Crystal packing analysis of murine VDAC1 crystals in a lipidic environment reveals novel insights on oligomerization and orientation

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Abbreviations: VDAC, voltage dependent anion channel; OMM, outer mitochondrial membrane; hVDAC1, human VDAC1; mVDAC1, mouse VDAC1; LDAO, n-dodecyl-N,N-dimethylamine-N-oxide; EM, electron microscopy; AFM, atomic force microscopy; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine

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All eukaryotic cells require efficient trafficking of metabolites between the mitochondria and the rest of the cell. This exchange is carried out by the dominant protein in the outer mitochondrial membrane (OMM), the Voltage Dependent Anion Channel (VDAC), which serves as the primary pathway for the exchange of ions and metabolites between the cytoplasm and the intermembrane space of the mitochondria. Additionally, VDAC provides a scaffold for the binding of modulator proteins to the mitochondria and has been implicated in mitochondrial-dependent cell death. We recently determined the structure of the murine VDAC1 (mVDAC1) at 2.3 Å resolution crystallized in a native-like bilayer environment. The high-resolution structure provided concise structural details about the voltage-sensing N-terminal domain and catalyzed new hypotheses regarding the gating mechanisms for metabolites and ions that transit the OMM. In this study, the crystal packing of mVDAC1 is analyzed revealing a strong antiparallel dimer that further assemble as hexamers mimicking the native oligomeric packing observed in EM and AFM images of the OMM. Oligomerization has been shown to be important for VDAC regulation and function, and mVDAC1 crystal packing in a lipidic medium reveals insights on how oligomerization is accomplished using protein-protein and protein-lipid interactions. Furthermore, orientation of VDAC in the OMM remains uncertain due to inconsistencies in antibody labeling studies. The physiological implications of a novel antiparallel arrangement are addressed that may clarify these conflicting biochemical data.

VDAC serves as an important node in the cellular crosstalk between mitochondria and the rest of the cell, facilitating free exchange of ions and metabolites including ATP across the OMM.1 In addition to its metabolic and energetic functions, VDAC appears to have a more complex role, serving as a scaffold for molecules and proteins that modulate the organelle’s permeability, and thereby its function.2-4 This cell death/survival role has implicated VDAC in the metabolic stresses of cancer and cardiovascular disease specifically as well as mitochondrial-dependent cell death in general.5-7 Thus, understanding the structure, function and protein interactions of VDAC constitutes a critical objective for basic and medical research.

Recently, three publications reported with increasing detail, the long sought after structure of VDAC. Hiller et al. resolved the solution structure of hVDAC1 by NMR spectroscopy in the presence of the detergent LDAO; Bayrhuber et al. applied a combinatorial approach of NMR spectroscopy and X-ray crystallography to obtain a medium resolution structure (4 Å) again of hVDAC1 in the detergent Cymal-5; and our group obtained a high-resolution structure (2.3 Å) of mVDAC1 from crystals grown in a more natural lipidic environment (bicelles) by X-ray crystallography.8-10

All three structures reveal an amphipathic β-barrel motif formed by 19 β-strands, representing a new fold of outer membrane β-barrel proteins with an odd number of strands. The high-resolution mVDAC1 crystal structure showed, with the most clarity, the orientation of the N-terminal voltage sensing domain that transverses the entire pore. The α-helix portion of the N-terminal segment is positioned halfway through the pore causing a narrowing of the cavity,
where it is ideally situated to regulate metabolite flux. Both N- and C-termini face the same side of the membrane, but inconsistencies in previous, as well as recent, antibody labeling studies preclude the accurate orientation of VDAC within the OMM.11–13

Analysis of the crystal packing of mVDAC1, grown within a native-like lipid medium, revealed a strong antiparallel dimer interface, which further assembles as hexamers matching the native arrangement observed in EM and AFM images of the OMM.14,15 The oligomerization of VDAC has been proposed to have important physiological roles for regulation of VDAC function, binding of proteins to the OMM and mitochondria-dependent apoptosis.16–18 We present crystal packing analysis of mVDAC1 and address a novel antiparallel arrangement that clarifies some conflicting biochemical data about topology and further reveals insights into the packing arrangement of VDAC oligomers.

Packing Analysis of mVDAC1 Crystals

The oligomerization of VDAC is widely viewed as an essential component for a number of fundamental mitochondrial functions—ranging from scaffolding for the binding of modulator proteins from both sides of the OMM to facilitating the release of cytochrome c during apoptosis.16–18 Biochemical studies have shown VDAC to conform to a number of oligomeric species.18,19 A recent AFM study, on freshly isolated OMM of potato tubers, revealed the most frequently identified VDAC oligomer to be hexamers that may serve as platforms for hexokinase binding to the mitochondria or result in mega-pore formation with pro-apoptotic proteins such as Bax or Bak resulting in the release of cytochrome c in the event of mitochondrial-dependent cell death.15

Greater insights into the interaction facilitating oligomerization of VDAC1 can be seen through packing analysis of mVDAC1 (PDB 3EMN) crystals, grown in a native-like bilayer environment using lipidic bicelles. A crystallographic-imposed antiparallel dimer formed by transmembrane β-strands 1, 2, 3, 4, 18 and 19 (Fig. 1A) is observed. The dimer interface has a buried surface area of 2,376 Å² composed of 127 inter-monomer contacts contributed by 36 residues per protomer.20 All contacts are formed by van der Waals interactions with the exception of a lone hydrogen bond, situated in the middle of the membrane. The lipid DMPC, shown using black spheres, is sandwiched between the dimers and stabilizes the interface.

