Chapter 7
Vibrio cholerae Cytolysin: Structure–Function Mechanism of an Atypical β-Barrel Pore-Forming Toxin

Anand Kumar Rai and Kausik Chattopadhyay

Introduction

Every living cell is surrounded by a plasma membrane composed of phospholipid bilayer. Plasma membranes allow selective passage of solvents, ions, small molecules, and macromolecules into and out of the cells, while at the same time preventing unwanted exchange of substances between the cells and the outside environment. Thus, plasma membranes serve an extremely crucial function in terms of acting as the selective permeability barrier for the living cells. Virulence mechanisms of many pathogenic bacteria involve breaching of this permeability barrier function of the target host cell membranes by employing a specialized class of toxins, known as pore-forming toxins (PFTs) (Alouf and Popoff 2006).

PFTs represent a unique class of membrane-damaging proteins that act to kill their target cells by forming pores in the cell membranes. PFTs are found in a wide array of organisms starting from bacteria to humans (Dunstone and Tweten 2012; Voskoboinik and Trapani 2006; Voskoboinik et al. 2006; McCormack et al. 2013), and are implicated in diverse biological functions that include bacterial pathogenesis processes (Alouf and Popoff 2006) as well as vertebrate immune responses (Voskoboinik et al. 2006; McCormack et al. 2013; Kondos et al. 2010; Voskoboinik and Trapani 2006). Some of the classic examples of the PFTs include the membrane attack complexes generated by the complement cascade of the vertebrate innate immune system (Borsos et al. 1964; Humphrey and Dourmashkin 1969; Bhakdi and Tranum-Jensen 1978; Mayer 1972), and the perforin protein produced by the cyto-

A.K. Rai • K. Chattopadhyay (✉)
Centre for Protein Science, Design and Engineering,
Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Mohali, Sector 81, Knowledge City, SAS Nagar, Mohali, Manauli 140306, Punjab, India
e-mail: kausik@iisermohali.ac.in

A. Chakrabarti, A. Surolia (eds.), *Biochemical Roles of Eukaryotic Cell Surface Macromolecules*, Advances in Experimental Medicine and Biology 842,
DOI 10.1007/978-3-319-11280-0_7

toxic T lymphocytes of the adaptive immune system (Dennert and Podack 1983; Podack and Dennert 1983; Blumenthal et al. 1984; Law et al. 2010). PFTs are known to be important components of the venoms of insects (Smith et al. 1994), snakes (Wu et al. 2012) and sea anemones (Kristan et al. 2009; Tejuca et al. 2009).

As mentioned above, PFTs are implicated in the virulence mechanisms of a wide array of bacterial pathogens (Los et al. 2013). In fact, one third of the bacterial toxins belong to the PFT family (Alouf 2001). In their generalized mode of action, bacterial PFTs are commonly generated as water-soluble molecules, which upon interacting with the target cell membranes convert into membrane-inserted water-filled pore(s) (van der Goot 2003; Parker and Feil 2005; Gonzalez et al. 2008; Iacovache et al. 2008, 2010). Depending on the specific PFT under consideration, membrane-inserted pore diameters can vary in the range from less than 1 nm up to 50 nm (Rosado et al. 2008; Parker and Feil 2005). PFT-mediated pore formation acts to punch holes in the target cell membrane, which in turn allow free diffusion of solvents, ions and other substances. In some cases, membrane pores formed by the PFT molecules are used by the pathogenic bacteria to inject toxic substances into the target host cells (Young and Collier 2007). Many bacterial pathogens, which survive within the subcellular organelles, are known to employ PFTs to rupture the organelle membranes for transmission into the cytoplasm toward exerting their pathogenesis process (Hamon et al. 2012; Schnupf and Portnoy 2007).

Mode of actions of the PFT family of proteins highlights a remarkable dimorphic nature of their structural property. With a unique primary amino acid sequence, PFTs are capable of adopting two distinct structural forms that can be accommodated into two discrete physicochemical environments: the hydrophilic environment of the aqueous phase and the hydrophobic environment of the membrane lipid bilayer. Such a property of the PFT family of molecules makes them unique models to address questions concerning the dynamics of protein structure and folding (Heuck et al. 2001; Chattopadhyay and Banerjee 2003).

Based on the structural and functional considerations, PFTs can be grouped into a number of distinct subclasses (Alouf and Popoff 2006). The most common way of classifying the PFT family members is on the basis of the structural mechanism used for the membrane pore formation process. Accordingly, PFTs are broadly classified into two structural subfamilies: α-PFTs and β-PFTs (Iacovache et al. 2010) (Fig. 7.1). α-PFTs employ α-helices to generate the transmembrane pore structures. Pore-forming Colicins (Wiener et al. 1997) and Cytolysin A (Mueller et al. 2009) produced by *Escherichia coli* and closely related bacteria are the archetypical examples in the α-PFT category. Members in the β-PFT subfamily are known to form transmembrane pores composed of β-strand-rich motifs, commonly termed as β-barrel structure (Heuck et al. 2001; Prevost et al. 2001; Menestrina et al. 2001). *Staphylococcus aureus* α-hemolysin is one of the most well-studied β-PFT family members (Song et al. 1996).

In the present review, we will discuss the structure–function relationship of *Vibrio cholerae* cytolysin, one of the prominent members in the β-PFT family of bacterial protein toxins.

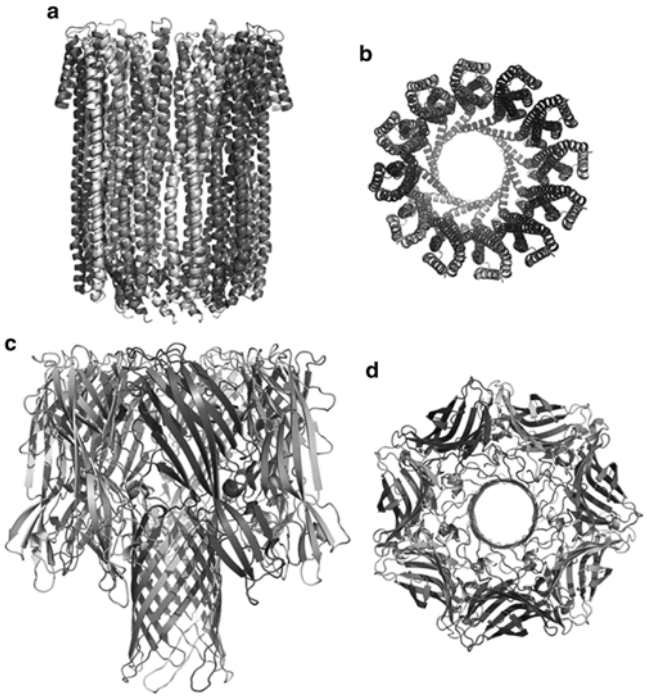

Fig. 7.1 Structural classification of α-PFTs and β-PFTs. (**a**, **b**) Oligomeric pore structure of Cytolysin A from *E. coli* (PDB: 2WCD) is shown as an example of α-PFT. (**c**, **d**) Oligomeric pore structure of the *S. aureus* α-hemolysin (PDB: 7AHL) represents the classic example of β-PFT. (**a**) and (**c**) show the *side views* of the pore structures, while *top views* of the pores are shown in (**b**) and (**d**). Structural models are visualized using the program PyMOL [DeLano WL, The PyMOL Molecular Graphics System (2002) found online (http://pymol.org)]

