Univalent Binding of the Cry1Ab Toxin of *Bacillus thuringiensis* to a Conserved Structural Motif in the Cadherin Receptor BT-R_1

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ABSTRACT: The Cry1Ab toxin produced by *Bacillus thuringiensis* (Bt) exerts insecticidal action upon binding to BT-R_1, a cadherin receptor localized in the midgut epithelium of the tobacco hornworm *Manduca sexta* [Dorsch, J. A., Candas, M., Griko, N. B., Maaty, W. S., 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 Biochem. Mol. Biol. 32,* 1025−1036]. BT-R_1 represents a family of invertebrate cadherins whose ectodomains (ECs) are composed of multiple cadherin repeats (EC1 through EC12). In the present work, we determined the Cry1Ab toxin binding site in BT-R_1 in the context of cadherin structural determinants. Our studies revealed a conserved structural motif for toxin binding that includes two distinct regions within the N- and C-termini of EC12. These regions are characterized by unique sequence signatures that mark the toxin-binding function in BT-R_1 as well as in homologous lepidopteran cadherins. Structure modeling of EC12 discloses the conserved motif as a single broad interface that holds the N- and C-termini in close proximity. Binding of toxin to BT-R_1, which is univalent, and the subsequent downstream molecular events responsible for cell death depend on the conserved motif in EC12.

Bacillus thuringiensis (Bt) Cry toxins exert lethal action on certain orders of insects, many of which are economically important crop pests. A key event in Cry toxin action is the binding of toxin molecules to specific receptors in the midgut epithelium of susceptible insect larvae. These receptors, represented by BT-R_1 of the lepidopteran (moth) *Manduca sexta* (*2*, *3*), constitute a family of homologous cadherins crucial to the binding and insecticidal action of various Cry toxins (*1*, *3*−*6*). Recently, we showed that binding of the Cry1Ab toxin to the cadherin BT-R_1 receptor triggers a signaling event that leads to oncotic-like cell death (*7*). Toxin action can be inhibited by blocking the binding of the toxin to BT-R_1 either in an insect (*1*) or in cultured insect cells (*7*, *8*), demonstrating that specific toxin−receptor interaction is the primary determinant of toxicity (*1*, *8*).

On the basis of the classification of cadherins, BT-R_1 is composed of four domains: ectodomain (EC),[1] membrane-proximal extracellular domain (MPED), transmembrane domain (TM), and cytoplasmic domain (CYTO) (*1*, *8*−*12*). The EC consists of 12 ectodomain modules (EC1−EC12) that are composed of β-barrel cadherin repeats, all of which are connected by interdomain linkers.

Previously, we discovered that a 169-amino acid segment encompassing EC11 and EC12 of BT-R_1 contains the binding site for the Cry1Ab toxin (*1*). This amino acid segment completely inhibited action of the Cry1Ab toxin in insect larvae when it was preincubated with the toxin at a 1:1 molar ratio (*1*). In the current study, gel filtration chromatography, along with affinity binding assays, demonstrate that the Cry1Ab toxin forms a univalent complex with BT-R_1 at a 1:1 stochiometric ratio. To further define toxin binding on BT-R_1 in the context of the cadherin repeats, various truncated peptide fragments of EC11 and EC12 were tested for their capacity to bind the Cry1Ab toxin. The results reveal that EC12 plus part of the EC11−12 interdomain linker contains a conserved motif for Cry1Ab toxin binding. The motif is comprised of N- and C-terminal amino acid sequences in EC12 that are required for binding Cry1Ab. Furthermore, there are sequence signatures within the N- and C-termini that incorporate both structural and functional information. Neither the N- nor C-terminal amino acid sequences alone are sufficient for toxin binding. Protein structure modeling reveals a broad structural interface that brings the N- and C-termini of EC12 into close proximity.

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[1] Abbreviations: BSA, bovine serum albumin; CYTO, cytoplasmic domain; EC, ectodomain; IPTG, isopropyl β-thiogalactoside; MBP, maltose-binding protein; MPED, membrane-proximal extracellular domain; PKA, protein kniase A; PVDF, poly(vinyl difluoride); SAS, sequences annotated by structure; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; TM, transmembrane domain; TBR, toxin-binding region.

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Binding of toxin to the N- and C-termini of EC12 constitutes the first step in triggering the cytotoxic events leading to oncotic cell death described previously (*7*).

