Biochimica et Biophysica Acta 1848 (2015) 1410–1416

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Phosphorylation, nitrosation and plasminogen K3 modulation make VDAC-1 lucid as part of the extrinsic apoptotic pathway—Resulting thesis: Native VDAC-1 indispensible for finalisation of its 3D structure

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ARTICLE INFO

Article history:
Received 20 December 2014
Received in revised form 17 February 2015
Accepted 25 February 2015
Available online 11 March 2015

Keywords:
Porin
VRAC
Cystic fibrosis
Alzheimers disease
Autism
Malaria

ABSTRACT

Native and recombinant VDAC preparations differ in their acetylation, phosphorylation and nitrosation state; additionally, proteineous modulators are missing in the latter. They thus vary in channel characteristics, as can be taken from comparative black lipid bilayer experiments. Furthermore, the multi-compartment expression makes expect even differing native VDAC-1 molecules.

Recent structural work on mammalian VDAC-1 has only used recombinant material, refolded from *Escherichia coli* inclusion bodies. While this approach established the basic three-dimensional structure of VDAC-1, a ß-barrel set up by nineteen ß-pleated sheets, dissent is on positioning and movements of its free N-terminal helical peptide stretch preceding ß-pleated sheet-1. A synopsis of data concerning posttranslational modifications, cytotopology and physiology of native VDAC-1, from my point of view, suggests that the finalisation of its three-dimensional structure will need native channel preparations to be studied.

Concerning relevance, recent evidence on the regulation of cell membrane-integrated VDAC-1 by posttranslational modifications and proteineous modulators, taken together with experimental demonstrations that VDAC-1 is involved in cell volume regulation, it thus may be part of the extrinsic apoptotic pathway can hopefully help to understand some relevant medical syndromes, e.g. cystic fibrosis, Alzheimer's disease, autism and malaria.

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1. Introduction

The intention of this minireview is to highlight two recent lines of VDAC research, that from my point of view are in a mutual relation. On the one hand, I recall and another time discuss studies that have demonstrated the inclusion of extra-mitochondrial VDAC-1 into the plasmalemma. This finding, for the moment, was hotly refused mostly because VDAC then had never been seen in a fully closed state. Recent data on VDAC-1 regulation by posttranslational modifications and proteineous modulation, however, not only point to plasmalemmal VDAC-1 but they help understand its integration into the cell membrane. The recent progress may furthermore help to accept the relevant role of plasmalemmal VDAC-1 in cell volume regulation and the

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http://dx.doi.org/10.1016/j.bbamem.2015.02.031
0005-2736/

extrinsic apoptotic pathway, and thus put it on the schedule whenever type-1 VDAC is studied.

On the other hand, according to a recent comparison of recombinant human type-1 VDAC with native mitochondria-derived bovine VDAC by artificial planar lipid bilayer experiments, the channels are close but differing. The differences seen, most likely, rest on differences in the post-translational modification of the channel preparations used. A fully closed VDAC state could not be detected in either case. Together, a summary of the data available, from my point of view, suggests the following: for the finalisation of the three-dimensional VDAC-1 structure, native channel preparations will be indispensible.

Tewari and colleges [1] recently reported on effects of Curcumin on mammalian type-1 VDAC in artificial lipid bilayer experiments. Accordingly, the agonist connects building blocks of the free N-terminal part of VDAC-1 with those of the channel wall. This results in a switch from the canonically open = anion-selective to closed = cation-selective channel states of type-1 VDAC, and in its fixation to the latter. The study refers another time to an interaction of VDAC-1 and the agonist. Taken together, the polyphenol blocks the channel and thus appears to be critical for either apoptotic pathway. Curcumin is established as a pharmakon in Chinese medicine [2–4].

Aside its putative pharmacological relevance, the paper touches another exciting aspect of recent VDAC research. In its Fig. 2, left panel, the report presents a direct graphical comparison of recombinant human VDAC-1 obtained by *Escherichia coli* overexpression and of native bovine VDAC prepared from mitochondria, as elaborated by black membrane measurements. This plot convincingly evidences both forms as rather close but differing in their channel characteristics. The data are in line with manifold endeavours to electro-physiologically characterise VDAC from different levels of eukaryotic evolution [5–7] and to elucidate the three-dimensional structure and channel characteristics of vertebrate VDAC by NMR spectroscopy, X-Ray crystallography and simulation approaches [8–16]. The latter still await finalisation.

