Biochimica et Biophysica Acta 1818 (2012) 1457–1465

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

VDAC structure, selectivity, and dynamics ☆

Marco Colombini *

Department of Biology, University of Maryland, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 7 September 2011
Received in revised form 2 December 2011
Accepted 22 December 2011
Available online 3 January 2012

Keywords:
Mitochondrion
Outer membrane
Channel
Voltage-gating
Selectivity
Permeability

ABSTRACT

VDAC channels exist in the mitochondrial outer membrane of all eukaryotic organisms. Of the different isoforms present in one organism, it seems that one of these is the canonical VDAC whose properties and 3D structure are highly conserved. The fundamental role of these channels is to control the flux of metabolites between the cytosol and mitochondrial spaces. Based on many functional studies, the fundamental structure of the pore wall consists of one α helix and 13 β strands tilted at a 46° angle. This results in a pore with an estimated internal diameter of 2.5 nm. This structure has not yet been resolved. The published 3D structure consists of 19 β strands and is different from the functional structure that forms voltage-gated channels. The selectivity of the channel is exquisite, being able to select for ATP over molecules of the same size and charge. Voltage gating involves two separate gating processes. The mechanism involves the translocation of a positively charged portion of the wall of the channel to the membrane surface resulting in a reduction in pore diameter and volume and an inversion in ion selectivity. This mechanism is consistent with experiments probing changes in selectivity, voltage gating, kinetics and energetics. Other published mechanisms are in conflict with experimental results. This article is part of a Special Issue entitled: VDAC structure, function, and regulation of mitochondrial metabolism.

Contents

☆ This article is part of a Special Issue entitled: VDAC structure, function, and regulation of mitochondrial metabolism.

* Tel.: +1 301 405 6925; fax: +1 301 314 9358.
E-mail address: colombini@umd.edu.

0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.12.026

1. Introduction

VDAC (voltage dependent anion-selective channel) is a small (~30 kDa) highly-conserved protein in the outer membrane of all mitochondria tested, regardless of species [1–7]. Its fundamental function as a membrane channel is to facilitate and regulate the flow of metabolites between the cytosol and the mitochondrial intermembrane space. Fig. 1 presents the results of an experiment [8] that embodies the essential function of VDAC. Here, VDAC channels reconstituted into a phospholipid membrane were controlled, through voltage, favoring either the open or closed state and the flux of ATP was measured at the same time. VDAC closure blocks the flux of ATP and reopening restores the flux.

The canonical isoform of VDAC isolated from species from all eukaryotic kingdoms has virtually the same properties of ion selectivity and voltage dependence [3]. Proteins with similar properties exist in other cellular membranes but it is not useful to confuse these with VDAC. Rather, this muddles the field, a field that is already overly muddled. Claims that VDAC is located in other cellular membranes in cells where it has not been overexpressed have NOT, so far, held up to careful scrutiny. When examined with care these claims have proved to be unfounded [9].

The use of the term "mitochondrial porin" when referring to VDAC, is unhelpful. It does remind the reader that VDAC is essentially a beta barrel channel like the family of channels found in the bacterial outer membrane. Beyond that, the use of this term has caused some to mistakenly attribute characteristics of some of the porins to VDAC, not realizing that these are distinct proteins with questionable evolutionary relationship. To classify all beta barrel pores as a family is not unreasonable and perhaps useful but one needs to be wary of the limitations of such a classification.

In this review I will focus on the generic structure and dynamics of VDAC. Although experiments were performed on VDAC from different species, these will be taken as describing the properties of one conserved protein. I will focus on the big picture, largely ignoring the interspecies variation. There are many indications that evolution has maintained the fundamental properties of VDAC in at least one isoform in the mitochondria of each organism [3,10,11]. These properties are therefore necessary for VDAC to perform functions that transcend differences among species and are likely related to the regulation of metabolic flux between the cytosol and mitochondria. When multiple isoforms are present, where studied, it is clear that VDAC isoforms are not redundant but have specialized properties [12,13]. Further, it is clear that VDAC has functions over and above the formation of voltage-gated channels that facilitate and control the flux of metabolites across the mitochondrial outer membrane. This review will focus on the channel-forming function of VDAC: the structure of the channel, its dynamics and its regulation.

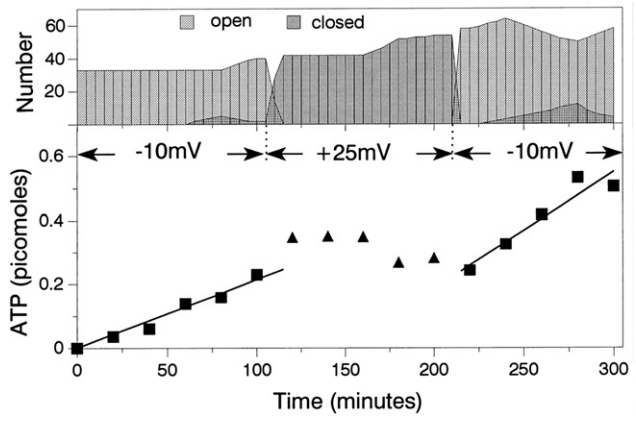

Fig. 1. The open state of VDAC mediates ATP flux and VDAC closure stops this flux despite the closed state being conductive and having an estimated pore radius of 0.9 nm. The channels were reconstituted into a planar phospholipid membrane under voltage clamp conditions. The number of open and closed channels was monitored continuously as was the flux of ATP. The presence of a large ATP gradient shifted the voltage dependent characteristics as described [74].
Reprinted from [8].

2. Structure

Information on the structure of the VDAC channel comes from various sources: the primary sequences [4–6], molecular modeling [11,14–18], circular dichroism estimates of the helical and beta sheet content [19], measurements of the tilt angle of the beta strands [20], measurements of pore size [10,21,22], electron-microscopic imaging and reconstruction [23–27], determination of the protein regions lining the inner wall of the pore [28,29], measurements of regions accessible to binding by soluble proteins [30–32] and regions accessible to protease action [31,32], and NMR and X-ray structures [33–35]. The 3-D structures from NMR and X-ray studies are insufficient because, being inconsistent with the results of many functional studies [36], these are unlikely to represent native structures.

The VDAC channel is composed of a single [26,37,38] 30 kDa polypeptide [4–6] forming fundamentally a beta barrel structure. This is supported by the 62% beta strand content measured by circular dichroism [19]. The alternating polar/non-polar pattern necessary to form beta strands that have the biophysical characteristics necessary to separate the apolar membrane environment from the aqueous environment in the pore of the channel exists in roughly 19 stretches of primary sequence [11]. Indeed, when VDAC was refolded from inclusion bodies, NMR and X-ray crystallography show the formation of a 19-stranded beta barrel [33–35]. However, experiments demonstrate that in the functional VDAC structure, the structure that forms voltage-gated channels, only 13 of these strands form the wall of the channel [36,39]. In addition, in the N-terminus there is sequence whose polar/non-polar pattern fits that of a "sided" alpha helix. This 20 amino-acid sequence, a length well suited to span the membrane, forms an alpha helix that is apolar on one side and polar on the other [11]. Thus it has the right biophysical characteristics to form part of the wall of the channel and functional studies are consistent with that conclusion [28]. In the refolded, non-functional structure, this helix is located within the lumen of the channel [33–35].

