Bivalent Sequential Binding Model of a *Bacillus thuringiensis* Toxin to Gypsy Moth Aminopeptidase N Receptor*

Jeremy L. Jenkins‡, Mi Kyong Lee§, Algimantas P. Valaitis¶, April Curtiss‡, and Donald H. Dean¶¶

Received for publication, September 22, 1999, and in revised form, January 10, 2000

---

**Specificity for target insects of *Bacillus thuringiensis***

Insecticidal Cry toxins is largely determined by toxin affinity for insect midgut receptors. The mode of binding for one such toxin-receptor complex was investigated by extensive toxin mutagenesis, followed by real-time receptor binding analysis using an optical biosensor (BIACore). Wild-type Cry1Ac, a three-domain, lepidopteran-specific toxin, bound purified gypsy moth (*Lymantria dispar*) aminopeptidase N (APN) bimERICALLY. Site 1 displayed fast association and dissociation kinetics, while site 2 possessed slower kinetics, yet tighter affinity. We empirically determined that two Cry1Ac surface regions are involved in *in vivo* toxicity and APN binding. Mutations within domain III affected binding rates to APN site 1, whereas mutations in domain II affected binding rates to APN site 2. Furthermore, domain III contact is completely inhibited in the presence of N-acetylgalactosamine, indicating loss of domain III binding eliminates all APN binding. Based upon these observations, the following model is proposed. A cavity in lectin-like domain III initiates docking through recognition of an N-acetylgalactosamine moiety on *L. dispar* APN. Following primary docking, a higher affinity domain II binding mechanism occurs, which is critical for insecticidal activity.

---

*Bacillus thuringiensis* (Bt) insecticidal crystal proteins (or Cry toxins) have been used worldwide in a number of transgenic crops and in sprays as a safer alternative to chemical pesticides (1). Upon ingestion by a feeding insect, the crystal proteins are solubilized in the midgut and activated by midgut proteases (2). The activated toxins then target molecules lining the epithelial cell membrane and disrupt membrane ionic potential (3) by forming pores. Understanding the mechanism of binding of Cry toxins to the midgut receptors is important for engineering Bt toxins with higher toxicity and insect specificity.

The binding kinetics of several Bt toxins with midgut receptors has been observed using optical biosensors (4–8). Biosensors, such as the BIACore (Biacore AB, Uppsala, Sweden), measure the affinity of a flowing molecule for another molecule immobilized on a surface as they form a real-time complex. As the molecule in solution is adsorbed by ligand, changes in mass on the surface are monitored using surface plasmon resonance (SPR) (9, 10). Real-time kinetic analysis can be an important utility when macromolecular interactions deviate from simple, monophasic binding (11–18). SPR studies conducted using Cry1A toxins specific for lepidopteran receptors have reported that Cry1Ac binds to two sites on purified *Manduca sexta* aminopeptidase N (APN) (4) and *Heliotis virescens* APN (6) with 2:1 toxin-receptor stoichiometries. Cry1Aa and Cry1Ab toxins (86% and 82% homologous to Cry1Ac, respectively (Ref. 19)) are also able to bind these insect APNs. However, purified *Lymantria dispar* APN binds Cry1Ac with a 1:1 stoichiometry (5) and does not bind Cry1Aa or Cry1Ab (20). This suggests structural differences among different insect APNs, despite sequence conservation (21).

