MUTAGENIC ANALYSIS OF SURFACE-EXPOSED LOOP RESIDUES IN THE RECEPTOR-BINDING DOMAIN OF THE Bacillus thuringiensis Cry4Ba TOXIN

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ABSTRACT

Critical surface-exposed loop residues (Pro\(^{389}\) in the \(\beta_6-\beta_7\) loop, Glu\(^{417}\) in the \(\beta_8-\beta_9\) loop, Tyr\(^{455}\) and Asn\(^{456}\) in the \(\beta_{10}-\beta_{11}\) loop) in the receptor-binding domain of the *Bacillus thuringiensis* Cry4Ba toxin have been demonstrated to be involved in larvicidal activity. The extended mutagenic analysis in this study was carried out to investigate a correlative effect among these critical loop residues on Cry4Ba toxicity. Several different double-loop mutants, P389A/E417A (\(\beta_6-\beta_7/\beta_8-\beta_9\) loops), P389A/Y455A, P389A/N456A (\(\beta_6-\beta_7/\beta_{10}-\beta_{11}\) loops), E417A/Y455A and E417A/N456A (\(\beta_8-\beta_9/\beta_{10}-\beta_{11}\) loops) were constructed via PCR-based mutagenesis and subsequently were highly expressed in *Escherichia coli* as 130-kDa protoxins at levels comparable to the wild type toxin. Each double mutant toxin was determined their toxicity against *Aedes aegypti* mosquito larvae. An almost complete loss in larvicidal activity was observed from all these double mutant toxins. Using immunohistochemical staining with a Cry4Ba specific monoclonal antibody, the double mutant toxins were able to bind to the apical microvilli of the susceptible *A. aegypti* larval midguts, albeit at lower-binding activity compared to the full-length active toxin. In addition, it was observed via electrochemical sensor that E417A/Y455A double-loop mutant was able to insert and permeabilize the liposomes leading to the release of the entrapped redox species comparable to the Cry4Ba wild-type. Therefore, the dramatic loss of larvicidal activity of E417A/Y455A is likely due to inability to bind to their receptors rather than to pore-forming activity. This study demonstrated that these critical loop residues in Cry4Ba-domain II: Pro\(^{389}\) in \(\beta_6-\beta_7\) loop, Glu\(^{417}\) in \(\beta_8-\beta_9\) loop, Tyr\(^{455}\) and Asn\(^{456}\) in \(\beta_{10}-\beta_{11}\) loop are dependently involved in Cry4Ba-receptor binding activity. These results also imply that the Cry4Ba-domain II activity requires more than one loop for the receptor-binding process. It is conceivable that the Cry4Ba-domain II could be divided into two sub-domains. The first sub-domain would consist of the residues which are located on the \(\beta_2\) to \(\beta_5\) (2 loops-\(\beta_2-\beta_3/\beta_4-\beta_5\)) and the other sub-domain would consist of the residues from \(\beta_1\) to \(\alpha_8\) and \(\beta_6\) to \(\beta_{11}\) (4 loops-\(\beta_1-\alpha_8/\beta_6-\beta_7/\beta_8-\beta_9/\beta_{10}-\beta_{11}\)).

KEY WORDS: MUTAGENIC ANALYSIS/ DOUBLE-LOOP RESIDUES/ *Bacillus thuringiensis*/ CRY TOXIN, RECEPTOR BINDING/ MOSQUITO- LARVICIDAL ACTIVITY/ IMMUNOHISTOCHEMICAL ASSAY/ MEMBRANE PERTURBATION ASSAY
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Pro389, Glu 417, Tyr 455, Asn 456 in a surface loop of Bacillus thuringiensis Cry4Ba; Pro389A/E417A, P389A/Y455A, P389A/N456A and E417A/N456A have lost toxicity in larvae of the mosquito. The modified Cry4Ba proteins were isolated and used as antigens. The results showed that the modified Cry4Ba proteins could be used in future research to develop new Cry4Ba-based pesticides.