Identification of the strands forming the wall of the channel requires a combination of theoretical analysis and experimental testing. The theoretical analysis first looked for 10–12 amino-acid stretches with an alternating polar/non-polar pattern of amino acid side chains to identify regions with the right biophysical characteristics for sided beta strands [40]. A simple approach is to use an algorithm that sums the hydropathy numbers of the side chains such that the value of the first side chain is subtracted from the second added to the third and so on. Since the hydropathy values for polar and non-polar side chains have opposite signs, these sums are large for an alternating patter forming what is referred to as a beta pattern [11]. Then these candidate sequences were examined to look for problems (charged side chains facing the apolar side, prolines in the midst of the sequence) or confirmation (prolines or aromatics at the end of the sequence, glycines in tight turns). Most importantly, site-directed mutagenesis was used to alter the charge of residues that would face the aqueous phase, usually replacing a charged amino acid with one with opposite charge [28]. Charged side chains are almost certainly in the water phase rather than the membrane lipid environment and thus if these are facing the lumen of the channel they must influence the selectivity for small ions. For a 10-fold KCl gradient the observed change in reversal potential was generally 3–4 mV per change in 1 unit of charge [28]. Replacing charged residues with residues of opposite charge produced twice the change in reversal potential. When a charge change in a candidate strand produced no change in reversal

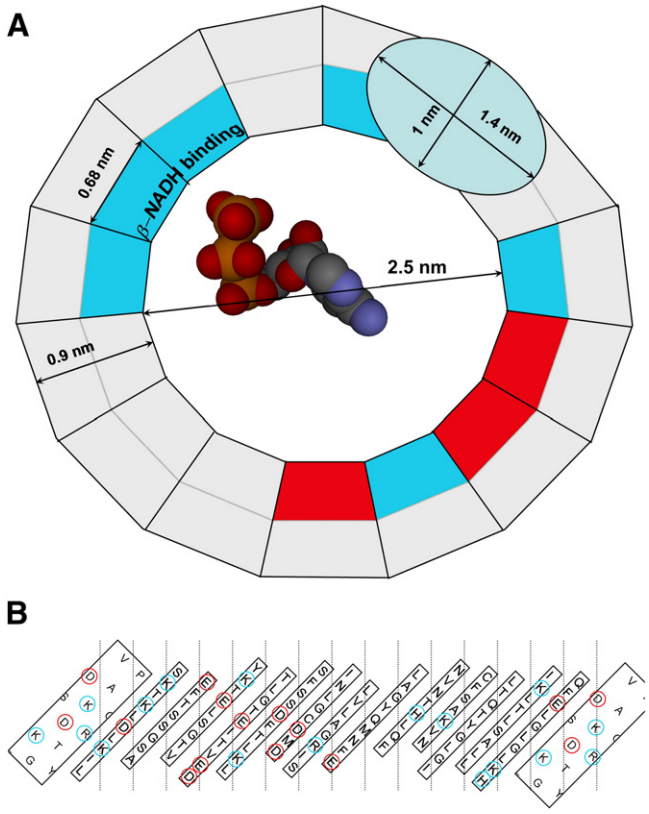

Fig. 2. A. A drawing of the top view of the open state of a VDAC channel using the constraints from experimental results [39]. Functional studies show that the pore is composed of 1 α helix and 13 β strands. Structural studies report that the β strands are tilted by 46° [20]. Thus from the top view, the apparent backbone to backbone distances for the β strands is 6.8 nm and the α helix is elongated in one dimension to 14 nm. The resulting pore diameter is 2.5 nm, in good agreement with experimental estimates. The colors on the inner wall refer to the net charge of that region based on the folding pattern shown in "B". Blue is positive, gray is neutral, and red is negative. The molecule of ATP in the center is scaled to the size of the channel and oriented according to the charge on the channel. B. The folding pattern of VDAC for human VDAC1 is based on results from the functional studies [39]. As these do not define the ends of each beta strand, the endings were defined based on the NMR structure [33]. The charged residues were color-coded as above. The dotted lines are the arbitrarily defined regions whose net charge is indicated in "A".

potential, the strand cannot form part of the wall of the channel. Of the 19 candidate beta strands, when tested 6 were found not to form the wall of the channel [28,30]. Ten separate point mutations covering all these strands showed that charge changes at each of these positions did not affect channel selectivity and thus these regions cannot be part of the wall of the functional channel. The positions of the remaining 13 strands are in excellent agreement between the functionally-derived structure and the NMR/X-ray structure [36]. This confirms the validity of the theoretical considerations.

An objection that can be raised is that any mutation can alter the structure of the channel resulting in changes in function that are not related to the charge change and thus are not informative. Unlike even small deletions that result in frame shifts that could wreak havoc [41] with, for example, the polar/non-polar pattern necessary for pore stability, single amino-acid substitutions are less disruptive, especially if conservative point mutations are used [28]. Conservative point mutations replace a polar or charged residue with one of opposite charge and replace residues expected to face the aqueous environment. This minimizes any electrostatic or steric effects. Experimental evidence of minimal or no structural change was twofold: 1) double and triple mutations resulted in changes in selectivity that were indistinguishable from the summation of changes induced by the simple mutations [28]; 2) conformational free energy changes between open and closed states calculated from the analysis of the voltage dependence showed relatively little change following the point mutations despite large changes in the steepness of the voltage dependence [42].

The location of the alpha helix in an otherwise beta barrel channel is problematic. One proposal [43] has the helix excluded from the channel lumen and only entering the channel to reduce its conductance upon voltage-dependent closure. Although this seems reasonable and has the elegance of a simple beta barrel without the problem of interfacing with the alpha helix, it is inconsistent with some compelling experimental results (Section 4.2.4). Electrophysiological studies on VDAC lacking part or all of the alpha helix report loss of voltage dependence [44–46] and general instability [44]. The difficulty here is that such a large structural change may alter the overall structure of the channel. The NMR/X-ray structures place the helix in the channel lumen. That reduces the effective size of the channel but with 19 beta strands it still leaves the pore large enough to satisfy experimental size constraints. In the functionally-derived structure, the alpha helix is proposed to be part of the wall of the pore, along with 13 beta strands. The helix is amphipathic and the hydrophobic surface would interact with the apolar lipid environment. This is, by far, the major component of free energy stabilization. The fact is that hydrogen bonding plays a small role in the stabilizing energy of protein folding because the energy of the protein–water hydrogen bonds are very similar to the protein's intramolecular hydrogen bonds. Still there is a structural problem in determining how the helix would interface with the beta strands. This would seem to introduce an unstable part of the structure. However, since the alpha helix is in the middle of the voltage sensor region, instability in that location might reduce the energy barrier to the gating process. Indeed, the refolded protein used to generate the NMR/X-ray structure does not respond to voltage[1] [33]. Perhaps the voltage sensor region is too stable. With the alpha helix and the greater tilt of the beta strands (46° [20]), as compared to the NMR/X-ray structure, the pore size of the functionally-derived structure is also consistent with experiments. Fig. 2 shows how using standard dimensions for an alpha helix and spacing between beta strands, a pore is formed that has an internal diameter of 2.5 nm, consistent with experimental size measurements [27,45]. Most importantly, point mutations that change the charge on the polar surface of the alpha helix change the selectivity as expected from the engineered mutation [28], demonstrating that the helix is in intimate contact with the ion stream within the channel. Thus it must line the wall of the channel.

