Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

VDAC electronics: 1. VDAC-hexo(gluco)kinase generator of the mitochondrial outer membrane potential

Victor V. Lemeshko *

Escuela de Física, Facultad de Ciencias, Universidad Nacional de Colombia, sede Medellín, Calle 59A, No 63-20, Medellín, Colombia

ARTICLE INFO

Article history:
Received 31 August 2013
Received in revised form 25 December 2013
Accepted 1 January 2014
Available online xxxx

Keywords:
VDAC
Hexokinase
Cancer
Aerobic glycolysis
Mitochondrial outer membrane potential
Computational model

ABSTRACT

The simplest mechanism of the generation of the mitochondrial outer membrane potential (OMP) by the VDAC (voltage-dependent anion channel)-hexokinase complex (VHC), suggested earlier, and by the VDAC-glucokinase complex (VGC), was computationally analyzed. Even at less than 4% of VDACs bound to hexokinase, the calculated OMP is high enough to trigger the electrical closure of VDACs beyond the complexes at threshold concentrations of glucose. These results confirmed our previous hypothesis that the Warburg effect is caused by the electrical closure of VDACs, leading to global restriction of the outer membrane permeability coupled to aerobic glycolysis. The model showed that the inhibition of the conductance and/or an increase in the voltage sensitivity of a relatively small fraction of VDACs by factors like tubulin potentiate the electrical closure of the remaining free VDACs. The extrusion of calcium ions from the mitochondrial intermembrane space by the generated OMP, positive inside, might increase cancer cell resistance to death. Within the VGC model, the known effect of induction of ATP release from mitochondria by accumulated glucose-6-phosphate in pancreatic beta cells might result not only of the known effect of GK dissociation from the VDAC-GK complex, but also of a decrease in the free energy of glucokinase reaction, leading to the OMP decrease and VDAC opening. We suggest that the VDAC-mediated electrical control of the mitochondrial outer membrane permeability, dependent on metabolic conditions, is a fundamental physiological mechanism of global regulation of mitochondrial functions and of cell death.

1. Introduction

VDAC, the most abundant protein of the mitochondrial outer membrane (MOM[1]) [1–4], is universally accepted as responsible for the control of metabolite fluxes between mitochondria and the cytosol [3–7]. This porin has been demonstrated to directly relate to many physiological processes and pathologies [5,7–9]. VDAC has even been considered as a governator of global mitochondrial functions both in health and disease [5]. However, experimental results related to the VDAC-mediated regulation of the MOM permeability and of cell death are confusing and contradictory, and the mechanisms responsible for this regulation remain poorly understood [4,10–12].

Although VDAC's electrical properties have been studied in detail [1–4,10,13], it has been generally considered as a permanently open pore under physiological conditions. Meanwhile, a large body of literature has been accumulated showing that VDAC conductance in living cells should be regulated [4,5,8–10,12–15]. It has been widely assumed that the MOM permeability is controlled by a partial or complete blockage of VDAC by various cytosolic proteins like tubulin [13–19], or in general, by various anti- and pro-apoptotic factors [5,7,8,10,12]. Many cases of apparently anomalous behavior of mitochondria and of global suppression of mitochondrial functions have been attributed to such reversible blockage-type regulation of the MOM permeability [5].

On the other hand, it is unlikely, according to Mannella et al. [20], that VDAC simply converts the MOM in a coarse sieve. We could add in this respect, that it is unlikely that the permeability of this sieve is simply regulated by only "molecular corks", as by hexokinase (HK) bound to VDAC, for example [21]. Meanwhile, the role of VDAC's highly conserved voltage gating properties remains to be the main unresolved question [4]. This question seems to be fundamental and can be answered possibly by finding the missing players of the MOM permeability regulation.

Earlier, we have proposed several steady-state mechanisms of the generation of metabolically-dependent OMP to demonstrate that the electrical closure-opening of VDAC might represent a physiological mechanism of regulation of the MOM permeability [22–24]. Experimental evidence of the generation of the negative OMP in living cells has been obtained by Porcelli et al. [25], although it is not yet clear, to

Abbreviations: VDAC, voltage-dependent anion channel; HK, hexokinase: GK, glucokinase; VHC, VDAC-HK complex; VGC, VDAC-GK complex; MIMS, mitochondrial intermembrane space; MOM, mitochondrial outer membrane; OMP, outer membrane potential; IMP, inner membrane potential; ANT, adenine nucleotide translocator; N_H, the percentage of VDACs bound to HK or to GK; N_{VS}, the percentages of voltage sensitive VDACs; N_{NS}, the percentage of voltage non-sensitive VDACs, TE, tubulin-like effectors; N_{TE}, the percentage of VDACs bound to TE; N_I, the percentage of VDACs completely blocked by an inhibitor

* Tel.: +57 4 4309378; fax: +57 4 4309327.
E-mail address: vvasilie@unal.edu.co.