The molecular mechanism of Cry1A toxins is best understood by examination of their three-domain structure (22, 23). Domain I is involved in pore formation in the membrane, following binding (24–30). Domain II has been shown to influence reversible binding and irreversible membrane insertion to insect brush border membrane vesicles (BBMVs) (31–41). Mutations in this domain have caused the greatest losses in toxicity. Domain III has several proposed functions. One of these roles is determining receptor specificity (8, 42–45). For example, in Cry1Ac, lectin-like domain III recognizes an N-acetylgalactosamine (GalNAc) moiety on *M. sexta* APN (8, 45). Based on homology modeling studies between Cry1Aa and Cry1Ac, it is proposed GalNAc docks in a surface cavity (46). Interestingly, this cavity is non-conserved in Cry1Aa and Cry1Ab, and the binding of these toxins to APN is not inhibited by preincubation with sugars (4, 6). To the contrary, Cry1Ac binding to *L. dispar* APN-1 is almost completely inhibited by preincubation with GalNAc (5), indicating carbohydrate recognition is essential for this toxin-receptor interaction. This suggests Cry1Ac domain III has a sugar-dependent mechanism of binding (47) unique to Cry1A toxins. In fact, Cry1Ac domain III sequence is notably divergent from all other Cry toxins (48, 49). The requirement of sugar recognition for binding, however, is common among many intestinal pathogens (50–55).

In this study, the nature of Cry1Ac binding to purified *L. dispar* APN was examined by comparing mutant toxin affinities on an optical biosensor. APN-binding epitopes were localized to specific residues in domain II and III. Our results suggest that Cry1Aa binds *L. dispar* APN in sequential steps, first by domain III, then domain II. Here we present an empir-
ical determination of both contact sites and a model for sequen-
tial receptor-binding steps by Cry1Ac insecticidal β-endotoxin.

MATERIALS AND METHODS

Molecular Visualization—Cry1Ac three-dimensional structure was homology-modeled from Cry1Aa (23) using SWISS-MODEL (56–58), and visualized in SWISS-pdbViewer v.3.1 with Q3D rendering (Glaxo Wellcome).

Construction of Mutants—Site-directed mutagenesis of cry1Ac1 gene (pOS4201) subcloned into pBlueScript KS+ (pOS112100) was performed with a Bio-Rad MutA-Gen gene phagemid in vitro mutagenesis kit. Mutagenic primers were purchased from Biosynthesis or Genomed. Automated DNA sequencing with a United States Biochemical Corp. kit was performed according to manufacturer’s instructions. The constructs of Cry1Ac-Cry1Aa domain-switched hybrids were described previously (59). Mutant constructs were expressed in Escherichia coli MV1190.

Bioassays—L. dispar eggs were supplied by the United States Department of Agriculture (Otis Methods Development Center, Beltsville, MD). LC50 (50% lethal concentration) values were measured by the magnesium precipitation method (61) occurring at the pH of a lepidopteran gut environment.

RESULTS

B. thuringiensis Cry1Ac “Hot Spots”—Site-directed mutagenesis was performed in Cry1Ac domains II and III by substituting alanine for selected surface amino acids. Follow-
ing size-exclusion column purification of toxins, the aggregation state of toxins in solution was examined by dynamic light scattering. Essentially all of the mass within purified fractions consisted of proteins with hydrodynamic radii corresponding to monomeric toxin.

Next, mutant toxins were purified and tested for biological activity against neonate L. dispar larvae. Alanine substitutions

2 J. L. Jenkins and D. H. Dean, unpublished observation.
that did not alter toxicity are shown in Fig. 1 (yellow). We found two different regions affecting toxicity to *L. dispar* larvae that are separated by up to 62 Å (from Asn 377 to Trp545). The first region includes residues surrounding a small depression in domain III (Fig. 1, blue), including Gln509, Arg511, and Tyr513. We have recently shown alanine substitutions at these residues greatly affected binding to *L. dispar* BBMVs with only minor reductions in toxicity (62). Another mutation in domain III, W545A, which is located on the upper lip of the cavity mouth, had the largest loss in toxicity for this region. The second region affecting toxicity was in domain II. This surface, larger than the one in domain III, was more critical for toxicity (Fig. 1, red). Domain II, loop 3 mutations from residue 438 to 443 caused the largest reductions in toxicity. Additionally, alanine substitutions of arginine residues at positions 281, 289, 368, and 369 and also at Asn377 caused reduced toxicity. Interestingly, I375A, located at the bottom of domain II, showed a slight increase in toxicity as compared with wild type, although their 95% confidence intervals were overlapping.

