

Thermodynamics of ATP synthesis in mitochondria

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Summary— In 1978, Peter D. Mitchell described, for the first time, the way in which energy is used within mitochondria to synthesize ATP. His discovery would soon be named the *chemiosmotic theory*, and it proves that there is a connection between the electron flow of the respiratory chain in mitochondria and the activity of the well-known ATP-synthase, situated in mitochondrial inner membranes. In this article, we analyse this concept from the thermodynamic point of view, going through the definition of proton motive force and the chemical potential and voltages associated to proton-specific channels within the mitochondrial inner membrane.

Keywords - Chemical potential, voltage, free energy, mitochondria, membrane, respiration.



1. INTRODUCTION

Mitochondria is the powerhouse of the cell, people say. This statement would make allusion to the ability of mitochondria to produce energy, which is stored in highly-energetic bonds within the molecular structure of ATP (adenosine triphosphate). However, how does this happen?

In the mitochondrial matrix, respiratory chain reactions are given. There, an electron transport chain takes place through the permanent reduction and oxidation of molecules. A donor molecule will first donate an electron to an acceptor, later becoming a second donor by giving up its electron to a second acceptor, and so on. It is important to notice that this chain occurs thanks to each molecule being more electronegative than the previous one: the initial electron donors are reducing power molecules such as NADH and FADH₂, whereas the final electron acceptor is a molecule of oxygen, which ends up being reduced to a water molecule. All this process occurs between different complexes that are anchored to

the mitochondrial inner membrane, a barrier that mediates the transport of ions and substances between the mitochondrial intermembrane space and the matrix. In these complexes, we can highlight molecules like coenzyme Q and cytochrome c as electron acceptor/donors.

2. THE CHEMIOSMOTIC THEORY

This theory, introduced by Peter D. Mitchell in 1978, aims to detail the relationship between the

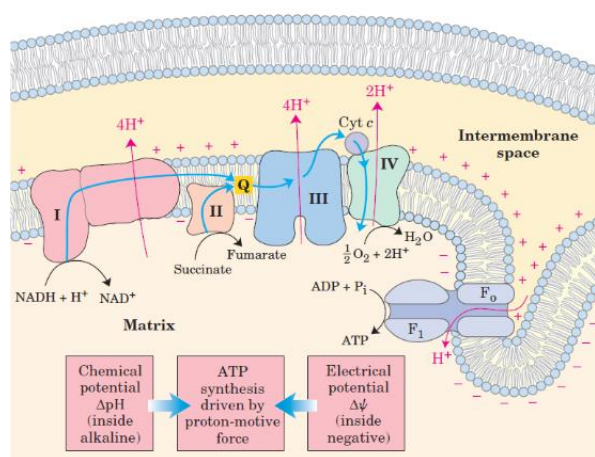


Fig. 1. Diagram of the electron transport chain in the mitochondria inner membrane. Image extracted from Reference [2].

electron transport chain and the ability of mitochondria to produce ATP (adenosine triphosphate), a molecule that is known to be the “energy storage” unit in cells, since it contains a high-energy bond that can be broken down in different cellular processes to release energy for metabolic needs.

In complexes I, III and IV from the electron transport chain, the oxidation of NADH and other substances lead to the introduction of protons by the complexes into the intermembrane space. This occurrence ends up generating what is known as the *proton-motive force*, an energy that arises from the electrochemical gradient across the inner mitochondrial membrane and that induces the transport of protons back into the matrix. In addition, there exists only one pathway for the protons to return, which is through a proton-specific channel cleaved to the membrane named F₀, also commonly referred to as an *ATP-synthase*. This channel is coupled to an F₁ proteic complex, that is able to catalyse the formation of ATP from ADP (adenosine di-phosphate) and free phosphate. For this reason, the electron flow from the electron transport chain is inevitably related to the synthesis of this energetic molecule.

Furthermore, experiments were carried out so as to prove the relationship between the electron flow and the ATP synthesis. When blocking the F₀ channel for protons to go back into the matrix (using toxic substances that bind to this complex, such as oligomycin or venturicidin), the electron flow was observed to stop. As protons get pumped into the intermembrane space by the different complexes of the electron transport chain, the proton-motive force keeps increasing as the gradient generated across the inner mitochondrial membrane becomes higher. Then, a point is reached where the energy released by the electron

transport chain is compensated or exceeded thermodynamically by the free energy that is needed so as to pump protons into the intermembrane space against its gradient. Once here, the system is at thermodynamic equilibrium, as the change in free energy turns into zero. Hence, the electron flow stops.

3. EXAMPLE EXERCISE

To better explain the concepts mentioned in this article, we proceed to solve a thermodynamic exercise:

The synthesis of ATP under standard conditions requires 7.7 kcal·mol⁻¹, and this is coupled to the movement of two protons across a mitochondrial membrane. What should be the pH difference across the inner mitochondrial membrane needed to drive ATP synthesis at 25 °C?