Vibrio cholerae Cytolysin

Vibrio cholerae cytolysin (VCC) is a membrane-damaging cytolytic/cytotoxic protein produced by many pathogenic strains of the Gram negative bacteria *V. cholerae*, the causative agent of severe diarrheal disease cholera (Kaper et al. 1995). VCC shows potent lytic activity against variety of erythrocytes and mammalian cells (Honda and Finkelstein 1979; Goldberg and Murphy 1984; Richardson et al. 1986; McCardell et al. 1985; Saka et al. 2008; Mitra et al. 2000; Coelho et al. 2000; Mukherjee et al. 2008; Figueroa-Arredondo et al. 2001; Zitzer et al. 1997a; Chakraborty et al. 2011). It is also shown to possess enterotoxic activity in terms of triggering bloody fluid accumulation in the rabbit ligated ileal loops (Ichinose et al. 1987). Based on such observations, VCC has been considered as a potential virulence factor of *V. cholerae*. VCC is particularly implicated in the pathogenesis process of those strains that lack 'cholera toxin', the major virulence factor responsible for causing the massive dehydrating diarrhea during *V. cholerae* infection (Saka et al. 2008; Kaper et al. 1995).

VCC is encoded by the *hlyA* gene in *V. cholerae* (Goldberg and Murphy 1984; Yamamoto et al. 1990; Rader and Murphy 1988). VCC is synthesized as an ~82 kDa molecule, Pre-Pro-VCC (Yamamoto et al. 1990). In the process of secretion, the N-terminal 25-residue signal peptide is cleaved to generate an inactive precursor of the toxin (~79.5 kDa), termed as Pro-VCC. Subsequently, ~15 kDa N-terminal region from Pro-VCC is proteolytically removed resulting in the generation of the cytolytically active mature form of VCC (Nagamune et al. 1996). Proteolytic maturation of VCC is believed to be mediated by the HA/protease, which represents the major extracellular proteolytic activity of *V. cholerae* (Nagamune et al. 1996). Conversion of Pro-VCC into the mature form of the toxin can also be achieved in vitro by other proteases like trypsin, chymotrypsin, and subtilisin (Nagamune et al. 1996). It has also been shown that the proteolytic activation of Pro-VCC can be triggered by the action of the proteases present on the surface of the target eukaryotic cells as well (Valeva et al. 2004).

Active form of VCC, in its purified form, has been shown to trigger lysis of erythrocytes, and other eukaryotic cells by forming transmembrane oligomeric pores of 1–2 nm diameters (Ikigai et al. 1996). Membrane pore-forming activity of VCC could also be mimicked in the lipid bilayer of model membranes (Ikigai et al. 1997). Apart from of its pore-forming cytolytic activity, VCC shows a potent lectin-like activity in interacting with complex glycoproteins and glycolipids containing terminal β1-galactosyl moiety (Saha and Banerjee 1997). Structural studies have characterized VCC as a β-PFT molecule, and suggest that it would follow the overall scheme of the generalized β-PFT mechanism (De and Olson 2011; Olson and Gouaux 2005; Valeva et al. 2005). However, VCC differs from the archetypical members of the β-PFT family in several aspects, particularly in the intricate details of the structure-function mechanism(s) associated with its membrane pore formation process. In the subsequent sections, we will discuss our current understanding regarding the structure-function relationship of VCC, in the context of its role as a β-PFT family of bacterial protein toxins.

Structural Features of VCC

As stated earlier, VCC is secreted as water-soluble monomeric precursor Pro-VCC, which upon removal of the Pro-domain gets converted into the mature active form of the toxin (Nagamune et al. 1996). Mature form of VCC causes lysis of its target cells by forming transmembrane oligomeric pores (Ikigai et al. 1996; Zitzer et al. 1995). High resolution three-dimensional structure has been determined for the water-soluble, monomeric precursor form Pro-VCC (Olson and Gouaux 2005) (Fig. 7.2). Structure of the oligomeric pore state of VCC has also been elucidated recently (De and Olson 2011) (Fig. 7.2). Structural studies have confirmed that VCC is indeed a β-PFT family of toxin, and it acts by forming transmembrane heptameric β-barrel pores in the membrane lipid bilayer. Consistent with the structural organization of the archetypical β-PFT pores, transmembrane heptameric pore

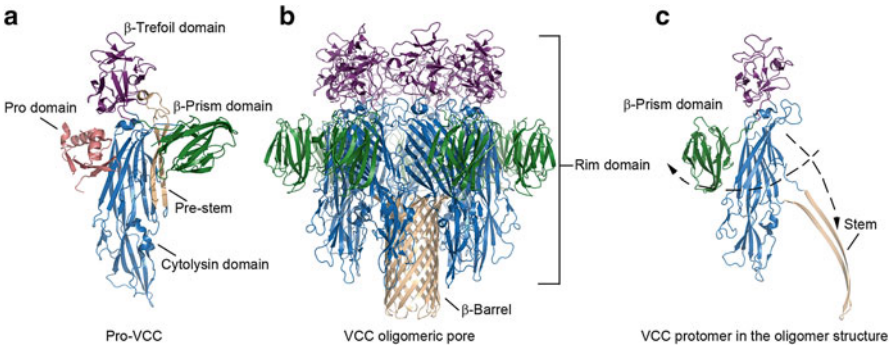

Fig. 7.2 Structures of the monomeric and oligomeric pore form of VCC. (**a**) Structure of the monomeric precursor form Pro-VCC (PDB: 1XEZ). (**b**) Heptameric β-barrel pore structure of VCC (PDB: 3O44). (**c**) VCC protomer in the oligomeric pore structure. Individual domains are marked. Structural rearrangements of the stem loop and the β-Prism domain are indicated in (**c**). Structural models are visualized using the program PyMOL [DeLano WL, The PyMOL Molecular Graphics System (2002) found online (http://pymol.org)]

complex of VCC depicts a mushroom-shaped architecture, which is grossly divided into two parts: (a) transmembrane β-barrel segment, and (b) membrane-proximal rim domain (Fig. 7.2). Analysis of the structures, however, highlights several unique features that are not commonly documented in the archetypical β-PFT family of bacterial protein toxins. Consistent with the conventional β-PFT molecular structures, VCC contains a central scaffold of cytolysin domain that constitutes the core structure of the mushroom-shaped oligomeric pore complex (Fig. 7.2). The cytolysin domain also encompasses the pore-forming motif of the toxin. In addition to the cytolysin domain, however, VCC documents presence of three additional domains that are not commonly found in the classical β-PFT molecules: an N-terminal Pro-domain present in the Pro-VCC precursor form of the toxin, and two lectin-like domains located at the C-terminal side of cytolysin domain (Fig. 7.2).

