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VDAC electronics: 2. A new, anaerobic mechanism of generation of the membrane potentials in mitochondria

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ABSTRACT

Mitochondrial hexokinase (HK) and creatine kinase (CK) known to form complexes with a voltage dependent anion channel (VDAC) have been reported to increase cell death resistance under hypoxia/anoxia. In this work we propose a new, non-Mitchell mechanism of generation of the inner and outer membrane potentials at anaerobic conditions. The driving force is provided by the Gibbs free energy of the HK and CK reactions associated with the VDAC–HK and the ANT (adenine nucleotide translocator)–CK–VDAC complexes, respectively, both functioning as voltage generators. In the absence of oxygen, the cytosolic creatine phosphate can be directly used by the ANT–CK–VDAC contact sites to produce ATP from ADP in the mitochondrial matrix. After that, ATP released through the fraction of unbound ANTs in exchange for ADP is used in the mitochondrial intermembrane space by the outer membrane VDAC–HK electrogenic complexes to convert cytosolic glucose into glucose-6-phosphate. A simple computational model based on the application of Ohm's law to an equivalent electrical circuit showed a possibility of generation of the inner membrane potential up to −160 mV, under certain conditions, and of relatively high outer membrane potential without wasting of ATP that normally leads to cell death. The calculated membrane potentials depended on the restriction of ATP/ADP diffusion in narrow cristae and through the cristae junctions. We suggest that high inner membrane potential and calcium extrusion from the mitochondrial intermembrane space by generated positive outer membrane potential prevent mitochondrial permeability transition, thus allowing the maintenance of mitochondrial integrity and cell survival in the absence of oxygen.

1. Introduction

Various aerobic cell types have been reported to survive for some time under hypoxia/anoxia conditions [1–6]. A high resistance to anoxia has been demonstrated for several tumor cell lines [4–6]. Anoxic damage in rat hippocampal slices has been shown to be prevented by their pre-incubation with creatine that enhanced the level of creatine phosphate in the neurons [2]. It has also been found that the presence of glucose in the perfused fluid during anoxia of an isolated rat heart was essential for its post-anoxic recovery [1]. Many other cases of resistance to anoxia/hypoxia have been reviewed in [3].

In this respect, hexokinase (HK), which is known to be commonly overexpressed in the brain and in many cancer cell lines and to be bound in high proportion to VDAC of the mitochondrial outer membrane (MOM), has been shown to greatly increase cell resistance to apoptosis or to hypoxic/anoxic death [3,7–13]. On the other hand, the knockdown of HK2 sensitizes human glioblastoma multiforme cells to apoptosis induced by hypoxia, radiation, or chemotherapy due to an increase in mitochondrial membrane permeability monitored by the potential-sensitive dye JC-1 [9]. The HK binding to VDAC has been found to decrease VDAC conductance [8,10] and to inhibit the flux of ATP into mitochondria [14]. The most important finding was that the glucose phosphorylation reaction associated with the VDAC–HK complex was needed to provide such a glucose-mediated cell resistance to death [15].

The mitochondrial creatine/creatine kinase system has also been shown to reduce the damage of both the heart and the brain induced by ischemia, anoxia, or inhibitors of mitochondrial respiration [2,16–20] and to increase the survival of some malignant cancer cell lines with an enhanced expression of mitochondrial octameric CK [21–23].

It has been suggested that the mitochondrial octameric creatine kinase system functions as a specific energy channel between mitochondrial matrix and the cytosol through the ANT–CK–VDAC intermembrane contact sites [24–27]. According to the developed hypothesis, these contact sites allow the production of cytosolic creatine phosphate directly using the mitochondrial matrix ATP at aerobic conditions.

The mechanism of the HK/CK-dependent protection of cells in anoxic conditions is not yet elucidated. It has been assumed that the

Abbreviations: VDAC, voltage-dependent anion channel; HK, hexokinase; CK, creatine kinase; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; MIMS, mitochondrial intermembrane space; OMP, mitochondrial outer membrane potential; IMP, mitochondrial inner membrane potential; ANT, adenine nucleotide translocator

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mitochondrial inner membrane (MIM) potential might be generated by a well known Mitchell mechanism due to hydrolysis of ATP produced in the cytosol by glycolysis [28]. However, anoxia seems to decrease the mitochondrial exchange of inorganic phosphate, ADP, ATP, and several respiratory substrates by VDAC closure [3]. In addition, the mitochondrial hydrolysis of cytosolic ATP has been reported to be strongly inhibited under anoxia [29], or if not inhibited, it leads to rapid depletion of ATP and to cell death [20,30]. Direct production of ATP in the mitochondrial matrix by the HK reaction associated with the ANT–VDAC–HK contact sites has also been proposed [31], i.e. reverse to what we have suggested earlier [32]. According to the authors, the cytosolic glucose-6-phosphate might be used as a substrate to produce mitochondrial ATP [31]. However, such a reverse process seems to be impossible due to a well-known fact that the HK reaction is essentially irreversible under physiological conditions that can be demonstrated by thermodynamic estimations.

On the other hand, it should be noted that the mitochondrial VDAC–HK and ANT–CK–VDAC complexes might function as transmembrane net charge carriers, and thus as direct generators of membrane potentials, without mitochondrial respiration or ATP hydrolysis.

