Overview of Mitochondrial Bioenergetics

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Chapter 1

Overview of Mitochondrial Bioenergetics

Vitor M. C. Madeira

Abstract

Bioenergetic Science started in eighteenth century with the pioneer works by Joseph Priestley and Antoine Lavoisier on photosynthesis and respiration, respectively. New developments were implemented by Pasteur in 1860s with the description of fermentations associated to microorganisms, further documented by Buchner brothers who discovered that fermentations also occurred in cell extracts in the absence of living cells. In the beginning of twentieth century, Harden and Young demonstrated that orthophosphate and other heat-resistant compounds (cozymase), later identified as NAD, ADP, and metal ions, were mandatory in the fermentation of glucose. The full glycolysis pathway has been detailed in 1940s with the contributions of Embden, Meyeroff, Parnas, Warburg, among others.

Studies on the citric acid cycle started in 1910 (Thunberg) and were elucidated by Krebs et al. in the 1940s.

Mitochondrial bioenergetics gained emphasis in the late 1940s and 1950s with the works of Lenhinger, Racker, Chance, Boyer, Ernster, and Slater, among others. The prevalent "chemical coupling hypothesis" of energy conservation in oxidative phosphorylation was challenged and replaced by the "chemiosmotic hypothesis" originally formulated in 1960s by Mitchell and later substantiated and extended to energy conservation in bacteria and chloroplasts, besides mitochondria, with clear-cut identification of molecular proton pumps.

After identification of most reactive mechanisms, emphasis has been directed to structure resolution of molecular complex clusters, e.g., cytochrome c oxidase, complex III, complex II, ATP synthase, photosystem I, photosynthetic water splitting center, and energy collecting antennæ of several photosynthetic systems.

Modern trends concern to the reactivity of radical and other active species in association with bioenergetic activities. A promising trend concentrates on the cell redox status quantified in terms of redox potentials.

In spite of significant development and advances of bioenergetic knowledge, major issues remain mainly related with poor experimental designs not representative of the real native cell conditions. Therefore, a major effort has to be implemented regarding direct observations in situ.

Key words: ATP syntase, Bioenergetics, Chloroplast, Chemical coupling hypothesis, Chemiosmotic hypothesis, Citric acid cycle, Complexes II and III, Cytochrome c oxidase, Energy collecting antennæ, Fermentation, Glycolysis, Mitochondria, Photosystem, Radical, Redox potential, Water splitting center

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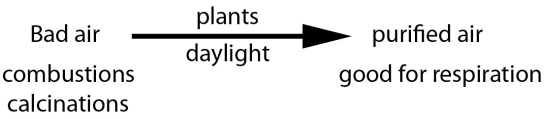
Very often, Bioenergetics is wrongly considered a modern science of the twentieth century. Rather, the pioneers started already in the middle of eighteenth century, with the works of Joseph Priestley, in London, and Antoine de Lavoisier, in Paris, in parallel with the development of Chemistry.

Joseph Priestley (1, 2) described several important gases, including oxygen, named *dephlogisticated* air. He discovered that plants, in the presence of daylight, could purify the bad air (unsuitable for respiration) resulted from combustions and metal calcinations (oxidations).



In Priestley's terminology, the bad or *phlogisticated* air (loaded with *phlogiston,* the principle of fire and calcinations) (cf. Phlogiston Theory) turned into *dephlogisticated* air. Later, Priestley concluded that *dephlogisticated* air was in fact a fraction of the total air.

Antoine de Lavoisier clearly established that Priestley's *dephlogisticated* air was an independent gas, initially named *vital air* and later *oxygine* (acid generator, in Greek), the acidifying principle of common acids (3, 4). Additionally, Lavoisier carried out precise experiments on animal and human respiration, showing that pure air turned into *acide crayeux* (lime acid, carbon dioxide) in lungs during respiration. He further demonstrated that there is a constant relationship between the heat released and the amount of air entered in lungs (4). Therefore, the first measurement of a metabolic rate has been reported by Lavoisier *ca*. 1780.

