BIOLOGICAL OXIDATION

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Biological oxidation is that oxidation which occurs in biological systems to produce energy.

Oxidation can occur by:

1-Addition of oxygen (less common)

2-Removal of hydrogen (common)

3-Removal of electrons (most common)

Electrons are not stable in the free state, so their removal form a substance (oxidation) must be accompanied by their acceptance by another substance (reduction) hence the reaction is called oxidation-reduction reaction or redox reaction and the involved enzymes are called oxido-reductases

Redox potential

It is the affinity of a substance to accept electrons i.e. it is the potential for a substance to become reduced.

Hydrogen has the lowest redox potential (-0.42 volt), while oxygen has the highest redox potential (+0.82 volt). The redox potentials of all other substances lie between that of hydrogen and oxygen.

Electrons are transferred from substances with low redox potential to substances with higher redox potential. This transfer of electrons is an energy yielding process and the amount of energy liberated depends on the redox potential difference between the electron donor and acceptor. These enzymes catalyze oxidation- reduction reactions.

- They are classified into five groups:
- 1- oxidases . 2- aerobic dehydrogenises.
- 3-anaerobic dehydrogenises.
- 4- hydroperoxidases and
- 5-oxygenases.





2. Aerobic Dehydrogenases

(Flavoprotein Linked Oxidases).



The coenzyme of aerobic dehydrogenases may be:

- •FMN (Flavin adenine mononucleotide) as in L-amino acid oxidase.
- •FAD (Flavin adenine dinucleotide) as in D-amino acid oxidase, xanthine oxidase, aldehyde dehydrogenase and glucose oxidase.

3. Anaerobic Dehydrogenases



They can use artificial substrate (dye) as hydrogen acceeptor.



Anaerobic dehydrogenases are further classified according to *their coenzymes* into:

•NAD+ linked anaerobic dehydrogenases e.g.

a)Cytoplasmic glycerol-3-phosphate dehydrogenase

b)Isocitrate dehydrogenase.

c)Malate dehydrogenase.

d)β- Hydroxy acyl CoA dehydrogenase.

e)β- Hydroxy butyrate dehydrogenase.

•NADP linked anaerobic dehydrogenases e.g.

a)Glucose-6-phosphate dehydrogenase.

b)Malic enzyme.

c)Cytoplasmic isocitrate dehydrogenase

• FAD linked anaerobic dehydrogenases e.g.

a)Succinate dehydrogenase.

b)Mitochondrial glycerol-3-phosphate dehydrogenase.

c)Acy1 CoA dehydrogenase.

•Cytochromes :-

All cytochromes are anaerobic dehydrogenases except <u>cytochrome oxidase</u> (cyt a3), which is an oxidase and <u>cytochrome P450</u> that is mono-oxygenase (hydroxylase).

•*Ubiquinol (coenzyme Q) dehydrogenase*, which is

present in the respiratory chain.

4. Hydroperoxidases

These enzymes use hydrogen peroxide (H_2O_2) as substrate changing it into water to get rid of its harmful effects.

They are further classified into peroxidases and catalases.

• <u>Peroxidases:</u> These enzymes need a reduced substrate as hydrogen donor peroxidase H2O2 + XH2 (reduced substrate) -----→ X(oxidized substrate) + 2 H2O

Example:-Glutathione peroxidase gets rid of H_2O_2 from red cells to protect them from haemolysis

Glutathione Peroxidase

 $H_2O_2 \qquad + \quad 2 \text{ G-S H} \qquad ----- \quad 2H_2O \quad + \quad \text{G-S-S-G}$

•*Catalases:* These enzymes act on 2 molecules of hydrogen peroxide; one molecule is hydrogen donor & the other molecule is hydrogen accepetor.

$2H2O2 + catalase \longrightarrow 2H2O + O2$

<u>N.B.</u> Hydrogen peroxide is continuously produced by the action of aerobic dehydrogenases and some oxidases. It is also produced by action of superoxide dismutase on superoxide (O_2). It is removed by the action of peroxidases and catalases to protect cells against its harmful effects.



Hydrogen peroxide metabolism

5. Oxgenases

These enzymes catalyze direct incorporation (addition) of oxygen into substrate.

They are further classified into dioxygenases and monooxygenases.

A. Dioxygenases (true oxgenases)

These enzymes catalyze direct incorporation of two atoms of oxygen molecule into substrate e.g. tryptophan pyrrolase, homnogentisic acid dioxygenase, carotenase and β - hydroxy anthranilic acid dioxygenase .

