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Origin of ion selectivity in *Phaseolus coccineus* mitochondrial VDAC

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ABSTRACT

The mitochondrial voltage-dependent a nion-selective channel (VDAC) is the major permeation pathway for small ions and metabolites. Although a wealth of electrophysiological data has been obtained on different VDAC species, the physical mechanisms of their ionic selectivity are still elusive. We addressed this issue using electrophysiological experiments performed on plant VDAC. A simple macroscopic electrodiffusion model accounting for ion diffusion and for an effective fixed charge of the channel describes well its selectivity. Brownian Dynamics simulations of ion permeation performed on plant and mammalian VDACs point to the role of specific charged residues located at about the middle of the pore.

1. Introduction

Ion channels are present in the inner and outer mitochondrial membrane (OMM) (Harsman et al., 2011; O'Rourke, 2007; Szewczyk et al., 2006; Zoratti et al., 2009) where they play a fundamental role in mitochondrial homeostasis and bioenergetics. The channels of the OMM belong to the β-barrel transmembrane protein family (e.g., SAM50, TOM40 and VDAC). They have important biological functions including metabolite exchange between mitochondria and cytoplasm (Colombini, 2012), protein and tRNA import (Bolender et al., 2008; Jeon et al., 2011; Murcha et al., 2014) as well as outer membrane β-barrel biogenesis (Wenz et al., 2014; Zeth, 2010).

The main functional role of one of these OMM channels, the voltage-dependent anion-selective channel (VDAC), is to allow and regulate the flux of metabolites and inorganic ions through the OMM (Colombini, 2012; Gincel et al., 2000; Hodge and Colombini, 1997; Homblé et al., 2012; Rostovtseva and Colombini, 1997; Yehezkel et al., 2006). Most of the data on the structure, function and regulation of VDAC arise from reports on mammalian and yeast VDAC proteins (Homblé et al., 2012) though plant VDACs have also been at the center of several studies. This channel is the most abundant protein of the OMM with a high surface density (about $2 \cdot 10^4$ protein/μm^2) (Mannella and Bonner, 1975) covering up about 25–40% of the membrane (Gonçalves et al., 2007; Hoogenboom et al., 2007). Electrophysiological characterization of different plant VDACs revealed functional similarities with the channels from other organisms: a weak anion selectivity (Permeability ratio $P_{Cl}/P_K \sim 2$), a relatively symmetrical bell-shaped voltage-dependence which characterizes the transition from the open to subconductances states upon applied voltages and a high conductance (~4 nS in 1 M KCl) in the fully open state (Abrecht et al., 2000a; Blumenthal et al., 1993; Colombini, 2012; Reina et al., 2013; Smack and Colombini, 1985).

Plant VDACs, however, have also their own specificities and play a key role in plant physiology (Homblé et al., 2012). In contrast to membranes from mammals and fungi, plant membranes contain a large variety of different sterol types, each of which affects VDAC's electrophysiology differently (Mlayeh et al., 2010). Recent studies suggest that plant VDACs also play a role in programmed cell death in response to biotic and abiotic stress (Godbole et al., 2013; Takahashi and Tateda, 2013) though how VDAC intervenes in this process is not known.

Atomic level 3D structures were determined only for the mammalian isoform 1 of VDAC (Bayrhuber et al., 2008; Hiller et al., 2008; Ujwal et al., 2008). These structures adopt a 19 β-strand barrel flanked by an N-terminal α-helix which is folded into the pore. There are data suggesting that plant VDACs possess structural features similar to those of the mammalian structure of VDAC1: 1) the pore size determined on potato VDAC by AFM (Hoogenboom et al., 2007) is about 2.7 nm consistent with the backbone–backbone diameter (~3.5 nm) of the mammalian 3D structures (Bayrhuber et al., 2008; Hiller et al., 2008; Ujwal et al.,

Abbreviations: BD, Brownian Dynamics; GHK, Goldman–Hodgkin–Katz; LCT, large channel theory; mVDAC1, mouse VDAC isoform 1; MD, molecular dynamics; NcVDAC, *Neurospora crassa* VDAC; OMM, outer mitochondrial membrane; ScVDAC1, *Saccharomyces cerevisiae* VDAC isoform 1; TMS, Teorell–Meyer–Sievers; PcVDAC, *Phaseolus coccineus* VDAC; VDAC, voltage-dependent anion-selective channel.

