Biochemical and Biophysical Research Communications 459 (2015) 24–28

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Channel characteristics of VDAC-3 from *Arabidopsis thaliana*

C. Berrier [a], R. Peyronnet [b, 1, 2], J.-M. Betton [c], G. Ephritikhine [b], H. Barbier-Brygoo [b], J.-M. Frachisse [b], A. Ghazi [a, *]

[a] Neuroscience Paris-Saclay Institute (Neuro-PSI) UMR 9197, CNRS-Université Paris-Sud, Bâtiment 430, F-91405 Orsay Cedex, France
[b] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Sciences Plant, Saclay, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France
[c] Unité de Microbiologie Structurale & CNRS UMR3528, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

ARTICLE INFO

Article history:
Received 3 February 2015
Available online 14 February 2015

Keywords:
VDAC
Cell-free synthesis
Arabidopsis
Channel

ABSTRACT

Four different isoforms of the Voltage-Dependent Anion Channel (VDAC) have been identified in *Arabidopsis* plant cells. The electrophysiological characteristics of several VDAC channels from animal as well as plant cells are well documented, but those of this model plant are unknown. One isoform, AtVDAC-3 was obtained either directly by cell-free synthesis or produced in *Escherichia coli*, as inclusion bodies, and re-natured. An electrophysiological study of the purified proteins in planar lipid bilayers showed that both methods yielded proteins with similar channel activity. The characteristics of AtVDAC-3 are that of a *bona fide* VDAC-like channel.

1. Introduction

VDAC (Voltage-Dependent Anion Channel) or mitochondrial porins are membrane proteins mostly localized on the outer membrane of mitochondria where they mediate the exchange of metabolites. VDAC has also been implicated in mitochondrial apoptosis. In particular, it has been suggested to play a critical role in the release of apoptotic factors from animal mitochondria. The structure of humanVDAC-1 [1,2] and of mouse VDAC-1 [3] was obtained a few years ago. The protein folds into a 19-stranded β-barrel with an N-terminal α-helix located midway inside the pore.

Electrophysiological studies of VDAC proteins from different species, purified and inserted in artificial lipid bilayers, have generally revealed the following pattern: these proteins form high conductance, weakly anion-selective channels which are open at low membrane potentials. Above a certain value (20–30 mV), positive or negative, of the membrane potential, the channels tend to switch to low conductance states that are cation-selective [4,5]. However exceptions have been reported. For instance, De Pinto and colleagues have described an isoform of VDAC from *Drosophila melanogaster* insensitive to membrane potential [6].

Extra-mitochondrial localization of VDAC has been well documented [7]. The presence of VDAC in the plasma membrane of animal cells was suggested by Thinnes and coworkers. In particular large anionic conductance channels with VDAC-like properties, which were detected in patch-clamp experiments were shown to be sensitive to an antibody directed against HVDAC1 [8]. Using a combination of biochemical and electrophysiological techniques, Zoratti and co-workers showed the presence of VDAC in caveolae [9]. It was also shown that usage of alternative exons in the gene of mouse VDAC1 leads to two proteins differing by their N-termini, one targeted to the mitochondria and the other to the plasma membrane [10].

In the model plant *Arabidopsis thaliana*, four nucleus-encoded VDACs (AtVDAC1-4) were identified by Tateda et al. [11] and by Robert et al. [12]. In both studies study, all four AtVDAC isoforms were detected, as expected, in the mitochondria fraction. Additionally extra-mitochondrial localizations were found by these two authors with different targeting according to the considered study. Indeed, Robert et al. [12] developing two complementary approaches of immunology shown also a localization of AtVDAC-1, -2 and -3 in the plasma membrane, a result consistent with previous proteomic studies of the plant plasma membrane [13,14], while Tateda et al. [11] mentioned an extra-mitochondrial localization of AtVDAC-2 and -4 without specifying the compartment. In any case, these two studies [11,12] revealed a function of AtVDACs

* Corresponding author.
E-mail address: alexandre.ghazi@u-psud.fr (A. Ghazi).
[1] Present address: National Heart and Lung Institute, Imperial College London, United Kingdom.
[2] Present address: The Magdi Yacoub Heart Science Centre, Harefield, United Kingdom.

http://dx.doi.org/10.1016/j.bbrc.2015.02.034
0006-291X/

linked with their mitochondrial localization. For AtVDAC3 that we electrophysiologicaly characterized in this study, mitochondrial functions were identified: (i) in AtVDAC-3 protein production in response to the bacterial pathogen *Pseudomonas syringae* [15], (ii) in respiration during seed germination in response to cold [16]. These results, in agreement with the decreased in *atvdac3* mutants mitochondrial membrane potential [11] suggest a mitochondrial function of AtVDAC-3 during abiotic or biotic stress.

