Molecular Characterization of *Bacillus thuringiensis* d-Endotoxin Receptor

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ABSTRACT

Introduction: *Bacillus thuringiensis* (Bt) crystal proteins are effective in controlling agriculturally and biomedically harmful insects. However, the mechanism of Bt protein pesticidal action is not well understood. It is assumed that the pesticidal protein has affinity for specific receptors in the midgut of the susceptible larvae and binds irreversibly to create holes in the gut leading to eventual death of the target larvae.

Objectives: The main aim of the research is to identify, purify and characterize *Bt* delta endotoxin receptor in agronomically important pest, American bollworm (*Helicoverpa armigera*). The study presented is endeavored to; (1) discovery of a receptor protein, (2) purification of the receptor, (3) cloning of the newly discovered protein gene in *E.coli,* (4) expression studies on recombinant protein, (5) Complete sequencing of the cloned gene.

Methodology: Biological activities of different *Bt* crystal proteins, namely Cry1Aa, Cry1Ab and Cry1Ac and Cry2A were determined by in vitro mortality assays on larvae of *H. armigera.* Presence of a 65 kDa Protein is reported here, in the extract of the larval midgut membrane of *Helicoverpa armigera* as putative receptor for *Bt* Cry1A delta-endotoxins, on the basis of binding affinity to Cry1Aa, Cry1Ab and Cry1Ac but not to Cry2A.

The protein has been highly purified by a combination of gel-filtration, protoxin affinity and anion exchange chromatography. When the purified proteins were assayed for amino-peptidase activity, it showed 2.5 fold enrichment when compared to the activity in crude extract of *Helicoverpa armigera* brush border membrane vesicles. The stability of receptor protein was analyzed.

Key Findings: The results showed that 65 kDa protein was resistant to trypsin and gut juice and has binding affinity to Cry1Ac in ligand blots. Binding assays were performed. Cry1Ac toxins produced saturable, high-affinity binding to *H. armigera.* BBMVs. The results suggest that Cry1Aa, Cry1Ab, and Cry1Ac recognize the same binding site, which is different from Cry2A. N-terminal sequence of the purified protein does not show any homology to protein sequences in the Gen bank (NCBI) protein database. Degenerate primers were designed based on N-terminal sequence of the purified protein and hybridization of Probe with mRNAs of *Helicoverpa armigera* indicated sequence complimentarity. The structural gene of this purified protein was cloned in pGEMT-easy vector and pGEX-4T-3 expression vector.

The cloned gene was characterized through sequencing and studying expression in *E.coli*. The expressed GST fusion protein was purified through Glutathione Sepharose 4 Fast Flow column. The cloned, expressed and purified receptor protein was found to have 3-fold increase in APN activity than crude lysate, exhibited binding properties, in Western & ligand blots and other characteristics of native protein.

Larval mortality to Cry1A toxin was considerably reduced when the larvae were prefed a diet containing antibodies to 65 kDa protein, presumably due to blocking of the receptor sites in BBMVs.

Conclusion: A new method for quick, easy detection of Bt-receptor in crude BBMVs extract, was established, based on sandwich ELISA technique. By applying that method, the types of Cry proteins in Bt

isolates and Bt Cry1A receptor varieties in crude BBMVs extracts of target pests can be detected within one hour, before going into the tedious work of receptor purification.

The predicted amino acid sequence of cloned gene possess APN conserved motif (zinc binding site) and N-glycosylation site. The sequence has novelty because it has no significant homology to already existing sequences. The N- terminal sequence of the purified Protein (65 kDa) has been entered in the Gen Bank (NCBI) protein database (Accession # A59445). On Sep 13; 2005, sequence version replaced as Gen Bank Accession Q7M4K6.

Keywords: *Bacillus thuringiensis*, Delta Endotoxin, Receptor, *Helicoverpa armigera*.

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