CD spectra were also analyzed for each mutant and compared with wild type to ensure differences in activity were not the result of structural changes. The mutant toxins had overlapping CD spectra to that of wild-type Cry1Ac at neutral pH, with the exception of mutations $D_{F371}$ (deletion), I373A, G546A, L583A, I586A, V587A, which were unstable during trypsin digestion at pH 10, and therefore not used. Additionally, the CD spectra of several mutants (see “Materials and Methods”) were examined at an alkaline pH to better reflect the lepidopteran gut environment. The spectra of wild-type Cry1Ac shifted at higher pH values, as observed previously (63). The mutants that were tested shifted identically, indicating a wild-type transition at basic pH (Fig. 2). It is, therefore, unlikely that differences in wild-type and mutant properties are the result of structural problems arising at the “active” pH.

Determining a Kinetic Model—Next, we explored the hypothesis that mutant toxins with reduced insecticidal activity were affected in their ability to bind *L. dispar* aminopeptidase N receptor. First, APN was column-purified from detergent-solubilized *L. dispar* BBMVs by anion-exchange and size-exclusion chromatography until a single peak was obtained (Fig. 3A). SDS-PAGE (10%) analysis revealed a single band at approximately 120 kDa (Fig. 3B). To further test for purity, the APN preparation was probed by ligand blotting with biotinylated Cry1Ac toxin, as well as biotinylated soybean agglutinin (Fig. 3C). Both the toxin and the GalNAc-specific lectin appeared to bind to a single band at 120 kDa. These results provided evidence that there are no isoforms of APN present in the sample preparation that might contribute to the toxin binding response.
To study the real-time binding kinetics of Cry1Ac to purified *L. dispar* APN, the receptor was immobilized on a biosensor chip surface and toxin-receptor complex formation was analyzed by surface plasmon resonance (SPR). Wild-type Cry1Ac binding curves were fit to various models (see “Materials and Methods”). The best-fitting models deviated from pseudo-first order kinetics. Cry1Ac response curves gave similar fits to two different binding models. The equation describing the first model involves two independent binding sites on the receptor (T + R1 \(\leftrightarrow\) TR1; T + R2 \(\leftrightarrow\) TR2). The equation for the second model describes a sequential binding, or “two-step” mechanism of binding, to a single receptor molecule (T + R \(\leftrightarrow\) TR \(\leftrightarrow\) TRx). The equation for sequential binding is identical to the conformational change equation used in BIAevaluation 3.0 software, since two binding events occur on one receptor in both scenarios. When the wild-type Cry1Ac binding curves were fit to both the “two-site” and sequential binding (“two-step”) models, the “goodness-of-fit” was similar, as indicated by \(\chi^2\) of \(<1.0\). Further demonstrating a similar goodness-of-fit, a statistical \(F\)-test comparing the fits of these two models indicated no significant preference. All other models had \(\chi^2 > 1\), indicating higher non-random deviation from the fitted curve.

Initially, the sequential binding model was chosen for evaluation since a 1:1 binding stoichiometry was previously demonstrated for Cry1Ac and *L. dispar* APN (5). Apparent rate constants of Cry1Ac obtained from this model were \(k_{a1} = 8.5 \times 10^4 \text{ M}^{-1} \text{s}^{-1}\), \(k_{d1} = 2.4 \times 10^{-2} \text{ s}^{-1}\), and \(k_{d2} = 3.9 \times 10^{-3} \text{ s}^{-1}\). The overall affinity (\(K_p\)) for Cry1Ac binding to APN can be calculated by \((k_{d2}/k_{d1}) \times (k_{a1}/k_{d1})\), yielding 208 nM. An example fitting of Cry1Ac (500 nM) using the sequential binding model (Fig. 4) displays the simulated component curves. Step 1 displays fast association and fast dissociation kinetics, while step 2 binding is slower, but enables adhesion to APN.