It is also worth noting that so far, no VDAC-like activity has been detected by patch-clamp at the level of Arabidopsis plasma membrane. Finally, the role of these extra-mitochondria targeted isoforms is still unclear.

Although the electrical characteristics of various VDAC channels isolated from different plant organisms have been documented (reviewed in Homble [17]), so far no AtVDAC protein has been characterized at the electrophysiological level. Starting with AtVDAC-3, we set to examine two methods to produce directly from their DNA sequence, proteins amenable to electrophysiological recordings. In one case the protein was produced by cell-free synthesis in the presence of detergent, in the other case it was produced in *Escherichia coli*, as inclusion bodies, and renatured. In both cases, the purified protein was studied in planar lipid bilayers.

2. Materials and methods

2.1. Plasmids, strains, and growth conditions

Expression plasmids were constructed by amplifying the coding region of AtVDAC-3 (At5g15090) gene with Platinium ® Pfx DNA polymerase (Invitrogen), fused to a T7 promoter, and cloned into pCRkan plasmid as previously described [18]. The resulting construct was used for cell-free expression as well as for cellular expression in *E. coli*.

E coli DH5α (Invitrogen) and BL21 (λDE3) (Novagen) strains were used as the host and expression strains, respectively, for the pCRkan-*Atvdac3* plasmids. Cultures were grown under aerobic conditions at 37 °C in LB medium. When appropriate, Kanamycin (30 μg/ml) and chloramphenicol (25 μg/ml, Appligene) were added to the medium. The expression of the recombinant VDAC-3 in BL21 cells was induced for 3 h with 1 mM of IPTG (Sigma).

2.2. In vitro expression and purification of the recombinant AtVDAC-3 protein

Cell free expression was carried out with the RTS system 500 HY (Roche). 15 μg of recombinant plasmid DNA were incubated in a final volume of 1 ml at 30 °C for 20–24 h with 0.2% Triton X-100, 18 μg *E. coli* lipids and 0.5% of cholesterol. The synthesis mixture was diluted in 5 ml of buffer A (50 mM NaH_2PO_4, 300 mM NaCl, 0.2% Triton X-100, pH 8) containing 10 mM imidazole. After dilution, the suspension was incubated for 1 h at room temperature with 1.2 ml of Ni-NTA matrix (Quiagen) equilibrated in the previous buffer. The matrix was packed in a 2.5 ml shell, extensively washed five times with 2 ml of buffer A supplemented with 20 mM imidazole. The bound proteins were eluted in six steps with 500 μl of buffer A containing 250 mM imidazole. After purification, eluted fractions were pooled and dialyzed twice for 1 h against 2 l of 100 mM NaCl, 0.2% Triton X-100, 10 mM Hepes KOH, pH 7 with slide A Lyzer (Pierce). Purified protein samples were analyzed by 15% SDS-PAGE and concentrations were determined using the bicinchoninic acid method (Pierce chemical).

2.3. In vivo expression, purification and refolding of the recombinant AtVDAC-3 protein

After induction, transformed BL21 (λD3) cells (0.5 g wet weight) were harvested by centrifugation and resuspended in 20 ml lysis buffer (50 mM NaH_2PO_4, 2 mM $MgSO_4$, 5% sucrose, 10 mM NaCl, 10 μg/ml DNase, pH 7.5). Cells were broken through a French press and centrifuged (10,000 g for 10 min). The resulting pellet, was suspended in 20 ml buffer B (100 mM NaH_2PO_4, 8 M urea, 10 mM Tris NaOH, pH 8). The suspension was incubated for 1 h at room temperature with 1.2 ml of Ni-NTA matrix (Qiagen) equilibrated in the previous buffer and the matrix was packed in a 2.5 ml shell. The column was washed successively with 4 ml buffer C (buffer B except that the pH is 6.3, adjusted using HCl), 2 ml buffer D (buffer B except the pH is 5.9) and 2 ml E (buffer B except the pH is 4.5) to elute the polyhistidine tagged recombinant protein. The last two fractions which contained the protein, as assessed by SDS-PAGE analysis, were pooled and diluted five-fold in buffer B devoid of urea and supplemented with 0.1% LDAO. After extensive dialysis against buffer B devoid of urea, and in order to concentrate it, the protein solution was incubated with 1.2 ml of Ni-NTA matrix equilibrated with buffer A containing 0.1% LDAO instead of Triton X-100. The protein was then purified as described above. Synthetized VDAC-3 proteins were pre-treated with 16% sterol as indicated by Popp et al. [19].