To promote the clarity of the review presented: artificial lipid bilayer studies of native [5–7] or recombinant [8–16] VDAC preparations, respectively, are used to operationally define several states of channel regulation depending on the transmembrane voltage (TV) applied. Canonically, one open = anion-selective state at 10 mV TV and a variety of partially closed = cation-selective states appearing above 30 mV TV are emphasised. Since 1989, increasing topochemical evidence of the extra-mitochondrial expression of VDAC-1, in particular its inclusion into the plasmalemma of vertebrate cells together with the demonstration of its involvement in cell volume regulation and thus apoptosis [17–23], argue in favour of a fully closed VDAC-1 state, where ions cannot pass the channel. For details, see below. Correspondingly, three differing VDAC states are discussed in recent structural work on type-1 and also type-2 vertebrate VDAC using varying biophysical or in silico approaches [8–16]; particularly 11].

These are the following: (1) open = anion-selective = N-terminal stretch of AAs 1–25 inside the barrel; (2) partially closed = cation-selective = N-terminal stretch of AAs 1–25 inside the barrel but in differing position; and (3) fully closed = collapsed = impermeable for ions = N-terminal stretch outside the ß-barrel [11], the latter accessible for antibodies. This is a postulate derived from the proof of cell membrane-standing VDAC-1 [17–23] (Fig. 1).

2. Phosphorylation, nitrosation and proteineous modulation of VDAC-1 help understand conductance switches and its involvement in the extrinsic apoptotic pathway

A series of recent papers on the phosphorylation of plasmalemma-standing human type-1 VDAC opens up a better understanding of the channel's switching between different conductance states and furthermore supports its involvement in the process of extrinsic apoptosis [24–27].

First, the exciting study recently presented by Fernandez-Echevarria et al. [24] adds some highly relevant new data concerning human type-1 VDAC. It shows that the channel is abundantly found in neuronal cell membrane lipid rafts, where the channel is associated in a complex with signalling proteins that trigger neuro-protective responses. There is indication that loss of lipid raft integrity may ultimately promote VDAC-1 activation, and some data had already demonstrated that VDAC-1 at the neuronal membrane might be involved in the mechanisms of amyloid beta (Aß)-induced neurotoxicity, through unknown mechanisms. The

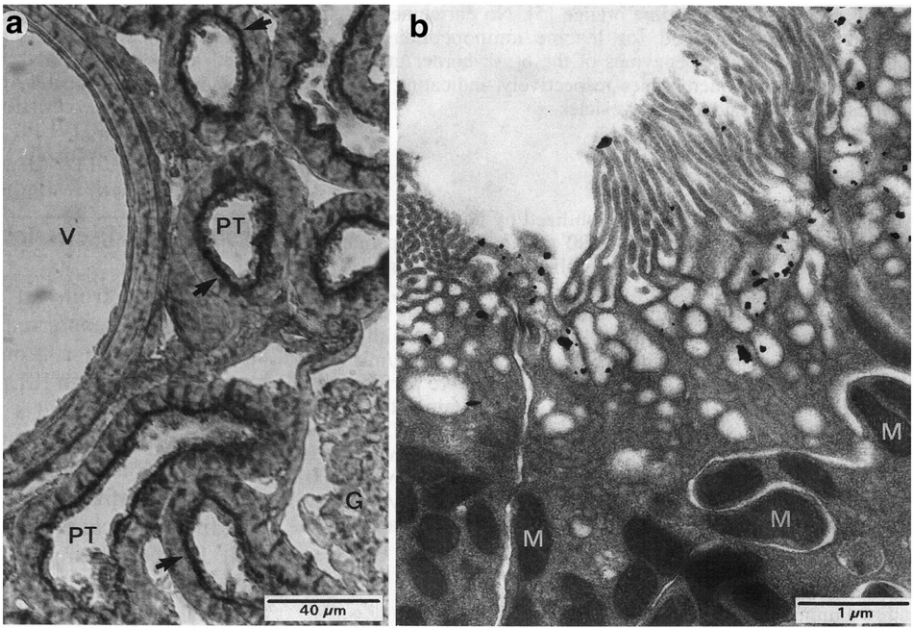

Fig. 1. Localisation of type-1 porin in rat renal cortex by indirect immunocytochemistry performed with a monoclonal anti-human type-1 porin antibody [19]. (a) Light micrograph of a perfusion-fixed cryostat section of rat renal cortex. By indirect immune-peroxidase labelling, the endocytotic vesicles underneath the brush-border membranes (arrows) are heavily stained. (G Glomerulus, PT proximal tubule, V renal vessel). (b) Electron micrograph of a thin section of rat renal cortex labelled by "pre-embedding immune-gold staining." Silver-enhanced gold particles are present mainly in the endocytotic vesicles underneath the microvillar plasma membranes of proximal tubule cells. (M, Mitochondrion). Noteworthy, mitochondria are not labelled due to their inaccessibility to antibodies in the pre-embedding immune-gold staining technique. The figure is taken from Reference [23].

