بسم الله الرحمن الرحيم

Basic Principles and Perspectives in Medical Chemistry and Biochemistry

Enzymes

Part 2

8th Medical and Biochemistry (BIQC-101) Lecture
Second Semester

by
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Enzyme specificity, isoenzyme, inhibitor& enzyme regulation

Learning Objectives

1. Explain what an enzyme inhibitor is.
2. Distinguish between reversible and irreversible inhibitors.
3. Differentiate between competitive and noncompetitive inhibitors.
4. Discuss the biological role of isoenzymes and their use in clinical diagnosis.
5. Understand the bases of enzyme catalysis and the mechanisms of enzyme regulation.
6. Know the role of regulatory enzymes in controlling metabolic pathways and cellular responses.

Enzyme Specificity

Enzymes have varying **degrees of specificity** for substrates .Enzymes may recognize and catalyze

1. **Absolute** : catalyze one type of reaction for a single substrate .Ex. urease catalyzes only the hydrolysis of urea.
2. **Group** : catalyze one type of reaction for similar substrates. Ex. Hexokinase adds a phosphate group to hexoses.
3. **Linkage**: catalyze one type of reaction for a specific type of bond. Ex. Chymotrysin catalyzes the hydrolysis of peptide bonds.
4. **Stereospecificity:** Ex. Phenylalanine hydroxylase uses L-Phe not D-Phe

Isoenzymese

- Enzymes isolated from different organisms , catalysing same reactions but have same number amino acid with different sequence.
- Even within a single species, there may exist different forms of enzyme catalysing the same reaction. Differences may be:

1. Amino acid sequence
2. Some covalent modification
3. 3-D structure

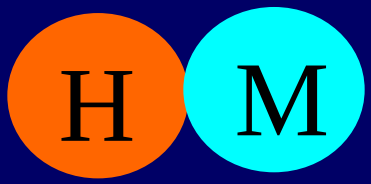
Lactate Dehydrogenase (LDH) Isoenzymes

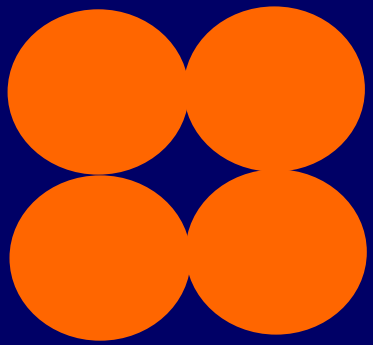
- Also known as lactic acid dehydrogenase. LDH plays an important role in making your body's energy. It is found in almost all the body's tissues.
- There are five types of LDH. They are known as **isoenzymes**. The five isoenzymes are found in different amounts in tissues throughout the body.

1. LDH-1: found in heart and red blood cells
2. LDH-2: found in white blood cells. It is also found in heart and red blood cells, but in lesser amounts than LDH-1.
3. LDH-3: found in lung tissue
4. LDH-4: found in white blood cells, kidney and pancreas cells, and lymph nodes
5. LDH-5: found in the liver and muscles of skeleton

Isoenzyme of lactose dehydrogenase

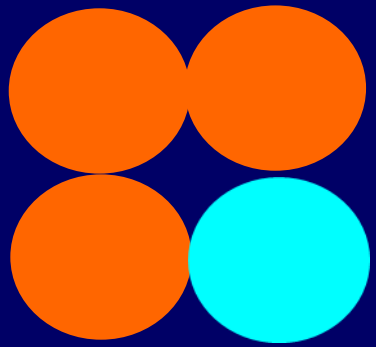
Highest levels found in the followings





H_4 (LDH_1)

Heart , red blood cells

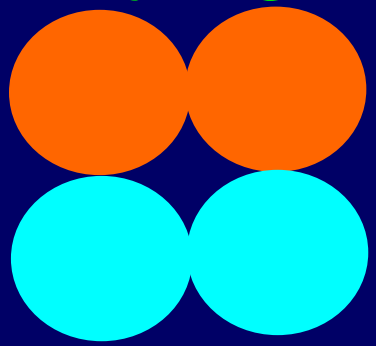


H_3M (LDH_2)

