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| Title: | Revisiting the oligomerization mechanism of Vibrio cholerae cytolysin, a beta-barrel pore-forming toxin |
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| Abstract: | Vibrio cholerae cytolysin (VCC) is a membrane-damaging beta-barrel pore-forming toxin (beta-PFT). VCC causes permeabilization of the target membranes by forming transmembrane oligomeric beta-barrel pores. Oligomerization is a key step in the mode of action of any beta-PFT, including that of VCC. Earlier studies have identified some of the key residues in VCC that are directly involved in the generation of the inter-protomer contacts, thus playing critical roles in the oligomerization of the membrane-bound toxin. Analysis of the VCC oligomeric pore structure reveals a potential hydrogen-bond network that appears to connect the sidechain of an asparagine residue (Asn582; located within an inter-domain linker sequence) from one protomer to the backbone CO- and NH-groups of the neighbouring protomer, indirectly through water molecules at most of the inter-protomer interfaces. In the present study, we show that the mutation of Asn582Ala affects the oligomerization and the pore-forming activity of VCC in the membrane lipid bilayer of the synthetic lipid vesicles, while the replacement of Asn582Gln results into the restoration of the oligomeric pore-forming ability of the toxin. Using a number of truncated variants of VCC, having deletion in the C-terminal region of the toxin starting from the Asn582 residue or beyond, we also show that the presence of Asn582 is critically required for the oligomerization of the truncated form of the protein. |
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