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2008) 2) spectroscopic and bioinformatic studies indicate that the secondary structure content of bean (*Phaseolus coccineus*) VDAC (PcVDAC) is close to that of the atomic resolution structures of the mammalian VDAC1 (Abrecht et al., 2000b; Hafez et al., 2012; Homblé et al., 2012) and 3) the VDAC supramolecular organization is the same in plants and in other organisms (Gonçalves et al., 2007; Hoogenboom et al., 2007; Zalk et al., 2005). Moreover, a recently built 3D model of PcVDAC was shown to display features such as electrostatic energy ion profile, β-strand dynamics and distribution of the hydrophobic and charged residues akin to those of the mouse VDAC isoform 1 (mVDAC1) crystal structure (Homblé et al., 2012).

The deciphering of the mechanism underpinning anion transport through the VDAC and of its selectivity is important as most of the chemical species entering or leaving the mitochondrion are anions that are directly involved in respiration. The origin of VDAC selectivity was first addressed by Blachly-Dyson et al. (1990), Peng et al. (1992) and Zambrowicz and Colombini (1993) using site directed mutagenesis and electrophysiology. These authors developed a model named the large channel theory (LCT) which divides VDAC pore in two compartments: one electrically neutral core and one outer cylindrical shell where ion diffusion is influenced by the electrostatic effect arising from the effective fixed charge of the pore wall (Zambrowicz and Colombini, 1993). This model was shown to correctly describe the change in selectivity of fungal VDAC upon variation of the salt concentration difference across the membrane. Another study showed however that the selectivity of *Dictyostelium*'s VDAC measured at different salt concentration gradients followed the classical Goldman–Hodgkin–Katz (GHK) equation valid for one-compartment channel (Troll et al., 1992). Recent molecular simulation studies performed on the crystal structure of mVDAC1 suggested that the dominant contribution to VDAC selectivity is indeed electrostatic (Krammer et al., 2011, 2013).

Altogether these data prompted for a more thorough investigation of VDAC electrophysiological properties. Here we examined the ionic selectivity of the open state of the plant PcVDAC experimentally and theoretically. For weakly selective ion channels the reversal potential, defined as the electric potential needed to zero the ion current induced by a concentration gradient, provides qualitative information about the selectivity of a channel, i.e. whether the channel is more selective to one ion species than another. The quantification of this selectivity is however model-dependent. The GHK equation (Hille, 1992) has often been used to calculate the ion permeability ratio at a single value of the concentration gradient (Abrecht et al., 2000a; Aljamal et al., 1993; Blumenthal et al., 1993; Elkeles et al., 1997; Mlayeh et al., 2010; Smack and Colombini, 1985). We showed in this study that this equation cannot however correctly predict the selectivity of plant VDACs over a range of concentration gradients. Instead we demonstrated that a simple one-compartment macroscopic electrodiffusion model including both ion diffusion and an effective fixed charge in the pore can properly describe the selectivity of the channel. By using Brownian Dynamics (BD) simulations we also proposed a comprehensive detailed model of the molecular mechanism of ion permeation.

2. Material and methods

2.1. Electrophysiological experiments

2.1.1. VDAC purification

Seeds from *P. coccineus* were soaked in tap water for 18 h and mitochondrial membranes were isolated from the cotyledons by differential centrifugation steps and further purified on a 28% Percoll gradient as described previously (Abrecht et al., 2000a). Purification of the most abundant PcVDAC isoform (32 kDa) was achieved using the chromatofocusing technique (Abrecht et al., 2000b).

