Model of the Outer Membrane Potential Generation by the Inner Membrane of Mitochondria

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ABSTRACT Voltage-dependent anion channels in the outer mitochondrial membrane are strongly regulated by electrical potential. In this work, one of the possible mechanisms of the outer membrane potential generation is proposed. We suggest that the inner membrane potential may be divided on two resistances in series, the resistance of the contact sites between the inner and outer membranes and the resistance of the voltage-dependent anion channels localized beyond the contacts in the outer membrane. The main principle of the proposed mechanism is illustrated by simplified electric and kinetic models. Computational behavior of the kinetic model shows a restriction of the steady-state metabolite flux through the mitochondrial membranes at relatively high concentration of the external ADP. The flux restriction was caused by a decrease of the voltage across the contact sites and by an increase in the outer membrane potential (up to +60 mV) leading to the closure of the voltage-dependent anion channels localized beyond the contact sites. This mechanism suggests that the outer membrane potential may arrest ATP release through the outer membrane beyond the contact sites, thus tightly coordinating mitochondrial metabolism and aerobic glycolysis in tumor and normal proliferating cells.

INTRODUCTION

Voltage-dependent anion channels (VDAC) of the outer mitochondrial membrane (OMM) are permeable to many nonelectrolytes with molecular masses less than 4 to 8 kDa (Colombini, 1980; Zalman et al., 1980). The VDAC permeability to anions strongly depends on the applied electrical potential (Shein et al. 1976; Colombini, 1979; Benz, 1985; Hodge and Colombini, 1997; Rostovtseva and Colombini, 1997). It has been suggested that VDAC may control mitochondrial metabolism (Liu and Colombini, 1992; Sorgato and Moran, 1993; Hodge and Colombini, 1997; Rostovtseva and Colombini, 1997; Lemeshko and Lemeshko, 2000), but mechanisms of the outer mitochondrial membrane potential (OMMP) generation are not known yet, except the Donnan potential (Liu and Colombini, 1991, 1992). Even the maintenance of any OMMP, if generated, has been considered doubtful (Benz et al., 1990; Sorgato and Moran, 1993) due to a high ionic conductance of the OMM. Recently, we have considered one of the possible mechanisms of the OMMP generation, resulting from the difference in the VDACs permeability to various charged metabolites passing through the mitochondrial membrane (Lemeshko and Lemeshko, 2000). The modeling of this mechanism showed that the value of generated potential depends on the rate of endergonic processes in the cytoplasm. In turn, the energy flux through the OMM was regulated by this metabolically derived potential. Other mechanisms of the outer membrane potential generation are not excluded, and the superposition of the OMM potentials generated by various mechanisms may take place, as it was recently considered (Lemeshko and Lemeshko, 2000), with respect to the Donnan potential and the metabolically derived potential across the OMM.

One mechanism of the OMMP generation might be expected taking into account the existence of the contact sites between the inner and outer membranes of mitochondria (Hackenbrock, 1968; Ohlendieck et al., 1986; Sandri et al., 1988; Brdiczka, 1991; Zoratti and Szabó, 1995; Brdiczka et al., 1998; Crompton, 1999). Adenine nucleotide translocators (ANT), which are the most abundant proteins of the inner mitochondrial membrane (IMM), and VDAC, which are the most abundant proteins of the OMM, are believed to form the contact sites (Benz et al., 1988; Brdiczka, 1991; Kinnaly and Tedeschi, 1994; Wilson, 1994; Beutner et al., 1997; Crompton, 1999). The data of kinetic analysis indicate that the functional interaction between ANT, VDAC, and kinases takes place in the contacts (Gots and Bessman, 1974; Weiler et al., 1985; Benz et al., 1988, 1990; Brdiczka, 1990; Wilson, 1994; Laterveer et al., 1995; Crompton, 1999). It was directly confirmed by reconstruction of ANT, VDAC, and hexokinase (HK) in phospholipid vesicles (Brdiczka et al., 1998). According to these data, the matrix ATP may be directly transported through the contact sites to

Submitted August 10, 2001, and accepted for publication October 26, 2001.

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List of symbols used: $\Delta\psi$, the IMMP; $\Delta\psi_c$, the intermembrane contact sites voltage; $\Delta\psi_o$, the OMMP; G-P_i^{1-}, glucose-6-phosphate; Pr, end products of glucose-6-phosphate metabolism; g_{AV}, the conductance of the intermembrane contact sites resulted from the ATP^{4-}/ADP^{3-} exchange, $R_{AV} = 1/g_{AV}$; g_V, the conductance of the VDAC beyond the contacts, $R_V = 1/g_V$; g_H, the proton conductance of the H^+-ATP synthase; g_L, the proton leak across the IMM; E_r, the redox potentials difference in the coupling sites of the respiratory chain; g_r, the inner conductance of the battery E_r; C_m, electric capacity of the IMM; J_L, the proton leak across the IMM; J_H, the IMM proton flux across the H^+-ATP synthase; J_{AV}, the flux of ATP^{4-} across the intermembrane contact sites in exchange for ADP^{3-} that is equal to the flux of P_i^{1-} across the OMM and IMM at steady state; J_a, the flux of P_i across the OMM and IMM, the rate of ATP synthesis, or ATP/ADP exchange through the intermembrane contact sites; a, the voltage sensitivity of VDAC according to Eq. 5.