In this work, we propose a new, non-Mitchell mechanism of VDAC-mediated generation of the inner and outer membrane potentials (IMP and OMP, respectively) that might function under anaerobic conditions using cytosolic ATP without its wasting. The VDAC–HK complex of the MOM and the ANT–CK–VDAC intermembrane contact sites of mitochondria were presented as electric batteries, with the voltages provided by the Gibbs free energy of corresponding kinase reactions. The computational analysis of this model was based on the known thermodynamic properties of both the HK and the CK reactions. The calculated IMP and OMP were sufficiently high to maintain mitochondrial integrity and thus cell survival in the absence of oxygen.

2. Materials and Methods

2.1. The model description

According to the model (Fig. 1A), creatine phosphate (CrP) in the cytosol is used by the ANT–CK–VDAC complex to synthesize ATP from ADP in the mitochondrial matrix. The complex functioning might be presented as a battery E_c (Fig. 1B), the voltage of which is applied to both the MIM and the MOM. The voltage V_c of the battery is directly related to the Gibbs free energy of the CK reaction as:

$$V_c = \frac{\Delta G_c^{o'}}{F} + \frac{RT}{F} \ln \frac{[Cr]_c \cdot [ATP]_m}{[CrP]_c \cdot [ADP]_m}. \quad (1)$$

Here, $\Delta G_c^{o'} = -12.7$ kJ/mol is the standard Gibbs free energy of creatine kinase reaction, F is the Faraday constant, R is the universal gas constant, $T = 310$ K is normal body temperature, $[CrP]_c$ and $[Cr]_c$ are cytosolic concentrations of creatine phosphate and creatine, respectively, and $[ATP]_m$ and $[ADP]_m$ are concentrations of ATP and ADP in the mitochondrial matrix. For calculations, we arbitrarily took $[ATP]_m/[ADP]_m = 1.0$, somewhat lower than for respiring mitochondria, in which $[ATP]_m/[ADP]_m = 3$, approximately [33]. According to Eq. (1), the voltage V_c of the CK battery at standard conditions should be equal to −132 mV (as $G_c^{o'}/F$).

The internal resistance of the CK battery may be presented as composed of the resistance R_c of the CK–VDAC complex and of the resistance R_i of ANTs bound to CK–VDAC. These two connected in series resistances (Fig. 1B) can be expressed through the respective conductances g_c and g_i, as $1/g_c$ and $1/g_i$. Taking as 100 arbitrary units (a.u.) the conductance of 100% VDACs in the MOM, all in the open state, the conductance g_c of the percentage N_c of VDACs bound to CK in the ANT–CK–VDAC contact sites may be presented as the N_c value multiplied by the normalized hyperbolic factor with the Michaelis–Menten constant for CrP, $K_{m,CrP} = 1.69$ mM for the mitochondrial octameric CK [34]:

$$g_c = \frac{N_c \cdot [CrP]_c}{K_{m,P} + [CrP]_c}. \quad (2)$$

Assuming the thermodynamic equilibrium of the creatine kinase reaction in the cytosol, we can write:

$$0 = \Delta G_c^{o'} + RT \ln \frac{[Cr]_c \cdot [ATP]_c}{[CrP]_c \cdot [ADP]_c}. \quad (3)$$

Here, $\Delta G_c^{o'} = -12.7$ kJ/mol, $[ATP]_c/[ATP]_c$ is the ratio of ATP and ADP concentrations in the cytosol that we set equal to 200, in a physiological

