Integrative Model for Binding of *Bacillus thuringiensis* Toxins in Susceptible and Resistant Larvae of the Diamondback Moth (*Plutella xylostella*)

VICTORIA BALLESTER,[1] FRANCISCO GRANERO,[1] BRUCE E. TABASHNIK,[2]
THOMAS MALVAR,[3] AND JUAN FERRÉ[1]*

*Departament de Genètica, Universitat de València, 46100 Burjassot, València, Spain[1];
Department of Entomology, University of Arizona, Tucson, Arizona 85721[2];
and Monsanto Co., St. Louis, Missouri 63198[3]*

Insecticidal crystal proteins from *Bacillus thuringiensis* in sprays and transgenic crops are extremely useful for environmentally sound pest management, but their long-term efficacy is threatened by evolution of resistance by target pests. The diamondback moth (*Plutella xylostella*) is the first insect to evolve resistance to *B. thuringiensis* in open-field populations. The only known mechanism of resistance to *B. thuringiensis* in the diamondback moth is reduced binding of toxin to midgut binding sites. In the present work we analyzed competitive binding of *B. thuringiensis* toxins Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F to brush border membrane vesicles from larval midguts in a susceptible strain and in resistant strains from the Philippines, Hawaii, and Pennsylvania. Based on the results, we propose a model for binding of *B. thuringiensis* crystal proteins in susceptible larvae with two binding sites for Cry1Aa, one of which is shared with Cry1Ab, Cry1Ac, and Cry1F. Our results show that the common binding site is altered in each of the three resistant strains. In the strain from the Philippines, the alteration reduced binding of Cry1Ab but did not affect binding of the other crystal proteins. In the resistant strains from Hawaii and Pennsylvania, the alteration affected binding of Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F. Previously reported evidence that a single mutation can confer resistance to Cry1Ab, Cry1Ac, and Cry1F corresponds to expectations based on the binding model. However, the following two other observations do not: the mutation in the Philippines strain affected binding of only Cry1Ab, and one mutation was sufficient for resistance to Cry1Aa. The imperfect correspondence between the model and observations suggests that reduced binding is not the only mechanism of resistance in the diamondback moth and that some, but not all, patterns of resistance and cross-resistance can be predicted correctly from the results of competitive binding analyses of susceptible strains.

Insecticides derived from the bacterium *Bacillus thuringiensis* are the most widely used biological pesticides (8). During sporulation, *B. thuringiensis* produces crystals containing toxins that are called insecticidal crystal proteins (ICPs) or δ-endotoxins (19, 23). The mode of action of *B. thuringiensis* includes the following steps in the insect midgut: solubilization of the crystals, enzymatic activation of protoxins, binding of activated toxins to target sites on midgut membranes, and pore formation (2, 14, 22, 36). *B. thuringiensis* toxins kill a specific set of insect pests but do not harm people, wildlife, or even most beneficial insects. The genes encoding ICPs have been incorporated into and expressed by plants so that the plants have become toxic to some insect pests (11, 43).

Evolution of resistance by pests is the greatest threat to the continued success of the *B. thuringiensis* toxins used in conventional sprays or in transgenic plants (20). Although laboratory selection has produced resistance to *B. thuringiensis* in many pests, only the diamondback moth (*Plutella xylostella*), a major pest of crucifer crops worldwide, has evolved resistance to *B. thuringiensis* in open-field populations (37). Resistance to *B. thuringiensis* has been documented in diamondback moth populations in Hawaii, Asia, the continental United States, and Central America (35, 37, 41, 48, 49). Reduced binding of ICPs

to midgut target sites is the best-documented mechanism of resistance in members of the Lepidoptera and the only known mechanism of resistance in the diamondback moth (10, 41, 42, 48). Nonetheless, some evidence suggests that other mechanisms, such as reduced activation of protoxins and increased degradation of toxins, can confer resistance to ICPs (12, 15, 28, 30, 32, 34).