EXPERIMENTAL PROCEDURES

Cloning, Expression, and Purification of Soluble EC Fragments. Cloning and characterization of BT-R_1 (GenBank accession no. AF310073) was accomplished previously in our laboratory (*1*−*3*). DNA fragments encoding the EC11 and EC12 domains and the inter-EC linker were generated by PCR amplification using primers that encode amino acid residues 1243−1460, 1243−1362, 1349−1373, 1374−1460, 1349−1447, 1349−1460, 1349−1403, 1349−1416, 1349−1425, and 1349−1440. The fragments were cloned directionally into the expression plasmid vector pMal (Novagen), which includes the *malE* gene for the production of fusion proteins in conjunction with maltose-binding protein (MBP). The inter-EC linker construct was produced by amplifying a portion of the MBP and continuing into the 1349−1460 construct up to the amino acid residue 1373. All plasmid DNAs were purified by plasmid preparation kits obtained from Promega or Qiagen. The nucleotide sequence of the DNA inserts in the recombinant plasmids were verified by sequencing. Cloned PCR products were expressed in *Escherichia coli* as MBP fusion proteins. The MBP fusion greatly facilitated expression and purification of the peptides in the host bacteria and significantly enhanced their solubility in buffer.

Expression and purification of the EC constructs were accomplished as follows. Selected recombinant bacterial colonies were grown overnight at 37 °C in 25 mL of LB medium containing ampicillin (50 μg/mL) to obtain a starter culture. A 1 L portion of LB medium was then inoculated with the 25 mL starter culture and grown at 37 °C to $OD_{600\ nm}$ = 0.5. Recombinant protein production was induced by the addition of isopropyl β-thiogalactoside (IPTG) to a final concentration of 100−500 μM. After incubation for 4 h at 37 °C, the bacteria were precipitated by centrifugation and resuspended in 20 mM Tris buffer (pH 8.0) supplemented with 200 mM KCl, 5 mM β-mercaptoethanol, and 1 mM PMSF. Resuspended bacteria were lysed by sonication. The recombinant proteins were purified using ion-exchange chromatography followed by affinity chromatography on amylose beads as the refining step.

Cry1Ab Toxin Purification. *B. thuringiensis* ssp. *berliner* was grown in trypticase broth. Harvesting of the cells for parasporal crystal purification was performed after 4 days, based on examination of the cultures under a phase contrast microscope to confirm sporulation and parasporal crystal formation. Crystal solubilization was carried out in carbonate buffer (50 mM Na_2CO_3, 0.1 M NaCl, and 10 mM dithiothreitol, pH 10.5) for 2 h at room temperature with constant shaking. After centrifugation to eliminate insoluble material, toxin activation was carried out with trypsin (type I from bovine pancreas, Sigma Chemical Co.), and the completion of the reaction was checked by sodium dodecyl sulfate−10% polyacrylamide gel electrophoresis (SDS−PAGE). Trypsin-activated Cry proteins were purified by anion-exchange chromatography with a MonoQ HR 10/10 column (AP Biotech FPLC system). All toxin protein quantitations were performed by the bicinchoninic acid method (Pierce) using bovine serum albumin (fraction V) as a standard.

Immunoligand Blot Experiments for Analysis of Toxin Binding to Fragments. Gel electrophoresis was carried out according to the procedure described by Laemmli (*13*). Proteins from SDS gels were blotted to poly(vinylidene difluoride) (PVDF) membranes (Millipore) using a semidry blotting apparatus (Owl Scientific). The blotted membranes were blocked for 1 h at room temperature with Tris·HCl-buffered saline (TBS; pH 8.0) containing 5% nonfat dry milk powder, 5% glycerol, and 0.5% Tween 20. Cry1Ab toxin was added to blocking buffer at a concentration of 3 nM and incubated with the membrane for 1 h at room temperature. Cry1Ab binding to the protein fragments was detected by anti-Cry1Ab polyclonal antibody (1:50000, 1 h) and visualized with a donkey anti-rabbit antibody coupled with horseradish peroxidase (Amersham Bioscience) (1:3000, 1 h) using an ECL Plus Western blotting detection system (Amersham Bioscience) according to the manufacturer's instructions.

Analysis of Toxin Binding to EC Fragments by Amylose Bead Affinity Chromatography. Binding of Cry1Ab toxin to EC domains was tested using an affinity column-based assay. MBP-linked EC fragments were bound to amylose matrix in small columns (approximately 150 μL) that were pre-equilibrated with a wash buffer composed of 20 mM Tris (pH 8.0) and 150 mM NaCl. The columns were then washed twice with 1 mL of wash buffer to remove any unbound proteins. The columns were loaded with Cry1Ab toxin and washed twice with 1 mL of the wash buffer. Proteins were eluted in two aliquots of 400 μL by applying 10 mM maltose to the wash buffer. Samples from each elution step were analyzed by SDS−PAGE followed by Coomassie staining.

Analysis of Toxin−Receptor Interaction by Gel Filtration Chromatography. Purified Cry1Ab toxin, MBP-EC12 protein, and a mixture of these proteins at various molar ratios were applied to a Superdex 200 HR 10/30 column attached to an FPLC system (AP Biotech) equilibrated with TBS (pH 8.0). The concentrations of purified toxin and MBP-EC12 were determined by UV absorbance using calculated molar extinction coefficients. Eluates were monitored at 280 nm, and 0.5 mL fractions were collected for analysis. The proteins were examined before and after gel filtration by SDS−PAGE.