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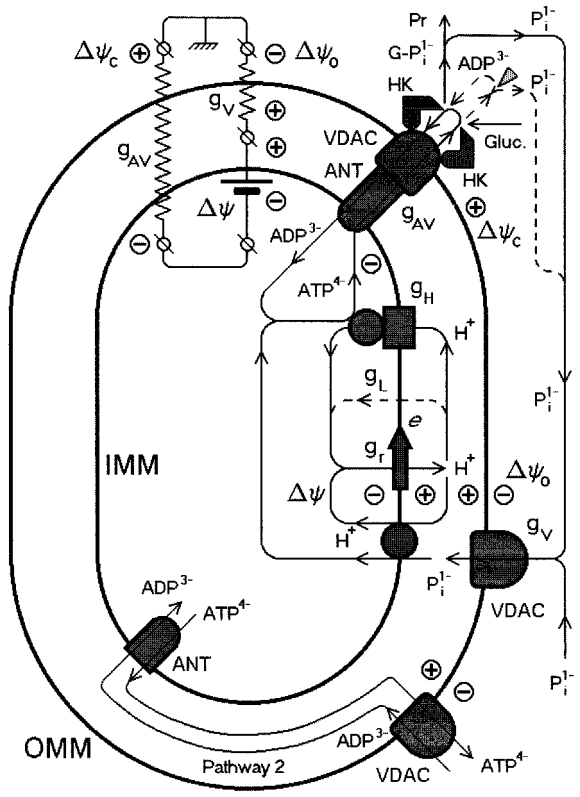

FIGURE 1 The main principle of the outer membrane potential generation in mitochondria, based on the inner membrane voltage ($\Delta\psi$) division between the intermembrane contact sites ($\Delta\psi_c$) and the outer membrane beyond the contacts ($\Delta\psi_o$). G-P_i^{1-}, glucose-6-phosphate; Pr, end products of glucose-6-phosphate metabolism; g_{AV}, conductance of the complexes ANT-VDAC(HK) in the contact sites; Gluc., glucose; g_V, conductance of the VDAC beyond the contact sites; g_H, proton conductance of the H^+-ATP synthase coupled to ATP synthesis; g_L, proton leak across the IMM;. *e*, the respiratory chain electron transport coupled to generation of $\Delta\psi$ and ΔpH across the IMM.

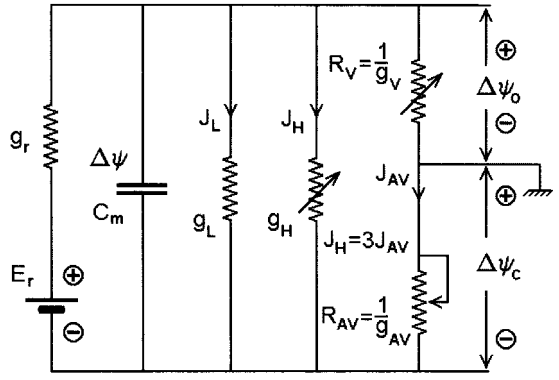

FIGURE 2 Simplified electric model of the mechanism of the outer membrane potential generation due to the inner membrane voltage ($\Delta\psi$) division between the intermembrane contact sites ($\Delta\psi_c$) and the outer membrane ($\Delta\psi_o$) in mitochondria. E_r, The redox potentials difference in the coupling sites of the respiratory chain; $1/g_r$, the inner resistance of the battery E_r; C_m, electric capacity of the IMM; g_{AV}, g_V, g_L, and g_H, see legends for Fig. 1; $R_{AV} = 1/g_{AV}$, $R_V = 1/g_V$.

the cytoplasm side, without passing through the mitochondrial intermembrane space (MIMS). If that is the case, the electric current should flow through the contacts sites at steady-state ATP^{4-}/ADP^{3-} exchange, taking into account the electrogenic character of ANT functioning (Brustovetsky et al., 1996). Inorganic phosphate (P_i^{1-}), yielded from hydrolysis of released ATP, can pass into the MIMS through the VDAC localized beyond the contacts, thus closing the electric circuit between the inner and outer sides of the IMM (Fig. 1). Thus the inner membrane potential (IMMP), generated by the respiratory chain, should partially drop across the OMM. This possibility of the OMMP generation was never discussed in the literature, although the idea that the IMMP might be transduced to the OMM inside the contact sites (but not beyond the contacts) due to the capacity coupling has been suggested (Benz et al., 1990).

Here, we have considered the OMMP generation as a result of the IMMP division on two sequentially connected resistances, the contact sites, formed by ANT and VDAC, and the free VDAC localized beyond the contacts. This concept was presented in the form of an equivalent electric circuit and a simplified kinetic model. The kinetic model behavior was studied computationally. The obtained data showed that a relatively high OMMP might be generated, restricting the metabolite flux across the mitochondrial membranes at high workloads on the contact sites.

DESCRIPTION OF THE MODELS

Electric model

The main principle of the proposed mechanism of the OMMP generation is shown in Fig. 1. If the contact site between the IMM and OMM functions as a bi-transmembrane electrogenic ATP^{4-}/ADP^{3-} antiporter, similar to ANT alone (Brustovetsky et al., 1996), then a net flux of negative charges should flow through the contacts at steady state. According to the model, the steady state is supported by direct or indirect hydrolysis of the released ATP. One of the indirect mechanisms of ATP hydrolysis may be carried out through metabolism of glucose-6-phosphate formed at the contact sites by the OMM bound HK in the presence of glucose. The liberated ADP^{3-} returns back into the matrix in exchange for a new molecule of ATP^{4-}. At the same time, P_i^{1-} passes from the external medium into the MIMS through the VDAC beyond the contacts, carrying the same flux of negative charges as that carried by the electrogenic ATP^{4-}/ADP^{3-} exchange across the contact sites. Thus, the electric circuit between the inner and outer sides of the IMM, i.e., between the negative and positive poles of the IMMP, will be closed (Fig. 1). For ATP resyntheses, P_i^{1-} and one proton are transported into the matrix by the phosphate carrier, in addition to ADP entering the matrix as a result of the ATP^{4-}/ADP^{3-} exchange through the contact sites. Thus, the electric circuit (Fig. 2) is formed by a conductance of the contact sites, g_{AV} (the total resistance is $R_{AV} = 1/g_{AV}$, related to one mitochondrion), and by a conductance of the VDAC localized beyond the contacts, g_V (the total resistance is $R_V = 1/g_V$, related to one mitochondrion). The two resistances, R_{AV} and R_V, connected in series, serve as a load on the IMMP ($\Delta\psi$). According to the Ohm's law, the voltage division