Cytolysin domain: VCC harbors a central ~325 residue cytolysin domain (Olson and Gouaux 2005; De and Olson 2011) that resembles overall architecture of the cytolysin domains present in the prototype β-PFTs like *S. aureus* α-hemolysin (Song et al. 1996). Consistent with the structural features of the transmembrane oligomeric pores of the conventional β-PFT molecules, cytolysin domain of VCC provides the central scaffold of the pore complex. VCC forms mushroom-shaped heptameric transmembrane pore structures that can be divided grossly into two parts: (a) transmembrane segment that creates the β-barrel pore, and, (b) membrane-proximal rim-domain that remains sited onto the membrane surface. The transmembrane β-barrel segment of the VCC pore complex is constituted solely from the contribution of the cytolsyin domain. Moreover, majority of the rim-domain is also constructed by the structural components of the cytolysin domain.

This cytolysin domain encompasses the 42-residue long, pore-forming segment of VCC that contributes to the generation of the transmembrane β-barrel pore

structure (Fig. 7.2). In the water-soluble monomeric state of the protein, this region assumes two-strand β-sheet structure, and remains compactly packed against the cytolysin domain, in the form of a so called 'pre-stem' configuration. The interactions that keep the pre-stem loop packed within the cytolysin domain include a range of polar and non-polar/hydrophobic residues. In the process of oligomeric pore formation, the 'pre-stem' loop from each of the toxin protomers undergoes distinct conformational change to attain a so called 'stem' configuration, and gets inserted into the membrane lipid bilayer in a synchronous manner. In this way, stem region from each toxin subunit contributes two β-strands toward generation of the stem region of the transmembrane β-barrel pore. In the transmembrane oligomeric pore structure, stem regions from the neighboring protomers make extensive interactions between each other, and thus presumably contribute toward remarkable stability of the oligomeric pore states (SDS-stability, resistance toward proteolysis etc.) (Ray et al. 2003).

While part of the VCC cytolysin domain contributes toward generation of the transmembrane segments, rest of it constitutes the membrane-proximal rim-domain of the oligomeric pore complex. Analysis of the structural models of the β-PFT pore structures (Song et al. 1996), including that of VCC (De and Olson 2011), suggests that the membrane-proximal rim-domain act as the structural scaffold for the β-PFT oligomeric pores. Rim-domain provides an interface that mediates interaction of the toxin with the lipid head-groups of the membrane lipid bilayer. It, therefore, appears that the cytolysin domain may also contribute toward interaction of the toxin with the membrane lipid head-groups. Indeed, a lipid-binding pocket in the cytolysin domain has been experimentally established within the membrane-proximal rim-domain of *S. aureus* α-hemolysin (Olson et al. 1999; Galdiero and Gouaux 2004). In case of VCC, however, no such lipid-binding pocket has been documented. Nevertheless, in one of our study, we have shown that a mutation within the membrane-proximal rim-domain of VCC confers compromised membrane-binding, and membrane pore-forming activity for the toxin (Paul and Chattopadhyay 2012). This observation, therefore, indicates the possibility of a potential lipid-binding motif in the cytolysin domain of VCC as well.

Pro-domain: As mentioned above, VCC is secreted by the bacteria in the form of an inactive precursor, Pro-VCC (Nagamune et al. 1996; Yamamoto et al. 1990). Structure of the Pro-VCC (Olson and Gouaux 2005) shows presence of ~15 kDa Pro-domain, which is attached to the N-terminus of the cytolysin domain via 29-residue long connecting flexible linker. This linker contains ~15 residue long sequence motif that acts as the cleavage site(s) for a battery of proteases (Nagamune et al. 1996). Consistent with such observation, proteolytic cleavage at this linker sequence results into the removal of the Pro-domain, and thus leads to the maturation of the VCC toxin.

Presence of the Pro-domain in the precursor form of VCC has been shown to be essential for efficient secretion and proper folding of the toxin molecule. One previous study has shown that the recombinant *V. cholerae* cells, harboring truncated variant of *hlyA* gene lacking the sequence for the Pro-region, fails to secrete the

toxin outside the bacterial cells (Nagamune et al. 1997). In vitro denaturation/renaturation experiments have demonstrated that in absence of the Pro-domain VCC fails to refold back to its active form, while Pro-VCC can achieve proper refolding (Nagamune et al. 1997). In one of our recent study, we have shown that the presence of the Pro-domain increases the unfolding propensity of the precursor molecule in response to various denaturing conditions, while mature form of the toxin shows considerable resistance toward unfolding (Paul and Chattopadhyay 2011). Altogether, these studies suggest an intramolecular chaperone-like activity of the Pro-domain in terms of providing sufficient extent of structural plasticity in the VCC molecular structure, which might be required for efficient secretion of the toxin in its precursor form across the bacterial membrane. It is, however, still not properly elucidated how exactly the presence of the Pro-domain keeps the VCC toxin in its inactive precursor state.

β-Trefoil lectin-like domain: VCC contains a β-Trefoil lectin-like domain (~15 kDa) at the C-terminal boundary of the cytolysin domain (Olson and Gouaux 2005). This β-Trefoil lectin-like domain is also present in the closely related cytolysins from the *Vibrionaceae* bacteria, but it is absent in the archetypical β-PFT molecules like *S. aureus* α-hemolysin (Olson and Gouaux 2005). The β-Trefoil domain is connected to the cytolysin domain via a short linker sequence composed of Gly-Gly-Arg-Pro. VCC β-Trefoil domain shows sequence and structural similarity to the carbohydrate-binding domain of plant toxin ricin, and highlights presence of the QXW conserved carbohydrate-binding motif(s) observed in the archetypical β-trefoil lectin domains (Rutenber et al. 1987; Montfort et al. 1987; Loris 2002; Sharon and Lis 2004). However, carbohydrate-binding activity of the β-trefoil domain of VCC has not been explored yet experimentally. Also, the implication of the β-trefoil domain in the structure-function mechanism of VCC remains to be investigated.

β-Prism lectin-like domain: VCC contains another ~15 kDa domain that is connected to the C-terminus of the β-Trefoil domain via relatively long linker sequence (Olson and Gouaux 2005). This domain is not commonly documented in other β-PFTs, including closely related cytolysins from *V. vulnificus* and *Aeromonas hydrophila* (Olson and Gouaux 2005). This C-terminal domain of VCC shows structural similarity to several prototype β-Prism lectins, like jacalin (Sankaranarayanan et al. 1996) and *Maclura pomifera* agglutinin (MPA) (Lee et al. 1998). VCC β-Prism-like domain highlights presence of a binding pocket similar to the carbohydrate-binding site of the jacalin and MPA lectins. In one of our recent studies, we have established the role of the β-Prism domain in the lectin-like activity of VCC (Rai et al. 2013). In this study, we have shown that the truncation of the β-Prism domain completely abolishes the lectin activity of VCC toward β1-galactosyl-terminated glycoconjugates. Using structure-guided mutagenesis approach we have also mapped the critical residues within the β-Prism domain that are essential for the lectin activity. The study shows that an amino acid triad (composed of Asp617, Tyr654, and Tyr679) positioned within the putative carbohydrate-binding pocket constitute the essential element for the VCC lectin activity. Altogether, it has been

conclusively established now that the β-Prism domain acts as the structural scaffold responsible for the lectin-like activity of VCC (Rai et al. 2013; Levan et al. 2013).

