Cadherin-like receptor from the European corn borer (*Ostrinia Nubilalis*) for *Bacillus thuringiensis* cry1A toxins

(Recived: 15.10.2002;Accepted: 23.03.2003)

Salah A. Mostafa[*], W.S.A. Maaty[*], M.A. Madkour[*] and L.A. Bulla, Jr[**]
**Department of Molecular and Cell Biology, The University of Texas at Dallas, Richardson, TX[2].
*Agricultural Genetic Engineering Research Institute, 9 Gamaa St. Giza,

ABSTRACT

Bacillus thuringiensis Cry1A toxins are lethal to the corn pest European corn borer (Ostrinia nubilalis) larvae. Cry1Aa, Cry1Ab and Cry1Ac bind to a protein of ~205-kDa in the brush border membrane vesicles. In addition, Cry1Ab binds to proteins of ~ 150 and 170-kDa and Cry1Ac binds to proteins of ~120 kDa. A competition ligand blot using unlabeled Cry1Ab to compete with ^{125}I-Cry1Ab shows that binding to the 150, 170 and 205-kDa proteins is compateted away by Cry1Ab. Furthermore, toxin-receptor dissociation constant K_d shows that the binding occurs with high affinity (K_d ~ 1.2 nM). These results suggest that Cry1Aa, Cry1Ab and Cry1Ac share a 205-kDa common membrane binding protein and they bind specifically with high affinity. The Southern blot and polymerase chain reaction analysis show that O. nubilalis contains a homologue to the 210-kDa Manduca Sexta BT-R_1 cadherin-like receptor which mediates toxicity for Cry1A toxins. Consequently, these results suggest that the 205-kDa protein band is a cadherin-like receptor homologous to the 210-kDa tobacco horn worm receptor. Therefore, it is more likely that the 205-kDa receptor is mediating toxicity to O. nubilalis but not the 120, 150 or 170-kDa proteins.

Keywords: *Cadherin/ Ostrinia nubilalis /Bacillus thuringiensis/Cry toxin*

* These authors contributed equally to this work § Corresponding author

INTRODUCTION

Microbial *Bacillus thuringiensis* (BT) based products have been used commercially for almost 40 years by growers, to control selected insect pests (Baum *et al.*, 1999). *Bacillus thuringiensis* is a gram-positive, spore-forming bacterium that forms a parasporal crystal which contains insecticidal toxins (Bulla *et al.*, 1980; Höfte and Whiteley, 1989). This observation led to the development of bioinsecticides that utilize *B. thuringiensis* for the control of many insect Species in the order Lepidoptera, Diptera, Coleoptera, Hymenoptera, Orthoptera and Mallophaga and against nematodes, mites and protozoa (Schnepf *et al.*, 1998). The toxin binds readily to specific receptors on the apical brush border of the midgut microvillae (BBMV) of susceptible insects (Hofmann *et al.*, 1988a;b). One of the most destructive pests in the world is the European corn borer. It causes severe damage to corn in Egypt and worldwide. We were interested to study the molecular mechanism by which the Bt toxins can control this insect. Our laboratories

previously reported the purification and cDNA cloning of a *B. thuringiensis* toxin receptor, Bt-R_1, present in midgut brush border membranes prepared from larvae of the lepidopteran *Manduca sexta* (Vadlamudi *et al.*, 1993 ; 1995). BT-R_1 is a cadherin-like glycoprotein, which has a molecular mass of approximately 210-kDa. BT-R_1 binds specifically with high affinity to Cry1Aa, Cry1Ab and Cry1Ac toxins in both heterologous and homologous competition binding experiments with *M. sexta* midgut protein preparations (Keeton and Bulla, 1997; Martinez-Ramirez *et al.*, 1994) and in heterologous cell cultures expressing the BT-R_1 cDNA (Keeton and Bulla, 1997). Truncation analysis of Bt-R_1 revealed that the only fragment capable of binding to the Cry1A toxins of *B. thuringiensis* was a contiguous 169-amino acid sequence adjacent to the membrane-proximal extracellular domain (Dorsch *et al.*, 2002).

Recently, two other cadherin-like proteins, 175- and 180-kDa, demonstrating amino acid sequence similarity to Bt-R_1 have been identified, purified and cloned from the lepidopteran *Bombyx mori* (silkworm) (Nagamatsu *et al.*, 1998a;b; Ihara *et al*, 1998). Moreover, a 210-kDa protein from the lepidopteran *L. dispar* (Gypsy moth) has been reported to bind Cry1Aa and Cry1Ab in ligand blot studies (Valaitis *et al.*, 1995, 1997). The 175-kDa protein from *B. mori* binds to Cry1Aa specifically with high affinity. The investigatios showed that the 210-kDa BT-R_1 and 175-kDa Bt-R 175 receptors were found exclusively in the *M. sexta* midgut (Midboe *et al.*, 2001; Nagamatsu *et al.*, 1998a). Several other binding proteins have been identified as aminopeptidase N (APN) with molecular masses that range from 100 to 170 kDa. In *M. sexta*, Cry1Ac and Cry1C bind to a 120- and 106-kDa APN, respectively (Knight *et al.*, 1994; Luo *et al.*, 1996). In *Plutella xylostella* (Luo *et al.*, 1997), and *L. dispar* (Valaitis *et al.*, 1995), a 120-kDa APN appears to bind Cry1Ac as well. In *B. mori*, Cry1Aa binds to a 120-kDa APN (Yaoi *et al.*, 1997). In general, these proteins showed low affinity for the toxin, and, the receptor assignments were difficult to reconcile with some of the ligand blot data (Lee and Dean, 1996).