Cytotoxicity Studies. Inhibition of Cry1Ab toxin action by soluble EC fragments was tested by using cultured High Five insect cells (Invitrogen) that were heterologously expressing BT-R_1 (*8*). Toxin action on the transfected cells was tested by adding Cry1Ab toxin into the growth medium at a concentration of 180 nM. Cell death was determined by Trypan blue exclusion analysis (*8*). Soluble EC fragments were tested for their ability to inhibit toxin action by preincubating the fragments with Cry1Ab toxin (1:1 molar ratio) prior to addition to the growth medium.

Structure Modeling of EC12. Structure modeling was performed using the PSIPRED protein structure prediction server (*14*, *15*) and Geno3D (*16*). A predicted three-dimensional model was produced by the fold recognition method GenTHREADER (*14*) using the sequence similarity (27%) between EC12 and EC1−2 of the classical mouse E-cadherin (PDB code 1edh). PDB entries available for N-cadherin as well as other E-cadherins also were confirmed to generate highly similar results with the EC12 model obtained using the spatial restraints of 1edh. Protein structure

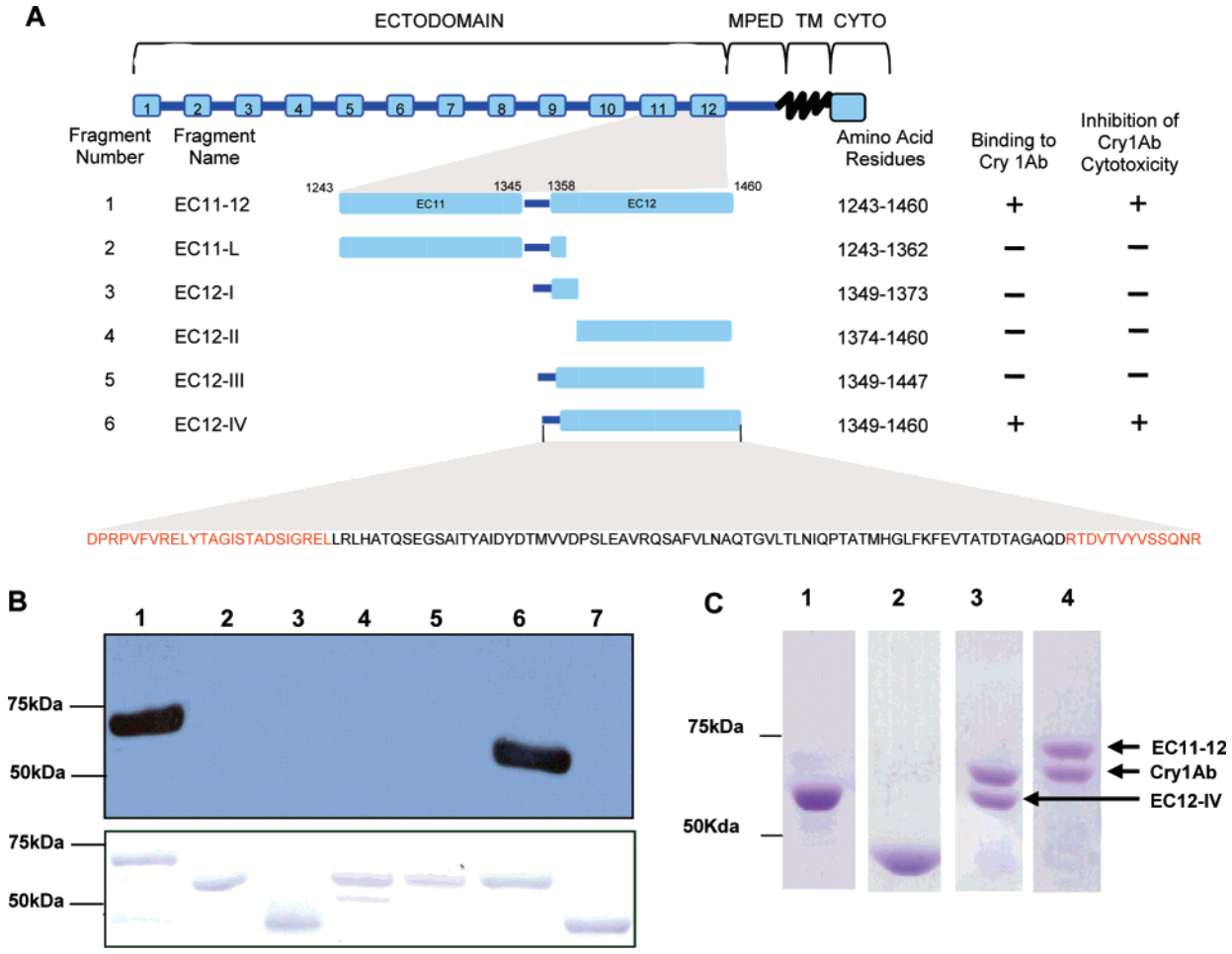

FIGURE 1: Cry1Ab toxin binding to truncated EC fragments of BT-R_1. (A) Molecular structural organization of BT-R_1. The full-length structure of BT-R_1 is based on nucleotide (GenBank accession no. AF319973) and deduced amino acid sequences using Pfam (*17*) and SAS (*18*). MPED = membrane-proximal extracellular domain, TM = transmembrane domain, and CYTO = cytoplasmic domain. The blue-shaded boxes represent EC modules, and the dark blue solid bars in between represent interdomain linkers. The amino acid residue numbers that specify the six truncated EC fragments as well as the results of toxin binding and inhibition of Cry1Ab cytotoxicity are summarized on the right. The amino acid residues (red letters) of EC12-IV represent those residues that are involved exclusively in binding Cry1Ab. (B) Western analysis of Cry1Ab binding to recombinant truncated EC fragments. Purified MBP-fused EC fragments were separated by SDS−PAGE, transferred to a PVDF membrane, and tested for their ability to bind toxin. The presence of toxin was detected using Cry1Ab antiserum (lane 1, fragment 1, EC11−12; lane 2, fragment 2, EC11-L; lane 3, fragment 3, EC12-I; lane 4, fragment 4, EC12-II; lane 5, fragment 5, EC12-III; lane 6, fragment 6, EC12-IV; lane 