2.4. Reconstitution of the recombinant AtVDAC-3 protein in lipid bilayer and electrophysiological recording

Channel activity was assayed by adding to the bilayer chamber either the pure protein in detergent or proteoliposomes reconstituted with the pure protein according to [20]. Bilayers were formed from a solution of asolectin lipids dissolved in *n*-decane (30 mg ml^{-1}) across a 250 μm diameter hole. The protein in detergent (250 ng/ml, final concentration) or proteoliposomes (166 ng of the *in vivo* produced protein per ml, or 500 ng of the *in vitro* produced protein per ml, final concentration) were added to the *cis* compartment. Fusion was induced by imposing a salt gradient between the two chambers as follows: 300 mM KCl, 10 mM Hepes-KOH, pH 7.4, in the *cis* compartment versus 100 mM KCl, 10 mM Hepes-KOH, pH 7.4, in the *trans* compartment. The bilayer set up was connected to the external circuit through salt bridges (1 M KCl) with Ag/AgCl electrodes. Unitary currents were recorded using an Axon 200B patch clamp amplifier. Recordings were filtered at 1 kHz, digitized at 3 kHz and analyzed with pClamp 8.1 and ORIGIN 8.0 software. The membrane potential refers to the potential of the *cis* side minus the potential of the *trans* side.

3. Results

3.1. AtVDAC-3 production

The *AtVDAC-3* gene was inserted into plasmids which allow synthesis of the gene product under the control of a T7 promoter and are optimized for *in vitro* synthesis. They are designed to produce a protein with a His6 tag on its C-terminus or its N-terminus, respectively. Cell-free expression of the *AtVDAC-3* gene was carried out in the presence of Triton X-100, a detergent previously shown to be compatible with membrane protein cell-free production [21]. The C-terminus construct yielded a sizable protein production. Using the exchange system, 300 μg of pure protein per ml of lysate could be recovered (Fig. 1A, B, C). By contrast, the N-tagged protein was very poorly produced. It was nevertheless purified. A total amount of 1–2 μg of pure protein per ml of lysate was estimated from immunoblot experiments. This amount was

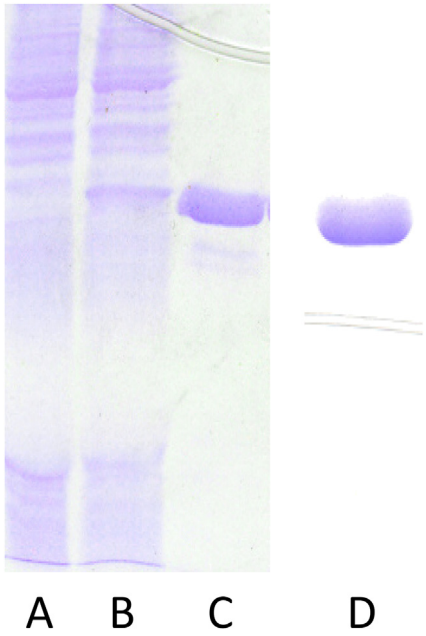

Fig. 1. *In vitro* (A,B,C) and *in vivo* (D) synthesis and purification of AtVDAC-3. A 10% SDS-PAGE gel after Coomassie blue staining shows cell lysate before (A) and after (B) synthesis of the protein. Purified AtVDAC-3 protein synthesized in the a-cellular system after purification on Ni-NTA column (C). AtVDAC-3 produced as inclusion bodies, denatured, purified on Ni-NTA column, and renatured (D).

however sufficient for a set of electrophysiological experiments. Since, we concluded that the tag has no influence on the protein activity.