It is important to note that the β-Prism domain of VCC adopts two distinct positions with respect to the cytolysin domain, in the monomeric precursor form and in the oligomeric pore state (De and Olson 2011; Olson and Gouaux 2005) (Fig. 7.2). In the water-soluble precursor Pro-VCC, β-Prism domain is located on the opposite side of the Pro-domain on top of the pre-stem loop, while in the oligomeric pore structure it is repositioned in place of the Pro-domain (Fig. 7.2). Such rearrangement of the β-Prism domain is absolutely essential for the membrane insertion, and oligomeric pore formation process. In absence of the reorganization of the β-Prism domain, it would be positioned in such a way that would in turn block oligomerization of the toxin protomers, simply because of steric clash. Also, in absence of β-Prism domain's rearrangement, pre-stem loop would not be able to open up toward membrane insertion. Based on our recent study (Rai et al. 2013) it appears that the lectin-like activity of the β-Prism domain might act as a triggering mechanism so as to prompt its structural rearrangement against the cytolysin domain.

Presence of the β-Prism domain has been shown to be critical for efficient membrane pore-formation of VCC (Rai et al. 2013; Mazumdar et al. 2011; Olson and Gouaux 2005). Truncated variant of VCC lacking the C-terminal β-Prism domain shows abortive membrane pore formation. It has been observed that in absence of the β-Prism domain, the truncated toxin can form membrane-bound oligomers, but does not display functional pore-forming activity (Rai et al. 2013). Cryo electron microscopy-based analysis of the oligomers formed by the truncated variant suggests that in absence of the β-Prism domain VCC might form an abortive oligomeric pore having obstructed hole in the *cis*-side of pore lumen (Dutta et al. 2010; He and Olson 2010). It has been proposed that such pore occlusion happens, presumably due to collapse of the β-Trefoil domain in absence of the β-Prism domain.

Structural reorganizations during oligomeric pore formation: Comparison of the structures of the monomeric and oligomeric form of VCC suggests massive structural reorganization within the toxin monomers during the oligomeric membrane pore formation process (De and Olson 2011; Olson and Gouaux 2005) (Fig. 7.2). As mentioned above, the most critical structural rearrangement is the opening up of the 'pre-stem' loop from the cytolysin domain, and its subsequent membrane insertion in the form of 'stem' configuration. In the water-soluble monomeric Pro-VCC state, 'pre-stem' loop remains sandwiched between the β-Prism domain and the cytolysin domain. Thus, for 'pre-stem' to 'stem' conversion to occur, it requires movement of the β-Prism domain. Indeed, during the whole process β-Prism domain reorients with respect to the central cytolysin domain by almost 180° angle, and occupies the position where originally Pro-domain is located in the Pro-VCC structure. Rearrangement of the β-Prism domain represents the second most prominent structural change associated with the membrane pore formation process of VCC. Movement of the β-Prism domain makes way for the 'pre-stem' loop to undergo the conformational change required for the subsequent membrane insertion and oligomeric pore formation process.

Structural Features of the VCC β-Barrel Pore

Transmission electron microscopy (TEM)-based studies have initially characterized the transmembrane oligomeric pore complexes of VCC as membrane lesions of typical ring-like structures having inner diameter of approximately 1–2 nm (Ikigai et al. 1996). Studies showing inhibition of lytic activity by the osmoprotectants of defined molecular sizes have also suggested similar range of pore diameters for VCC (Ikigai et al. 1996). Single channel conductance measurement(s) using VCC pores generated in the supported lipid bilayer system have suggested that VCC forms anion-selective diffusion channels (Menzl et al. 1996; Ikigai et al. 1997). Single channel measurement studies have also suggested asymmetric lumen geometry for the VCC pores: larger opening in the *cis*-side than in the *trans*-side, with narrow constriction at the central part of the lumen (Yuldasheva et al. 2001). Crystal structure of the VCC oligomeric pore state confirms such 'cup-shaped' lumen geometry (De and Olson 2011). Crystal structure also elucidates that the lining of the VCC pore lumen is constituted by a combination of charged, as well as hydrophobic/aromatic amino acid residues. Analysis of the structural model also explains that the narrow constriction near the central region of the pore lumen is caused by the aromatic ring of a tryptophan residue contributed by each of the VCC protomer. Similar architecture has been documented in another β-PFT member, anthrax protective antigen (Krantz et al. 2005; Katayama et al. 2010; Sun et al. 2008). Implication of the lumen geometry of the VCC membrane pore has not been explored yet in the context of VCC mode of action.

Mechanism of Membrane Pore Formation

Membrane pore formation mechanism of β-PFT family of bacterial protein toxins, in general, has been proposed to involve three distinct steps: (i) binding of the water-soluble toxin monomers to the target cell membrane; (ii) assembly of the membrane-bound toxin monomers to generate a transient 'pre-pore' oligomeric intermediate on the membrane surface; (iii) conversion of the pre-pore intermediate into the functional transmembrane pore (Menestrina et al. 2001; Parker and Feil 2005). In the sequence of events, as mentioned above, stem-loops from each of the toxin protomers insert into the membrane lipid bilayer in a concerted manner, and generate the transmembrane β-barrel. Structural studies on a number of β-PFTs have validated overall generalization of such scheme (Song et al. 1996; Gilbert 2010; Dunstone and Tweten 2012; Hotze and Tweten 1818; Rossjohn et al. 2007). However, individual β-PFTs quite often deviate from such generalized scheme in terms of displaying significant variations in the details of the mechanism. For example, process of membrane interaction shows wide range of diversity in terms of receptor specificity, role of membrane lipid components, and so on. In many cases, discrete intermediate events are described only to a limited extent. More importantly, the mechanism(s) that regulate the discrete steps leading toward functional membrane pore-formation are not properly understood for most of the β-PFTs.

Membrane pore-formation process of VCC has been explored in large number of studies. Membrane pore-formation by VCC can be mimicked in the membrane lipid bilayer of synthetic lipid vesicles or liposomes suggesting that the membrane interaction process does not critically require any non-lipid components (Ikigai et al. 1997). However, membrane pore formation is found to be more efficient in biomembranes as compared to that in liposomes, suggesting accessory role of additional molecules present in the cellular membranes (Zitzer et al. 1999). For example, erythrocytes are significantly more susceptible compared to liposome. More interestingly, erythrocytes of different species show different extent of susceptibility against the lytic activity of VCC. Rabbit erythrocytes are found to be more sensitive as compared to human erythrocytes (Zitzer et al. 1997b). Previous studies have indicated role of different cell surface proteins (e.g., glycophorin B on human erythrocytes) as the potential receptor(s) for VCC (Zhang et al. 1999). Since, VCC contains specific lectin-like activity, it has also been speculated that cell surface glycoprotein/glycolipid molecules can act as probable receptor(s) for the toxin (Rai et al. 2013). However, exact identity of the specific cell surface receptor for VCC has not been elucidated yet.