7, MBP alone). The purified fragments separated by SDS−PAGE are portrayed immediately below the Western blot. (C) Interaction of EC11−12 and EC12-IV with Cry1Ab toxin analyzed by affinity chromatography. MBP-fused EC fragments were immobilized on amylose affinity matrix columns (see the Experimental Procedures). Cry1Ab toxin was added to the columns, and unbound toxin was removed with TBS. The MBP fusion proteins and bound toxin were eluted with maltose buffer and analyzed by SDS−PAGE (lane 1, EC11-L; lane 2, MBP; lane 3, Cry1Ab and EC11-IV; lane 4, Cry1Ab and EC11−12). Coelution of Cry1Ab toxin with MBP-fused EC12-IV (lane 3) and EC11−12 (lane 4) indicates binding of the toxin to these proteins.

comparison was done using databases and approaches described in Pfam (*17*) and SAS (*18*). Multiple sequence alignments were performed using CLUSTAL W (*19*, *20*).

RESULTS

Analysis of Toxin−Receptor Interaction by Immunoligand Blotting and Amylose Bead Affinity Chromatography. Toxin binding to various fragments originated from EC11 and EC12 was analyzed by immunoligand blot analysis and affinity chromatography (Figure 1). A schematic illustration of the fragments with respect to the entire BT-R_1 molecule is shown in Figure 1A. The fragments (fragments 1−6, Figure 1A) were generated by bacterial expression in *E. coli* as fusion proteins linked to MBP and purified by amylose chromatography. As seen in Figure 1B, Cry1Ab toxin bound only to fragments 1 and 6, which are composed of those amino acids that define the EC11−12 modules (residues 1243−1460) and EC12 plus the EC11−12 interdomain linker (residues 1349−1460), respectively (Figure 1B, lanes 1 and 6). Thus, the Cry1A binding region includes residues 1349−1460. The toxin did not bind to fragment 4 (residues 1374−1460), which is composed of EC12 minus the first 15 amino acids of the N-terminus of the domain (Figure 1B, lane 4), or to fragment 3, which contains the first 15 amino acid residues of the N-terminus of EC12 together with the EC11−12 interdomain linker (Figure 1B, lane 3, residues 1349−1373). Obviously, residues 1349−1373 are required but not sufficient for binding. Toxin also did not bind to fragment 2, which contains EC11 and the EC11−12 interdomain linker (Figure 1B, lane 2, residues 1243−1362). Furthermore, Cry1Ab toxin did not bind to fragment 5, which has the 13 amino acid residues 1448−1460 deleted from the C-terminus

