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Molecular insights into pore formation and target specificity of mosquito-active toxins from *Bacillus thuringiensis*

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We have been devoted to discovering a multitude of toxic mechanisms of bacterial insecticidal proteins, i.e. Cry4Aa & Cry4Ba toxins from *Bacillus thuringiensis* (*Bt*), which are highly toxic to larvae of *Aedes* and *Anopheles* species, vectors of dengue viruses and malaria, respectively. The insecticidal feature of these Cry mosquito-active toxins is generally attributed to their capability to form oligomeric pores, causing lysis of target mid-gut cells. However, molecular description of their oligomerization process and target specificity has not been clearly defined. In this report, we employed two direct rendering techniques, i.e. single-particle negative-stain electron microscopy and high-speed atomic force microscopy, for visualizing 3D structure and trimeric assembly of the membrane-associated Cry4Ba toxin. We clearly showed that a membrane-induced state of toxin monomers is a critical prerequisite for the formation of a potential pre-pore trimer. Moreover, the polarity of the Cry4Ba α4-α5 loop residue-Asn^{166} was found to be important for ion permeation and pore-opening. We further demonstrated that single-reversal charge in the β_{10}-β_{11} receptor-binding loops, Cry4Aa-Lys^{514} or Cry4Ba-Asp^{454}, reflected their different toxicity against target mosquito-larvae. Furthermore, we demonstrated that Cry4Ba utilizes two aromatic loop-residues, Tyr^{332} and Phe^{364} which are respectively located in β_2-β_3 and β_4-β_5 loops, comprising the receptor-binding domain, for synergistic interactions with its alternative receptor–Cyt2Aa2 from *Bt* subsp. *darmstadiensis*. We further showed that Thr^{328} and Thr^{369} could form H-bonding responsible for holding together these two receptor-binding hairpins (i.e., β_2-β_3 and β_4-β_5) in relevant to toxicity synergism with Cyt2Aa2. Altogether, we now feel able to tackle the key steps viz., their insecticidal mechanism, particularly on toxin-receptor interactions and the events following insertion of part of the toxin into membrane phase to form a trans-membrane leakage pore. Comprehensive understandings of the actual underlying toxic mechanism would bolster the future development of a better engineered bio-pesticide for control of such disease-carrying vectors.

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