Dual mode of action of Bt proteins: protoxin efficacy against resistant insects

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Transgenic crops that produce Bacillus thuringiensis (Bt) proteins for pest control are grown extensively, but insect adaptation can reduce their effectiveness. Established mode of action models assert that Bt proteins Cry1Ab and Cry1Ac are produced as inactive protoxins that require conversion to a smaller activated form to exert toxicity. However, contrary to this widely accepted paradigm, we report evidence from seven resistant strains of three major crop pests showing that Cry1Ab and Cry1Ac protoxins were generally more potent than the corresponding activated toxins. Moreover, resistance was higher to activated toxins than protoxins in eight of nine cases evaluated in this study. These data and previously reported results support a new model in which protoxins and activated toxins kill insects via different pathways. Recognizing that protoxins can be more potent than activated toxins against resistant insects may help to enhance and sustain the efficacy of transgenic Bt crops.

Insecticidal proteins from the common soil bacterium Bacillus thuringiensis (Bt) are used extensively in sprays and transgenic plants to control insects that attack crops and vector diseases1,2. These Bt proteins are especially valuable because they kill some of the world’s most harmful pests, yet are not toxic to people and most other organisms3–5. Global planting of crops genetically engineered to produce Bt proteins increased to 78 million hectares in 2014, with a cumulative total of 648 million hectares since 19966. Although Bt sprays and Bt crops have provided substantial economic and environmental benefits7–9, rapid evolution of pest resistance to Bt proteins is eroding these advantages10–14.

Understanding the mode of action of Bt proteins is critical for enhancing and sustaining their efficacy against pests. In particular, many studies have examined the mode of action of the crystalline Bt proteins Cry1Ab and Cry1Ac, which kill lepidopteran pests and are produced by widely adopted transgenic Bt corn, cotton, and soybean1,2,15–18. All models of Bt mode of action agree that the full-length forms of Cry1Ab and Cry1Ac proteins called protoxins are converted by insect midgut proteases to activated toxins that bind to insect midgut receptors (Fig. 1)15–18. This activation entails removal of approximately 40 amino acids from the amino terminus and 500 amino acids from the carboxyl terminus, converting the protoxins of approximately 130 kDa to activated toxins of approximately 65 kDa15–18. Although competing models differ in post-binding events that eventually kill insects, the currently accepted paradigm

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asserts that protoxins do not bind to midgut receptors and must be converted to activated toxins of approximately 65 kDa to bind to larval midgut receptors and exert toxicity 15–18.

Contrary to this paradigm, however, in vitro experiments with *Pectinophora gossypiella* showed that both the protoxin and activated toxin forms of Cry1Ac bind to fragments of cadherin, a key midgut receptor protein, and to brush border membrane vesicles prepared from insect midguts 19. These unexpected results raised the intriguing possibility that binding of protoxins to midgut receptors can kill insects via a toxic pathway different from the primary pathway initiated by binding of activated toxin to midgut receptors. In both models, binding to midgut receptors triggers post-binding events that eventually kill the insect.

Crystallography recently revealed that, like activated toxins, the carboxyl half of Cry1Ac protoxin that is removed during activation is organized into distinct structural domains 20 (Fig. 1). Domains V and VII of this portion of Cry1Ac protoxin resemble carbohydrate-binding modules and are structurally similar to domains II and III of the activated toxin 20 that mediate binding to midgut receptors 16,17. In addition, recent in vitro experiments showed that both the protoxin and activated toxin forms of Cry1Ab bind to the same cadherin fragment from *Manduca sexta*, with only slightly lower binding affinity for protoxin relative to activated toxin 21. Moreover, both forms promoted post-binding events in the toxic pathway, including oligomerization and pore formation, with one oligomer formed by protoxin and a different oligomer formed by activated toxin 21.