White blood cell, heat, red blood Cells lesser then LDH-1

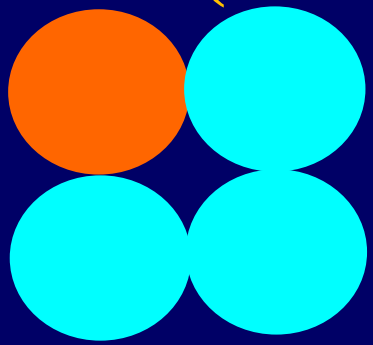
Isoenzyme of lactose dehydrogenase

Highest levels found in the followings



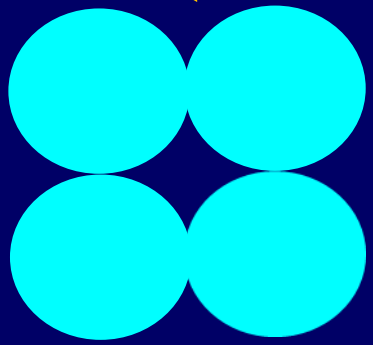
H_2M_2 (LDH_3)

Lung tissue



HM_3 (LDH_4)

White blood cells, kidney, pancreas cells, lymph nodes



H_4 (LDH_5)

liver , muscles of skeleton

Lactate Dehydrogenase (LDH) Isoenzymes Test

- This test measures the level of the different lactate dehydrogenase (LDH) isoenzymes in the blood.
- When tissues are damaged or diseased, they release LDH isoenzymes into the bloodstream. The type of LDH isoenzyme released depends on which tissues are damaged. This test can help your provider find out the location and cause of your tissue damage.
- An LDH isoenzymes test is used to find out the location, type, and severity of tissue damage. It can help diagnose a number of different conditions including:

1. Recent heart attack
2. Anemia
3. Kidney disease
4. Liver disease, including hepatitis and cirrhosis
5. Pulmonary embolism, a life-threatening blood clot in the lungs

Enzyme inhibition

Inhibitor (I) are molecules that cause a loss of enzyme activity when binds to an enzyme and prevents the formation of ES complex or breakdown it to E + P.

In a tissue and cell there are different chemical agents (**metabolites, substrate analogs, toxins, drugs, metal complexes** etc) can inhibit the enzyme activity.

Enzyme Inhibition

- An enzyme inhibitor is a molecule that disrupts the normal reaction pathway between an enzyme and a substrate

Types of Enzyme Inhibition

Inhibition of enzymes may be either reversible or irreversible depending on the specific effect of the inhibitor being used:-

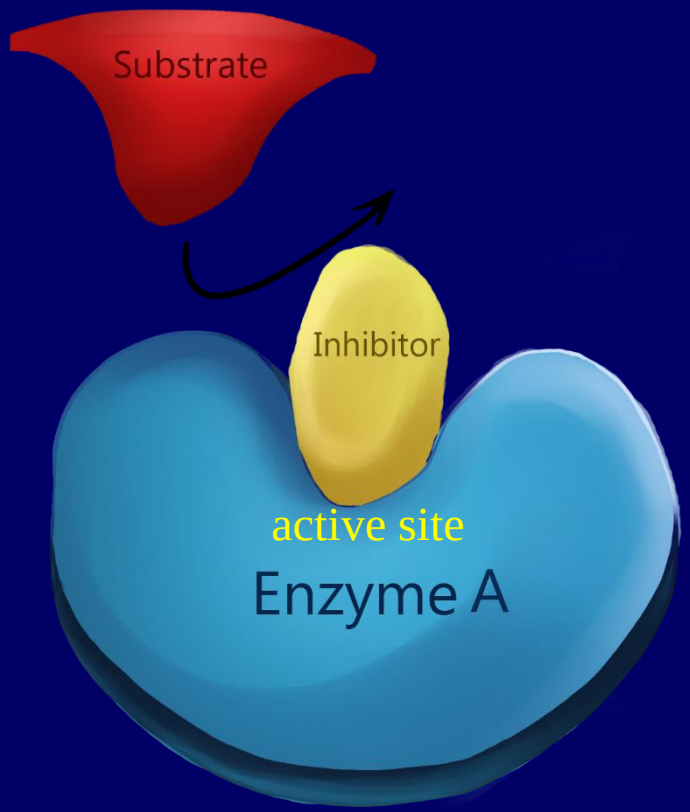
1. Reversible inhibitor :-

- Reversible inhibitors bind to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding.
- There are three kinds of reversible enzyme inhibitors:-