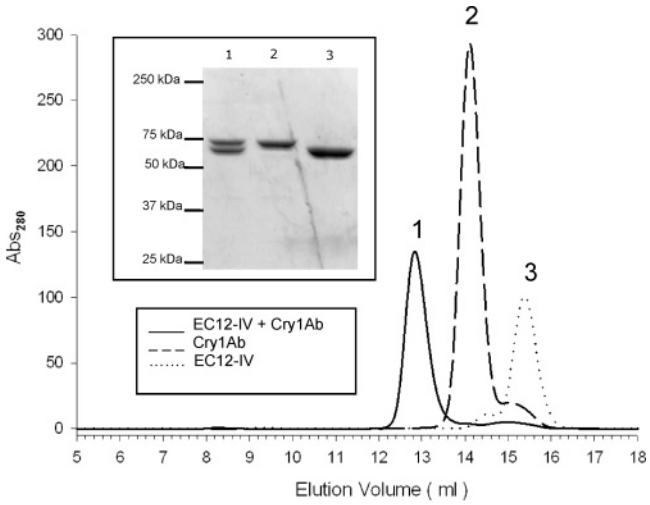

FIGURE 2: Analysis of the Cry1Ab−EC12-IV complex by gel filtration chromatography. The equimolar interaction between Cry1Ab toxin and the EC12-IV construct was examined by analyzing the chromatographic elution profiles of the molecules from a Superdex 200 HR size exclusion column. Samples from the eluted protein fractions corresponding to peak 1 (EC12-IV + Cry1Ab), peak 2 (Cry1Ab), and peak 3 (EC12-IV) were analyzed by SDS−PAGE (inset; lane 1, EC12-IV + Cry1Ab; lane 2, Cry1Ab; lane 3, EC12-IV). Peaks 1−3 represent the results of three separate experiments and are superimposed for comparative analysis.

of EC12 (Figure 1B, lane 5). These results demonstrate that Cry1Ab binding requires both the N-terminal (residues 1349−1373) and C-terminal (residues 1448−1460) regions of EC12.

To confirm the specificity of Cry1Ab binding, fragments 1 and 6 along with fragment 2 as a control were subjected to amylose bead affinity column chromatography and their ability to bind toxin was analyzed (Figure 1C). This approach takes advantage of the fact that specific ligands interact with specific proteins through recognition of particular molecular structural properties, including folding, and that such interaction can be captured in solution using affinity-tagged recombinant proteins immobilized on a selective resin. Accordingly, MBP-linked fragments 1, 2, and 6 were enriched on an amylose matrix, as described in the Experimental Procedures, and their interaction with Cry1Ab was examined. As can be seen in Figure 1C, fragments 1 (EC11−12) and 6 (EC12-IV) bound Cry1Ab toxin as is demonstrated by coelution of the fragments with the toxin (Figure 1C, lanes 4 and 3, respectively). Neither fragment 2 (EC11-L, lane 1) nor MBP alone (lane 2) interacted with the toxin. These results along with those obtained by immunoligand blotting (Figure 1B) establish that EC12 is responsible for specifically binding Cry1Ab toxin.

Analysis of Toxin−Receptor Interaction by Gel Filtration Chromatography. The interaction between Cry1Ab toxin and EC 12-IV (fragment 6) at various molar ratios was analyzed by gel filtration chromatography. Examination of the chromatographic elution profiles of an equimolar mixture of toxin and EC12-IV are shown in Figure 2. Elution profiles of the EC12-IV−toxin complex (peak 1, Figure 2), Cry1Ab toxin (peak 2, Figure 2), and EC12-IV (peak 3, Figure 2) demonstrate that the complex can be resolved differentially on the basis of its size. Association of the Cry1Ab toxin with MBP-linked EC12-IV resulted in formation of a macromolecular complex that acquired an apparent larger Stokes radius than that of either the toxin or EC12-IV alone (Figure 2). Importantly, gel electrophoretic analysis (inset, Figure 2) of the proteins after gel filtration revealed that the interaction between Cry1Ab and EC12-IV (fragment 6) is univalent (lane 1, Figure 2, inset). When molar excesses of the toxin were used, the EC12-IV−toxin complex eluted precisely as seen in Figure 2, with excess unbound toxin appearing as an extra peak at 14 mL (data not shown). Indeed, Cry1Ab binds to BT-R_1 at a molar ratio of 1:1, even in the presence of excess toxin.

Inhibition of Cry1Ab Cytotoxicity by Truncated BT-R_1 Fragments. Previously, we showed that inhibiting the binding of Cry1Ab toxin to midgut epithelial cells by a soluble truncation fragment of BT-R_1 blocks the toxin action in insect larvae (*1*). We also established a direct link between BT-R_1