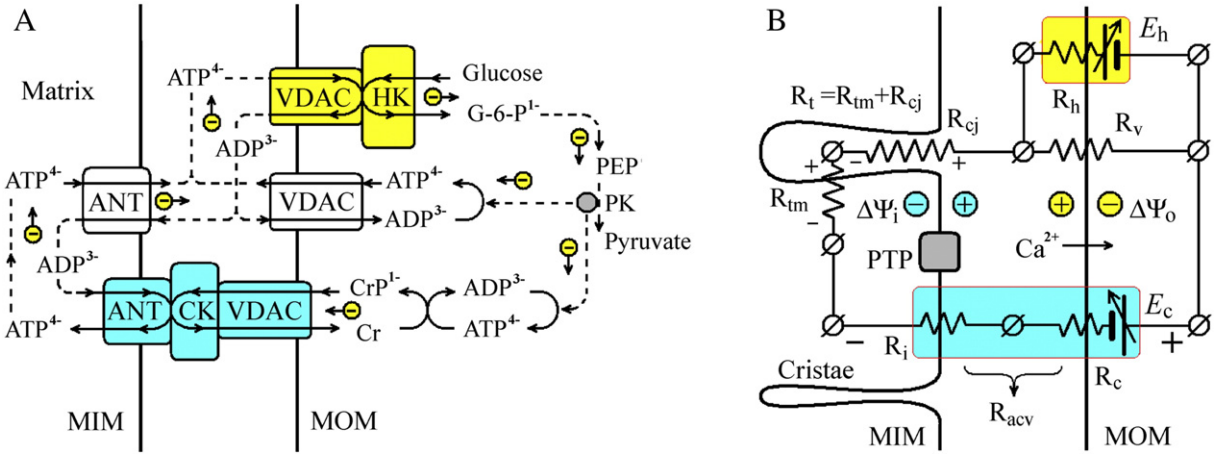

Fig. 1. The VDAC–kinase mechanism of the IMP ($\Delta\psi_i$) and the OMP ($\Delta\psi_o$) generation coupled to aerobic glycolysis (A) and its equivalent electrical circuit (B). MIM — mitochondrial inner membrane; MOM — mitochondrial outer membrane; G-6-P^{-1} — glucose-6-phosphate; CrP^{-1} — creatine phosphate in the cytosol; Cr — creatine in the cytosol; PEP — phosphoenolpyruvate; PK — pyruvate kinase; R_c — the resistance of the CK–VDAC complex as an internal resistance of the CK battery E_C; R_i — the resistance of ANTs forming the ANT–CK–VDAC contact sites; R_{acv} — the resistance of the ANT–CK–VDAC contact sites; R_t — the free ANT–cristae combined resistance presented as the sum of the resistance R_{tm} of ATP/ADP exchange through the ANTs and the resistance R_{cj} of ATP/ADP diffusion in narrow cristae and through the cristae junctions; R_v — the resistance of free VDACs, beyond the VDAC–kinase complexes; R_h — the resistance of the VDAC–HK complexes as an internal resistance of the HK battery E_H; PTP— the permeability transition pore. IMP and OMP result from voltage drops on the resistances R_t and R_v, respectively.

range. The model allows changing this ratio to evaluate its influence on calculated parameters. The total concentration of Cr plus CrP (CC) in the cytosol is expressed as:

$$CC = [Cr]_c + [CrP]_c. \quad (4)$$

The ratio $[Cr]_c/[CrP]_c$ is derived from Eq. (3) at a selected $[ATP]_c/[ATP]_c$ ratio.

The amount of ANTs in the heart mitochondria is known to be 10 times higher than that of VDACs [35]. For other cell types it is significantly lower, thus we can assume it to be 5 times the amount of VDACs to be applicable to more different cell types. The total conductance of all ANTs to ATP^{4-}/ADP^{3-} electrogenic exchange in comparison with the conductance of all VDACs in the open state is not known, but at least it seems to be less than the total VDAC conductance. For calculations, we took the total conductance of all ANTs to ATP^{4-}/ADP^{3-} exchange as 50 a.u., as a half of the total VDAC conductance of the MOM in the open VDAC state. Altogether these approximations mean that the ATP^{4-}/ADP^{3-} exchange conductance of 1 open VDAC is equal to the conductance of 10 ANTs. If in addition we assume that 1 octameric CK is bound to 4 ANTs and to 1 VDAC, the conductance g_i of free ANTs in the MIM (Fig. 1B, $R_i = 1 / g_i$) may be expressed as:

$$g_i = 0.4 \cdot N_c. \quad (5)$$

Most of the free ANTs in the MIM are localized in the narrow cristae [36,37], which strongly restrict ATP/ADP accessibility to VDACs in the MOM (additional resistance R_{cj} in Fig. 1B) and thus it might be regulated by condensed-orthodox configuration changes, as postulated by Mannella [36]. Taking this into account, we finally presented the free ANT–cristae combined conductance g_t (Fig. 1B, $R_t = 1 / g_t$) in a.u. as:

$$g_t = k_r \cdot (50 - g_i). \quad (6)$$

Here, k_r is the cristae diffusion restriction coefficient of the conductance g_t.

The mitochondrial intermembrane space (MIMS) ATP and cytosolic glucose are used by the electrogenic VDAC–HK complex to produce glucose-6-phosphate in the cytosol, thus the VDAC–HK complex functions as an electric battery, E_h (Fig. 1B). The voltage V_h of this HK battery, positive in the MIMS, can be presented as:

$$V_h = -\left(\frac{\Delta G_h^{o'}}{F} + \frac{RT}{F} \ln \frac{[G6P]_c \cdot [ADP]_s}{[Gluc]_c \cdot [ATP]_s}\right). \quad (7)$$

Here, F is the Faraday constant, R is the universal gas constant, $T = 310$ K is normal body temperature, and $[G6P]_c$ and $[Gluc]_c$ are cytosolic concentrations of glucose-6-phosphate and glucose, respectively. $[ADP]_s/[ATP]_s$ is the ratio of ADP and ATP concentrations in the MIMS: we set $[ATP]_s/[ADP]_s = 200$, as in the cytosol for simplicity. We also used a fixed concentration of glucose-6-phosphate in the cytosol, $[G6P]_c = 0.1$ mM, in the known physiological range. If $\Delta G_h^{o'} = -16.7$ kJ/mol, we obtain $V_h^{o'} = 173$ mV ($-\Delta G_h^{o'} / F$). According to Eq. (7), the total voltage of the HK battery is equal to 430 mV, for the hypothetical conditions of $[ADP]_s/[ATP]_s = 0.005$, $[G6P]_c = 0.1$ mM and $[Gluc]_c = 10$ mM, which are close to the physiological.

The internal resistance R_h of the HK battery can be presented through the conductance g_h of the VDAC–HK complex (Fig. 1B, $R_h = 1 / g_h$). As the conductance of all 100% of VDACs in the open state was set to be equal to 100 a.u., the conductance of the percentage N_h of VDACs bound to HK may be expressed as the N_h value multiplied by the normalized hyperbolic factor containing the Michaelis–Menten constant for glucose, $K_{m,g} = 0.1$ mM:

$$g_h = \frac{N_h \cdot [Gluc]_c}{K_{m,g} + [Gluc]_c}. \quad (8)$$

It means that at a zero glucose concentration, the conductance is equal to zero. Here we assume, for simplicity, that the MIMS concentration of ATP is not a limiting factor for the rate of the HK reaction, and that the concentration of the mitochondrial matrix ADP is not a limiting factor for the CK reaction catalyzed by the ANT–CK–VDAC complex.

