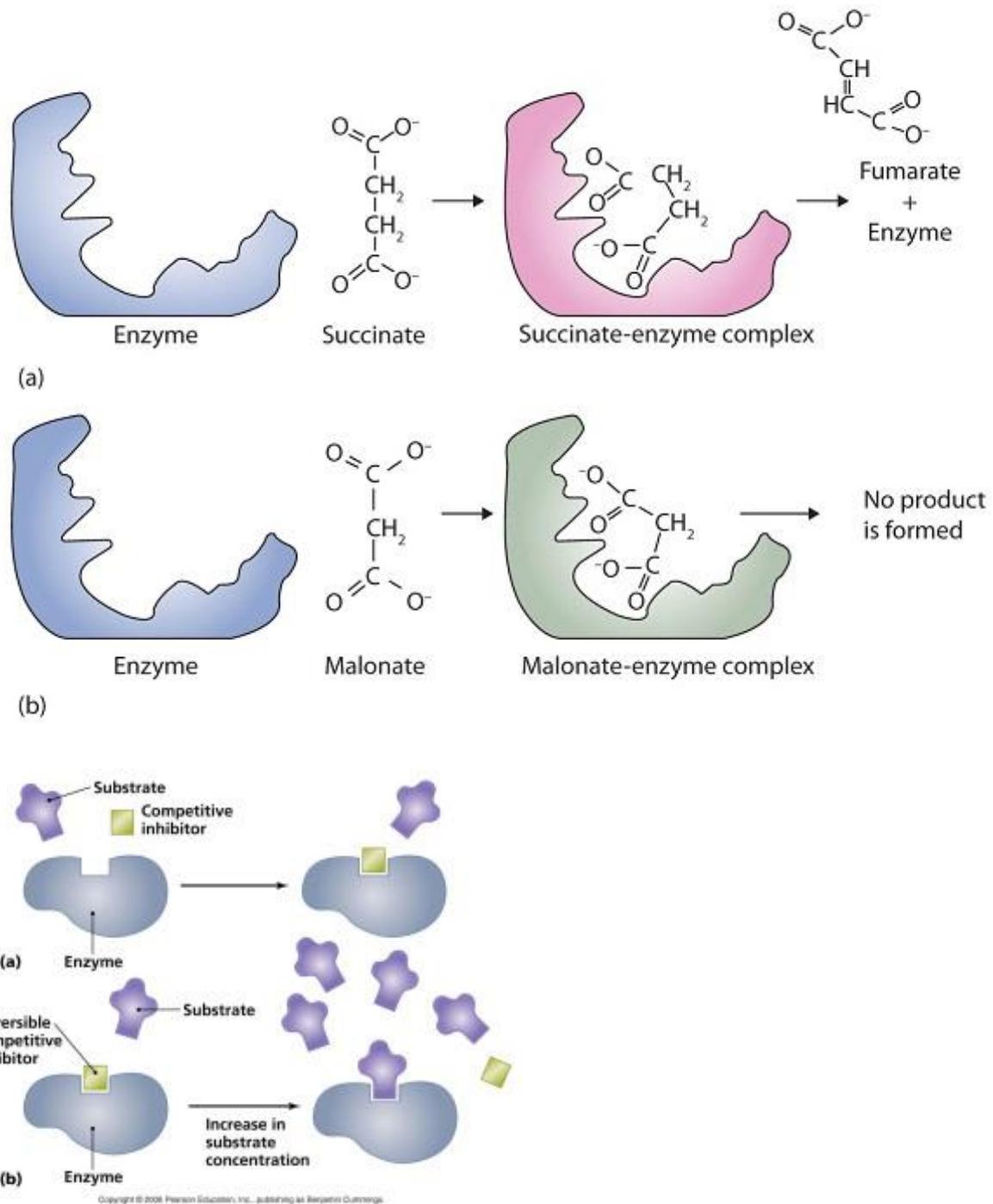
An example of this behaviour is inhibition of the enzyme succinate dehydrogenase by malonic acid. succinate dehydrogenase catalyse the oxidation succinate to fumarate in the kreb's cycle. in the presence of malonate reaction rate is slowed down malonate has a molecular structure very similar to that of succinate. Inhibition occurs because the active centre of

some of the enzyme molecules become occupied by malonate rather than by succinate. In fact, both malonate and succinate are competing for the active site of the enzyme.