2.1.2. VDAC reconstitution and electrophysiology

The purified PcVDAC was reconstituted in planar lipid bilayers as described previously (Homblé et al., 2010). The bilayer was made of soybean phospholipid extract, purchased from Avanti Polar Lipid (Alabaster, AL). It was chosen as its phospholipid composition is close to that found in plant membranes. Lipids were dissolved in hexane to a final concentration of 2% (w/v). Planar lipid bilayers were formed by folding two lipid monolayers over a hole (110–150 μm in diameter) made in a 25 μm thick Teflon partition that separated two Teflon experimental chambers. Before each experiment the partition was treated with a solution of hexadecane/hexane (2.5%, v/v). Ag/AgCl electrodes connected in series with a salt bridge (1 M KCl in 1% agar) were used to connect the experimental chambers to the electronic equipment. The *trans* compartment is defined as the one connected to the ground and the voltage was applied to the *cis* compartment. For channel reconstitution into a planar lipid bilayer, proteins were added to the *cis* compartment. KCl solutions of specified molality (M) were buffered with 10 mM HEPES at pH 7.5.

Current recordings were performed as described previously (Mlayeh et al., 2010) using a BLM 120 amplifier (BioLogic, France). Data were filtered at 300 Hz (5-poles linearized Tchebichev filter). Single channel experiments were performed for reversal potential measurements. The reversal potential (zero-current potential) was set to zero in presence of identical KCl molality on both sides of the membrane. The *cis* compartment was afterwards perfused three times its volume with a solution of different KCl molality and the change in reversal potential (E_{rev}) was recorded. The values of E_{rev} were corrected for the liquid junction potential at salt bridges.

The single channel conductance was calculated from the current amplitude flowing through the channel in response to a 10 mV voltage pulse using Ohm law.

2.1.3. Data analysis

Nonlinear least square regressions were performed using a homemade program written in the MATLAB environment (The MathWorks, The Netherlands). Experimental data are shown as the mean ± standard error of the mean (N = number of experiments).

2.2. Brownian Dynamics simulations

All BD inputs were created with the GCMC/BD module (Lee et al., 2012) of the CHARMM GUI server (Jo et al., 2008) and all simulations were performed using the GCMC/BD program (Im et al., 2000) on either the mVDAC1 crystal structure (Ujwal et al., 2008) or on a previously constructed 3D model of PcVDAC (Homblé et al., 2012). To describe the diffusion profile of the ions inside the pore a position-dependent scaling of the diffusion coefficient was applied (Lee et al., 2012; Paine and Scherr, 1975). This led to an enhanced agreement between our calculated conductance and reversal potential values with the experimental data as shown in a comparison of the results from this study and from a previous work (Krammer et al., 2013). All other parameters were as described elsewhere (Krammer et al., 2013).

The conductance of PcVDAC was determined at 0.1 M and 1.0 M KCl from 1 and 0.1 μs BD trajectories respectively and its selectivity was computed from non-equilibrium 0.1 μs BD trajectories performed in 0.1 M/1.0 M KCl gradient. The conductance of mVDAC1 was determined from 1, 0.4, 0.2, 0.125 and 0.1 μs BD trajectories in a 0.1, 0.25, 0.5, 0.8, and 1.0 M KCl concentration respectively on both sides of the membrane and with an applied voltage of ±50 mV. The reversal potential and the conductance values of mVDAC1 were also computed from non-equilibrium 0.1 μs BD trajectories performed in 0.025 M/1.0 M (trans/cis), 0.033 M/1.0 M, 0.05 M/1.0 M, 0.166 M/1.0 M, 0.1 M/1.0 M, 0.25 M/1.0 M, 0.3 M/1.0 M, and 0.5 M/1.0 M, 0.2 μs trajectories in 0.25 M/0.5 M, 0.3 μs in 0.15 M/0.3 M, 0.4 μs in 0.025 M/0.25 M, 0.8 μs in 0.15 M/0.3 M, 1 μs in 0.125 M/0.25 M and 2.5 μs in 0.05 M/0.1