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Mathematical Simulation of Energy Coupling in Mitochondria within the Framework of the Proton–Chemical Hypothesis

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Abstract – The effect imposed by the method of transmembrane potential change on the rates of ATP synthesis and respiration in mitochondria is discussed within the framework of the proton-chemical hypothesis of coupling. Electrical and mathematical models are proposed to simulate energy coupling. A theoretical dependence of the rates of oxidative phosphorylation and mitochondrial respiration on the transmembrane potential was derived on the basis of computer analysis of the mathematical model. The theoretical dependence fits the experimental findings inconsistent with the chemiosmotic theory of energy coupling

Key words: mitochondria, ATP, transmembrane potential, energy coupling, model.

INTRODUCTION

The chemiosmotic theory of energy coupling advanced by Mitchell [1] is widely recognized as the most comprehensive description of the mechanism of energy coupling in biological membranes [2]. However, the actual research has accumulated a large body of experimental findings that are either inconsistent or in clear contradiction with this theory [3-9]. This gave rise to a number of modifications of the chemiosmotic theory of energy coupling [10-12]. The chemical hypothesis of oxidative phosphorylation [4] has also been slightly modified. According to the modified chemical hypothesis, there is indeed a protonmotive force ($\Delta\bar{\mu}_{H^+}$) across the coupling membrane (as suggested by Mitchell), although the protonmotive force plays only a minor function in the mechanism of energy coupling. The chemiosmotic theory of energy coupling can hardly explain the following experimental observations: (1) the rate of oxidative phosphorylation as a function of the transmembrane potential depends on the method of changing the transmembrane potential [3,4,6]; (2) the extent of activation of mitochondrial respiration induced by a decrease in the protonmotive force may vary depending on the method of reduction of the protonmotive force (the value of the protonmotive force reduction is maintained at the same level whatever the method of reduction is used).

According to the chemiosmotic theory of energy coupling, the rates of both oxidative phosphorylation and respiration depend on the $\Delta\bar{\mu}_{H^+}$ value but do not depend on the method of its change. These data, as well as some other results concerning the system of oxidative phosphorylation, can be consistently explained within the framework of the proton-chemical hypothesis [13-16]. This hypothesis is based on the chemical hypothesis and includes elements of the chemiosmotic theory.

The goal of this work was to construct a mathematical model designed to provide a quantitative description of the rates of ATP synthesis and respiration in mitochondria as functions of $\Delta\bar{\mu}_{H^+}$. The value of $\Delta\bar{\mu}_{H^+}$ can be varied by adding uncouplers or respiration inhibitors and by changing the rate of ATP consumption.

Abbreviation: RP, redox potential.

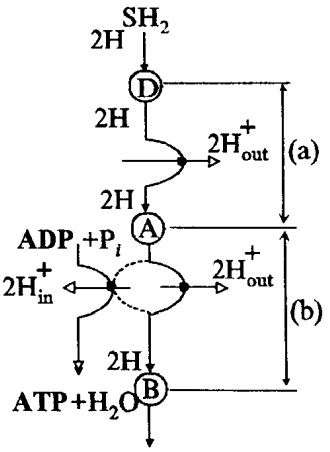

Fig. 1. General scheme of a coupling site: (D), (B), and (A) are the redox centers of respiratory chain; ⟶ denotes the electron flow in the respiratory chain; ----- denotes the electron flow in the chemical route; ⟶ denotes the transmembrane transport of protons; ⟶ ATP synthesis route; ● denotes the coupling between flows.

ELECTRICAL AND MATHEMATICAL MODELS OF PROTON-CHEMICAL COUPLING

The mathematical description of the proton-chemical hypothesis is based on an equivalent electrical circuit of one site of proton-chemical coupling of the respiratory chain [16]. Energy coupling simulation with equivalent electrical circuits was used by many authors as a method of qualitative and quantitative testing of the hypotheses of oxidative phosphorylation [11, 12, 16]. For the sake of simplicity of description of the equivalent electrical circuit of the coupling site, consider the two main consequences of the proton-chemical hypothesis [13-16]:

(1) The electron flow bifurcates at the coupling site, one part of it passing through the Mitchell route, in which the energy of the redox potential (RP) difference is spent to generate the transmembrane gradient of protons, and part of it being involved in direct chemical synthesis of ATP.

