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## Structural analysis of mitochondrial pores

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*Summary.* Structural information about the channel in the mitochondrial outer membrane, derived from sequence analysis and electron microscopy of two-dimensional crystals, is summarized. A model for the channel is presented, consisting of a cylindrical beta-barrel that is formed by one or two 30-kDa polypeptides, with an alpha-carbon backbone diameter of 3.8 nm. The radial distributions of basic amino acids and lipid-contact regions on the projected cylinder are mapped relative to interchannel bonding sites inferred from channel packing in the arrays. Speculation on the kinds of conformational changes that the channel might undergo is also presented.
*Key words.* Mitochondrial outer membrane; channels; membrane crystals; electron microscopy; image processing.

Voltage-gated ion channels play a central role in several biological processes. Yet, it is not an overstatement to say that we are generally ignorant of the molecular mechanisms underlying the operation of these channels. Basic unanswered questions include: How may polypeptides fold to make a polar, transmembrane compartment? What are the physical parameters that control the selectivity of channels? And what kinds of conformational changes are involved in gating? Answering these questions will require detailed understanding of the structure and dynamics of these intriguing cellular components.

Much of our current conception of the higher order structure of gated channels comes from two sources: polypeptide folding schemes inferred from sequence analysis[15], and low-resolution models of the channels in gap junctions and acetylcholine receptors derived from X-ray scattering[23] and electron microscopic data[4,54]. The picture taking shape is one of homologous protein domains (four to six, contributed by one or more polypeptides) interacting laterally in the membrane to form one transverse polar compartment. The predominant structural elements are bundles of alpha-helices aligned normal to the membrane, twenty or more coming together to form a single pore. The total protein mass involved in making one of these channels is generally 200 kDa or greater.

There is another major class of eukaryotic pore that displays some of the same general functional characteristics as the alpha-helical-bundle channels but which falls in a very different structural category. These channels are the voltage-dependent pores in the mitochondrial outer membrane, initially called VDAC (voltage-dependent anion selective channels)[5,50] and later referred to as mitochondrial porin[8,14,20,48]. In terms of numbers, this class of channel probably ranks first in most eukaryotic cells, the constituent proteins occurring at densities of several thousand per square micrometer on the mitochondrial surface[8,14]. In terms of physiological importance, this channel controls the access of metal ions, metabolites and xenobiotics to the mitochondrial inner membrane, the site of oxidative phosphorylation in eukaryotes.

While the VDAC pore is larger than those formed by the alpha-helical-bundle channels, the amount of protein mass per pore is substantially less: only 30–60 kDa (see below). Also, the predominant secondary structural unit does not appear to be transmembrane alpha-helices. Instead, these channels may be designed along the lines of bacterial porins, with beta-sheet the dominant structural element[18].

This review summarizes the available structural information about the mitochondrial outer membrane pore, most of which has been obtained by electron microscopy and image analysis of two-dimensional crystals. A model is presented which is consistent with available data and mechanisms are suggested for the conformational changes involved in the functioning of this important eukaryotic pore.

## *Functional characteristics*

The mitochondrial outer membrane channel is very permeable to small inorganic ions, showing a step conductance of 700 pS (pico Siemens) in 150 mM KCl. (Contrast this with a step conductance of 20 pS for rat brain sodium channel[1].) The VDAC channel is not ion-specific, as are many of the alpha-helical-bundle channels, but it does (despite its large size) display selectivity. It is generally more permeable to smaller than to larger solutes of like charge and it is more permeable to anions than to cations of similar size[5,50].

Large numbers of these channels on the mitochondrial outer membrane would account for the permeability properties of this membrane. For example, conductance measurements made by patch-clamping techniques are consistent with channel densities in the range expected from the pore-protein content of this membrane[51]. The mitochondrial outer membrane appears not to limit diffusion of respiratory substrates and adenine nucleotides, which have molecular weights in the range 100–400 and are generally negatively charged. At the other extreme of size and charge, the outer membrane is impermeable to holocytochrome c, a cationic polypeptide of molecular weight 12,000[55]. Moreover, there is recent evidence that the mitochondrial outer membrane may be impermeable to even smaller (mol. wt < 1000) organic cations, such as spermidine and adriamycin[32]. Thus, the VDAC channel may render the mitochondrial outer membrane generally more permeable to anionic compounds, such as respiratory substates, than to organic cations, which tend to disrupt oxidative phosphorylation.