should take place at steady state of the system, satisfying the following equation:

$$\Delta\psi = \Delta\psi_c + (-\Delta\psi_o), \quad (1)$$

in which $\Delta\psi_c$ and $\Delta\psi_o$ are the potentials across the contact sites and the OMM beyond the contacts, respectively. Polarities of these potentials are related to zero potential in the cytoplasm (Fig. 2). The voltage division may be expressed as:

$$\Delta\psi_c = \Delta\psi \times g_V/(g_{AV} + g_V), \quad (2)$$

$$-\Delta\psi_o = \Delta\psi \times g_{AV}/(g_{AV} + g_V). \quad (3)$$

Dependence of conductance g_V on the OMMP may be expressed mathematically similar to that described earlier for the VDACs permeability (Lemeshko and Lemeshko, 2000). The conductance g_{AV} depends not only on the ADP and ATP concentrations in the matrix and on the cytoplasmic side of the contact sites, but it may also depend on the voltage across the contacts. On the other hand, there are no experimental data confirming that the voltage sensitivity of the VDAC associated with ANT and HK is conserved. For simplicity, we assume that the VDACs permeability does not restrict the ATP^{4-}/ADP^{3-} exchange at any voltage across the contact sites.

To study the model behavior, various workloads on the mitochondrial contact sites were simulated by changing the local concentration of ADP on the cytoplasmic side of the contact sites. This concentration may depend on the activity of HK or glycerol kinase attached to the contacts. In this work, HK or glycerol kinase activities are not included in the modeling, and the local ADP and ATP concentrations within the VDAC in the contact sites are set to be equal to those in the cytoplasm. The concentrations of ATP in the matrix and cytoplasm, the concentration of ADP in the matrix, and the average concentration of P_i in the system were set as constants, for simplicity. Under these conditions, the change in the ADP concentration in the cytoplasm (understanding it, as it would be the local concentration of ADP within the VDAC, which is located in the contact sites) modulates the conductance g_{AV}, according to the electric model (Fig. 2).

The equivalent electric circuit (Fig. 2) shows that the respiratory chain generates the IMMP ($\Delta\psi$). Its value depends on: 1) the redox potential difference (E_r) between the electron acceptor and donor sites in the coupling units of the respiratory chain; 2) the inner resistance ($1/g_r$) of the battery E_r; 3) the proton leak across the IMM (J_L); and 4) the cytoplasmic concentration of ADP. Higher g_{AV} corresponds to higher concentrations of ADP in the cytoplasm. The proportion between $\Delta\psi_o$ and $\Delta\psi_c$ depends on the g_V/g_{AV} ratio. In turn, g_V depends on $\Delta\psi_o$, reflecting the permeability-voltage dependence of the VDAC beyond the contacts. The IMM proton conductance through the H^+-ATP synthase (g_H) is proportional to the rate of ATP synthesis. If we assume that $3H^+$ are transported through the H^+-ATP synthase per one synthesized ATP, then the IMM proton flux across the H^+-ATP synthase (J_H) is three times bigger than the flux of P_i across the OMM and IMM, or of ATP^{4-} across the contact sites in exchange for the external ADP^{3-} (J_{AV}), that is $J_H = 3 \times J_{AV}$ (Fig. 2), yielding the final ratio H/ATP = 4 for synthesis and transport of ATP out of mitochondria (Hinkle, 1995). At steady state, J_{AV} is equal to the flux of P_i^{1-} across the OMM and IMM.

The presented electric model was described by a system of mathematical equations and analyzed computationally. The change of $\Delta\psi_o$ was observed when g_{AV} was varied according to a hyperbolic function of ADP concentration in the cytoplasm (data not shown).

Kinetic model

The steady-state process shown in Fig. 1 was also described by a simplified kinetic model. Four different fluxes have to be equal at steady state: ATP (ADP) flux through the contact sites, P_i flux across the VDAC beyond the contacts, P_i flux across the IMM, which is mediated by the phosphate carrier, and the rate of ATP synthesis in mitochondria. The Goldman equation for the P_i^{1-} flux across the VDAC beyond the contacts may be used at the first approximation:

$$J_{po} = P \times \frac{F \times \Delta\psi_o}{RT} \times \frac{[P_i]_i - [P_i]_o \times e^{F\times\Delta\psi_o/RT}}{1 - e^{F\times\Delta\psi_o/RT}}. \quad (4)$$

Here, F is the Faraday constant, R is the universal gas constant, $T = 310$ K is normal body temperature, P is the P_i^{1-} permeability of the VDAC beyond the contacts related to one average mitochondrion. P depends on the OMMP ($\Delta\psi_o$) according to the equation describing a bell-shaped permeability-voltage characteristic of VDAC (Lemeshko and Lemeshko, 2000):

$$P = a_0 \times [p_c + (p_o - p_c) \times e^{-(a\times\Delta\psi_o)^2}]. \quad (5)$$

The parameters p_o and p_c are the OMM relative permeabilities for the open and closed states of VDAC, set at 1.00 and 0.11, respectively, according to the experimental data (Hodge and Colombini, 1997). The absolute permeability coefficient a_0 was set at 3.6 fl/s per one average mitochondrion, as in the model of the metabolically derived potential (Lemeshko and Lemeshko, 2000). The parameter a in Eq. 5 allows to change the steepness of the permeability-voltage characteristic of VDAC, i.e., its voltage sensitivity.