As the use of transgenic crops containing Bt toxin genes increases, insect resistance almost certainly will become a problem. A few species of insects already have demonstrated increased tolerance for the toxins (Tabashnik *et al.* 1994; Muller-Cohn *et al.*, 1996; Moar *et al.*, 1995). Not surprisingly, it has been demonstrated by laboratory selection experiments that the use of Bt formulations and transgenic plants can provoke the development of resistance in European corn borer ECB (Venette *et al.*, 2000). Therefore, it is imperative to understand the mechanism of toxicity for this important insect. The characterization and cloning of the receptor, characterizing its binding site(s) for the toxin, and determining its relationship in binding different toxins will be of great value in preventing or delaying the development of resistance to bioinsecticides based on Bt. In the present study, we report the identification and characterization of toxin binding proteins in ECB for Cry1A toxins. We have studied the binding parameters of three Cry1A proteins toxic to ECB using brush border membrane vesicles (BBMV) and compared the results with their corresponding toxicities. We have identified a common binding protein in the size of 205 kDa as a cadherin-like receptor. In addition, we report the characterization of the other binding proteins: 150 and 170 kDa as an amino-peptidase-N.

MATERIALS AND METHODS

Toxin purification

Recombinant toxins Cry1Aa, Cry1Ab, and Cry1Ac (*Bacillus* Genetic Stock Center, The Ohio State University) were prepared from *E. coli* JM-103 and trypsinized essentially as described by Lee *et al.* (1992). In addition, the soluble trypsinized 60-kDa toxins were subjected to FPLC NaCl salt gradient purification over an HR-5/5 Mono-Q anion exchange column (Pharmacia) prior to quantitation, radio-iodination, and use in bioassays. All toxin protein quantitations were performed using the bicinchoninic acid method (Pierce Chemical Co.) with bovine Serum albumin (BSA, Fraction V) as a standard.

Insect toxicity assays

Toxicity assays were performed with neonate larvae of *O. nubilalis*. Multiwell-24 plates were filled with an artificial diet. Pro/toxins were diluted in phosphate-buffered saline (PBS). Five different concentrations of the toxin were tested on 30 larvae each. Sample dilutions (55 μl) were applied uniformly onto the diet surface and allowed to dry. Mortality scoring was determined after 5 days. The toxicity data were analyzed by probit analysis (Finney, 1971).

Preparation of brush border membrane vesicles (BBMV)

Early fourth-instar larvae were kept on ice for 1 h and midguts were surgically removed from the larvae. BBMV were prepared from midgut tissues by the differential magnesium precipitation method of Wolfersberger *et al.* (1987) in the presence of protease inhibitors (5 mg/ml pepstatin, antipain, aprotonin, leupeptin, 1 mM PMSF, and 5 mM benzamidine). The final pellet was resuspended in buffer A (300 mM mannitol, 5 mM EGTA, and 17 mM Tris-HCl, pH 7.5) containing the protease inhibitors, flash frozen in liquid nitrogen, and stored at 85°C.

Protein iodination

All Cry toxins used in this work were radioiodinated using the chloramine-T method (Hunter and Greenwood, 1962) with ^{125}I-Na (NEN Dupont). Ten μg of toxin were mixed with 5μl of ^{125}I-Na (0.5 mCi) in 100 μl of $NaHPO_4$ buffer (0.5 M, pH 7.4) with 25 μl of Chloramine T (4 mg/ml). The reaction mixture was agitated for 20-25 sec at 23°C and the reaction was stopped by adding 50 μl of $Na_2S_2O_5$ (4.4 mg/ml). Free iodine was removed by gel filtration on an Excellulose desalting column (Pierce) equilibrated with PBS containing 10 mg/ml BSA.

Toxin binding assays

Homologous competition inhibition binding assays were performed as described by Keeton and Bulla (1997). Twinty five μg of BBMV were incubated with 1.2 nM ^{125}I-Cry1Ab toxin in the presence of increasing concentrations (0 to 1000 nM) of the appropriate unlabeled homologous toxin (Cry1Ab). Incubations were in 100 μl of binding buffer (PBS/0.2% BSA) at 25° C for 30 min. Radiolabeled and unlabeled toxins were mixed together before adding them to the BBMV. Unbound toxins were separated from BBMV-bound toxin by centrifugation at 14,000-x g for 10 min. The pellet containing bound toxin was washed three times in ice cold binding buffer by a gentle mixing and radioactivity in the final pellet was measured using a Beckman Gamma 5500 counter. Binding data were analyzed by the PRISM program (GraphPad Software Inc., San Diego). The dissociation constant (K_d) of labeled toxins was calculated from three separate

experiments. The equilibrium binding parameters were estimated by analyzing the data with the PRISM computer program.

