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Review

Acidic lipids, H^+-ATPases, and mechanism of oxidative phosphorylation. Physico-chemical ideas 30 years after P. Mitchell's Nobel Prize award

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ABSTRACT

Peter D. Mitchell, who was awarded the Nobel Prize in Chemistry 30 years ago, in 1978, formulated the chemiosmotic theory of oxidative phosphorylation. This review initially analyzes the major aspects of this theory, its unresolved problems, and its modifications. A new physico-chemical mechanism of energy transformation and coupling of oxidation and phosphorylation is then suggested based on recent concepts regarding proteins, including ATPases that work as molecular motors, and acidic lipids that act as hydrogen ion (H^+) carriers. According to this proposed mechanism, the chemical energy of a redox substrate is transformed into nonequilibrium states of electron-transporting chain (ETC) coupling proteins. This leads to nonequilibrium pumping of H^+ into the membrane. An acidic lipid, cardiolipin, binds with this H^+ and carries it to the ATP-synthase along the membrane surface. This transport generates gradients of surface tension or electric field along the membrane surface. Hydrodynamic effects on a nanolevel lead to rotation of ATP-synthase and finally to the release of ATP into aqueous solution. This model also explains the generation of a transmembrane protonmotive force that is used for regulation of transmembrane transport, but is not necessary for the coupling of electron transport and ATP synthesis.

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1. Introduction

Adenosine triphosphate, or ATP, is the molecule universally used to store chemical energy in a biological cell. Expressed in typical biochemical units, the rate of ATP synthesis by mitochondria is near 10 nmole/s per mg of mitochondrial proteins. Active ATP-synthase constitutes only 1–2% of these proteins.

Cellular respiration involves the transport of electrons while the synthesis of ATP is a reaction of chemical condensation with the removal of water. It has now been proven that these two fundamentally different chemical processes are coupled via H^+ ions, which are delivered to the ATP-synthase from respiratory chain proteins. The opposite process, hydrolysis of ATP, can lead to the

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active transport of protons across the membrane, which can then be used in the processes of secondary active transport.

The importance of research in this area was always recognized, and Peter D. Mitchell, who formulated the chemiosmotic theory of oxidative phosphorylation, was awarded the Nobel Prize in Chemistry in 1978. Later, P. Boyer and J. Walker were awarded a 1997 Nobel Prize in Chemistry for the elucidation of the mechanisms underlying the synthesis of ATP. Their major results and concepts are described in modern biochemistry and biophysics textbooks (Cotterill, 2003; Garret and Grisham, 2005; Nicholls and Ferguson, 2002; Wikstrom, 2006). This paper will briefly review the historical aspects and currently accepted theory, including unresolved problems, in this area. Some data regarding the functional mechanisms of redox active membrane proteins will be presented. ATP-synthase will be described as a molecular machine that works with almost 100% efficiency. Finally, the mechanisms of a membrane-based coupling of oxidation and ATP synthesis will be discussed, where several molecular machines are working together. According to the new mechanism suggested in this review, acidic lipids and especially cardiolipin play the key role in these processes. Only selected papers and reviews necessary to support the main ideas and hypotheses, or the publications describing lesser known and more specific results and facts, will be cited. A few of these papers were published by the author in Russian or international nonbiological journals. Experiments were done on artificial biomimetic membranes and are not usually familiar to biologists, but nevertheless they can be useful in understanding the physical chemistry aspects discussed below.

2. Chemiosmotic theory of oxidative phosphorylation and ubiquinone as an electron and H^+ carrier

Oxidative phosphorylation occurs in the second, inner membrane of mitochondria. This membrane can be fractionated to yield four separate supramolecular complexes of the respiratory chain components and one of the ATP-synthase. The first one is NADH dehydrogenase (called complex I). Then there are succinate dehydrogenase (complex II), cytochrome *c* reductase, which has low and high potential cytochromes b_h and b_l, Rieske protein with 2Fe/2S centre and c_1 (complex III) and, finally, cytochrome *c* oxidase (complex IV). NADH and succinate come from the inner side while cytochrome *c*, which serves as an electron mediator between complexes III and IV, is located on the outer interface of this membrane. Oxygen also evidently diffuses from outside. Three of the four oxidoreductase complexes (I, III and IV) couple electron transport with the transport of H^+, which can be used to synthesize ATP.