The mitochondrial outer membrane VDAC channel is also voltage-gated, i.e. it switches to lower-conducting substrates (approx. 300 pS) when exposed to transmembrane potentials as low as 20 mV. Not only is the voltage-induced 'closed' state less conducting than the normal 'open' state, it is reported to have opposite charge selectivity, i.e. it is more permeable to cations than anions. There is recent evidence that the same closed state is induced in VDAC by synthetic anionic polymers[2,7] and by an endogenous mitochondrial modulator[16]. Thus, the prospect is raised that the permeability of the mitochondrial outer membrane may be actively regulated, either by transmembrane potential or by binding of macromolecular ligands to VDAC. The effect of the 'open' to 'closed' VDAC transition on mitochondrial metabolism may be profound. For example, it has been reported that the polyanionic modulator of VDAC inhibits the diffusion of ATP across the mitochondrial outer membrane[2].

## *Analysis of primary structure*

The VDAC (mitochondrial porin) polypeptides are not particularly hydrophobic. Polarity indices based on amino acid composition are about the same as those of water-soluble, globular proteins[14,20]. In fact, a water-soluble form of VDAC can be produced by organic solvent extraction of bound lipid[47].

The amino acid sequences of VDAC from yeast (*Saccharomyces cerevisiae*)[12,42] and fungus (*Neurospora crassa*)[17] have been inferred from cDNA sequences. When subjected to 'hydropathy' analysis[19], these sequences do not show the predominantly hydrophobic, 20-residue runs which are commonly considered to be indicators of transmembrane helices. Instead, there is a general pattern of alternating polar/nonpolar amino acids along much of the VDAC sequences. Beta-sheets formed from these sequences would be amphipathic, with distinct polar and nonpolar sides, well suited to forming an interface between lipid and aqueous compartments[12]. Assuming that much of the VDAC sequence is used to form transmembrane beta-sheets, the high polarity index of the polypeptide is understandable. As many residues may be in contact with the aqueous inner compartment of the wide pore as with the lipid phase in which the protein is embedded.

Computer algorithms have been used to search for similarities between the amino acid sequences of mitochondrial pore proteins and other classes of transport proteins. No similarities have been detected between the

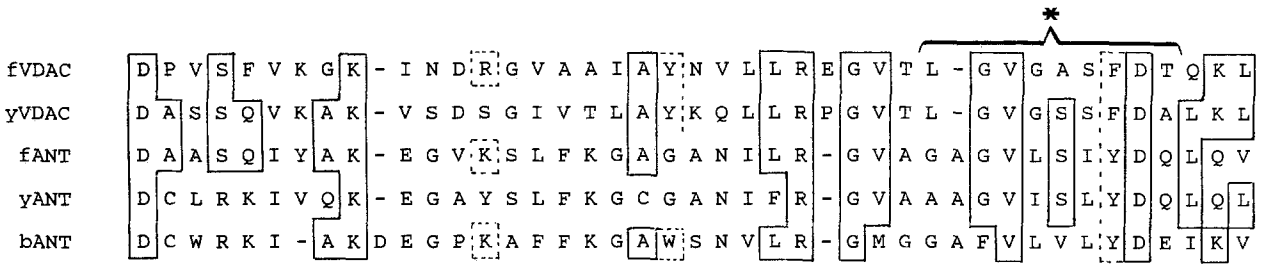


Figure 1. Local comparison of amino acid sequences near the C-termini of mitochondrial outer membrane channels (VDAC) and adenine nucleotide translocators (ANT)[17,30,42]. Prefixes: f, fungal (*N. crassa*); y, yeast (*S. cerevisiae*); b, bovine. The number of the first residue in each subsequence is fVDAC, 228; yVDAC, 228; fANT, 262; yANT, 262; bANT, 255. Residues enclosed in solid boxes are identical in at least one VDAC and one ANT sequence. Broken boxes enclose non-identical residues that share either basic or very hydrophobic character. The percent identity between yVDAC and fANT in this region is 38%. Bracket with * indicates putative DCCD binding site of yVDAC[44].

VDAC sequences and bacterial (*E. coli* and *Salmonella*) porin sequences available in the data banks[12,17,30]. Thus, either the two types of pore proteins are not related or any evolutionary link has become irretrievably blurred over the eons since the branching of mitochondria from eubacteria. It may be of interest to compare mitochondrial pore sequences with those of porins from bacterial species closer to the postulated branch point, i.e. the alpha group of purple photosynthetic bacteria.