The protein was also produced *in vivo*, by transforming BL21 cells with the above plasmids. Only the C-terminus construct yielded a detectable production. Cell fractionation showed that the protein was entirely produced as inclusion bodies. The C-terminus tagged protein was denatured in 8 M urea, purified on affinity column, and renatured by urea dialysis in the presence of detergent (Fig. 1D). This procedure yielded 1 mg of pure protein for 20 ml of cell suspension.

3.2. Electrophysiological characteristics of AtVDAC-3

The electrophysiological activity of AtVDAC-3 was assayed using the planar bilayer technique. No meaningful difference in electrophysiological activity was observed between the C-tagged and N-tagged protein produced *in vitro*. Also no difference could be found between the protein directly produced *in vitro* in the presence of detergent and the protein re-natured from inclusion bodies. Below we present electrophysiological data obtained with the C-tagged protein obtained indifferently by cell-free synthesis or cellular synthesis.

AtVDAC-3 inserted in the lipid bilayer as an open channel and could be closed by positive as well as negative voltage. As shown in Fig. 2 when the voltage was stepped from 0 to ± 120 mV, the channels which were open at low voltage, closed following a slow kinetics. Most steps had the same conductance (440 pS in 100/300 mM KCl asymmetrical media) but substates with conductances ranging from 200 to 650 pS were occasionally observed. In symmetrical 300 mM KCl media, the main conductance was 500 pS. Closures were usually reversible: upon return to 0 mV, the channels re-opened, as observed from a following pulse. However, from time to time, some channels did not re-open, getting trapped in a long-lived closed state.

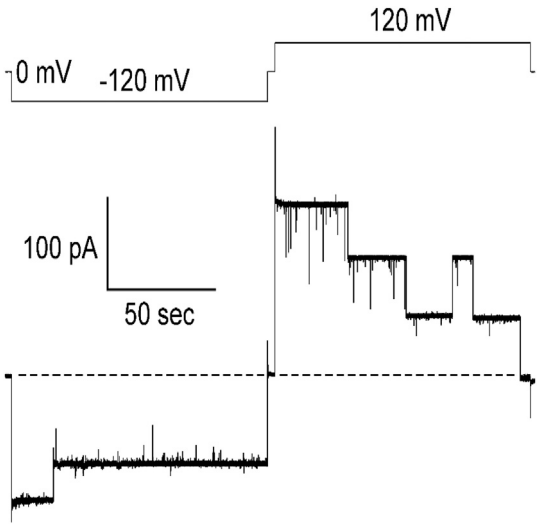

Fig. 2. Dissymmetrical closure of AtVDAC-3 channels in response to voltage. After insertion of AtVDAC-3 proteins in the lipid bilayer, the potential was stepped to −120 mV or +120 mV as indicated. Ionic conditions are: KCl 300 mM *cis* versus 100 mM *trans* (details in Materials and Methods section). The protein was produced by cell-free synthesis.

The response of AtVDAC-3 to voltage was clearly dissymmetrical. Generally, channels closed faster at positive potentials than at negative potentials (Fig. 2). The opposite behaviour (faster closure at negative voltage) was rarely observed, suggesting that most of the channels were inserted with the same orientation in the bilayer. This dissymmetrical sensitivity is also illustrated in Fig. 3A. The voltage was stepped to different values and the ratio of the conductance of the bilayer at the onset of the pulse to its value after 30 s was plotted as a function of the applied potential. Typical traces are shown in Fig. 3B, the dissymmetrical response is especially visible at high potentials. All the channels were almost closed at positive potentials above 160 mV while applying −180 mV does not close all the inserted channels. Below 50–60 mV, positive or negative, no closure could be observed.