peptide generated from the amyloid precursor protein (APP) is released to the extracellular space where it forms senile plaques. Amyloid Aß appears body wide in the circulation system, too. Excitingly, the study demonstrates that VDAC-1 associates with APP and Aß in lipid rafts of neurons. Furthermore, Aß exposure is shown to enhance the dephosphorylation of VDAC-1, in other words, to facilitate channel opening, and this in correlation with cell death. Both effects were reverted in the presence of tyrosine phosphatase inhibitors. Noteworthy, VDAC-1 dephosphorylation is corroborated in lipid rafts of AD brains. Together, these results demonstrate that Aß is involved in changes of the phosphorylation state of VDAC-1 in neuronal lipid rafts, a putative mechanism in the pathogenesis of Alzheimer's Disease.

Second, very recently, Tewari SG et al. [25] presented an analysis of posttranslationally modified gating kinetics of VDAC purified from rat mitochondria. Accordingly, single channel currents of wild-type, nitrosated and phosphorylated VDAC were analysed using a generalised continuous-time Markov chain Monte Carlo method. The method described three distinct conducting states, open, half-open and closed, of VDAC activity. Lipid bilayer experiments were also performed to record single VDAC activity under un-phosphorylated and phosphorylated conditions and were analysed using the developed stochastic search method. Significant alterations in VDAC gating kinetics and conductance were observed as a result of posttranslational modifications. The effect of PTMs on VDAC kinetics was captured in the parameters associated with the identified Markov model. Stationary distributions of the Markov model suggested that nitrosation of VDAC not only decreased its conductance but also significantly locked VDAC in a closed state. Compared with that, stationary distributions of the model associated with un-phosphorylated and phosphorylated VDAC indicated a reversal in channel conformation from relatively closed state to an open state. Model analyses of the nitrosated data suggested that faster reaction of nitric oxide with Cys-127 thiol group might be responsible for the biphasic effect of nitric oxide on basal VDAC conductance.

Third, another recent study by Li et al. [26] impressively widened the knowledge concerning the reaction partners of human plasminogen kringle 5 (K5) and VDAC-1. Here, the K5 molecule induced endothelial cell (EC) apoptosis and thus showed a potent anti-angiogenesis effect. While VDAC-1 had already been identified as a receptor of K5, the channel has now been shown to increase the protein level of VDAC-1, too, thus initiating the mitochondrial apoptosis pathway of ECs. The study has also shown that K5 inhibits the ubiquitin-dependent degradation of VDAC-1 by promoting phosphorylation of VDAC-1. Hence, phosphorylated VDAC-1 was attenuated by the AKT agonist GSK3ß inhibitor or corresponding siRNA, suggesting that K5 increases VDAC-1 phosphorylation via the AKT-GSK3 pathway. In addition, K5 promoted cell surface translocation of VDAC-1, resulting in binding between K5 and VDAC-1 on the plasma membrane. Hexokinase I blocked the impact of K5 on the AKT-GSK3 pathway by competitively inhibiting the interaction of K5 and cell surface VDAC-1. Moreover, K5-induced EC apoptosis was suppressed by VDAC-1 antibody. Together, these data show that K5-induced apoptosis of ECs is mediated by the positive feedback loop of VDAC1-AKT-GSK3ß-VDAC-1, thus another time putting the channel in touch with extrinsic apoptosis [20]. For a recent review on VDAC phosphorylation concerning cell fate, see [27].

3. Differences concerning native and recombinant VDAC make native VDAC-1 preparations indispensible for the finalisation of its three-dimensional structure.

Aside their relevance concerning the pathogenesis of Alzheimer's disease, these studies [24–27] point to another important aspect of current VDAC research that needs discussion. While demonstrating that native cell membrane-integrated VDAC-1 of human neuronal cells and tissues are highly phosphorylated and may furthermore be nitrosated, recent structural work on the channel uses recombinant VDAC, refolded from *E. coli* inclusion bodies containing mammalian VDAC-1 or fish VDAC-2, respectively [8–16]. Here, a physiologic translational processing of the molecules cannot been expected. No doubt, the approach has meanwhile established the basic three-dimensional structure of vertebrate VDAC. Accordingly, the barrels are built up by nineteen loop-connected ß-pleated sheets, sheet-1, in addition carrying a free and mostly helical N-terminal stretch of about twenty amino acids that moves inside the barrel, and this in dependence with the channel's status of regulation. An adequate demonstration of the fully closed state of VDAC-1 has not yet been achieved going this way. However, immune-topochemical data concerning vertebral plasmalemma-integrated VDAC-1 here argue in favour of a pore-outside positioning of its free N-terminal peptide stretch. For a discussion of alternative VDAC models, e.g., postulating that VDAC is open when its free N-terminal part is found outside the pore see [28,29].