Here we report the results of competitive binding tests performed with ICPs in a susceptible strain of diamondback moth (strain LAB-V) and in resistant strains of diamondback moth obtained from the Philippines (strain PHI), Hawaii (strain NO-QA), and Pennsylvania (strain PEN). Each of the three resistant strains evolved resistance to three ICPs present in *B. thuringiensis* sprays (Cry1Aa, Cry1Ab, and Cry1Ac) while remaining susceptible to Cry1C and some other ICPs not present in the sprays (39, 41). NO-QA and PEN evolved cross-resistance to two ICPs that were not in the sprays (Cry1F and Cry1J), whereas PHI did not (39, 41). Previous analyses of noncompetitive binding provided a preliminary description of ICP-target site interactions in the four strains of diamondback moth which we used (41). Previous results also show that the ICPs Cry1Aa, Cry1Ab, and Cry1Ac bind to a common target site in diamondback moth and other moths (1, 7, 25, 44) and that Cry1F also binds to this common target site in diamondback moth (16). In the present study, we used results from our competitive binding assays along with previously published data (9, 16) to produce an integrative model for binding of Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, and Cry1F in a sus-

* Corresponding author. Mailing address: Departament de Genètica, Universitat de València, 46100 Burjassot, València, Spain. Phone: 34-96-386-4506. Fax: 34-96-398-3029. E-mail: juan.ferre@uv.es.
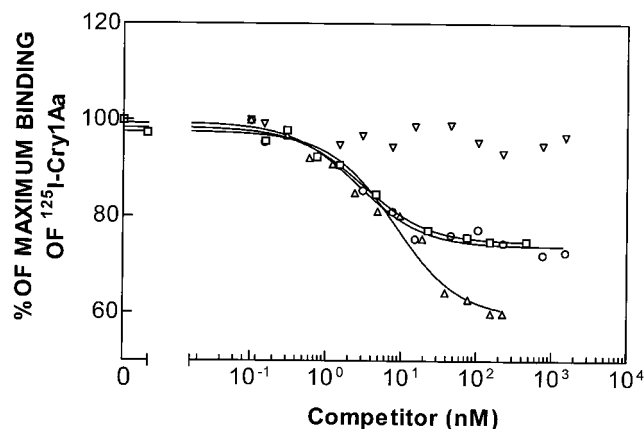
FIG. 1. Binding of ^{125}I-labeled Cry1Aa to BBMV of the susceptible strain (LAB-V) at different concentrations of unlabeled competitor. Symbols: △, Cry1Aa; ○, Cry1Ab; □, Cry1Ac; ▽, Cry1F.

FIG. 2. Binding of ^{125}I-labeled Cry1Ab to BBMV of the susceptible strain (LAB-V) at different concentrations of unlabeled competitor. Symbols: △, Cry1Aa; ○, Cry1Ab; □, Cry1Ac.

ceptible strain and variations on the model that explain the patterns observed in the three resistant strains.

MATERIALS AND METHODS

Insects. The three resistant strains of diamondback moth were started from field populations that had been sprayed repeatedly with commercial formulations of *B. thuringiensis* subsp. *kurstaki* containing the toxins Cry1Aa, Cry1Ab, and Cry1Ac (41). Before we compared the three resistant strains, each was exposed to *B. thuringiensis* in the laboratory to eliminate susceptible individuals. Susceptible strain LAB-V originated from The Netherlands and had been reared in the laboratory for more than 10 years (9).

Source of ICPs. Trypsin-activated Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F were kindly provided by Luke Masson (National Research Council of Canada, Montreal, Canada). The Cry1A proteins were obtained from recombinant *Escherichia coli* HB101, and Cry1F was obtained from recombinant *E. coli* EG1945 (from Ecogen Inc., Langhorne, Pa.).

Iodination of ICPs. Cry1A proteins (25 μg each) were labeled with Na[125]I by using the chloramine-T method (44). Cry1Aa was labeled twice, once with 0.5 mCi of Na[125]I (1 Ci = 37 GBq) and once with 1 mCi of Na[125]I, and the two preparations were used in independent experiments. Cry1Ab and Cry1Ac were labeled with 1 mCi of Na[125]I. Labeled ICPs were separated from free iodine by using a Bio-Gel P30 (Bio-Rad) column. The specific activities were 0.63 mCi/mg for Cry1Aa labeled with 0.5 mCi of Na[125]I, 5.2 mCi/mg for CryAa labeled with 1 mCi of Na[125]I, 1.9 mCi/mg for Cry1Ab, and 2.1 mCi/mg for Cry1Ac.

