

12 cm (4.75 inches)



STRUCTURE-FUNCTION RELATIONSHIPS IN PERFRINGOLYSIN O

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Perfringolysin O (PFO) is a member of the large family of cholesterol-dependent cytolysins (CDCs). These toxins share the characteristics of being absolutely dependent of the presence of membrane cholesterol and of forming large, pore-forming oligomeric complexes on lipid bilayers. The mechanism by which these toxins insert into and disrupt the membrane are 16 cm of current interest. The structure of the membrane-penetrating domain of PFO has been resolved and has been found to exhibit unique characteristics. We have substituted cysteine residues for amino acids suspected to be involved in membrane penetration, labeling these cysteines with the environmentally-sensitive fluorescent probe NBD via the sulfhydryl group and then used fluorescence intensity, lifetime and collisional quenching analyses of the NBD-modified cysteines to elucidate their position within the bilayer. Using these methods we have determined that each PFO monomer contributes two transmembrane beta-hairpins (TMHs) to the membrane-spanning beta-sheet. Interestingly these two TMHs assume an alpha-helical conformation in the soluble monomer, based on the crystal structure of PFO, and are converted to the beta-hairpin structure at some point in the assembly of the membrane oligomer and its insertion into the bilayer. By the application of these fluorescence-based techniques, and a novel application of SDS-agarose gel electrophoresis to measure the membrane oligomer distribution of PFO, we have also determined that PFO assembles into a large prepore complex prior to the insertion of the TMHs. Furthermore, we have trapped PFO in this prepore complex by the introduction of specific disulfide bridges between one of the TMHs and domain 2 of PFO. These studies indicate that PFO, and likely all CDCs, insert their large, membrane-spanning beta sheet into the lipid bilayer via the formation of a large oligomeric prepore complex on the surface of the membrane.



16 cm
(6.5
inches)