Much of the mechanism(s) associated with the membrane pore formation process of VCC have been studied using synthetic liposome vesicles. As stated above, oligomeric β-barrel pore formation can be efficiently triggered in the membrane lipid bilayer of liposomes (Ikigai et al. 1996). In some earlier studies, association of VCC with the membrane lipid bilayer of liposomes has been suggested to be a non-specific process, driven mostly by global amphiphilicity of the water-soluble monomeric form of the toxin molecules (Chattopadhyay et al. 2002). However, oligomerization and membrane pore formation has been shown to be more specific events requiring assistance of distinct membrane components. Notably, presence of cholesterol has been shown to be absolutely essential for functionality of VCC (Ikigai et al. 1996). Cholesterol appears to regulate the mode of action of VCC in a stereospecific manner; enantiomeric form of cholesterol does not support the efficient activity of VCC in the liposome membrane (Zitzer et al. 2003). It therefore appears that cholesterol regulates VCC activity, not by regulating the physicochemical properties of the membrane environment, rather by physically interacting with the toxin molecule. In fact, requirement of specific structural elements present in cholesterol has been implicated for membrane-binding and oligomerization property of VCC (Ikigai et al. 2006). Nevertheless, specific cholesterol-binding structural motif present within the VCC structure has not been established yet. In one recent study, we have shown that a single point mutation within the potential membrane-binding rim domain of VCC critically abrogates cholesterol-dependent membrane pore-formation mechanism of VCC (Paul and Chattopadhyay 2012). More detail studies would be required, however, to conclusively elucidate the structural mechanism(s) of cholesterol-dependency in the context of VCC mode of action.

In addition to the requirement of cholesterol, efficiency of membrane pore formation by VCC varies depending on the lipid composition of the membrane. For example, membrane permeabilization activity of VCC is found to be more pronounced in

presence of sphingolipids (Zitzer et al. 1999). In particular, ceramide moiety has been shown to be critically implicated for efficient membrane-damaging activity (Zitzer et al. 1999). It has also been demonstrated that the presence of specific cone-shaped lipids in conjugation with cholesterol favors efficient membrane pore-forming activity (Zitzer et al. 2001). It has been suggested that these specific lipids regulate VCC activity, not by directly interacting with the toxin molecule, rather by promoting favorable interaction of the membrane-bound toxins with cholesterol (Zitzer et al. 2001). Altogether, it appears that VCC employs complex lipid-dependent mechanism to exert its membrane pore-forming activity, the exact nature of which is still not fully understood. Once again, no information is available at present whether VCC utilizes any specific structural motif within its molecular structure to mediate interaction with the lipid components of the target membrane.

While the role(s) of membrane lipid components in regulating the VCC functionality have been studied extensively in the past, the dynamics of its membrane pore-formation events has been explored only in recent years. Existence of the pre-pore oligomeric intermediate in the process of pore formation for VCC has been documented only recently. It has been shown that the trapping of the membrane-spanning stem loop in its pre-stem configuration via engineered disulfide linkage could arrest the pre-oligomeric intermediate of VCC (Lohner et al. 2009). Also, a truncated variant of VCC lacking the pre-stem structure is found to remain trapped as the pre-pore oligomer on the membrane surface (Paul and Chattopadhyay 2014). These studies conclusively show that VCC follows the archetypical β-PFT mechanisms in terms of displaying involvement of the pre-pore oligomeric intermediates.

It has been suggested previously that the membrane-binding step precedes oligomerization of VCC (Zitzer et al. 2000). It has also been shown that the membrane oligomerization and functional pore formation for VCC require more stringent criteria as compared to the membrane binding step. For example, membrane binding can occur even at low temperature of 4 °C, whereas oligomerization and pore formation get critically arrested at temperature range less than 10 °C (Zitzer et al. 1997b, 2000). Such observation clearly suggests that the binding step is definitely distinct from the subsequent oligomerization and functional pore-formation events. However, membrane insertion event could not be delineated earlier from the binding and/or oligomerization steps of VCC. In a very recent study, we have now shown that the membrane insertion step depends critically on the prior oligomerization of the membrane-bound VCC monomers (Rai and Chattopadhyay 2014). Arresting of the VCC molecule in its membrane-bound monomeric state abrogates oligomerization, membrane insertion, as well as functional pore formation (Rai and Chattopadhyay 2014). These results provide valuable insights regarding the dynamics of the membrane pore-formation process employed by VCC as a prototype in the β-PFT family. Such issues are relevant not only in the context of the VCC mode of action, but are also crucial to understand and elaborate the generalized mechanisms of the β-PFT family of proteins. It is also important to note that such aspects are also relevant to understand the dynamics of the protein-membrane interactions in a broader canvas of membrane protein functionalities.

Conclusion

Existing information regarding the membrane pore formation process of VCC highlights an intriguing mechanism of enormous complexity. The mechanism of functional pore formation appears to be the outcome of a complex cross-talk between the structural elements of the protein's molecular structure and the components of the target membrane. It is really fascinating how exactly such mechanism is regulated in presence of the membrane lipid bilayer of the target cell systems.

Valuable insights have been obtained regarding the mechanism of actions of a large number of β-PFTs, including VCC. In particular, for VCC structures have been described for the monomeric and oligomeric pore form of the toxin. Information is also available regarding some of the intermediate steps involved in the process of membrane pore formation. However, the regulatory mechanisms that control the dynamics of the process have been elucidated only to a limited extent. Also, structural basis of the regulatory mechanisms imposed by the membrane lipid components needs to be elaborated in detail in future studies. Such information would be critical to enrich our insights regarding the membrane pore-formation mechanism of VCC in the context of its implications for the *V. cholerae* pathogenesis process. Also, such insights would enrich our knowledge regarding the generalized mechanism of β-PFT family of bacterial pore-forming toxins.

Acknowledgements We acknowledge Department of Biotechnology (DBT), India for financial support (DBT Grant No. BT/PR13350/BRB/10/751/2009). We also thank IISER Mohali for support.

