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JONGRAK KITTIWORAKARN : MOLECULAR STUDIES OF A PUTATIVE PORE-FORMING FRAGMENT OF THE *Bacillus thuringiensis* Cry4B TOXIN. THESIS ADVISORS : CHANAN ANGSUTHANASOMBAT, Ph.D., SAKOL PANYIM, Ph.D., GERD KATZENMEIER, Ph.D. 120 p. ISBN 974-661-772-9

The Cry4B δ-endotoxin, produced as a crystalline inclusion by *Bacillus thuringiensis* subsp. *israelensis*, is variously toxic to mosquito larvae. The common mechanism of action of δ-endotoxins is proposed to be the formation of lytic pores in the susceptible larval midgut epithelium. The pores cause osmotic lysis of the cell, leading to the death of those larvae. Two published crystal structures of lepidopteran-specific Cry1Aa and coleopteran-specific Cry3A toxins reveal a possible apparatus for pore-formation in the form of a seven-amphipathic helix bundle in which five helices (α3, α4, α5, α6, and α7) that are longer than 30 Å could span the membrane to form a transmembrane pore. The possibility that this structure is essential for pore-formation is supported by the prediction that it is conserved in all Cry toxins. Several studies with synthetic peptides of α5 demonstrated their pore-forming activity in phospholipid bilayers. Also, site directed mutagenesis has shown that α4 and α5 are involved in pore-formation rather than in receptor recognition.

In this study, the peptides corresponding to the five-helix bundle (α1~α5) and the helical hairpin (α4~α5) were successfully expressed in *E. coli* as insoluble inclusions by employing glutathione-S-transferase (GST)-fusion system. Both proteins were unfolded by guanidine HCl denaturation and subsequently refolded to soluble proteins via a buffer exchange with urea followed by stepwise dialysis. Upon trypsin digestion, the five-helix bundle, was released from its fused GST tag, but the completed α4~α5 fragment could not be obtained. The peptides corresponding to α4 and α5 of Cry4B, termed as H4 and H5, respectively, were also chemically synthesized and characterized. The α-helical contents of the synthetic peptides, assessed in methanol by circular dichromism, were 90% and 96% for H4 and H5, respectively. The H4 (20.3 μM) apparently showed hemolytic activity against canine erythrocytes, whilst the the trypsin-digested GST-fused α1~α5 and H5 did not show observable lytic activity under the tested conditions. These data suggest that α4 may be important in pore-forming activity.