As shown in Figs. 2 and 3, AtVDAC-3 closure followed a typical slow kinetics (in the range of several seconds). However, in some cases the channels entered a fast gating mode superimposed on the slow kinetics (Fig. 4A). This rapid flickering was generally obtained when high voltages were applied to the membrane. VDAC channels are usually described as channels which do not close completely. As shown in Fig. 2, AtVDAC-3 could be completely closed by voltage. Fig 4B describes a more complex picture. The channels were initially subjected to a voltage step to +120 mV, before returning to 0 mV. Since the recordings were performed in asymmetrical media (300 mM/100 mM KCl), cationic and anionic states could be easily distinguished. After return to 0 mV, the channel oscillated between a cationic state and a fully closed state before reaching an anionic open state. The reversal potentials of these cationic and anionic open states were independently determined to be respectively 6.2 ± 0.3 mV (n = 4) and +9.1 ± 0.4 mV (n = 4). Upon direct insertion in the bilayer, and in the absence of applied potential, we observed that AtVDAC-3 channels were most of the time in the anionic state. Insertion in the cationic state was more rarely observed.

4. Discussion

The electrophysiological characteristics of AtVDAC-3 can be summarized as follows: 1) the channel that has a large conductance

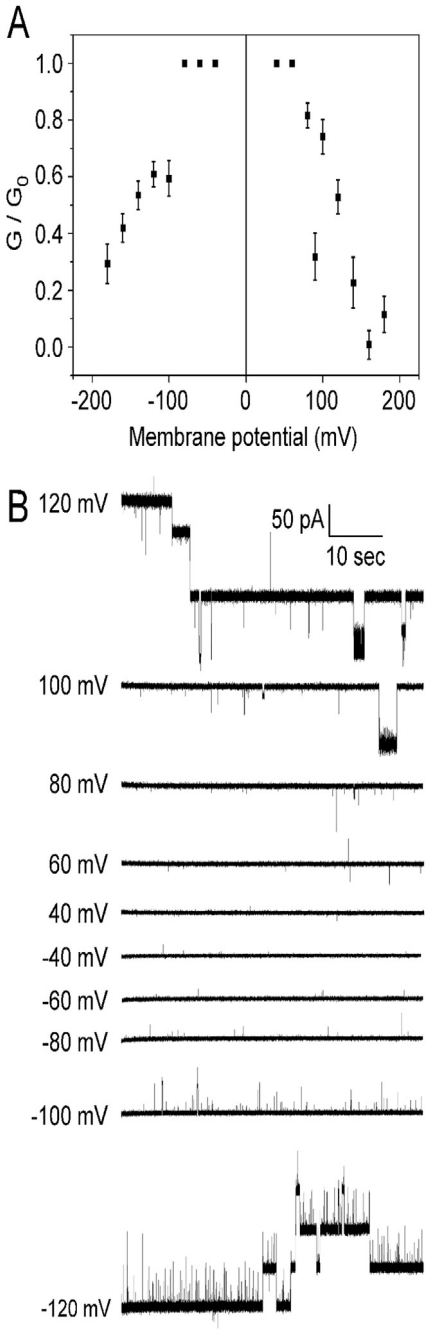

Fig. 3. AtVDAC-3 presents a dissymmetrical voltage dependence. (A) Bell-shaped curve for G/G_0 measured as a function of the membrane potential. Between each pulse the membrane potential was maintained at 0 mV; G/G_0 was measured 30 s after applying pulse. Each point is the average of six experiments. (B) Representative recording showing AtVDAC-3 closure at high potentials and weak voltage dependency for voltages below 100 mV. Same ionic conditions as in Fig. 2. The protein was re-natured from inclusion bodies.

