BIOCHEMISTRY- BP203T

UNIT : 1 METABOLISM OF CARBOHYDRATES

CLASS:6

TOPIC: OXIDATIVE PHOSPHORYLATION

OXIDATIVE PHOSPHORYLATION:

The transport of electrons through the ETC is linked with the release of free energy.

The process of synthesizing ATP from ADP and Pi coupled with the electron transport chain is known as oxidative phosphorylation.

The complex V of the inner mitochondrial membrane is the site of oxidative phosphorylation.

P : O Ratio

The P : O ratio refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed.

More appropriately, P : O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC.

The mitochondrial oxidation of NADH with a classical P : O ratio of 3 can be represented by the following equation :

$NADH + H+ + 1\ 2\ O2 + 3ADP + 3Pi \rightarrow NAD+ + 3ATP + 4H2O$

Further, a P : O ratio of 2 has been assigned to the oxidation of FADH2.

There is a strong evidence now to suggest a P:O ratio of 2.5 for NADH, and 1.5 for FADH2, since ten protons (for NADH) and six protons (for FADH2) are pumped across mitochondrial membrane.

Synthesis of one ATP requires four protons.

Sites of oxidative phosphorylation in ETC

There are three sites in the ETC that are exergonic to result in the synthesis of 3 ATP molecules:

1. Oxidation of FMNH2 by coenzyme Q.

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2. Oxidation of cytochrome b by cytochrome c1.

3. Cytochrome oxidase reaction.

Each one of the above reactions represents a coupling site for ATP production. There are only two coupling sites for the oxidation of FADH2 (P : O ratio 2), since the first site is bypassed.

Energetics of oxidative phosphorylation

The transport of electrons from redox pair NAD+/NADH (E0 = – 0.32) to finally the redox pair 1/ 2 O2/H2O (E0 = + 0.82) may be simplified and represented in the following equation
1/ 2 O2 + NADH + H+ → H2O + NAD+

The redox potential difference between these two redox pairs is 1.14 V, which is equivalent to an energy 52 Cal/mol.

Three ATP are synthesized in the ETC when NADH is oxidized which equals to 21.9 Cal (each ATP = 7.3 Cal).

The efficiency of energy conservation is calculated as

21.9 ×100/ 52= 42%.

Therefore, when NADH is oxidized, about 42% of energy is trapped in the form of 3 ATP and the remaining is lost as heat.

The heat liberation is not a wasteful process, since it allows ETC to go on continuously to generate ATP.

Further, this heat is necessary to maintain body temperature.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Several hypotheses have been put forth to explain the process of oxidative phosphorylation.

The most important among them—namely, chemical coupling, and chemiosmotic—are discussed below

Chemical coupling hypothesis

This hypothesis was put forth by Edward Slater (1953).

According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of phosphorylated high-energy intermediates are first produced which are utilized for the synthesis of ATP.

These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle.

However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

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Chemiosmotic hypothesis

This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted.

It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi.

The concept of chemiosmotic hypothesis is comparable with energy stored in a battery separated by positive and negative charges.

Proton gradient:

The inner mitochondrial membrane, as such, is impermeable to protons (H+) and hydroxyl ions (OH–).

The transport of electrons through ETC is coupled with the translocation of protons (H+) across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space.

The pumping of protons results in an electrochemical or proton gradient.

This is due to the accumulation of more H+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side .

The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi.

Enzyme system for ATP synthesis:

ATP synthase, present in complex V, utilizes the proton gradient for the synthesis of ATP.

This enzyme is also known as ATPase since it can hydrolyse ATP to ADP and Pi.

ATP synthase is a complex enzyme and consists of two functional subunits, namely F1 and F0. Its structure is comparable to 'lollipops'.

The protons that accumulate in the intermembrane space re-enter the mitochondrial matrix, leading to the synthesis of ATP.

Rotary motor model for ATP generation:

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP.

The original Boyer hypothesis, now considered as a rotary motor/engine driving model or binding change model, is widely accepted for the generation of ATP.

a. The enzyme ATP synthase is the F0F1 complex (of complex V).
b. The F0 subcomplex is composed of channel protein 'C' subunits to which F1-ATP synthase is attached.

c. F1-ATP synthase consists of a central J subunit surrounded by alternating D and E subunits (D3 and E3). In response to the proton flux, the J subunit physically rotates.
d. This induces conformational changes in the E3 subunits that finally lead to the release of ATP.
e. According to the binding change mechanism, the three E subunits of F1-ATP synthase adopt different conformations.
f. One subunit has open (O) conformation, the second has loose (L) conformation while the third one has tight (T) conformation.
g. By an unknown mechanism, protons induce the rotation of J subunit, which in turn induces conformation changes in E subunits.
h. The substrates ADP and Pi bind to E subunit in L-conformation.
i. The L site changes to T conformation, and this leads to the synthesis of ATP.
j. The O site changes to L conformation which binds to ADP and Pi. The T site changes to O conformation, and releases ATP.
k. This cycle of conformation changes of E subunits is repeated. And three ATP are generated for each revolution .
l. It may be noted that the ATP release in O conformation is energy dependent (and not ATP synthesis) and very crucial in rotary motor model for ATP generation.
m. The enzyme ATP synthase acts as a protondriving motor, and is an example of rotary catalysis.

Thus, ATP synthase is the world's smallest molecular motor.

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