Based primarily on the in vitro findings summarized above, Gomez et al. 21 proposed a new model for Bt mode of action, which we refer to here as the “dual model,” where both the protoxin and activated toxin forms can kill insects, with each form exerting its toxic effect via a different pathway (Fig. 1). This

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**Figure 1. Bt protein mode of action.** In the classical model (black arrows), inactive Cry1Ac protoxin (domains I-VII; PDB 4W8J) must be converted to activated toxin (domains I-III; PDB 4ARY) before binding to insect midgut receptors to exert toxicity. In the dual model, conversion of protoxin to activated toxin is the primary toxic pathway, but either intact protoxin or part of the protoxin other than the activated toxin also contribute to toxicity in a secondary toxic pathway (red arrow) that can be especially important in resistant insects with disruptions in the primary pathway, such as reduced binding of activated toxin to midgut receptors. In both models, binding to midgut receptors triggers post-binding events that eventually kill the insect.
contrasts with what we refer to hereafter as the “classical model” described above in which protoxins are inactive. The data cited above that spurred the dual model are valuable because the in vitro approach eliminated activation of protoxins by proteases in the insect midgut. However, experiments in vivo are essential to determine which model more accurately describes what happens inside live insects.

Here we test the classical and dual models using nine sets of bioassays that compare responses to protoxins versus activated toxins against seven resistant strains and three susceptible strains of three major pests (*Helicoverpa armigera*, *Helicoverpa zea*, and *Diatraea saccharalis*) from two families of Lepidoptera (Noctuidae and Crambidae) (n = 11,520 insects tested in 34 bioassays, Tables S1–S5). The results show that protoxins were generally more potent than activated toxins against resistant strains, which contradicts the classical model and supports the dual model.

**Results**

**Resistance lower to protoxins than activated toxins.** According to the classical model, protoxins must be converted to activated toxins to kill insects, and disruption of this step causes higher resistance to protoxins than to activated toxins. The classical model also predicts that reduced binding of activated toxins to midgut receptors, the most common and most potent mechanism of resistance, causes similar resistance to protoxins and activated toxins. Thus, the classical model predicts that resistance will not be lower to protoxins than activated toxins.

Contrary to this prediction, resistance to Cry1Ab or Cry1Ac was lower to protoxins than the corresponding activated toxins in eight of the nine new data sets reported in this study (Fig. 2, Tables S2–S4). We calculated the resistance ratio as the concentration of activated toxin (or protoxin) killing 50% of larvae (LC$_{50}$) for a resistant strain divided by the LC$_{50}$ of activated toxin (or protoxin) for a conspecific susceptible strain. We measured the reduction in resistance ratio for the protoxin relative to its activated toxin as the resistance ratio for the activated toxin divided by the resistance ratio for the corresponding protoxin. For the nine cases, the median resistance ratio was 140 for activated toxins versus 21 for protoxins, with a median 10-fold reduction in resistance ratio for protoxins relative to activated toxins (paired t-test of log-transformed data, t = 4.9, df = 8, P = 0.001).

In the sole exception to the pattern of higher resistance ratios for activated toxin than protoxin in the nine cases from this study, the resistance ratio was 7.9 for trypsin-activated Cry1Ac and 17 for Cry1Ac protoxin against the GA strain of *H. zea*. GA was derived from a population that was exposed to Bt proteins in the field, but had been reared subsequently in the laboratory for more than 40 generations without exposure to Bt proteins and was the least resistant of the seven resistant strains tested here (Fig. 2, Table S4). For the GA-R strain, which was derived from GA and selected with Cry1Ac protoxin in the laboratory, the resistance ratio was 82 for trypsin-activated Cry1Ac versus 6.4 for Cry1Ac protoxin (Table S4). For both GA and GA-R, resistance to Cry1Ac protoxin was lower than resistance to Cry1Ac activated with midgut juice from a susceptible strain of *H. zea* (Fig. 2, Table S4). Across all nine cases, the reduction in resistance ratio for protoxin relative to activated toxin increased significantly as
the resistance ratio for activated toxin increased (regression of log-transformed data, \( r^2 = 0.71 \), \( df = 7 \), \( P = 0.004 \), Figure S1).