Preparation of BBMV. Brush border membrane vesicles (BBMV) were prepared from whole last-instar larvae by the differential magnesium precipitation method (5, 46). BBMV were frozen in liquid nitrogen and kept at −80°C until they were used. The concentration of proteins in BBMV preparations was determined with the Bio-Rad reagent (3) by using bovine serum albumin as the standard.

Binding of ^{125}I-ICPs to BBMV. Binding experiments were performed essentially as described previously (9). BBMV (5 to 10 μg of vesicle protein per assay) were incubated in 0.1 ml (final volume) of binding buffer (8 mM Na₂HPO₄, 2 mM KH₂PO₄, 150 mM NaCl [pH 7.4], 0.1% bovine serum albumin) containing 1.1 nM ^{125}I-labeled Cry1Aa, 1.5 nM ^{125}I-labeled Cry1Ab, 1.0 nM ^{125}I-labeled Cry1Ac, and various concentrations of unlabeled competitor. Incubations were carried out at room temperature for 30 min. Bound ICPs were separated from free ICPs by filtration through glass fiber filters (type GF/F; Whatman). The filters were washed with 5 ml of cold binding buffer, and the radioactivity retained in the filters was measured with a model 1282 Compugamma CS gamma-counter (LKB). A 150- to 1,000-fold excess of unlabeled toxin was used to determine the extent of nonspecific binding. The maximum specific binding was 4 to 6% for Cry1Aa, 3% for Cry1Ab in strain LAB-V, 9% for Cry1Ac in strain LAB-V, and 3% for Cry1Ac in strain PHI (41).

Statistical analyses. Data from the competition experiments were analyzed by using the LIGAND (33) and PRISM (17) computer programs. Both programs were used to analyze homologous competition (competition of a labeled ligand and its unlabeled analogue for binding to the receptor) curves to see if they fit a one-binding-site model or a two-binding-site model. In both programs, the null hypothesis assumes that the two binding sites really are the same. If the *P* value obtained from the analysis is less than 0.05, then the null hypothesis is rejected, which indicates that the experimental curve does not fit a one-binding-site model. The results obtained with the two programs were always in agreement. Binding parameters (dissociation constant [K_d] and concentration of receptors [R_t]) were estimated from homologous competition curves, as well as
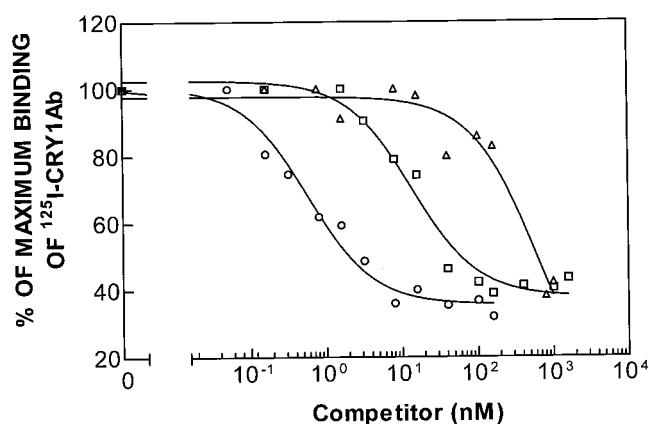
heterologous competition curves (when the unlabeled ligand was not an analogue of the labeled ligand), by using the LIGAND program. Heterologous competition also revealed whether different ICPs bound to the same binding site.