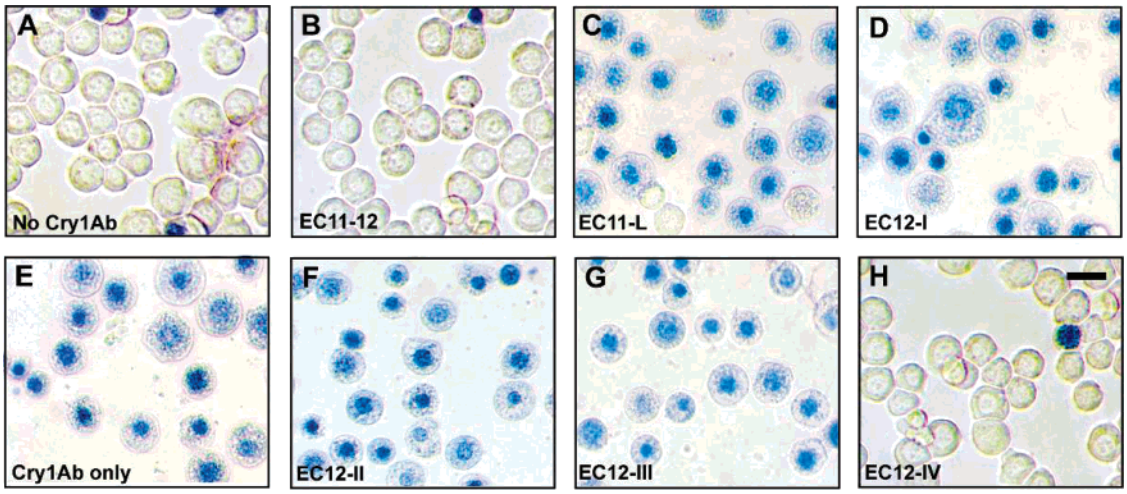

FIGURE 3: Inhibition of cytoxicity by truncated EC fragments. Truncated EC fragments were tested for there ability to inhibit Cry1Ab toxin action on High Five (H5) cells stably expressing BT-R_1 cDNA (GenBank accession no. AF319973). Such transfected cells are designated as S5 cells (*8*). EC fragments 1−6 (Figure 1) were preincubated with Cry1Ab toxin (180 nM) and applied to the S5 cells. Toxicity was assessed using Trypan blue, which stains dead cells blue, whereas living cells remain clear (*8*). S5 cells in the absence of Cry1Ab remained viable (panel A) as did those treated with Cry1Ab pretreated with fragments 1 (EC11−12, panel B) and 6 (EC12-IV, panel H). S5 cells died in the presence of Cry1Ab only (panel E). Likewise, those cells treated with Cry1Ab preincubated with fragments 2−5 (panels C, D, F, and G, respectively) also died.

