Susceptibility of a Field-Derived, *Bacillus thuringiensis*-Resistant Strain of Diamondback Moth to In Vitro-Activated Cry1Ac Toxin

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Resistant and susceptible populations of the diamondback moth (*Plutella xylostella*) were tested with crystalline, solubilized, and partially and fully activated forms of the *Bacillus thuringiensis* Cry1Ac δ-endotoxin. Fully activated toxin greatly reduced the resistance ratio (ratio of the 50% lethal concentration for the resistant population to that for the susceptible population) of the resistant population, suggesting that a defect in toxin activation is a major resistance mechanism.

Bacillus thuringiensis crystalline (Cry) protoxins are solubilized and cleaved by proteinases in the insect midgut to form active toxins which then bind to specific sites on midgut brush border membranes, leading to pore formation and cell lysis (8). Changes that cause disruption of any of these steps could confer resistance. The diamondback moth (*Plutella xylostella*) is the only insect in which resistance to *B. thuringiensis* has been selected in the field, and the only significant resistance mechanism described for *P. xylostella* involves the loss of toxin binding, although there is indirect evidence for alternative mechanisms of resistance in some field populations (3, 10, 11). One possible alternative mechanism has been described in a *B. thuringiensis*-resistant strain of *Plodia interpunctella*, where the loss of a specific proteinase was believed to be responsible for the observed resistance (6). Reduced production of active toxin has also been proposed as a mechanism of resistance in *Heliothis virescens* (1). Proteolytic activation of Cry1 protoxins

involves the removal of peptide sequences from both N and C termini. The observations that a mutant toxin in which N-terminal cleavage is prevented has reduced toxicity (4) and that N-terminally truncated toxins are toxic to *Escherichia coli* expression hosts (4, 9) suggest that N-terminal cleavage may be an important part of the toxic mechanism.

An N-terminally activated Cry1Ac was constructed by deleting the DNA encoding amino acids 2 to 29. This and full-length Cry1Ac were expressed in *E. coli* or *B. thuringiensis* and used to bioassay *P. xylostella* using a leaf dip assay as described previously (7). A Cry1Ac-resistant field population of *P. xylostella* from Serdang, Malaysia (SERD5; collected in August 1999), was divided into two subpopulations. One subpopulation was left unselected (UNSEL); the other (Cry1Ac-SEL) was reselected with in vitro-activated Cry1Ac from F_2 to F_9. Compared with the susceptible strain (ROTH), UNSEL and Cry1Ac-SEL showed resistance ratios (ratio of the 50% lethal concentration

TABLE 1. Toxicity of crystalline Cry1Ac for ROTH, UNSEL, and Cry1Ac-SEL populations of *P. xylostella*

Strain	Form of toxin	LC_{50} (95% FL)[a]	Avg slope (SE)	RR[b]
ROTH	Crystal[c]	0.0020 (0.0001–0.0085)	0.69 (0.18)	
	Spore-crystal[d]	0.0013 (0.0003–0.0039)	0.85 (0.19)	
	NAT spore-crystal[d,e]	0.0020 (0.0008–0.004)	2.12 (0.53)	
UNSEL	Crystal	0.0880 (0.034–0.19)	1.29 (0.24)	44
	Spore-crystal	0.0920 (0.03–0.2)	1.23 (0.24)	71
	NAT spore-crystal	0.1110 (0.0003–0.315)	1.64 (0.61)	55
Cry1Ac-SEL	Crystal	2.33 (0.14–29.62)	0.54 (0.22)	1,170
	Spore-crystal	3.60 (1.1–15.0)	0.81 (0.24)	2,770
	NAT spore-crystal	2.08 (0.56–6.01)	0.64 (0.16)	1,040

[a] Expressed in micrograms per milliliter. At least 150 larvae (F_{11}) were used in each experiment. LC_{50}, 50% lethal concentration FL, fiducial limits.
[b] Resistance ratio of 50% lethal concentrations for the UNSEL and Cry1Ac-SEL populations to those for the ROTH population.
[c] Expressed in *E. coli*.
[d] Expressed in *B. thuringiensis* IPS78/11 and applied as a spore-crystal mixture.
[e] NAT, N-terminally activated toxin.