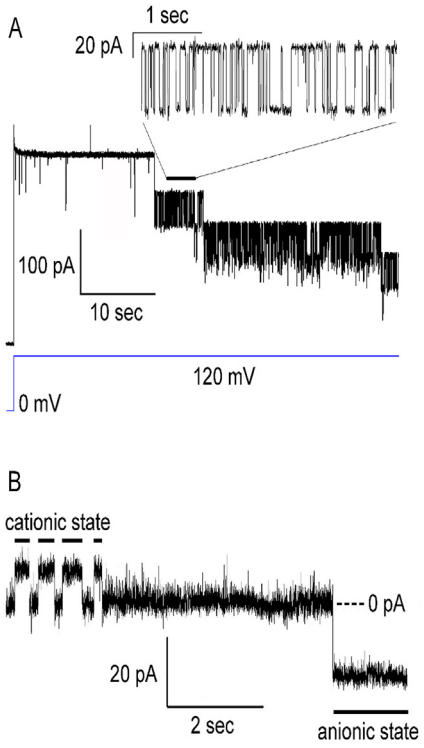

Fig. 4. Gating characteristics of AtVDAC-3 channels. (A) Fast gating kinetics of AtVDAC-3. When high potentials are applied, AtVDAC-3 can exhibits flickerings superimposed on normal slow kinetic. Same ionic conditions as described in Fig. 2. The protein was re-natured from inclusion bodies. (B) Cationic and anionic open states of AtVDAC-3. Recording at 0 mV, after a pulse from 120 mV to 0 mV. under asymmetric conditions (300 m M KCl *cis* versus 100 mM KCl *trans*), reveals three different states, two open states with cationic or anionic selectivity, and a fully closed state. The protein was produced by cell-free synthesis.

is open at or around 0 mV and closes at positive or negative voltages 2) voltage dependence is asymmetric 3) the channel exhibit both fast and slow kinetics, and 4) the channel exhibit several conductance states. This type of behaviour is characteristic of β−barelled channels such as bacterial or mitochondrial porins [22–25].

A symmetrical dependence on voltage of VDAC has generally been emphasized [9,26]. We show here that AtVDAC-3 is characterized by a dissymmetry in the voltage response which is less common but has also been clearly documented for animal VDACs [26–28] and for TAVDAC-2 and TAVDAC-3 from wheat [29]. One specificity of this channel is to enter a fast gating mode superimposed on the slow kinetics. A few studies report this feature for mitochondrial VDACs from rat heart, liver and brain [27,28,30]. This fast kinetic mode was also observed in response to the pro-apoptotic oligonucleotide, G3139 [31]. Interestingly, if this fast gating mode occurs briefly for AtVDAC-3, a wheat VDAC (TAVDAC2) seems to have only this gating mode [29]. It is generally assumed that the VDAC pore does not close completely. However, a fully closed state has been reported for animal VDAC by Zoratti and coworkers [28]. We report here that AtVDAC-3 can also enter such a fully closed state which is clearly a requirement if the channel is located in the plasma membrane. However, one characteristic of AtVDAC-3 is relatively unusual: it is closed at potentials higher than 50–60 mV, while other VDAC channels are usually reported to close at potentials as low as 20 mV [4,24]. This peculiar property was not reported in plant (reviewed in Ref. [17])

Channels such as mitochondrial porins have to be produced, purified and reconstituted for an electrophysiological study. In the case of β-barrelled membrane proteins, it could be shown that, when expressed in *E. coli*, these proteins accumulate as inclusion bodies in the cytoplasm. After denaturation of these inclusion bodies, the protein can be purified and re-natured for functional or structural studies [32]. Cell-free synthesis in the presence of detergent or preformed liposomes is another interesting technique for the production of membrane proteins, in particular ion channels. It can be used for α-helices ion channels [21,33], but it has also been shown to be effective for β-barrelled structures [34]. In our case, both techniques led to the production of an active protein.

Clearly, the yield is higher with the first technique for this type of protein. However, given the availability of commercial kits, cell-free synthesis is a simpler and faster procedure. Moreover, it is now possible to improve the yield of synthesis by using synthetic DNA specifically designed for improved synthesis of protein [35]. Therefore it can be a useful approach for the rapid functional characterization of a family of proteins such as plant VDACs.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was funded by the Centre National de la Recherche Scientifique and by Agence Nationale de la Recherche Grant BLAN06-2-135035.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.034.