The total conductance (percentage) of the N_{vs} fraction of the voltage-sensitive VDACs in the MOM beyond the complexes (free VDACS) in their open state may be presented in a.u. as:

$$N_{vs} = 100 - N_c - N_h - N_{ns}, \quad (9)$$

where N_c is the percentage of VDACs bound to CK in the ANT–CK–VDAC contact sites, N_h is the percentage of VDACs forming the VDAC–HK complexes, and N_{ns} is the percentage of the voltage-insensitive fraction of VDACs, recently reported in the literature as a relatively small fraction of VDACs with low sensitivity to the membrane potential [38]. Here we set $N_{ns} = 5\%$, the same as the conductance $g_{ns} = 5$ a.u., for all calculations.

The dependence of the conductance g_{vs} of the fraction N_{vs} on the OMP can be expressed as described earlier [32,39,40], using a voltage-sensitivity parameter "S":

$$g_{vs} = N_{vs} \cdot P_c + N_{vs} \cdot (1 - P_c) \cdot \exp\left(-(S \cdot OMP)^2\right). \quad (10)$$

Setting $S = 40\ V^{-1}$, the almost complete VDAC closure takes place at OMP = ±40 mV. The parameter P_c is the VDAC relative conductance in the closed state of VDAC. We used $P_c = 0.25$ as the lowest conductance of reported low-conducting "closed" states of VDAC [41].

The total conductance g_v of the free VDACs in the MOM is the sum of the conductances g_{vs} and g_{ns}:

$$g_v = g_{vs} + g_{ns}. \quad (11)$$

In accordance with Ohm's law applied to the equivalent electrical circuit shown in Fig. 1B, the voltages of the CK and HK batteries (V_c and V_h, respectively) can be expressed as:

$$V_c = \frac{I_c}{g_i} + \frac{I_c}{g_o} + \frac{I_c + I_h}{g_v} + \frac{I_c}{g_t} \quad (12)$$

and

$$V_h = \frac{I_h}{g_h} + \frac{I_h + I_c}{g_v}. \quad (13)$$

Here, I_c and I_h are the currents supported by the CK and HK batteries, respectively. The OMP is expressed as:

$$OMP = \frac{I_h + I_c}{g_v}. \quad (14)$$

The IMP can be presented as:

$$IMP = \frac{I_c}{g_t}. \quad (15)$$

At the steady-state generated OMP, Ca^{2+} ions, known to be permeable through even closed VDACs in the MOM [42], will achieve their electrochemical equilibrium described by the Nernst's equation:

$$OMP = \frac{RT}{2F} \ln \frac{[Ca^{2+}]_c}{[Ca^{2+}]_s}, \quad (16)$$

where $[Ca^{2+}]_c$ and $[Ca^{2+}]_s$ are calcium concentrations in the cytoplasm and in the MIMS, respectively.

2.2. Calculations

The calculations were made by numerical methods using the licensed software Mathcad Professional 2001i (MathSoft, Cambridge, MA).

3. Results

A possibility of generation of mitochondrial membrane potentials by a proposed anaerobic mechanism (Fig. 1) was analyzed using the computational model described by Eqs. (1)–(16). The computational model allows calculations in a wide range of concentrations of glucose. The calculations shown in Fig. 2 were made at 5 mM glucose in the cytosol, as the function of the percentage N_c of VDACs forming the ANT–CK–VDAC contact sites, taking the percentage $N_h = 3\%$ of VDACs bound to HK and the cristae coefficient $k_r = 0.1$. A low value of k_r allows modeling a decrease in the effective conductance g_t by the restriction of ADP/ATP diffusion in narrow cristae of mitochondria in the orthodox configuration [36,37,43]. The calculated IMP increased with an increase in N_c at both, 25 mM and 0.25 mM CC in the system (CC = [Cr] + [CrP]). At 25 mM CC, higher IMPs (Fig. 2A, a) and lower OMPs (Fig. 2A, c) were calculated than those determined at 0.25 mM CC (Fig. 2A, b and d, respectively).

As a result of an increase in the OMP-gating closure of free VDACs at an increasing N_c, the MOM conductance g_v decreased as linear and as sigmoid parts, respectively (Fig. 2B, curve a for 25 mM CC and curve b for 0.25 mM CC). The MOM conductance at 25 mM CC was in general higher than at 0.25 mM CC.

At 25 mM CC, it was also calculated that there were relatively high kinase currents, I_h for the VDAC–HK (Fig. 2C, a) and I_c for the ANT–CK–VDAC (Fig. 2C, c) complexes, in comparison with those calculated at 0.25 mM CC (Fig. 2C, b and d, respectively).

Due to the OMP generation, positive inside (Fig. 2A, c and d), the equilibrium concentration of calcium ions in the MIMS, in its compartment external to the cristae, was decreased by more than one order of magnitude (Fig. 2D) at the highest calculated OMPs (Fig. 2A). This effect of calcium extrusion from the MIMS by generated positive OMPs was more expressive at 0.25 mM CC (Fig. 2D, b) than at 25 mM CC (Fig. 2D, a).

Very high OMPs were also calculated at any percentage N_c of the ANT–CK–VDAC contact sites in the range of 10–60%, when the percentage N_h of the VDAC–HK complexes was increased to $N_h = 5\%$ (Fig. 3A, curve d for 0.25 mM CC, and curve c for 25 mM CC). Here, we used $k_r = 0.2$, corresponding to an even lower restriction of ATP/ADP diffusion in the cristae space than at $k_r = 0.1$ in the former case (Fig. 2). The IMPs up to −160 mV at 25 mM CC (Fig. 3A, a) and up to −100 mV at 0.25 mM CC (Fig. 3A, b) were calculated under these conditions, with $N_h = 5\%$. High calculated OMPs resulted in a complete electrical closure of VDACs, thus causing strong restriction of the MOM conductance g_t at both CC concentrations: 25 mM CC (Fig. 3B, a) and 0.25 mM CC (Fig. 3B, b). As a result, the equilibrium concentration of calcium ions in the MIMS was decreased by almost two orders of magnitude in the case of 25 mM CC (Fig. 3C, a), and by 2–3 orders in the case of 0.25 mM CC (Fig. 3C, b).