Dioxygenase

 $A \quad + \quad O_2 \qquad \dashrightarrow \qquad AO_2$

B. Mono-oxygenases (pseudo-oxygenases; hydroxylases; mixed

function oxygenases):

 $AH + O_2 + XH_2 \longrightarrow A-OH + H_2O + X$

Fuctions of cytochrome P450

Functions of microsomal cytochrome P450

1-It is important for <u>detoxication of xenobiotics</u> by hydroxylation. e.g. insecticides, carcinogens, mutagens and drugs.
2-It is also important for <u>metabolism of some drugs</u> by hydroxylation e.g. morphine, aminopyrine, benzpyrine and aniline.

drug-H + O_2 + $XH_2 \rightarrow drug-OH + H_2O + X$

Function of mitochondrial cytochrome P450

1- It has a role in biosynthesis of steroid hormones from cholesterol in adrenal cortex, testis, ovary and placenta by hydroxylation

2-It has a role in biosynthesis of bile acids from cholesterol in the liver by hydroxylation at C26 by 26 hydroxylase.

3- It is important for activation of vitamin D

It is a group of hydroxylases which are collectively referred to as cytochrome P450.

They are so called because their reduced forms exhibit an intense absorption band at wavelength 450 nm when complexed to carbon monoxide.

They are *conjugated protein containing haeme (haemoproteins).*

According to their intracellular localization they may be classified into:

• Microsomal cytochrome P450.

It is present mainly in the microsomes of liver cells. It represents about 14% of the microsomal fraction of liver cells.

•Mitochondrial cytochrome P450.

It is present in mitochondria of many tissues but it is particularly abundant in liver and steroidogenic tissues as adrenal cortex, testis, ovary, placenta and kidney.

Electron Transport Chain

<u>Electron transport chain</u> is a chain of catalysts (enzymes & coenzymes)arranged in stepwise of increasing redox potential. It collects reducing equivalents (hydrogen atoms and electrons) from substrates transferring it stepwise to be oxidized in a final reaction with oxygen to form water and energy. It is also known as <u>redox chain</u> or <u>respiratory chain</u>

The electron carrier are found within four membrane-bound enzyme-complexes, which are imbedded in the inner mitochondrial membrane

Components of the electron transport chain

The electron transport chain is formed of :-

• Hydrogen and electron carriers.

• Four membrane-bound enzyme complexes

Hydrogen and electron carriers of the electron transport chain

1- NAD+:-

It receives two hydrogen atoms (2H) from substractes as isocitrate, malate, β -hydroxy acyı CoA and β -hydroxy butyrate. Its reduced form (NADH+H⁺) passes its hydrogen to flavoprotein containing FMN and iron sulfur protein (FeS).

2-Flavoproteins

FAD and FMN serve as hydrogen carriers, which are tightly bound to flavoproteins in a manner that prevents its reduced form reacting with oxygen directly.

There are many types of flavoproteins that have a role in electron transport chain Flavoprotein Fp1 containing FMN receives two hydrogen atoms from reduced NAD+ passing them to coenzyme Q.

Flavoprotein Fp2 containing FAD receives two hydrogen atoms from substrates as succinate, acyl CoA passing them to coenzyme Q.

3- Ubiquinone (Coenzyme Q) :-

Ubiquinones are a group of compounds containing quinine ring but vary according to number of isoprene units at the side chain.

It is a small molecule collecting reducing equivalents from the more fixed component of the respiratory chain.

Ubiquinone can carry two hydrogen atoms forming ubiquinol (reduced coenzyme Q) or one hydrogen atom forming semiquinone. So, it forms a bridge between flavoproteins, which can carry 2 hydrogen atoms, and cytochrome b.

Reduced coenzyme Q passes the electrons to cytochrome b and releases 2H⁺ into the mitochondrial matrix.

The oxidation of ubiquinol involves the successive action of 2 enzymes:

1- Ubiquinol (coenzyme Q) dehydrogenase which transfers electrons to cytochrome c. it needs cyt b, FeS protein and cyt c1 as coenzymes .

2- Cytochrome oxidase, which transfers electrons from cyt c to oxygen. It needs cyt a as coenzymes.

4- Cytochromes :-

They are electron carrier transferring electrons from coenzyme Q to oxygen. They have given letter designation a, b and c according to their order of discovery. <u>All cytochromes are haemoproteins but they differ in redox potential</u>. <u>The haeme in cytochromes differs from that of haemoglobin as the iron</u> atom oscillates between oxidation and reduction during the physiological action of cytochromes, while the iron of haemoglobin remains in the reduced form during its physiological action.

Cytochrome c is a water soluble, peripheral membrane protein. It is relatively mobile.