(a)
Enzyme — Succinate → Succinate-enzyme complex → Fumarate + Enzyme

(b)
Enzyme — Malonate → Malonate-enzyme complex → No product is formed

(a) Substrate, Competitive inhibitor, Enzyme

(b) Reversible competitive inhibitor, Substrate, Enzyme, Increase in substrate concentration

The degree of inhibition by a competitive inhibitor is less if the ratio of substrate to inhibitor is high.

2.Non-Competitive Inhibitors:

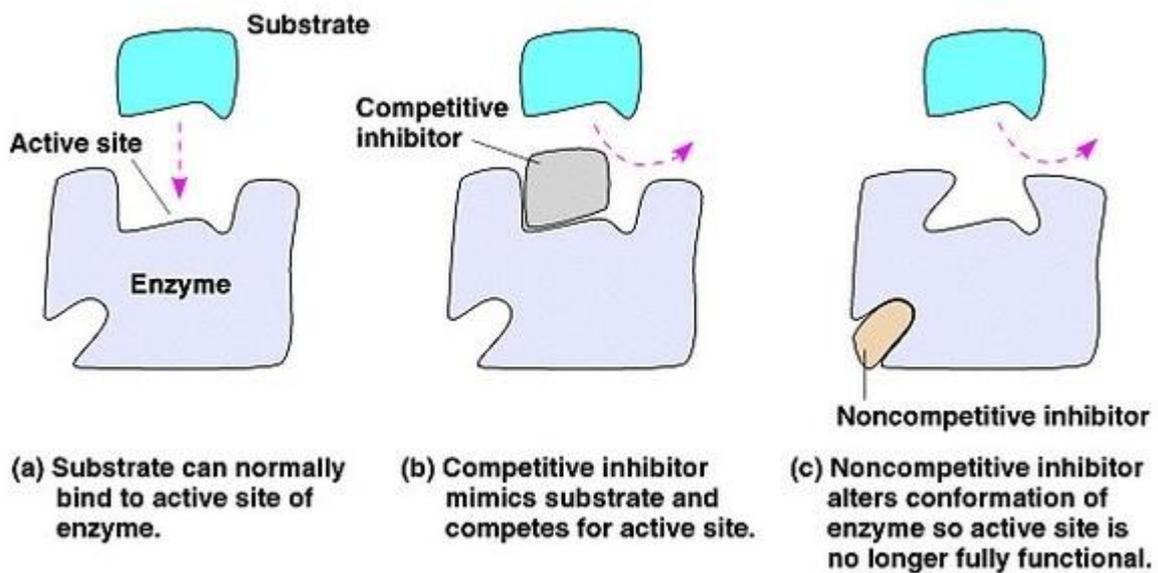
In this case, the inhibitor becomes attached to the enzyme at a site other than the active site. As a result of this binding a change is caused in the structure of the enzyme, especially around the active site.

The substrate is still able to find to the active site, but the enzyme cannot catalyse the reaction when the inhibitor is bound to it. Thus, the enzyme, the substrate or possibly both become changed so that enzyme activity stops.

Disulphide bridges are important in maintaining the tertiary structure of enzyme molecules. if the disulphide bridges are broken in three-dimensional shape of enzyme changes. The ions of heavy metals such as Mercury silver copper affect the enzyme in this way.Hg^{2+}, Ag^+ and Cu^{2+} ions combine with thiol(-SH) groups in enzymes. They denature enzyme molecule and thus, inhibit enzyme activity. Cyanide is another non-competitive inhibitor. It blocks the action of some enzymes by combining with iron which may be present in a prosthetic group or which may be required as an enzyme activator. For this reason, salts of heavy metals and cyanide are potent to living organisms. However, non-competitive inhibitors do not bind strongly to enzymes and can be removed by dialysis. Enzyme activity is then restored.

(a) Substrate can normally bind to active site of enzyme.

