College of pharmacy

Biochemistry I

Third stage

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Lecture 7

Enzyme Inhibition, Regulation of enzyme activity, Isoenzymes, and Bi-Bi reactions

Enzyme Inhibition

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called an inhibitor. In general, enzyme inhibitors either irreversible or reversible.

1. Irreversible inhibition

These inhibitors act irreversibly by chemically modifying the enzyme. It bind to enzymes through covalent bonds. Since these covalent changes are relatively stable, an enzyme that has been "poisoned" by an irreversible inhibitor remains inhibited even after removal of the free inhibitor from the surrounding medium.

Example: Diisopropylphosphofluoridate (**DIPF**) inhibits the enzyme acetyl cholinesterase. Acetyl cholinesterase catalyzes the hydrolysis of acetylcholine (acetylcholine is important in the transmission of nerve impulses) to acetic acid and choline, a neurotransmitter substance functioning in certain portions of the nervous system.

2. Reversible inhibition

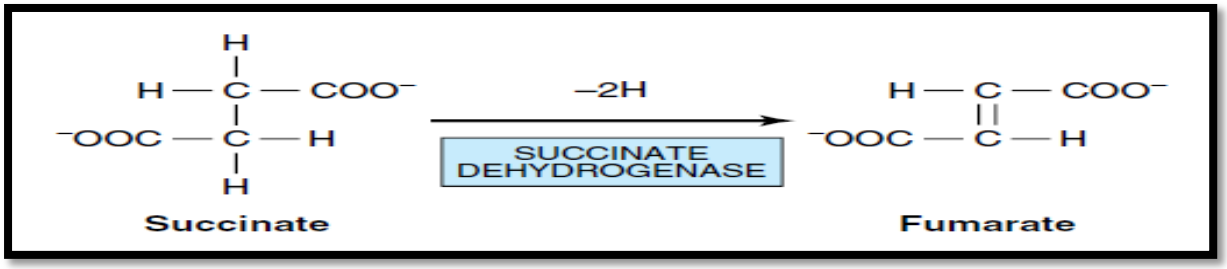
This type of inhibitor typically bind to enzymes through non-covalent bonds. The two most commonly encountered types competitive and noncompetitive inhibition:

A. Competitive Inhibition

This type of inhibition occurs when the inhibitor binds **reversibly** to the **same site** that the **substrate** would normally occupy, therefore, **competes** with the **substrate for that active site** as a result of **similarity in structure**. The enzyme substrate complex will be broken dawn to products, whereas enzyme inhibitor complex (EI) will not be broken down to P:

$$E+S \rightleftharpoons ES \rightarrow E+P$$
$$E+I \rightleftharpoons EI$$

Example: Inhibition of the enzyme succinate dehydrogenase by malonate illustrates competitive inhibition by a substrate analog. Succinate dehydrogenase catalyzes the removal of one hydrogen atom from each of the two methylene carbons of succinate. Both succinate and its structural analog malonate ($^{-}OOC{-}CH_2{-}COO^{-}$) can bind to the active site of succinate dehydrogenase, forming an ES or an EI complex, respectively. However, since malonate contains only one methylene carbon, it cannot undergo dehydrogenation.



B. Non-competitive inhibition

In non-competitive inhibition, the inhibitor binds at different site rather than the substrate binding site [non-competitive inhibitors bind reversibly either to the free enzyme or the ES complex to form the inactive complexes EI and ESI (enzyme substrate inhibition)]. When the inhibitor binds at this site there will be a change in conformation of the enzyme molecules, which leads to the reversible inactivation of the catalytic site.

Regulation of enzyme activity

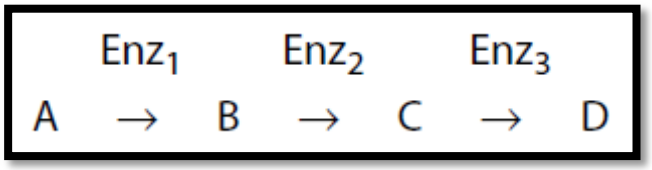
There are several means by which the activity of a particular enzyme is specifically regulated.

1. Covalent modification

Many enzymes may be regulated by covalent modification, most frequently by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme.