A recent review article [47] begins with a historical perspective, glosses over most of the relevant experiments and purports to provide a strong case for the 19-stranded structure as being the native structure. For those not intimately familiar with the relevant literature, it seems to make a convincing case. Indeed it does a good job as deceiving the reader by selecting the evidence consistent with

[1] Protein samples from Wagner's lab were tested on planar membranes and these produced channels that did not respond to voltage [33]. The protein could be treated with cholesterol and Triton and produce normal-looking voltage-gated channels. The refolded structure thus spontaneously converts into what looks like a functional native structure. This conversion occurs but the parameters by which it occurs have not been studied. A hypothetical model for how the conversion could occur has been published and explains beautifully differences in strand orientation at two foci flanked by sites of strand insertion [36]. Abramson and co-workers [34] report a single voltage gated channel formed by the protein whose structure was determined by NMR/X-ray. Others have also reported voltage gating. However, only an infinitesimal fraction of the added protein reconstitutes into the membrane and it may not represent the properties of the population. Thus, those results are not convincing. The fact is that functional studies measure the properties of the very channels upon which structural inferences are made. The proteins studied by NMR or X-ray are never the same proteins upon which electrophysiological studies are performed. Thus if functional studies exclude a particular region from forming a portion of the wall of the channel and NMR/X-ray structures indicate otherwise, the latter must be studying an altered conformation.

the 19 stranded model and studiously ignoring the rest.[2] It uses the concept "everybody does it" as an argument that refolded proteins are the same as native proteins. It pollutes the case by not distinguishing between the more problematic membrane proteins and soluble proteins. Indeed it is a fine example of an attempt at deception by propaganda. In sum, the hard evidence presented in favor of the 19-stranded model consists of only 2 facts: the measured size of the channel from low-resolution electron microscopy and the ability of DCCD to label glutamate 73 [48], actually 72 if one recalls that the N-terminal methionine is removed in the mature protein. The former evidence is consistent with the functional structure if the alpha helix is part of the wall of the channel and the tilt of the beta strands is as measured (rather that the tilt in the 19-stranded structure). The latter does not provide structural information other than that glutamate is in a hydrophobic environment. In the functional structure the glutamate is located in a connecting loop and thus its environment could be either polar or non-polar. Indeed that loop contains 4 strongly hydrophobic residues and thus the glutamate could very well be in a hydrophobic environment. Thus the reaction with DCCD does not help to distinguish between the structural models. The other interactions with molecules such as NADH are totally or predominantly in regions where the models agree. Thus they are also not useful in distinguishing between models. The percent beta sheet content from CD measurements [19] is also not useful at distinguishing between competing models because beta structure need not be solely in the transmembrane strands.

3. Molecular basis for selectivity

From the crude to the refined, the selectivity of membrane channels starts from steric constraints to electrostatic effects to intimate interactions with the inner wall of the channel. I believe that this is generally applicable to all channels, both large and small. The study of VDAC has taught me that the evolutionary process does not stop at crude and therefore the notion of a "general diffusion pore" is totally naïve. The relentless process of natural selection continuously refines the structure of channels to optimize function. Thus the selectivity properties of channels are intimately linked to their function. This refinement is abundantly clear in VDAC.

Of the metabolites that must translocate through the mitochondrial outer membrane, ATP and ADP are probably the most important. Thus one might expect that the inner wall of the VDAC channel has an interacting surface ideally suited for the translocation of these adenine nucleotides. This should be more than just a binding site but rather an interacting path. Yet this path must also allow the flow of both larger and smaller metabolites and ions in harmony with cellular requirements. At the large end of the scale we have dinucleotides such as NADH. At the small end are the simple ions.

[2] In considering published evidence for or against a particular structural model or feature, it is not useful and, in fact, deceptive to lump all together rather than to distinguish between compelling evidence, suggestive observations, inconclusive findings, and theoretical predictions. To merely point out the existence of conflicting evidence and conflicting models without an effort at distinguishing between those based on hard evidence and those based on soft evidence or merely theoretical arguments, is to engage in obfuscation. The less reliable evidence that I am not reviewing here (I have reviewed it in a recent article [36]) falls into one of these categories: 1) Antibodies against peptide stretches of VDAC were used to analyze the channel topology. Unfortunately most of these peptides ended up spanning the membrane making deductions about topology difficult to interpret without knowing the precise epitope. 2) Proteases were used to study topology but these resulted in structural changes making the results difficult to interpret. 3) Large deletions were made in VDAC and the resulting effects on function were examined. These could cause structural changes and thus the findings are difficult to interpret objectively. Deletions of even a few amino acids at a time could result in major structural changes. 4) Theoretical arguments alone or arguments based on the structure of unrelated beta barrel proteins provide only ideas to consider. These ideas must be tested experimentally to provide any useful information.

3.1. Selectivity based on steric restrictions

Clearly the size of the permeant is a limiting factor because of the strong energy barrier against the overlap of electron orbitals. The limiting size of VDAC, the steric limit, was measured using polymers lacking both a net charge and any strong dipoles. Dextrans, polyethylene glycols, and cyclodextrins were used for vesicle swelling assays [10,21,49] and negative stain [23,25] for size estimates using the electron microscope. Based on the ability of these polymers to permeate into VDAC-containing liposomes, a molecular mass cut-off of about 4000 is reasonable. Channel closure induced by the addition of König's polyanion reduces the pore size so that gamma cyclodextrin (radius of 0.9 nm) just barely permeates the channel [49]. ATP has a Stokes–Einstein radius of 0.48 nm (based on a diffusion constant of 4.5×10^{-6} cm^2/s [50]) and therefore should, on steric grounds, be able to permeate through both the open and closed state. However, the presence of charge drastically changes the permeation.

3.2. Selectivity based on electrostatic effects

The presence of net charge on the permeant adds much more long-range interactions resulting in the possibility that small ions face a greater energy barrier than large molecules. For instance, VDAC is far less permeable to Ca^{2+} than to the Ca–EDTA^{2-} complex. Both have an equal amount of net charge but the sign of the charge is opposite. As expected, the effect of charge is greater for polyvalents and for larger ions that, because of their size, come in closer proximity to the walls of the channel.

For small ions the rate of translocation through VDAC depends on their mobility in water and the electrostatics of the channel interior. By using KCl to probe VDAC's selectivity one focuses of the properties of the channel because the mobilities of these ions is almost the same. The properties of the channel include effects of the surrounding phospholipids as these have a strong effect on the results [51]. This is not surprising given the thinness of the wall of the channel (1 nm or less [27]) compared to the Debye length. For a KCl gradient, the permeability ratio calculated from reversal potential measurements is usually quoted as 2:1 Cl^-:K^+ based on experiments with a 10-fold gradient (1 M vs 0.1 M). Although good for purposes of comparison between different VDAC molecules, this result seriously underestimates the selectivity of the channel. The use of a 2-fold gradient (0.2 M vs 0.1 M KCl) yielded a permeability ratio of 5 based on calculated flux measurements [44,52]. For small ions the overall selectivity of the channel is determined by an ionic environment within the channel that varies with the nature of the nearby protein surface and the distance from that surface. For relatively large channels, the selectivity is not described by classical Goldman/Hodgkin/Katz theory even though this theory is typically used to report permeability ratios [53]. Instead the values of the reversal potential can fit rather well to theory by considering the solution inside the channel to be composed of two regions: a cylindrical shell of solution next to the inner wall of the channel and a central cylinder of nearly bulk solution [53]. The cylindrical shell contains the counterions to the fixed charges on the channel wall as well as ions from the bulk solution all in dynamic equilibrium with the surface potential. The thickness of the cylindrical shell is taken as the point where the surface potential decays to 1/e of the value at the protein surface. Therefore this thickness will depend on the ionic strength. Although this approach seems crude, recall that the inner wall of the channel is not a smooth surface but composed of amino-acid side chains and thus the added complexity of a continuum model is not warranted. In any case, this simple model accounts quite well of how the reversal potential varies with transmembrane ionic activity ratio, the magnitude of the activity, changes in the charge of the inner surface of the channel and the mobility of the ions used [53]. Thus the selectivity among small ions is dominated by the surface charge of the protein lining the inner wall of the channel and how this

charge influences the concentration and distribution of ions in solution within the aqueous pore. Upon channel closure,[3] the drastic change in the nature of the wall of the pore [29,30,42] results in a drastic change in selectivity among small ions. The closed states favor cations [51,54,55] because the net charge in the pore becomes negative. This relatively simple understanding of the selectivity displayed by VDAC channels is not valid for organic anions that interact with the inner wall of the pore at multiple points and are thus sensitive to the actual distribution of charges and dipoles.