Figure 1. (A) Cartoon representation of the mVDAC1 antiparallel dimer. The protomers are shown in blue and yellow displaying opposite topologies. The N-terminus of each protomer is indicated. The yellow protomer has its N- and C-termini facing the inter-membrane space of the mitochondria while the blue protomer has its N- and C-termini facing the cytoplasm. Ser-43 from each protomer is shown forming a hydrogen bond in the middle of the membrane plane. (B) Surface representation of the mVDAC1 dimer-dimer interface. Antiparallel mVDAC1 dimers associate laterally within the membrane plane to generate a hexamer arrangement.

Is the Asymmetry Physiological?

It is intriguing to speculate as to whether the antiparallel dimer and the resulting hexamer formation seen in the mVDAC1 crystal packing are physiologically relevant. There are a number of comprehensive arguments in support of this scenario: First, mVDAC1 was crystallized in lipidic bicelles that mimic the physiological bilayer environment and possibly represent the native arrangement within the OMM. Second, the antiparallel mVDAC1 dimer interface is considerably tighter than the observed parallel interface of hVDAC1 dimer. Third, the hexamer packing blo...
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identified in mVDAC1 crystals mimics the native arrangement observed by EM and AFM studies on the OMM. Thus, an antiparallel arrangement of VDAC1 may be a physiological requirement for a compact dimer suitable for composing a hexameric arrangement seen in native membranes.

Further evidence from previous scientific reports suggests a dual-directionality of VDAC in the outer membrane of the mitochondria. Early studies from Pinto et al. labeled intact mitochondria with antibodies generated against the N-terminus of VDAC indicating a cytoplasmic orientation while Stanley et al. showed a contradictory result where the N-terminus faces the intermembrane space. It has been widely speculated that the differences between the studies were the result of improper handling and isolation of the mitochondria in the former study. A more recent study further complicates the issue where McDonald et al. probed the membrane orientation of yeast VDAC1 with FLAG-epitopes designed using the hVDAC1 and mVDAC1 structures. While epitopes FLAG1 and FLAG5 located on the N-terminal region and C-terminus respectively, suggested an orientation of VDAC with the C-terminus exposed to the cytosol; epitopes FLAG2 and FLAG3 located on loop regions facing opposite sides of the OMM, were more ambiguous (Fig. 3). Both VDAC-FLAG2 and VDAC-FLAG3 bound antibody in intact mitochondria, but binding increased significantly upon membrane solubilization, suggesting that both sides of VDAC are exposed to the cytosol and the inter-membrane space. The authors attributed these conflicting results to dynamic behavior of this region formed by β-strands 1–6. It would appear that the topological orientation of VDAC in the OMM is still an ongoing debate.

Dual-Topology Membrane Proteins

It is generally believed that integral membrane proteins have only a single orientation within the membrane. Recently, however, dual-topology proteins displaying opposite orientations in the membrane have been reported. The most well documented of these is the Escherichia coli proton-coupled multidrug transporter EmrE. Supporting evidence for this observation came from structural studies on 2-D and 3-D crystals of EmrE that show an asymmetric dimer with the protomers in an antiparallel topological orientation. Additionally, von Heijne's group found using a comparative genomics approach that EmrE has a dual-topology. Importantly, recent biochemical studies from the Schuldiner group show that EmrE displays unique promiscuous behavior where protomers can adopt different topologies relative to each other and to the lipid bilayer (personal communication by Professor Schuldiner), and yet are still capable of transport. The authors show that two EmrE protomers can associate in a parallel Cin - Cin orientation, parallel Cout - Cout orientation or an antiparallel Cin - Cout orientation and remarkably all retain functionality as a homodimer.

In addition to EmrE, von Heijne's group identified four other dual-topology candidates, SugE (multi-drug resistance protein), CrcB (camphor resistance protein), YdgC (associated with alginate biosynthesis) and YnfA. It has been suggested that the flip-flopping of membrane proteins to adopt a mixture of topologies may serve as an effective strategy to satisfy functional requirements.

Furthermore, it may represent a stage in the topological evolution of membrane proteins resulting from gene duplications.
and mutations.\textsuperscript{29} Although the list of identified dual-topology membrane proteins is small, the idea that proteins can adopt multiple topologies in the membrane has been established.

There is increasing evidence to support an antiparallel topology for VDAC in the mitochondria. A dual-topology model will help to explain the inconclusive results of topology studies and will link the hexamers observed in the OMM with those seen in mVDAC1 crystals grown in lipidic bicelles. The hypothesized bi-orientation of VDAC discussed here establishes a model to be tested biochemically.

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