Radioligand blotting

Two hundred μg of BBMV proteins were solubilized, separated by 7.5% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane as described by Francis and Bulla (1997). Blots were blocked with TBS (10 mM Tris-HCl and 0.9 % NaCl) containing 5% non-fat dry milk powder, 5% glycerol, 0.5% Tween-20, and 0.025% sodium azide for 2 h at 25°C. Blocking buffer was removed and membranes were incubated for 2 h at 25°C in an equal volume of fresh blocking buffer containing 2 x 10^5 cpm/ml (1-1.25 nM) of ^{125}I-Cry1Aa, b or c toxins. Finally, membranes were washed three times with fresh blocking buffer for 10 min each, rinsed once with TBS, dried, and exposed to Kodak X-ray film at 80°C. To determine the specificity of binding of Cry1Ac toxin to the 200- and 120-kDa protein, blots of ECB BBMV proteins were incubated with ^{125}I-Cry1Ab toxin in the presence of increasing concentrations of unlabeled Cry1Ab toxin.

Southern blot analysis

Forty μg of *PvuII* digested genomic DNA from *O. nubilalis* or *M. sexta* were separated on a 0.8% 1X TBE-agarose gel and blotted onto a nylon membrane (BIO-RAD, Zeta-probe® GT). The analysis was carried out according to Sambrook *et al.* (1989). The filter was hybridized with ^{32}P-labeled, random-primed, C-terminal of BT-R_1 cDNA (HincII fragment, 0.5 kb).

Electrophoretic elution and protein sequencing

The electroeluted 150 and 170 kDa proteins were reduced and alkylated with N-isopropyliodoacetamide and then digested with endoproteinase Lys-C. Peptides were fractionated on HPLC followed with peptide sequencing for one peptide from each band (Fig .1A).

PCR analysis

Total RNA was prepared from the midgut tissue of the fourth instar larvae by the Guanidinium Thiocyanate method (Chomczynki and Sacchi, 1987). First strand cDNA was synthesized using oligo-dT with Reverse transcriptase superscript II RNase H$^-$. The cDNA was used as a template in PCR amplification using Gene specific primers GSP1 (ATGGCAGTTGACGTCCGAATC), GSP2 (AGGTGGTCACTCTGATAGCCA), GSP3 (GTAGTGACCTAGTCCTCCTGA) and GSP4 (GGACAGTTCAGAAAGAT-CTAG) that were driven from the Bt-R_1 sequence.

RESULTS

Identification of ^{125}I-Cry1A binding proteins

BBMV proteins of *O. nubilalis* ranged in molecular size from 400 kDa to 25 kDa (Fig. 1A) as determined by SDS-PAGE. ^{125}I-labeled Cry1Aa, Cry1Ab and Cry1Ac were used in ligand blots to identify which *O. nubilalis* BBMV proteins bind to the respective toxins. Proteins that had been separated by SDS-PAGE were transferred to PVDF membranes and incubated with each radiolabeled toxin separately. ^{125}I-Cry1Aa, -1Ab and -1Ac bound to a protein of ~ 205-kDa (Fig. 1B). ^{125}I-Cry1Ab bound to a protein bands in the range of 120 to 170 kDa. ^{125}I-Cry1Ac bound also to protein band at ~ 120-kDa.

Toxicity of Cry1A proteins

The specific toxicities of purified Cry1Aa, Cry1Ab, and Cry1Ac were tested using neonate *O. nubilalis* larvae (Table 1). The results showed that all three Cry1A toxins are highly toxic, with LC_{50} values ranging from 50-1250 ng/cm^3 of artificial diet.

Competition inhibition binding assays

^{125}I-labeled Cry1Ab was used in homologous competition binding assays with *O. nubilalis* BBMV. This was carried out in the presence of increasing concentrations of unlabeled Cry1Ab. Fifty-percent inhibition of Cry1Ab binding was observed at 10 nM of unlabeled Cry1Ab (Fig. 2A). These data indicate that each of the three toxins binds specifically with high affinity. The dissociation constant, K_d of toxin was calculated from the three separate homologous competition inhibition experiments by analyzing data with the GraphPad computer program.

Specificity of ^{125}I-Cry1Ab toxin binding in ligand blots

Radioligand blots of *O. nubilalis* BBMV proteins were carried out with ^{125}I-Cry1Ab toxin in the presence of increasing concentrations of unlabeled Cry1Ab toxin. Autoradiography (Fig. 3) of these blots revealed significant reduction in the intensity of all bands (120-205kDa). Indeed, it was undetectable at a Cry1Ab toxin concentration of 10 nM.

Protein sequencing

The amino acid sequence from the lower band (150 kDa) is A T F X I T L R R P V G F (K), while the obtained sequence from the higher band (170 kDa) is (I) V L L Q V L G C T P H E (A). The two sequences were used in a BLAST search for homology with any proteins in the databases. The two peptides came up with great similarity to the membrane alanyl aminopeptidase from tobacco budworm (*Heliothis virescens*).

