INHIBITORS OF ETC (Electron Transport Chain)

Introduction:

Mitochondria generate ATP through oxidative phosphorylation in eukaryotic microorganisms. Oxidative Phosphorylation is the process whereby the free energy that is released when electrons are transferred along the electron transport (respiratory) chain is **coupled** to the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (P_i). Oxidative phosphorylation is the main **source of energy in aerobic cells**.

In uncoupled mitochondria, because electron transport may still occur, free energy may still be released as the electrons are transferred down the transport chain. However, this energy is not trapped as ATP and appears instead as heat.

Mitochondria play an important role in satisfying the requirement of cellular energy through oxidative phosphorylation. Apart from this it plays many other important roles which are

- ✓ It buffers the cytoplasmic calcium
- ✓ It creates and liberates reactive oxygen
- ✓ It liberates metabolites that control crucial pathways such as succinate and α- ketoglutarate
- ✓ It helps in controlling apoptosis
- ✓ It assists the cell to adopt change in substrate availability by signaling pathways

They modify their organization and dynamics for quality control. So role of mitochondria indicates that it plays a vital task in maintaining the balance with in the cell. It requires a strong regulation. Regulation of mitochondrial activity through expression, transcription and translation. Recent studies indicate that regulation of mitochondrial activity is also achieved by dynamic assembly/ organization of the respiratory protein complexes in the inner mitochondrial membrane

The mitochondrial electron transport chain i.e. mitochondrial respiratory chain consist of five protein complexes:

Complex I : NADH-ubiquinone oxidoreductase

Complex II : succinate-ubiquinone oxidoreductase

Complex III: Ubiquinone cytochrome- c oxidoreductase

Complex IV: cytochrome-c oxidase

Complex V: ATP synthase

Electrons are released from NADH2 enters in to Complex I. Then electron is transferred through a series of electron and hydrogen carrier i.e. complex II to V. This transfer of electron and proton generates the gradient. This gradient drives the synthesis of ATP through ATP synthase (Complex V).

The energy derived from the movement of these protons is used to synthesize ATP from ADP and phosphate. Formation of ATP by this mechanism is referred to as oxidative phosphorylation.

What are inhibitors?

Inhibitors are the inhibiting agents. They bind with the specific electron carriers.

The inhibitors bind to different components of the electron transport chain and block the carrier. After binding with the carrier it does not allow to change in a reversible form from an oxidized state to a reduced state. It leads to and this results in the accumulation of reduced forms prior to the inhibitor point, and oxidized forms of the components of the ETC down the line of inhibition point.

The synthesis of ATP stops due to cease of energy release. The most important known inhibitors of the ETC are Amytal, Rotenone, Antimycin A, CO, Sodium Azide, and Cyanides.

What are Uncouplers?

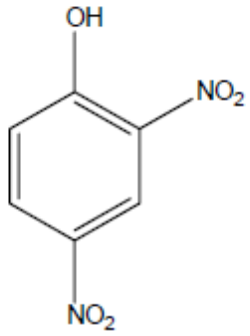
In intact mitochondria and in special preparations of sub mitochondrial particles, the transport of electrons and the phosphorylation of ADP are tightly coupled reactions.

In damaged mitochondria, respiration (i.e., electron transport) may occur unaccompanied by oxidative phosphorylation. When this happens the mitochondria are said to be uncoupled.

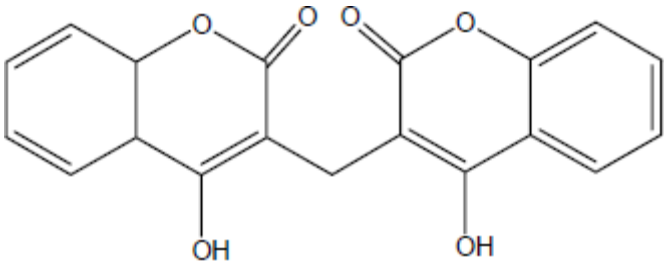
Chemical agents which uncouple the electron transport with ATP synthesis is known as uncouplers of electron transport chain. Such agents will stop the synthesis of ATP but transport of electron will continues. Example: Dintrophenol

These uncoupling agents are lipid-soluble. These agents bind with the hydrogen ions and transport them across the membranes. During electron transport hydrogen ions are pumped across the mitochondrial membrane but these uncoupling agents carries it back to the mitochondria. It will stop the generation of proton gradient. It will stop the synthesis of ATP by oxidative phosphorylation. Rather the energy derived from electron transport is released as heat.