3.1. DATA AND UNITS

We will start by organizing all the data given in their proper units.

- The energy given by the problem constitutes the *chemical potential* ($\Delta\mu$), which is the free energy released or absorbed per mole of ion due to a change in its particle number or absorbed per mole of ion due to a change in its particle number or state. In this problem, this energy is associated to the transport of ions through the inner mitochondrial membrane. We change its units to joules:

$$\Delta\mu = 7.7 \frac{\text{kcal}}{\text{mol}} \cdot \frac{4184 \text{ J}}{1 \text{ kcal}} = 32216.8 \frac{\text{J}}{\text{mol}}$$

- Given that the problem asks for the calculations in the movement of two protons, the charge (z) with which we will work will be +2.
- We will also need *Faraday's* (F) and the *ideal gas* (R) constants: $F = 96500 \text{ C}$; $R = 8.31 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$.
- The *temperature* (T) at which the process takes place is $25^\circ\text{C} = 298 \text{ K}$.
- Finally, we will make use of research data. In liver mitochondria, the inner membrane voltage (ΔV) was measured to be 0.17 V when the convention is ($V_{inter.space.} - V_{matrix}$) (Data extracted from Reference [2]). This membrane voltage arises from the fact that there are different concentrations of ions at each side of the membrane, generated by the free passing of these through ion channels as well as their active transport by transmembrane proteins. Each side of the membrane is charged with an electric potential, and the voltage represents the difference in these. We also know that $1 \text{ V} = 1 \text{ J} \cdot \text{C}^{-1}$.

3.2. FORMULAE

Once we have all our data in the corresponding units, we find a formula that relates them between each other. It will be the following:

$$\Delta\mu = R \cdot T \cdot \ln \left(\frac{[Ion]_{in}}{[Ion]_{out}} \right) + z \cdot F \cdot \Delta V (V_{out} - V_{in}) \quad (\text{Eq. 1})$$

In our case, instead of just any ion, we will treat protons. We can write:

$$\ln \left(\frac{[Ion]_{in}}{[Ion]_{out}} \right) = \ln \left(\frac{[H^+]_{matrix}}{[H^+]_{inter.space}} \right) \quad (\text{Eq. 2})$$

If we take into consideration that $\text{pH} = -\log [H^+]$ and that $\ln(x) = 2.3 \log(x)$, we can express the right side of Eq. 2 as:

$$-2.3 \cdot \ln \left(\frac{[H^+]_{matrix}}{[H^+]_{inter.space}} \right) = -2.3 \cdot (\text{pH}_{matrix} - \text{pH}_{inter.space}) = 2.3 \cdot (\text{pH}_{inter.space} - \text{pH}_{matrix}) \quad (\text{Eq. 3})$$

Combining the result obtained in Eq. 3 with Eq. 1, we arrive to:

$$\Delta\mu_{H^+} = R \cdot T \cdot 2.3 \cdot \Delta\text{pH} + z \cdot F \cdot \Delta V \quad (\text{Eq. 4})$$

Where the convention we use is $\Delta\text{pH} = (\text{pH}_{inter.space} - \text{pH}_{matrix})$ and $\Delta V = (V_{inter.space.} - V_{matrix})$.

3.3. RESULTS AND CONCLUSIONS

We will introduce our data for the chemical potential needed to produce one ATP molecule into Eq. 4 and solve for ΔpH :

$$32216.8 \frac{\text{J}}{\text{mol}} = 8.31 \frac{\text{J}}{\text{mol} \cdot \text{K}} \cdot 298 \text{ K} \cdot 2.3 \cdot \Delta\text{pH} + (+2) \cdot 96500 \text{ C} \cdot 0.17 \frac{\text{J}}{\text{C}}$$

$$\Delta\text{pH} = -0.10$$

Looking at our final result and the convention used for the change in pH, we arrive to the conclusion that in order to have two protons pumped to produce one molecule of ATP, the intermembrane space must be 0.10 units of pH more acid than the mitochondrial matrix.

Contrasting this result with experimental data extracted from different text books (Reference 1 and 2), we found out that the real pH change in the mitochondrial matrix is approximately 8, whereas the real pH of the intermembrane space is 7. This supports the idea that there exists an

active transport of protons between the mitochondrial matrix and the intermembrane space, whereas on the other hand there is no active transport between the intermembrane space and the cell's cytosol (across the outer mitochondrial membrane), as there exists no gradient since both compartments are at the same concentration of protons (both at pH 7).

The result obtained in our exercise implies that the slightest change in pH across the inner mitochondrial membrane would thrive the transport of two protons. As explained previously, the proton-motive force generated by the gradient in the concentration of protons across the inner membrane drives the protons back into the matrix. Since this is a process thermodynamically favoured due to the system aiming to reach an equilibrium across the membrane, even the smallest change in pH will favour this transport of protons. The crossing of protons through the ATP-synthase will keep on going as long as the electron transport chain keeps pumping protons into the intermembrane space and the proton-motive force is kept generated.

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