RESULTS

Binding assays performed with the susceptible strain (strain LAB-V). Binding of ^{125}I-labeled Cry1Aa was determined at different concentrations of unlabeled Cry1Aa, Cry1Ab, Cry1Ac, or Cry1F (Fig. 1). Results obtained from both the LIGAND and PRISM program analyses showed that the data for the homologous competition curve (labeled Cry1Aa versus unlabeled Cry1Aa) fit a two-binding-site model better than they fit a one-binding-site model. The K_d values for Cry1Aa indicated that a high-affinity binding site (K_{d1}, 0.1 ± 0.1 nM) and a low-affinity binding site (K_{d2}, 17.7 ± 1.0 nM) were present (Table 1). Considerable binding of labeled Cry1Aa was detected in the presence of the homologous competitor at a concentration of 200 nM (Fig. 1), which indicated that a substantial portion of the labeled Cry1Aa bound nonspecifically to the BBMV. Cry1Ab and Cry1Ac competed with labeled Cry1Aa but did not completely impede specific binding of labeled Cry1Aa. These results suggest that the three ICPs compete for binding to one of the Cry1Aa binding sites but not the other. Cry1F apparently did not compete for binding with labeled Cry1Aa.
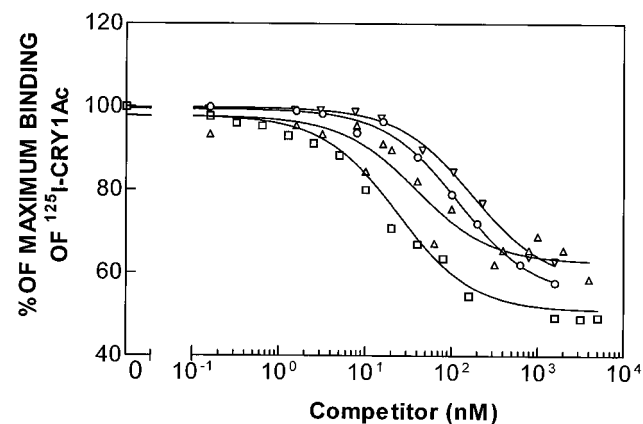

FIG. 3. Binding of ^{125}I-labeled Cry1Ac to BBMV of the susceptible strain (LAB-V) at different concentrations of unlabeled competitor. Symbols: △, Cry1Aa; ○, Cry1Ab; □, Cry1Ac; ▽, Cry1F.

TABLE 1. Equilibrium K_d and R_t values for *B. thuringiensis* crystal proteins for BBMV of susceptible strain LAB-V of *P. xylostella* obtained with different ^{125}I-labeled ICPs

[125]I-labeled ICP	Binding characteristics of ICPs[a]									
	Cry1Aa				Cry1Ab		Cry1Ac		Cry1F	
	K_{d1} (nM)	R_{t1} (pmol/mg of protein)[b]	K_{d2} (nM)	R_{t2} (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]
Cry1Aa	0.1 ± 0.1	0.2 ± 0.2	17.7 ± 1.0	6.2 ± 0.1	0.8 ± 0.2	2.7 ± 0.4	0.6 ± 0.2	2.0 ± 0.3		
Cry1Ab	27.0 ± 5.7	0.3 ± 0.1			0.4 ± 0.2	0.3 ± 0.1	5.5 ± 2.0	0.8 ± 0.4		
Cry1Ac	17.2 ± 2.8	2.3 ± 0.6			5.0 ± 0.4	1.3 ± 0.1	17.0 ± 0.2	1.0 ± 0.1	13.0 ± 1.0	2.0 ± 0.1

[a] Values are means ± standard deviations for four experiments performed with two independently prepared batches of BBMV for labeled Cry1Aa and for two experiments performed with the same batch of BBMV for the other labeled ICPs.

[b] Picomoles per milligram of vesicle protein.

The results obtained for binding of ^{125}I-labeled Cry1Ab and ^{125}I-labeled Cry1Ac at different concentrations of unlabeled Cry1Aa, Cry1Ab, and Cry1Ac are shown in Fig. 2 and 3. Analysis of the homologous competition data indicated that the data fit a one-binding-site model better than they fit a two-site model. In both cases, the heterologous competitor inhibited specific binding of the labeled ligand almost completely, suggesting that the three ICPs bound to the single receptor recognized by the labeled ICPs. Although different values were obtained depending on the labeled ICP used, Cry1Ab was the ICP that showed the highest affinity for the binding site, followed by Cry1Ac and Cry1Aa (Table 1). These results suggest that the shared binding site is the low-affinity binding site for Cry1Aa.