While there is broad consent on the arrangement of the nineteen ß-sheets of VDAC-1, the scale of movement and final positioning of the N-terminal peptide stretch and its correlation with channel function still await definition. However, the papers under discussion here point to multiple phosphorylation and nitrosation processes as critical steps in the regulation of native VDAC-1 incorporated into the cell membrane and thus insinuates to recall biochemical data on this issue (see below). Those data, from my point of view, suggest that these days biochemical data exceed the results of structural and simulation work on recombinant VDAC refolded from *E. coli* inclusion bodies. Simulation studies concerning VDAC-1 phosphorylation and its nitrosation may fail clarity, too.

However, upcoming laser-based approaches will hopefully work with small amounts of native VDAC-1 in solutions. Endeavours are on their way to establish X-ray free electron lasers (XFEL) centres trying to promote techniques that allow determine the structure of biologically relevant molecules and their interaction in complexes, too, and this in crystals and in solution. They furthermore search for methods to capture intermediate states that might be lost in crystals. These efforts promise benefits also for channel proteins. For example, while 2D crystals of membrane proteins usually show lower resolution, they need less protein and furthermore allow that distinct conformations of the protein, e.g., open and closed state, of an ion channel may be detected. Small size nano-crystals of native VDAC-1 may help to leave the dead end VDAC-1 structure research is currently locked in refs. [30,31]; http://www.embl-hamburg.de/XFEL/annex.html].

Taken together, to care for native vertebrate VDAC preparations seems to be the order of the day. Suitable preparation methods are at hand [17,32]. First, starting from highly enriched human B-lymphocyte cell membranes solubilised in NP40, my laboratory prepared milligram amounts of channel-active Porin 31HL = VDAC-1 [17] several times; the material after precipitation was used for the elaboration of the complete primary structure of the molecule. To note, other VDAC isotypes were not seen in our sequence work. Endeavours to get crystals from those preparations failed in the first step of detergent change (Dr. K. Zeth, then working in the laboratory of Wolfram Welte). Second, starting from enriched mammalian mitochondria, De Pinto et al. [32] presented a single-step VDAC purification scheme resulting in channel-active preparations. This approach was later used to elaborate the complete primary structure of human mitochondria-derived VDAC-1, the most abundant in OMM, thus proving its molecular identity with plasmalemmal VDAC-1 [33].

4. Varying phosphorylation and nitrosation states of native VDAC-1 appear to result in multiple populations of channel molecules—putatively correlated to differing cell compartments

Theses: the multi-topologic = multi-compartment expression of mammalian VDAC-1—in whole cell analyses—makes expect the appearance of several phosphorylation and nitrosation states, resulting in differing VDAC-1 molecule populations inside cells in parallel.

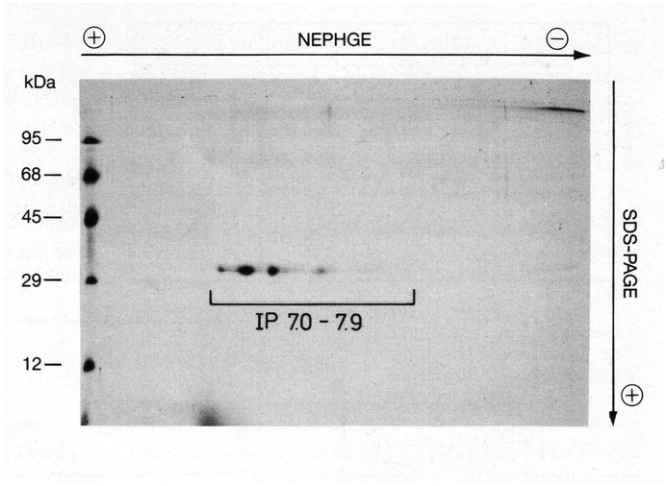

Fig. 2. Two-dimensional electrophoretic demonstration of sequence grade Porin 31HL = VDAC-1 obtained by three steps of classic ion-exchange chromatography from highly enriched B-lymphocyte membranes solubilised in NP40 at pH 5.0. The spots labelled by Coomassie brilliant blue refer to several isoelectric points of native plasmalemmal type-1 VDAC, putatively representing several VDAC-1 populations that differ in their phosphorylation state. Data are taken from ref. [18].