**Potency of protoxins relative to activated toxins.** Based on the assumption that protoxins must be converted to activated toxins to kill insects, the classical model predicts that activated toxins will be more potent than their protoxin counterparts. More specifically, it has been hypothesized that Cry1A protoxins will be about half as potent as their activated toxins, because the active portion of these protoxins weighs about half (65 kDa) of the total of the protoxin (130 kDa)\(^{30} \). To test this hypothesis, we evaluated potency, which is inversely related to the LC\(_{50}\) value\(^{31} \). We calculated the potency of protoxin relative to activated toxin as the LC\(_{50}\) of an activated toxin divided by the LC\(_{50}\) of the corresponding protoxin\(^{31} \).

The results from analyzing potency for the new data reported here are consistent with the classical model for susceptible strains, but not for resistant strains (Fig. 3, Table S5). Against susceptible strains, the mean potency of protoxins relative to activated toxins was 0.70 (range = 0.17 to 1.6), which does not differ significantly from the predicted value of 0.50 (one sample t-test of log-transformed data, \( df = 7 \), \( t = 1.5 \), \( P = 0.18 \)). However, contrary to the classical model, protoxins were more potent than activated toxins in eight of nine pairwise comparisons for resistant strains (Fig. 3, Table S5). The lone exception again involved the GA strain tested with trypsin-activated Cry1Ac (potency of protoxin relative to activated toxin = 0.72). Nonetheless, consistent with the overall trend, Cry1Ac protoxin was 4.3 times more potent than Cry1Ac activated with midgut juice against this strain (Fig. 3, Table S5). Against all resistant strains, the mean potency of protoxins relative to activated toxins was 5.7 (range = 0.72 to 39), which is more than 10 times greater than the predicted value of 0.50 (one-sample t-test of log-transformed data, \( df = 8 \), \( t = 6.8 \), \( P = 0.0001 \)). Moreover, the potency of protoxins relative to activated toxins was significantly greater for resistant strains than susceptible strains (paired t-test of log-transformed data, \( df = 8 \), \( t = 4.8 \), \( P = 0.001 \)).

**Discussion**

The responses to Cry1A protoxins and activated toxins in bioassays with 10 resistant and susceptible strains of three species reported here generally support the dual model, but not the classical model. Previous results from five additional resistant and susceptible strains of two species also support the dual model, but not the classical model. Similar to our findings, the resistance ratio was higher for activated toxin than protoxin for the Europe-R and RSTT-R strains of *Ostrinia nubilalis*\(^{32} \). The resistance ratio for Cry1Ab was 108 for activated toxin versus 5.7 for protoxin (a 19-fold difference) for Europe-R; and 484 for activated toxin versus 15 for protoxin (a 32-fold difference) for RSTT-R\(^{32} \). Also similar to the results reported here, Cry1Ab protoxin was less potent than activated toxin against a susceptible strain (relative potency = 0.27), but the protoxin was more potent than activated toxin against both resistant strains.
(relative potency = 13 for Europe-R and 15 for RSTT-R)\(^2\). Likewise, results with the resistant AR strain of \(H.\ zea\) show higher resistance to activated toxin than protoxin, as well as greater potency of protoxin than activated toxin against the resistant strain\(^2\).