(b) Competitive inhibitor mimics substrate and competes for active site.

(c) Noncompetitive inhibitor alters conformation of enzyme so active site is no longer fully functional.

Competitive inhibition	Non-competitive inhibition
1. In this type of inhibition, the chemical structure and shape of substrate and inhibitor are quite similar.	1. In this type of inhibition, the chemical structure and shape of substrate and inhibitor are different.
2. In this, inhibitors bind to the active site of enzyme.	2. In this, inhibitors bind to the allosteric site of enzyme.
3. Here, inhibitor does not change the shape of the active site of enzyme.	3. Here, inhibitor changes the shape of the active site of enzyme.
4. If substrate concentration is increased, then inhibition rate is decreased.	4. Here, no effect of substrate concentration is on the inhibition rate of enzyme.
5. Example is succinate dehydrogenase substrate is inhibited by malonate inhibitor.	5. Example is pyruvate kinase is inhibited by alanine inhibitor.

Some enzymes possess additional sites known as allosteric sites beside the active site such enzymes are known as allosteric enzymes. The allosteric sites are unique places on the enzyme molecules; enzymes have one or more allosteric sites.

PROPERTIES OF ALLOSTERIC ENZYME

- Allosteric enzyme have one or more allosteric sites

- Allosteric sites are binding sites distinct from an enzyme active site or substrate binding site

- Molecule that bind to allosteric sites are called effector or modulator

- Effector may be positive or negative, this effector regulate the enzyme activity. The enzyme activity is increased when a positive allosteric effector binds at the allosteric site known as activator site. On the other hand, negative allosteric effector bind at the allosteric site called inhibitor site and inhibit the enzyme activity.

- Binding to allosteric sites alter the activity of the enzyme, this is called cooperative binding. Allosteric enzymes display sigmoidal plot of V_0 vs [S].

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Allosteric Inhibition Inhibits Enzymatic Activity

To control the speed of metabolic reactions, we have what is called **allosteric inhibition.** Allosteric inhibitors slow down enzymatic activity by deactivating the enzyme. An allosteric inhibitor is a molecule that binds to the enzyme at an **allosteric site.** This site is not at the same location as the active site. Upon binding with the inhibitor, the enzyme changes its 3D shape.

Allosteric inhibition is a class of enzyme inhibition in which allosteric inhibitor inhibits enzyme activity by binding to the allosteric site of enzyme.

Allosteric inhibition is a form of **non-competitive inhibition.** This means that the inhibitor is not directly competing with the substrate at the active site. Instead, it is indirectly changing the composition of the enzyme.

After changing its shape, the enzyme becomes inactive. It can no longer bind with its corresponding substrate. This will then slow down the formation of subsequent products. Think of the allosteric inhibitor as a locksmith. The locksmith (i.e. allosteric inhibitor) changes the lock (i.e. enzyme) so that the key (i.e. substrate) will no longer be able to open the lock (i.e. enzyme).

Active site Enzyme

Distorted active site

Substrate

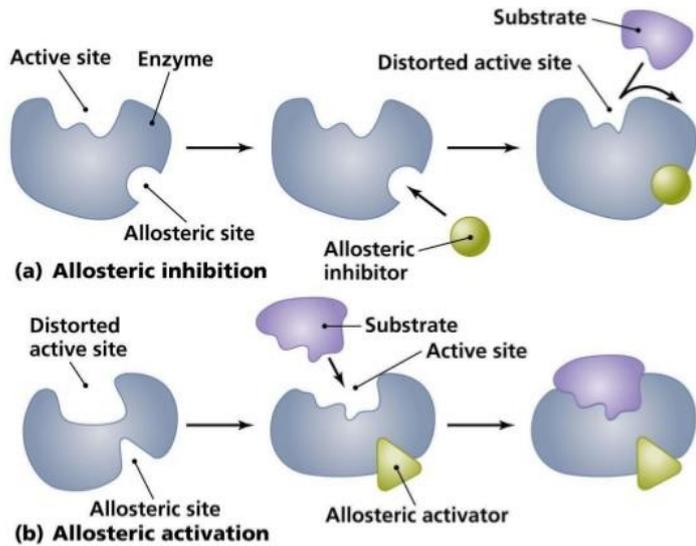
Allosteric site
(a) Allosteric inhibition