1. Competitive inhibition.
2. Non-competitive inhibition.
3. Mixed inhibition.

1. Competitive Inhibition

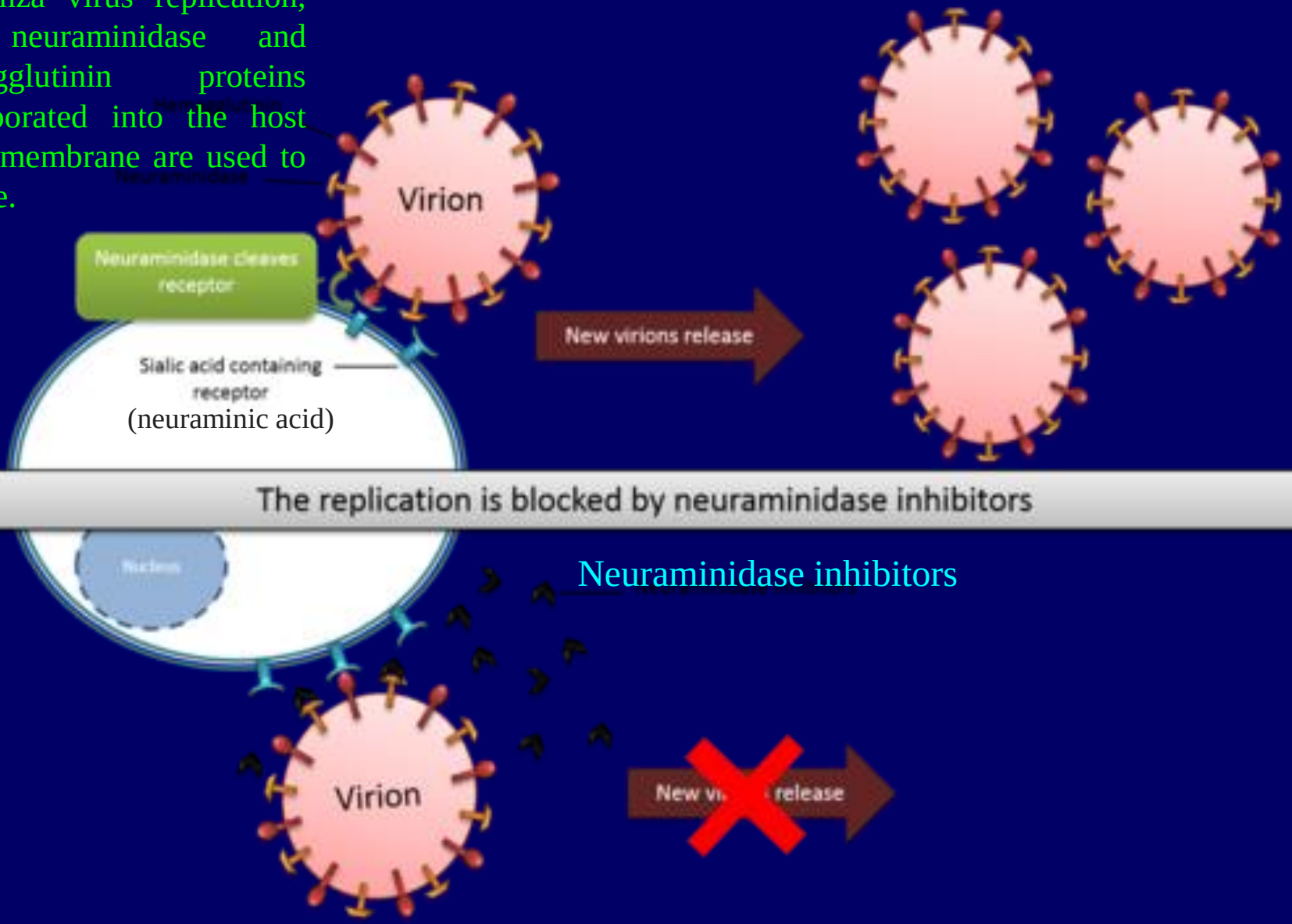
- Competitive inhibition involves a molecule, other than the substrate, binding to the enzyme's *active site*
- The molecule (inhibitor) is structurally and chemically similar to the substrate (hence able to bind to the active site)
- The competitive inhibitor blocks the active site and thus prevents substrate binding
- As the inhibitor is in competition with the substrate, its effects can be reduced by increasing substrate concentration