Considering the new data reported here together with previous results, evidence from 10 resistant strains and five susceptible strains of four major pest species (\(D.\ saccharalis\), \(H.\ armigera\), \(H.\ zea\), and \(O.\ nubilalis\)) tested in five different laboratories support the dual model, but not the classical model. The 10 resistant strains are diverse in terms of how they were selected for resistance as well as their genetic basis and mechanism of resistance (Table S1). Both strains of \(O.\ nubilalis\) were selected with Cry1Ab protoxin in the laboratory\(^3\), the GA and GA-R strains were exposed to Bt crops in the field and selected with Cry1Ac protoxin in the laboratory\(^4\), the RR strain of \(D.\ saccharalis\) was selected with Bt corn leaf material in the laboratory, and the AR strain of \(H.\ zea\)\(^5\) as well as all four resistant strains of \(H.\ armigera\) here tested were selected with Cry1Ac activated toxin in the laboratory. In the four strains of \(H.\ armigera\), resistance is caused by a recessive extracellular cadherin mutation in one strain (SCD-r1), a partially dominant intracellular cadherin mutation in another (SCD-r15), as well as partially dominant and dominant mutations affecting genes other than cadherin in the two remaining strains, respectively (SCD-423 and AY2)\(^6\)–\(^9\). Reduced binding of Cry1Ac is associated with resistance in SCD-r1, but not in SCD-r15 where resistance entails interference with post-binding events\(^10\) (Table S1). Resistance in the Bt-RR strain of \(D.\ saccharalis\) is associated with reduced expression of three aminopeptidase N genes and cadherin, but not with mutations in the genes encoding these proteins\(^11\)–\(^13\). In \(O.\ nubilalis\), resistance is associated with reduced binding of toxin in the Europe-R strain, but not the RSTT-R strain\(^14\). In the AR and AR1 strains of \(H.\ zea\), resistance is associated with increased alkaline phosphatase in the midgut lumen, but not with reduced binding of toxin\(^15\).

The results summarized above imply that the classical model provides an adequate description for Bt toxin mode of action in susceptible insects, but apparently not in at least 10 resistant strains of insects. The evidence from both susceptible and resistant insects is consistent with the dual model as follows: In susceptible insects, conversion of protoxins to activated toxins is the primary toxic pathway, whereas intact protoxin or portions of protoxin other than the 65 kDa activated toxin cause mortality via a secondary pathway. However, in these 10 resistant strains, where the potency of activated toxins has been reduced by severe disruption of the primary toxic pathway, protoxins acting via a secondary pathway can be more potent than activated toxins (Fig. 1).

We considered an alternative hypothesis under which more of the data would fit the classical model: Reduced potency of activated toxin caused by removal of activated toxin from the toxic pathway (via degradation, sequestration, or excretion) is greater in resistant than susceptible insects when insects are fed activated toxin, but not when activated toxin is generated more gradually by conversion of protoxin within the insects. The second part of this hypothesis is essential to explain the observed patterns, because if enhanced removal also occurs for activated toxin generated by conversion of protoxin inside insects, this mechanism cannot explain why protoxins were more potent than activated toxins against 10 resistant strains. Although this alternative hypothesis is difficult to exclude in some cases, it does not explain the higher potency of protoxins than toxins in all of the 10 resistant strains described above. For example, in the resistant SCD-r1 and SCD-r15 strains of \(H.\ armigera\) tested in this study, the primary cause of resistance is mutant cadherin alleles \(r1\) and \(r15\), respectively, which were introgressed into susceptible strain SCD\(^16\). Given that the major mechanism of resistance in these strains does not entail enhanced removal of activated toxin, the data do not support the alternative hypothesis for these strains. In addition, increased degradation of activated toxin was ruled out as a resistance mechanism in the Europe-R and RSTT-R strains of \(O.\ nubilalis\)\(^17\). In general, despite some evidence that increased degradation and sequestration can contribute to resistance\(^18\)–\(^20\), these are not primary mechanisms of field-evolved resistance\(^21\)–\(^23\). Moreover, we know of no data supporting the idea that any resistance mechanism increases removal of activated toxin when insects are fed activated toxin, but not when activated toxin is generated by conversion of protoxin within insects.