**Substitutions in the Binding Sites Alter Step-specific Rate Constants**—The response curves for mutant toxins were analyzed, and individual rate constants for each mutant were compared with wild type (Table I). For each mutant, rate constants for step 1 and for step 2 were determined. Changes in rate constants with respect to wild type are presented from curve fittings using the sequential binding model. In general, all mutants with decreased toxicity had a lower overall affinity for APN. The mutations can be categorized into two groups, those made in domain II and those made in domain III. This is illustrated in Table I, where boldface type denotes changes in binding greater than 2-fold. Domain II mutations affected step 2 rate constants of the sequential binding model. In contrast, domain III mutations affected step 1 rate constants.

Within domain II, we observed that mutations mostly caused decreases in \(k_{a1}\) (association) or increases in \(k_{d2}\) (dissociation). Replacing Arg at 281, 289, 368, and 369 by Ala or Glu tended to slow on-rates. Changes made in loop 3 were detrimental to \(k_{d2}\), causing increased off-rates. These loop 3 mutants displayed the most dramatic losses in biological activity against *L. dispar*. However, mutating Ile at 375 to Ala decreased its off-rate. The delayed adherence time reflects its slightly augmented toxicity (Table I), emphasizing the biological importance of this kinetic step.

Domain III mutations more severely affected association and dissociation rates of step 1 when compared with domain II mutants. Analysis of W545A indicates a dependence of step 2 binding on step 1 binding. This is suggested since this domain III mutation completely disrupts toxin binding to the APN receptor. Thus, step 1 binding of domain III may be a prerequisite to step 2 binding.

For visual comparison, relative changes in apparent rate constants were plotted for each mutation (Fig. 5). Mutations...
FIG. 5. Relative changes in individual rate constants of mutant toxins. Relative changes in association rates are \( k_{a(mut)/a(wt)} \), and relative changes in dissociation rates are \( k_{d(mut)/d(wt)} \). Mutations are identified by residue number of Cry1Ac and are assumed to be alanine substitutions except 368/369 where noted. Mutations occurring in domain II or domain III are divided by vertical dashed line. Relative rates <1.0 indicate slower association or faster dissociation compared with wild type APN-binding. Mutants with greater than 2-fold changes in relative rates are colored gray.

with 2-fold or greater changes in rate constants are shown (Fig. 5, gray bars). For mutant toxins that altered binding more than 2-fold, the domain in which the mutation was made appears to correlate with which set of rate constants are affected.

Step-specific alteration of rate constants can also be observed by overlaying response curves from each mutant type (Fig. 6), as obtained from SPR experiments. Domain II mutations that alter step 2 binding only moderately affect total RU's bound during the association phase. After 240 s of association time, the \( k_{a2} \) mutants were the only type to have a similar amount bound as wild type. Alanine substitutions in loop 3, such as F440A (curve b, Fig. 6), fall off APN faster after toxin injection is replaced by buffer flow. Mutant R281A decreases \( k_{a2} \) (curve c, Fig. 6). Mutant R368A/R369A nearly eliminates step 2 binding (Table I), and approximately half of the binding signal (curve d, Fig. 6), but has no effect on step 1 binding. Conversely, when step 1 binding is decreased by Y513A (curve e, Fig. 6), total binding was diminished. W545A in the step 1 binding epitope eliminated binding to APN (curve f, Fig. 6). Other domain III mutants affecting step 1 also showed great reductions in APN binding.

The mutant toxins that most affected binding and toxicity were also compared with wt Cry1Ac using SPR at pH 9.7. We found that the affinities obtained for wild-type and mutant toxins were the same as those obtained at pH 7.4 (Fig. 7). Interestingly, the total binding response to APN of every toxin tested at this pH was lower than the total response observed at neutral pH, despite having the same affinity at both pH values. The observation of a reduced \( R_{max} \) without reduced \( K_D \) is similar to what has been found in binding studies using brush border membrane vesicles, where increasing pH from 7.4 to 10 reduced the binding site concentration for toxin (\( R_{max} \)) by more than half, while insignificantly affecting affinity (64). In this study, the reduction of total binding at higher pH values was correlated with decreased nonspecific binding. An alternative explanation for the reduced signal response is that increasing pH may induce repulsion from residual free carboxyl groups on the surface of the sensor chip. However, the behavior of wild type and these mutant toxins is conserved at varying pH values, and thus the reductions in toxicity and binding of these mutant toxins is not a reflection of unique damage inflicted in a basic environment.