ATP^{4-}/ADP^{3-} exchange through the contact sites may be expressed as a net flux of one-charge anions using the Goldman equation:

$$J_{td} = P_{td} \times \frac{F \times \Delta\psi_c}{RT} \times \frac{P_{tx} \times P_{do} - P_{to} \times P_{dx} \times e^{F\times\Delta\psi_c/RT}}{1 - e^{F\times\Delta\psi_c/RT}}, \quad (6)$$

in which P_{td} is the maximal rate of the ATP^{4-}/ADP^{3-} exchange through the contact sites, $\Delta\psi_c$ is the voltage across the contacts. Coefficients P_{to}, P_{dx}, P_{tx}, and P_{do} are the probabilities of occupation of the adenine nucleotide binding centers of the ANT in the ANT-VDAC intermembrane contact sites: by ATP on the cytoplasmic side (P_{to}), ADP on the matrix side (P_{dx}), ATP on the matrix side (P_{tx}), and ADP on the cytoplasmic side (P_{do}). Thus, the product $P_{to} \times P_{dx}$ is the probability of the simultaneous loading of the ANT with the cytoplasmic ATP and the matrix ADP. The product $P_{tx} \times P_{do}$ is the probability of the simultaneous loading of the ANT with the matrix ATP and the cytoplasmic ADP. These probabilities may be described as:

$$P_{to} = \frac{[ATP]_o}{K_{to} + [ATP]_o}, \quad (7)$$

$$P_{dx} = \frac{[ADP]_x}{K_{dx} + [ADP]_x}, \quad (8)$$

$$P_{tx} = \frac{[ATP]_x}{K_{tx} + [ATP]_x}, \quad (9)$$

$$P_{do} = \frac{[ADP]_o}{K_{do} + [ADP]_o}, \quad (10)$$

in which K_{to}, K_{dx}, K_{tx}, and K_{do} are the dissociation constants of the adenine nucleotide binding centers of the ANT within the contact sites for ATP on the cytoplasmic side (K_{to}), ADP on the matrix side (K_{dx}), ATP on the matrix side (K_{tx}), and ADP on the cytoplasmic side (K_{do}). Here, $[ATP]_o$ and $[ADP]_o$ are concentrations of ATP and ADP in the cytoplasm, respectively (understanding that they are the local concentrations of ATP and ADP within the contact sites), and $[ATP]_x$ and $[ADP]_x$ are their concentrations in the matrix. We consider the symmetric approximation, assuming that $K_{to} = K_{tx}$ and $K_{dx} = K_{do}$. They were set at $K_{to} = 1.0$ mM, i.e., near

the K_m for ATP hydrolysis by the intact mitochondria; $K_{do} = 20$ μM, to obtain the K_m value for the ANT near 17 to 20 μM ADP. $[ATP]_o$ was set at 9.0 mM, close to that in the cytoplasm of cardiomyocytes (Saks and Aliev, 1996). The proton-motive force for mitochondria in muscle cells is near 180 mV with $[ATP]_x/[ADP]_x$ close to 3 (Korzeniewski and Mazat, 1996). We set $[ATP]_x = 7.5$ mM and $[ADP]_x = 2.5$ mM, making $[ATP]_x/[ADP]_x$ as a constant at any workloads, for simplicity. The IMMP was set at -150 mV, and $\Delta pH = 0.75$ across the IMM. The rate of ATP transport through the contact sites (ATP^{4-}/ADP^{3-} exchange) under these conditions will depend on $[ADP]_o$, on the maximal activity of ANT (P_{td}), and the voltage across the contact sites, $\Delta\psi_c$ (Eq. 6).

ATP synthesis in mitochondria may be considered as an essentially reversible reaction, and its rate may be expressed by the following equation [see (Lemeshko and Lemeshko, 2000) and references therein for details]:

$$J_t = \frac{V_{max} \times \left(\frac{[ADP]_x \times [P]_x}{K_{dx} \times K_{px}} - \frac{[ATP]_x}{K_{tx}} \right)}{1 + \frac{[ATP]_x}{K} + \frac{[ADP]_x}{K_{dx}} + \frac{[ADP]_x \times [P]_x}{K_{dx} \times K_{px}}} \quad (11)$$

Here, V_{max} is the maximal rate that we take to be equal for the forward and reverse reactions. It was set at $V_{max} = 0.0067$ fmol/s for one average mitochondrion (Lemeshko and Lemeshko, 2000), taking into account the experimental data reported by Saks and Aliev (1996). The other constants for the cardiomyocytes were also as those used by these authors: $K_{tx} = 0.5$ mM ATP, $K_{dx} = 0.1$ mM ADP, $K_{px} = 2.5$ mM P_i^-. Setting $[ATP]_x = 7.5$ mM and $[ADP]_x = 2.5$ mM as constants at any workloads, the rate of ATP synthesis will depend only on the concentration of inorganic phosphate in the mitochondrial matrix, $[P_i]_x$ (Eq. 11).

The inorganic phosphate transport across the IMM is pH dependent, and its flux may be described by the following equation for the simplest case (Korzeniewski and Mazat, 1996):

$$J_{pi} = k_p \times ([P_i]_i \times [H]_i - [P_i]_x \times [H]_x), \quad (12)$$

in which k_p is the rate constant, $[P_i]_i$ is the P_i^{1-} concentration in the MIMS, $[P_i]_x$ is the P_i^{1-} concentration in the matrix, $[H]_i$ is the concentration of protons in the MIMS, and $[H]_x$ in the matrix. According to the previously set $\Delta pH = 0.75$ across the IMM at any workloads, Eq. 12 becomes:

$$J_{pi} = k_p \times ([P_i]_i \times [H]_i - [P_i]_x \times 0.178[H]_i), \quad (13)$$

Here, $k_p = 0.1$ $\mu l\ s^{-1}\ M^{-1}$, to obtain the range of the flux values close to that evaluated for the rates of ATP synthesis, ATP^{4-}/ADP^{3-} exchange, and the P_i permeability across the VDAC beyond the contacts.

Assuming an unlimited permeability of the OMM for protons, the Nernst equation may be described for the relation of proton concentrations in the cytoplasm, $[H]_o$, and in the MIMS, $[H]_i$:

$$\Delta\psi_o = \frac{RT}{F} \ln \frac{[H]_o}{[H]_i} \quad (14)$$

The concentration of inorganic phosphate in the sarcoplasm of cardiomyocytes is known to change in a narrow range ~3 mM at a wide range of workloads (Saks and Aliev, 1996). We took the average concentration of P_i^- in the system, $[P_i]_s$, as constant and equal to 3.5 mM. It may be expressed as:

$$[P_i]_s = \frac{[P_i]_x \times V_x + [P_i]_i \times V_i + [P_i]_o \times V_o}{V_x + V_i + V_o}, \quad (15)$$

in which V_x, V_i, and V_o are the volumes of the matrix, MIMS, and cytoplasm that were set at 0.06, 0.03, and 0.36 fl, respectively, related to one average mitochondrion [see (Lemeshko and Lemeshko, 2000) and references therein for details].