(2) In mitochondria, the elementary act of biosynthesis of one molecule of ATP requires the transmembrane transfer of a proton through ATP synthase to the matrix and transfer of two hydrogen atoms (two electrons and two protons) through ATP synthase from an electron donor to an electron acceptor of the respiratory chain. These two processes are coupled to one another so as to provide for the chemical mechanism of ATP synthesis, although the energy of $\Delta\bar{\mu}_{H^+}$ is also used in this reaction. In addition, a fraction of the energy should be spent to transport the phosphorylation substrates through the mitochondrial membrane (one H^+ per one ATP molecule). Thus, the energy of simultaneous transfer of two protons through the coupling site and two hydrogen atoms between the redox centers of the electron transport chain is required to synthesize one molecule of ATP. The phosphate potential generated in the system (ΔG_p) is determined by the summation of the two types of energy: $\Delta G_p = 2F\left(\Delta\bar{\mu}_{H^+} + \Delta E\right)$, where F is the Faraday number, $\Delta\bar{\mu}_{H^+}$ is the transmembrane gradient of electrochemical proton potential (protonmotive force suggested by Mitchell), ΔE is the difference between the RP levels of the electron donor and the electron acceptor components of the coupling site.

Consider the mitochondrial proton-chemical coupling site in more detail (Fig. 1). In accordance with the model suggested in the preceding work [16], the model of the site consists of two functional components (*a* and *b*). The electron transport in component *a* is coupled only to the proton efflux from mitochondria, whereas the electron flow at point *b* bifurcates, one part of it passing through the Mitchell route, in which the electron transport is coupled to proton efflux, and the other part of it being involved in direct chemical synthesis of ATP and transmembrane transfer of two protons to the matrix. Rather trivial calculations give a stoichiometric ratio of $4H^+/2\bar{e}$ per one coupling site (component *a* plus component *b*) in the absence of ATP synthesis or $1ATP/2\bar{e}$ under conditions of completely coupled ATP synthesis. Therefore, synthesis of one ATP molecule at the coupling site requires the energy released during the transfer of two electrons through the chemical route (provided that the two-electron chemosynthesis is active) and the energy of the transmembrane transfer of two protons

In the preceding works [13, 14, 16], the phosphite-thiol mechanism of oxidative phosphorylation was considered as an example of implementing the principles of proton-chemical coupling. According to this mechanism, the rate of ATP synthesis declines exponentially with the decrease in the membrane potential and the RP difference

