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SARINTHIP RUANGWETDEE : EXPANDING THE PREDICTED INTERHELICAL LOOP BETWEEN α3 AND α4 OF THE *Bacillus thuringiensis* CRY4B TOXIN FOR PROTEOLYTIC CLEAVAGE. THESIS ADVISORS : CHARTCHAI KRITTANAI, Ph.D., CHANAN ANGSUTHANASOMBAT, Ph.D., GERD KATZENMEIER, Ph.D. 105 p. ISBN 974-663-328-7

This study was a continuing attempt to introduce an additional proteolytic cleavage site into the interhelical loop between α3 and α4 of the Cry4B toxin. The *Bacillus thuringiensis* Cry4B δ-endotoxin is over produced in *Escherichia coli* as an inactive and insoluble protoxin in the form of inclusion bodies. The 130 kDa protein inclusion can be solubilized in Na_2CO_3 buffer pH 9.0 and activated by proteolytic processing. This processing has been suggested to be essential for a mechanism of action of the toxin. A treatment of Cry4B by trypsin or mosquito larval gut extracts indicates a removal of the C-terminal half and some amino acids on the N-terminal part of the protoxin resulting in a 65 kDa active toxin. On the active molecule of Cry4B, another cleavage site has also been identified at the amino acid R203 located on the predicted interhelical loop between α5 and α6 of the domain I.

Previously engineered Cry4B toxin containing an amino acid sequence change on the targeted loop from E_{136}PNNQ to S_{136}SRNP was reexamined for a proteolytic processing. In addition to the existing cleavage site on the interhelical loop between α5 and α6, the new cleavage site was generated on the targeted loop between α3 and α4. However, the efficiency for the cleavage on this engineered loop is very low and the processing is incompleted. After an insertion of an amino acid asparagine (N136) to expand the loop size, the cleavage is greatly enhanced. The products from the trypsin processing analyzed by SDS-PAGE reveal three fragments of approximately 47, 13 and 4.8 kDa. These fragments were found to remain associated when analyzed by a size-exclusion chromatography under non-denaturing condition. The expression level, solubility and mosquito larvicidal activity of the mutant toxin are lower than those of the wild type. The size of the inclusion from the mutant was found smaller than that of the wild type by electron microscope.

This study suggests that the loop size is crucial for the proteolytic processing on the helical hairpin loop between α3-α4 of Cry4B toxin.