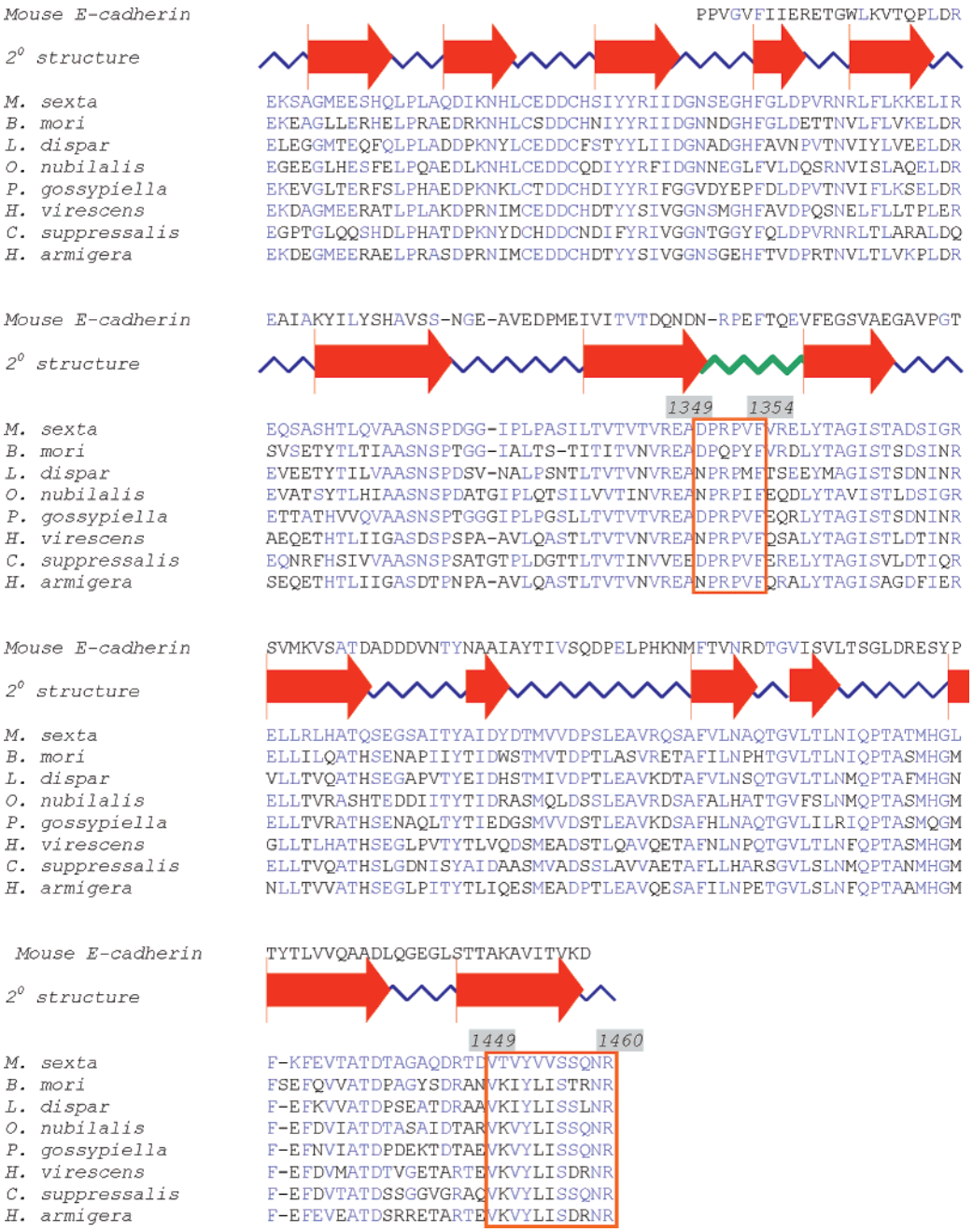

FIGURE 4: Analysis of multiple sequence alignments of EC11−12 and corresponding regions in lepidopteran cadherin receptors. Multiple sequence alignment and the predicted secondary structure of EC11−12 of BT-R_1 from *M. sexta* and related regions of other lepidopteran insects as well as mouse E-cadherin were compared using the Pfam (*17*) and SAS (*18*) databases. Amino acids shown in blue denote invariant residues. The two red boxes contain the conserved residues in the upstream and downstream regions of the Cry toxin-binding motif. Red arrows and blue squiggly lines highlight predicted β-strand and loop organization, respectively, and indicate adaptation of similar secondary structural elements in the aligned sequences. The green squiggly line indicates the EC11−12 interdomain linker.

and Cry toxin-induced cytotoxicity by demonstrating dramatic morphological effects when the Cry1Ab toxin binds to both mammalian and insect cells expressing BT-R_1 (*1*−*3*, *8*). In the present study, we tested truncated BT-R_1 fragments 1−6 (see Figure 1) for their ability to block the cytotoxic action of Cry1Ab on High Five cells expressing BT-R_1 (S5). Cry1Ab (180 nM) was highly toxic to S5 cells as can be seen by Trypan blue staining (Figure 3, panel E). However, in the presence of equimolar amounts of fragments 1 (EC11−12, panel B) and 6 (EC12-IV, panel H), binding of toxin to BT-R_1 was blocked and cytotoxicity was prevented (note the clearness of the cells). Fragments 2−5, which did not bind toxin (Figure 1b), also did not inhibit the toxin action on the S5 cells as evidenced by the blue-stained cells (panels C, D, F, and G, Figure 3).

Comparative Sequence Analysis of EC11−12 in BT-R_1. The EC11 and EC12 modules each contain approximately 100 amino acid residues that are organized in highly conserved structural folds composed of up-and-down, seven-strand β-barrels (Figure 4). Because EC11−12 contains the toxin-binding motif, which includes EC12 plus the EC11−12 interdomain linker, we wanted to determine whether this motif has conserved structural features among other lepidopteran insect cadherins identified as Cry toxin receptors. EC11 and EC12 are defined to include amino acid residues 1243−1345 and 1358−1460, respectively (see Figure 1). The

region between EC11 and EC12 (1345−1358) is referred to as the EC11−12 interdomain linker (Figure 4, green squiggly line). Multiple sequence alignments combined with a comparison of predicted protein folding regions in EC11−12 to corresponding regions in seven homologous lepidopteran proteins and to EC1−2 of the epithelial cadherin from mouse (E-cadherin) revealed high conservation of secondary structure (Figure 4). Interestingly, within the N- and C-termini are sequence signatures (extremely conserved amino acid residues, 1349−1354 and 1449−1460, which are outlined in the red boxes in Figure 4) that provide an identification scheme for Cry1Ab toxin binding to all of the homologous cadherins.

DISCUSSION

The BT-R_1 receptor and its homologues are the principal determinants for Cry1A toxin action in various lepidopteran insects (*1*−*4*, *6*, *21*, *22*). The high affinity and specific binding of Cry1Ab toxin to the cadherin receptor BT-R_1 have established the cadherin as the cell surface ligand recruited by Bt for targeting insect host cells (*7*, *8*). This binding provokes cell death by triggering a signaling pathway involving the G protein α subunit, adenylyl cyclase, increased cAMP levels, and activation of protein kinase A (*7*). In the current study, we demonstrate that a conserved structural motif within EC12 is essential for Cry toxin binding. Specifically, residues 1349−1373 (N-terminus of EC12 plus the EC11−12 linker, Figure 1) and residues 1448−1460 (C-terminus of EC12, Figure 1) constitute the conserved motif. Furthermore, the stoichiometry of the interaction between toxin and receptor in solution is strictly 1:1 (Figure 2). The exclusive dependency of Cry1Ab toxin on univalent binding to BT-R_1 indicates that toxin action relies on binding of toxin monomers to the receptor. Indeed, our recent study using a cell-based system demonstrated that the interaction of Cry1Ab toxin monomers with BT-R_1 is directly related to cytotoxicity (*8*). Likewise, the presence of the receptor is critical not only to Cry toxin action but also to the susceptibility of insect cells to the toxin because the receptor establishes specificity and selectivity of toxin binding on the cells as well as in target insects (*1*, *8*).