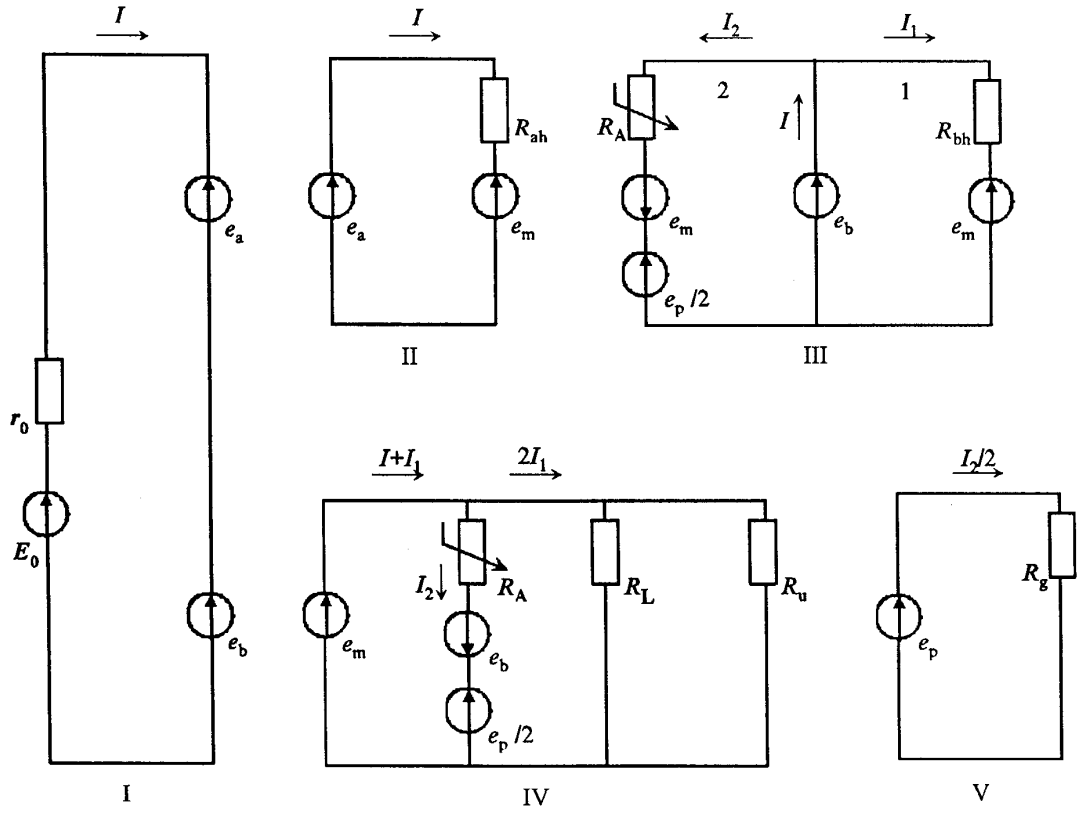

Fig. 2. Equivalent electrical circuit of two proton-chemical coupling sites (explanation in text).

between the electron donor and electron acceptor components of the coupling site [16]:

$$J_p \sim \frac{[P]^{3+}}{[P]^{5+}} \sim 10^{\frac{const - \Delta\bar{\mu}_{H^+} - \Delta E_{AB}}{2.3RT/nF}}, \qquad (1)$$

where $[P]^{3+}$ and $[P]^{5+}$ are the phosphite and the phosphate concentrations, respectively.

The energy effect of complete reduction of phosphate to phosphite is essentially negative [18], because of the very low RP value of this redox pair [16]. This makes very unlikely the involvement of phosphite in the mechanism of energy transformation, although does not completely discard such a probability. However, it is the phosphite mechanism that allows the simplest introduction of the proton-chemical coupling [16]. Therefore, by analogy with equation (1), the exponential dependence of the rate of ATP synthesis on $\Delta\bar{\mu}_{H^+}$ and ΔE can be deduced from any other redox mechanism of ATP synthesis at the proton-chemical coupling site (including one-electron or free radical mechanisms) [15]. The radical hypothesis of energy coupling has been put forward later by Schole and Schole [19]. According to this hypothesis, the role of the macroergic intermediate is played by cardiolipin, which is converted into cardiolipin-*enol*-phosphate during the redox reaction.

Most generally, the electron flow distribution between the chemiosmotic and the chemical branches of component *b* can be described within the framework of the proton-chemical principle of coupling as follows: as the coupling membrane is charged to the maximum level by the chemiosmotic mechanism, the electric current through the membrane (i.e., a kind of an electric capacitor) stops, and the RF difference ΔE_{BA} at component *b* also attains its maximum level. This activates the electron transfer through the chemical route. Thus, the toggling is in essence thermodynamic, and may specifically involve a molecular switchover, e.g., one of the mechanisms regulating the functional activities of biological membranes by the transmembrane electric potential [17].

