THEORETICAL DEPENDENCE OF PHOSPHATE POTENTIAL ON MAGNITUDE OF PROTONMOTIVE FORCE FOR TWO-ELECTRON PROTON-CHEMICAL COUPLING POINT MODEL.

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In the work the electrical model of a two-electronic proton-chemical coupling point is considered. The computer analysis of the model shows that for mitochondria in respiration state 4 (by Chance) the ratio of the phosphate potential to the proton-motive force grows as the concentration of an uncoupler in the medium increases. The obtained theoretical dependence correlates qualitatively with experimental data contradicting to the chemiosmotic hypothesis.

KEYWORDS: respiration, oxidative phosphorylation, coupling mechanism, mathematical modelling.

At the present t ime the overwhelming majority of works concerned with the study of energy coupling in biomembranes use Mitchell's chemiosmotic hypothesis [1] as a theoretical basis. Nevertheless, some experimentally measured characteristics of energy coupling system cannot be described in terms of chemiosmosis [2, 3]. The proton-chemical hypothesis of energy coupling gives possible variants of the explanation of the mentioned experimental data [4 - 7].

Among the most substantial objections to the orthodox chemiosmotic coupling principle is the variable ratio of phosphate potential to the magnitude of protonmotive force [8, 9]. This ratio grows with the reduction of membrane potential in the process of a titration of mitochondria in respiration state 4 (by Chance) by uncoupler-protonophore. The purpose of the present work is the analysis of the theoretical dependence of the ratio of the phosphate potential to the membrane potential resulting from the proton-chemical mechanism of ATP synthesis. This dependence is considered on the example of a two-electron proton-chemical coupling point. For this purpose the corresponding electrical model of the system of mitochondrial oxidative phosphorylation is used.

MODEL

In fig.1 the general system of reactions in the considered model of oxidative phosphorylation system is shown. Since the detailed description of a two-electronic proton-chemical coupling point and the principles of composing of the equivalent electrical circuits was published earlier [6, 7], it seems expedient only to mention briefly some key moments of the proton-chemical point organization. The coupling point is divided in two subunits "a" and "b" according to [5, 6]. In the subunit "a" the transfer of the electron pair ($2\bar{e}$) is coupled only with pumping out $2H^+$ from a mitochondrion, and in the subunit "b" $2\bar{e}$ can pass either by Mitchell's way coupled with pumping out $2H^+$, or by chemical way, coupled with synthesis 1 ATP and transfer of $2H^+$ into mitochondrial matrix.

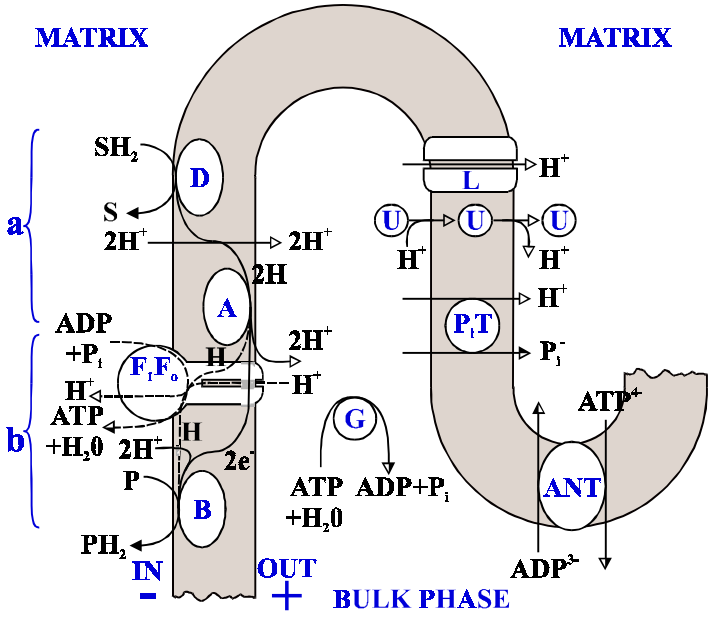

Fig. 1. The system of reactions of oxidative phosphorylation in a two-electronic proton-chemical coupling point. Symbols: D, A, B, - redox-centers of a respiratory chain; SH2 and P - respectively, donor and acceptor of hydrogen atoms; ▬ ▬ ▬ - flow of electrons through the ATP-synthase (F_1F_o) by the chemical way; ANT adeninnucleotidetranslocase; G - ferments hydrolysing ATP; PiT - phosphate carrier; U - uncoupler; L - natural leakage of protons.