Experimental evidence comes from electro focussing and two-dimensional electrophoreses (see Figs. 4 and 6 of [24]; Fig. 2 of this review). Unfortunately, there is not much information on transmembrane potentials aside from plasmalemma and inner mitochondrial membrane. However, its phosphorylation and nitrosation can be assumed as basic in VDAC-1 regulation, an aspect worth to keep in mind.

In 1989, first data on an extra-mitochondrial expression entered the VDAC field by demonstrating that Porin 31HL = VDAC-1 is expressed in the plasmalemma of mammalian cells [17,33–38]—data another time supported by the studies under discussion here. This made VDAC-1 regulation a key problem, and also opened hunting for its expression and function(s) in different cell compartment. In trials to explain cell membrane integration of the VDAC-1 channel, interactions with protein-like and low molecular weight VDAC-1 modulators have been postulated from the beginning on ref. [34].

Concerning mouse, it has been furthermore proposed that, per usage of two alternative first exons of the murine VDAC-1 gene, two channel forms differing within their N- termini should be expressed. One of them, plasmalemmal VDAC-1, carries a hydrophobic leader peptide and is primarily targeted through the Golgi apparatus to the cell membrane. In contrast, the second form lacking the N-terminal leader, mitochondrial VDAC-1, is trans-located more efficiently into the outer mitochondrial membrane [39]. To notice, sequence analyses by bioinformatics did not identified a pl-VDAC-1 splice site in the human gene [40].

Characterisation of native VDAC solubilised from mitochondria is usually gained from black membranes experiments that always map just open or partially closed channel states, respectively, depending on the transmembrane voltage applied; concerning human VDAC-1 that nicely represents eukaryotic porin, see [6]. While recent work on the three-dimensional structure of recombinant vertebrate VDAC also reports almost exclusively on those two channel states [8–16], data from different laboratories point to a third regulation state of native VDAC-1 called: fully closed = collapsed = impermeable for ions = N-terminal stretch at least partially outside the ß-barrel and accessible for antibodies.

First, the plethora of topologic studies from different laboratories worldwide using antisera as well as monoclonal antibody preparations raised against complete native VDAC-1 or selected peptide stretches of the molecule, respectively, have demonstrated the incorporation of the channel in the outer membranes of mitochondria, in the plasmalemma and also in the endoplasmic reticulum membranes [5,6,17,20,21].

Second, VDAC-1 in the plasmalemma of HeLa cell, human neuronal cells and also mouse epithelial cells has been shown to be involved of the regulatory volume decrease (RVD) after hypotonic stimuli [40–43]. From here, cell membrane-integrated VDAC-1 is part of the cell volume regulatory system and thus apoptosis. However, native VDAC-1 in cell membranes needs to be fully closed in resting cells. To note, in situ cell volume regulation must be seen in the context of cell's turnover inducing permanent changes in cell isotonicity. Channels engaged in RVD can thus be expected as flickering, and in accordance with the studies discussed here [24–27], quick changes in phosphorylation and nitrosation states of plasmalemma-integrated VDAC-1 can be assumed to be part of the biochemical basis in the process. However, while former data just pointed to plasmalemmal VDAC-1 as forming the channel part of volume regulated anion channels (VRAC, ORDIC, VSOR, Icl_{swell}) [17,20,21,38–45], now a biochemical understanding emerges.

Third, monoclonal antibodies reacting with epitopes inside the N-terminally acetylated peptide stretch AAs 1–11 of human VDAC-1 proved the accessibility of this channel part in a channel-active preparation of VDAC-1 after solubilisation at pH 5 in NP40 [6].

In a first set of experiments, three monoclonal antibodies presented by Babel et al. [19] were applied after channel reconstitution. No effect on the channel characteristics or voltage dependence of incorporated human type-1 VDAC was observed. The channels, inserted at zero transmembrane potential, and afterwards figured as what traditionally is called the open = anion-selective state. Here, the N-terminal channel stretch is assumed to be buried inside the channel and thus inaccessible for the antibodies. Notably, further channel incorporation was abolished when antibodies were added at the cis-side of the black membranes, while trans-application was without effect.