In the course of these 'homology' searches, a region of moderate similarity (31% identity over 57 amino acids) was found to occur near the C-termini of yeast VDAC and *Neurospora* mitochondrial adenine nucleotide transporter[30] (fig. 1). This was later confirmed with the *Neurospora* VDAC sequence[17]. The statistical significance of this agreement is borderline, but should be considered in light of the modest overall amino acid identity (43%) between the two ascomycete VDAC sequences[17]. VDAC may be an example of strong structural (and functional) conservation with a minimum of sequence retention. For example, polyclonal antibodies to mitochondrial pore proteins of higher and lower eukaryotes do not cross-react[8,33], despite the fact that the functional characteristics of the channels are almost indistinguishable. (This weak sequence conservation, of course, lowers the odds of linking the VDAC sequences to putative ancestral bacterial pore sequences.) The limited similarity between VDAC and the adenine nucleotide transporter may be spurious or it may be real, reflecting a common derivation of the two types of mitochondrial transport proteins.

Another interesting aspect of the primary structure of VDAC is the occurrence of two regions near the C-terminus of the yeast sequence that resemble DCCD (dicyclohexylcarbodiimide) binding sites in other proteins[44]. One of these short segments occurs in the region of the VDAC sequence that shows strongest agreement with that of the adenine nucleotide transporter (fig. 1). DCCD has been shown to bind to the channel in the mammalian mitochondrial outer membrane[9]. While this binding does not effect the permeability properties of VDAC, it inhibits the binding of hexokinase to the channel[45]. (VDAC has been identified as the hexokinase receptor of mammalian mitochondria[11,21]).

*Pore arrays in the outer membranes of plant mitochondria*

Early electron microscopic studies of negatively stained plant mitochondria indicated the presence of numerous close-packed, stain-accumulating subunits in the outer membrane[46]. The presence of these in-plane subunits was later confirmed by X-ray scattering studies and associated with a major class of trypsin-resistant 30-kDa polypeptides in this membrane[31]. It was subsequently demonstrated that the same polypeptides induced the formation of large pores when reconstituted in liposomes[56]. Thus, the connection was established between the pore-like subunits in the mitochondrial outer membrane and the channel-forming activity of VDAC or mitochondrial porin.

X-ray scattering data from centrifugally oriented specimens of the plant membrane were consistent with the presence of randomly packed, in-plane subunits with a mean outer diameter of 4–5 nm[31]. Maxima in the vicinity of 0.52 nm, diagnostic for alpha-helical structure, were not seen in the diffraction patterns. More detailed analysis of the arrays seen in electron microscopic images of the plant membranes was not attempted. Such analysis would be complicated by the tight packing of the subunits and the overlapped information in projection images of membrane layers in collapsed vesicles.

*Periodic arrays of fungal VDAC: Electron image analysis*

In examining electron images of other kinds of mitochondrial outer membranes, it was found that those from the fungus *N. crassa* occasionally contained ordered arrays of stain-accumulating subunits like those seen in the plant membrane[24]. The main protein component of the fungal outer membranes is a 30-kDa trypsin-resistant polypeptide with in vitro pore-forming activity[14]. Antibodies to the 30-kDa pore protein bound specifically to the periodic outer membrane arrays, indicating that the arrays are composed of the pore protein[33].

It was subsequently found that crystallization of the fungal mitochondrial outer membrane channels could be reproducibly induced by treatment of the membranes with soluble phospholipase $A_2$[25]. Since this is an en-

dogenous mitochondrial activity, it has been suggested that ordering of the channels might occur in vivo, perhaps in response to elevated levels of $Ca^{+2}$, an activator of the mitochondrial enzyme[28]. In any event, the availability of two-dimensional crystalline arrays of VDAC has proven to be critically important for the structural analysis of this channel. In particular, it has allowed the application of low-dose electron microscopic and computer image processing techniques in the study of these channels.

The main analytical techniques employed in these studies have been:

a) quasi-optical Fourier filtration[43], which takes advantage of the separation of periodic and random image components in Fourier space to produce averages of individual crystalline layers in electron microscopic projection images, and

b) correlation averaging[13,49], in which averages are formed by summation of image subfields located at precise unit cell coordinates. (This technique can yield higher resolution averages than Fourier filtration since it corrects for lattice disorder in the membrane arrays).

Both techniques (which are described in detail elsewhere[40]) may be applied to images of overlapped periodic arrays to yield independent averages of the different layers. The only condition is that maxima on the different reciprocal lattices do not overlap in Fourier space.

*Geometry of the fungal VDAC arrays*

The channel arrays observed in outer membranes of Neurospora mitochondria are polymorphic[26,33,40]. The most common form (fig. 2A) is a parallelogram (oblique) array with dimensions 13 × 11.5 nm and a lattice angle of 109°. The unit cell of this array contains six circular negative-stain-accumulating sites (each 2–3 nm in diameter, 4.5–5 nm apart) arranged on a hexagon with a central axis of two-fold rotational symmetry. As will be described below, each of these heavily stained features is the projection of a stain-filled cylinder that traverses the membrane[39], i.e. a channel. Lattice geometry changes with increased phospholipid removal from the membranes. First, a contracted version of the parallelogram lattice forms, apparently derived from the more oblique array by sliding of adjacent rows as indicated in figure 2B. Eventually, a very different array is observed, with near-rectangular geometry (fig. 2C). The smaller unit cell in the near-rectangular array (8.5 × 5 nm) holds only two channels.