Both complexes I and II transfer electrons to ubiquinone (coenzyme Q), which is available in a considerable molar excess. At least part of it is able to freely diffuse within the membrane. According to the Singer–Nicholson model (Singer and Nicolson, 1972) it is usually assumed that all complexes I–IV also diffuse in the membrane and electron transfer is based on their random collisions with each other or coenzyme Q.

Historically, the initial proposed mechanisms of the coupling of oxidation and phosphorylation were based on ideas from classical biochemistry. The existence of a high energy intermediate compound that would be the means of conveying the energy necessary for ATP synthesis from oxidation to the ATPase was postulated. This intermediate was never found (Boyer et al., 1977).

Mitchell's chemiosmotic mechanism in its original form had several components (Mitchell, 1961, 1968):

a) The electron-transfer components are arranged vectorially across the membrane in such a manner that electron transfer is linked to proton translocation across the membrane. In other words, the energy of redox processes is transformed into the transmembrane difference of electrochemical potentials of H^+.
b) The membrane forms a closed structure and it is not easily permeable for H^+;
c) H^+ can return only through the ATPase, which uses them to drive ATP synthesis. The membrane also has proton-linked transporters that are necessary for osmotic stabilization and transport of metabolites, for example, H^+/P_i cotransport, which is extremely active in mitochondria.

Developing and improving his theory, Mitchell has suggested many new terms, including proticity (analogue of electricity for H^+), simport and antiport (coupled cotransport and coutertransport of ions), etc. Many of these terms are seldom used in modern literature, but the term "chemiosmotic" can be found practically in any biochemistry textbook. The historical and philosophical background behind this term can be much better understood after reading one of the last of Mitchell's reviews with the title "Foundations of vectorial metabolism and osmochemistry" (Mitchell, 1991). In his own terms, 'the bag-of-enzymes view of cell metabolism was prevailing' when Mitchell started thinking about role of membranes. The separation of cells into several compartments immediately leads from homogeneous to transmembrane vectorial transport processes, and even to vectorial chemical reactions between substances separated by the membrane. "Chemiosmotic theory is more broadly based than bioenergetics. It is more informative because it maps the spatially directed forces and displacements of ligands along pathways, rather than matching the energies that are the scalar products of those co-linear forces and displacements". When concentrations in aqueous solutions are changed as the result of transmembrane reactions and transport ("vectorial metabolism"), it means that the transmembrane osmotic pressure also changes, hence the terms osmochemistry and chemiosmotic theory. As it is known since van't Hoff, relatively small changes of concentration easily can lead to several meters of water column hydrostatic pressure, which is available to do useful work, including chemical.

He also understood that in bacteria, chloroplast and mitochondrial transport of H^+ and its reactions play the key role. For dilute solutions at constant pressure and temperature, the energy of 1 mol of H^+ ions is described by electrochemical potential:

$$\mu_{H^+} = \mu_0 + RT \ln[H^+] + zFE$$

where μ_0 is a standard chemical potential, which depends on the solvent and is the same in both aqueous solutions, separated by the membrane. *R* is the gas constant, *T* is absolute temperature, *F* is the Faraday number, *E* is electric potential, and for monocharged H^+, $z = 1$. The difference, $\Delta\mu_{H^+}$, which does not depend on μ_0, serves as a source of energy for different transmembrane transport processes and the synthesis of ATP.

Instead of H^+ transmembrane electrochemical potential difference $\Delta\mu_{H^+}$ Mitchell preferred to use the equivalent value expressed in volts, i.e., $\Delta\mu/zF$, which he called the protonmotive force (PMF). For protons and $\Delta\mu$ expressed in J/mol, the ratio of these two values is simply 10^5. The PMF value on the inner mitochondrial membrane can be near 220 mV, which corresponds to 22 kJ/mol. In other units it is 5.26 kcal/mol or 8.8 k_BT or 0.22 eV, and is formed by both pH and transmembrane electric potential differences. In terms of the PMF, the value of the transmembrane electric potential difference could be 80 mV. An additional component related to the pH difference is near 140 mV, but it varies for different cells and metabolic states.

Mitchell has also suggested a simple mechanism of vectorial redox loops, which could lead to charge separation through the membrane. According to this mechanism, an electron moves from