3.3. Selectivity based on the 3-dimensional nature of the VDAC channel

The influence of electrostatic interactions on the ability of small ions to permeate through the VDAC channel will also apply to the larger charged organic molecules. Clearly their larger size means that it is more difficult or perhaps impossible for these to travel through the channel without interacting with the charges and dipoles on the channel wall. Thus the influence of electrostatic interactions will be greater. This expectation explains very well the finding that ATP translocates easily through VDAC in the open state but does not do so in the closed state [8,56]. This was done in a defined system where the state of the channels and the flux of VDAC were monitored at the same time (Fig. 1). The reversal in net charge within the channel, as measured by the selectivity to small ions, explains this finding. Steric effects do not account for this because, as indicated in Section 3.1, the closed state is still much larger than the size of the ATP molecule. Nevertheless, a smaller channel will result in a more intimate interaction between the permeant and the walls of the channel.

The surprise comes for the observation that organic molecules of the same overall size and charge as ATP are unable to translocate through the open state [50]. The molecules tested had virtually identical size, charge and diffusion coefficient as ATP and yet were excluded from the open VDAC channel. The only conclusion one can make is that the distribution of charge in these molecules differs from that of ATP, and it does, resulting in an energy barrier to translocation because the charge distribution did not match that of the channel. The lack of a 3D structure for the *native* VDAC channel makes it impossible to test this conclusion but a "hand-in-glove" interaction between ATP and any proposed 3D structure of the channel should be a litmus test for the validity of such a structure.

Further evidence for the importance of the "hand-in-glove" interaction between ATP and the channel came from experiments in which multiple mutations were used to generate a channels whose open state reversal potential (in the presence of a KCl gradient) was indistinguishable from that of the closed state of wild-type VDAC [57]. In this mutant, the electrostatic nature of the channel felt by small ions favored cations and thus naively one would expect that organic anions like ATP would be excluded. However, this mutant channel still allowed ATP to permeate. Even NADH was able to permeate to some extent. Thus, despite the change in overall charge, the mutant retained critical interactions necessary for translocation of adenine nucleotides.

A comparison of the primary sequences of VDAC from different organisms shows quite a bit of variation. Yet the amino acids at certain sites are highly conserved [11]. Indeed, it is remarkable that lysines and arginines are not interchangeable if all that mattered were net charge. The fact that lysines are strongly preferred at specific sites argues for specific functions and binding to ATP may be one of these functions.

Fig. 2 shows how the functional structure results in a folding pattern that produces an asymmetrical charge in the channel that matches, to some extent, the asymmetrical charge of ATP. The β-NADH binding site [33,58], a modified Walker B nucleotide binding motif, may be involved in facilitating ATP flux.

3.4. Overall message on selectivity

The insights gained from the experiments point to a conclusion that is obvious by hindsight. Natural selection will tend to optimize channel size and selectivity to facilitate the flux of the appropriate metabolites and interfere with the flux of others. In the case of VDAC, optimization favors the flow of adenine nucleotides and anionic metabolites. The flow of cations, including Ca^{+2}, must proceed so the selectivity cannot be too high. VDAC closure increases Ca^{+2} flux as much as 10 fold but it is unclear whether this is physiologically significant [59]. A mixture of open and closed channels and the control of total number of channels may be an effective way to regulate the flux of both cations and metabolites. There is no credible evidence of VDAC facilitating the flux of folded proteins through the outer membrane.

4. Dynamics: structural changes associated with gating

4.1. Overview

VDAC channels can exist in a variety of structural states exhibiting a range of conductances and selectivities. This is the case for each individual channel as shown by prolonged recordings of single channels probed with triangular voltage waves [45,52,60]. Low transmembrane voltages (say 10 mV) result in the highest conductance, the open state. Elevated voltages, generally above 40 mV, cause the channel to transition to one of a range of lower conducting states, termed closed states because these have a drastically reduced permeability to anionic metabolites [8,52]. The introduction of a modulating factor causes the channels to respond to an elevated potential by preferentially closing to a much narrower range of closed states [61] (also for Bcl-xL, unpublished). Clearly the interaction favors some conformations and disfavors others.

There are two sets of closed states, one set favored by positive potentials and another by negative potentials. This is clearly demonstrated by closing a group of channels with a high positive potential and then applying a large negative potential, changing the voltage very rapidly so that the channels should not have time to open (faster than the opening kinetics). What is observed is that all the channels open and then close again [62]. The fast rates of opening and the slow rates of closure make this experiment easy to perform. Thus channel closure is not the result of "electrostriction", the electric field squeezing the membrane and in turn applying pressure on the channel favoring closure. One cannot exclude some influence of electrostriction but evidently the primary effect is the movement of charged regions of the channel in the direction of the electric field.

The gating kinetics are very revealing. The opening rate is fast [1], in the millisecond time scale, and largely voltage independent [63]. The closing rate is slower by 2 to 5 orders of magnitude and voltage dependent [63]. Thus the energy barrier in the opening process is largely outside the transmembrane electric field whereas that for the closing process is within the field. The large difference in the rates of opening and closure reflects the large difference in the size of the energy barrier for each process.

One factor that complicates the study of the dynamics of VDAC is "memory". The channel undergoes slow changes in its properties that show up as changes in kinetics. It adapts to a particular conformational state thus reducing the rates of transition to other states. Thus, after entering a particular conformational state, slow structural changes occur that stabilize that state. There are many of these because the stabilization appears to be continuous. Typically these changes do not result in obvious changes in the properties of the

[3] There are a variety of "closed" conducting states, not all of which show both a reduction in conductance and an inversion in selectivity. The complex set of states has not been addressed either structurally or mechanistically. The results refer to typical closed states, ignoring the variation.

channels and are detected as changes in the kinetics of transitions. For example, the rate of channel opening is fast but only if the channel was closed for a brief period (a few seconds or a minute). If the channel is held in the closed state for an extended period by maintaining a high voltage and if the channel does not decide to open during the test period, then reducing the voltage results in a rate of reopening that depends on how long the channel was held in the closed state. This memory explains why VDAC channels tend to oscillate between states only soon after an elevated voltage is applied and then tend to remain in one state for an extended period of time. This adaptation requires that special care be taken when generating and interpreting steady state (near equilibrium) conductance/voltage relationships. Clearly, analysis of single-channel dwell times is not a useful way of collecting information about channel kinetics. Neither is the strategy of applying fixed voltages and letting channels achieve a steady-state conductance useful to generate conductance-voltage plots. What has proved useful is to apply slow triangular voltage waves. The rate of voltage change is chosen so that changing the frequency of the wave does not change the conductance change in the reopening portion of the wave. There is still hysteresis in that the opening process differs from the closing process. However, the closing process depends on the frequency of the triangular wave whereas the opening process does not (at the appropriate range of frequencies: 1 to 5 mHz). The conductance/voltage plot obtained in this way measures the voltage dependence of the states accessible in this time scale. All the analyses performed in my lab refer to these "rapidly accessible" states. An additional important point is that these are the states rapidly accessible from the channel adapted to the open state conformation. The situation is likely to be very different if the channels were adapted to one of the closed conformations. In the cell, the time scales can be very long and this memory will influence the behavior of the channel (in addition to the influence of other modulating factors).

The phospholipid content has strong effects on the gating properties of VDAC [51]. The molecular basis for these effects needs further investigation.

4.2. Voltage gating

The conformational changes in VDAC that have been observed are voltage dependent. VDAC gating is voltage gating. That means that the energy level of the conformational states of VDAC is influenced by the transmembrane voltage. That requires that these conformational changes result in either the movement of charge through the transmembrane electric field or the alignment of substantial dipoles with the field. Proposed models of voltage gating based on structural information that do not involve a voltage-dependent energy change are just plain wrong even if they are published in PNAS [34]. This is a theoretical argument based on the fundamental principles of thermodynamics and it is supported by detailed experimental results. The conformational changes associated with VDAC gating are well defined in broad terms even if the molecular details are still needed.