References

[1] S. Hiller, R.G. Garces, T.J. Malia, V.Y. Orekhov, M. Colombini, G. Wagner, Solution structure of the integral human membrane protein VDAC-1 in detergent micelles, Science 321 (2008) 1206–1210.
[2] M. Bayrhuber, T. Meins, M. Habeck, S. Becker, K. Giller, S. Villinger, C. Vonrhein, C. Griesinger, M. Zweckstetter, K. Zeth, Structure of the human voltage-dependent anion channel, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 15370–15375.
[3] R. Ujwal, D. Cascio, J.P. Colletier, S. Faham, J. Zhang, L. Toro, P. Ping, J. Abramson, The crystal structure of mouse VDAC1 at 2.3 A resolution reveals mechanistic insights into metabolite gating, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17742–17747.
[4] R. Benz, Permeation of hydrophilic solutes through mitochondrial outer membranes: review on mitochondrial porins, Biochim. Biophys. Acta 1197 (1994) 167–196.
[5] M. Colombini, E. Blachly-Dyson, M. Forte, VDAC, a channel in the outer mitochondrial membrane, Ion. Channels 4 (1996) 169–202.
[6] R. Aiello, A. Messina, B. Schiffler, R. Benz, G. Tasco, R. Casadio, V. De Pinto, Functional characterization of a second porin isoform in *Drosophila melanogaster*. DmPorin2 forms voltage-independent cation-selective pores, J. Biol. Chem. 279 (2004) 25364–25373.
[7] G. Bathori, I. Parolini, I. Szabo, F. Tombola, A. Messina, M. Oliva, M. Sargiacomo, V. De Pinto, M. Zoratti, Extramitochondrial porin: facts and hypotheses, J. Bioenerg. Biomembr. 32 (2000) 79–89.
[8] F.P. Thinnes, H. Gotz, H. Kayser, R. Benz, W.E. Schmidt, H.D. Kratzin, N. Hilschmann, Identification of human porins. I. Purification of a porin from human B-lymphocytes (Porin 31HL) and the topochemical proof of its expression on the plasmalemma of the progenitor cell, Biol. Chem. Hoppe Seyler 370 (1989) 1253–1264.
[9] G. Bathori, I. Parolini, F. Tombola, I. Szabo, A. Messina, M. Oliva, V. De Pinto, M. Lisanti, M. Sargiacomo, M. Zoratti, Porin is present in the plasma membrane where it is concentrated in caveolae and caveolae-related domains, J. Biol. Chem. 274 (1999) 29607–29612.
[10] R. Buettner, G. Papoutsoglou, E. Scemes, D.C. Spray, R. Dermietzel, Evidence for secretory pathway localization of a voltage-dependent anion channel isoform, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3201–3206.
[11] C. Tateda, K. Watanabe, T. Kusano, Y. Takahashi, Molecular and genetic characterization of the gene family encoding the voltage-dependent anion channel in Arabidopsis, J. Exp. Bot. 62 (2011) 4773–4785.
[12] N. Robert, I. d'Erfurth, A. Marmagne, M. Erhardt, M. Allot, K. Boivin, L. Gissot, D. Monachello, M. Michaud, A.M. Duchene, H. Barbier-Brygoo, L. Marechal-Drouard, G. Ephritikhine, S. Filleur, Voltage-dependent-anion-channels (VDACs) in Arabidopsis have a dual localization in the cell but show a distinct role in mitochondria, Plant Mol. Biol. 78 (2012) 431–446.
[13] J. Morel, S. Claverol, S. Mongrand, F. Furt, J. Fromentin, J.J. Bessoule, J.P. Blein, F. Simon-Plas, Proteomics of plant detergent-resistant membranes, Mol. Cell. Prot. MCP 5 (2006) 1396–1411.
[14] G.H. Borner, D.J. Sherrier, T. Weimar, L.V. Michaelson, N.D. Hawkins, A. Macaskill, J.A. Napier, M.H. Beale, K.S. Lilley, P. Dupree, Analysis of detergent-resistant membranes in Arabidopsis. Evidence for plasma membrane lipid rafts, Plant Physiol. 137 (2005) 104–116.
[15] A.M. Jones, V. Thomas, M.H. Bennett, J. Mansfield, M. Grant, Modifications to the Arabidopsis defense proteome occur prior to significant transcriptional change in response to inoculation with Pseudomonas syringae, Plant Physiol. 142 (2006) 1603–1620.
[16] X.Y. Yang, Z.W. Chen, T. Xu, Z. Qu, X.D. Pan, X.H. Qin, D.T. Ren, G.Q. Liu, Arabidopsis kinesin KP1 specifically interacts with VDAC3, a mitochondrial protein, and regulates respiration during seed germination at low temperature, Plant Cell. 23 (2011) 1093–1106.