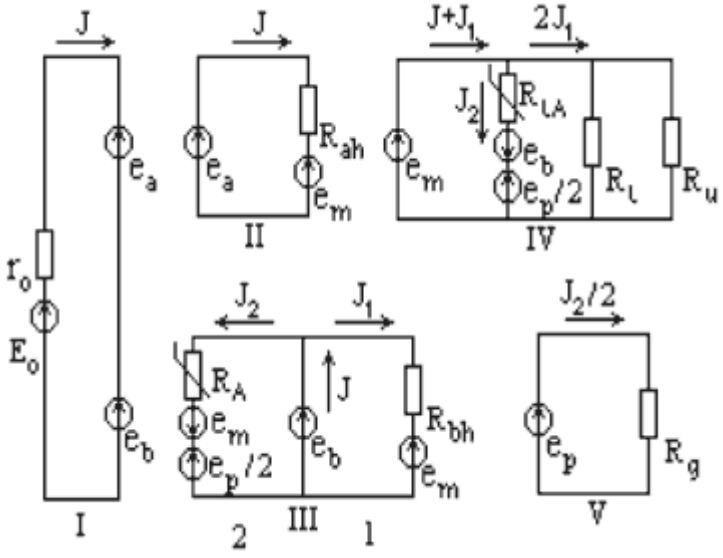

Fig. 2. The equivalent electrical circuit of a two-electron proton-chemical coupling point

The given system of energy coupling can be modelled by the equivalent electrical circuit given in fig. 2. The circuit consists of five contours. The contour I describes a flow of electrons through the whole coupling point. The current J corresponds to a flow of electrons, the voltage E_o reflects the difference of redox potentials ΔE_{BD}, r_o corresponds to internal resistance of E_o source. The voltage e_a and e_b in contour I and other contours reflects respectively redox potential decrease ΔE_{AD} and ΔE_{BA} in the subunits "a" and "b" of a proton-chemical point.

Contour II shows the coupling of electron flow with pumping out of protons in the subunit "a". The resistance R_{ah} characterizes the work of the appropriate proton pump. The voltage e_m corresponds to the transmembrane potential $\Delta\bar{\mu}_{H^+}$.

Contour III describes the coupling of electrons transfer in the subunit "b" of a respiratory chain with pumping out of protons (flow J_1) and ATP synthesis (flow J_2). In the given contour the resistance R_{bh} corresponds to H^+-pump of the subunit "b", and the voltage e_p describes the phosphate potential in nonmitochondrial bulk phase. The resistance R_A reflects the work of the ATP-synthase. According to the redox nature of the formation of "high-energy" bond in the proton-chemical mechanism of ATP synthesis [4 - 7], R_A exponentially depends on the magnitude of $\Delta\bar{\mu}_{H^+}$ and redox potential e_b. It is reflected in the equation (6), where R_{A0} - normalising multiplier.

Contour IV describes the distribution of proton flows in the simulated system. In the given contour

resistance R_L reflects natural leakage of protons through a membrane, and R_U describes the leakage caused by an added uncoupler. The resistance R_{LA} describes the leakage of protons through the ATP-synthase and system of phosphorylation substrates transport coupled with ATP synthesis.

Contour V displays ATP hydrolysis in energy-consuming processes determined by the corresponding resistance R_g.