Factors other than increased phospholipase $A_2$ treatment may also induce transitions in the planar VDAC crystals. In particular, the polyanion which modulates VDAC's gating characteristics appears to induce the oblique-to-contracted transition (see below).

*Size and shape of the fungal pore*

Three-dimensional reconstruction of the oblique VDAC array (fig. 2A) was obtained by combining electron microscopic projections from tilted, negatively stained specimens[39]. The result indicates that each of the stain centers is the projection of a stain-filled cylinder that is normal to and traverses the membrane.

Assuming that the channel lumens may be approximated by right cylinders, the diameters of their projections in negative stain should be a direct measure of mean inner diameter. In practice, the projected channel diameters were found to be stain-dependent[35,40]. Larger diameters were obtained with the anionic stain, phosphotungstate, than with weakly ionized uranyl salts (3.5 vs 2.5 nm). This indicated that positive staining effects might be occurring (see below). To eliminate such complications, averages were computed for VDAC arrays negatively stained with aurothioglucose, an uncharged, low-contrast embedding medium. The channel projections observed were consistent with the smaller diameters obtained with uranyl[37,40]. This channel bore, 2.5 nm, falls midway between predictions made on the basis of

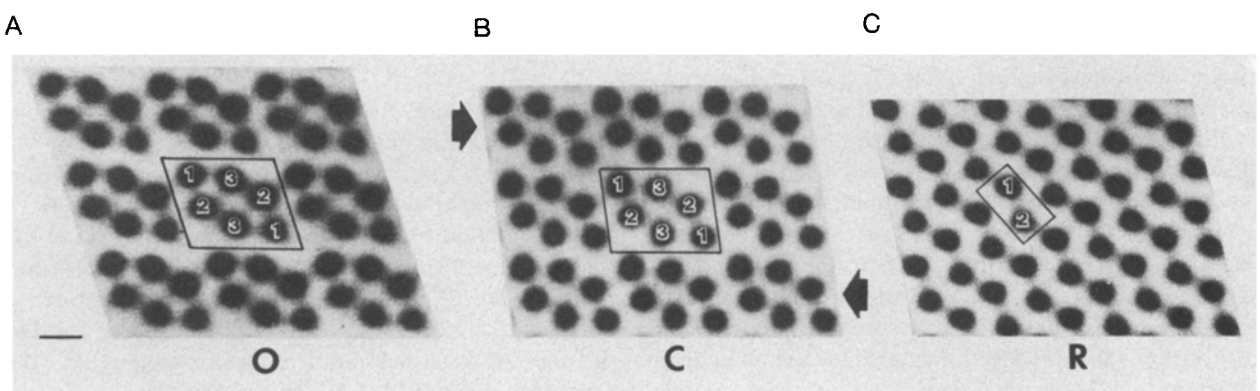


Figure 2. Polymorphic forms of the fungal VDAC membrane crystals. *O* Oblique parallelogram array; *C* contracted parallelogram array; *R* near-rectangular array. In the *O* and *C* arrays, there are six channels (densely stained circles) per unit cell. The crystallographic asymmetric unit consists of any combination of the three non-symmetry-related channels (1-2-3). In the *R* array, there are 2 channels in the unit cell. The transition from the *O* to *C* arrays corresponds to sliding of adjacent rows of the crystal in the directions indicated by the large arrows; coordination of the channels within each row is unchanged. The transition to the *R* array involves more significant changes in channel coordination.

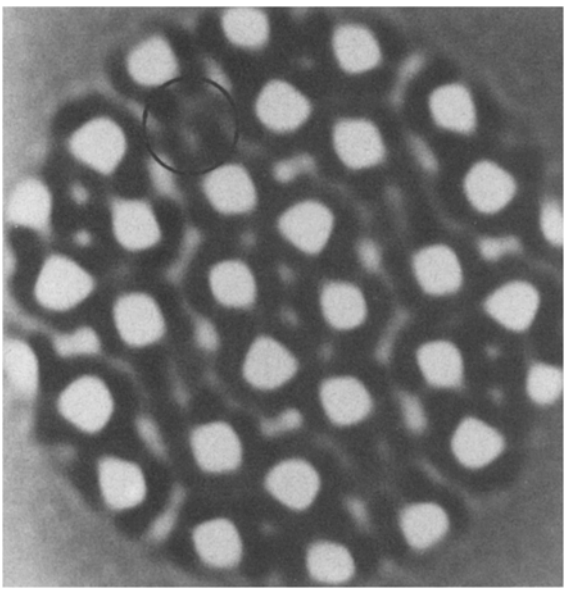

Figure 3. Correlation average of unstained, frozen-hydrated oblique array of *N. crassa* VDAC. Dark corresponds to high density (protein), white to low-density (e.g. water, lipid). The effective resolution of this average, summed over 400 unit cells from two different membrane crystals, is approx. 2.0 nm. (p2 symmetry has been enforced and density thresholding has been applied to enhance details outside the channel lumens). Circle encloses a region containing dense (protein) arms extending between the channel complexes.