Next, we extended the calculations for the conditions described in Fig. 1 ($N_h = 3\%$), scanning the concentration of CC in the range of 0.2–20 mM and the cristae coefficient k_r in the range of 0.1–0.6, taking the percentage of the contact sites to be fixed at $N_c = 60\%$. The calculations were made for two concentrations of glucose in the cytosol, 5 mM (Fig. 4, A–C) and 0.05 mM (Fig. 4, D–F). At 5 mM glucose, the OMP decreased from approximately 55 mV, positive inside, to almost zero level with an increase in the CC concentration and in the cristae coefficient k_r (Fig. 4A). As a result of the OMP decrease, the MOM conductance increased from very low values to the conductance corresponding to the completely open free VDACs, beyond the VDAC complexes (Fig. 4C). At the low, 0.05 mM glucose concentration, the OMP changed from an almost zero level to negative values, up to −30 mV approximately, when the CC concentration and the cristae coefficient k_r increased (Fig. 4C), leading to a decrease in the MOM conductance (Fig. 4F) due to the VDAC closure by relatively high negative OMPs (according to Eq. (10)).

High IMPs, up to levels near −160 mV, were calculated for the presence of 5 mM glucose at very low values of the coefficient k_r and relatively high concentrations of CC (Fig. 4B). A similar tendency was

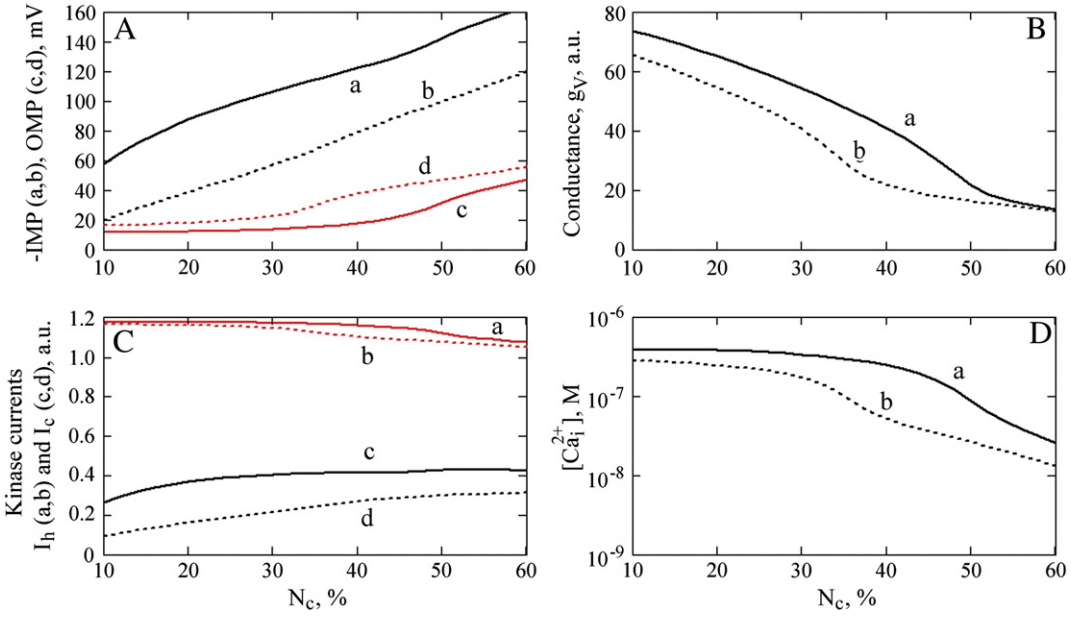

Fig. 2. Generation of the IMP (minus inside) and the OMP (A) by the VDAC–kinase mechanism shown in Fig. 1, and corresponding changes of the MOM conductance g_v (B), of the HK and CK currents (C) and of the MIMS concentration of calcium ions (D) as the function of the percentage N_c of VDACs forming the ANT–CK–VDAC contact sites at the fixed percentage $N_h = 3\%$ of VDAC–HK complexes and the cristae coefficient $k_r = 0.1$. *a,c*— in the presence of 25 mM CC ([Cr] + [CrP]), *b,d*— in the presence of 0.25 mM CC. The cytosolic concentration of calcium ions was set as 1 μM.