References

Alouf JE (2001) Pore-forming bacterial toxins: an overview. In: van der Goot FG (ed) Pore-forming toxins. Springer, Heidelberg, pp 1–14

Alouf JE, Popoff MR (2006) The comprehensive sourcebook of bacterial protein toxins, 3rd edn. Academic, New York

Bhakdi S, Tranum-Jensen J (1978) Molecular nature of the complement lesion. Proc Natl Acad Sci U S A 75(11):5655–5659

Blumenthal R, Millard PJ, Henkart MP, Reynolds CW, Henkart PA (1984) Liposomes as targets for granule cytolysin from cytotoxic large granular lymphocyte tumors. Proc Natl Acad Sci U S A 81(17):5551–5555

Borsos T, Dourmashkin RR, Humphrey JH (1964) Lesions in erythrocyte membranes caused by immune haemolysis. Nature 202:251–252

Chakraborty DC, Mukherjee G, Banerjee P, Banerjee KK, Biswas T (2011) Hemolysin induces Toll-like receptor (TLR)-independent apoptosis and multiple TLR-associated parallel activation of macrophages. J Biol Chem 286(40):34542–34551. doi:10.1074/jbc.M111.241851. M111.241851 [pii]

Chattopadhyay K, Banerjee KK (2003) Unfolding of Vibrio cholerae hemolysin induces oligomerization of the toxin monomer. J Biol Chem 278(40):38470–38475. doi:10.1074/jbc.M305965200. M305965200 [pii]

Chattopadhyay K, Bhattacharyya D, Banerjee KK (2002) Vibrio cholerae hemolysin. Implication of amphiphilicity and lipid-induced conformational change for its pore-forming activity. Eur J Biochem 269(17):4351–4358. 3137 [pii]

Coelho A, Andrade JR, Vicente AC, Dirita VJ (2000) Cytotoxic cell vacuolating activity from Vibrio cholerae hemolysin. Infect Immun 68(3):1700–1705

De S, Olson R (2011) Crystal structure of the Vibrio cholerae cytolysin heptamer reveals common features among disparate pore-forming toxins. Proc Natl Acad Sci U S A 108(18):7385–7390. doi:10.1073/pnas.1017442108. 1017442108 [pii]

Dennert G, Podack ER (1983) Cytolysis by H-2-specific T killer cells. Assembly of tubular complexes on target membranes. J Exp Med 157(5):1483–1495

Dunstone MA, Tweten RK (2012) Packing a punch: the mechanism of pore formation by cholesterol dependent cytolysins and membrane attack complex/perforin-like proteins. Curr Opin Struct Biol 22(3):342–349

Dutta S, Mazumdar B, Banerjee KK, Ghosh AN (2010) Three-dimensional structure of different functional forms of the Vibrio cholerae hemolysin oligomer: a cryo-electron microscopic study. J Bacteriol 192(1):169–178. doi:10.1128/JB.00930-09. JB.00930-09 [pii]

Figueroa-Arredondo P, Heuser JE, Akopyants NS, Morisaki JH, Giono-Cerezo S, Enriquez-Rincon F, Berg DE (2001) Cell vacuolation caused by Vibrio cholerae hemolysin. Infect Immun 69(3):1613–1624

Galdiero S, Gouaux E (2004) High resolution crystallographic studies of alpha-hemolysin-phospholipid complexes define heptamer-lipid head group interactions: implication for understanding protein-lipid interactions. Protein Sci 13(6):1503–1511

Gilbert RJ (2010) Cholesterol-dependent cytolysins. Adv Exp Med Biol 677:56–66

Goldberg SL, Murphy JR (1984) Molecular cloning of the hemolysin determinant from Vibrio cholerae El Tor. J Bacteriol 160(1):239–244

Gonzalez MR, Bischofberger M, Pernot L, van der Goot FG, Freche B (2008) Bacterial pore-forming toxins: the (w)hole story? Cell Mol Life Sci 65(3):493–507

Hamon MA, Ribet D, Stavru F, Cossart P (2012) Listeriolysin O: the Swiss army knife of Listeria. Trends Microbiol 20(8):360–368

He Y, Olson R (2010) Three-dimensional structure of the detergent-solubilized Vibrio cholerae cytolysin (VCC) heptamer by electron cryomicroscopy. J Struct Biol 169(1):6–13

Heuck AP, Tweten RK, Johnson AE (2001) Beta-barrel pore-forming toxins: intriguing dimorphic proteins. Biochemistry 40(31):9065–9073

Honda T, Finkelstein RA (1979) Purification and characterization of a hemolysin produced by Vibrio cholerae biotype El Tor: another toxic substance produced by cholera vibrios. Infect Immun 26(3):1020–1027

Hotze EM, Tweten RK (1818) Membrane assembly of the cholesterol-dependent cytolysin pore complex. Biochim Biophys Acta 4:1028–1038

Humphrey JH, Dourmashkin RR (1969) The lesions in cell membranes caused by complement. Adv Immunol 11:75–115

Iacovache I, van der Goot FG, Pernot L (2008) Pore formation: an ancient yet complex form of attack. Biochim Biophys Acta 1778(7–8):1611–1623

Iacovache I, Bischofberger M, van der Goot FG (2010) Structure and assembly of pore-forming proteins. Curr Opin Struct Biol 20(2):241–246

Ichinose Y, Yamamoto K, Nakasone N, Tanabe MJ, Takeda T, Miwatani T, Iwanaga M (1987) Enterotoxicity of El Tor-like hemolysin of non-O1 Vibrio cholerae. Infect Immun 55(5):1090–1093

Ikigai H, Akatsuka A, Tsujiyama H, Nakae T, Shimamura T (1996) Mechanism of membrane damage by El Tor hemolysin of Vibrio cholerae O1. Infect Immun 64(8):2968–2973

Ikigai H, Ono T, Iwata M, Nakae T, Shimamura T (1997) El Tor hemolysin of Vibrio cholerae O1 forms channels in planar lipid bilayer membranes. FEMS Microbiol Lett 150(2):249–254

Ikigai H, Otsuru H, Yamamoto K, Shimamura T (2006) Structural requirements of cholesterol for binding to Vibrio cholerae hemolysin. Microbiol Immunol 50(10):751–757

