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PANIDA LUNGCHUKIET: MUTAGENESIS INVESTIGATION OF THE ARGININE RESIDUE ON HELIX 5 OF THE *Bacillus thuringiensis* CRY4B TOXIN. THESIS ADVISORS : CHARTCHAI KRITTANAI, Ph.D., CHANAN ANGSUTHANASOMBAT, Ph.D., GERD KATZENMEIER, Ph.D.106 p. ISBN 974-664-648-6

The engineered Cry4B protein, S136NSSRNP, has been constructed recently to have two proteolytic cleavage sites on the interhelical loop between helix 3 and 4, and helix 4 and 5. Mass spectrophotometric analysis indicated that the released helical hairpin of α4 and α5 is truncated at the arginine located on the C-terminal of S136NSSRNP. To investigate the role of this arginine, we have used a PCR-based site-directed mutagenesis to substitute the arginine at either position 190 of Cry4B and position 191 of S136NSSRNP with glutamine (Q), alanine (A) and lysine (K). All mutant toxins expressed in *Escherichia coli* strain JM 109 as a cytoplasmic inclusion. The solubility of the protoxin inclusion in carbonate buffer, pH 9.0, is dramatically decreased. Biochemical characterization by trypsin digestion shows a drastic increase in proteolytic degradation. In addition, the activities of mutant toxins against *Aedes aegypti* mosquito larvae are completely demolished except for the R190A mutant, which still maintains some toxicity. The results suggest that the arginine residue located on helix 5, either at the position 190 of Cry4B or position 191 of S136NSSRNP has a critical role for structural folding and stability of the Cry4B toxin.