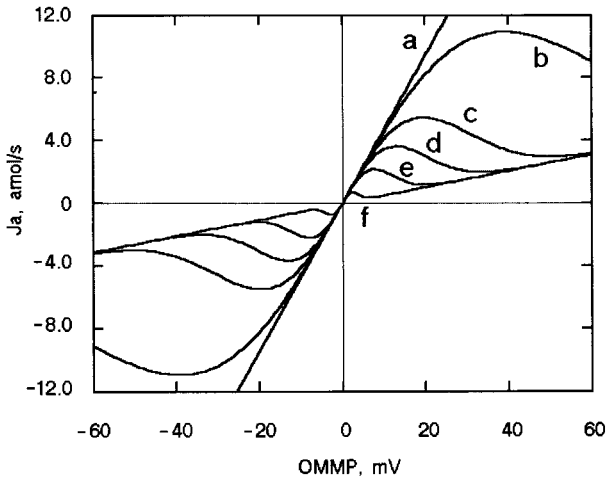

FIGURE 3 The flux of inorganic phosphate through the VDAC beyond the contact sites in the outer membrane of one average mitochondrion as the function of the OMM potential, according to Eq. 4. The concentration of P_i in the MIMS and cytoplasm was set 3.5 mM; $a_0 = 3.6$ fl/s; (*a*) $a = 0$; (*b*) $a = 20\ V^{-1}$; (*c*) $a = 40\ V^{-1}$; (*d*) $a = 60\ V^{-1}$; (*e*) $a = 100\ V^{-1}$; (*f*) $a = 300\ V^{-1}$ (see Eq. 5).

At steady state, all fluxes have to be equal:

$$J_t = J_{pi} = J_{po} = -J_{td} \quad (16)$$

The steady-state metabolite flux for the described conditions depends on $[ADP]_o$ that was varied in the range 1 to 200 μM to model various workloads on the contact sites. The pathway 2 in Fig. 1 is discussed but not included in the modeling. This pathway can be considered as a part of an integrated model that may be developed to describe the superposition of the OMMP generated by various mechanisms.

The system of Eq. 1 and Eqs. 4–16 was solved by a numeric method using the standard software Mathcad 2000 (MathSoft, Cambridge, MA).

RESULTS

The possibility of the OMMP generation, resulting from the IMM voltage division between the contact sites and the OMM beyond the contacts, was analyzed using the simplified kinetic model shown in Fig. 1. At first, the separate behavior of each of the four fluxes was determined: the fluxes of P_i through the OMM and through the IMM, the rate of ATP synthesis in mitochondria and the flux of ATP (ADP) through the contact sites.

The dependence of the P_i flux through the OMM on the OMMP is demonstrated in Fig. 3, according to Eq. 4 at 3.5 mM P_i in the cytoplasm and in the MIMS. The permeability P in Eq. 4 depends on the VDACs voltage sensitivity parameter a and on the maximal absolute permeability of VDAC in the open state a_0, set at 3.6 fmol/s per one average mitochondrion (Eq. 5). The dependence of the relative VDACs permeability on the OMMP for different voltage sensitivities a is shown in Fig. 4. The data in Fig. 3 demonstrate that the P_i^{1-} electrodiffusion across the OMM is

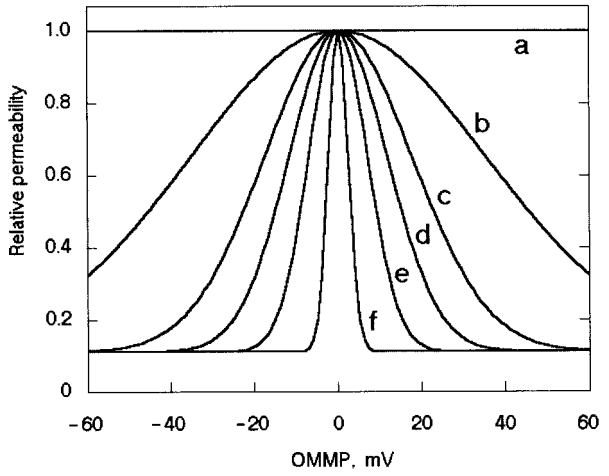

FIGURE 4 The relative permeability of VDAC to inorganic phosphate (P_i^{1-}) as the function of the outer membrane potential in mitochondria according to Eq. 5. a_0 = 1.0 fl/s; (*a*) a = 0; (*b*) a = 20 V^{-1}; (*c*) a = 40 V^{-1}; (*d*) a = 60 V^{-1}; (*e*) a = 100 V^{-1}; (*f*) a = 300 V^{-1}.

modulated by the OMMP, where the VDACs permeability-voltage characteristic plays a crucial role.

The flux of P_i across the IMM, described by Eq. 13 for the phosphate carrier, is shown in Fig. 5 as a function of P_i concentration in the matrix, $[P_i]_x$, at four different pH values, and 3.5 mM P_i in the MIMS. The rate of ATP synthesis by mitochondria also depends on $[P_i]_x$, according to Eq. 11, as shown in Fig. 6 for three different values of V_{max}. At $[ATP]_o$ = 9.0 mM and the constant values of $[ATP]_x$ and $[ADP]_x$, the rate of ATP^{4-}/ADP^{3-} exchange through the contact sites depends on the concentration of ADP in the cytoplasm, as shown in Fig. 7 for different values of the voltage across the contact sites, according to Eq. 6.

