Poster

Structural and functional analysis of a pore forming toxin from *Streptococcus mitis*

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Cholesterol dependent cytolysins (CDCs) are pore forming toxins produced mostly by Gram positive bacteria and are responsible for bacterial pathogenesis. The CDCs are secreted as soluble monomers and upon interaction with cholesterol containing membrane; they undergo oligomerization to form large pores.

Fluorescence and mutagenic studies on perfringolysin (CDC of *Clostridium perfringens*) demonstrated that the conserved Tyrosine (Y181) and phenylalanine (F318) residues are essential for stabilizing the neighbouring monomers by the π-π stacking interaction during pore formation [1]. However, the phenylalanine residue contributing towards the π-π stacking interaction is replaced by non-aromatic residues in some CDCs (Fig. 1). This suggested that the π-π stacking interaction is not universal in stabilizing the pore complex. In line with this, a recent study from our group has shown an important cation-π interaction between tyrosine (Y150) and lysine (K288) in pneumolysin (CDC of *Streptococcus pneumoniae*) [2]. The tyrosine and cationic residue responsible for the cation-π interaction are also present in mitilysin (Mly) (CDC of *Streptococcus mitis*)and other CDCs (Fig. 1) posing question whether they belong to a subfamily of CDCs where cation-π interaction stabilizes the neighbouring monomers.

Our study focuses on understanding the structural changes occurring during the pore formation and the interactions stabilizing the pore complex by CDCs using Mly and related toxins. We have identified few residues critical for the pore complex formation in Mly. Also mutating the residues involved in proposed cation- π interaction in Mly leads to loss of the pore forming ability. Further, we are analysing the pore complex structure formed by Mly using Cryo-EM. Our data clearly demonstrates that CDCs can be sub-classified into a new class which forms pore by stabilizing the β-barrrel through cation-π interaction.

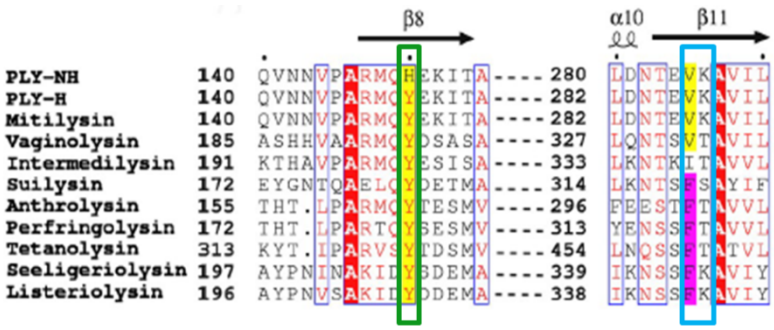

Figure 1. The sequence alignment of various CDCs highlighting the conserved tyrosine (green rectangle) and the residues involved in the π-π stacking interaction or may form cation-π interaction (blue) [2]

[1] Ramachandran, R., Tweten, R. K., & Johnson, A. E. (2004). Membrane-dependent conformational changes initiate cholesterol-dependent cytolysin oligomerization and intersubunit β-strand alignment. Nature structural & molecular biology, 11(8), 697-705.

[2] Badgujar, D. C., Anil, A., Green, A. E., Surve, M. V., Madhavan, S., Beckett, A., A., Prior I. A., Godsora B. K., Patil S. B., More P., Banerjee R., Bhaumik P. & Banerjee, A. (2020). Structural insights into loss of function of a pore forming toxin and its role in pneumococcal adaptation to an intracellular lifestyle. PLoS pathogens, 16(11), e1009016.