Competition of Cry1F with labeled Cry1Ab for the same binding site has been observed previously in LAB-V (16). Here we found that Cry1F also competes with labeled Cry1Ac (Fig. 3), with an affinity similar to the affinity observed when Cry1F was competing with labeled Cry1Ab (Tables 1 and 2), which is consistent with the observation that Cry1Ab and Cry1Ac share the same binding site.

Binding assays performed with three resistant strains (strains PHI, NO-QA, and PEN). In a previous study, we demonstrated that specific binding of ^{125}I-labeled Cry1Aa occurs in resistant strains PHI, NO-QA, and PEN (41). Here, we expanded the study with competition analyses. With PHI, the homologous competition data for Cry1Aa fit a two-binding-site model better than they fit a one-binding-site model (Fig. 4A), and the

two K_d values were essentially the same as the K_d values obtained with the susceptible strain (Table 3). This indicates that binding of Cry1Aa is not affected in this resistant strain. In contrast, with strains NO-QA and PEN, the homologous competition data fit a one-binding-site model better than they fit a two-binding-site model (Fig. 4B). The K_d and R_t values of Cry1Aa for NO-QA (4.8 ± 2.7 nM and 1.9 ± 0.8 pmol/mg of protein, respectively) and PEN (4.0 ± 2.8 nM and 1.9 ± 0.8 pmol/mg of protein, respectively) are similar to each other and are intermediate between the values obtained for the two binding sites of LAB-V and PHI (Tables 1 and 3).

Binding was low or nil in all three resistant strains for Cry1Ab (41) and in NO-QA and PEN for Cry1Ac (41). However, Cry1Ac exhibits specific binding to BBMV from strain PHI (41). Analysis of the homologous competition data (Fig. 5) gave a better fit with a one-binding-site model than with a two-binding-site model. K_d and R_t values were calculated from both the homologous and heterologous competition data (Table 3). The values of K_d and R_t depended on which ICP was labeled. However, for a particular labeled ICP, the K_d and R_t values for Cry1Ac did not differ between strain PHI and strain LAB-V, which indicates that binding of Cry1Ac in strain PHI was not altered compared to binding of Cry1Ac in strain LAB-V.

Heterologous competition experiments confirmed the homologous competition results showing that binding of Cry1Aa and Cry1Ac is not altered in strain PHI. The results of competition of Cry1Ab, Cry1Ac, and Cry1F with labeled Cry1Aa

TABLE 2. Previously reported values for K_d and R_t of *B. thuringiensis* crystal proteins for BBMV from susceptible *P. xylostella* strains (strains LAB-V, LAB-P, and ROTH) and resistant *P. xylostella* strains (strains Philippines, NO-QA, SERD3, and Bta-Sel)

Strain	Reference	Binding characteristics of ICPs[a]									
		Cry1Aa		Cry1Ab		Cry1Ac		Cry1C		Cry1F	
		K_{d1} (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]
LAB-V	9			4.2	1.6			6.5	10.8		
Philippines[c]	9							7.6	2.9		
LAB-V	1	22[d]	0.8[d]	1.4	0.8	3.7[d]	0.8[d]				
LAB-P	38					2.0	1.1	8.8	3.2		
NO-QA	38							8.5	3.5		
LAB-V	16			1.2	0.7					7.4[d]	0.5[d]
ROTH	48					22.4	2.7	8.9	9.2		
SERD3	48					27.3	3.3				
Bta-Sel	48							8.7	9.0		

[a] Unless indicated otherwise, values were obtained from homologous competition experiments.

[b] Picomoles per milligram of vesicle protein.

[c] Strain Philippines was derived from the same field location as strain PHI, but the culture was started 3.5 years earlier than the PHI culture (1).