- An example of a use for a competitive inhibitor is in the treatment of influenza via the neuraminidase inhibitor, Relenza™
- Relenza is a synthetic drug designed by Australian scientists to treat individuals infected with the influenza virus
- Virions are released from infected cells when the viral enzyme neuraminidase cleaves a docking protein (haemagglutinin)
- Relenza competitively binds to the neuraminidase active site and prevents the cleavage of the docking protein
- Consequently, virions are not released from infected cells, preventing the spread of the influenza virus

The action of neuraminidase in replication of virions in influenza infection.

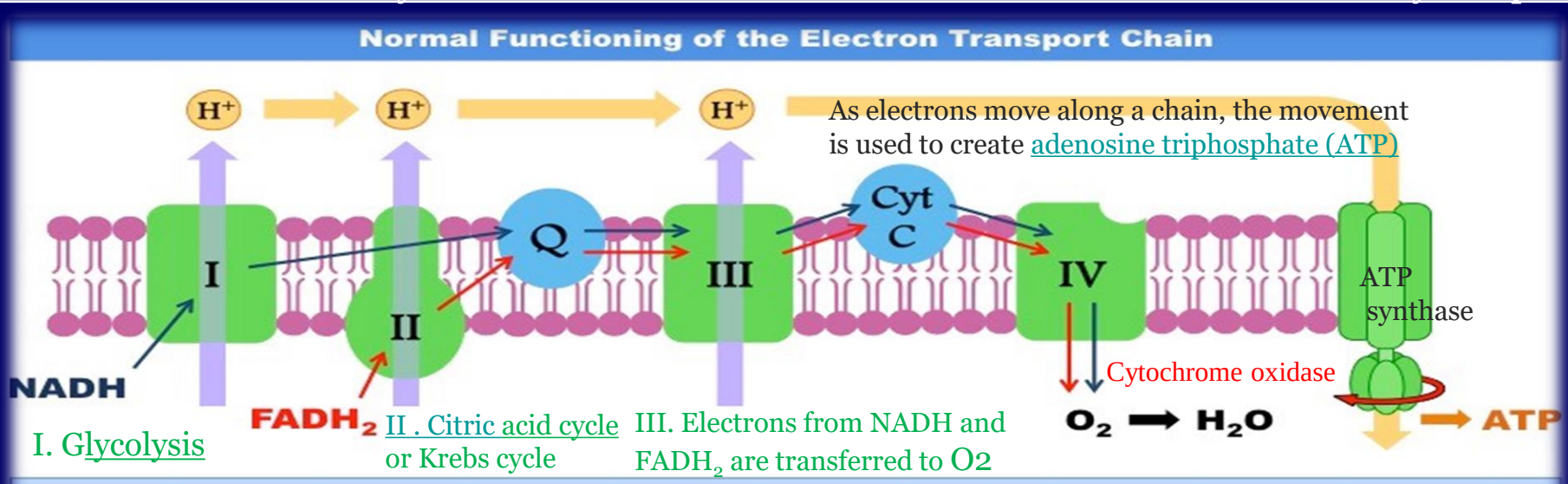
Influenza virus replication, the neuraminidase and hemagglutinin proteins incorporated into the host cell's membrane are used to escape.