[17] F. Homble, E.M. Krammer, M. Prevost, Plant VDAC: facts and speculations, Biochim. Biophys. Acta 1818 (2012) 1486–1501.
[18] J.M. Betton, Cloning vectors for expression-PCR products, BioTechniques 37 (2004) 346–347.
[19] B. Popp, D.A. Court, R. Benz, W. Neupert, R. Lill, The role of the N and C termini of recombinant Neurospora mitochondrial porin in channel formation and voltage-dependent gating, J. Biol. Chem. 271 (1996) 13593–13599.
[20] B. Ajouz, C. Berrier, M. Besnard, B. Martinac, A. Ghazi, Contributions of the different extramembranous domains of the mechanosensitive ion channel MscL to its response to membrane tension, J. Biol. Chem. 275 (2000) 1015–1022.
[21] C. Berrier, K.H. Park, S. Abes, A. Bibonne, J.M. Betton, A. Ghazi, Cell-free synthesis of a functional ion channel in the absence of a membrane and in the presence of detergent, Biochemistry 43 (2004) 12585–12591.
[22] V. De Pinto, A. Messina, R. Accardi, R. Aiello, F. Guarino, M.F. Tomasello, M. Tommasino, G. Tasco, R. Casadio, R. Benz, New functions of an old protein: the eukaryotic porin or voltage dependent anion selective channel (VDAC), Ital. J. Biochem. 52 (2003) 17–24.
[23] V. Shoshan-Barmatz, D. Gincel, The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death, Cell. Biochem. Biophy. 39 (2003) 279–292.
[24] M. Colombini, VDAC: the channel at the interface between mitochondria and the cytosol, Mol. Cell. Biochem. 256–257 (2004) 107–115.
[25] C. Berrier, M. Besnard, A. Ghazi, Electrophysiological characteristics of the PhoE porin channel from Escherichia coli. Implications for the possible existence of a superfamily of ion channels, J. Membr. Biol. 156 (1997) 105–115.
[26] E.J. Marques, C.M. Carneiro, A.S. Silva, O.V. Krasilnikov, Does VDAC insert into membranes in random orientation? Biochim. Biophys. Acta 1661 (2004) 68–77.
[27] G. Bathori, I. Szabo, I. Schmehl, F. Tombola, A. Messina, V. De Pinto, M. Zoratti, Novel aspects of the electrophysiology of mitochondrial porin, Biochem. Biophys. Res. Commun. 243 (1998) 258–263.
[28] I. Szabo, V. De Pinto, M. Zoratti, The mitochondrial permeability transition pore may comprise VDAC molecules. II. The electrophysiological properties of VDAC are compatible with those of the mitochondrial megachannel, FEBS Lett. 330 (1993) 206–210.
[29] A. Elkeles, A. Breiman, M. Zizi, Functional differences among wheat voltage-dependent anion channel (VDAC) isoforms expressed in yeast – indication for the presence of a novel VDAC-modulating protein? J. Biol. Chem. 272 (1997) 6252–6260.
[30] T. Mirzabekov, C. Ballarin, M. Nicolini, P. Zatta, M.C. Sorgato, Reconstitution of the native mitochondrial outer membrane in planar bilayers. Comparison with the outer membrane in a patch pipette and effect of aluminum compounds, J. Memb. Biol. 133 (1993) 129–143.
[31] J.C. Lai, W.Z. Tan, L. Benimetskaya, P. Miller, M. Colombini, C.A. Stein, A pharmacologic target of G3139 in melanoma cells may be the mitochondrial VDAC, P Natl. Acad. Sci. U. S. A. 103 (2006) 7494–7499.
[32] B. Schmid, M. Kromer, G.E. Schulz, Expression of porin from Rhodopseudomonas blastica in Escherichia coli inclusion bodies and folding into exact native structure, FEBS Lett. 381 (1996) 111–114.
[33] C. Berrier, I. Guilvout, N. Bayan, K.H. Park, A. Mesneau, M. Chami, A.P. Pugsley, A. Ghazi, Coupled cell-free synthesis and lipid vesicle insertion of a functional oligomeric channel MscL MscL does not need the insertase YidC for insertion in vitro, Biochim. Biophys. Acta 1808 (2011) 41–46.
[34] C. Klammt, D. Schwarz, K. Fendler, W. Haase, V. Dotsch, F. Bernhard, Evaluation of detergents for the soluble expression of alpha-helical and beta-barrel-type integral membrane proteins by a preparative scale individual cell-free expression system, FEBS J. 272 (2005) 6024–6038.
[35] A. Villalobos, M. Welch, J. Minshull, In silico design of functional DNA constructs, Methods Mol. Biol. 852 (2012) 197–213.