Kaper JB, Morris JG Jr, Levine MM (1995) Cholera. Clin Microbiol Rev 8(1):48–86
Katayama H, Wang J, Tama F, Chollet L, Gogol EP, Collier RJ, Fisher MT (2010) Three-dimensional structure of the anthrax toxin pore inserted into lipid nanodiscs and lipid vesicles. Proc Natl Acad Sci U S A 107(8):3453–3457
Kondos SC, Hatfaludi T, Voskoboinik I, Trapani JA, Law RH, Whisstock JC, Dunstone MA (2010) The structure and function of mammalian membrane-attack complex/perforin-like proteins. Tissue Antigens 76(5):341–351
Krantz BA, Melnyk RA, Zhang S, Juris SJ, Lacy DB, Wu Z, Finkelstein A, Collier RJ (2005) A phenylalanine clamp catalyzes protein translocation through the anthrax toxin pore. Science 309(5735):777–781
Kristan KC, Viero G, Dalla Serra M, Macek P, Anderluh G (2009) Molecular mechanism of pore formation by actinoporins. Toxicon 54(8):1125–1134
Law RH, Lukoyanova N, Voskoboinik I, Caradoc-Davies TT, Baran K, Dunstone MA, D'Angelo ME, Orlova EV, Coulibaly F, Verschoor S, Browne KA, Ciccone A, Kuiper MJ, Bird PI, Trapani JA, Saibil HR, Whisstock JC (2010) The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature 468(7322):447–451
Lee X, Thompson A, Zhang Z, Ton-that H, Biesterfeldt J, Ogata C, Xu L, Johnston RA, Young NM (1998) Structure of the complex of Maclura pomifera agglutinin and the T-antigen disaccharide, Galbeta1,3GalNAc. J Biol Chem 273(11):6312–6318
Levan S, De S, Olson R (2013) Vibrio cholerae cytolysin recognizes the heptasaccharide core of complex N-glycans with nanomolar affinity. J Mol Biol 425(5):944–957. doi:10.1016/j.jmb.2012.12.016. S0022-2836(12)00949-7 [pii]
Lohner S, Walev I, Boukhallouk F, Palmer M, Bhakdi S, Valeva A (2009) Pore formation by Vibrio cholerae cytolysin follows the same archetypical mode as beta-barrel toxins from gram-positive organisms. FASEB J 23(8):2521–2528. doi:10.1096/fj.08-127688. fj.08-127688 [pii]
Loris R (2002) Principles of structures of animal and plant lectins. Biochim Biophys Acta 1572(2–3):198–208
Los FC, Randis TM, Aroian RV, Ratner AJ (2013) Role of pore-forming toxins in bacterial infectious diseases. Microbiol Mol Biol Rev 77(2):173–207
Mayer MM (1972) Mechanism of cytolysis by complement. Proc Natl Acad Sci U S A 69(10):2954–2958
Mazumdar B, Ganguly S, Ghosh AN, Banerjee KK (2011) The role of C-terminus carbohydrate-binding domain of Vibrio cholerae haemolysin/cytolysin in the conversion of the pre-pore beta-barrel oligomer to a functional diffusion channel. Indian J Med Res 133:131–137. IndianJMedRes_2011_133_2_131_78112 [pii]
McCardell BA, Madden JM, Shah DB (1985) Isolation and characterization of a cytolysin produced by Vibrio cholerae serogroup non-O1. Can J Microbiol 31(8):711–720
McCormack R, de Armas L, Shiratsuchi M, Podack ER (2013) Killing machines: three pore-forming proteins of the immune system. Immunol Res 57(1–3):268–278
Menestrina G, Serra MD, Prevost G (2001) Mode of action of beta-barrel pore-forming toxins of the staphylococcal alpha-hemolysin family. Toxicon 39(11):1661–1672
Menzl K, Maier E, Chakraborty T, Benz R (1996) HlyA hemolysin of Vibrio cholerae O1 biotype E1 Tor. Identification of the hemolytic complex and evidence for the formation of anion-selective ion-permeable channels. Eur J Biochem 240(3):646–654
Mitra R, Figueroa P, Mukhopadhyay AK, Shimada T, Takeda Y, Berg DE, Nair GB (2000) Cell vacuolation, a manifestation of the El tor hemolysin of Vibrio cholerae. Infect Immun 68(4):1928–1933
Montfort W, Villafranca JE, Monzingo AF, Ernst SR, Katzin B, Rutenber E, Xuong NH, Hamlin R, Robertus JD (1987) The three-dimensional structure of ricin at 2.8 A. J Biol Chem 262(11):5398–5403
Mueller M, Grauschopf U, Maier T, Glockshuber R, Ban N (2009) The structure of a cytolytic alpha-helical toxin pore reveals its assembly mechanism. Nature 459(7247):726–730
Mukherjee G, Biswas A, Banerjee KK, Biswas T (2008) Vibrio cholerae hemolysin is apoptogenic to peritoneal B-1a cells but its oligomer shepherd the cells for IgA response. Mol Immunol 45(1):266–270

Nagamune K, Yamamoto K, Naka A, Matsuyama J, Miwatani T, Honda T (1996) In vitro proteolytic processing and activation of the recombinant precursor of El Tor cytolysin/hemolysin (pro-HlyA) of Vibrio cholerae by soluble hemagglutinin/protease of V. cholerae, trypsin, and other proteases. Infect Immun 64(11):4655–4658

Nagamune K, Yamamoto K, Honda T (1997) Intramolecular chaperone activity of the pro-region of Vibrio cholerae El Tor cytolysin. J Biol Chem 272(2):1338–1343

Olson R, Gouaux E (2005) Crystal structure of the Vibrio cholerae cytolysin (VCC) pro-toxin and its assembly into a heptameric transmembrane pore. J Mol Biol 350(5):997–1016. doi:10.1016/j.jmb.2005.05.045. S0022-2836(05)00600-5 [pii]

Olson R, Nariya H, Yokota K, Kamio Y, Gouaux E (1999) Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel. Nat Struct Biol 6(2):134–140

Parker MW, Feil SC (2005) Pore-forming protein toxins: from structure to function. Prog Biophys Mol Biol 88(1):91–142

Paul K, Chattopadhyay K (2011) Unfolding distinguishes the Vibrio cholerae cytolysin precursor from the mature form of the toxin. Biochemistry 50(19):3936–3945. doi:10.1021/bi200332g

Paul K, Chattopadhyay K (2012) Single point mutation in Vibrio cholerae cytolysin compromises the membrane pore-formation mechanism of the toxin. FEBS J 279(21):4039–4051. doi:10.1111/j.1742-4658.2012.08809.x

Paul K, Chattopadhyay K (2014) Pre-pore oligomer formation by Vibrio cholerae cytolysin: insights from a truncated variant lacking the pore-forming pre-stem loop. Biochem Biophys Res Commun 443(1):189–193. doi:10.1016/j.bbrc.2013.11.078. S0006-291X(13)01979-7 [pii]

Podack ER, Dennert G (1983) Assembly of two types of tubules with putative cytolytic function by cloned natural killer cells. Nature 302(5907):442–445

Prevost G, Mourey L, Colin DA, Menestrina G (2001) Staphylococcal pore-forming toxins. Curr Top Microbiol Immunol 257:53–83

Rader AE, Murphy JR (1988) Nucleotide sequences and comparison of the hemolysin determinants of Vibrio cholerae El Tor RV79(Hly+) and RV79(Hly-) and classical 569B(Hly-). Infect Immun 56(6):1414–1419

Rai AK, Chattopadhyay K (2014) Trapping of Vibrio cholerae Cytolysin in the Membrane-bound Monomeric State Blocks Membrane Insertion and Functional Pore Formation by the Toxin. J Biol Chem 289(24):16978–16987. doi:10.1074/jbc.M114.567099

Rai AK, Paul K, Chattopadhyay K (2013) Functional mapping of the lectin activity site on the beta-prism domain of vibrio cholerae cytolysin: implications for the membrane pore-formation mechanism of the toxin. J Biol Chem 288(3):1665–1673. doi:10.1074/jbc.M112.430181. M112.430181 [pii]

Ray A, Chattopadhyay K, Banerjee KK, Biswas T (2003) Macrophage distinguishes Vibrio cholerae hemolysin from its protease insensitive oligomer by time dependent and selective expression of CD80-CD86. Immunol Lett 89(2–3):143–147. S0165247803001354 [pii]

Richardson K, Michalski J, Kaper JB (1986) Hemolysin production and cloning of two hemolysin determinants from classical Vibrio cholerae. Infect Immun 54(2):415–420