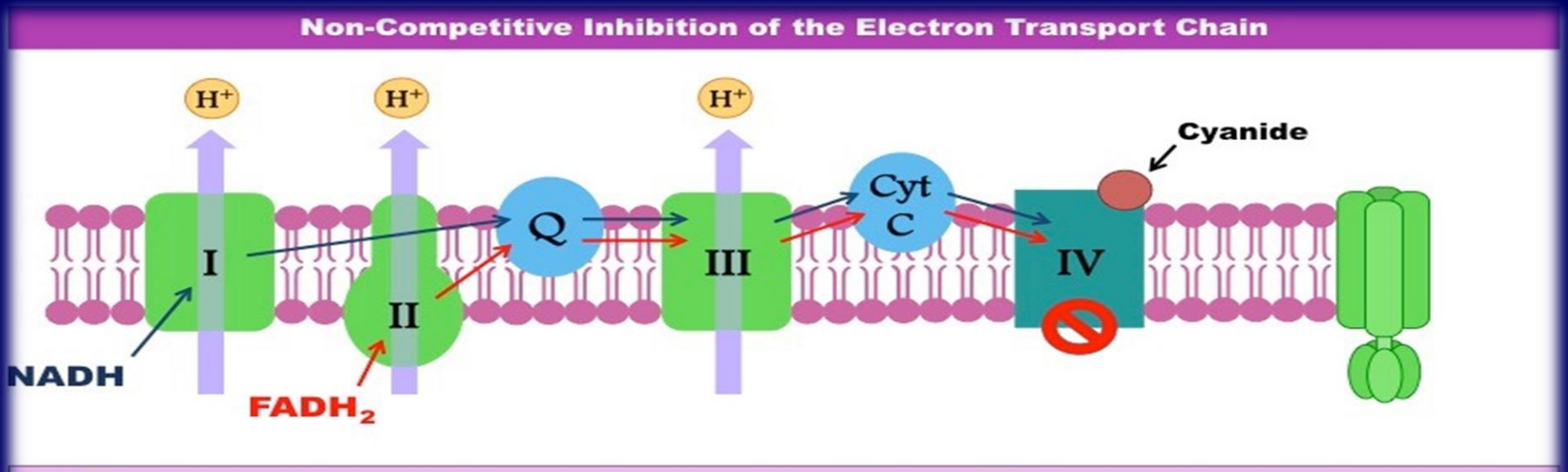


2. Noncompetitive Inhibition

- Non-competitive inhibition involves a molecule binding to a site other than the active site (an *allosteric site*)
- The binding of the inhibitor to the allosteric site causes a conformational change to the enzyme's active site
- As a result of this change, the active site and substrate no longer share specificity, meaning the substrate cannot bind
- As the inhibitor is not in direct competition with the substrate, increasing substrate levels cannot mitigate the inhibitor's effect

- An example of a use for a non-competitive inhibitor is in the use of cyanide as a poison (prevents aerobic respiration)
- Cyanide is a poison which prevents ATP production via aerobic respiration, leading to eventual death
- It binds to an allosteric site on cytochrome oxidase – a carrier molecule that forms part of the electron transport chain
- By changing the shape of the active site, cytochrome oxidase can no longer pass electrons to the final acceptor (oxygen)
- Consequently, the electron transport chain cannot continue to function and ATP is not produced via aerobic respiration


Normal Functioning of the Electron Transport Chain
H+
H+
H+
As electrons move along a chain, the movement is used to create adenosine triphosphate (ATP)
I
II
Q
III
Cyt C
IV
ATP synthase
NADH
FADH2
Cytochrome oxidase
O2 → H2O
ATP
I. Glycolysis
II . Citric acid cycle or Krebs cycle
III. Electrons from NADH and FADH2 are transferred to O2
The electron transport chain uses the shuttling of electrons to transport H+ ions into the intermembrane space.
The ions are returned to the matrix via ATP synthase and the electrons are removed from the chain by oxygen.