permeability properties of the VDAC pore, 1.7 to 4.0 nm[6,8,14,48].

Recently, projection images of *unstained,* ice-embedded VDAC arrays have been obtained by cryo-electron microscopic techniques[38,40] (fig. 3). The contrast in these images arises from inherent density differences among the components of the arrays (protein, lipid, water). Thus correlation averages computed from these image fields correspond to maps of the projected density of crystalline VDAC. These density maps agree (to an effective resolution of 2 nm) with a simple model of thin-walled, 3.8-nm-diameter, hollow cylinders aligned normal to the membrane[38].

Assuming that the walls of the channel are formed by twisted beta-sheets, the diameter stated above would be that measured at the alpha-carbon backbone. Inner and outer channel diameters would depend on the distribution of amino acid residues that extend from the carbon backbone cylinder. Assuming that the residues form layers about 0.5 nm thick on both sides of the cylinder, mean inner and outer channel diameters would be 2.8 and 4.8 nm, respectively. These values are consistent with the lumen diameter derived from negative stain exclusion and the spacings between channels in the membrane crystals (see above).

The issue of the number of 30-kDa polypeptides that form each VDAC channel is presently unsettled. Based on space-filling considerations, there is room for either one or two polypeptides per channel in the unit cell of the oblique fungal array, depending whether lipid occupies 50 or 20% of the membrane volume[26,27]. Biochemical and functional data tend to support a dimer channel[3,6,22,47]. However, a monomer channel cannot presently be excluded from structural considerations alone. The cylinder diameter inferred from the density map of unstained crystalline VDAC, 3.8 nm, is close to that of the largest beta-barrel that can be formed from one 30-kDa polypeptide[12,38]. To form a pore this wide, essentially all of the sequence must be used to form transmembrane beta strands except for the first 20 residues at the N-terminus[12], which may form an amphipathic alpha-helix[17].

### *Structural model derived from array geometry and electron microscopic mapping*

The complex geometry of the oblique arrays of mitochondrial outer membrane channels can be explained by a fairly simple packing model[29]. In this model, only interchannel contacts that are invariant with lattice contraction are taken into account. Variable contacts are considered weak and ignored, as are changes associated with transition to the near-rectangular array (see below). The model (fig. 4 A) represents the channel as a projected cylinder divided into four sectors by radii A, B, C, D, each of which represents a point of interchannel contact on the outer surface of the cylinder. The channel packing in the oblique array can be accounted for by allowing only two types of lateral interactions ('bonds') between channels, A–B and C–D (fig. 5 A).

Considerable information about the location of biochemically important sites on the projection map of crystalline VDAC has been obtained from electron microscopic studies. This data is interpreted below in terms of the packing scheme of figure 5 A.

*a) Phospholipid domains* (fig. 5 B). The probable location of phospholipid regions in the channel arrays can be inferred from the projected density maps of unstained,

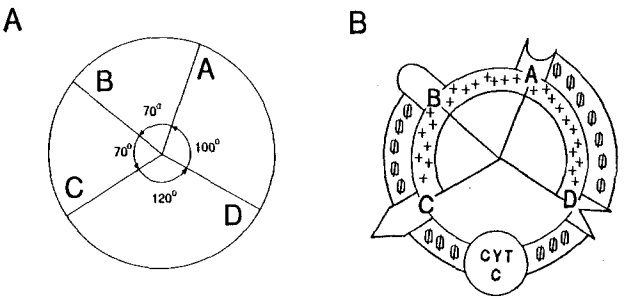


Figure 4. *A* Proposed distribution of interchannel contact points (labeled A through D) around one channel cylinder, inferred from the packing geometry of channels in rows of the parallelogram arrays. *B* Model of the channel derived from electron microscopic results. The two pairs of bonding points are represented as complementary geometric shapes. Sectors whose inner surfaces are rich in basic amino acids are indicated with +. Sectors whose outer surfaces contact lipid are indicated with ∅. The region in closest proximity to bound cytochrome is also indicated. Figures reproduced with permission from Mannella[29].