Rosado CJ, Kondos S, Bull TE, Kuiper MJ, Law RH, Buckle AM, Voskoboinik I, Bird PI, Trapani JA, Whisstock JC, Dunstone MA (2008) The MACPF/CDC family of pore-forming toxins. Cell Microbiol 10(9):1765–1774

Rossjohn J, Polekhina G, Feil SC, Morton CJ, Tweten RK, Parker MW (2007) Structures of perfringolysin O suggest a pathway for activation of cholesterol-dependent cytolysins. J Mol Biol 367(5):1227–1236

Rutenber E, Ready M, Robertus JD (1987) Structure and evolution of ricin B chain. Nature 326(6113):624–626

Saha N, Banerjee KK (1997) Carbohydrate-mediated regulation of interaction of Vibrio cholerae hemolysin with erythrocyte and phospholipid vesicle. J Biol Chem 272(1):162–167

Saka HA, Bidinost C, Sola C, Carranza P, Collino C, Ortiz S, Echenique JR, Bocco JL (2008) Vibrio cholerae cytolysin is essential for high enterotoxicity and apoptosis induction produced by a cholera toxin gene-negative V. cholerae non-O1, non-O139 strain. Microb Pathog 44(2):118–128

Sankaranarayanan R, Sekar K, Banerjee R, Sharma V, Surolia A, Vijayan M (1996) A novel mode of carbohydrate recognition in jacalin, a Moraceae plant lectin with a beta-prism fold. Nat Struct Biol 3(7):596–603

Schnupf P, Portnoy DA (2007) Listeriolysin O: a phagosome-specific lysin. Microbes Infect 9(10):1176–1187

Sharon N, Lis H (2004) History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology 14(11):53R–62R

Smith R, Separovic F, Milne TJ, Whittaker A, Bennett FM, Cornell BA, Makriyannis A (1994) Structure and orientation of the pore-forming peptide, melittin, in lipid bilayers. J Mol Biol 241(3):456–466

Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274(5294):1859–1866

Sun J, Lang AE, Aktories K, Collier RJ (2008) Phenylalanine-427 of anthrax protective antigen functions in both pore formation and protein translocation. Proc Natl Acad Sci U S A 105(11):4346–4351

Tejuca M, Anderluh G, Dalla Serra M (2009) Sea anemone cytolysins as toxic components of immunotoxins. Toxicon 54(8):1206–1214

Valeva A, Walev I, Weis S, Boukhallouk F, Wassenaar TM, Endres K, Fahrenholz F, Bhakdi S, Zitzer A (2004) A cellular metalloproteinase activates Vibrio cholerae pro-cytolysin. J Biol Chem 279(24):25143–25148

Valeva A, Walev I, Boukhallouk F, Wassenaar TM, Heinz N, Hedderich J, Lautwein S, Mocking M, Weis S, Zitzer A, Bhakdi S (2005) Identification of the membrane penetrating domain of Vibrio cholerae cytolysin as a beta-barrel structure. Mol Microbiol 57(1):124–131. doi:10.1111/j.1365-2958.2005.04684.x. MMI4684 [pii]

van der Goot FG (2003) Membrane-damaging toxins: pore-formation. In: Burns DL, Barbieri JT, Iglewski BH, Rappuoli R (eds) Bacterial protein toxins. ASM, Washington, pp 189–202

Voskoboinik I, Trapani JA (2006) Addressing the mysteries of perforin function. Immunol Cell Biol 84(1):66–71

Voskoboinik I, Smyth MJ, Trapani JA (2006) Perforin-mediated target-cell death and immune homeostasis. Nat Rev Immunol 6(12):940–952

Wiener M, Freymann D, Ghosh P, Stroud RM (1997) Crystal structure of colicin Ia. Nature 385(6615):461–464

Wu PL, Chiu CR, Huang WN, Wu WG (2012) The role of sulfatide lipid domains in the membrane pore-forming activity of cobra cardiotoxin. Biochim Biophys Acta 1818(5):1378–1385

Yamamoto K, Ichinose Y, Shinagawa H, Makino K, Nakata A, Iwanaga M, Honda T, Miwatani T (1990) Two-step processing for activation of the cytolysin/hemolysin of Vibrio cholerae O1 biotype El Tor: nucleotide sequence of the structural gene (hlyA) and characterization of the processed products. Infect Immun 58(12):4106–4116

Young JA, Collier RJ (2007) Anthrax toxin: receptor binding, internalization, pore formation, and translocation. Annu Rev Biochem 76:243–265

Yuldasheva LN, Merzlyak PG, Zitzer AO, Rodrigues CG, Bhakdi S, Krasilnikov OV (2001) Lumen geometry of ion channels formed by Vibrio cholerae EL Tor cytolysin elucidated by nonelectrolyte exclusion. Biochim Biophys Acta 1512(1):53–63

Zhang D, Takahashi J, Seno T, Tani Y, Honda T (1999) Analysis of receptor for Vibrio cholerae El tor hemolysin with a monoclonal antibody that recognizes glycophorin B of human erythrocyte membrane. Infect Immun 67(10):5332–5337

Zitzer A, Walev I, Palmer M, Bhakdi S (1995) Characterization of Vibrio cholerae El Tor cytolysin as an oligomerizing pore-forming toxin. Med Microbiol Immunol 184(1):37–44

Zitzer A, Wassenaar TM, Walev I, Bhakdi S (1997a) Potent membrane-permeabilizing and cytocidal action of Vibrio cholerae cytolysin on human intestinal cells. Infect Immun 65(4):1293–1298

Zitzer A, Palmer M, Weller U, Wassenaar T, Biermann C, Tranum-Jensen J, Bhakdi S (1997b) Mode of primary binding to target membranes and pore formation induced by Vibrio cholerae cytolysin (hemolysin). Eur J Biochem 247(1):209–216

Zitzer A, Zitzer O, Bhakdi S, Palmer M (1999) Oligomerization of Vibrio cholerae cytolysin yields a pentameric pore and has a dual specificity for cholesterol and sphingolipids in the target membrane. J Biol Chem 274(3):1375–1380

Zitzer A, Harris JR, Kemminer SE, Zitzer O, Bhakdi S, Muething J, Palmer M (2000) Vibrio cholerae cytolysin: assembly and membrane insertion of the oligomeric pore are tightly linked and are not detectably restricted by membrane fluidity. Biochim Biophys Acta 1509(1–2):264–274. S0005-2736(00)00303-5 [pii]

Zitzer A, Bittman R, Verbicky CA, Erukulla RK, Bhakdi S, Weis S, Valeva A, Palmer M (2001) Coupling of cholesterol and cone-shaped lipids in bilayers augments membrane permeabilization by the cholesterol-specific toxins streptolysin O and Vibrio cholerae cytolysin. J Biol Chem 276(18):14628–14633. doi:10.1074/jbc.M100241200. M100241200 [pii]

Zitzer A, Westover EJ, Covey DF, Palmer M (2003) Differential interaction of the two cholesterol-dependent, membrane-damaging toxins, streptolysin O and Vibrio cholerae cytolysin, with enantiomeric cholesterol. FEBS Lett 553(3):229–231