An electrical equivalent to the double site of proton-chemical coupling (Fig. 1, see also [16]) is shown as the five circuits in Fig. 2. The electron flow in a form of a flow of hydrogen atoms from the donor D to the acceptor B (Fig. 1) is induced by the RP difference between D and B (ΔE_{BD}). The RP Difference between D and B is also the driving force of the oxidative phosphorylation in general. In Fig. 2, the electric current in loop I corresponds to the electron flow through the entire coupling site, whereas the voltage source E_0, with the internal resistor r_0, represents the steady-state RP difference ΔE_{BD} between D and B (Fig. 1). At point *a*, there is a steady-state RP difference between D and A (ΔE_{AD}), which is represented in loop I as a voltage source e_a; and at point *b*, there is the RP difference ΔE_{BA}, which is represented in loop I as a voltage source e_b. The electric current J in loop I is equal to the rate of electron transfer through the coupling site. This value is determined by the variables E_0, e_a and e_b, as

$$J = \frac{E_0 - e_a - e_b}{r_0}. \qquad (2)$$

It is obvious from Fig. 1 that the transfer of an electron from the donor D to the acceptor A in point *a* is accompanied by the transmembrane transfer of one H^+ and generation of $\Delta\bar{\mu}_{H^+}$. In the electrical circuit shown in Fig. 2, $\Delta\bar{\mu}_{H^+}$ is represented by a voltage source e_m, of loop II. In addition to e_m, loop II also includes the resistance R_{ah}, of the H^+-pump. The electric current driven in loop II by the voltage difference (e_a - e_m) is equal to the rate of proton transport by the H^+-pump of point *a* and to the electric current J passing through the entire coupling site:

$$J = \frac{e_a - e_m}{R_{ah}}. \qquad (3)$$

The electric current passing through point *b* is determined as a sum of electric currents passing through the chemiosmotic route and a parallel chemical route of ATP synthesis (Fig. 1). It is seen in loop III of the electrical circuit in Fig. 2 that the voltage e_b drives the electric current through both the right (chemiosmotic) and the left (chemical) arms of the loop (loop III.1 with current J_1, and loop III.2 with current J_2, respectively). The sum of currents J_1, and J_2 passing through point *b* is equal to the total electric current J passing through the entire coupling site in loop I:

$$J = J_1 + J_2. \qquad (4)$$

The electric current J_1 is associated with the active proton transport through point *b* mediated by the H^+-pump. The amplitude of the current is determined by the voltage difference (e_b - e_m) and the pump resistance R_{bh}. According to the method of circuit currents, we can put down:

$$J_1 = \frac{e_b - e_m}{R_{bh}}. \qquad (5)$$

In accordance with the two-electron mechanism of proton-chemical coupling considered in this work, synthesis of one molecule of ATP is coupled to a simultaneous transfer of two electrons and two protons along the transmembrane gradient of electrochemical potential. Hence, the amplitude of electric current J_2 in loop III.2 is determined by the sum of voltages (e_b + e_m) and by the resistance R_A, of the ATP synthase. In addition, the rate of chemical synthesis of ATP and the amplitude of electric current J_2 depend on the phosphate potential e_p. This dependence can be taken into account by adding an additional source of voltage $e_p/2$ to loop III.2, the polarity of the additional source being opposite to the polarity of e_b and e_m. The divider 2 below e_p represents the lATP/2$\bar{e}$ stoichiometry of the single site of the proton-chemical coupling. In this case, the value of the phosphate potential is measured in the extramitochondrial space.

Thus, according to the method of circuit currents, the electric current in loop III.2 is

$$J_2 = \frac{e_b + e_m - e_p/2}{R_A}. \qquad (6)$$

On the one hand, the amplitude of current J_2 is equal to twice the rate of the chemical synthesis of ATP, and on the other hand, it is equal to the rate of the proton influx to the matrix coupled to ATP synthesis.