Southern blot analysis

To detect the presence of the Cry1A receptor in *O. nubilalis*, genomic DNA was hybridized against two probes from *M. sexta* BT-R_1 cDNA (SacI probe, 4.8 kb) or (HincII probe, 0.5 kb). The two probes bound intensively to the PvuII digested fragments of *M. sexta* genomic DNA (Fig. 4, lane 2) and to a less extent with the *O. nubilalis* genomic DNA (lane 1). Furthermore, a 507-bp fragment from BT-R_1 cDNA (HincII fragment, 0.5 kb) did not hybridize with ECB genomic DNA (data not shown). These results suggest that the full-length sequence of the ECB new receptor will have a significant level of nucleotide similarity to Bt-R_1 full length but not to the Bt-R_1 minimum-binding fragment.

PCR analysis

A strong confirmation to the Southern blot analysis came from the PCR analysis for the Bt receptor in ECB. The gene- specific primers drived from Bt-R_1 sequence were able to amplify DNA fragments with identical sizes to the predicted from the Bt-R_1 receptor of tobacco hornworm. The resulted receptor DNA fragments were as follows: 1.9, 2.7 and 5.2 kbp, respectively (Fig 5).

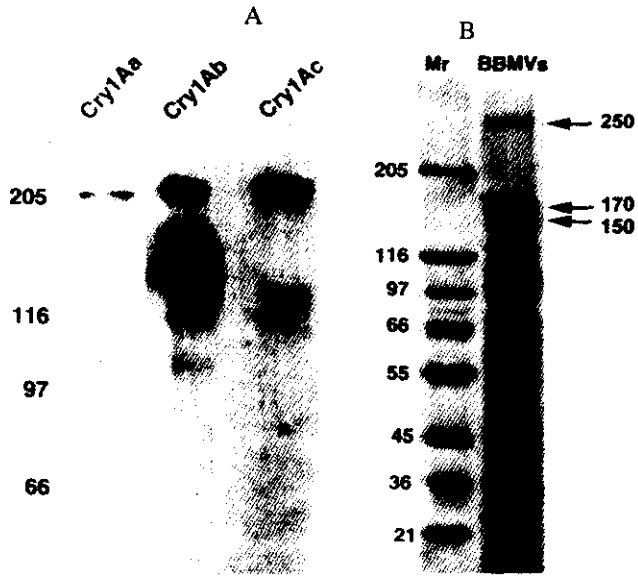

Fig. (1): SDS-PAGE ligand blots of O. nubilalis midgut proteins. 100 µg Membrane proteins were prepared by the Wolfersberger method and separated by SDS-PAGE (panel A, BBMVs), or blotted to PVDF filters and incubated with ^{125}I-labeled Cry1Aa, Ab, and Ac, respectively (Panel B). Protein molecular size marker (in kDa) is indicated on the left (Mr).

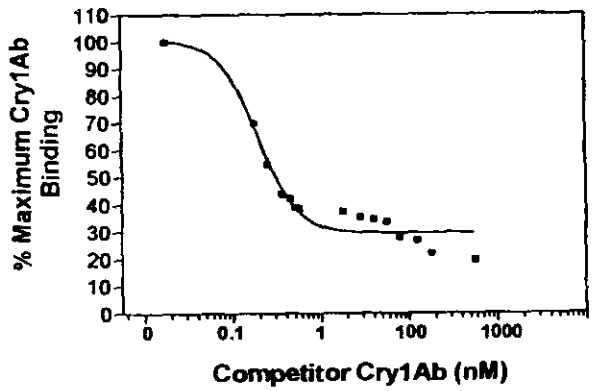

Fig. (2): Homologous competition binding of ^{125}I-labeled Cry1Ab toxins to BBMV proteins. BBMV (25 µg) were incubated with 1 nM ^{125}I-labeled toxin in the presence of increasing concentrations of the corresponding unlabeled toxin Cry1Ab (0.01-1µM). Each point represents the mean of three independent experiments.

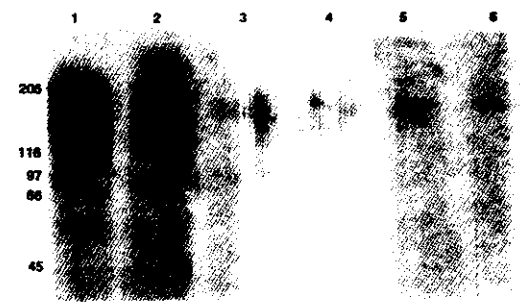

Fig. (3): Competition ligands blot of O. nubilalis midgut proteins and Cry1Ab. O. nubilalis midgut proteins (100 µg) prepared as shown in materials and methods and blotted to PVDF filters. Lanes were hybridized with ^{125}I-labeled Cry1Ab (lane 1) or a combination of ^{125}I-labeled Cry1Ab and an approximately 1, 10, 50, 100, or 1,000-fold excess of unlabeled Cry1Ab (lanes 2, 3, 4, 5, and 6 respectively). Positions of molecular size markers (in kilo Daltons) are indicated on the left.

