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Identification, cloning and expression of a Cry1Ab cadherin receptor from European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)

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Abstract

Transgenic corn expressing the Cry1Ab toxin from *Bacillus thuringiensis* is highly toxic to European corn borer, *Ostrinia nubilalis*, larvae. A putative Cry1Ab receptor (OnBt-R_1) molecule was cloned and sequenced from a cDNA library prepared from midgut tissue of *O. nubilalis* larvae. The 5.6 Kb gene is homologous with a number of cadherin genes identified as Cry1 binding proteins in other lepidopterans. Brush border membrane vesicles were prepared using dissected midguts from late instars. A 220-kDa protein was identified as a cadherin-like molecule, which bound to Cry1Ab toxin and cross-reacted with an anti-cadherin serum developed from recombinant expression of a partial *O. nubilalis* cadherin peptide. Two additional proteins of smaller size cross-reacted with the anti-cadherin serum indicating that Cry1Ab binds to multiple receptors or to different forms of the same protein. *Spodoptera frugiperda* (SF9) cells transfected with the OnBt-R_1 gene were shown to express the receptor molecule which caused functional susceptibility to Cry1Ab at concentrations as low as 0.1 μg/ml. These results in combination suggest strongly that a cadherin-like protein acts as receptor and is involved with Cry1Ab toxicity in *O. nubilalis*.

Keywords: Lepidoptera; *Bacillus thuringiensis*; Binding analysis; Cadherin-like protein; Midgut; Cry toxins

1. Introduction

Bacillus thuringiensis (Berliner) (Bt) is a Gram-positive, spore-forming bacterium that produces crystalline inclusion bodies during sporulation that contain insecticidal δ-endotoxins. Lepidopteran insects are particularly susceptible to Cry1 toxins, which bind specifically to midgut receptors and are highly toxic after ingestion. Solubilization of the crystal releases a 130-kDa protoxin, which is activated by proteases in the insect midgut to form the truncated 65-kDa toxin. The target of the activated toxin is the apical (brush border) membrane of larval midgut cells (Bravo et al., 1992). Binding of the activated toxin to midgut-specific receptors causes the toxin conformation change, which allow its insertion and formation of ion channels or pores in the midgut apical membrane, leading to osmotic imbalance of the insect gut (Gill et al., 1992; Knowles, 1994; Schnepf et al., 1998).

Cry1-binding proteins detected on ligand blots of insect brush border membrane vesicles (BBMV) have been identified as members of the aminopeptidase N and cadherin families although the relative role of the two putative receptor molecules in insects has yet to be conclusively determined. A 210-kDa cadherin-like glycoprotein has been identified as a Cry1Ab binding protein in BBMV prepared from the midguts of *Manduca sexta* larvae (Vadlamudi et al., 1993, 1995).

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Although initially detected with Cry1Ab, other toxins such as Cry1Aa and Cry1Ac also bind the cadherin-like protein. In *Bombyx mori*, a 175 kDa cadherin-like protein was identified as a Cry1Aa binding protein (Nagamatsu et al., 1998a, b). Gahan et al. (2001) reported that Cry1Ac resistance in the tobacco budworm, *Heliothis virescens*, was tightly linked to a cadherin-encoding gene but not to genes encoding aminopeptidases. More recently, Morin et al. (2003) reported three different cadherin alleles from the pink bollworm, *Pectinophora gossypiella*, linked with resistance to Cry1Ac and survival on transgenic Bt cotton.

An epitope involved in Cry toxin–receptor interactions has been identified in a cadherin-like protein from *M. sexta* (Gomez et al., 2001). Previously, Nagamatsu et al. (1999) determined a region in the *B. mori* cadherin BtR_{175} which included the cadherin domain 9 and part of the membrane proximal region (MPR) that bound to Cry1Aa. Likewise, a region comprising the cadherin domain 11 in *M. sexta*, also adjacent to the membrane-proximal extracellular domain, was shown to bind Cry1A toxins (Dorsch et al., 2002). Both binding regions in these homologous proteins differed from that reported by Gomez et al. (2001). In addition to these studies, Hua et al. (2004) showed that both cadherin domains 11 and 12 from BtR_1 are important for Cry1A toxin binding, but that binding occurs first to domain 12, which mediates the subsequent binding to domain 11.