Cytochrome a & cyt a3 contains copper in addition to the haeme group.

N.B. the mobile components of the electron transport chain include <u>coenzyme Q</u> and cytochrome c. they collect reducing equivalents from the other fixed components.

5-Iron sulfur protein :-

- It is an additional component found in the electron transport chain. It is also called FeS or none- haeme iron. It consists of a cluster of cysteine residues which complex iron through covalent bonds with the sulfur of cysteine.
- It is associated with the <u>flavoproteins(FAD &FMN) and cytochrome b</u>. The sulfur and iron are thought to take part in the oxidationreduction mechanism between flavoprotein and coenzyme Q as the iron atom in these complexes oscillates between oxidation and reduction that allow them to either give up or accept electrons.

Enzyme Complexes of the Electron Transport Chain



The enzymes of the electron transport chain are organized in the inner mitochondrial membrane in the form of four enzyme complexes. The four enzyme complexes of the electron transport chain are: <u>Complex I:</u> NADH dehydrogenase (NADH-uniquinone oxidoreductase) It is a flavoprotein that contains FMN as well as FeS protein as coenzymes. It transfers hydrogen atoms from NADH+H⁺ to uniquinone.

<u>Complex II</u>: Succinate dehydrogenase (succinate-uniquinone oxidoreductase)

It is a flavoprotein that contains FAD as well as FeS protein as coenzymes. It transfers hydrogen atoms from succinate to uniquinone.

<u>**Complex III:</u>** Ubiquinol dehydrogenase (ubiquinol-cytochrome c oxidoreductase).</u>

It transfers electrons from ubiquinol to cytochrome c using cyt b and cyt c1 as coenzymes.

<u>Complex IV:</u> Cytochrome oxidase (cytochrome-oxygen oxidoreductase) It transfers electrons from cytochrome c to oxygen. It needs cyt a and cyt a3 as coenzymes. In addition to these four enzyme complexes, there is fifth complex (complex V) which is the ATP synthase that responsible for biosynthesis of ATP from ADP and inorganic phosphate.



Sequence of events in the electron transport chain

The hydrogen atoms produced from oxidation of substrates can enter the chain through FAD or NAD+.

The hydrogen atoms are then successively transferred through the respiratory chain to oxygen to produce water and energy. the sequence of events that occurs in the electron transport chain will be shown in the following diagram



Oxidative Phosphorylation

It means coupling of the electron transport in respiratory chain with phosphorylation of ADP to form ATP. It is a process by which the energy of biological oxidation is ultimately converted to the chemical energy of ATP.

There are 3 sites of the chain that can give enough energy for ATP synthesis. These sites are:

Site I between FMN and coenzyme Q at enzyme complex I. Site II between cyt b and cyt c1 at enzyme complex III.

Site III between cyt a and cyt a3 at enzyme complex IV.

The number of ATP generated depends on the site at which the substrate is linked to the respiratory chain:

If the substrate is linked to chain through FAD, 2 ATP are formed for each molecule oxidized. If the substrate is linked to chain through NAD⁺, 3 ATP are formed for each molecule oxidized.



- It is ratio of the number of molecules of ADP converted to ATP
- to the number of oxygen atoms utilized by respiratory chain.
- It is a measure to the efficiency of oxidative phosphorylation.
- It is 3/1 if NADH+H⁺ is used and 2/1 if FADH₂ is used.

Mechanism of oxidative phosphorylation

There are 2 theories explaining this mechanism :-

<u>1- Chemical theory :-</u>

It suggests that there is a direct chemical coupling of oxidation and phosphorylation through high-energy intermediate compounds. This theory is not accepted, as postulated high-energy intermediate compounds were never found.

2- Chemiosomotic theory :-

It suggest that the transfer of electrons through the electrons transport chain causes protons to be translocated (pumped out) from the mitochondrial matrix to the intermembrane space at the three sites of ATP production (I, III, IV) (i.e. it acts as a proton pump) resulting in an electrochemical potential difference across the inner mitochondrial membrane. *The electrical potential difference is due to accumulation of the positively charged hydrogen ions outside the membrane and the chemical potential difference is due to the difference in pH, being more acidic outside the membrane.*

In order for oxidative phosphorylation to proceed, two principal conditions must be met. *First, the inner mitochondrial membrane must be physically intact so that protons can only reenter the mitochondrion by a process coupled to ATP synthesis. Second, a high concentration of protons must be developed on the outside of the inner membrane*.