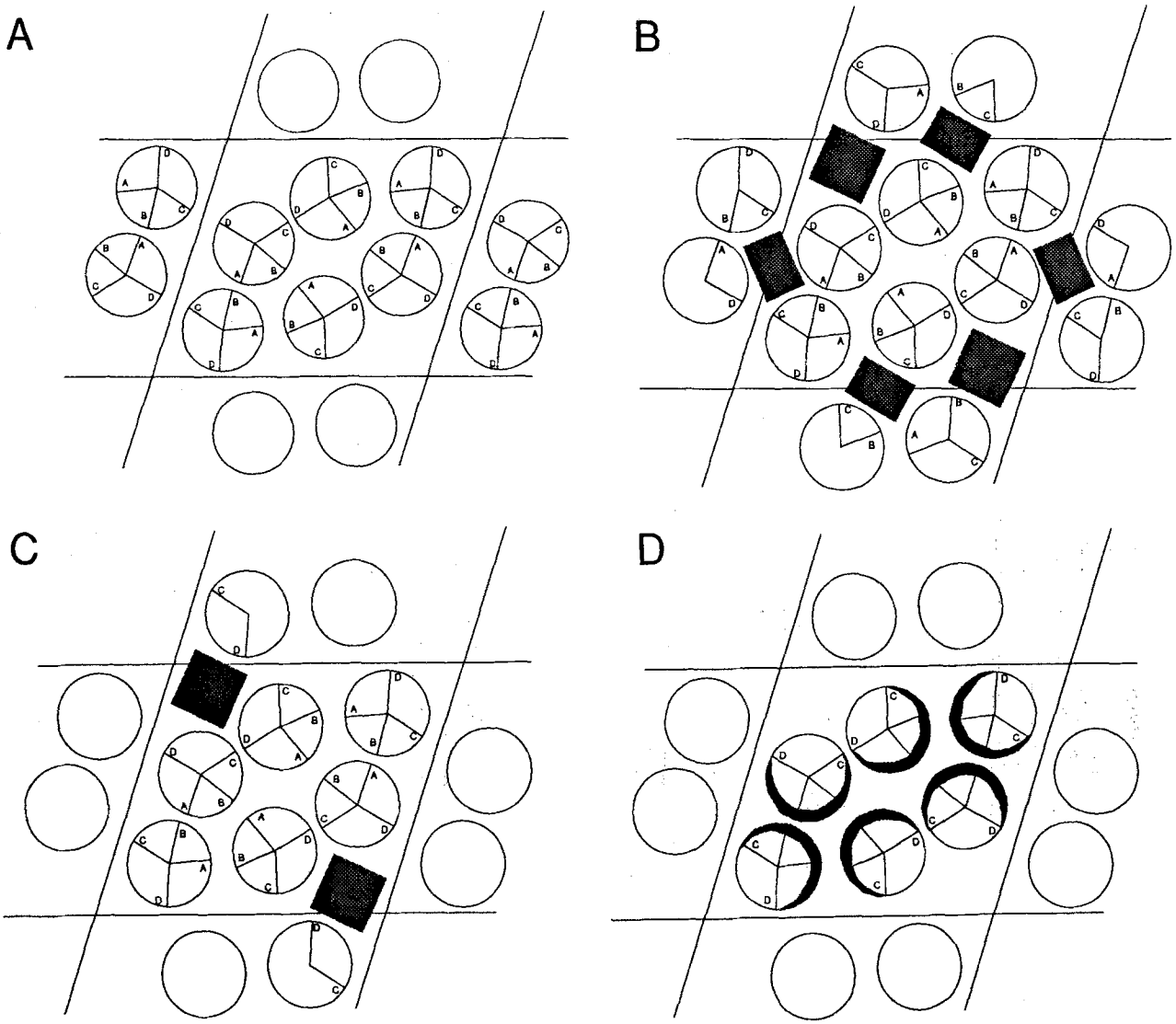


Figure 5. *A* Schematic version of the oblique VDAC array with contacts drawn according to the model of figure 4 A. Note that there are only two types of interchannel 'bonds', A–B and C–D. *B–D* Summary of electron microscopic mapping results (see text). Shaded regions correspond to *B* lipid domains, *C* cytochrome c binding sites, and *D* accessible basic amino acids. Figures reproduced with permission from Mannella[29].

frozen-hydrated arrays (fig. 3). There are six low-density sites located outside the channel 'hexagon' in the unit cell. These same sites in negatively stained specimens stain lightly with uranyl, which has an affinity for the polar head groups of phospholipids[52]. By the packing scheme of figure 5 A, these putative phospholipid domains fall into two classes: 1) those formed between B–C and D–A sectors of neighboring channels and 2) those bounded only by C–D sectors.