The given equivalent electrical circuit can be described by the following system of eight equations, in which there are eight unknown variables.

$$J = \frac{E_o - e_a - e_b}{r_o} \quad (1);$$

$$J = \frac{e_a - e_m}{R_{ah}} \quad (2);$$

$$J = J_1 + J_2 \quad (3);$$

$$J_1 = \frac{e_b - e_m}{R_{bh}} \quad (4);$$

$$J_2 = \frac{e_b + e_m - e_p / 2}{R_A} \quad (5);$$

$$R_A = R_{A0} \times 10^{-\frac{e_b + e_m}{30}} \quad (6);$$

$$2J_1 = e_m \frac{R_u + R_L}{R_u R_L} \quad (7);$$

$$J_2 / 2 = \frac{e_p}{R_g} \quad (8).$$

The choice of numerical values of resistances was based on the general qualitative characteristics of mitochondrial oxidative phosphorylation system, such as low proton conductivity of a coupling membrane, approximate value of the respiratory control ratio, the maximum degree of respiration activation, and others. For some parameters, such as a normalising multiplier of the ATP-synthase resistance, which are not obviously determined by general reasons the selection was carried out empirically with the subsequent computer analysis of the model behaviour. (The more detailed description of the parameters' values choice see in work [6].) The values of resistances given below are normalized per one coupling point and have a relative character, though they are expressed in ohms.

The following values of parameters of the electrical circuit were chosen: E_o = 400 mV; r_o = 1 ohm, R_{ah}, R_{bh} = 10 ohms; $R_{A0} = 10^9$ ohms, R_L = 100 ohms; R_g. = 1000 ohms. The titration of system by uncoupler was simulated by the resistance R_U change from value 1000 ohms corresponding to state 4 of respiration (by Chance), up to value 1 ohm at the maximum concentration of uncoupler.

RESULTS AND DISCUSSION

The solving of the equations system (1) - (8) was carried out by the computer. The computer solving of the equations system allowed us to receive the dependences given in fig. 3. As can be seen, with the decrease of the membrane potential the ratio $\Delta G_P/$ [illegible] grows. From the point of view of the proton-chemical coupling principle it can be explained as follows. For ATP synthesis both the energy and directly the energy of redox potentials difference in a respiratory chain are used. At the uncoupler addition the part of energy received from

$\Delta\bar{\mu}_{H^+}$ is reduced much more than the part of energy received from the redox-potentials difference. Due to this the ratio of the phosphate potential (including both these parts) to the membrane potential grows.

Thus, the redox status of the redox-centers of a respiratory chain directly influences the characteristics of ATP formation system. This is inherent qualitaty of a proton-chemical coupling principle in contrast to delocalized chemiosmosis. The obtained curves (fig. 3) qualitatively correlate with real experimental data [8, 9], contradicting to the chemiosmotic hypothesis. Also good agreement with the experimental data, incompatible with the delocalized chemiosmosis principle, was shown earlier for theoretical dependences of ATP synthesis and respiration rates on the membrane potential when $\Delta\bar{\mu}_{H^+}$ was changed in various ways [6, 7]. Thus, the analysis of the model of the proton-chemical coupling mechanism can be considered as an argument in favor of a principle possibility of its existence.

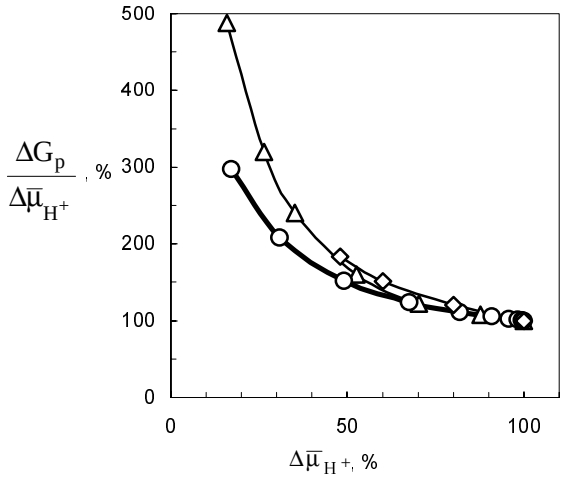

Fig. 3 Dependences of the ratio of the phosphate potential to the membrane potential ($\Delta G_P/\Delta\bar{\mu}_{H^+}$) on the magnitude of $\Delta\bar{\mu}_{H^+}$ at the uncoupler titration for the model of two-electronic proton-chemical coupling unit. Symbols: ◇, △ - experimental data (taken from [8] and [9] accordingly).; ○ - theoretical dependence for a two-electronic coupling unit. All values are normalized relative to the respiration state 4 (by Chance). (Parameters of the electrical circuits see in the text).

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