Allosteric inhibitor

Distorted active site

Substrate
Active site

Allosteric site
(b) Allosteric activation

Allosteric activator

B.Non-Reversible inhibitors

Non-reversible or irreversible inhibitors reacts with the enzyme to produce a protein that is not enzymatically active and from which original enzyme cannot be regenerated. The enzymes undergo irreversible inactivation when they are treated with agents capable of permanently modifying a functional group required for catalysis, making the enzyme molecule inactive.

Organophosphorus insecticide such as malathion are good examples of non-reversible inhibitors. they become firmly bound to active centre so that substrate molecules cannot bind to enzymes and activity of enzyme is permanently stopped. Insecticides of this type inactive the enzyme cholinesterase, which is essential for the functioning of the nervous system.

2) Irreversible inhibitor:

- Inhibitor binds at or near the active site of the enzyme irreversibly, usually by covalent bonds, so it can't dissociate from the enzyme.
- Irreversible inhibitors combine with the functional groups of the amino acids in the active site, irreversibly.
- Irreversible inhibitors occupy or destroy the active sites of the enzyme permanently and decrease the reaction rate.
- Enzyme activity is not regained on dialysis.

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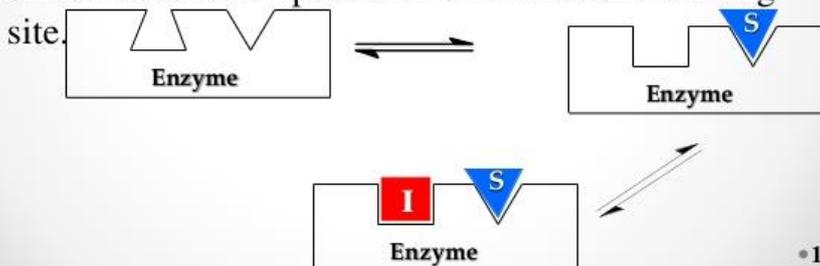
c) Uncompetitive inhibitor

- Uncompetitive inhibitors do not bind to the free enzyme. They bind only to the enzyme-substrate complex to yield an inactive E. S. I complex.
- Uncompetitive inhibitors frequently observed in multi substrate reaction.
- Inhibition can't be reversed by increasing the [S] since I doesn't compete with S for the same binding site.

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REVERSIBLE ENZYME INHIBITION
VERSUS
IRREVERSIBLE ENZYME INHIBITION

REVERSIBLE ENZYME INHIBITION	IRREVERSIBLE ENZYME INHIBITION
The process of binding inhibitors to the enzyme through monovalent interactions so that, once removed, they allow the restoring of the enzyme function	The process of binding inhibitors to the enzyme through covalent interactions so that, their dissociation takes a long time, permanently removing the enzyme action
Reversible inhibitors bind to the enzyme through non-covalent interactions such as hydrogen bonds, hydrophobic interactions, and ionic bonds in reversible enzyme inhibition	Irreversible inhibitors bind to the enzyme through covalent interactions, which modify amino acid residues by reactive functional groups in irreversible enzyme inhibition
The enzyme-inhibitor complex dissociates quickly	Dissociates very slowly in the irreversible enzyme inhibition
Enzymatic action can be restored	It takes a long time to restore the enzymatic action
Types: Competitive, uncompetitive, non-competitive, and mixed inhibition	Occurs through the covalent inactivation of the active site of the enzyme

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Reduce the catalytic activity of enzymes	Reduce the catalytic activity of enzymes
Slow dissociation of enzyme-inhibitor complex	Rapid dissociation of enzyme-inhibitor complex
Classified into three categories: group-specific reagents, substrate analogs, and suicide inhibitors	Classified into two categories: competitive and non-competitive inhibitors
Substrate analogs imitate enzyme substrate and irreversibly modify the active site of the enzyme	Competitive inhibition can be reversed by increasing substrate concentration

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