4.2.1. Information from selectivity changes resulting from point mutations

Just as the identification of transmembrane strands forming the wall of the channel could be achieved by determining whether point mutations influenced the ion selectivity of the channel, such mutations were also able to determine if the same protein segments still formed transmembrane strands in the closed state [29]. The fact that the closed state is conductive allows this probing to take place. Again, a change in charge in a segment that forms the inner wall of the channel must affect the selectivity to small ions. A distal mutation, far from the ion stream, might cause a selectivity change by inducing a change in conformation but this change need not be of the correct sign or correct magnitude. Five types of results were obtained [29] when the selectivity of channels containing single point mutations were examined: 1) no selectivity change in either the open or closed state; 2) selectivity change in both the open and closed states; 3) selectivity change in the open state but not in the closed state; 4) selectivity change in the open state but a weaker change in the closed state; 5) no selectivity change in the open state but some change in selectivity in the closed state. Cases 3 and 4 identified regions of the channel that moved away from the ion stream and thus were proposed to move to or toward the surface of the membrane. Clearly such movement would be a voltage-dependent conformational change if the region that moved had a net charge… and indeed it did. Furthermore the net charge was positive and this accounted both for the sign of the voltage-dependent energy change and for the selectivity change upon VDAC closure. Obviously it would also naturally account for the reduction in channel conductance and require a reduction in the volume of the pore. Note that this is consistent with the measured reduction of the volume of the pore upon channel closure measured by Zimmerberg and Parsegian [64] using polymers to shift the voltage gating of VDAC (another constraint ignored by some gating models).

4.2.2. Information from quantitative analysis of voltage-gating of VDAC mutants

Naturally, the movement of regions of the channel from forming the inner walls of the channel to the membrane surface would require that point mutations in these regions influence the steepness of the voltage dependence by the correct amount and by the correct sign. This is a critical test. One could observe effects on voltage gating arising from changes in the energy barrier if the mutation merely interfered with the conformational change. Such effects would not be informative. One might also observe changes in voltage gating that are out of proportion to the charge change engineered into the channel. These would cast doubt on the proposed mechanism. However, the results were amazingly consistent [42] with the simple mechanism of translocation of a region of the channel from forming the inner wall of the channel to the membrane surface. They were consistent with the region moving through half the transmembrane field. Mutations in other regions had no effect on voltage gating. Even more satisfying was the observation that the mutation had little effect on the energy difference between the open and closed state. The voltage gating process was analyzed [42] by fitting to the Boltzmann distribution yielding two parameters: the steepness of the voltage dependence, n, and the voltage at which half the channels are open and half are closed, V_0. "n" is the number of charges that would need to move through the entire potential difference to account for the voltage dependence. Since the measured value of n was approximately half the engineered charge change, the charges moved through half the potential change [42]. The product of n, V_0 and Faraday's constant yields the conformational energy difference between the two states. For this to remain constant, an increase in n would need to be accompanied by a compensatory decrease in V_0. That was generally the case, demonstrating that the mutation resulted in little change in the overall energies of these states. This is an exquisite test that the mutations did not change the structure of the channel. It must also be emphasized that the same regions that were proposed to move out of the channel based on selectivity changes [29] were also those that influences the steepness of the voltage dependence. Thus both experiments identified the SAME region of the channel as the voltage sensor!

4.2.3. Information from biotinylation and streptavidin accessibility experiments

Although both changes in selectivity and analysis of voltage gating require motion of the voltage sensor region by the same amount, further evidence of this motion was deemed necessary because the large-scale motion did not fit in with the structural changes deemed acceptable by researchers then and now. Direct demonstration of the proposed motion was achieved by the use of cysteine

mutagenesis [30]. The introduction of a single cysteine into *Neurospora crassa* VDAC that lacks cysteines allowed for specific biotinylation of that site and determination of the accessibility of the site to added streptavidin in the medium. Single biotinylated channels were reconstituted into planar membranes and examined for their ability to gate normally prior to streptavidin addition to one or the other side of the membrane. When streptavidin was added to the proper side, a small but abrupt conductance change occurred when the streptavidin bound to the biotinylated site resulting in one of two types of changes in channel properties. Type 1 change resulted in a reduction in conductance but gating continued. In this change, application of a positive potential on the streptavidin side resulted in normal gating kinetics whereas a negative potential resulted in gating with much slower kinetics. A positive potential would drive the voltage sensor to the side opposite from that with the bound streptavidin whereas a negative potential would drive the sensor toward the streptavidin and steric hindrance could interfere with this movement. Type 2 change resulted in a larger drop in conductance and the channel was locked in a closed state. The conductance remaining no longer responded to the applied potential. A type 2 change was facilitated by applying a potential that drove the sensor to the streptavidin side (i.e. a negative potential on that side) but would occur spontaneously at 10 mV indicating that the channel spontaneously explores the different structural states and the electric field is simply favoring one state over another by changing the energy level of the conformational states. Thus a type 1 change was interpreted as a site on the channel that was not on the voltage sensor whereas a type 2 change indicated that the cysteine modification was at a site on the voltage sensor or at least on a region that became exposed to the surface upon VDAC closure. The results were, by in large, consistent with the voltage gating and selectivity measurements in terms of identifying the voltage sensor. Other motions are detected but this is not a problem because the channel could undergo additional motions.

4.2.4. Congruence with other experimental measurements

The voltage gating mechanism (Fig. 3) is consistent with measured changes in conductance, pore size [49], selectivity [52] and pore volume [64]. A conductance drop upon channel closure is obvious and seems perhaps unlikely to serve as a criterion to distinguish among models of voltage gating. While that is largely true, the fact that there is a residual conductance [3,55] does require a model consistent with such a result and indeed the proposed gating mechanism does require a residual conductance. The changes in pore size, as measured by non-electrolyte permeation [49], are consistent with the model. The most compelling is the change in selectivity. That does require a change in the protein surface interacting with the ion stream. Simple covering the surface with a segment of the protein, like the alpha helix, will not work unless one proposes large changes in the dissociation constants of the amino acid side chains. Such changes, if they were to occur in a large channel, would require drastic action, like the movement of charged residues into a hydrophobic environment. The enthalpy changes associated with that would be large and unfavorable. The measured large drop in pore volume upon VDAC closure strongly supports the working mechanism but does not exclude a process such as the insertion of the alpha helix into the channel. What excludes the helix insertion mechanism are selectivity changes resulting from charge-change mutations in the alpha helix [29]. In the open state, two mutations in the alpha helix, D15K and K19E, have strong effects on selectivity, the former increasing anion selectivity and the latter reducing it [28]. In the closed state, K19E has a much weaker effect and D15K has no effect [29], consistent with the helix moving out of the channel upon channel closure. The results of voltage-gating measurements are also in agreement. Both of these mutations affect the steepness of the voltage dependence but in opposite directions [42]. As expected, K19E reduces the "n" value whereas D15K increases it. Regardless, the alpha helix is not the entire voltage sensor. In fact the alpha helix from yeast has no net charge and the charge it does have is distributed in such a way that it counters the dipole moment of the backbone hydrogen bonds. Thus the charge on the voltage sensor comes from the beta strands that are part of the entire mobile domain.

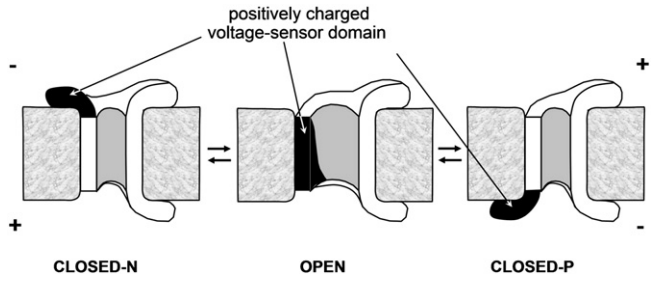

Fig. 3. The gating process of VDAC consisting of one open state and two closed states: one of these achieved at positive potentials and the other at negative potentials. The black region is the positively-charged, mobile, voltage-sensor domain. Its movement out of the channel results in a reduced diameter, reduced volume, and inverted ion selectivity. Each closed state is actually a population of closed states.