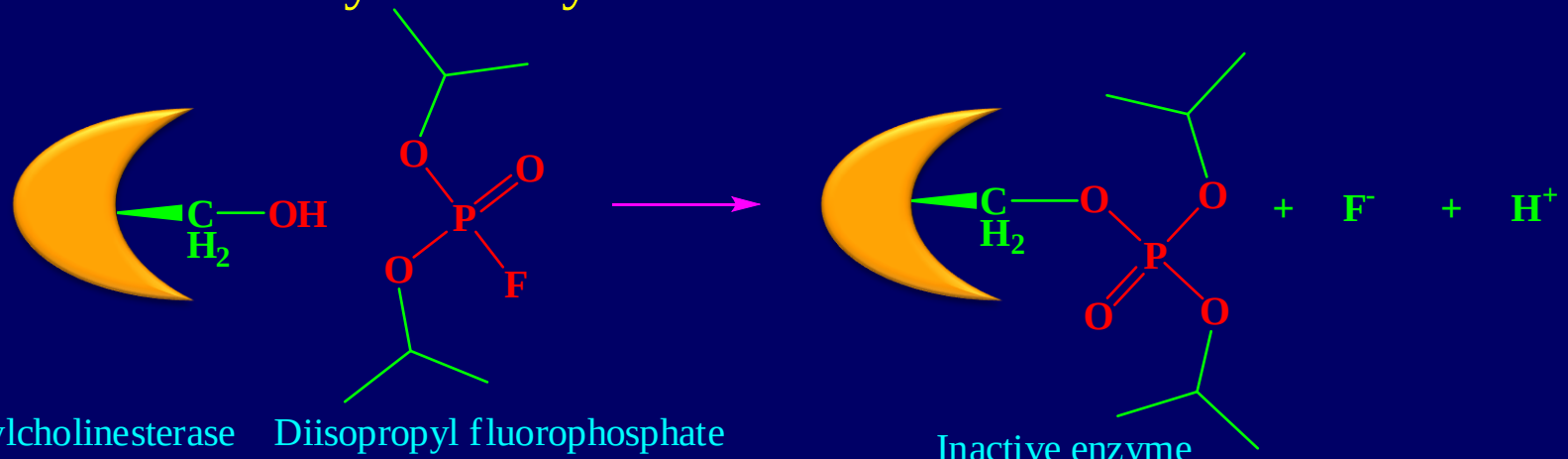

Non-Competitive Inhibition of the Electron Transport Chain
H+
H+
H+
Cyanide
I
II
Q
III
Cyt C
IV
NADH
FADH2
Cyanide binds non-competitively to cytochrome oxidase (complex IV) and alters the shape of its active site.
Consequently, electrons cannot be released to oxygen and the electron transport chain shuts down.

3. Mixed inhibition

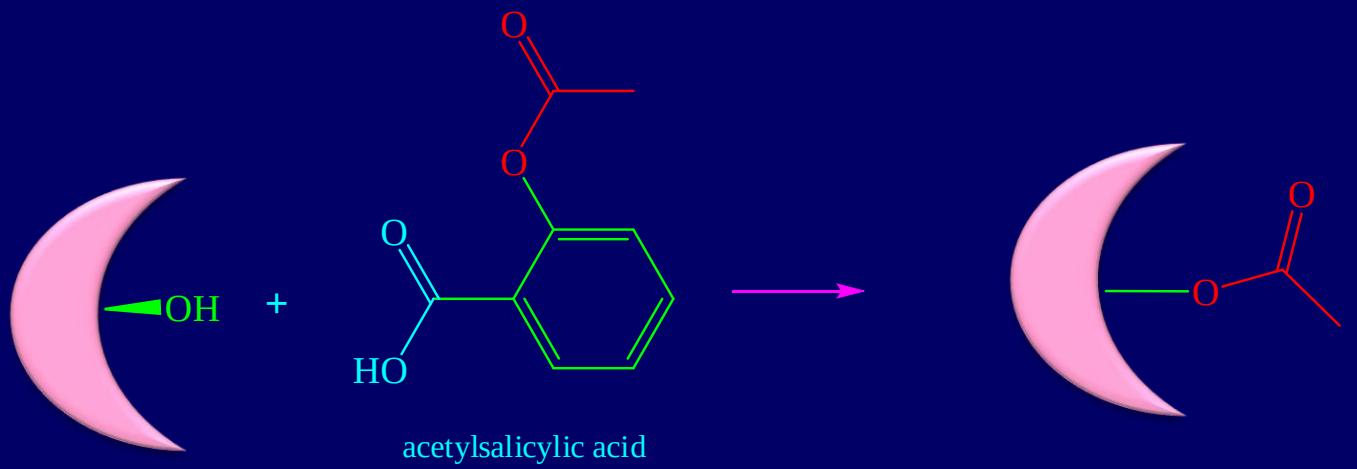
- The inhibitor can bind to the enzyme at the same time as the enzyme's substrate. However, the binding of the inhibitor affects the binding of the substrate, and vice versa.
- This type of inhibition can be reduced, but not overcome by increasing concentrations of substrate.
- Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect where the inhibitor binds to a different site on an enzyme.
- Inhibitor binding to this allosteric site changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced.