In summary, the results of our mutant toxin analysis by SPR provided evidence that domain III appears necessary for recognition of APN since mutations in this domain can eliminate all binding. This agrees well with the findings of Lee et al. (42), who showed domain III determines receptor specificity in \( L.\) dispers for Cry1A toxins. Domain II binding is secondary, but also necessary. In combination with previous findings of a 1:1 stoichiometry of Cry1Ac binding to \( L.\) dispers APN (5), our results favor a two-step binding process of toxin to a single receptor molecule.

Sugar-binding Domain III Is Initially Required for Any Binding—Since our Cry1Ac binding steps to \( L.\) dispers APN are domain modulated, we analyzed the binding response of domain-switch hybrids to APN after preincubation with N-acetylgalactosamine (GalNAc). One hybrid used, hybrid 4109, consisted of domains I and II of Cry1Aa and domain III of Cry1Ac (1Ac/1Aa/1Aa). Hybrid 4209 had a complementary construction (1Aa/1Ac/1Aa) (31). After a 30-min preincubation in increasing concentrations of GalNAc, toxins (500 nM) were injected over immobilized APN (Fig. 8). The IC\(_{50}\) of GalNAc for Cry1Ac was 4.5 mM, which agrees with GalNAc inhibition constants previously observed (4, 5, 7). Hybrid 4109 possessed a slightly lower inhibition constant (IC\(_{50}\) = 1 mM). It was expected that hybrid 4209 would not bind APN since the domain III of Cry1Aa does not recognize APN (42). As expected, hybrid 4209 was unable to bind \( L.\) dispers APN at all. Mutant Q509A, which exhibited a...
reduced \( k_{s1} \), was also tested for GalNAc inhibition. Although total binding of Q509A to APN after 4 min of association is only 12% of the total binding of wild type, it is still completely inhibited by increasing GalNAc concentrations, affirming step 2 binding relies on step 1 (Fig. 8, open squares).

Finally, we tested galactose and epimeric GlcNAc for inhibition of binding. Talose and gulose (epimers of galactose at the C2 and C3 positions, respectively) were also tested (data not shown). None of these sugars was able to inhibit Cry1Ac binding to APN. Our results confirm previous reports, which found sugar binding requires a galactoside orientation at the C-4 hydroxyl (4, 7), and also suggest that Cry1Ac prefers an acetamido group at the C-2 position.

**Computer-simulated Docking**—The results of our GalNAc competition studies prompted us to test docking of GalNAc to domain III by computational methods as well. Electrostatic calculations of Cry1Ac indicate a strong, positive field around domain II loops, and near the domain III GalNAc-binding epitope, while the domain I \( \alpha \)-helical bundle is negatively charged (Fig. 9). Given the alkaline environment of the insect gut (65), the orientation of positive surface charges on this and other Bt toxins may serve to direct toxin receptor-binding epitopes toward the negatively charged surface of the insect brush border membrane. Using a computer-simulated docking method, we observed that GalNAc binding in the putative cavity of domain III was favorable in steric and energy-pairing analyses. Multiple docking positions were possible for GalNAc. The least potential energy obtained for binding was ~24 kcal/mol (Fig. 9). Potential hydrogen bonds between the GalNAc-Cry1Ac complex were calculated for various rotamers of GalNAc at the lowest energy positioning. Potential hydrogen bonding occurred between the GalNAc acetamido group and Cry1Ac residues Gln509, Asn544, and the backbone of Asn547. These interactions may account for the specificity of Cry1Ac for GalNAc, but not galactose. Additionally, the backbones of...
Asn\(^{544}\) and Gly\(^{546}\) interact with C-3 and C-4 hydroxyls, indicating a preference for a galactoside orientation. Thus, the geometry around the C-4 hydroxyl may account for Cry1Ac’s preference for GalNAc, but not GluNAc (a C-4 epimer). Our results also indicated the C6 hydroxyl might serve as a hydrogen bond donor to protein atoms at the Gly\(^{512}\) backbone and Arg\(^{511}\). Finally, Trp\(^{450}\) or Tyr\(^{513}\) may contribute by stacking against the sugar ring, as observed of aromatic residues in other GalNAc-binding pockets (66). Conversely, in docking simulations with Cry1Aa and GalNAc, no favorable annealings were found. This agrees with previous reports that Cry1Aa binding to \(M.\ sexta\) APN is not inhibited by GalNAc (4).