The 2 electrons from NADH generate a 6-proton gradient. The energy of the gradient is used to drive ATP synthesis as the protons are transported back down

Electrons return to the mitochondrion through the integral membrane protein known as ATP synthase (or Complex V).



Inhibitors of oxidative phosphorylation

The inhibitors of oxidative phosphorylation are classified into:

I- Specific-site inhibitors that inhibit oxidation.

II- Non specific-site inhibitors that inhibit phosphorylation

A-Specific-site inhibitors

They block the oxidation process at specific sites on the respiratory chain (at one of the 3 sites of ATP production).

1. Site 1 inhibitors :-

These are substances that inhibit electron transport from reduced FMN to coenzyme Q.

They include:

- 1- Rotenone (insecticide and fish poisoning).
- 2- Chloropromazine (tranquilizer).
- 3- Barbiturates (hypnotic).
- 4- Alkyl guanidine (hypotensive).

2- Site II inhibitors :-

These are substances that inhibit electron transport from reduced cyt b to cyt c1. They include:

- 1- Antimycin A (antibiotic).
- 2- BAL (British Anti Lewisite). It is dithioglycerol, an antagonist for an old war gas.
- 3- Dimercaprol.
- 4- Phenformine (hypoglycemic).
- 5- Napthoquinone.

<u>3- Site III inhibitors :-</u>

These are substances that inhibit electron transport from reduced cyt ba to cyt a3. They include:

- 1- Cyanide.
- 2- Carbon monoxide (CO).
- 3- Hydrogen sulphide (H_2S).
- 4- Sodium azide.

B- Non specific-site inhibitors :-

They function primarily by blocking phosphorylation, but they prevent the whole process of oxidative phosphorylation e.g.

- Oligomycin (antibiotic) that inhibits ATP synthase enzyme .
- Atractyloside (herbicide) that inhibits ADP/ATP transporter which is responsible for the transport of ADP into the mitochondria and the transport ATP out of the mitochondria.

Inhibitors of Oxidative Phosphorylation

Name	Function	Site of Action
Rotenone	e- transport inhibitor	Complex I
barbiturates	e- transport inhibitor	Complex I
Antimycin A	e- transport inhibitor	Complex III
Cyanide	e- transport inhibitor	Complex IV
Carbon Monoxide	e- transport inhibitor	Complex IV
Azide	e- transport inhibitor	Complex IV
2,4,-dinitrophenol	Uncoupling agent	transmembrane H+ carrier
Pentachlorophenol	Uncoupling agent	transmembrane H ⁺ carrier
Oligomycin	Inhibits ATP synthase	ATP synthase

Uncouplers

<u>They are substances that dissociate oxidation from</u> <u>phosphorylation leading to loss of the resulting energy as heat</u>. There is normal oxygen consumption without ATP generation and P/O ratio becomes zero.

Uncoupling agents act as lipophilic weak acids, associating with protons on the exterior of mitochondria, passing through the membrane with the bound proton, and dissociating the proton on the interior of the mitochondrion. These agents cause maximum respiratory rates but the electron transport generates no ATP, since the translocated protons do not return to the interior through ATP synthase.

Example of the uncouplers include:

- •2,4 dinitrophenol.
- •Dinitrocrisol.
- •Pentachlorophenol.
- •Calcium injection.
- •Thyroid hormones.

Brown Adipose Tissue and Heat Generation

The uncoupling of proton flow releases the energy of the electrochemical proton gradient as heat. This process is a normal physiological function of brown adipose tissue. Brown adipose tissue gets its color from the high density of mitochondria in the individual adipose cells. Newborn babies contain brown fat in their neck and upper back .

The process of thermogenesis in brown fat is initiated by the release of free fatty acids from the triglycerides stored in the adipose cells. The mitochondria in brown fat contain a protein called uncoupling protein 1, UCP1 (also called thermogenein)which responsible for fatty acid oxidation & heat production in brown adipose tissue of mammals in response to cold sensation . UCP1 acts as a channel in the inner mitochondrial membrane to control the permeability of the membrane to protons



Oxidation of Cytoplasmic NADH+H⁺

NADH+H⁺ is continuously formed in the cytoplasm by glycolysis and it must be oxidized to regenerate cytoplasmic NAD⁺ which is important for the process of glycolysis to proceed normally.

In the absence of oxygen :-

To regenerate NAD⁺ under anaerobic conditions, two electrons are transferred from each NADH+H⁺ molecule to pyruvic acid forming lactic acid by the action of the lactate dehydrogenase enzyme (LDH).

