

Vibrio cholerae cytolysin (vcc) membrane pore formation



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Vibrio cholerae cytolysin (VCC) is a potent membrane pore forming protein toxin secreted by many pathogenic strain of the *V. cholerae*. In its membrane pore-formation mode of action, VCC belongs to the family of the β barrel pore-forming toxin (β -PFTs) proteins. VCC is secreted by the pathogenic bacteria *V. cholerae* in the form of water-soluble, monomeric, inactive precursor form of the protein, known as Pro-VCC. Proteolytic deletion of the N-terminal Pro-domain from this inactive precursor molecule generates the active, mature form of the VCC toxin. VCC induces colloid-osmotic lysis of the target eukaryotic by generating the transmembrane heptameric β barrel channels/pores. The high-resolution molecular structural information is available for the water-soluble monomeric Pro-VCC form as well as for the transmembrane heptameric pore structure of the VCC. Structural analysis of the water-soluble monomeric form and the transmembrane structure suggest that the VCC follows the overall pattern of the archetypical β -PFTs mechanism of pore-formation. However, the distinct intermediate steps leading the generation of the functional membrane pore formation by VCC have only been characterized to a limited extent.

Consistent with the generalized β -PFTs mode of action, the membrane pore formation mechanism of the VCC is recommended to follow three different steps: interaction of the VCC monomeric unit towards the target cell membrane; formation of the metastable, transient prepore oligomeric intermediates on the cell membrane; and finally conversion of the prepore oligomeric assembly into the functional transmembrane oligomeric β barrel channels. Previous research work on the members of β -PFTs including VCC suggest that the generation of the functional transmembrane oligomeric

pore structure comprises the membrane insertion of the pore-forming stem region from each of the toxin protomer towards formation of the transmembrane β barrel portions of the toxin. However, it has not been examined experimentally, in particular in the case of VCC toxin, whether the membrane insertion of the stem region could occur in the membrane-associated monomeric state of the toxin before the prepore oligomer generation or whether the prepore oligomer generation precedes the membrane insertion of the stem region of the VCC toxin. Even in the case of generalized β -PFTs membrane pore-formation mechanism, such sequence of pore formation events has not been established precisely. Previous studies demonstrated that the engineered β -PFTs (for example, VCC and staphylococcal LukF) not able to inserting their pore-forming stem region into the membrane lipid bilayer of the target cell membrane. Such protein variants, having their stem loop in a closed structure through the engineered disulfide linkage, are remaining trapped in their nonfunctional prepore oligomeric state. However from this observation it not clear whether the membrane oligomerization is absolutely necessary to trigger the membrane insertion or whether membrane insertion could be initiated before the prepore generation. Such perception can only be investigate by trapping the β -PFTs toxin molecule in its membrane-bound monomeric form without allowing the generation of the transmembrane oligomeric structures. In this order, a direct correlation between the membrane oligomerization and the membrane insertion has been demonstrated in Staphylococcal α -toxin, an archetypical member of the β -PFTs family. Staphylococcal α -toxin containing a single point mutation has been shown to displayed defective membrane oligomerization of the membrane-bound protein and blocking the membrane

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insertion of the pore-forming stem loop of the toxin. This investigation suggests that, in case of Staphylococcal α -toxin, the membrane insertion event of the toxin depend critically on the prior membrane oligomerization step of the toxin. A similar pore-forming mechanism has been reported in the case of perfringolysin O, a prominent member of the subclass of the cholesterol-dependent cytolysin (CDC) under β -PFTs family. Interestingly, streptolysin O, another prominent member in the cholesterol-dependent cytolysin type β -PFTs, follows a different mechanism of membrane pore formation that may have involve distinct sequence of events. In the membrane pore formation mechanism of streptolysin O, it has been proposed that the progressive assembly of the membrane-inserted monomeric unit of the toxin may act towards the formation of the transmembrane oligomeric pore structures of different pore sizes. A similar membrane oligomeric assembly has also been reported on another cholesterol-dependent cytolysin (CDC) class of β -PFTs, pneumolysin. Thus, it emerge that the β -PFTs member may not necessarily follow a common generalized procedure for the membrane pore formation mechanism of the toxin. It is, therefore, important to examine the sequence of the membrane oligomerization and insertion events for each particular member of the β -PFTs family to elucidate the molecular mechanistic details of their membrane pore-formation action of the toxin.

Many previous studies explored the molecular mechanism(s) of the membrane oligomerization process involved with the membrane pore formation action of the member of the β -PFTs family including VCC. It is generally proposed that the association of the β -PFTs protein monomer with

the membrane component of the target host cells work as the triggering step to initiate the following events resulting towards membrane oligomerization, membrane insertion, and functional transmembrane β barrel pore formation. In particular, lipid components of the membrane like cholesterol have been extensively involved in the regulating of membrane pore formation process. In the mode of action of VCC, the presence of cholesterol in the target membrane lipid bilayer has been reported to be an imperative requirement for the efficient oligomerization and functional membrane pore formation by the protein. Cholesterol appears to regulate the membrane pore-formation of the toxin by physically binding with the protein molecule and not by modulating the physicochemical environment of the target host cell membrane. The molecular mechanism of the membrane oligomerization in VCC has been explored only to a limited extent. In particular, the membrane interaction mechanism of the monomeric units of the VCC toxin, which probably acting to regulate the membrane oligomerization process of the VCC toxin, has not been explored so far. Structure analysis of the VCC oligomeric highlights the major interprotomer interactions between the neighboring monomer units. The most significant interactions are observed between the amino acid residues within the membrane pore forming stem loop of the toxin. More importantly, blocking the stem loop in its prestem configuration has been to abrogate the functional transmembrane oligomeric pore formation (SDS-stable oligomeric assembly) without any effect on the generation of the prepore oligomeric species (SDS-labile oligomers). It has been reported that, even in the physically absence of the stem loop, a truncated variant of VCC can generate prepore oligomeric assembly on the membrane. These observations precisely suggest that the interprotomer

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interactions involved in formation of the functional transmembrane oligomeric pore assembly without playing any critical role in initiating the membrane oligomerization event of the membrane-bound protein molecules. Therefore, it appears that the additional molecular interaction between the monomer of the toxin might be playing the significant role in inducing the functional membrane oligomerization of the VCC toxin.

In this part of study, to explore the details of the molecular mechanism of the membrane oligomerization process of the VCC toxin, we have mapped the key amino acid residue in the VCC molecular structure that are crucial to trigger the membrane oligomerization of the membrane-associate monomeric toxin molecules. Mutation of such key amino acid residues abrogates the membrane oligomerization step, trap the toxin in its membrane-bound monomeric state, and does not allow membrane insertion of the pore-forming stem loop form the VCC monomers. Our study, dissect the membrane interaction step from the following membrane oligomerization and insertion steps of VCC as a prototype in the b-PFTs family, This study also conclusively established that the membrane insertion critically required the functional oligomerization of the membrane-associate VCC toxin monomers on the target cell membrane.