Rather trivial calculations [16] show that in the case of the two-electron mechanism of proton-chemical coupling, the rate of ATP synthesis declines exponentially as the values of $\Delta\bar{\mu}_{H^+}$ and/or ΔE_{BA} decrease (see equation (1)). In the electrical circuit shown in Fig. 2, loop III, this exponential function is represented by a nonlinear depen-

dence of the ATP synthase resistance R_A, on e_b and e_m:

$$R_A = R_{A0} \cdot 10^{-\frac{e_b + e_m}{30}}, \quad (7)$$

where R_{A0} is the scaling factor.

In equivalent electrical circuits, $\Delta\bar{\mu}_{H^+}$ is represented by a voltage source e_m,. The value of $\Delta\bar{\mu}_{H^+}$ is determined by the ratio of the rate of the proton efflux by H^+ pumps and the proton conductance of the coupling membrane. Loop IV in Fig. 2 represents the fact that the rate of proton efflux is equal to the sum of the rates of the proton efflux by H^+ pumps at point *a* (current *J* in loop II, Fig. 2) and point *b* (current J_1 in loop III.1, Fig. 2). Therefore, the total rate of the proton efflux is $J + J_1$. In the general case, the proton conductance (loop IV, Fig. 2) is determined by: (1) spontaneous proton leakage through the membrane (resistor R_L; (2) leakage induced by addition of uncoupling agents (resistor R_U); and (3) proton transfer through ATP synthase and phosphorylation substrate transport system (resulting resistance is numerically equal to R_A).

According to the two-electron mechanism of proton-chemical coupling, the resulting transmembrane flow of protons, which is coupled to ATP synthesis and transport of phosphorylation substrates, is numerically equal to the corresponding electron flow J_2. Therefore, the total electric current passing through resistors R_U, and R_L, in loop IV is $(J + J_1 - J_2)$; with account of equation (3), this current is equal to $2J_1$. On the other hand, the electric current passing through resistors R_U, and R_L, can be expressed as a function of e_m:

$$2J_1 = e_m \frac{R_U + R_L}{R_U R_L}. \quad (8)$$

The value of the phosphate potential is represented in this circuit by a voltage source e_p and is determined by the ratio of the rates of ATP synthesis at the coupling site and ATP hydrolysis in endergonic reactions. Let us assume that all endergonic reactions proceed in the extramitochondrial space. Therefore, the value of the phosphate potential in the case considered is determined for the extramitochondrial space. Hydrolysis of ATP is simulated in the electrical circuit by the resistor R_g, in loop V. In the steady state, the rate of ATP hydrolysis is equal to the rate of ATP synthesis, i.e., $J_2/2$. This rate can also be expressed as the quotient of the phosphate potential divided by the resistance R_g:

$$J_2 / 2 = \frac{e_p}{R_g}. \quad (9)$$

This brings us to a set of eight nonidentical simultaneous equations (2)-(9), which contain eight unknown variables: J, J_1, J_2, e_a, e_b, e_m, e_p, and R_A. In a more convenient form, this set of simultaneous equations can be recast as:

$$J_2 = E_0 x_1 / [R_g (x_1 + R_{ah} + r_0)/4 + (R_{ah} + r_0) R_A + (R_{ah} + r_0 + R_A) x_1], \quad (10)$$

$$J_1 = \frac{E_0 - J_2 (R_{ah} + r_0 + R_A)}{R_{ah} + r_0}, \quad (11)$$

$$J = J_1 + J_2, \quad (12)$$

$$e_m = 2J_1 \frac{R_U R_L}{R_U + R_L}, \quad (13)$$

$$e_a = J R_{ah} + e_m, \quad (14)$$

$$e_b = E_0 - e_a - J r_0, \quad (15)$$

$$e_p = J_2 R_g / 2, \quad (16)$$

$$R_A = R_{A0} \cdot 10^{-\frac{J_2 R_A + e_p/2}{30}}, \quad (17)$$

where $x_1 = 4 \frac{R_U R_L}{R_U + R_L} + R_{bh}$.