Despite the discrepancy between the classical model and the results from the 10 resistant strains of four species summarized above, both the dual and classical models correctly predict that reduced conversion of protoxin to activated toxin can yield higher resistance to protoxins than activated toxins, as seen in at least seven other resistant strains of five species: the KS-SC strain of \(O.\ nubilalis\), the SERD5 strain of \(P.\ xylostella\), the 198’ and Dpl’ strains of \(P.\ interpunctella\), the Akola-R and LF5 strains of \(H.\ armigera\) and the MR strain of \(M.\ unipuncta\)\(^24\)–\(^28\). However, reduced conversion of protoxin to activated toxin is usually a relatively weak mechanism that is not common in field-selected strains, whereas reduced binding of toxin to midgut receptors confers high levels of resistance and is the most common field-selected mechanism of resistance to Bt toxins\(^29\)–\(^31\). Aside from the 17 resistant strains discussed above, the responses to protoxins versus activated toxins remain to be evaluated in some other resistant strains that show reduced binding of toxin and a resistance ratio >500 for at least one Cry1A protoxin (e.g., the NO-QA strain of \(P.\ xylostella\), the YHD2 strain of \(H.\ virescens\), the AZP-R strain of \(P.\ gossypiella\), and the GLEN-Cry1Ac strain of \(T.\ ni\))\(^32\)–\(^34\). The resistance to protoxin relative to activated toxin in these other strains may differ from the seven resistant strains studied here (Fig. 2, Tables S2–24) and the three strains of \(O.\ nubilalis\)\(^35\) and \(H.\ zea\)\(^36\) described above in which resistance ratios for Cry1A protoxins were at most 120.
With a few notable exceptions\textsuperscript{19,21,48}, previous work has largely ignored the potential binding to midgut receptors and contributions to toxicity of intact protoxins or portions of protoxins other than activated toxins. In addition, most studies of activated toxins have used mammalian trypsin or chymotrypsin to convert protoxin to activated toxin, which does not necessarily mimic activation by the mixture of proteases in larval midguts\textsuperscript{17,21}. However, in one of the few previous direct comparisons for Cry1A proteins, mortality of susceptible \textit{M. sexta} larvae was only slightly higher when Cry1Ab was activated with midgut juice from susceptible \textit{M. sexta} larvae than with bovine trypsin\textsuperscript{48}. In the comparisons here with the resistant GA and GA-R strains of \textit{H. zea}, the potency of protoxin relative to activated toxin was lower for trypsin-activated Cry1Ac than Cry1Ac activated by midgut juice from susceptible \textit{H. zea} larvae (Fig. 3 and Tables S4 and S5). These results with \textit{H. zea} suggest that the data from resistant strains of other species tested only with trypsin-activated toxin may underestimate the potency of protoxins relative to activated toxins. Resistance to Cry1Ac protoxin of GA and GA-R relative to LAB-S was lower in this study (Fig. 2 and Table S4) than reported previously\textsuperscript{29}. Factors that may have caused this pattern include differences between the two studies in the method of toxin preparation and the bioassay method (diet surface overlay here versus diet incorporation previously), and changes in the strains over time.

Although bioassay results indicate that in at least 10 resistant strains of four major lepidopteran pests, the intact protoxin or some part of the protoxin other than the activated toxin contributes to toxicity, much work remains to elucidate the details of the putative secondary toxic pathway. For both resistant strains of \textit{O. nubilalis} mentioned above, \textit{in vitro} experiments reveal that a substantial amount of protoxin remained intact after incubation with their larval midgut juice for 30 minutes at 30 °C\textsuperscript{32}. After 60 minutes, the quantity of intact protoxin was markedly lower, but intermediate peptides larger than activated toxin and smaller than intact protoxin remained\textsuperscript{32}. We hypothesize that the higher potency of protoxins relative to activated toxins against some resistant insects is caused by intact protoxin, peptides of intermediate size, or both. Although additional experiments are needed to distinguish between these possibilities, the available evidence from crystallography and bioassays, as well as \textit{in vitro} assays of protein processing, binding, oligomerization and pore formation suggest that Bt protoxins contribute to insect mortality via a different toxic pathway than activated toxins.