2. Allosteric modulation

Feedback inhibition refers to inhibition of an enzyme in a biosynthetic pathway by an end product of that pathway. For example, for the biosynthesis of D from A catalyzed by enzymes Enz_1 through Enz_3, high concentrations of D inhibit conversion of A to B. Typically, D binds at an **allosteric site** spatially distinct from the catalytic site of the target enzyme. In this example, the feedback inhibitor D acts as a negative allosteric effector of Enz_1. Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors.



3. Irreversible zymogens activation

Some enzymes are secreted in an inactive form called proenzymes or zymogens. At the site of action specific peptide bonds are hydrolyzed either enzymatically or by pH changes to convert it into active form, e.g. pepsinogen to pepsin, trypsinogen to trypsin, plasminogen to plasmin. After hydrolysis when it is activated, it cannot be reconverted into proenzyme form.

4. Induction and repression of enzyme synthesis

The cells can regulate the amount of enzyme present by altering the rate of enzyme degradation or, more typically, the rate of enzyme synthesis. The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites.

Isoenzymes (Isozymes)

These are enzymes having similar catalytic activity, act on the same substrate and produces the same product but originated at different site and exhibiting different physical and chemical characteristics such as electrophoretic mobilities and amino acid composition.

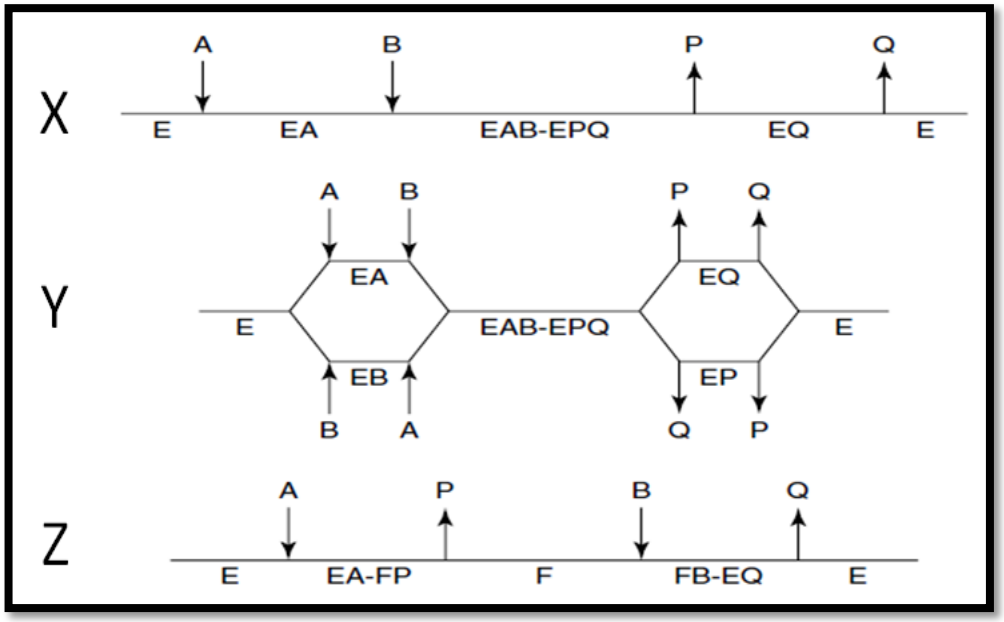
Example: L-Lactate dehydrogenase is a tetrameric enzyme whose four subunits occur in two isoforms, designated H (for heart) and M (for muscle). The subunits can combine as shown below:

Isozymes	Subunits
I_1	H H H H
I_2	H H H M
I_3	H H M M
I_4	H M M M
I_5	M M M M

Bi-Bi reactions

Many enzymes have a single substrate, many others have two and sometimes more than two substrates and products. **Two substrate and two product reactions** termed "**Bi-Bi**" reactions. **Bi-Bi** reactions include **sequential** and **ping-pong** Bi-Bi reactions. In **sequential** reactions, both substrates must combine with the enzyme to form a ternary complex before catalysis can proceed. **Sequential** reactions are sometimes referred to as **single displacement reactions**, this reactions either **ordered** Bi-Bi or **random** Bi-Bi reactions.

Ping-pong Bi-Bi reactions (double displacement reactions) this term "ping-pong" applies to mechanisms in which one or more products are released from the enzyme before all the substrates have been added.



Three classes of Bi-Bi reaction mechanisms.

X: An ordered Bi-Bi, Y: A random Bi-Bi, Z: A ping-pong reactions