Resistors r_0, R_{ah}, R_{bh}, R_U, R_L, R_g, and R_{A0}, represent individual model parameters of the system of energy coupling as they may appear in the actual experimental settings. For example, malonate-induced inhibition of succinate-dependent respiration of mitochondria can be simulated by a change in the internal resistance r_0, of the voltage source E_0. Addition of an uncoupler to the incubation medium is simulated by varying the resistance R_U, within a certain range. Activation of ATP synthesis caused by a decrease in the phosphate potential (e.g., as a result of addition of ADP or hexokinase to the incubation medium) can be simulated by a decrease in resistance R_g.

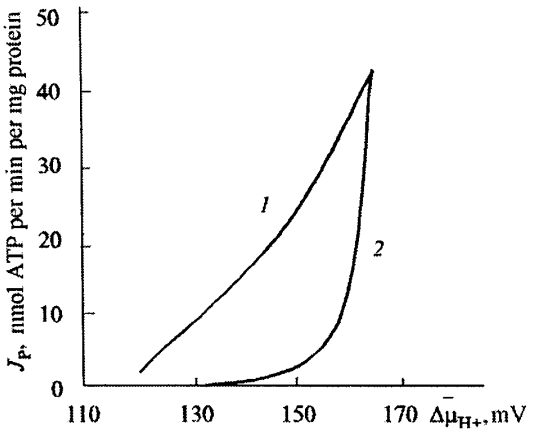

Fig. 3. Dependence of the rate of ATP synthesis on the transmembrane potential value: (1) titration with uncoupler; (2) titration with respiration inhibitor.

SELECTION OF PARAMETERS OF MODEL SIMULATING THE PROTON-CHEMICAL COUPLING SITE

Consider the rates of ATP synthesis and respiration at one site of proton-chemical coupling in mitochondria as functions of $\Delta\bar{\mu}_{H^+}$, the latter being changed by two different methods. Let the parameters of the system be defined as follows.

Let the voltage E_0, be 400 mV, i.e., ~1/3 of the total RP difference between the oxygen and the hydrogen electrodes.

The values of resistance are selected using the criteria of the order of magnitude of the respiratory control, the maximum extent of the uncoupler-induced activation of respiration, the low proton conductance of coupling membrane, etc. The choice of the model parameters was empirical, subject to refinement by computer simulation. The resistance values used in the model were brought to normal levels typical of one coupling site. Although the resistances are expressed in ohms, these are relative rather than absolute values.

Let the values of r_0, R_{ah}, and R_{bh}, be equal to 1 Ω each, and the value of spontaneous proton leakage through membrane R_L, be 100 Ω (i.e., significantly higher than the H^+ pump resistance). Let also the scaling factor R_{A0} of the ATP synthase resistance R_A, be $R_{A0} = 2 \cdot 10^{11}$ Ω (the actual value of R_A, during ATP synthesis is about 10 Ω). The initial leakage resistance R_U, caused by addition of uncoupling agents can be taken 10 kΩ. To the first approximation, this value can be regarded as an infinitely large resistance R_U, in the absence of uncouplers. The value of resistance R_g, which corresponds to the mitochondrial respiration state 4 (by Chance), can be taken as large as the initial leakage resistance in the absence of uncouplers (R_g = 10 kΩ). This corresponds to a negligible rate of ATP hydrolysis in the extramitochondrial space.

COMPUTER ANALYSIS OF A MODEL OF PROTON-CHEMICAL COUPLING

The set of simultaneous equations (10) - (17) cannot be solved explicitly, because equation (17) is selflinked and nonlinear (variable R_A, depends on its own value as an exponent index). Therefore, the method of iteration by the variable R_A was used to solve the set of simultaneous equations. The initial parameters of the equivalent electrical circuit listed above were taken as the initial parameters of the model. Calculations were performed numerically using a computer. The rates of ATP synthesis and respiration were calculated as functions of the transmembrane potential $\Delta\bar{\mu}_{H^+}$. The value of $\Delta\bar{\mu}_{H^+}$ for the two dependences was changed by two different methods.