*b) Cytochrome c binding sites* (fig. 5 C). The issue of the possible involvement of VDAC in the import of mitochondrial proteins was addressed in a negative-stain study[41]. It was found that cytochrome c causes uranyl acetate to be excluded from specific sites on the array, which were thereby inferred to be loci at which the protein binds to the array surface. These binding sites were identified as phospholipid domains by the criteria in the previous paragraph. In fact, the sites of attachment of cytochrome c correspond only to the type 2 phospholipid regions. In the initial study[41], it was suggested that phospholipid domains organized by mitochondrial outer membrane proteins might serve as initial receptors for cytochrome c (and other imported proteins). The fact that only certain phospholipid domains bind the cytochrome suggests a specificity in these interactions (see paragraph below).

*c) Basic amino acids* (fig. 5 D). The anionic stain phosphotungstate has an affinity for basic amino acids[53]. Positive staining of exposed basic amino acids by this stain was proposed to account for its heavier accumulation at the channel openings in VDAC arrays, compared with other stains[35,40]. (There are 28 basic amino acids in the *N. crassa* sequence[17].) To test this hypothesis, comparisons were made of projection averages of normal and

succinylated arrays[36,40]. Succinylation of proteins changes the charge on exposed lysines from +1 to −1 and so should inhibit binding of anions, such as phosphotungstate. Succinylation has pronounced effects on the functional properties of VDAC, causing reduced voltage dependence and reversing the ion selectivity[10]. Thus, basic amino acids are implicated in both mechanisms.

After succinylation of VDAC arrays, phosphotungstate accumulation at the mouth of the channel projections decreased, expected if the accumulation was due to positive staining of basic amino acids. The decrease was not radially uniform, but was greatest at the inside of the channel 'hexamer'. In the packing scheme of figure 5A, the C–D sector of the VDAC channel is always turned to the outside of the hexagonal groups, suggesting that this sector is deficient in basic amino acids.

*d) The VDAC model.* The above results are summarized in the model of figure 4B[29]. VDAC is represented as an axially projected cylinder (beta-barrel) divided radially into four sectors by contact points A through D. The cylinder has inner and outer surfaces, composed of inward and outward facing residues, respectively. Three of the sectors contact lipid, suggesting that the corresponding regions on the outer cylinder surface contain numerous hydrophobic residues. Three sectors that face inside the unit cell contain most of the accessible basic amino acids, represented as lining the inner cylinder surface. The sector lacking basic amino acids is also the one in closest proximity to bound cytochrome c. (The apparent specificity of cytochrome c for phospholipid domains bordered by this part of the channel may actually reflect electrostatic repulsion of the basic protein from the vicinity of the other channel sectors.)

While the structural model derived from the above considerations is far from complete, it provides important constraints on predictions of folding schemes for the VDAC polypeptide.

### *Structural effects of the polyanionic modulator*

As noted above, a synthetic anionic polymer has been shown to cause VDAC to switch to its 'closed' state and to increase the voltage dependence of the channels remaining 'open'[2,7]. This polyanion has several pronounced structural effects on crystalline VDAC[37]. Incubation of the arrays with the polyanion results in: 1) disordering of oblique arrays and increased occurrence of the contracted polymorph, 2) decrease in the mean projected diameter of the channel lumen from 2.5 to 1.7 nm, and 3) the appearance of new, sinuous zones of stain exclusion around the outside of the channels.

The new zones of stain exclusion probably represent the binding sites of the polyanion, which is a co-polymer of styrene, maleate and methacrylate. Its amphiphilic character may account for its apparent affinity for protein/lipid boundaries. The two other structural changes induced by polyanion binding, i.e. crystal phase transition and decreased pore size, do not appear to be directly related. In particular, the projected pore diameters of contracted arrays decrease from 2.5 to 1.7 nm as do those of oblique arrays, with polyanion treatment[37]. It is presently unclear whether this decreased lumen diameter (which is consistent with the observed conductance decrease) is due to a conformational change in the channel or to blockage of the channel by the polyanion.

### *Speculation on the nature of conformational changes in VDAC*

The available sequence and structural information about VDAC poses as many questions as it answers. The data point to a relatively (perhaps deceptively) simple fundamental structure, namely that of a transmembrane cylinder formed by twisted beta-sheets. There are likely to be other structural domains in the protein as well, such as an amphipathic alpha-helix predicted to occur at the N-terminus[17]. This helix may be embedded in the membrane, perhaps stabilizing the beta-barrel[3]. Alternatively, it may extend across the surface of the bilayer, a possibility suggested by the 'arms' which extend between the channels in projection images of frozen-hydrated specimens (fig. 3). The areas in the array which these arms occupy shrink in the transition from oblique to contracted lattice geometry. Thus, this crystal phase transition (induced by the polyanionic modulator) may correspond to movement of the amphipathic helices off the bilayer surface (fig. 6). Once detached, the helical arm might move to a position that alters the ion selectivity of the channel (e.g. by screening fixed charges at the mouth). It might also bind to the channel cylinder in a way that makes subsequent closure more likely.

