Title:	Structure-function analysis of the core cytolysin domain of Vibrio cholerae cytolysin
Authors:	Singh, Dilraj (/jspui/browse?type=author&value=Singh%2C+Dilraj)
Keywords:	Vibrio cholerae cytolysin Protein Molecular Biology
Issue Date:	23-Jul-2014
Publisher:	IISER M
Abstract:	Vibrio cholerae cytolysin (VCC) is a membrane-damaging cytolytic protein toxin belonging to the family of β-barrel pore-forming protein toxins. It is secreted by Vibrio cholerae, the causative organism of severe diarrhoeal disease cholera. It is secreted by the pathogen in the form of ~79 kDa precursor molecule, Pro-VCC. Upon removal of the N-terminal Pro-domain, the toxin is converted into the mature form of VCC. In its mode of action, VCC is shown to form transmembrane oligomeric pores in the membrane lipid bilayer of the target eukaryotic cells. In this process, the central cytolysin domain of the VCC molecule contributes the pore- forming stem region that participates toward formation of the transmembrane β- barrel. Apart from the core cytolysin domain, VCC harbors two additional lectin-like domains, which are unique to this toxin: a β-Trefoil lectin-like domain and β-Prism lectin-like domain. Presence of the β-Prism lectin-like domains appears to facilitate the mode of action of the VCC protein, presumably via regulating the membrane- binding process of the toxin. It is, however, still not clear how the β-Trefoil domain contributes toward the VCC mode of action. In this context, the overall aim of our present study was to investigate whether the core cytolysin domain of the VCC protein could function in absence of the accessory lectin-like domains of the protein. In this direction, we generated a double-deletion construct of VCC lacking both the lectin-like domains of the protein. The recombinant protein was expressed and purified to homogeneity using the heterologous protein expression system in Escherichia coli. Intrinsic tryptophan fluorescence spectra of the mutant variant showed red-shifted tryptophan fluorescence emission maxima, as compared to that of the wild type VCC. Such tryptophan fluorescence profile of the truncated protein was consistent with the extent of surface exposure of the tryptophan residues present in the core cytolysin domain. Comparison of the thermal melting profiles showed that the mutant molecule mimicked wild type VCC in terms of overall stability of the tertiary structural elements. The truncated variant of VCC protein showed lack of any hemolytic activity against human erythrocytes, thus suggesting complete loss of functional membrane pore-forming activity in absence of the lectin- like domains. Our data suggested that the core cytolysin domain of VCC was able to fold by its own, but required the accessory lectin-like domains for functional channel forming property.
URI:	http://hdl.handle.net/123456789/405 (http://hdl.handle.net/123456789/405)
Appears in Collections:	MS-08 (/jspui/handle/123456789/270)

Files in This Item:

File	Description	Size	Format	
Dilraj MS08018.pdf (/jspui/bitstream/123456789/405/3/Dilraj%20MS08018.pdf)		852.12 kB	Adobe PDF	View/Open (/jspui/bitstream/123456789/405/3/I

Show full item record (/jspui/handle/123456789/405?mode=full)

(/jspui/handle/123456789/405/statistics)