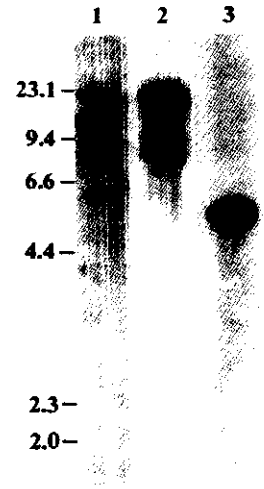

Fig. (4): Southern blot analysis for O. nubilalis genomic DNA. Forty µg of PvuII digested genomic DNA from O. nubilalis (lane 1) and M. sexta (lane 2) were separated on a 0.8% 1 X TBE-agarose gel and blotted onto a nylon membrane. The filter was hybridized with ^{32}P-randomly labeled Bt-R$_1$ cDNA (SacI fgment, 4.8 kb). The full-length Bt-R$_1$ cDNA (SacI fragment, 4.8 kb) was used as positive control (lane 3). Marker positions (kbp) are indicated on the left.

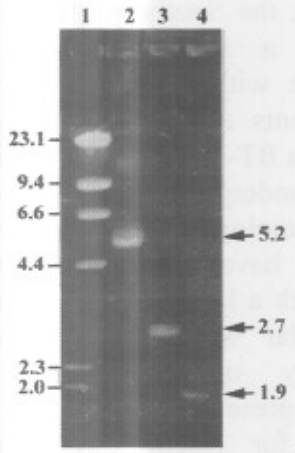

Fig. (5): The polymerase chain reaction (PCR) detection for BT-R_1 homologue in ECB. Gene specific primers GSP1, GSP2, GSP3 and GSP4 corresponding to different regions of BT-R_1 cDNA were used to detect and amplify the BT-R_1 homolouge from ECB. One distinct band with each pair of primers was obtained (5.2, 2.7 and 1.9 kbp in lanes 2, 3 and 4, respectively). A Lambda Hind III DNA size marker is shown on the left (lane 1).

DISCUSSION

Corn insects reduce yields by a significant percentage across the corn fields allover the world. One group in particular, the corn borers complex was the most damaging, causing a 4% loss with a financial loss of about $1billion. Biological control of the corn pests using *B. thuringiensis* formulations and transgenic plants has been in use for a number of years and is growing rapidly. Concerns that BT formulations or transgenic plants expressing the *cry1A* genes of *B. thuringiensis* may evoke emergence of either resistant or tolerant strains of corn insects such as *O. nubilalis*, as has occurred with some other insects, have prompted the search for a better understanding of the interaction between the Bt toxin proteins and their respective insect receptors. A better understanding of the mode of action of Cry proteins is needed to develop strategies that may prevent or slow the occurrence of resistance.

The research described herein was targeted toward the identification and characterization of the Cry toxin receptor for the ECB. In this study, we examined the toxicity of three different Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac) against neonate ECB larvae. The lepidopteran-specific toxins (Cry1Aa, Cry1Ab and Cry1Ac) showed high toxicity toward ECB larvae with a LC_{50} ranging from 50-1250 ng/cm^3 of insect diet (Table 1). The results demonstrated the exceptional specificity and potency of the three Cry1Ab toxins. Therefore, we examined the binding of this lepidopteran specific Cry1Ab toxin to the BBMV of ECB. Ligand blot experiments showed that proteins of 120-170 kDa bind only the Cry1Ab toxin whereas a 205-kDa protein binds to Cry1Aa, Cry1Ab and Cry1Ac toxins (Fig. 1). Thus, the 205-kDa protein from the ECB is a common binding protein for the lepidopteran-specific Cry1A toxins. These results suggest that Cry1Ab binding to BBMV proteins could be more extensive than binding of Cry1Aa or Cry1Ac. In addition, we found that Cry1Ac binds to 120-kDa band. These observations are similar to those reported for *M. sexta* (Keeton and Bulla 1997, Keeton et al., 1998). Moreover, it has been reported that binding of Cry1Ac to a 120-kDa aminopeptidase in *M. sexta* is dependent upon specific sequences in domain III of Cry1Ac and binding to BT-R_1 is dependent on specific sequences in domains I and/or II (De Maaged et al., 1996). In an

analysis of the phylogeny of the three domains of the Cry1A toxins, only the origins of domains I and II appear to be shared. Domain III of Cry1Ac resides on a unique branch in the phylogenetic tree described by Bravo (1997).