Consensus alignment of amino acid sequences corresponding to the EC11−12 portion of homologous cadherins in various lepidopteran insects along with EC1−2 of mouse E-cadherin reveals strong conservation of secondary structural elements throughout the EC11−12 region (Figure 4). Particularly, there are stretches of amino acids within the N- and C-terminal residues of EC12 and the corresponding modules that are the most highly conserved. These sequences are highlighted in the red boxes (Figure 4) and as such represent sequence signatures that mark the toxin-binding function in BT-R_1 and the corresponding homologous proteins.

The toxin-binding motif is displayed in a predicted three-dimensional model of EC12 (Figure 5) based on the crystal structures of domain 2 of an E-cadherin (PDB code 1edh), an N-cadherin (PDB code 1ncj), and a C-cadherin (PDB code 1l3w) (*23*−*25*). The EC12 sequence was templated to the structures and energy minimized using SAS (*18*) and the SWISS-MODEL protein structure homology-modeling server (*26*). The model shows the N- and C-termini at the ends of

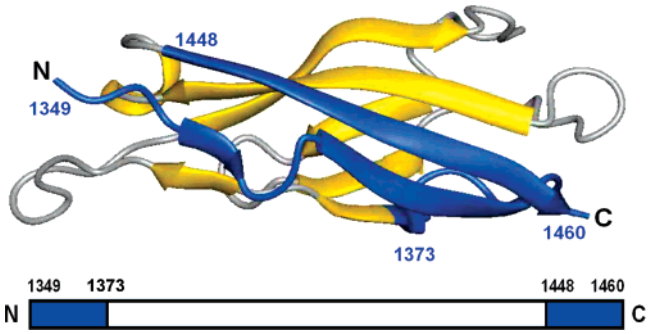

FIGURE 5: Predicted three-dimensional model of EC12 (residues 1349−1460) displaying the conserved structural motif for toxin binding. Note that residues 1349−1372 (N-terminus of EC12 plus the EC11−12 interdomain linker) and residues 1448−1460 (C-terminus of EC12) constitute the conserved motif for Cry toxin binding.

two adjacent strands within the β-barrel fold of the cadherin repeat (Figure 5). As a result, the two essential sequences for toxin binding (residues 1349−1372 and residues 1448−1460) form a single contiguous surface spanning adjacent β-strands and loops (blue-shaded region in Figure 5). The specific interface for toxin binding likely resides within this surface and must include residues from both the N- and C-terminal sequences, since deleting either sequence abolishes toxin binding (fragments 4 and 5, EC12-II and EC12-III, Figure 1). This result may extend to other cadherin−toxin interactions. Identical results for Cry1Ac toxin binding were obtained when similar truncation experiments were done using the cadherin receptor BT-R_2 from the pink bollworm *Pectinophora gossypiella* (*27*). A recent study with a homologous cadherin receptor, HevCaLP, in the tobacco budworm *Heliothis virescens* showed that mutations of specific amino acids at the C-terminal portion of EC12 resulted in decreased toxin binding in insects that were resistant to Cry toxin (*4*, *28*).

Generally, the active sites in proteins are better conserved than the overall fold (*29*). Indeed, it has been clearly demonstrated that enzyme active sites are more highly conserved than other parts of the protein (*30*). Although ligand-binding sites have not been identified using inexact models (*31*), it is apparent from the results of our present study that the Cry toxin-binding region is extremely conserved in a number of lepidopteran cadherins (Figure 4). Moreover, the region is highlighted by a sequence signature that is part of the conserved structural motif of EC12 (see the model in Figure 5). Certainly, the functionality of EC12, i.e., binding of Cry1Ab toxin, was demonstrated by its ability to block toxin action on High Five cells stably expressing BT-R_1 cDNA (Figure 3). It is noteworthy that such a structure−function relationship is apparent among the lepidopteran cadherins (Figure 4), but when functional threading is done for all other publicly reported proteins, no "active site" match can be found either in amino acid sequence or in structural and functional site descriptor libraries.

Obviously, the structural features of the toxin-binding region on BT-R_1 are critical to the specificity, selectivity, and affinity of toxin. The univalent binding of Cry1Ab toxin to EC12 is stable in solution (Figure 2), and this relationship demonstrates that the conformation and structure of the two molecules mutually support their interaction. Thus, Cry1Ab toxin binding and the subsequent molecular events respon-

sible for cell death depend on the conserved motif in EC12. Determining the chemistry of this structure−function relationship will be a significant step toward better understanding Cry toxin action.

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