The latter observation fits to the results of a second set of experiments: here Porin 31HL was pre-incubated with the monoclonal antibodies and afterwards added to the aqueous phase of the black lipid membrane equipment. Now, the reconstitution rate was heavily reduced, while channels entering the membrane showed normal eukaryotic porin behaviour.

The experiments did not allow for a decision whether channels in contact with the monoclonal antibodies were inserted in fully closed state or did not enter the membrane at all; however, they can be read as indicating that VDAC-1 molecules solubilised in NP40 at pH 5 are in the fully closed = collapsed states, and their N-terminus thus accessible for antibodies. To note, a recent study applying rat liver mitochondria-derived VDAC in black membrane experiments demonstrated that acidification of the setting reversibly increases VDAC's voltage-dependent gating and thus promotes channel closure. Furthermore, both anion selectivity and single channel conductance increased with acidification [46]. However, a fully closed VDAC state could not be documented in the experiments. Nevertheless, the results are in line with the accessibility of the N-terminal free part of VDAC-1 for corresponding antibodies in solution at pH 5.0.

To get an idea of what may happen whenever VDAC-1 opens up while entering black membranes, two lines of evidence exist: On the one hand, it has been shown that native VDAC solubilised in detergents includes cholesterol in a ratio of five molecules of cholesterol/polypeptide chain [47]. In line, native Porin 31HL solubilised from highly enriched cell membrane preparations of human B-lymphocytes in the detergent NP40 at pH 5.0 totally lost channel activity by pre-incubation with cyclodextrin. This indicates that stripping off cholesterol might mediate the channels entering in black membranes (Thinnes, unpublished). To notice, vertebrate mammalian VDAC-1 in positions 20 to 24 of its free N-terminal peptide stretch and other positions, too, carries several GxxxG motifs, good candidates for cholesterol binding [20,21]. However, solubilised native cell membrane derived and fully closed VDAC-1/cholesterol complexes appear to open up as open = anion-selective VDAC-1 phenotypes when entering artificial lipid bilayers.

On the other hand, the NMR solution structure of recombinant human VDAC-1 reconstituted in detergent micelles has been presented. Accordingly, VDAC-1 β-barrel on its hydrophobic outside carries detergent molecules in belt-like fashion [8]. Here, in the presence of cholesterol but absence of phosphorylation, recombinant VDAC-1 forms voltage-gated channels in phospholipid bilayers similar to the native protein. Anyway, while Fernandez-Echevarria et al. [24] have shown that in intact cells the regulation of native VDAC-1 in the plasmalemma depends on the phosphorylation status of the channel, black membrane experiments with native and recombinant VDAC-1 point to a high relevance of cholesterol in native VDAC-1/cholesterol complexes. Additional studies may help to better understand this enigma [45].

5. On the orientation of type-1 VDAC in the plasmalemma and the outer mitochondrial membrane

There is still a debate on this issue, from my point of view, detrimentally focussed on OMM. However, it has been shown by several groups using different approaches that free acetylated N-terminal peptide stretches of native VDAC-1 (Ac-1-24) can be labelled by differing antibody preparations on the surface of vertebrate cells [5,6,17,20,21,23,24,26,35–39].

To note, concerning topochemical work on VDAC-1: The inaugural study on the extra-mitochondrial expression of Porin 31HL = type-1 VDAC presented in 1989 used a series of rabbit antisera raised against sequence grade native Porin 31HL or synthetic peptides of it, respectively; the latter included AAs 1–19 two of them acetylated N-terminally [17,18]. The monoclonal anti-Porin 31HL antibodies, reported in 1991 [19] and used in topographical studies concerning OMM and other cell compartments in several laboratories worldwide [20,21] had been elaborated in a collaboration with Behringwerke Marburg, Germany; they have been meanwhile taken from the market. To define the epitopes seen, we had shown them to react with the N-terminal tryptic peptide of native type-1 VDAC: Ac-AVPPTYGDLGK comprising eleven AAs; in corresponding experiments, the N-terminal acetylation had also been shown to be constitutive for the epitope [22].

By corresponding immune-topographic and biochemical studies on the transmembrane topology of VDAC at the OMM of bovine mitochondrial porin, De Pinto's laboratory demonstrated in 1991 that the N-terminal region of the channel, here too, is not embedded in the lipid bilayer of the OMM but is exposed to the cytosolic side. Furthermore, no difference in the reactivity of the antisera used was observed between intact and broken mitochondria. The group furthermore summarised its 2007 study on the conformation and function of the VDAC-1N-terminus by stating that the free peptide plays a role in the proper function of the protein during apoptotic events. Finally, the data on the orientation of VDAC-1 in the OMM were recently another time confirmed [47–49].