**DISCUSSION**

Toxicity and receptor binding studies with our Cry1Ac mutants provided evidence of functional epitopes on Cry1Ac domains II and III. Each domain contributed its own set of rate constants during binding. Biosensor analysis of domain II and III mutant toxins indicated domain III binds and releases quickly \(k_{a1}\) and \(k_{d1}\), while domain II binds slower and tighter \(k_{a2}\) and \(k_{d2}\). Loss of domain III binding by mutagenesis or domain-exchanges eliminates APN binding, demonstrating the dependence of secondary domain II binding on initial domain III binding. Domain III binding is specifically inhibited by GalNAc. Given these results and the 1:1 binding stoichiometry of Cry1Ac to APN, we propose a two-step, or sequential binding model, rather than a two-site model. In this model, APN recognition is determined by Cry1Ac domain III binding through a GalNAc moiety, followed by contact of domain II loop residues (Fig. 10).

Previously, we have observed that, under our experimental conditions, the effects of mass transport and analyte rebinding do not contribute to Cry1Ac’s deviation from simple bimolecular binding.\(^6\) We note that there are some possible alternative explanations of our findings. First, a heterogeneous surface of APN molecules may result from amine coupling. However, it is unlikely that our two sets of rate constants are induced by constrained receptor positions since mutations in separate domains of Cry1Ac affect both toxicity and APN binding. Two separate structural and functional regions make monophasic binding improbable, and thus, amine coupling is an unlikely source of heterogeneity. Second, the two Cry1Ac domains could bind independently to APN. Such bivalency is also seen in antibody-antigen interactions, but separate antibody fragments are monovalent (12). Cry1Ac is different from an antibody since the second binding site depends on the first one. In addition, the bivalent analyte model did not fit our biosensor curves as well as the sequential binding model (data not shown). A third alternative is that APN receptors dimerize on the biosensor surface in a sequential fashion, like the human growth hormone interaction with its receptor hGH\(\alpha\) (67). This could occur by domain III binding to one APN and domain II binding to a second APN. Curve fittings with a receptor dimerization model (see “Materials and Methods”) did not fit better than the sequential binding model (data not shown), and our predicted 1:1 stoichiometry does not suggest dimerization occurs. Additionally, our apparent kinetic rate constants do not vary with different densities of receptor immobilized, indicating ligand interactions do not affect complex formation.\(^2\) One final possibility is that a conformational change actually occurs upon APN binding. It has been suggested that Cry1Ac does undergo a conformational change upon binding to \(M.\ sexta\) APN in a lipid monolayer (7). This would initiate membrane insertion of domain I \(\alpha\)-helices involved in pore formation. It remains to be determined if binding to a functional toxin receptor, alone, can trigger this event.