In the model analysis of the curve of the dependence of the ATP synthesis rate on the $\Delta\bar{\mu}_{H^+}$ value, as changed experimentally by adding an uncoupler, the change of the transmembrane potential value was simulated by decreasing the R_U resistance from the initial level of 10 kΩ to 1 Ω. The lower limit of the R_U, value (1 Ω) was chosen to be two orders of magnitude less the value of spontaneous proton leakage through the membrane, R_L. The value of R_g, was taken equal to 1 Ω which corresponds to ΔG = 0.

In the model analysis of the curve of the ATP synthesis rate dependence on the $\Delta\bar{\mu}_{H^+}$ value, as changed experimentally by the addition of a competitive inhibitor of substrate oxidation, the change in the transmembrane potential value was simulated by varying the value of the internal resistance r_0, from 1 to 1000 Ω.

The curves of the ATP synthesis rate dependence on $\Delta\bar{\mu}_{H^+}$ for the two cases are shown in Fig. 3. It is seen that the theoretical curves decline exponentially as the transmembrane potential decreases, and are divergent (i.e., one value of $\Delta\bar{\mu}_{H^+}$ corresponds to two different values of

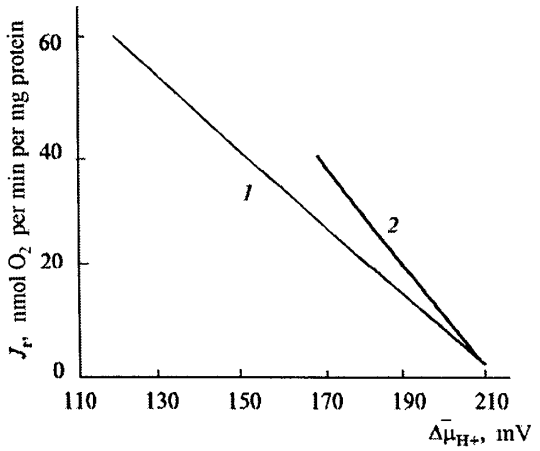

Fig. 4. Dependence of the respiration rate on the transmembrane potential value: (1) titration with uncoupler; (2) titration with hexokinase.

the ATP synthesis rate). There is a qualitative fit of the computer simulation with the experimental data obtained for mitochondria [6].

It does not follow from the chemiosmotic theory that an abrupt decrease in the ATP synthesis rate can be caused by an insignificant decrease in the transmembrane potential. Even if this were assumed to be associated with the specific kinetic characteristics of the ATP synthase, it would be impossible to explain within the framework of the chemiosmotic theory the existence of two differing values of the ATP synthesis rate at the same value of $\Delta\bar{\mu}_{H^+}$ changed by different methods. However, this artifact is a mathematically rigorous corollary to the model of proton-chemical coupling.

Two modes of $\Delta\bar{\mu}_{H^+}$ changing (titration with a protonophore uncoupler or titration with hexokinase) were considered during the theoretical simulation of the curve of the mitochondrial respiration rate dependence on the transmembrane potential. As noted above, titration with an uncoupler was simulated by decreasing the R_U, resistance from the initial level of 10 kΩ to 1 Ω. Hexokinase titration was simulated by decreasing the R_g, value (resistance to ATP hydrolysis) from the initial level of 10 kΩ to 1Ω. Two diverging straight lines were obtained as a result of simulation (Fig. 4), which is in a qualitative agreement with certain experimental data contradicting the Mitchell's hypothesis [3, 9].

The mathematical and electrical models of proton-chemical coupling considered in this work allow a number of thermodynamic parameters of the system of oxidative phosphorylation to be brought in correlation with each other. Computer simulation of these models provides interpretation of a large body of experimental results from the standpoint of the proton-chemical coupling. The experimental findings inconsistent with the chemiosmotic theory of energy coupling can also be explained. The possibility of simulating the physicochemical characteristics of the system of energy coupling that cannot be measured by the currently available experimental methods is an advantage of the model suggested in this work.

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