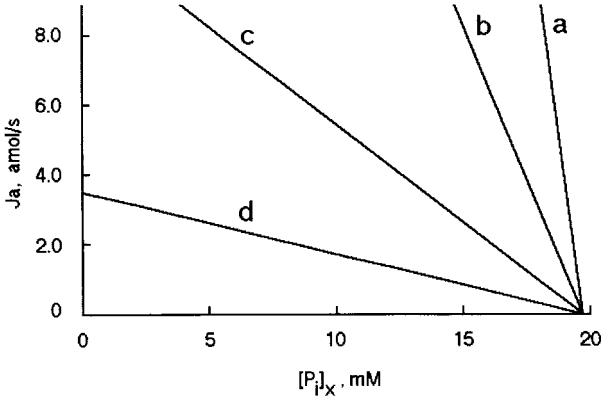

FIGURE 5 The flux of inorganic phosphate (P_i^{1-}) through the inner membrane as a function of the P_i concentration in the mitochondrial matrix, according to Eq. 13, at $[P_i]$ = 3.5 mM in the MIMS and ΔpH = 0.75 across the IMM. The values of pH in the MIMS: (*a*) pH = 6.5; (*b*) pH = 7.0; (*c*) pH = 7.5; (*d*) pH = 8.0.

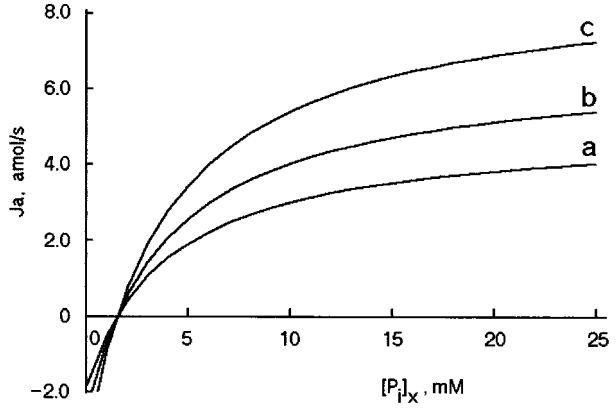

FIGURE 6 The rate of ATP synthesis by mitochondria as a function of inorganic phosphate concentration in the matrix, according to Eq. 11, at $[ATP]_x$ = 7.5 mM, $[ADP]_x$ = 2.5 mM. The maximal rate of ATP synthesis (V_{max}): (*a*) 0.005 fmol/s; (*b*) 0.0067 fmol/s; (*c*) 0.009 fmol/s per one average mitochondrion.

The data in Fig. 3 and Figs. 5 through 7 demonstrate that all four fluxes may change in the same range of magnitudes under the given conditions. At steady state, all these fluxes have to be equal (Eq. 16). At the given concentration of $[ADP]_o$, the steady state may be reached due to the IMM voltage division between the contact sites ($\Delta\psi_c$) and the OMM ($\Delta\psi_o$), as well as due to the adequate distribution of P_i among the cytoplasm, MIMS, and the matrix. The modeling shows that the ATP flux through the contact sites hyperbolically increases with an increase in ADP concentration in the cytoplasm (Fig. 8), when the VDACs permeability is insensitive to the OMMP (a = 0 in Eq. 5) or the VDACs voltage sensitivity is relatively low (a = 20 V^{-1} or

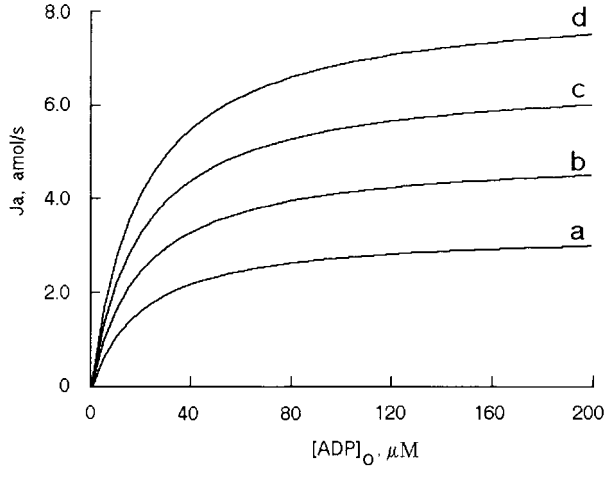

FIGURE 7 The rate of the adenine nucleotide transport through the contact sites in one average mitochondrion as the function of ADP concentration in the cytoplasm, according to Eq. 6 (it was taken, P_{td} = 0.001 fmol/s). The voltage across the contact sites ($\Delta\psi_c$): (*a*) 0.10 V; (*b*) 0.15 V; (*c*) 0.20 V; (*d*) 0.25 V.

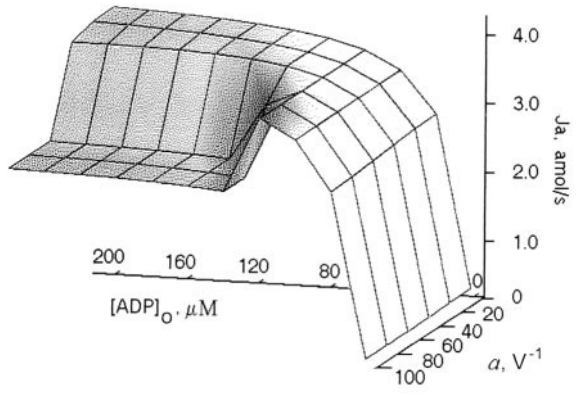

FIGURE 8 The influence of the ADP concentration in the cytoplasm and the VDACs voltage sensitivity (parameter a in Eq. 5) on the steady-state adenine nucleotide flux through the contact sites in one average mitochondrion.

$a = 40$). At higher voltage sensitivities ($a = 60\ V^{-1}$ and higher in Eq. 5), a significant restriction of the ATP steady-state flux was observed when ADP concentration in the cytoplasm reached above 60 μM.