Pathogenic bacterial toxins that target cell membranes appear to possess a similar functional construction. It has been observed in well characterized toxins, such as cholera and shigella, that a “B” domain functions in binding to cell surface receptors, while an “A,” or activity, domain exerts the toxin’s specific biological activity (68, 52). A and B domains may be synthesized together or separately. It is further postulated that regions of hydrophobicity on one of the domains or on a separate domain called “E” (entry domain), plays a role in facilitating insertion of the toxin after receptor binding (68). The ABE model of toxin structure may be analogous to the domains of Cry1Ac. Our data suggest that \(L.\ dispers\) APN specificity is determined by sugar binding of domain III. Mutations around the domain III cavity affect initial binding rates. This lectin-like, jellyroll structure acts like a B domain. Domain II mutations affect rate constants of the subsequent step. It is tempting to speculate secondary domain II binding is critical for facilitating entry into the membrane, acting as an E domain. It is not known if this would involve binding to a second receptor site or initiating a conformational change. However, the loss of exposed, hydrophobic Phe\(^{440}\) caused the most dramatic effects on \(k_{d2}\) fitting with its potential role as an E domain. Interestingly, changing charged arginine residues to alanine resulted in slower on-rates during the second step. Positively charged arginines might serve to orient hydrophobic loops to an APN binding site or toward the membrane surface. Finally, the
ability of domain I α-helices to form membrane pores suggests it may be an A domain.

A comparison of mutagenic toxicity and APN binding affinity in this study yields a strikingly contradictory result. Complete loss of APN binding caused by domain III mutation W545A only results in 50-fold decreased activity. Cry1Ac appears to this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.

ability of domain I

results in 50-fold decreased activity. Cry1Ac appears to

this study yields a strikingly contradictory result. Complete

it may be an A domain.
60. Andrade, M. A., Chacon, P., Merelo, J. J., and Moran, F. (1993) Protein Eng. 6, 383–390
61. Woltersberger, M., Luthy, P., Maurer, A., Parenti, P., Sacchi, V. F., Giordana, B., and Honozet, G. M. (1987) Comp. Biochem. Physiol. 86A, 301–308
62. Lee, M. K., You, T. H., Gould, F. L., and Dean, D. H. (1999) Appl. Environ. Microbiol. 65, 4513–4520
63. Feng, Q., and Becktel, W. J. (1994) Biochemistry 33, 8521–8526
64. Van Rie, J., Jansens, S., Hufle, H., Degheele, D., and Van Mellaert, H. (1989) Eur. J. Biochem. 186, 239–247
65. Dow, J. A. T. (1986) Adv. Insect Physiol. 19, 188–246
66. Rao, V. S., Lam, K., and Qasba, P. K. (1998) J. Biomol. Struct. Dyn. 15, 835–860
67. Cunningham, B. C., Ultsch, M., de Vos, A. M., Mulkerrrin, M. G., Clauser, K. R., Wells, J. A. (1991) Science 254, 821–825
68. Middlebrook, J. L., and Kohn, L. D. (1981) Receptor-mediated Binding and Internalization of Toxins and Hormones. p. 95, Academic Press, Inc., New York
69. Lee, M. K., You, T. H., Young, B. A., Cotrill, J. A., Valaitis, A. P., Dean, D. H. (1996) Appl. Environ. Microbiol. 62, 2845–2849
70. Carroll, J., Woltersberger, M. G., and Ellar, D. J. (1997) J. Cell Sci. 110, 3099–3104
71. Vadlamudi, R. K., Ji, T. H., and Bulla, L. A., Jr. (1993) J. Biol. Chem. 268, 12334–12340
72. Valaitis, A. P., Lee, M. K., and Dean, D. H. (1999) in Society for Invertebrate Pathology XXXII Meeting Program & Abstracts, Irvine, August 23–27, 1999, p. 76
Bivalent Sequential Binding Model of a *Bacillus thuringiensis* Toxin to Gypsy Moth Aminopeptidase N Receptor
Jeremy L. Jenkins, Mi Kyong Lee, Algimantas P. Valaitis, April Curtiss and Donald H. Dean

*J. Biol. Chem.* 2000, 275:14423-14431.
doi: 10.1074/jbc.275.19.14423

Access the most updated version of this article at http://www.jbc.org/content/275/19/14423

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 68 references, 30 of which can be accessed free at
http://www.jbc.org/content/275/19/14423.full.html#ref-list-1