In *1860,* Louis Pasteur described fermentation of sugar into alcohol strictly linked to living yeast cells (5). By the time, it was believed that the ferments (enzymes) could only be active inside the living cell "organized" by the "vital principle" (Vitalism Theory). However, by the end of nineteenth century, the chemists Hans Buchner and Eduard Buchner (6, 7*)* clearly showed that filtered yeast juice (cells absent) was very active in the fermentation of sugar into alcohol, meaning that the ferments are active when "disorganized", i.e., without the need of any "vital principle". This basic idea opened the way of modern Biochemistry, allowing detailed studies of metabolic pathways carried out later in twentieth century.

Details on fermentation were provided in 1905 by Harden and Young who demonstrated that yeast extracts rapidly fermented glucose into alcohol and that orthophosphate is required and consumed in the phosphorylation of hexose (8, 9). They separated the yeast extract in two types of compounds: *zymase,* non dialyzable and easily inactivated by heat; *cozymase,* a dialyzable fraction resistant to heat. The *zymase* fraction contained the glycolytic enzymes and *cozymase,* besides orthophosphate, contained NAD^+, ADP, and metal ions.

Full glycolysis pathway has been detailed in 1940s with the major contributions of Embden, Meyeroff, Parnas, Warburg, and other researchers (10).

Studies on citric acid cycle started in 1910 with the work of Thunberg, followed by important achievements of Szent-Györgyi in 1935 (5). However, full elucidation has been provided by Krebs (11) who established the condensation reaction as the cycle closing step.

Mitochondrial bioenergetics gained emphasis on late 1940s and 1950s with the relevant work of eminent scientists, e.g., Lehninger, Racket, Chance, Boyer, Ernster, and Slater (12). It has been shown that oxidations of NADH, $CoQH_2$, formed at expense of oxidation of citric acid cycle intermediates, resulted in energy yielding effective in ATP synthesis. The coupling of oxidations and ATP synthesis has been explained on the basis of the "chemical coupling hypothesis" (5) proposing that energy of oxidations could be conserved in "high energy compounds" e.g., phosphate esters as in the case of "substrate level phosphorylations" occurring in glycolysis and the citric acid cycle (5). However, these compounds were never demonstrated or isolated.

In the 1960s, Mitchell in his "chemiosmotic hypothesis" proposed that the energy of oxidations is transduced into physicochemical states through the establishment of a transmembrane proton gradient generating a transmembrane electric potential and/or a pH gradient (13) described by the famous equation of the "protonmotive force":

$$\Delta p = \Delta\Psi - Z\Delta\text{pH} \quad (Z = 59 \text{ at } 25°C),$$

Where the transmembrane potential $\Delta\Psi = \Psi\text{in} - \Psi\text{out}$ and the transmembrane $\Delta\text{pH} = \text{pHin} - \text{pHout}$.

The protonmotive force (Δp) is expressed in mV and represents the electrochemical potential (free energy change) of the transmembrane proton electrochemical gradient divided by the Faraday's constant. It is analogous to the electromotive force in electricity.

This hypothesis is generally accepted and explains conveniently the energy transduction in mitochondria, bacteria, and chloroplasts (14).

Δp is provided by protonmotive force generators at the expense of transmembrane proton transport: from matrix to cytoplasm in mitochondria; from cytoplasm to the intermembrane space in bacteria; from stroma to thylakoid space in chloroplasts (14).

Protonmotive force development has been detected in all known membrane redox systems (13), viz. mitochondria, bacteria, chloroplasts, and Archaea (15).

Protonmotive force generators function in the basis of molecular proton pumps and the ubiquinone pool associated with the

activity of mitochondrial complex III or equivalent in bacteria and chloroplasts, as proposed originally by Mitchell in his looping mechanisms (14).

Molecular proton pumps have been identified in mitochondria (16) and bacterial cytochrome c oxidases (17). Proton pumps have been also putatively assigned to complex I (18).