2. Irreversible inhibitors:-

- An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site.
- The inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate.
- The nerve gases, especially Diisopropyl fluorophosphate (DIFP), irreversibly inhibit biological systems by forming an enzyme-inhibitor complex with a specific OH group of serine situated at the active sites of certain enzymes. The peptidases trypsin and chymotrypsin contain serine groups at the active site and are inhibited by DIFP. Nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase.



Aspirin is an example of an irreversible inhibitor that actually forms a covalent bond with the enzyme. The aspirin (acetylsalicylic acid) transfers its acetyl group onto a serine residue on cyclooxygenase-2 (COX-2). This stops the production of in ammation-producing prostaglandins and thromboxanes by COX-2.



acetylsalicylic acid

Active cyclooxygenase-2 (COX-2)

In active cyclooxygenase-2 (COX-2)

Regulation of enzyme activity

- Regulation means controlling the activity of enzymes.
- Regulation of enzyme activity can be achieved by two general mechanisms:

1. **Control of enzyme quantity**

- As enzymes are protein in nature, they are synthesized from amino acids under gene control and degraded again to amino acids after doing its work.
- For example, rate of synthesis and degradation (ex. Liver arginase enzyme increases after protein rich meal).

2. Altering the catalytic efficiency of the enzyme

❖ Catalytic efficiency of enzymes is controlled by:

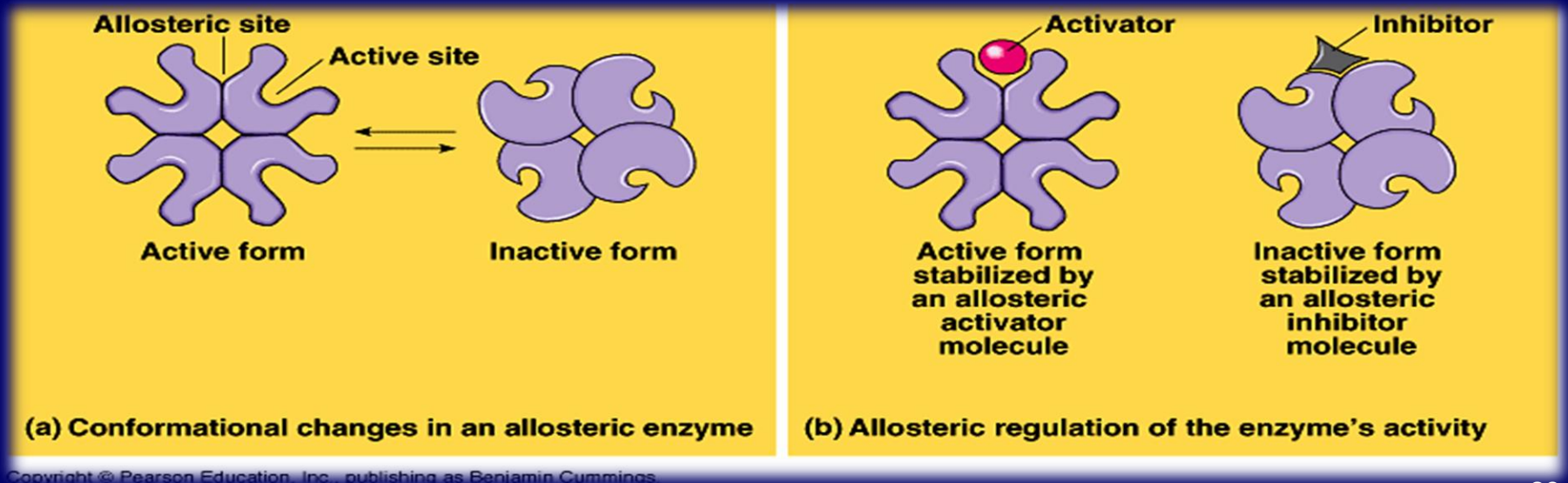
1. Allosteric regulation

Action at "another site" then the active site

The binding of an effecter to the regulatory site causes conformational change of the protein and influences the activity of the catalytic site.