As noted above, the oblique-to-contracted transition of the array probably does not correspond to channel closure itself. The average projected pore diameter is about the same in both polymorphs. Also, this transition only minimally effects the coordination of the channels in the membrane plane, suggesting that the corresponding structural change in the channels is small. By contrast, measurements of VDAC's response to osmotic stress suggest that the conformational change associated with closure involves a large decrease in lumen volume[57].

Unlike the oblique-to-contracted crystal transition, the transition of the VDAC array to near-rectangular geometry involves significant changes in channel coordination, suggesting a major conformational change. Also, three-dimensional reconstruction of negatively stained rectangular VDAC arrays indicates that the channel lumens may be shorter and tilted about 10° with respect to those in the oblique array (Mannella, unpublished data). There is presently no hard evidence about the nature of the conformational change associated with VDAC closure. It has been proposed that it might involve removal of strands from the beta-barrel[3]. Alternatively, the barrel

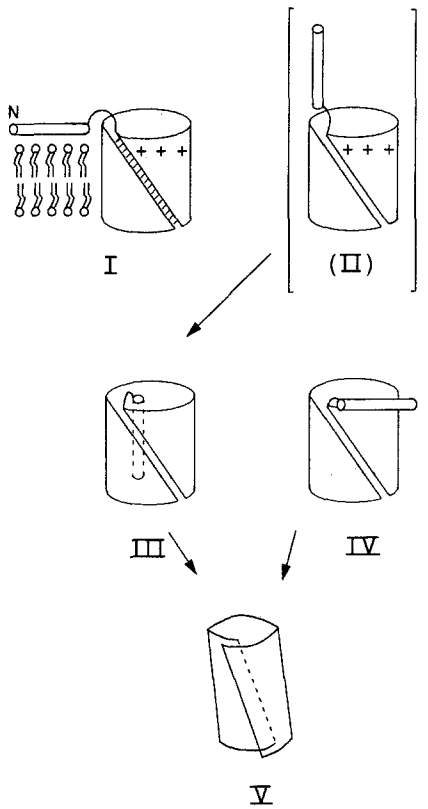


Figure 6. Speculation on the nature of the structural changes that VDAC may undergo. The channel is represented as a cylinder formed by a twisted beta-sheet (i.e. beta-barrel) with an alpha-helical 'arm' at the N-terminus (state I). The H-bonded 'seam' connecting the opposite ends of the sheet is indicated by hatching, showing the tilt of the beta-strands (38° for a monomer channel)[38]. Fixed positive charges are indicated on one side of the channel entrance. In this conformation, corresponding to that in the oblique array, the helical arm is extended on the bilayer surface. Loss of phospholipid or presence of the polyanionic modulator causes the arm to detach from the bilayer (state II) and transition of the crystal to the contracted lattice. As drawn, state II is a kind of 'transition state', from which the arm may either enter the channel (perhaps interacting with the 'seam', state III), or bind to the mouth of the channel (perhaps screening the fixed charges at the entrance, state IV). Finally, the cylinder itself narrows, perhaps by partially untilting its beta-strands and/or wrapping on itself (state V), corresponding to the crystal transition to near-rectangular geometry.

diameter might be narrowed by decreasing the tilt of the beta-strands and/or partially rolling the beta-sheet on itself (like a jelly roll). Both types of mechanisms would be expected to significantly reduce channel volume and alter the alignment of interchannel contact points.

The above speculations are summarized in figure 6, in which the channel is represented as a monomer for simplicity. The same conformational changes are also possible with a dimer channel, in which case more protein would be outside the channel cylinder.

Clearly, much remains to be learned about the structure of the voltage-dependent channels in the mitochondrial outer membrane. The prospect for a high-resolution 3-D reconstruction of the unstained channel is heightened by recent results with frozen-hydrated specimens. (Maxima out to 1 nm are detectable in Fourier transforms of crystal projections after correlation averaging[38].) Such a reconstruction of the three crystal forms of VDAC would provide considerable information about the fundamental structure of the channel and the conformational changes which it undergoes. This data together with insights gained from molecular modeling[12,15] and site-directed mutagenesis of the VDAC polypeptide[3], should begin to elucidate the mechanisms underlying the operation of this important channel.

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