Title:	Exploring the implication of cholesterol in regulating pore-formation mechanism of Vibrio cholerae cytolysin, a □-barrel pore-forming toxin
Authors:	Kathuria, Reema
Keywords:	Cholesterol Vibrio cholerae cytolysin Pore-forming toxin
Issue Date:	28-Jul-2021
Publisher:	IISERM
Abstract:	Vibrio cholerae cytolysin, a □-barrel pore-forming toxin constitutes an important virulence factor of Vibrio cholerae, contributing towards its pathogenesis. The mode of action of Vibrio cholerae cytolysin (VCC) involve three distinct steps as initially the toxin monomers need to interact and bind to the target host membrane, the membrane-associated seven monomeric subunits then interact with each other to generate the oligomeric assemblies and finally concomitant insertion of pre-stem loop from monomers takes place, leading to generation of a functional pore. In order to efficiently form the functional trans-membrane pores, VCC binds to the host membrane by exploiting the presence of membrane cholesterol which is exclusively present in the eukaryotic cells. However, there is no detailed evidence of how cholesterol regulates the pore-forming activity of VCC. Thus, in this study, we have examined the critical role of cholesterol in cellular membranes for functional pore-formation by VCC. For this, an array of experiments using artificial membrane system and biomembranes were employed. The liposome membranes were constituted having controlled cholesterol concentration, ranging from 0 to 50% in these liposomes. In this study, we have observed that the presence of optimal level of membrane cholesterol governs the efficient interaction of toxin to the target membranes that affects the subsequent oligomeric species formation and further generation of functional pores. The membrane cholesterol regulates initial binding of VCC to liposomes, leading to abrogation of functional pore-formation in artificial membranes having limited cholesterol or completely devoid of cholesterol. For understanding the physiological relevance of cholesterol in context of eukaryotic cells, human erythrocytes were treated with methyl □-cyclodextrin to sequester cholesterol from erythrocyte membranes that clearly indicated the compromised activity of VCC in membranes devoid of cholesterol. In erythrocytes membranes where cholesterol is being dislodged by employing methyl □-cyclodextrin, the formation of functional channel by VCC is affected. Further in the same direction, we explored the segregation and binding of VCC to cholesterol-enriched membrane microdomains, known as lipid rafts. VCC appears to sequester in these cholesterol-rich detergent-resistant membranes (DRMs) fractions. Surprisingly, even the biochemical evidence indicates that the variants of VCC having mutation in specific regions that are responsible for defining different steps of pore-formation associate with lipid rafts in the same way as wild-type VCC. Hence, the results from this study suggest the obligatory presence of cholesterol in target cellular membranes for functional pore- formation and the partitioning of VCC to liquid-ordered membrane domains takes place during pore- formation event.
URI:	http://hdl.handle.net/123456789/3719
Appears in Collections:	MP-12

Files in This Item:

File	Description	Size	Format	
PhD_Thesis_ReemaKathuria.pdf		5.5 MB	Adobe PDF	View/Open

Show full item record