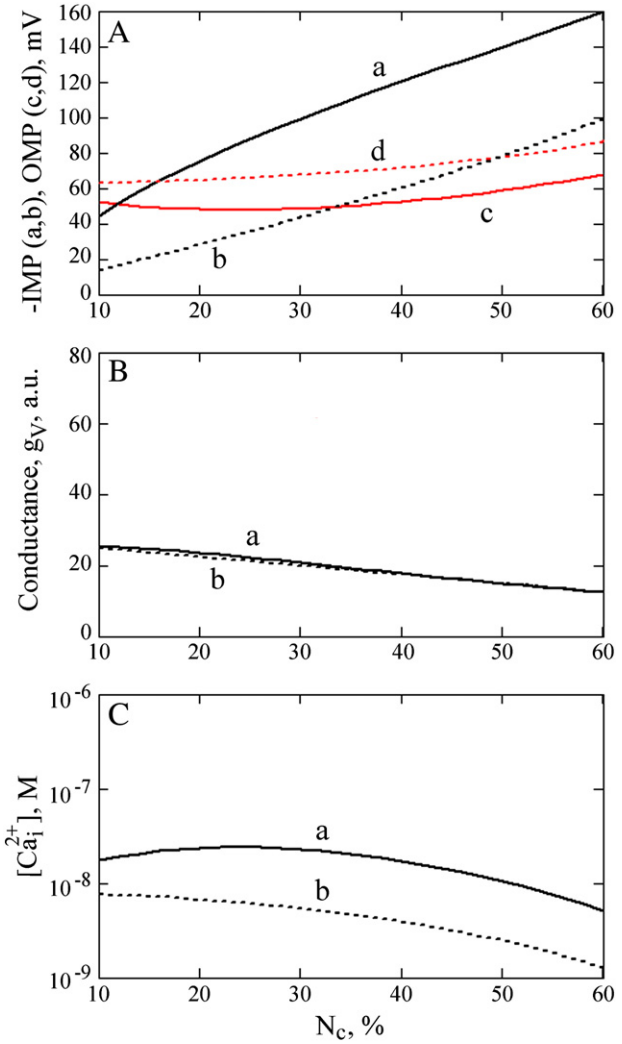

Fig. 3. Generation of the IMP (minus inside) and the OMP (A) by the VDAC–kinase mechanism shown in Fig. 1, and corresponding changes of the MOM conductance g_v (B) and of the MIMS concentration of calcium ions (C) as the function of the percentage N_c of VDACs forming the ANT–CK–VDAC contact sites, at the fixed percentage $N_h = 5\%$ of VDAC–HK complexes and the cristae coefficient $k_r = 0.2$. *a,c* — in the presence of 25 mM CC ([Cr] + [CrP]); *b,d* — in the presence of 0.25 mM CC; The cytosolic concentration of calcium ions was set as 1 μM.

observed for the presence of low, 0.05 mM glucose, but the calculated IMPs in general were significantly lower (Fig. 4E) than those determined at 5 mM glucose concentration (Fig. 4B).

The presented computational analysis clearly showed a possibility of the generation of both the inner and the outer membrane potentials of mitochondria in the absence of oxygen, using ATP produced by glycolysis in the cytosol and the Gibbs free energy of the kinase reactions. Note that it is not an ATP wasting mechanism, because ATP generated in the mitochondrial matrix in a CrP-dependent mode is subsequently used in the HK reaction associated with the VDAC–HK complex contributing to the first step of glycolysis.

4. Discussion

The mitochondrial membrane potentials, both the IMP and the OMP, seem to be important for the maintenance of mitochondrial integrity and cell survival under hypoxic/anoxic conditions. The decrease in the IMP, normally generated by the respiratory chain, is known to induce the permeability transition pore (PTP) [44,45] that has been recently suggested to be most probably associated with only the MIM [46,47]. The PTP opening leads to mitochondria swelling, outer membrane rupture and liberation of pro-apoptotic factors from the MIMS ([45,47] and the references therein), besides the ATP wasting by uncoupled mitochondria. The threshold value of the IMP to open the PTP has been shown to depend on the SH/S-S ratio [44] and thus on the oxidative stress. In addition, calcium overload of mitochondria is another powerful factor inducing the PTP opening [44–47]. Below the threshold concentration, calcium in the mitochondrial matrix is known to play a crucial role in activation of mitochondrial metabolism [48]. Thus, modulation of calcium concentration in the MIMS through an electrical extrusion or capture from/into the MIMS, depending on the sign of generated OMP, might play an important role in the regulation of mitochondrial metabolism and cell death.

The computational analysis of the mechanism shown in Fig. 1 demonstrated that the thermodynamic properties of the hexokinase and creatine kinase reactions allow generation of the IMP in the absence of oxygen. The magnitudes of calculated IMP under some metabolic conditions were comparable with those known for normal respiring mitochondria. The calculations showed that the IMP was higher at relatively high concentrations of glucose and CC, as well as at a relatively high percentage N_c of the mitochondrial ANT–CK–VDAC contact sites (Figs. 2A and 3A) corresponding to mitochondria with a highly expressed octameric creatine kinase.

The calculations of the IMP are consistent with various experimental data [16,19,20,23]. The environment inside even a small tumor is characterized by total anoxia or hypoxia [49]. It is also well known that the cytosolic CK-BB is elevated in many tumors, particularly in breast cancer [50]. Overexpression of ubiquitous mitochondrial CK (uMtCK), in addition, in the invasive ductal carcinomas of breast tissue has been recently shown to be associated with a poor prognosis because of the promotion of tumor growth by inhibiting apoptosis of tumor cells through stabilizing IMP [23]. Many cases of the protective effects of the creatine kinase system have been also reported for the brain and heart [2,16–20], although a possible contribution of a CrP-dependent reverse synthesis of intramitochondrial ATP and of generation of the IMP by the ANT–CK–VDAC contact sites in a tandem functioning with the VDAC–HK complex have not yet been studied.

A factor favored generation of high IMPs by a suggested mechanism was the orthodox configuration of mitochondria. We modeled this structural state of mitochondria taking $k_r = 0.1$ to reflect strong restriction of ADP/ATP diffusion in narrow cristae reported in [36,37,43]. According to Mannella at al. [37], about 93% of mitochondrial fluxes occur within the cristae. The cristae volume is also essentially separated from the external space of the MIMS by cristae junctions [36,37,43]. It has been assumed that the cristae configuration changes might be an important factor in regulation of the mitochondrial metabolism [36,37]. According to our calculations of the IMP generated by the kinase reactions, the cristae configuration factor might be important for cell survival under anoxia.