4.2.5. Congruence with kinetic measurements

The kinetics of voltage gating of VDAC are strongly supportive of the working model. In its most basic, a region of the channel is driven to the membrane surface and that requires that two sets of hydrogen bonds (2×10 for 10 amino acid residues needed for a beta strand to span the membrane) connecting that region to the rest of the barrel be broken and only one set be formed to restore a complete but smaller barrel. The reopening of the channel would now require that only one set of hydrogen bonds (10) be broken in order to reinsert the segment of the channel wall resulting in the larger barrel. Thus the activation energy barrier should be twice as great for channel closure as channel reopening. Using standard transition state theory, the rate constant can be related to the energy barrier of the transition state: $k = k^{\ddagger}K^{\ddagger}$; where $k^{\ddagger} = k_BT/h$ (k_B is Boltzman's constant, T is the absolute temperature and h is Plank's constant) and $\ln K^{\ddagger} = -\Delta G^{\ddagger}/RT$ ($\Delta G^{\ddagger}$ is the height of the energy barrier and RT is thermal energy). The theoretical relation between the natural log of the rate constant and the height of the energy barrier is plotted as a straight line in Fig. 4B and the measured values of the rate constants for VDAC channel opening (solid circles) and closing (open circles) are plotted as well (data from Ref. [63]). The opening process is not voltage dependent (Fig. 4A) and so the energy barrier is purely the conformational energy barrier. The closing rate is highly voltage dependent (Fig. 4A) and thus the voltage used to induce closure subtracts part of the conformational energy barrier. After compensating for this externally-applied voltage-dependent energy component (nFV) one obtains a measure of the conformational energy barrier for the closing process (open squares, Fig. 4B). Note that these are at an energy level that is double that of the opening process, as expected from the model of the gating process. Other mechanisms do not even attempt to explain the kinetics. Further, the closing process requires that the energy barrier be overcome while the sensor is moving through the electric field where in the reopening process the sensor is on the surface, largely outside the electric field. Thus the closure rate should be voltage dependent and the reopening rate largely voltage independent. That is exactly what was measured (Fig. 4A) [63].

4.2.6. Congruence with energetic measurements

Measurements of the temperature dependence of the equilibrium constant between the open and closed state yielded a positive enthalpy change between 35 and 40 kJ/mol [45]. Since the free energy change is 7 to 8 kJ/mol for *N. crassa* VDAC [3], the large unfavorable enthalpy change is largely balanced by a favorable entropy change.

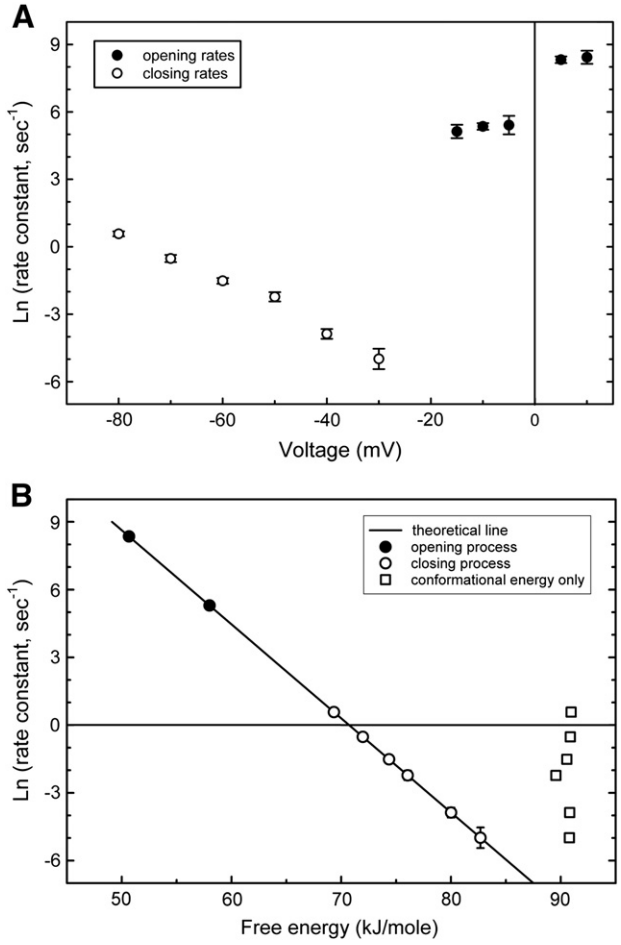

Fig. 4. Energetics and voltage-dependent kinetics of the VDAC gating process. The data was obtained from Ref. [63] and these are the results of experiments performed on VDAC from *N. crassa* reconstituted into planar membranes made from solvent-free soybean phospholipid monolayers. A. The rate constants are the means ± SE of 5 and 4 experiments at each voltage for the closing and opening rates resp. B. The data in "A" were converted to free energy of activation, as described in the text. The values for the rates of opening at positive and negative potentials were pooled into two points (filled symbols; one for negative and one for positive potentials) because there is no voltage dependence of this rate except for the sign of the potential used. The error bars were included but are almost always smaller than the symbol. The straight line is merely the relationship between the rate constant and the activation energy. For the closing rates, the voltage-dependent energy, nFV, was added to the calculated energy barrier to obtain the square symbols. These square symbols represent the true energy barrier for the conformational change from the open to the closed conformation.

The working model with the sensor driven out of the channel might be expected to have an unfavorable enthalpy change (as described in Section 4.2.5 for the number of hydrogen bonds broken and made) and an increase in disorder. The large number of closed states is certainly consistent with an increase in entropy. The magnitude of the enthalpy change is not inconsistent with a net breakage of 10 intramolecular hydrogen bonds and the formation of hydrogen bonds with water. Although generally both types of hydrogen bonds have similar energies, the latter being more disordered could be at a higher energy level (i.e. somewhat weaker bonds). The mechanism does not propose that the hydrophobic regions be removed from their apolar environment but that they remain in that environment as the sensor region slides from forming part of the wall of the channel to being part of the outer leaflet of the membrane (the lipids being moved aside).

4.2.7. Summary of VDAC dynamics

The large number and large variety of experimental evidence supporting the gating mechanism of VDAC act as strong constraints on any proposed gating process. The gating process illustrated in Fig. 3 is supported by all this evidence. It was first proposed in 1992 and then tested and retested by additional experiments [30,42], which supported the mechanism. It is therefore quite troubling when ad-hoc mechanisms are proposed and published ignoring just about all the published data. Having said that, I must point out that I have not discussed published results aimed at probing the gating process by deleting portions of the VDAC protein [41]. Simply deleting segments of the protein and examining whether conductance changes are detected is not an effective way to produce interpretable results. It's far better to observe graded changes in the voltage-gating parameters than total loss or qualitative changes in the voltage dependence. Loss of voltage dependent gating could arise simply from increasing the energy barrier between the states or overall changes in protein folding. Qualitative changes indicate major changes in conformation. Both of these are virtually impossible to interpret without detailed structural information. Thus they do not provide any useful information and are not discussed in this review.

5. Fascinating properties of VDAC not discussed in this review

There are a variety of remarkable properties of VDAC not discussed in this review or this collection of reviews. I will list these for completeness and to give the reader guidance to further reading.

Ultra-steep voltage dependence: Treating VDAC with anyone of a variety of polyanions (both natural and synthetic) results in a voltage-dependent behavior with a steepness greater than that of any channel so far reported in the literature [42,65,66].

Auto-directed insertion: VDAC inserted into a phospholipid membrane can determine both the direction of insertion of other VDAC channels to a high degree of fidelity and accelerate the rate of insertion by 10 orders of magnitude [67–69].

