

Dissecting the Actinoporin Pore-Forming Mechanism

Crystal structures of the eukaryotic pore-forming toxin sticholysin II, its complex with phosphocholine, and electron microscopy of 2D crystals on lipid monolayer give insights into the sequential steps of the actinoporin membrane penetration.

Pore-forming toxins (PFTs) are an example of proteins that insert spontaneously into membranes. In contrast to regular membrane proteins, they are converted from a soluble form to become integral membrane residents without the aid of chaperones and translocons. They often oligomerize on the membrane surface before puncturing the lipid bilayer, thereby corrupting the cell integrity (Figure 1). The crystal structure determination of the heptameric staphylococcal α -toxin was a vital step in understanding the conformational changes correlated with binding, insertion and oligomerization of PFTs. α -toxin is a paradigm for β -PFTs, which use amphipathic β -hairpins to build a stable transmembrane β -barrel pore (reviewed by Heuck et al., 2001). On the other hand, structural data on α -PFTs, toxins that use α helices to perforate the membrane (for example colicins; Lakey and Slatin, 2001), are lacking essentially because water-filled bundles of transmembrane helices are unstable.

A unique group of the α -PFTs are the 20 kDa actinoporins from sea anemones (reviewed by Anderluh and Maček, 2002). These toxins have been shown to require a phosphocholine lipid headgroup for binding; however, membrane permeabilization was promoted by sphingomyelin and lipids producing negative membrane curvature (de los Rios, 1998; Valcarcel et al., 2001). Recently solved crystal and solution structures of equinatoxin II (EqII) from *Actinia equina* (Athanasiadis et al., 2001; Hinds et al., 2002) and sticholysin II (StnII) from *Stichodactyla helianthus* (Hermoso et al., 2003) revealed remarkable features of the actinoporins structure: a compact β sandwich of ten β strands flanked on each side by two short α helices, the N-terminal stretch (1–29) bearing an amphipathic helix 1, and a cluster of aromatic amino acids situated on loops on one side of the β sandwich and on the helix 2. A well-conserved melittin-like amphipathic segment within the N terminus of actinoporins has been shown to have an important role in pore-formation (Malovrh et al., 2003). In this issue of *Structure*, Hermoso et al. (2003) have now made a significant progress by solving the crystal structure of StnII with bound phosphocholine, the headgroup of the acceptor membrane lipids. The cavity with bound phosphocholine is surrounded by side chains from helix 2, the tip of the β sandwich and a residue from the aromatic cluster. It is remarkable that the residues involved in phosphocholine binding are also strictly conserved in

the 3D structure of EqII. Furthermore, the phosphocholine binding site is colocalized to a Trp and Tyr-rich cluster that was shown to provide initial contact with the lipid membrane (Hong et al., 2002 and refs. therein). Finally, the actinoporin β sandwich, equipped with the phosphocholine binding site and aromatic cluster is structurally similar to domain 4 of a bacterial pore-forming toxin perfringolysin, a domain shown to interact with lipid bilayers (Heuck et al., 2001).

Besides solving the X-ray structure of StnII, Hermoso et al. (2003) also provide an analysis of TEM micrographs of negatively stained 2D crystals of StnII grown on phosphatidylcholine monolayers. Micrographs to 15 Å resolution revealed regular tetrameric assemblies on the lipid monolayer surface, each of them with a central cavity of 50 Å in diameter. A model of the StnII tetramer on the lipid monolayer was constructed by docking the atomic model of the soluble StnII into the 3D reconstruction of the micrographs. An excellent fit could be obtained simply by a pseudo-rigid body movement of the N-terminal region 1–28 around the loop between helix 1 and strand β 2 while the β sandwich fold remained intact. The combined 3D structural data lead them to a putative model of a tetrameric transmembrane pore. They propose that the monomers are attached to the bilayer by the aromatic cluster on the β sandwich plus helix 2, while the N terminus, extends to a longer helix, which protrudes the bilayer. The model predicts a toroidal type of pore consisting of four amphipathic helices tilted at an angle of 30° from the membrane normal with lipid headgroups intervening between them. A very similar model, predicting a helix tilt angle of 23°, has been derived recently for EqII by using mutational analysis and biophysical analysis (Malovrh et al., 2003).

That nature solves new tasks by recombination of classic polypeptide structural elements is thus also seen in the case of actinoporins. Combination of the N-terminal melittin-like amphipathic helix and the β sandwich fold armed with the aromatic cluster makes actinoporins very efficient membrane-damaging proteins with a unique mechanism of membrane penetration. However, there are still unanswered questions. What are the contact surfaces driving oligomerization, if any at all? Do membrane lipid rafts serve as platforms to foster pore formation? Is there any role for the RGD motif found in actinoporins? Finally, this clear model of the actinoporin toroidal pore in the bilayer membrane opens the door to direct experimental verification.

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Selected Reading

- Anderluh, G., and Maček, P. (2002). *Toxicon* 40, 111–124.
Athanasiadis, A., Anderluh, G., Maček, P., and Turk, D. (2001). *Structure* 9, 341–346.

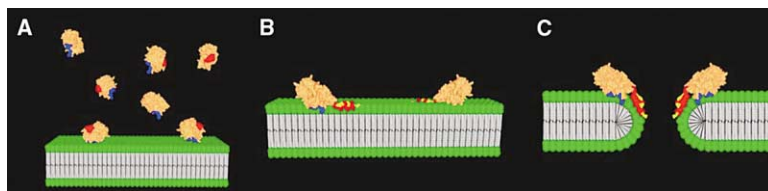


Figure 1. Mechanism of Pore Formation by Actinoporins

(A) Toxin monomers bind to the membrane surface by an aromatic rich surface (blue). (B) The N-terminal segment with amphipathic α helix (red) dislocates from the β sandwich core and anchors into the membrane-water interface, exposing polar residues toward solution.