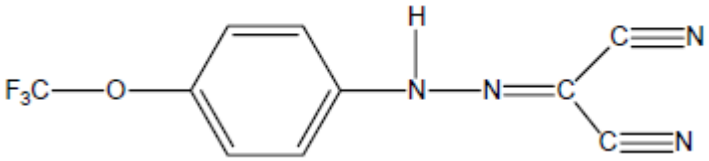
Energy derived from electron transport is released as heat during uncoupling. Such kind of heat generation is known as nonshivering thermogenesis. It is vital in many biological situations. For example, uncoupling occurs naturally in brown adipose tissue. The inner mitochondrial membranes of such tissue contain a protein called thermogenin (uncoupling protein). Thermogenin is an endogenous protein found in the brown adipose tissue. Thermogenin act as an uncoupler which uncouples the ATP synthesis from ETC by creating a passive proton pump (UCP-1) with in the inner mitochondrial membrane. E.g. Such tissues found in new born animals and hibernating animals.



Dinitrophenol



Dicumarol



Carbonyl cyanide-p-trifluoromethoxyphenol hydrozone
FCCP

Examples of uncouplers:

Chemical Uncouplers: 2,4-Dinitrophenol, dicumarol , CCCP (carbonyl cyanide m-chloro phenyl hydrazone) and FCCP (p-trifluoromethoxy carbonyl cyanide phenyl hydrazone). CCCP is a lipid-soluble weak acid. CCCP is a very potent mitochondrial uncoupling agent.

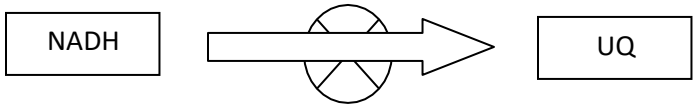
Physiological uncouplers: It includes long chain fatty acids, Thyroxin, Thermogenin (protein), Calcium ions.

These uncouplers have hydrophobic nature which makes them soluble in the lipid bilayer of membrane. These uncouplers have dissociable protons which allow them to bring protons from the inter membrane space to the matrix. It will disintegrate pH gradient. This energy lost as heat.

INHIBITORS

Site specific inhibitors of ETC have been identified. These compounds prevent the transfer of electrons by binding to a various complex of the chain which blocks the redox reactions.

ROTENONE: Rotenone is a plant product. Rotenone is extracted from roots of plant Derris elliptica. It is an insecticide. It is a strong inhibitor of complex I of the electron transport chain. It inhibits the transport of electron through the NADH-CoQ reductase complex. Certain tribes use it as a fish poison which paralyse the fish.



Amobarbital (Amytal) : Amytal is a barbiturate. It inhibits the transport of electron through the NADH-CoQ reductase complex.

Demerol: Demerol also inhibits complex I.

Piericidin A: It is an antibiotic of microbial origin. Its mode of action is similar to Rotenone.

Antimycins: It is an antibiotic from Streptomyces species. It blocks the electron flow at complex III of electron transport chain. It inhibits the flow of electrons from cytochrome b to cytochrome c1.

Cyanide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It blocks at cytochrome oxidase which prevents both coupled and uncoupled respiration. Cyanide binds with iron within this protein complex and prevent the regular activity of the complex system. It blocks the transport of electron to oxygen which stops the further passage of electron through the electron transport chain. As a result, the person is deprived of energy to carry out the many numerous processes that sustain life and the person dies.

Carbon monoxide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It binds with the cytochrome oxidase (Complex IV) which blocks the transfer of electrons to oxygen. Carbon monoxide binds with the reduced form of iron in the hem groups (Fe++) in Cytochrome Oxidase (Complex IV).

Azide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It blocks the electron flow between the cytochrome oxidase complex and oxygen. It reacts with the ferric form (Fe3+) of the complex IV of electron transport chain.

Hydrogen Sulphide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It is toxic.

Inhibitors of ATP synthase complex (Phosphorylation inhibitor):

These inhibitors prevent the synthesis of ATP by binding to the ATP synthase complex. It prevents the inflow of protons. Example: Oligomycin and dicyclo hexyl carbo diimide (DCCD).

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Oligomycin is polypeptide in nature act as an antibiotic which is obtained from the Streptomyces species. It stops the transfer of high energy phosphate group to adenine diphosphate (ADP). Oligomycin binds to a 23kd polypeptide in the F_0 base plate and blocks ATP synthesis by the F_0 /F_1 ATPase. It prevents respiration in mitochondria and all ATP-driven functions in sub-mitochondrial particle.

Diclyclohexyl carbo diimide (DCCD): It makes covalent bonds with amino acid glutamate of the c subunit of F_0. When it binds covalently, it stops the proton channel, which prevents the formation ATP synthesis

Inhibitors of ATP-ADP translocase (Transport inhibitor)

Atractyloside (a plant glycoside) or Bongregate (an antibiotic from a mold) is able to inhibit the ATP-ADP translocase with very small concentrations. Atractyloside is a plant glycoside while Bongregate is an antibiotic obtained from a mold. Absence of ATP also leads to inhibition in the process of ATP formation due to tight coupling between oxidation and phosphorylation. It means oxidation cannot proceed via the respiratory chain without simultaneous phosphorylation of ADP