0005-2736/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbamem.2014.01.001

what extent the metabolically-dependent inner membrane surface potential influenced the reported data, as it has been analyzed earlier [26].

According to one of the possible mechanisms of OMP generation, suggested earlier, the inner membrane potential (IMP) might be partly applied to the MOM through the intermembrane contact sites composed of adenine nucleotide translocator (ANT) and VDAC [23,24]. The same idea has recently been expressed by Pedersen [9]. In cancer cells, in addition, the resistance of the ANT-VDAC-HK contact sites has been suggested to decrease due to the free energy of the HK reaction applied to the contact sites, thus increasing the OMP [23,24]. The possible explanation of the Warburg effect has been proposed on the basis of global electrical closure of VDACs beyond the contact sites, due to the generated OMP. A similar concept has been developed in the last years by other authors [5,27], although without pointing to the electrical character of the MOM permeability suppression in cancer cells.

The simplest of the proposed mechanisms of the OMP generation has been based on the VDAC-HK complex only [23,24], because, for example, the mitochondrial intermembrane contact sites have not been found in the subpopulation HT29 Glc^+ of adenocarcinoma cells, although HK was predominantly bound to mitochondria [28]. Computational analysis of this model could represent certain interest for understanding possible mechanisms of the regulation of aerobic glycolysis and cell death. It has been discovered that in cancer cells, a large proportion of HK is associated with mitochondria [29,30 and reference therein] that has also been found to increase cancer cell resistance to death [8,9,11,12,31,32]. In addition, cancer cells have been characterized by a high rate of aerobic glycolysis and by a mitochondrial HK activity up to more than two orders of magnitude higher than in normal cells [33,34]. It has been found that both the HK binding to VDAC and the glucose phosphorylation reaction contribute to the protective effects of HK-I and HK-II against cell death [35]. It might be related to a decrease of the calcium concentration in the MIMS due to the positive OMP generation by VHC, according to the physical principles described earlier [23,24].

In the present work, we developed the VHC and VGC models of generation of the OMP, with the Gibbs free energy of kinase reactions as a driving force, as a battery in an equivalent electrical circuit. The calculations showed that the OMP value directly depends on the percentage of VDACs bound to HK, on the glucose concentration, and on the presence of tubulin-like effectors (TE). The calculated OMPs were high enough to electrically close VDAC. The positive sign of the OMP generated by the VHC might explain a high resistance of cancer cells to death as a result of calcium extrusion from the mitochondrial intermembrane space (MIMS). The model can be applied to pancreatic beta cells, for mitochondria of which high values of the OMP were calculated using the VGC model. Development and computational analysis of such and similar models seems to be an important approach to the further understanding of cell energy metabolism regulation, as well as of many cases of apparently anomalous behavior of mitochondria reviewed and analyzed in detail in [5].

2. Materials and methods

2.1. The VDAC-hexokinase complex model

According to the VHC model shown in Fig. 1A, ATP from the MIMS and glucose from the cytoplasm are used by HK bound to VDAC in the MOM, liberating ADP back into the MIMS and producing cytoplasmic glucose-6-phosphate. 100% of all VDACs in the MOM can be expressed as the sum of the percentage of VDACs bound to HK (N_H), of the percentages of the voltage sensitive VDACs (N_{VS}), and of voltage non-sensitive VDACs (N_{NS}), as well as of the percentage of VDACs bound to tubulin-like effectors (TE) influencing VDAC voltage sensitivity and/or partially blocking it (N_{TE}), and even of the percentage of