(C) After aggregation of four monomers, a transmembrane funnel-shaped pathway is set up by lipid headgroups and helices, exposing side chains of acidic amino acids (yellow) in the lumen of the toroidal pore. Residues 1–10 are not shown.

de los Rios, V., Mancheño, J.M., Lanio, M.E., Oñaderra, M., and Gavilanes, J.G. (1998). *Eur. J. Biochem.* 252, 284–289.

Heuck, A.P., Tweten, R., and Johnson, A.E. (2001). β -barrel pore-forming toxins: intriguing dimorphic proteins. *Biochemistry* 40, 9065–9073.

Hermoso, J.A., Mancheño, J.M., Martín-Benito, J., Martínez-Ripoll, M., and Gavilanes, J.G. (2003). *Structure*, 11, this issue, 1319–1328.

Hinds, M.G., Zhang, W., Anderluh, G., Hansen, P.E., and Norton, R.S. (2002). *J. Mol. Biol.* 315, 1219–1229.

Hong, Q., Gutiérrez-Aguirre, I., Barlič, A., Malovrh, P., Kristan, K.,

Podlesek, Z., Maček, P., Turk, D., Goñzález-Mañas, J.M., Lakey, J.H., et al. (2002). *J. Biol. Chem.* 277, 41916–41924.

Lakey, J.H., and Slatin, S.L. (2001). *Curr. Top. Microbiol. Immunol.* 257, 131–161.

Malovrh, P., Barlič, A., Dalla Sera, M., Podlesek, Z., Lakey, J.H., Maček, P., Menestrina, G., and Anderluh, G. (2003). *J. Biol. Chem.* 278, 22678–22685.

Valcarcel, C.A., Dalla Sera, M., Potrich, C., Bernhart, I., Tejuca, M., Martinez, D., Pazos, F., Lanio, M.E., and Menestrina, G. (2001). *Biophys. J.* 80, 2761–2774.

Let's Sco1, Oxidase! Let's Sco!

The solution structure of Sco1 from *Bacillus subtilis* is the first structure of a protein important in the assembly of cytochrome *c* oxidase (CcO). The assembly of CcO requires the insertion of multiple cofactors. Sco1 is a conserved protein implicated in formation of the binuclear Cu_A center.

Copper is an essential nutrient serving as a cofactor in numerous redox enzymes and enzymes involved in oxidative reactions. Copper ions are shuttled to sites of utilization by protein-mediated transfer reactions involving metallochaperones (Huffman and O'Halloran, 2001; Rosenzweig, 2001). Copper transfer is mediated by protein:protein interactions between the metallochaperone and the respective target molecule. Copper insertion into the Cu,Zn-superoxide dismutase (Sod1) requires the function of the CCS metallochaperone. Secretory cupro-enzymes are metallated within post-Golgi vesicles by copper ions translocated across the membrane by P-type ATPases. Cu(I) ions are presented to the ATPase translocase by the Atx1 metallochaperone. Atx1 and CCS share a conserved structural motif that is the Cu(I) binding domain. The Cu(I) binding site is near the surface of the protein being partially solvent shielded, allowing access for the target molecule yet protection for the cell (Arnesano et al., 2002). The metallochaperone data suggest that individual molecules exist for the loading of cupro-enzymes.

A major cupro-enzyme is cytochrome *c* oxidase (CcO), which resides within the mitochondrial inner membrane (IM). The mechanism of Cu(I) routing to the mitochon-

dron for assembly of CcO is unknown. As Cu delivery to Sod1 and the P-type ATPase transporters is protein mediated, the prediction is that Cu insertion into nascent CcO chains will also be protein mediated. Although Sco1 is not a copper shuttle analogous to Atx1 and CCS, it is postulated to function as a cometallochaperone in the insertion of Cu into CcO. Mammalian CcO consists of a 13 subunit complex embedded within the IM. Three core subunits of the complex, Cox1-3, are encoded by the mitochondrial genome, whereas the remaining 10 subunits are nuclear encoded. Copper ions exist in two CcO centers designated Cu_A and Cu_B enfolded by two mitochondrial-encoded subunits (Tsukihara et al., 1995). The Cu_A center in Cox2 is a binuclear center within a domain that protrudes into the soluble intermitochondrial membrane space (IMS). This domain is the docking site for cytochrome *c* for electron transfer to the Cu_A center. The Cu_B center is buried within Cox1 forming a binuclear site with one of the two heme A cofactors.

Copper metallation of CcO involves at least three proteins, Sco1, Cox11, and Cox17. Cox17 is implicated in the delivery of copper ions to the mitochondrion, whereas Sco1 and Cox11 are believed to be cometallochaperones assisting Cox17 in the metallation of CcO (Carr and Winge, 2003). Cox11 appears to be specific for Cu_B site formation. Sco1 was first implicated in Cu delivery to CcO by the observation that the respiratory-deficient phenotype of a *cox17* yeast mutant can be suppressed by overexpression of SCO1 or a related gene designated SCO2 (Glerum et al., 1996). Sco1 and Sco2 are highly similar yeast proteins associated with the mitochondrial IM. Both proteins contain a single transmembrane helix anchoring a soluble domain that projects into the IMS. Yeast cells lacking Sco1, but not Sco2, are respiratory deficient and show diminished