Abstract

Pore forming toxins (PFTs) are lytic membrane binding proteins that are ubiquitous across the animal kingdom and disrupt the structural integrity of the host cell membrane. A unique characteristic of these toxins is that they are normally secreted as water soluble monomers which undergo a conformational change into transmembrane proteins upon contact with the host plasma membrane. PFTs are classified into a-PFTs and B-PFTs, based on whether their transmembrane domains consist of amphipathic a-helices or amphipathic B-strands. Our understanding of a-PFT conformational transition, oligomerization and pore formation is much more incomplete as compared to B-PFTs. In this thesis, we examine the process of pore formation of the a-helical bacterial pore forming toxin, Cytolysin A (ClyA), in molecular detail using molecular dynamics (MD) simulations. Atomistic MD simulations of ClyA n-mer transmembrane oligomerization intermediates in DMPC and POPC membranes, show that the n-mer ClyA arcs are stable structures and form transport-capable proteo-lipidic complexes consisting of protein arcs with toroidal lipids lining the free edges. A single transmembrane protomer (which is a necessary step for pore formation) also possesses the ability to permeabilize the membrane and create a continuous channel transporting water and ions across the membrane. For the first time to our knowledge, we present strong in-silico evidence for a membrane mediated growing pore pathway for ClyA pore formation.

The conformational change of ClyA from the water-soluble monomer to the assembly competent membrane-inserted protomer is not well understood because the timescales involved prevent structural investigations of the intermediates. The mechanism of this conformational transition was elucidated using molecular dynamics simulations of coarse-grained models of ClyA and a membrane mimic. It was observed that a membrane is necessary for the conformational conversion because membrane-protein interactions counteract the loss of the many intra-protein hydrophobic interactions that stabilize the membrane-inserting segments in the ClyA monomer. Of the two membrane-inserting segments, the flexible and highly hydrophobic B-tongue inserts first while the insertion of helix A1 is membrane assisted. We conclude that the a-tongue is designed to behave as a quick response membrane sensor, while helix a-A1 improves target selectivity for cholesterol containing cell membranes by acting as a fidelity check.

Lastly, our understanding of the factors underlying the structural stability of completely oligomerized PFT pores, and differences between classes of PFTs remains incomplete. We report the presence of a comprehensive network of electrostatic interactions in both the a-PFT ClyA pore and the B-PFT AHL transmembrane pores, particularly in their solvent exposed extra-cellular domains, which play a significant part in their stabilization. Mutagenesis of some of the amino acids participating in high occurrence frequency salt bridges observed in our study is correlated with a decline in the haemolytic activity of ClyA, as shown in previous studies. Hence, we emphasize that observing salt bridges from only the crystal structure may not be sufficient, and MD simulations may yield new mutagenesis targets based on an ensemble of structures rather than a single crystal structure. Together, our study provides a molecular understanding of the pore forming mechanism of the a-PFT ClyA, and the different factors a effecting the stability of PFT pores.