Modulation of the voltage gating of VDAC by a highly-conserved protein in the intermembrane space: An as yet unidentified protein located in the mitochondrial intermembrane space increases the voltage dependence of VDAC rather dramatically [61,70–73].

References

[1] M. Colombini, Candidate for the permeability pathway of the outer mitochondrial-membrane, Nature 279 (1979) 643–645.
[2] S.J. Schein, M. Colombini, A. Finkelstein, Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from *Paramecium* mitochondria, J. Membr. Biol. 30 (1976) 99–120.
[3] M. Colombini, Voltage gating in the mitochondrial channel, VDAC, J. Membr. Biol. 111 (1989) 103–111.
[4] K. Mihara, R. Sato, Molecular-cloning and sequencing of cDNA for yeast porin, an outer mitochondrial-membrane protein — a search for targeting signal in the primary structure, EMBO J. 4 (1985) 769–774.
[5] H. Kayser, H.D. Kratzin, F.P. Thinnes, H. Gotz, W.E. Schmidt, K. Eckart, N. Hilschmann, To the knowledge of human porins. 2. Characterization and primary structure of a 31-kDa porin from human b-lymphocytes (porin 31 HL), Biol. Chem. Hoppe Seyler 370 (1989) 1265–1278.
[6] R. Kleene, N. Pfanner, R. Pfaller, T.A. Link, W. Sebald, W. Neupert, M. Tropschug, Mitochondrial porin of *Neurospora-crassa* — cDNA cloning, in vitro expression and import into mitochondria, EMBO J. 6 (1987) 2627–2633.
[7] M. Colombini, Anion channels in the mitochondrial outer-membrane, Current Topics in Membranes, in: W.B. Guggino (Ed.), Chloride Channels, 42, Academic Press, New York, 1994, pp. 73–101.
[8] T. Rostovtseva, M. Colombini, ATP flux is controlled by a voltage-gated channel from the mitochondrial outer membrane, J. Biol. Chem. 271 (1996) 28006–28008.
[9] R.Z. Sabirov, T. Sheiko, H.T. Liu, D.F. Deng, Y. Okada, W.J. Craigen, Genetic demonstration that the plasma membrane maxianion channel and voltage-dependent anion channels are unrelated proteins, J. Biol. Chem. 281 (2006) 1897–1904.
[10] M. Colombini, Pore-size and properties of channels from mitochondria isolated from *Neurospora-crassa*, J. Membr. Biol. 53 (1980) 79–84.
[11] J.M. Song, M. Colombini, Indications of a common folding pattern for VDAC channels from all sources, J. Bioenerg. Biomembr. 28 (1996) 153–161.
[12] E.H.Y. Cheng, T.V. Sheiko, J.K. Fisher, W.J. Craigen, S.J. Korsmeyer, VDAC2 inhibits BAK activation and mitochondrial apoptosis, Science 301 (2003) 513–517.