Based on the prevailing perspective of the classical model of Bt mode of action, engineering plants to produce activated toxins rather than protoxins has been proposed as a way to defeat resistance mediated by reduced conversion of protoxins to activated toxins\textsuperscript{24,26}. However, this approach eliminates any potential toxicity contributed by the putative secondary pathway, which might be particularly important against insects with reduced binding of activated toxins to midgut receptors that confers high levels of resistance to activated toxins. In addition, in a rare direct comparison based on a laboratory diet selection experiment, \textit{H. zea} evolved resistance faster to activated toxin than protoxin\textsuperscript{30}. Some cultivars of Bt crops produce activated toxins while others produce protoxins\textsuperscript{3,30}. The findings reported here and some previous results\textsuperscript{30} suggest that Bt crops producing protoxins or both protoxins and activated toxins could be more effective and durable than those producing only activated toxins. The results also suggest that production of protoxins rather than activated toxins could be a natural bacterial strategy for delaying resistance in insects. For plants producing protoxins, the extent to which plant proteases convert protoxins to activated toxins may affect efficacy\textsuperscript{41}. Better understanding of the higher potency of protoxins than activated toxins against some resistant insects may help to enhance the efficacy and durability of transgenic Bt crops.

**Methods**

**Insect strains.** We tested seven resistant strains and three susceptible strains of three lepidopteran species: \textit{D. saccharalis}, \textit{H. armigera}, and \textit{H. zea} (Table S1). All susceptible strains were reared in the lab without exposure to Bt toxins or other insecticides.

\textit{Diatraea saccharalis}. The susceptible strain (Bt-SS) was established using larvae collected from corn fields near Winnboro in northeastern Louisiana during 2004. A Bt-resistant strain (Bt-RR) was developed from a single isolate family using an F\textsubscript{2} screen\textsuperscript{52}. Bt-RR larvae completed development on commercial Cry1Ab corn hybrids\textsuperscript{52}. Before the current study, the Bt-RR strain was backcrossed three times with the Bt-SS strain and reselected for resistance with Cry1Ab corn leaf tissue in the F\textsubscript{2} generation after each backcross.

\textit{Helicoverpa armigera}. The susceptible strain of \textit{H. armigera} (SCD) originated from the Cote D’Ivoire in the 1970s and was obtained from Bayer CropScience in 2001. We tested four previously described resistant strains of \textit{H. armigera} (SCD-r1, SCD-r15, SCD-423 and AY2) that were each derived from northern China and selected by exposing larvae in the laboratory to diet overlaid with Cry1Ac activated toxin\textsuperscript{34–37,39}.

SCD-r1 and SCD-r15 were created by introgressing resistant cadherin alleles \textit{r1} and \textit{r15}, respectively, into the susceptible SCD strain via repeated backcrossing and selection, as described by Yang \textit{et al.}\textsuperscript{34} for SCD-r1. The recessive \textit{r1} allele has a premature stop codon that disrupts the extracellular region of cadherin and interferes with binding of Cry1Ac\textsuperscript{34}. The non-recessive \textit{r15} allele has a deletion of 55 amino acids disrupting the intracellular region of cadherin, which is not directly involved with binding, but disrupts post-binding events\textsuperscript{35}. The \textit{r1} allele was derived from the GYBT strain, which was obtained...
We recorded mortality after 7 days at 27 °C ± 1 °C, 16L:8D, and 60% RH. Larvae were scored as dead if they died or if they weighed < 0.1 mg based on visual estimation. For H. armigera, we used diet surface overlay bioassays to test Cry1Ac protoxin and activated toxin against second instars that had been starved for 4 hours. Cry1Ac protoxin and trypsin-activated toxin were purchased from Marianne Pusztai-Carey, who obtained Cry1Ac protoxin from E. coli cells transformed with the cry1Ac gene from the HD1 strain of Bt subsp. kurstaki. She produced trypsin-activated Cry1Ac using the same method as described above for Cry1Ab. Cry1Ac protoxin and activated toxin were diluted with a phosphate buffer solution (PBS; 0.01 M, pH 7.4). We put 900 μl of liquid artificial diet in each well of a 24-well plate. After the diet cooled and solidified, we added 1.5 ml of PBS with an appropriate concentration of protoxin or activated toxin. After the bioassay tray was rotated for 1 h to allow the dilution to spread evenly and dry, we put one neonate in each well and covered each tray with a ventilated plastic cover. We recorded mortality after 7 days at 27 ± 1 °C, 14L:10D, and 60 ± 10% RH.