The protonmotive force energy may be used in several activities: ATP synthesis, ion transport, orthophosphate transport, nucleotide exchange, transhydrogenase, heat generation, and flagellar motion (10, 14).

Redox processes are carried out in complex protein clusters: I (NADH-CoQ oxidoreductase), II (succinate-CoQ oxidoreductase), III ($CoQH_2$-cytochrome c oxidoreductase), and IV (cythochrome c-O_2 oxidoreductase) or the equivalents in bacteria. In chloroplasts, complexes I, II, and IV are absent and photosynthetic PSI and PSII clusters are present.

Complexes diffuse laterally in the lipid membrane (19) with relatively low diffusion constants for the big complexes (I, II, III, and IV) and the transfer of reducing equivalents is achieved randomly during effective encounters. These are significantly facilitated and accelerated by the ubiquinone pool (associating complexes I and II with III) and cytochrome c (associating complexes III and IV), owing to the fast diffusion constants (19).

This strategy involving big complex clusters and small fast components (ubiquinone and cytochrome c or equivalents) is a common motif in all known redox systems: mitochondria, bacteria, and chloroplasts. In several complexes (I and II), redox sequences occur in two distinct segments: a two-electron event (e.g., oxidation of NADH to NAD^+ and succinate to fumarate) is followed by an one-electron event (iron-sulfur centers of complexes I and II). The two events are coupled by a flavin center able to process either two or one electron at a time, undergoing intermediate semiquinone species (10). This strategy is a common motif in all redox systems where a redox segment of two-electrons is followed by an one-electron segment, e.g., bacteria (20), photosynthetic bacteria (20) (synthesis of NADH), chloroplasts (10) (synthesis of NADPH), methanogens (20), and nitrogenase systems in *Rhizobium* (21).

After identification of basic reactive mechanisms, emphasis has been directed to the structure resolution of complex clusters. Detailed structures of cytochrome c oxidases (22, 23), complex III (24), and complex II (25) have been described. Complex I has been also partially resolved (18). Structure of ATP synthase (26) and its clustering with the orthophosphate carrier and the adenine nucleotide carrier (27) (ATP synthasome) have been established. Available also are the structures of photosystem I (28, 29), water splitting center (30), and energy-collecting antennae (31-33) of several photosynthetic systems. The structural trend is still in progress for other bioenergetic assemblies.

Modern research trends are concerned with oxidative processes related with the formation and reactivity of radical species (oxygen related and other) in association with mitochondrial and other cell activities. A promising trend, attempting a quantitative description, regards the integration of the radical chemistry with the cell redox status in terms of NAD^+/NADH, $NADP^+$/NADPH, GSSG/GSH, and other redox balances in relation to redox potentials (34). These efforts may effectively contribute to a clear quantitative appraisal how the redox balances affect the radical chemistry and its involvement in major cell functions, viz. mitochondrial transition permeability, apoptosis, necrosis, enzyme, and gene activities.

In spite of the relevant progress in bioenergetic knowledge, there are still major issues and challenges to be accomplished. Most issues relate to poor experimental conditions that roughly deviate from the native cell conditions. Most available data has been collected with isolated preparations which contain fragments of the original chondriom framework. Therefore, it should be not surprising that relevant functions got lost and other severely modified during the crude isolation procedures. Hence, drawn conclusions must be evaluated with caution. Furthermore, most experimental setups and reaction media are diverse from in situ conditions, e.g., temperature is generally set at 25°C (for technical reasons), instead the actual cell temperature (37°C), in experiments of oxygen tracing by electrometry. The chemical composition of media (sucrose, salt concentrations, buffers, pH) often does not minimally relate with cytoplasm condition. Furthermore, oxygen experiments are carried out at saturation (240 μM, at 25°C), a situation far from the expected low oxygen activity in the living cell. Therefore, data on oxygen radicals may be severely questioned.

It is of general concern that a significant effort has to be implemented regarding direct observations in situ, looking at bioenergetic activities directly in living cells. This is a tremendous and difficult challenge for the near future. If not accomplished, we will never be certain if the observations are facts or artifacts.

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