The detailed mechanisms of the Cry1A toxin interactions with the midgut BBMV of the European corn borer also have been presented. The equilibrium dissociation constants (K_d) calculated from the homologous competition assays (Fig, 2) is 1.2 nM for Cry1Ab. These results indicate high affinity binding of Cry1A toxins to the BBMV receptors. Our observations using ECB BBMV proteins suggest that the 205-kDa protein is more likely to be mediating Cry1A toxicity in this insect whereas the 120-170 kDa proteins act as low-affinity or nonspecific binding sites (sink) for the Cry1Ab toxin. Competition assays for Cry1Ab binding to ECB BBMV show that binding of labeled Cry1Ab is almost completely inhibited by unlabeled Cry1Ab at 1 μM (Fig. 2). A ligand blot with labeled Cry1Ab shows that binding of ^{125}I-Cry1Ab to the BBMV proteins is almost completely eliminated in the presence of 100-fold excess of unlabeled Cry1Ab (Fig. 3). Similar results have been previously observed with *M. sexta* using the same three toxins (Keeton and Bulla, 1997), 175-kDa cadherin-like Cry1Aa receptor from *Bombyx mori* (Nagamatsu et al., 1998a); and 200-kDa cadherin-like Cry1A receptor from the cotton pest pink bollworm (Maaty et al., in preparation). Thus, it now appears that ECB, like *M. Sexta, B. mori and* Pink Bollworm, contains high-affinity receptor for Cry1A toxin. To confirm the presence of BT-R_1 homologue in ECB we designed gene specific primers based on BT-R_1 sequence. The primers were able to amplify DNA fragments with the expected molecular weight. Furthermore, the Southern blot analysis-using BT-R_1 as a probe showed significant hybridization with the ECB genomic DNA. This represents another confirmation to the presence of a BT-R_1 like receptor in ECB. The research is undergoing to clone and sequence the BT-R homologue from ECB. Certainly, the four insects have high-affinity cadherin-like receptors with a large molecular mass. These high-molecular weight proteins comprise a new family of cadherin-like proteins. Cadherins represent a family of glycoproteins responsible for calcium-dependent cell-cell adhesion (Takeichi, 1990). Cadherins preferentially interact with themselves in a homophilic manner to physically connect cells, thus acting as both receptor and ligand. A large number of cadherins and cadherin-related proteins are expressed in different tissues of a variety of multicellular organisms. At this time, we can only speculate on the function of these cadherin-like proteins in the mode of action of *B. thuringiensis* toxins, which are generally thought to disrupt ionic balance in the midgut epithelium (Knowles, 1994). However, at least one type of cadherin has been shown to be the crucial receptor for the binding of the gram-positive intracellular pathogen *Listeria monocytogenes* to the plasma membrane of nonphagocytotic epithelial cells (Mengaud et al., 1996). It is conceivable that in acting as a receptor for the Cry1A toxins, BT-R is responsible, either directly or indirectly, for mediating the intercalation of the lepidopteran-specific toxins into the brush border membranes of intoxicated larvae. The physiological function of these receptors in the midgut is under investigation. The understanding of toxin-receptor interaction mechanism will facilitate the proper use of BT toxins to minimize selection pressure on ECB populations.

Table (1): Toxicity of non-activated (protoxins) and activated (toxin) B. thuringiensis crystal proteins against Ist instar larvae of O. nubilalis.

BT toxin	LC_{50} (ng/cm^3) Protoxin	toxin
Cry1A a	1325	1250
Cry1A b	62	50
Cry1A c	730	615

LC_{50} values were calculated by probit analysis. Doses are expressed in nanograms of protoxin applied per square centimeter of artificial medium.

REFERENCES

Baum, J.A., Gilmer, A.J, Light Mettus, A.M. (1999). Multiple roles for TnpI recombinase in regulation of Tn5401 transposition in *Bacillus thuringiensis*. J. Bacteriol., 20:71-77.

Bravo, A. (1997). Phylogenetic relationships of *Bacillus thuringiensis* delta-endotoxin family proteins and their functional domains. J. Bacteriol., 179:2793-801.

Bulla, L.A. Jr., Bechtel, D.B, Kramer, K.J, Shethna, Y.I, Aronson, A.I, and Fitz-James, P.C. (1980). Ultrastructure, physiology, and biochemistry of *Bacillus thuringiensis*. Crit. Rev. Microbiol., 8:147-204.

Chomczynki, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analyt. Biochem., 162:156-159.

De Maagd, R. A., Van der Klei, Bakker, H, Stiekema, W.J, and Bosch, D. (1996). Different Domains of *Bacillus thuringiensis* δ-endotoxins can bind to insect midgut membrane proteins on ligand blots. Appl. Environ. Microbiol., 62:2753-2757.

Dorsch, J. A., Candas, M., Griko, N.B., Maaty, W.S.A., Midboe, E.G., Vadlamudi, R.K., and Bulla, L.A. Jr. (2002). Cry1A toxins of *Bacillus thuringiensis* bind specifically to a region adjacent to the membrane-proximal extracellular domain of BT-R_1 in *Manduca sexta*: involvement of a cadherin in the entomopathogenicity of *Bacillus thuringiensis*. Insect Biochemistry and Molecular Biology. 32:1025-1036.

Finney, D. Y. (1971). Probit Analysis 3rd ed. Cambridge univ. Press, Cambridge.

Francis, B. R., and Bulla, L.A. Jr. (1997). Further characterization of BT-R_1, the cadherin-like receptor for Cry1Ab toxin in tobacco hornworm (*Manduca sexta*) midguts. Insect. Biochem. Mol. Biol., 27:541-50.

Hofmann, C., Luthy, P., Hutter, R., and Pliska, V. (1988a). Binding of the delta endotoxin from *Bacillus thuringiensis* to brush- border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). Eur. J. Biochem., 173:85-91.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S., and Van Mellaert. H. (1988b). Specificity of *Bacillus thuringiensis* delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc. Natl. Acad. Sci., 85:7844-8.