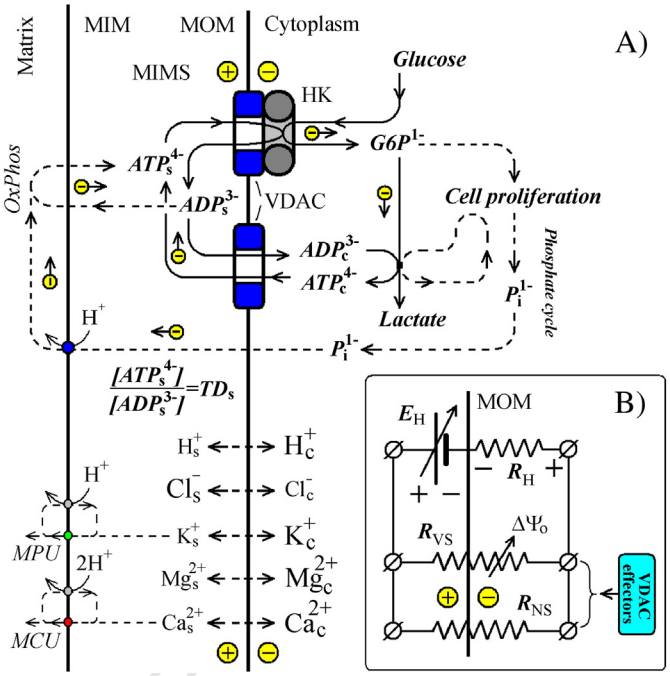

Fig. 1. The main principle of the OMP generation by the VDAC-hexokinase complex. A — VHC functioning leads to a charge separation across the MOM. The generated potential leads to the free VDAC closure strongly restricting ADP release from the MIMS to recover ATP in the cytoplasm. It represents the suggested anti-turbo mechanism of regulation of aerobic glycolysis allowing usage of mitochondrial ATP for the first stage of glycolysis in cancer cells (dotted lines). Ions H^+, Cl^-, K^+, Mg^{2+}, Ca^{2+} permeate through free VDACs in the MOM, achieving their electrochemical equilibrium. MPU — mitochondrial potassium uniporter; MCU — mitochondrial calcium uniporter. B — An equivalent electrical circuit of the VHC model: the battery E_H represents Gibbs free energy of the hexokinase reaction. The battery internal resistance, R_H, depends on the percentage of VDACs bound to HK. The resistance of the fraction of free voltage non-sensitive VDACs (as low sensitive VDAC3, for example) is presented as the resistance R_{NS} connected in parallel to the resistance R_{VS} of the remaining voltage gating VDACs. According to Ohm's law, the OMP generation results from voltage division on the equivalent resistance of free VDACs (R_{NS} and R_{VS}), that might be influenced by various VDAC effectors, and on the internal resistance of the battery R_H. Note, as the VDACs begin to close, their increasing resistance leads to further OMP increase by the mechanism of positive feedback control, allowing the MOM permeability regulation that depends on the glucose concentration and on the $[ATP]_s/[ADP]_s$ ratio.

VDACs completely blocked by some inhibitors (N_I):

$$100 = N_H + N_{VS} + N_{NS} + N_{TE} + N_I \quad (1)$$

The conductance g_{NS} (resistance $R_{NS} = 1/g_{NS}$ in Fig. 1B) of the N_{NS} fraction is not affected by the OMP, thus we can write $g_{NS} = N_{NS}$, expressing conductance in arbitrary units, a.u. The maximum conductance of the MOM was taken as 100 a.u., for 100% of all VDACs in the open state.

The conductance g_{VS} (resistance R_{VS} in Fig. 1B) of the fraction N_{VS} can be expressed as the function of the OMP ($\Delta\psi_o$) using an equation similar to that published earlier [22–24], at an arbitrary voltage-sensitivity parameter "*S1*":

$$g_{VS} = N_{VS} \cdot P_{c1} + N_{VS} \cdot (1 - P_{c1}) \cdot \exp\left(-(S1 \cdot \Delta\psi_o s)^2\right). \quad (2)$$

We used here $S1 = 40\ V^{-1}$ allowing almost complete VDAC closure at $\Delta\psi_o = \pm 40$ mV, as shown in Fig. 2a and b. The parameter P_{c1} is the VDAC relative conductance in the closed state, which was set in the range of 0.25–0.50 for various calculations [15,36–38].

Please cite this article as: V.V. Lemeshko, VDAC electronics: 1. VDAC-hexo(gluco)kinase generator of the mitochondrial outer membrane potential, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamem.2014.01.001