Transgenic corn expressing the Cry1Ab toxin has been deployed for control of the European corn borer, *Ostrinia nubilalis*, and has become an important component of corn production systems throughout the US. Cry1Ab has been shown to recognize a single population of binding sites on the brush border epithelium of *O. nubilalis* larvae (Denolf et al., 1993). Additionally, Hua et al. (2001) identified the presence of both aminopeptidases and a cadherin protein from the BBMVs, speculating that isoforms of both aminopeptidase and cadherin in the brush border membrane serve as binding proteins. The present study reports the cloning and expression of a cDNA that encodes a cadherin-like protein (OnBt-R_1) present in the midgut of *O. nubilalis* larvae. The receptor binds the Cry1Ab protein and is believed to be the major factor in mediating Cry1Ab toxicity in this insect.

2. Material and methods

2.1. Construction of cDNA library

Total RNA was extracted from *O. nubilalis* midgut tissue and used to create a lambda phage library. Briefly, total RNA was isolated from 4th-larval stage midgut tissue using the Messenger RNA Isolation Kit (Stratagene). The first strand cDNA was synthesized using StrataScript RT (Stratagene) and a poly(dT) oligo: 5′-GAGAGAGAGAGAGAGAGAGAACTAGTCTCG-AGTTTTTTTTTTTTTTTTT-3′. Second strand synthesis was accomplished via nick translation after the addition of DNA polymerase I. The termini of the cDNA were blunted by adding *pfu* DNA polymerase (Stratagene) and the following adapters containing *EcoR* I cohesive termini were ligated to the cDNA: 5′-OH-AATTCGGCACGAGG-3′ and 5′-CCTCGTGCCGp-3′. The cDNA was digested with Xho I and size fractionated. Fractions that contained fragments $\geqslant 1$ kb in length were ligated to the UNI-ZAP XR arms and packaged using the Gigapack III Gold packaging system (Stratagene).

2.2. cDNA cloning of OnBT-R_1

Based on the cDNA sequence for the *M. sexta* cadherin (Vadlamudi et al., 1995), a pair of degenerative primers were synthesized by Sigma Genosys for use in PCR reactions: 2A: 5′-CTTGGAATTCGAACAT/GTCCA/GTGC and 4S: 5′-TTGTACACAG/CGCA/TGGG/CATA/TTCCAC. PCR reactions were performed by standard techniques (Sambrook et al., 1989) using *Pwo* DNA polymerase (Roche). PCR products were cloned into pCR-Blunt II TOPO (Invitrogen) and sequenced using an ABI 3700 capillary electrophoresis unit and fluorescent dye termination chemistry (Foster City, CA).

The 280 base pair cadherin fragment generated by PCR was then used to screen the *O. nubilalis* midgut cDNA library previously described. Oligonucleotide probes were 3′ end-labeled with [α-^{32}P] dCTP using Rediprime™ II DNA Labeling System (Amersham Biosciences) in accordance with manufacturer recommendations. Approximately 6×10^9 recombinants were screened and several clones were found to hybridize to the probe. Positive clones were subjected to subsequent rounds of screening and plaque-purified. The cDNA from positive clones was sequenced in both directions by dideoxy chain termination.

2.3. Sequencing protocol

Sequencing reactions were performed using 1/8th reactions of v3.1 BigDye dye terminator chemistry (Applied Biosystems, Foster City, CA) in 20 μl reaction volumes under the following conditions: 10 s melting at 96 °C, 5 s annealing at 50 °C, 4 min extension at 60 °C for 25 cycles, followed by a 4 °C hold. Reactions were precipitated with 30 μl of 100% EtOH and resuspended in 30 μl dH_2O prior to loading on Applied Biosystems (Foster City, CA) 3700 capillary electrophoresis automated DNA analyzers, using the Pop5 polymer and a