The calculations showed that the IMP can be enhanced by an increase in the percentage N_h of the VDAC–HK complexes in the MOM, which is another powerful factor favoring the IMP generation, as it was calculated for the case of $N_h = 5\%$, even with $k_r = 0.2$. A significantly higher IMP was calculated for the same conditions but with $k_r = 0.1$ (calculations not shown). An increase in k_r, corresponding to mitochondria transition to the condensed configuration, was accompanied with a decrease in calculated IMP (Fig. 4, B and E).

These results support the suggestion that the cristae configuration changes might play a key role in regulation of mitochondrial metabolism [37], even under the here considered anaerobic conditions. Both high glucose and CC concentrations favored IMP generation by the mechanism shown in Fig. 1, and the calculated magnitudes of the IMP were high enough to maintain the PTP in the closed state using the Gibbs free energy of the mitochondria-associated kinase reactions. If we present the free ANT–cristae combined resistance R_t of the MIM ($Rt = 1 / g_t$) as the sum of the resistance R_{tm} to ATP/ADP exchange

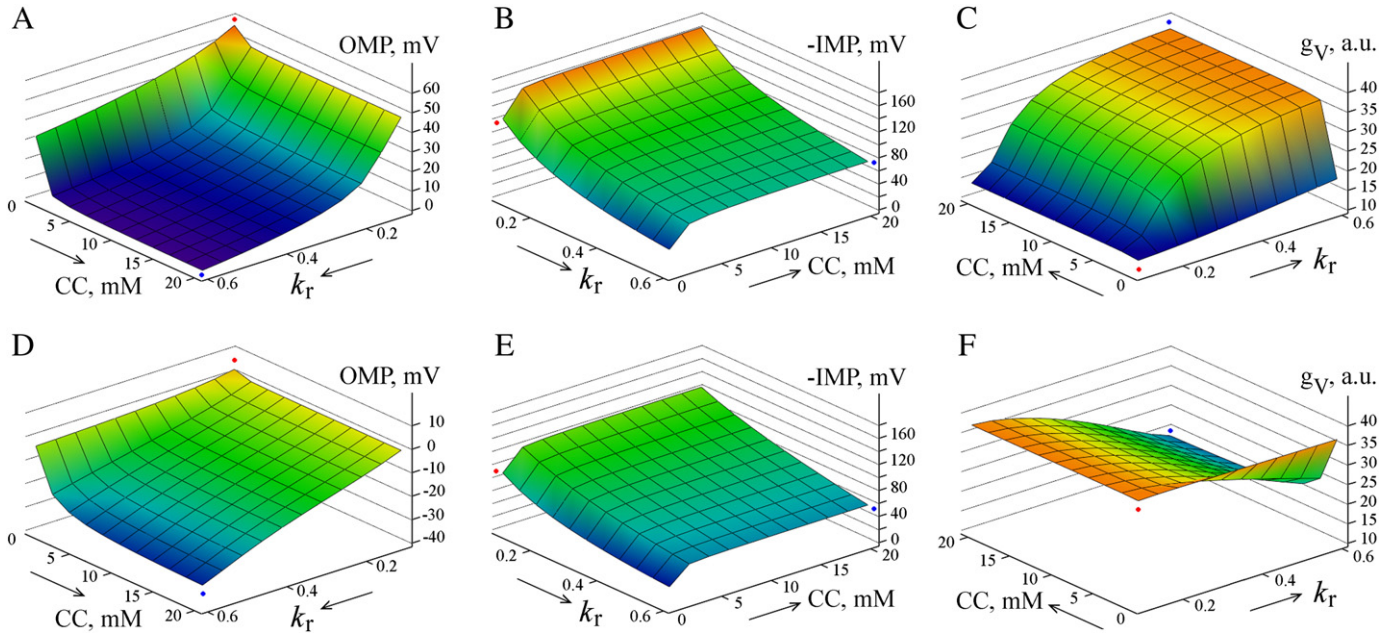

Fig. 4. Generation of the OMP (A,D) and the IMP (B,E) by the VDAC–kinase mechanism shown in Fig. 1, and the MOM conductance g_v changes (C,F) as the functions of CC concentration in the cytosol and the cristae coefficient k_r at the fixed percentage $N_c = 60\%$ of VDACs forming the ANT–CK–VDAC contact sites and the percentage $N_h = 5\%$ of VDAC–HK complexes. A–C — at 5 mM glucose; D–F — at 0.05 mM glucose; CC = [Cr] + [CrP].

through the ANTs and the resistance R_{cj} for ATP/ADP diffusion in narrow cristae and through the cristae junctions, the intra-cristae negative potential might be generated as a result of the voltage drop on the resistance R_{cj} (Fig. 1B), thus increasing the proton concentration in cristae with respect to the external compartment of the MIMS. A possibility of pH gradient existence in narrow cristae has been earlier considered in [37]. It means that in the narrow cristae, the pH component might represent a significant part of the MIM proton-motive force.

The VDAC–kinase mechanism suggested here allows the generation of the OMP (Fig. 1). If the OMP is positive, it might cause calcium extrusion from the MIMS thus protecting mitochondria against the PTP opening and preventing cell death at elevated calcium concentrations in the cytosol. It might be a natural alternative to the direct inhibition of the calcium MIM uniporter to increase cell resistance to death under the influence of factors increasing the cytosolic concentration of calcium [51].