Hofte, H. and Whiteley, H.R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev., 53:242-55.

Hunter, W. and Greenwood, F. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature., 194:495-496.

Ihara, H., Uemura, T., Masuhara, M., Ikawa, S., Sugimoto, K., Wadano, A. and Himeno. M. (1998). Purification and partial amino acid sequences of the binding protein from *Bombyx mori* for CrylAa delta-endotoxin of *Bacillus thuringiensis*. Comp Biochem. Physiol. B Biochem. Mol. Biol., 120:197-204.

Keeton, T.P. and Bulla, L.A. Jr. (1997). Ligand specificity and affinity of BT-R_1, the *Bacillus thuringiensis* Cry1A toxin receptor *from Manduca sexta*, expressed in mammalian and insect cell cultures. Appl. Environ. Microbiol., 63:3419-25.

Keeton, T.P., Francis, B.R., Maaty, W.S.A. and Bulla, L.A. Jr. (1998). Effects of midgut-protein-preparative and ligand binding procedures on the toxin binding characteristics of BT-R_1, a common high-affinity receptor in *Manduca sexta* for Cry1A *Bacillus thuringiensis* toxins. Appl. Environ. Microbiol., 64:2158-65.

Knight, P.J., Crickmore, N. and Ellar, D.J. (1994). The receptor for *Bacillus thuringiensis* CrylA(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase N. Mol. Microbiol., 11:429-36.

Knowles, B.H. (1994). Mechanism of action of *Bacillus thuringiensis* insecticidal δ-endotoxins. Adv. Insect. Physiol., 24:275-308.

Lee, M.K. and Dean, D.H. (1996). Inconsistencies in *determining Bacillus thuringiensis* toxin binding sites relationship by comparing competition assays with ligand blotting. Biochem. Biophys. Res. Commun., 220:575-80.

Lee, M.K., Milne, R.E., Ge, A.Z. and Dean, D.H. (1992). Location of *Bombyx mori* receptor binding region of a *Bacillus thuringiensis* δ-endotoxin. J. Biol. Chem., 267:3115-3121.

Luo, K., Lu, Y.J. and Adang, M.J. (1996). A 106 kDa form of aminopeptidase is a receptor for *Bacillus thuringiensis* Cry1C δ-endotoxin in the brush border membrane of *Manduca sexta*. Insect Biochem. Molec. Biol., 26:783-791.

Luo, K., Tabashnik, B.E. and Adang, M.J. (1997). Binding of *Bacillus thuringiensis* Cry1Ac toxin to aminopeptidase in susceptible and resistant Diamondback moths *(Plutella xylostella)*. Appl. Environ. Microbiol., 63:1024-1027.

Maaty, W.S.A., Stewart, F.D., Osman, Y.A., Madkour, M.A. and Bulla, L.A. Jr. Identification and purification of a high affinity receptor from *Pectinophora gossypiella* (Pink Bollworm) for Cry1A toxins of *Bacillus thuringiensis*. (In preparation).

Martinez-Ramirez, A.C., Gonzalez-Nebauer, S., Escriche, B. and Real, M.D. (1994). Ligand blot identification of a *Manduca sexta* midgut binding protein specific to three *Bacillus thuringiensis* CrylA-type ICPs. Biochem. Biophys. Res. Commun., 201:782-7.

Mengaud, J., Ohayon, H., Gounon, P., Mège, R.M. and Cossart, P. (1996). E-cadherin is the receptor required for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. Cell. 84:923-932.

Midboe, E.G., Candas, M., Dorsch, J.A. and Bulla, L.A. Jr. (2001). Susceptibility of *Manduca sexta* larvae to the Cry1Ab toxin of *Bacillus thuringiensis* correlates inversely with the developmental

expression of the toxin receptor BT-R_1 *SAAS Bulletin Biochem. Biotech.* 14:73-80.

Moar, W. J., Pusztai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., Luo, K. and Adang, M.J. (1995). Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Appl. Environ. Microbiol., 61:2086-2092.

Muller-Cohn, J., Chaufaux, J., Buisson, C., Gilois, N., Sanchis, V. and Lereclus, D. (1996). *Spodoptera littoralis* (Lepidoptera: Noctuidae) resistance to Cry1C and cross-resistance to other *Bacillus thuringiensis* crystal toxins. J. Econ. Entomol., 89:791-797.

Nagamatsu, Y., Toda, S., Koike, T., Miyoshi, Y., Shigematsu, S. and Kogure, M. (1998a). Cloning, sequencing, and expression of the *Bombyx mori* receptor for *Bacillus thuringiensis* insecticidal CryIA(a) toxin. Biosci. Biotechnol. Biochem., 62:727-34.

Nagamatsu, Y., Toda, S., Yamaguchi, F., Ogo, M., Kogure, M., Nakamura, M., Shibata, Y. and Katsumoto, T. (1998b). Identification of *Bombyx mori* midgut receptor for *Bacillus thuringiensis* insecticidal CryIA(a) toxin. Biosci Biotechnol Biochem., 62:718-26.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean D.H. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev., 62:775-806.

Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K. and Adang, M.J. (1994). Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. Proc. Natl. Acad. Sci., 91:4120-4.