The restriction of the steady-state flux of ATP and P_i under high workloads on the contact sites (Fig. 8) was caused by the VDACs permeability modulation under the generated OMMP (Fig. 9 *A*). The flux restriction was also accompanied by a decrease in the voltage across the contact sites ($\Delta\psi_c$) (Fig. 9 *B*). Somewhat unexpected result was obtained at $[ADP]_o$ less than 15 μM, when high negative OMMP was generated. The voltage across the contact sites in this case was significantly higher than the value of the IMMP. Still, the algebraic sum of these two potentials ($\Delta\psi_o$ and $-\Delta\psi_c$) was always equal to the IMMP (−150 mV) as it was defined in the model.

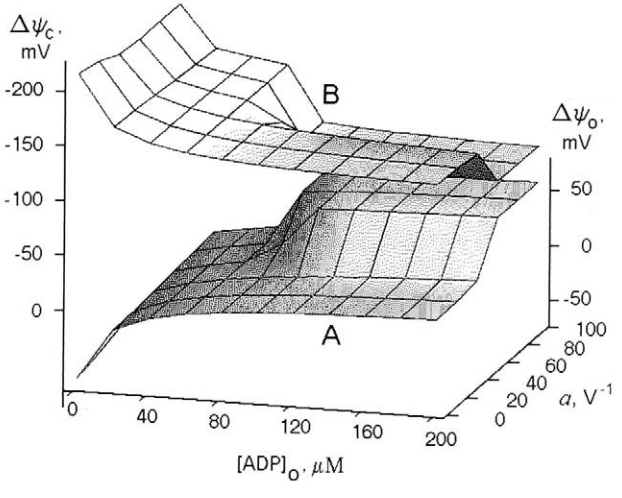

FIGURE 9 The influence of the ADP concentration in the cytoplasm and the VDACs voltage sensitivity (parameter a in Eq. 5) on the inner membrane voltage ($\Delta\psi$) division between the intermembrane contact sites ($\Delta\psi_c$) and the outer membrane ($\Delta\psi_o$) at steady-state adenine nucleotide flux through the contacts sites in mitochondria.

DISCUSSION

The presence of the pore protein, VDAC, in the outer mitochondrial membrane makes it permeable to many low weight substances. Although the physiological role of the OMM is not yet clear, it is unlikely, according to Mannella et al. (1992), that VDAC simply convert the OMM in a coarse sieve. For VDAC to be voltage gated at physiological conditions (Shein et al., 1976; Colombini, 1989; Liu and Colombini, 1992; Hodge and Colombini, 1997), some mechanisms of the OMMP generation probably exist to modulate the VDACs permeability in metabolically dependent manner. On the other hand, a common opinion was that the maintenance of any electrical potential on the OMMP is doubtful, taking into account the high ionic conductance of VDAC and relatively high concentration of salts in the cytoplasm. Benz et al. (1990) concluded, for example, that "the existence of an electrochemical potential across the outer membrane can't be expected," answering the question "whether a membrane potential exists at the outer membrane." Instead, a capacity coupling mechanism of the electrical potential generation across the OMM inside the contact sites, but not outside, was suggested (Benz et al., 1990). As a result, the VDAC localized in the contact sites between the IMM and OMM were expected to stay in closed (regulated) state, whereas those outside the contacts would be "unregulated" (Benz et al., 1990; Benz and Brdiczka, 1992), because any OMMP beyond the contacts has been considered impossible.

We have developed a different mechanism for the possible participation of the contact sites in the regulation of the metabolite exchange across the OMM. First of all, the above conclusion that an electrochemical potential across the OMM cannot be expected does not mean that the electrical potential cannot be generated; it simply may be equilibrated by the chemical potential making the resulting electrochemical potential equal to zero. The electrical part of the electrochemical potential is sufficient to modulate the VDACs permeability and it may be generated (and maintained) at some steady-state processes. The permeable ions Cl^-, K^+, Na^+, etc., if present, may not participate in the steady-state process but will achieve their electrochemical equilibrium. After that, their net flux across the OMM will be equal to zero. In this study, we have considered a possible mechanism of the OMMP generation based on the idea that the IMMP may partially drop across the OMM according to Ohm's law. This mechanism may be called the steady-state "resistance coupling mechanism," or the "voltage division mechanism."

According to different authors (Weiler et al., 1985; Benz et al., 1990; Brdiczka, 1990; Wilson, 1994; Golshani-He-

broni and Bessman, 1997; Mathupala et al., 1997; Mazurek et al., 1997; Smith, 2000; Crompton, 1999), ATP^{4-}/ADP^{3-} exchange through the contact sites of mitochondria allows the matrix ATP to directly access the cytoplasm, where it may be preferentially used by kinases attached to the contact sites, by HK for example. Inorganic phosphate derived from the subsequent metabolism of glucose-6-phosphate or from the direct hydrolysis of the released ATP will be liberated into the cytoplasm (Fig. 1). Before that, any other molecule of inorganic phosphate present in the cytoplasm at a concentration around 3 mM (Saks and Aliev, 1996) may keep the electrical current across the VDAC beyond the contacts, closing the electric circuit (Fig. 1).

To evaluate the proposed mechanism of the OMMP generation, the simplified kinetic model (Fig. 1) was described by a minimal number of mathematical equations, and its behavior was computationally studied under various workloads on the contact sites, varying the ADP concentrations in the cytoplasm in the range 1 to 200 μM. The obtained data show that a significant restriction of the ATP flux through the contact sites, and the P_i flux across VDAC beyond the contacts takes place at relatively high $[ADP]_o$ (Fig. 8), if the VDACs voltage sensitivity is sufficiently high. The metabolite flux restriction is reached, because the contact sites voltage decreases (Fig. 9 *B*) and the OMMP increases (Fig. 9 *A*).

