JCB Journal of Cell Biology

ARTICLE

Single-particle cryo-EM reveals conformational variability of the oligomeric VCC β-barrel pore in a lipid bilayer

Nayanika Sengupta[1], Anish Kumar Mondal[2], Suman Mishra[1], Kausik Chattopadhyay[2], and Somnath Dutta[1]

***Vibrio cholerae* cytolysin (VCC) is a water-soluble, membrane-damaging, pore-forming toxin (PFT) secreted by pathogenic *V. cholerae*, which causes eukaryotic cell death by altering the plasma membrane permeability. VCC self-assembles on the cell surface and undergoes a dramatic conformational change from prepore to heptameric pore structure. Over the past few years, several high-resolution structures of detergent-solubilized PFTs have been characterized. However, high-resolution structural characterization of small β-PFTs in a lipid environment is still rare. Therefore, we used single-particle cryo-EM to characterize the structure of the VCC oligomer in large unilamellar vesicles, which is the first atomic-resolution cryo-EM structure of VCC. From our study, we were able to provide the first documented visualization of the rim domain amino acid residues of VCC interacting with lipid membrane. Furthermore, cryo-EM characterization of lipid bilayer–embedded VCC suggests interesting conformational variabilities, especially in the transmembrane channel, which could have a potential impact on the pore architecture and assist us in understanding the pore formation mechanism.**

Introduction

The pore-forming toxins (PFTs) are a class of secreted virulent proteins produced by pathogenic bacteria. PFTs assemble on the host cell plasma membrane and permeabilize the target cells by punching transmembrane holes. PFTs adopt various strategies to traverse the lipid bilayer for creating pores in biomembranes. Individual protomers of PFTs are secreted as soluble protein by pathogenic bacteria that then accumulate on the surface of the target cell membranes to form a prepore (Iacovache et al., 2012). Subsequently, the pore-forming motifs of the membrane-associated prepore span through the lipid bilayer to punch holes (Dal Peraro and van der Goot, 2016). Generally, PFTs are classified as α-PFT and β-PFT based on the 3D structure of their membrane-inserted transmembrane region (Yamashita et al., 2014; Gouaux, 1997; Parker and Feil, 2005). The transmembrane channel of α-PFTs is spanned by α-helices, while the β-PFTs are able to construct the pore by a β-barrel structure (Iacovache et al., 2010). PFTs do not just puncture holes and permeabilize cells; many also hijack housekeeping functions of the host and lead to various pathophysiological conditions, as in the case of diphtheria, anthrax, or cholera.

Vibrio cholerae cytolysin (VCC) belongs to the β-PFT family, where a β-hairpin structure of the individual protomer assembles to form a β-barrel structure. VCC is encoded by the *hlyA* gene in enterotoxic strains of *V. cholerae*. It initially folds into an 80-kD protoxin, which is processed by endogenous or exogenous proteases to form the mature toxin. Proteolytic cleavage occurs in the N-terminus of the protoxin and removes ∼15 kD of "pro-domain" (Paul and Chattopadhyay, 2011). Thus, hemolytically active VCC is an ∼65-kD monomer that can bind the host cell membrane and oligomerize into a heptameric pore of an outer diameter of ∼8 nm and inner diameter between 1 and 2 nm. It has a preference to penetrate cholesterol-rich membranes and form a seven-membered ring-like oligomeric pore structure (Harris et al., 2002).

The crystal structure of the VCC oligomeric pore solved one decade ago revealed a heptameric assembly of protomers remarkably similar to the staphylococcal toxin α-hemolysin (De and Olson, 2011). Each protomer of VCC contributes β-trefoil lectin-like domain, β-prism lectin-like domain, cytolysin domain, and the membrane-proximal rim domain, which come together and build the extracellular mushroom head-like architecture. The membrane-spanning element of the toxin is a rigid barrel composed of 14 antiparallel β-strands (De and Olson, 2011). Sequence analyses of α-hemolysin, leukocidin F, VCC, and

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India; [2]Centre for Protein Science, Design and Engineering, Department of Biological Sciences, Indian Institute of Science Education and Research Mohali, Punjab, India.

Correspondence to Somnath Dutta: somnath@iisc.ac.in.

https://doi.org/10.1083/jcb.202102035