Takeichi, M., Inuzuka, H., Shimamura, K., Matsunaga, M. and Nose, A. (1990). Cadherin-mediated cell-cell adhesion and neurogenesis. Neurosci. Res. Suppl., 13:S92-6.

Vadlamudi, R.K., Ji, T.H. and Bulla, L.A. Jr. (1993). A specific binding protein from *Manduca sexta* for the insecticidal toxin of *Bacillus thuringiensis subsp. berliner*. J. Biol. Chem., 268:12334-40.

Vadlamudi, R.K., Weber, E., Ji, I., Ji, T.H. and Bulla, L.A. Jr. (1995). Cloning and expression of a receptor for an insecticidal toxin *of Bacillus thuringiensis*. J. Biol. Chem., 270:5490-4.

Valaitis, A.P., Mazza, A., Brousseau, R. and Masson L. (1997). Interaction analyses of *Bacillus thuringiensis* Cry1A toxins with two aminopeptidases from gypsy moth midgut brush border membranes. Insect Biochem. Mol. Biol., 27:529-539.

Valaitis, A.P., Lee, M.K., Rajamohan, F. and Dean D.H. (1995). Brush border membrane aminopeptidase-N in the midgut of the gypsy moth serves as the receptor for the CryIA(c) delta-endotoxin *of Bacillus thuringiensis*. Insect Biochem. Mol. Biol., 25:1143-51.

Venette R.C., Hutchison W.D. and Andow D.A. (2000). An in-field screen for early detection and monitoring of insect resistance to *Bacillus thuringiensis* in transgenic crops. J. Econ. Entomol., 93:1055-64.

Wolfersberger, M., Luethy, P., Maurer, A., Parenti, P., Sacchi, F.V., Giordana, B. and Hanozet, G.M. (1987). Preparation and partial characterization of amino acid transporting brush border membrane vesicles from the larval midgut of the

cabbage butterfly (*Pieris brassicae*). Comp. Biochem. Physiol., 86A:301-308.

Yaoi, K., Kadotani, T., Kuwana, H., Shinkawa, A., Takahashi, T., Iwahana, H. and Sato, R., (1997). Aminopeptidase N from *Bombyx mori* as a candidate for the receptor of *Bacillus thuringiensis* Cry1Aa toxin. Eur. J. Biochem., 246:652-657.

الملخص العربي

تعريف وتوصيف للمستقبل Cadherin like receptor من حشرة ثاقبة الذرة الأوروبية (*Ostrinia nubilalis*) عالي الارتباط مع البروتين السام (Cry1A) من بكتريا باسيلس ثيرنجنسيس

صلاح على مصطفى* – وليد سيد معاطى* – مجدى احمد مدكور* – لى بولا جى ار**

*معهد بحوث الهندسة الوراثية الزراعية – مركز البحوث الزراعية– الجيزة

**قسم الخلية و البيولوجيا الجزيئية – جامعة تكساس– دلاس بالولايات المتحدة الأمريكية

من المعروف أن البروتينات من نوع (Cry1A) شديدة السمية ليرقة ثاقبة الذرة الأوروبية. وجد أن ثلاثة أنواع من هذه السموم وهى Cry1Aa, Cry1Ab and Cry1Ac ترتبط مع بروتينات ذات وزن جزيئى حوالى ٢٠٥ كيلو دالتون موجودة فى الأغشية المبطنة لخميلات المعى المتوسط وبالإضافة الى ذلك يرتبط البروتين Cry1Ab مع بروتينين إضافيين لهما وزن جزيئى ١٥٠ ، ١٧٠ كيلو دالتون، كما يرتبط البروتين Cry1Ac مع بروتين وزنه الجزيئى ١٢٠ كيلو دالتون. أظهرت تجربة Competition ligand blot باستخدام بروتين Cry1Ab الموسوم باليود المشع ١٢٥ قدرته على التنافس وطرد البروتين غير الموسوم من مكانه على المستقبل، وقد تم حساب kd (toxin-receptor dissociation constant حيث كان قيمته١,٢ نانومول من البروتين السام مما يظهر مدى تخصص الارتباط.

من خلال النتائج السابقة يمكن القول أن البر وتينات السامة Cry1Aa, Cry1Ab, Cry1Ac لها مستقبل عام يشترك فى الارتباط به؛ وهو بروتين غشائي وزنه الجزئي ٢٠٥ كيلو دالتون حيث يرتبط به بدرجة عالية من التخصص. وقد أظهرت تجارب التهجين النووي (Southern blot) وتفاعل البلمرة المتسلسل (PCR) باستخدام بادئات متخصصة لمستقبل يرقة الدخان ذات القرون BT-R1 وجود تشابه كبير بين كل من مستقبل يرقة ثاقبة الذرة الأوربية ومستقبل يرقة الدخان ذات القرون. وفى النهاية يمكن القول أن البروتين السام Cry1A يرتبط مع المستقبل ٢٠٥ وأن هذا الارتباط يؤدى إلى موت الحشرة أكثر من ارتباطه مع البروتينات ١٢٠أو ١٥٠ أو ١٧٠ كيلو دالتون.