In line, a recent study from Varda Shoshan-Barmatz's laboratory, using site-directed mutagenesis and cysteine residue substitution together with thiol-specific cross-linking, revealed that the N-terminal VDAC-1 region exists in a dynamic equilibrium, located within the pore or exposed outside the β-barrel, respectively. The free VDAC-1 stretch furthermore proved to be relevant for channel dimerisation and the docking of hexokinase, Bcl2 and Bcl_{XL} molecules. Noteworthy, replacing glycine residues in a GxxxG motif in positions 20–24 of the native VDAC-1 molecule referred to the motif as a prerequisite for the translocation process under discussion. [50].

Contrary to this work, in a corresponding study on the transmembrane topology of *Neurospora crassa* VDAC, presented by Mannella's laboratory in 1995 [51,52], the reactivity of the antiserum against a synthetic peptide of its N-terminal end (AAs 1–20) increased with the lysis of the outer membrane of suspended mitochondria. The authors discussing possible explanations for the discrepancy to De Pinto's data referred to differences of the antigens applied for antibody production, and to the positioning of the putative epitopes seen by the antisera used in the two laboratories. As demonstrated by Fig. 3, the antibodies used quite likely recognised epitopes at opposite ends of the N-terminal peptide stretches of human VDAC-1 or fungal VDAC, respectively.

There is a significant difference between vertebrate and plant VDAC, including *Neurospora crassa* and yeast. While vertebrate VDAC1 carries a GxxxG motif in positions 21 to 25, here marking the end of the free N-terminal peptide stretch [8–16], this motif is missing in plants [53]. It is tempting to speculate that plant VDACs have shorter free N-terminal stretches, necessitating OMM lysis in the study discussed [51,52]. There is another interesting aspect of the GxxxG motif in position 20 to 24 of native mammalian VDAC-1: aside its putative relevance as peptide dimerisation/aggregation/membrane perturbation motif it has been shown to figure as an ATP binding site [54,55]. To my knowledge, there are no data on the three-dimensional structure of plant VDAC, but might be rather useful.

6. Conclusion and outlook

Recent studies in focus here, from my point of view, mark great moments of VDAC research. The minireview thus tries to accentuate some aspects of these papers including their medical relevance, points to controversies now vanishing and finally establishes the claim for native VDAC-1 preparations to finalise its three-dimensional structure.

1. The studies of Fernandez-Echevarria et al. [24], Tewari et al. [25] and Li et al. [26] give strong support to cell membrane-expression, more precisely plasmalemma lipid raft integration of mammalian VDAC-1. The data on VDAC-1 posphorylation of cells in culture, this in correlation to channel opening and closing, help understand differing regulation states even in different compartments, and they also argue in favour of an involvement in cell volume regulation and thus apoptosis; recent findings concerning VDAC nitrosation point in the same direction. The study of Li et al. [26], furthermore, puts type-1 VDAC another time in touch with the extrinsic apoptotic pathway. Taken together, the results recently presented appear to be in line with much evidence indicating that plasmalemmal VDAC-1 forms the channel part of a volume regulated anion channel complex (VRAC, ORDIC, VSOR, Icl_{swell}) and thus apoptosis.
   There are increasing data pointing to the medical relevance of cell membrane-standing VDAC-1 by its involvement in the pathogenesis of several syndromes via apoptosis, e.g. cancer, cystic fibrosis, Alzheimer's Disease, autism and malaria [56–60].
2. The ongoing dispute on the accessibility of the N-terminus of VDAC at cell surfaces or outer mitochondrial membranes by antisera or monoclonal antibodies, respectively, from my point of view may profit from an evident difference between animal and plant VDAC. While animal VDAC-1 at the proximal end of its free N-terminal peptide stretch carries a GxxxG motif, this motif is missing in plants [53]. From here it may well pay to elaborate first three-dimensional structure date on plant VDAC, the more as new ideas to understand VDAC cell membrane incorporation may arise.
3. Endeavours to characterise mammalian VDAC-1 by artificial lipid bilayers experiments, obtained either from detergent solubilised plasmalemma, outer mitochondrial membrane or from recombinant channel preparations refolded from *E. coli* inclusion bodies, respectively, just capture the canonical VDAC channel phenotypes open = anion selective and closed = cation selective. The fully closed VDAC-1 state, i.e., closed for anions and cations, cannot be studied by this approach. The same holds true for more recent NMR- or crystallisation-based approaches. However, upcoming laser techniques may work on native VDAC-1 in solutions. Thus, improvements to gain solubilised native VDAC preparation may pay to keep on the schedule.