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TABLE 2. Toxicity of in vitro solubilized and activated Cry1Ac for ROTH, UNSEL, and Cry1Ac-SEL populations of *P. xylostella*

Strain	Form of toxin[c]	LC$_{50}$ (95% FL)[a]	Avg slope (SE)	RR[b]
ROTH	Crystal	15 (8–64)	0.69 (0.18)	
	Solubilized[d]	10 (8–22)	1.11 (0.29)	
	Activated[d]	15 (15–45)	1.18 (0.39)	
UNSEL	Crystal	660 (255–1425)	1.29 (0.24)	44
	Solubilized	3,150 (975–6300)	2.02 (0.43)	315
	Activated	375 (135–750)	1.99 (0.44)	25
Cry1Ac-SEL	Crystal	17,475 (1,050–222,150)	0.54 (0.22)	1,170
	Solubilized	>150,000	0.44 (0.15)	>15,000
	Activated	1,410 (465–3,150)	0.65 (0.14)	94

[a] Values are expressed as picomolar concentrations due to differences in mass between the protoxin and activated forms of Cry1Ac. At least 150 larvae (F$_{11}$) were used in each experiment. LC$_{50}$, 50% lethal concentration. FL, fiducial limits.
[b] Resistance ratio of 50% lethal concentrations for the UNSEL and Cry1Ac-SEL populations to those for the ROTH population.
[c] Expressed in *E. coli*.
[d] Solubilized in 50 mM Na$_2$CO$_3$ and, where indicated, activated with 1 mg of trypsin per ml.

for the resistant population to that for the susceptible population) of 44 and 1,170, respectively, towards crystalline Cry1Ac protoxin synthesized in *E. coli* (Table 1) . To test whether the resistant strain might be defective in toxin solubilization or activation, bioassays were performed using Cry1Ac toxin which had been either solubilized or both solubilized and activated in vitro (Table 2). For ROTH, the activity of both forms of the toxin was equivalent to that of the crystalline protoxin. For the SERD5 populations, the activated form of toxin was significantly ($P < 0.01$) more toxic than the solubilized, but nonactivated, form. In the case of Cry1Ac-SEL, the activated toxin was at least 100-fold more toxic than the solubilized toxin. Activation of Cry1Ac involves removal of peptide fragments from both N and C termini. To test whether removal of the N-terminal peptide is an important step, an engineered protein lacking the N-terminal peptide was used in bioassays. No significant difference was found between the N-terminally truncated and full-length protoxins for any of the insect populations (Table 1), suggesting that N-terminal activation is not a limiting step.

The results described above suggest that reduced availability of active toxin is a major factor explaining the increased resistance in the Cry1Ac-SEL subpopulation. The resistance phenotype could be due to a specific defect in the activation step, as has been proposed for a resistant strain of *P. interpunctella* (6). Alternatively, a mechanism not directly involved in this step could be responsible. Prevention of protoxin activation by degradation or precipitation of the solubilized toxin, for example, could result in the observed phenotype. Both of these possibilities have been observed in other insect larvae (1, 2, 5) and have been put forward as explanations for reduced susceptibility to *B. thuringiensis* toxins. The SERD5 strain is believed to have initially developed resistance through exposure to *B. thuringiensis* sprays; the reselected population was maintained through exposure to in vitro-activated Cry1Ac. The high levels of resistance generated through this reselection would argue against a specific defect in the conversion of protoxin to toxin. More likely is a mechanism that reduces the availability of protoxin or toxin for pore formation, the kinetics being such that protoxin is removed more rapidly than it is converted into toxin. The reduced toxicity of the solubilized toxin compared to that of the crystalline form with the SERD5 populations might be an effect of the leaf dip bioassay, with solubilized toxin not

being presented to the larvae as efficiently as crystalline toxin is; this effect may not be so significant for the much more susceptible ROTH strain. Alternatively, it might represent the fact that crystalline protoxin is less of a target for resistance. Concomitant solubilization and activation may partially bypass the resistance mechanism.

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