[13] K. Anflous-Pharayra, N. Lee, D.L. Armstrong, W.J. Craigen, VDAC3 has differing mitochondrial functions in two types of striated muscles, Biochim. Biophys. Acta 1807 (2011) 150–156.
[14] M. Forte, H.R. Guy, C.A. Mannella, Molecular-genetics of the VDAC ion channel — structural model and sequence-analysis, J. Bioenerg. Biomembr. 19 (1987) 341–350.
[15] C.A. Mannella, A.F. Neuwald, C.E. Lawrence, Detection of likely transmembrane beta-strand regions in sequences of mitochondrial pore proteins using the Gibbs sampler, J. Bioenerg. Biomembr. 28 (1996) 163–169.
[16] V. Depinto, F. Palmieri, Transmembrane arrangement of mitochondrial porin or voltage-dependent anion channel (VDAC), J. Bioenerg. Biomembr. 24 (1992) 21–26.
[17] R. Casadio, I. Jacoboni, A. Messina, V. De Pinto, A 3D model of the voltage-dependent anion channel (VDAC), FEBS Lett. 520 (2002) 1–7.
[18] D.C. Bay, D.A. Court, Origami in the outer membrane: the transmembrane arrangement of mitochondrial porins, Biochem. Cell Biol. 80 (2002) 551–562.
[19] L. Shao, K.W. Kinnally, C.A. Mannella, Circular dichroism studies of the mitochondrial channel, VDAC, from *Neurospora crassa*, Biophys. J. 71 (1996) 778–786.
[20] H. Abrecht, E. Goormaghtigh, J.M. Ruysschaert, F. Homble, Structure and orientation of two voltage-dependent anion-selective channel isoforms — an attenuated total reflection Fourier-transform infrared spectroscopy study, J. Biol. Chem. 275 (2000) 40992–40999.
[21] L.S. Zalman, H. Nikaido, Y. Kagawa, Mitochondrial outer-membrane contains a protein producing nonspecific diffusion channels, J. Biol. Chem. 255 (1980) 1771–1774.
[22] I. Vodyanoy, S.M. Bezrukov, M. Colombini, Measurement of ion channel access resistance, FASEB J. 6 (1992) A114-A114.
[23] C.A. Mannella, A. Ribeiro, J. Frank, Structure of the channels in the outer mitochondrial-membrane — electron-microscopic studies of the periodic arrays induced by phospholipase-a2 treatment of the *Neurospora* membrane, Biophys. J. 49 (1986) 307–318.
[24] C.A. Mannella, X.W. Guo, B. Cognon, Diameter of the mitochondrial outer-membrane channel — evidence from electron-microscopy of frozen-hydrated membrane crystals, FEBS Lett. 253 (1989) 231–234.
[25] C.A. Mannella, X.W. Guo, Interaction between the VDAC channel and a polyanionic effector — an electron-microscopic study, Biophys. J. 57 (1990) 23–31.
[26] L. Thomas, E. Kocsis, M. Colombini, E. Erbe, B.L. Trus, A.C. Steven, Surface-topography and molecular stoichiometry of the mitochondrial channel, VDAC, in crystalline arrays, J. Struct. Biol. 106 (1991) 161–171.
[27] C.A. Mannella, M. Forte, M. Colombini, Toward the molecular-structure of the mitochondrial channel, VDAC, J. Bioenerg. Biomembr. 24 (1992) 7–19.
[28] E. Blachlydyson, S.Z. Peng, M. Colombini, M. Forte, Selectivity changes in site-directed mutants of the VDAC ion channel — structural implications, Science 247 (1990) 1233–1236.
[29] S. Peng, E. Blachlydyson, M. Forte, M. Colombini, Large-scale rearrangement of protein domains is associated with voltage gating of the VDAC channel, Biophys. J. 62 (1992) 123–135.
[30] J.M. Song, C. Midson, E. Blachly-Dyson, M. Forte, M. Colombini, The sensor regions of VDAC are translocated from within the membrane to the surface during the gating processes, Biophys. J. 74 (1998) 2926–2944.
[31] S. Stanley, J.A. Dias, D. D'Arcangelis, C.A. Mannella, Peptide-specific antibodies as probes of the topography of the voltage-gated channel in the mitochondrial outer membrane of *Neurospora crassa*, J. Biol. Chem. 270 (1995) 16694–16700.
[32] V. Depinto, G. Prezioso, F. Thinnes, T.A. Link, F. Palmieri, Peptide-specific antibodies and proteases as probes of the transmembrane topology of the bovine heart mitochondrial porin, Biochemistry 30 (1991) 10191–10200.
[33] 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.
[34] R. Ujwal, D. Cascio, J.P. Colletier, S. Faham, J. Zhang, L. Toro, P.P. Ping, J. Abramson, The crystal structure of mouse VDAC1 at 2.3 Angstrom resolution reveals mechanistic insights into metabolite gating, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17742–17747.
[35] 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.
[36] M. Colombini, The published 3D structure of the VDAC channel: native or not? Trends Biochem. Sci. 34 (2009) 382–389.
[37] C.A. Mannella, Electron-microscopy and image-analysis of the mitochondrial outer-membrane channel, VDAC, J. Bioenerg. Biomembr. 19 (1987) 329–340.
[38] S.Z. Peng, E. Blachlydyson, M. Colombini, M. Forte, Determination of the number of polypeptide subunits in a functional VDAC channel from *Saccharomyces-cerevisiae*, J. Bioenerg. Biomembr. 24 (1992) 27–31.
[39] J.M. Song, C. Midson, E. Blachly-Dyson, M. Forte, M. Colombini, The topology of VDAC as probed by biotin modification, J. Biol. Chem. 273 (1998) 24406–24413.
[40] E. Blachlydyson, S.Z. Peng, M. Colombini, M. Forte, Probing the structure of the mitochondrial channel, VDAC, by site-directed mutagenesis — a progress report, J. Bioenerg. Biomembr. 21 (1989) 471–483.
[41] G. Runke, E. Maier, W.A.T. Summers, D.C. Bay, R. Benz, D.A. Court, Deletion variants of *Neurospora* mitochondrial porin: electrophysiological and spectroscopic analysis, Biophys. J. 90 (2006) 3155–3164.
[42] L. Thomas, E. Blachlydyson, M. Colombini, M. Forte, Mapping of residues forming the voltage sensor of the voltage-dependent anion-selective channel, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 5446–5449.
[43] C.A. Mannella, Structural-analysis of mitochondrial pores, Experientia 46 (1990) 137–145.
[44] D.A. Koppel, K.W. Kinnally, P. Masters, M. Forte, E. Blachly-Dyson, C.A. Mannella, Bacterial expression and characterization of the mitochondrial outer membrane channel: effects of N-terminal modifications, J. Biol. Chem. 273 (1998) 13794–13800.
[45] M. Colombini, E. Balchly-Dyson, M. Forte, VDAC, a channel in the outer mitochondrial membrane, in: T. Narahashi (Ed.), Ion Channels, Vol. 4, Plenum Press, New York, 1996, pp. 169–202.
[46] 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.
[47] S. Hiller, J. Abramson, C. Mannella, G. Wagner, K. Zeth, The 3D structures of VDAC represent a native conformation, Trends Biochem. Sci. 35 (2010) 514–521.
[48] V. Depinto, J.A. Aljamal, F. Palmieri, Location of the dicyclohexylcarbodiimide-reactive glutamate residue in the bovine heart mitochondrial porin, J. Biol. Chem. 268 (1993) 12977–12982.
[49] M. Colombini, C.L. Yeung, J. Tung, T. Konig, The mitochondrial outer-membrane channel, VDAC, is regulated by a synthetic polyanion, Biochim. Biophys. Acta 905 (1987) 279–286.
[50] T.K. Rostovtseva, A. Komarov, S.M. Bezrukov, M. Colombini, VDAC channels differentiate between natural metabolites and synthetic molecules, J. Membr. Biol. 187 (2002) 147–156.
[51] T.K. Rostovtseva, N. Kazemi, M. Weinrich, S.M. Bezrukov, Voltage gating of VDAC is regulated by nonlamellar lipids of mitochondrial membranes, J. Biol. Chem. 281 (2006) 37496–37506.
[52] T. Hodge, M. Colombini, Regulation of metabolite flux through voltage-gating of VDAC channels, J. Membr. Biol. 157 (1997) 271–279.
[53] E.B. Zambrowicz, M. Colombini, Zero-current potentials in a large membrane channel — a simple theory accounts for complex behavior, Biophys. J. 65 (1993) 1093–1100.
[54] R. Benz, M. Kottke, D. Brdiczka, The cationically selective state of the mitochondrial outer-membrane pore — a study with intact mitochondria and reconstituted mitochondrial porin, Biochim. Biophys. Acta 1022 (1990) 311–318.
[55] M. Colombini, Structure and mode of action of a voltage-dependent anion-selective channel (VDAC) located in the outer mitochondrial membrane, Ann. N. Y. Acad. Sci. 341 (1980) 552–563.
[56] T. Rostovtseva, M. Colombini, VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function, Biophys. J. 72 (1997) 1954–1962.
[57] A.G. Komarov, D.F. Deng, W.J. Craigen, M. Colombini, New insights into the mechanism of permeation through large channels, Biophys. J. 89 (2005) 3950–3959.
[58] M. Zizi, M. Forte, E. Blachlydyson, M. Colombini, NADH regulates the gating of VDAC, the mitochondrial outer-membrane channel, J. Biol. Chem. 269 (1994) 1614–1616.
[59] W.Z. Tan, M. Colombini, VDAC closure increases calcium ion flux, Biochim. Biophys. Acta 1768 (2007) 2510–2515.
[60] D.W. Zhang, M. Colombini, Group-IIIA-metal hydroxides indirectly neutralize the voltage sensor of the voltage-dependent mitochondrial channel, VDAC, by interacting with a dynamic binding-site, Biochim. Biophys. Acta 1025 (1990) 127–134.
[61] M.J. Holden, M. Colombini, The mitochondrial outer-membrane channel, VDAC, is modulated by a soluble-protein, FEBS Lett. 241 (1988) 105–109.
[62] M. Colombini, Voltage gating in VDAC: toward a molecular mechanism, in: C. Miller (Ed.), Ion Channel Reconstitution, Plenum Publishing, New York, 1986, pp. 533–552.
[63] E.T. Dill, Probing the nature of the voltage-sensing mechanism of the mitochondrial outer membrane channel, VDAC: initial kinetic analysis and aluminum chloride-induced alterations, Doctoral Thesis, Dept. Zoology, University of Maryland, College Park, Maryland, 1987. http://hdl.handle.net/1903/12145
[64] J. Zimmerberg, V.A. Parsegian, Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel, Nature 323 (1986) 36–39.
[65] P.S. Mangan, M. Colombini, Ultrasteep voltage dependence in a membrane channel, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 4896–4900.
[66] M. Colombini, M.J. Holden, P.S. Mangan, Modulation of the mitochondrial channel VDAC by a variety of agents, in: A. Azzi, K.A. Nalecz, M.J. Nalecz, L. Wojtczak (Eds.), Anion Carriers of Mitochondrial Membranes, Springer-Verlag, Berlin, 1989, pp. 215–224.
[67] M. Zizi, L. Thomas, E. Blachlydyson, M. Forte, M. Colombini, Oriented channel insertion reveals the motion of a transmembrane beta-strand during voltage gating of VDAC, J. Membr. Biol. 144 (1995) 121–129.
[68] X.F. Xu, M. Colombini, Self-catalyzed insertion of proteins into phospholipid membranes, J. Biol. Chem. 271 (1996) 23675–23682.
[69] X.F. Xu, M. Colombini, Autodirected insertion: preinserted VDAC channels greatly shorten the delay to the insertion of new channels, Biophys. J. 72 (1997) 2129–2136.
[70] M.Y. Liu, M. Colombini, Voltage gating of the mitochondrial outer-membrane channel VDAC is regulated by a very conserved protein, Am. J. Physiol. 260 (1991) C371–C374.
[71] M.Y. Liu, M. Colombini, A soluble mitochondrial protein increases the voltage dependence of the mitochondrial channel, VDAC, J. Bioenerg. Biomembr. 24 (1992) 41–46.
[72] M.Y. Liu, M. Colombini, Regulation of mitochondrial respiration by controlling the permeability of the outer-membrane through the mitochondrial channel, VDAC, Biochim. Biophys. Acta 1098 (1992) 255–260.
[73] M.Y. Liu, A. Torgrimson, M. Colombini, Characterization and partial-purification of the VDAC-channel-modulating protein from calf liver-mitochondria, Biochim. Biophys. Acta 1185 (1994) 203–212.
[74] M. Zizi, C. Byrd, R. Boxus, M. Colombini, The voltage-gating process of the voltage-dependent anion channel is sensitive to ion flow, Biophys. J. 75 (1998) 704–713.