At $[ADP]_o$ less than 15 μM, the calculations show a high value for the OMMP as well but with an opposite polarity to that observed at high $[ADP]_o$. These data indicate that under these conditions the restriction of the P_i flux is reached not only due to the VDAC closing, but also electrophoretically, according to the negative electrical potential across the OMM. As a result, a higher voltage is now applied to the contact sites being the sum of the negative IMMP and the negative OMMP. Consequently, electrophoretic acceleration of the electrogenic transport of ATP (of ATP^{4-}/ADP^{3-} exchange) through the contact sites takes place. Thus, at low $[ADP]_o$, the steady state is reached due to the electrophoretic restriction of the P_i influx into the MIMS across the partially or completely closed VDAC beyond the contacts and by the electrophoretic acceleration of the ATP release through the contacts.

In frames of the voltage division mechanism of the OMMP generation, it would be attractive to speculate its possible relation to aerobic glycolysis of tumor and normal proliferating cells characterized by the high rate of lactate production regardless of oxygen tension (Golshani-Hebroni and Bessman, 1997; Mathupala et al. 1997; Mazurek et al., 1997). There are many factors that may contribute to the origin of aerobic glycolysis (Golshani-Hebroni and Bessman, 1997; Mathupala et al. 1997; Mazurek et al., 1997). One of the main metabolic events in malignant transformation of cells is activation of hexokinase gene transcription and the enhanced level of this enzyme bound to mitochondrial porins, thus gaining preferential and more direct access to the matrix ATP (Golshani-Hebroni and Bessman, 1997; Mathupala et al., 1997; Mazurek et al., 1997; Smith, 2000). Particularly, the initiation of liver tumors is accompanied with a shift of expression from HKIV isoenzyme to HKI and HKII, with increased binding of hexokinase to mitochondria (Smith, 2000), and more than a 100-fold increase in hexokinase activity (Nakashima et al., 1988). Significantly higher transcript levels of the three VDAC isoforms were also demonstrated for the malignant tumor cell line AH130, in comparison with that of normal liver (Shinohara et al., 2000). It was suggested that the malignant tumor cell mitochondria have a high HK-binding capacity due to a higher number of HK-binding sites.

Interestingly, the amount of VDAC, in heart mitochondria for example, is only 10% of ANT (Crompton, 1999), indicating that ANT is probably not a limiting factor for increasing the number of contact sites, whereas the enhancement of VDAC transcription (Shinohara et al., 2000) and the significant increase in HKI and HKII expression (Smith, 2000) may lead to an increase in the absolute and relative number of ANT-VDAC-HK complexes in mitochondria of malignant tumor cells. With respect to our model, it means an augmentation of the parameter P_{td} in Eq. 6 and conductance g_{AV} (Fig. 1). Consequently, the OMMP is expected to increase, closing the VDAC localized beyond the contacts, and thus inhibiting the ATP release from the mitochondria through the pathway 2 (Fig. 1). This explanation is quite different to that postulated by Warburg (1956), who assumed that there must be a respiratory defect in tumor cells, whereby glucose cannot be fully oxidized to CO_2. According to our model, the inhibition of the ATP release from mitochondria by the OMMP-dependent closure of the VDAC beyond the contact sites may mimic a respiratory defect. Similarly, the Crabtree effect, i.e., the glucose-induced inhibition of mitochondrial oxidative phosphorylation (Ibsen, 1961; Pedersen, 1978; Rodríguez-Enríquez et al., 2001) may also be explained in frames of this model.

On the other hand, although HK was shown to be enriched in the intermembrane contact sites, where it is associated with the OMM by interacting with porin (Brdiczka, 1991; Brdiczka et al., 1998; Crompton, 1999), the contact sites were not found in the subpopulation HT29 Glc+ of adenocarcinoma cells, but HK was predominantly bound to mitochondria (Denis-Pouxviel et al., 1987). These data may suggest that in these cells HK forms only VDAC-HK duplexes. It is not excluded that in VDAC-HK duplexes, the bound HK conserves its channeling properties described for ANT-VDAC-HK contact sites (Laterveer et al., 1995). For VDAC-HK duplexes, the channeling could mean that the bound HK uses the intermembrane ATP^{4-} and returns ADP^{3-} back, liberating glucose-6-phosphate^{1-} in the cytoplasm. In this case, the VDAC-HK duplex could function as an active electrogenic translocator that uses the free energy of the essentially irreversible hexokinase reaction. The OMMP may be directly generated by this duplex in the

presence of glucose. Thus, the HK-dependent generation of the OMMP resulted from the IMM voltage division, when the ANT-VDAC-HK complexes are present, or/and from the functioning of VDAC-HK duplexes could be an important element of the Crabtree effect that was considered to be a multifactorial phenomenon (Pedersen, 1978; Rodríguez-Enríquez et al., 2001).

Three VDAC isoforms are known (Shinohara et al., 2000) that may have a different capacity for ANT-VDAC-HK and VDAC-HK complexes formation, providing a genetic basis for programming a different proportion among the ANT-VDAC-HK, VDAC-HK, and free VDAC quantities in the OMM. Various apoptosis-inducing or apoptosis-preventing factors could have a different affinity for these three structural forms of VDAC (triplex, duplex, or free form), influencing their physico-chemical properties, and thus the probability of the OMMP generation by various mechanisms. This aspect seems to be important for a future study of the problem. Recently, very interesting data were obtained with this respect, that the antiapoptotic factor Bcl-xL prevents VDAC closure (Vander Heiden et al., 2001).

In conclusion, the presented electric and kinetic models show a clear possibility of the OMMP generation as a result of the IMM voltage division between the contact sites and the OMM beyond the contacts. The magnitude and polarity of generated OMMP according to the kinetic model (from −60 mV to +60 mV) depend on the local concentration of ADP within the contact sites. Although at real conditions, when ions K^+, Na^+, Cl^-, etc. are present, the OMMP seems to be diminished due to the electrodynamic compartmentation effect (Lemeshko and Lemeshko, 2000), its value may still be high enough to regulate the metabolite exchange across the OMM. Taking into account that various mechanisms of the OMMP generation may exist (Liu and Colombini, 1991, 1992; Lemeshko and Lemeshko, 2000), the superposition of the potentials, provided from different mechanisms, might play an important role in the energy channeling regulation in normal and malignant cells.

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