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Project Title:

Molecular Structure and Assembly of *Bacillus thuringiensis* Toxin-Induced Pores in Lipid Membranes

การศึกษาโครงสร้างและการก่อตัวของรูรั่วในผนังไขมันโดยโปรตีนสารพิษจากแบคทีเรีย *Bacillus thuringiensis*

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Abstract

Cry4Aa and Cry4Ba δ-endotoxins produced from *Bacillus thuringiensis* are specifically toxic to mosquito-larvae by forming ion-permeable pores in the midgut cell membrane. One proposed toxic mechanism *via* an "umbrella-like" model involves membrane penetration and pore formation of the α4-loop-α5 hairpin. In this report, functional importance of the Cry4Aa α4-α5 loop structure, especially the Pro-rich sequence ($Pro^{193}Pro^{194}\_Pro^{196}$) was examined. Val-substitutions (P193V, P194V and P196V) were found to reduce toxicity against *Aedes aegypti* mosquito-larvae. In addition, enhancing the flexibility of the α4-α5 loop through P193G, P194G and P196G mutations adversely affected the larvicidal activity, suggesting that loop rigidity resulted from the unique cyclic structure of Pro is important for toxicity. Structural analysis of Pro^{193} showed that this most critical residue is in close contact with the surrounding residues, thus playing an additional role in maintaining tertiary structure of the toxin. MD simulations of the 65-kDa Cry4Aa structure in solution revealed that the α4-α5 loop is substantially stable, suggesting that an important implication in toxin activity of the Cry4Aa α4-α5 loop structure. Structurally stable-Cry4Ba mutant toxins of both larvicidal-active (N166D) and inactive (N166A and N166I) were also characterized for their relative activities in liposomal-membrane permeation and single-channel formation. Similar to the wild type, the N166D bio-active mutant was still capable of releasing entrapped calcein from lipid vesicles. Conversely, the two other bio-inactive mutants showed a dramatic decrease in causing membrane permeation. When the N166D mutant was incorporated into planar lipid bilayers, it produced single-channel currents with a maximum conductance of ~425 pS comparable to the wild type. However, maximum conductances for single K^+-channels formed by both bio-inactive mutants were reduced to approximately 165-205 pS. Structural dynamics of 60-ns simulations of a trimeric α4-α5 pore model in a fully hydrated-DMPC system revealed that an open-pore structure could be observed only for the simulated pores of the wild type and N166D. The number of lipid molecules surrounding both wild-type and N166D pores are relatively higher than those of N166A and N166I pores. Altogether, our results signify that the polarity at the α4-α5 loop residue–Asn^{166} is directly involved in ion permeation through the Cry4Ba toxin-induced pore and pore opening at the membrane-water interface.

Keywords **:** Ion channel; MD simulations; Larvicidal activity; Loop rigidity; Polarity; Pro-rich sequence; Mutagenesis; Trimeric pore structure