In contrast, a high negative OMP has been experimentally demonstrated for mitochondria in living cells [52]. Although VDAC might be essentially closed under such conditions, even a completely closed VDAC has been shown to be highly permeable to calcium ions [53]. Hence, a high negative OMP [52] should increase the MIMS concentration of calcium, thus favoring its entrance into the mitochondrial matrix and increasing the mitochondrial metabolism [48]. On the other hand, after the calcium threshold concentration is reached, this calcium overload is known to lead to cell death through mitochondrial apoptosis/necrosis mechanisms. The OMP-mediated control of calcium signaling might be additional to the described mechanisms of the MOM permeability regulation by various permeabilizing factors [21,24,47] and by "molecular corks" of several types, suppressing mitochondrial metabolism [3].

As the simplest and most direct mechanism of the OMP generation, we have earlier suggested the functioning of the VDAC–HK complex [32,39]. In this complex, the MIMS ATP^{4-} and the cytosolic glucose are converted into the MIMS ADP^{3-} and into the cytosolic glucose-6-phosphate anion, thus moving one net negative charge through the MOM using the Gibbs free energy of the HK reaction. Here we estimated the contribution of the free energy of both the HK and the CK kinase reactions to generation of the OMP and the IMP (Fig. 1). High OMPs, positive inside, were calculated even at the relatively low percentage N_h (3%) of the VDAC–HK complexes relative to the total number of VDACs, but at high percentages N_c of the intermembrane ANT–CK–VDAC contact sites (Fig. 2A), or at an enhanced number of the VDAC–HK contact sites, as $N_h = 5\%$ for example, at any N_c (10–60%) (Fig. 3A).

The exhaustion of glucose that was modeled by a decrease of its concentration to 0.05 mM, demonstrated a significant decrease in calculated IMPs (Fig. 4, B and E, respectively). Interestingly, an increase in the concentration of CC decreased the OMP even in the presence of 5 mM glucose (Fig. 4A), and even caused generation of negative OMPs at the low, 0.05 mM glucose concentration, if the cristae coefficient k_r was relatively high (Fig. 4D), corresponding to the condensed configuration of mitochondria.

Strong suppression of the MOM conductance g_v by the generated positive OMP (Fig. 4A) was revealed in the presence of 5 mM glucose at low values of the coefficient k_r in a wide range of CC concentrations (Fig. 4C), or in the presence of low glucose concentration (0.05 mM) at high values of the cristae coefficient k_r in a range of relatively high concentrations of CC (Fig. 4F). All this means that the MOM permeability for ATP might be almost completely blocked due to the OMP generation by the suggested kinase mechanism. These results are consistent with the observations that ATP hydrolysis by the protonic F_OF_1 ATPase of mitochondria was strongly prevented in muscle cells under anoxic conditions [20]. Although it might be explained by an inhibition with the natural inhibitor IF1, by transport limitation at the level of ANT and by modifications of the kinetic properties of the ATPase [20], the OMP-dependent electrical suppression of mitochondria turnover of adenine nucleotides represents a new possibility for preventing ATP wasting under hypoxic/anoxic conditions. In anoxic resistant tissues, the generated OMP might be higher than in non-resistant cells and tissues. Interestingly, it has been recently reported that the CK-mediated energy flux from the subsarcolemmal space was able to sustain the IMP at the anoxic core of cardiomyocytes, thus avoiding ATP wasting and necrotic cell death [20]. We assume that this IMP might be sustained by the mechanism shown in Fig. 1 through the ANT–CK–VDAC contact sites.

Using the immunogold technique, it has been demonstrated that CK is colocalized with the mitochondrial intermembrane contact sites, as well as being localized inside the mitochondrial cristae [24,54], forming complexes with cristae ANT [21]. In this respect, the possibility exists that the IMP might also be generated by the cristae ANT–CK complexes using the free energy released from the dephosphorylation of cristae

creatine phosphate. Even the formation of quaternary ANT–CK–VDAC–HK contact sites has been assumed as a mechanism controlling mitochondrial outer membrane permeabilization by anti- and pro-apoptotic proteins [11]. The structure and the enzymatic activity of mitochondrial contact sites, related to a possible energy channeling [24–27] and generation of mitochondrial membrane potentials, evoke great interest and still have to be further elucidated.

In conclusion, it is important to highlight that the suggested non-Mitchell mechanism of generation of the mitochondrial membrane potentials was only to demonstrate the main principles by which the Gibbs free energy of the kinase reactions associated with mitochondrial VDACs might be involved in this process. The computational analysis of the model showed that the magnitude of the IMP generated in the absence of oxygen might be sufficiently high to maintain mitochondrial integrity. The generated OMP might modulate the MIMS concentrations of calcium ions, ADP, ATP and of other charged metabolites, and, in general, it might regulate the MOM permeability and the mitochondrial metabolism. A possibility of the VDAC closure by various factors and by "molecular corks" has been earlier considered to explain mitochondrial suppression under anoxia/hypoxia, ischemia and other conditions [3]. The suggested VDAC–kinase mediated mechanism of the OMP generation and of the electrical suppression of mitochondria might inhibit ATP/ADP turnover through the mitochondrial outer membrane, thus preventing ATP wasting and increasing cell resistance to death in the absence of oxygen. This mechanism may also explain the physiological role of the highly conserved voltage gating properties of VDAC [55].

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