LDH

pyruvic acid + NADH+H⁺ \rightarrow lactic acid + NAD⁺

Anaerobic bacteria and yeasts reduce pyruvic acid to ethanol and CO₂ with oxidation of NADH+H⁺ to NAD⁺ by the alcohol dehydrogenase enzyme, thereby regenerating NAD⁺.

In the presence of oxygen

The inner mitochondrial membrane is impermeable to NADH+H⁺. Therefore, NADH+H⁺ produced during glycolysis cannot pass directly into the mitochondria to be oxidized. Two shuttles serve to transport reducing equivalent from NADH+H⁺ in cytosol to the electron teansport chain in mitochondria; glycerol-3-phosphate shuttle and malate aspartate shuttle. 1- *Glycerol-3-phosphate shuttle:*-



2- Malate aspartate shuttle



Energy from oxidation of cytosolic NADH+H⁺

•Cytosolic NADH+H⁺ is oxidized by lactate dehydrogenase in absence of oxygen and gives no energy but serves to regenerate NAD⁺.

•Glycerol-3-phosphate shuttle generates 2 ATP for every cytosolic NADH+H⁺ molecule oxidized, as FADH₂ bypasses the first phosphorylation site in the electron transport chain.

•Malate aspartate shuttle generates 3 ATP for every cytosolic NADH+H⁺ molecule oxidized. So, it is more efficient than the glycerol-3-phosphate shuttle.

Mitochondrial transport system:

ATP-ADP - transport: The inner mitochondrial membrane requires specialized carriers to transport ADP & P_i from the cytosol into mitochondria where ATP can be resyntheasized. An adenine nucleotide carrier transports one molecule of ADP from the cytosol into mitochondria & exporting one ATP from mitochondria to cytosol. This carrier protein is strongly inhibited by herbicides. The result is no ADP inside mitochondria so no ATP production.
 Transport of reducing equivalents

•Pyruvate transport system utilizes H⁺ that neutralizes -ve charges of Pyruvate.

•Carnitine transport system for long chain fatty acids.

It is adenosine triphosphate, a nucleotide formed of adenine, ribose and 3 inorganic phosphates. It is a high energy phosphate compound.

<u>ATP production :-</u>

The energy needed for ATP synthesis is mostly obtained from:

1- Oxidative phosphorylation :-

in which the electrons produced by oxidation of foodstuffs are transferred through the electron transport chain to react finally with oxygen and the produced energy is utilized for ATP synthesis.

2- Substrate level phosphorylation:-

ATP can be formed by transferring the high energy from substrates directly to ADP. ATP is formed at substrate levels from:

- 1-1,3 diphosphoglycerate by phosphoglycerate kinase (in glycolysis).
- 2-Phosphoenol pyruvate by pyruvate kinase (in glycolysis).
- 3-Succinyl CoA by succinate thiokinase (in citric acid cycle).
- 4-Creatine phosphate by creatine kinase enzyme.
- 5- ADP by myokinase (adenyl kinase) enzyme

Biological Importance of ATP

- It is the source of energy for:
- •Biosynthetic reactions.
- •Muscle contraction.
- •Nerve conduction.
- •Active absorption and secretion.
- •Active transport across biological membranes.
- •Activation of monosaccharides, fatty acids and amino acids.
- •Formation of creatine phosphate, which is the energy store in muscles.
- •Biosynthesis of cAMP.

Low energy bonds

They are compounds that contain low energy bonds. Low energy bond is that bond which on hydrolysis gives energy less than 4000 calories.

- Examples of low energy bonds include:
- •Ester bonds in lipids.
- •Glycosidic bonds in carbohydrates.
- •Peptide bonds in proteins.

•Phosphate ester bond in glucose 6 phosphate and fructose 6 phosphate.

High energy bonds

They are compound that contain one or more high-energy bonds.

High energy bond is that bond which on hydrolysis gives energy more than 7000 calories.

Type of high energy bonds:

High energy bonds may be classified into:High energy phosphate bonds.High energy sulfur bonds.

A- High energy phosphate bonds

1- Pyrophosphate bond which occurs in ATP, GTP and ADP, GDP, CDP and UDP.

2- Carboxyl- phosphate bond that occurs in 1,3 diphosphoglycerate.

3- Enol- phosphate bond that occurs in phosphoenol pyruvic acid.

4- Guanidine- phosphate that occurs in creatine phosphate and arginine phosphate.

B-High energy sulfur bonds.

1- Thioester bonds present in acetyl CoA (active acetate) and succinyl CoA (activate succinate).

2- Sulfur bond in active methionine (S-adenosyl methionine).3- Sulfur bond in active sulfate (PAPS, phosphor-adenosyl-phospho-sulfate).