We conducted three sets of bioassays with H. zea. In all three sets, we used Cry1Ac protoxin provided by Jie Zhang. He obtained Cry1Ac protoxin by growing the HD73 strain of Bt subsp. kurstaki in 1/2 Luria-Bertani medium using the repeated crystal solubilization method. In the first and second sets
of bioassays, we compared Cry1Ac protoxin with trypsin-activated Cry1Ac purchased from Marianne PusztaI-Carey that she produced as described above. In the third set, we compared Cry1Ac protoxin with Cry1Ac activated by midgut extract as follows: We chilled five fourth instars of the susceptible LAB-S strain of *H. zea* on ice, removed their midguts, and put the midguts in ice cold NaCl (0.15 M) solution. Each midgut was ground by hand with a glass homogenizer and the soluble extract was separated as the supernatant after centrifuging at 100,000 g for 10 min at 4 °C. with BSA as the standard to measure the concentration of protein in the midgut extract and Cry1Ac protoxin. Cry1Ac protoxin was incubated with midgut extract (25 protoxin:1 extract by weight) at 37 °C for 6 h. Cry1Ac activated toxin was precipitated by adding acetic acid to adjust pH to 4.5, followed by centrifugation at 10,000 g for 10 min. The pellet was washed three times using ice cold double distilled water, dissolved in 50 mM pH 10.0 buffer, and precipitated by adding acetic acid to adjust pH to 4.5, followed by centrifugation at 100,000 g for 10 min. Each midgut was ground by hand with a glass homogenizer and the soluble extract was separated as the supernatant after centrifuging at 100,000 g for 10 min at 4 °C. with BSA as the standard to measure the concentration of protein in the midgut extract and Cry1Ac protoxin. Cry1Ac protoxin was incubated with midgut extract (25 protoxin:1 extract by weight) at 37 °C for 6 h. Cry1Ac activated toxin was precipitated by adding acetic acid to adjust pH to 4.5, followed by centrifugation at 10,000 g for 10 min.

### Analysis of data and crystal structures

Supplementary Methods provides details of the analysis of the data and the crystal structures of Cry1Ac protoxin and activated toxin.

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Author Contributions
B.E.T., M.Z., Y.W., F.H., Y.C. and X.L. contributed to research design; M.Z., M.G., F.H. and J.W. conducted bioassays; J.Z., M.S. and A.B. provided toxins; B.E.T., M.Z., Y.W. and F.H. analysed bioassay data; J.F. analysed protein structures; and A.Y. prepared figures. B.E.T. wrote the paper. All authors discussed the results and commented on the manuscript.
Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: B.E.T., M.S. and A.B. are coauthors of a patent on modified Bt toxins, “Suppression of Resistance in Insects to Bacillus thuringiensis Cry Toxins, Using Toxins that do not Require the Cadherin Receptor” (patent numbers: CA2690188A1, CN101730712A, EP2184293A2, EP2184293A4, EP2184293B1, WO2008150150A2, WO2008150150A3). J.A.F. is coauthor of a patent “Cadherin Receptor Peptide for Potentiating Bt Biopesticides” (patent numbers: US20090175974A1, US8354371, WO2009067487A2, WO2009067487A3). Bayer CropScience, Dow AgroSciences, DuPont Pioneer, Monsanto, and Syngenta did not provide funding to support this work, but may be affected financially by publication of this paper and have funded other work by some of the authors.

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