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Title:	Single point mutation in Vibrio cholerae cytolysin compromises membrane pore-formation mechanism of the toxin
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Abstract:	Vibrio cholerae cytolysin (VCC) belongs to the family of β-barrel pore-forming protein toxins. VCC is secreted by the bacteria as water-soluble monomers, which upon binding to target eukaryotic cells form transmembrane heptameric β-barrel channels. High-resolution 3D structures are described both for the water-soluble monomeric form and the transmembrane oligomeric pore; albeit that our understanding of the mechanistic details of the membrane pore-formation process remains incomplete. Here, we report the characterization of a nonfunctional VCC variant harboring a single point mutation of Ala425Val positioned within a potential membrane-interacting loop in the VCC structure. The mutation appears to affect interaction of the toxin with erythrocytes as well as cholesterol-containing liposome membrane, without affecting the oligomerization ability of the membrane-bound toxin molecules. The membrane-bound oligomers formed by this VCC mutant do not appear to represent the functional pore assembly of the toxin; rather, such assembly could be considered as being trapped in an abortive, nonfunctional oligomeric state. Our results suggest that the Ala425Val mutation in VCC critically compromises its cholesterol-dependent membrane-interaction mechanism and also abrogates the process of functional membrane pore formation by the toxin.
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