Title:	Elucadating the role of the pore-forming motif in the structure-function mechanism of vibrio cholerae cytolysin (VCC), a β-barrel pore-forming toxin
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Abstract:	β-barrel pore-forming toxins (β-PFTs) kill target cells by generating β-barrel oligomeric pores on the target cell membranes. PFTs are secreted as monomeric water-soluble molecules that are converted into the oligomeric pore assemblies upon membrane interaction. PFT molecule has to undergo multiple structural rearrangements during this structural metamorphosis. One of the most important structural reorganizations during the β-barrel pore-formation is the structural transition of the pore-forming motif into the membrane-inserted form that generates the β-barrel scaffold of the β-PFTs. However, the molecular mechanism regulating the structural reorganization of the pore-forming motif remains elusive. Using Vibrio cholerae cytolysin (VCC) as a prototype β-PFT, we investigated the triggering mechanism behind the structural transition of the pore-forming motifs, and explored the key residues in the pore-forming motif that play a crucial role in the β-barrel pore-formation mechanism of VCC. In the first part of our study, we targeted aromatic amino acid residues of the pore-forming motif, as the aromatic residues are known to play pivotal roles in the structure-function mechanism of transmembrane proteins. We performed structure-guided mutagenesis, and explored their implications in the pore-formation mechanism of VCC. We identified a critical tyrosine residue at position 321 (Y321) located at the hinge region of the pore-forming motif that plays a crucial role in the pore-formation mechanism of VCC. Mutation of this residue abrogated the oligomerization of the toxin monomers and compromised the pore-forming activity. Based on our study, it also appears that the mutation of Y321 possibly induces long-range defects in the protein structure that would affect the communications between the distinct structural modules in the VCC protein. Based on our study, it is possible to speculate that the mutation of Y321 presumably perturbs the structural constraints in the hinge region of the pore-forming motif, and arrests its partial collapse toward generating. Our study suggests that the presence of Y321 in the hinge region of the pore-forming motif is crucial for the toxin molecule to sense membrane-binding and to trigger essential structural rearrangements required for the subsequent oligomerization and pore-formation process. In the second part of this study, we targeted the polar/charged amino-acid residues of the pore-forming motif that are positioned in the lumen of the β-barrel scaffold of VCC. These polar/charged residues play a crucial role in generating water-filled β-barrel pores within the hydrophobic core of the lipid bilayer. In this study, we identified two residues, glutamate at position 289 (E289) and lysine at position 304 (K304), that are crucial for the pore-formation mechanism of VCC. Mutation of K304 resulted in an altered tertiary structural disposition of the toxin, and attenuated its functional interaction with the target membrane. In contrast, mutation of E289 was found to compromise the pore-formation mechanism of VCC, by arresting membrane-bound toxin molecules in a pre-pore-like intermediate state, presumably by elevating the energy barrier of the process. Altogether, our studies identified crucial structural features in the VCC β-PFT structure that triggers the structural transition of the pore-forming motif. We also identified multiple key residues in the pore-forming motif of VCC that play distinctly versatile roles in the different stages of the pore-formation mechanism. Overall, our study provides critical new insights into the enigmatic β-PFT structure-function mechanism.
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