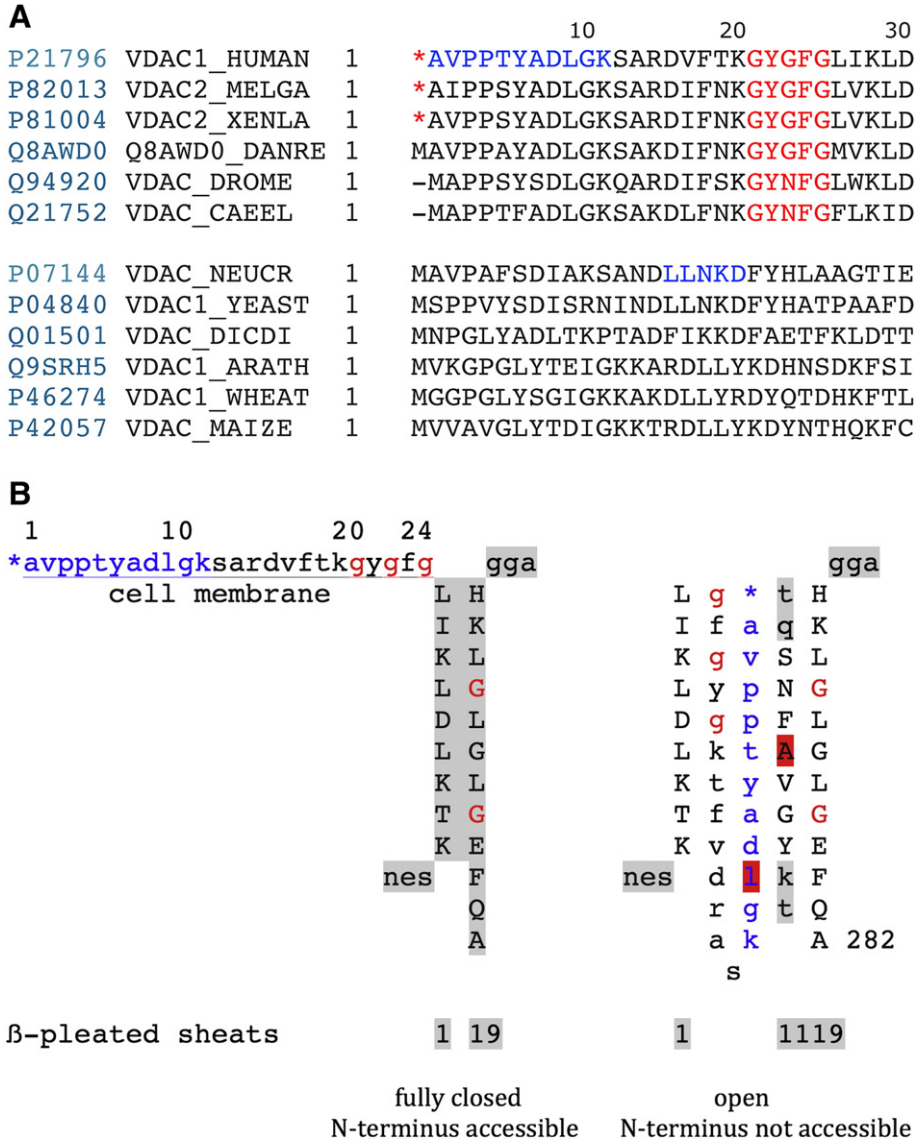

Fig. 3. (A) Alignment of several animal or vegetal VDAC channels according to Swiss Prot. The alignment, on the one hand, points to a significant difference concerning the free N-terminal part VDAC of animals and plants. While animal VDAC channels carry a GxxxG motif that is missing in plants. On the other hand, it may help to dispel contradictions discussed in the VDAC community [47–52]. * Initiator methionine replaced by an acetylation. *AVPPTYADLGK: epitope seen by monoclonal anti-Porin 31HL = VDAC-1 antibodies [19]; *Neurospora crassa* N-terminal peptide used for antibody building MAVPAFSDIAKSANDLLNKD: epitope seen in the study of Stanley et al. 1995 [51]. (B) Highly schematic two-dimensional projections of human type-1 VDAC trying to summarise data from my own lab [17] and the laboratories of Vito De Pinto [47-49], Roland Benz [6], Varda Shoshan-Barmatz [50], Carmen Mannella [51,52], and Jeff Abramson [11]. The fully closed state and the open state of native VDAC-1 are compared.

Conflict of interest

There are no conflicts of interests.

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