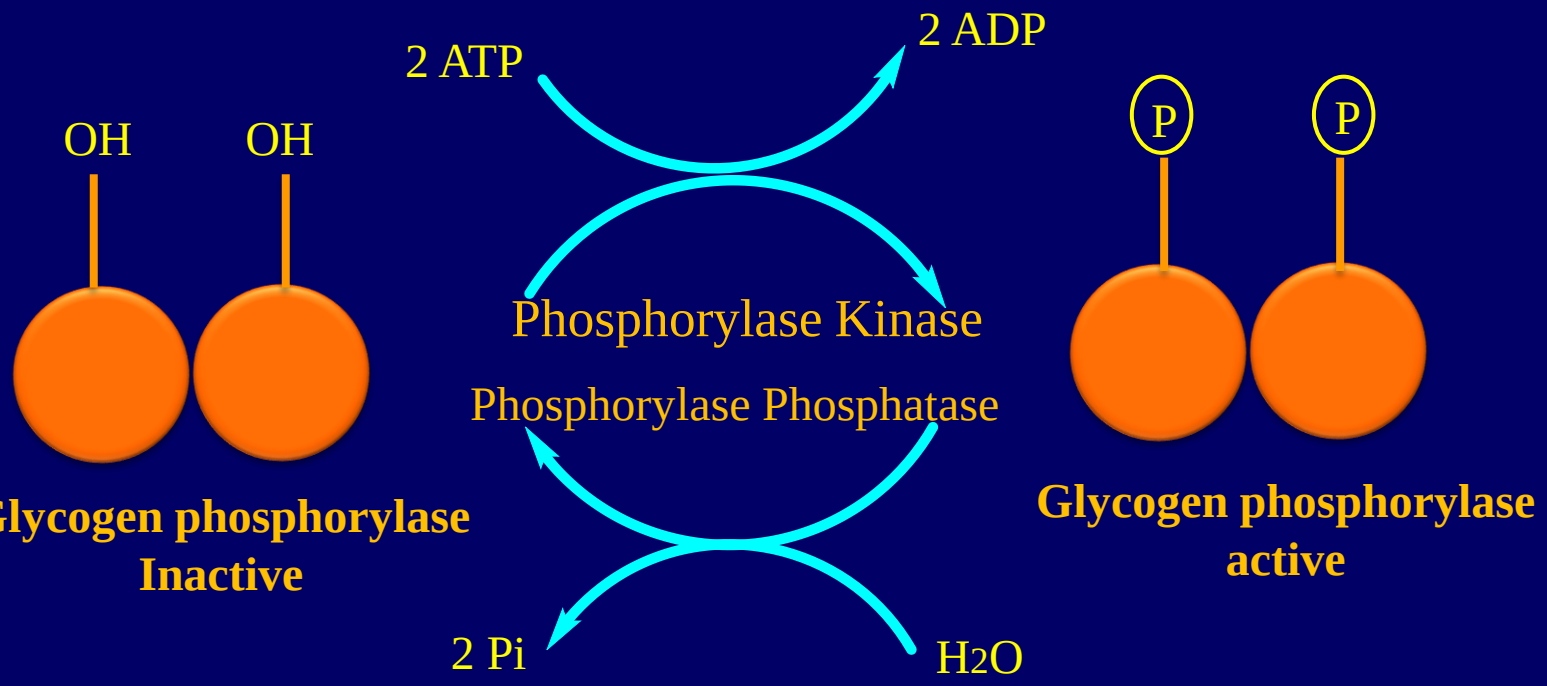
Positive effectors (activator)

Negative effectors (inhibitor)



2. Covalent modification

- Covalent attachment of a molecule to an amino acid side chain of a protein can modify activity of enzyme
- Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis in by releasing glucose-1-phosphate from the terminal alpha-1,4-glycosidic bond.
- Glycogen phosphorylase is studied as a model protein regulated by both reversible phosphorylation



3. Proteolytic activation

- Many enzymes are synthesized as **inactive precursors** (**zymogens**) that are activated by proteolytic cleavage.
- Proteolytic activation only occurs once in the life of an enzyme molecule
- An example is trypsin, a digestive enzyme it is synthesized and stored as trypsinogen, which has no enzyme activity. It becomes active only after a six-amino acid fragment is hydrolyzed from the N-terminal end of its chain removal of This small fragment changes allow the molecule to achieve its active form

thank you!