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Structural basis of nonhaemolytic nature of pneumolysin from strain ST-306

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Cholesterol-dependent cytolysins (CDCs), a family of β-barrel pore-forming toxins produced by various pathogens create pores on membranes of mammalian cells. Inhibition of pore formation by these toxins is considered as an effective prevention method of bacterial infection. However, the structural and mechanistic determinants of pore formation are not completely understood. Pneumolysin (Ply), a member of CDC family, is one of the important virulence factors produced by many strains of S. pneumoniae. The sequence type 306 (ST306) of serotype-1 is highly pathogenic and globally disseminated strain. Ply from ST306 (Ply-306) is a non-haemolytic variant with six amino acids substitutions compared to the widely studied hemolytic Ply from TIGR4 strain (Ply-4). The electron microscopy studies suggested similar pore architectures formed by these two Ply variants which corroborated with the SDS-agarose experiment using liposomes implying that Ply-306 and Ply-4 both forms higher order oligomers. Calcein leakage assay revealed that Ply-306 is unable to permeabilise the calcein molecule from calcein encapsulated liposomes.

Structural analysis of Ply-306 reveals that H150 is not engaged in cation-pi interactions with K268 residues from β5 of domain 3. While Ply-4 structure shows that Y150 is stabilized by a cation-pi interaction. The pore formation process involves the large domain movement and hence, the hydrophobic interactions around Y150 (Ply-4) favours proper domain movement over H150 (Ply-306). The second important substitution in Ply-306 is T172 to I172; which is found to be stabilized by hydrophobic interaction from other D3 residues. On contrary in case of Ply-4 the T172 being a polar residue is unstable in hydrophobic pocket and favourable for domain motion. Structurally, Y150 and T172 are found to be essential for pore formation. Mutations of these residues on Ply-306 to wild type amino acids such as H150Y and I172T must regain the haemolytic activity. Interestingly, the double mutant (H150Y and I172T) of ST306 shows approximately 80 % gain in haemolytic activity (Figure-1). These two mutations are found to control the haemolytic activity in Ply-306. Further spectroscopic experiments are in progress to identify the conformational changes (disengagement of D3 from D2 and β5 from β4) that are essential steps in pore formation.

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