[d] Values were obtained from heterologous competition experiments in which labeled Cry1Ab was used.
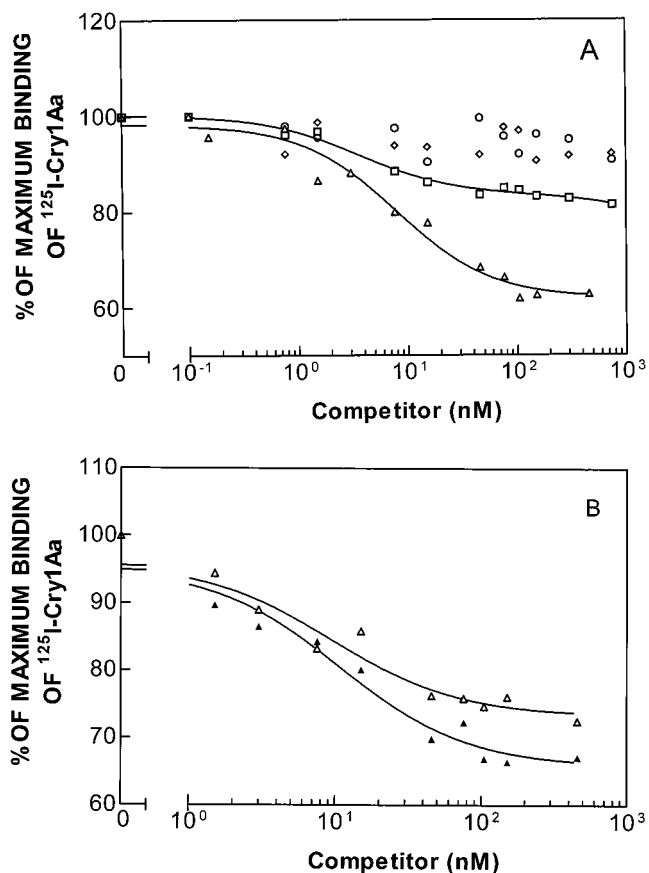
FIG. 4. (A) Binding of ^{125}I-labeled Cry1Aa to BBMV of resistant strain PHI at different concentrations of unlabeled competitor. Symbols: △, Cry1Aa; ○, Cry1Ab; □, Cry1Ac; ◇, Cry1F. (B) Binding of ^{125}I-labeled Cry1Aa to BBMV of resistant strains NO-QA (▲) and PEN (△) at different concentrations of unlabeled Cry1Aa.

for binding sites in BBMV of PHI larvae are shown in Fig. 4A. As in susceptible strain LAB-V, Cry1F did not compete with labeled Cry1Aa for binding, and Cry1Ab did not compete either (as expected, since this ICP does not bind to BBMV of this strain), but there was some competition with Cry1Ac (as a consequence of the shared binding site). The results of competition of Cry1Aa, Cry1Ab, and Cry1F with labeled Cry1Ac are shown in Fig. 5, which shows that the main difference compared with strain LAB-V is the lack of competition by Cry1Ab. Cry1F competed with labeled Cry1Ac for binding (Table 3) with essentially the same affinity as the affinity in strain LAB-V (Table 1).

DISCUSSION

If we considered the results of competition binding experiments performed with strain LAB-V in this work along with the results of other studies in which binding of Cry1B and binding of Cry1C were also determined (9, 16), an integrative model for the sites involved in binding to ICPs in the diamondback moth could be developed. We propose a model which includes at least four binding sites involved in binding of Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, and Cry1F (Fig. 6). In this model Cry1A proteins and Cry1F compete for binding to a common binding site, and Cry1Aa binds with low affinity to the common binding site and binds with high affinity to a different binding site not shared with any of the other ICPs.

Cry1B and Cry1C each bind to different binding sites. This model explains the finding that the homologous competition data for Cry1Aa fit a two-site model, whereas the homologous competition data for Cry1Ab, Cry1Ac, and Cry1C fit a one-site model. As far as we know, labeled Cry1B has not been tested in a competition experiment.

The model also explains the heterologous competition data. Cry1Ab and Cry1Ac cannot completely displace specific binding of labeled Cry1Aa. In the case of Cry1F, we interpret the lack of competition with labeled Cry1Aa as the result of Cry1F competing with low affinity to the common binding site that in turn Cry1Aa also binds to with low affinity. The low level of binding apparently escaped detection in our assay. In contrast, binding of labeled Cry1Ab or Cry1Ac can be eliminated by competition with the three Cry1A proteins and Cry1F, which indirectly suggests that Cry1Aa and Cry1F bind to the same common site. Alternatively, these two toxins could bind to different determinants in the binding site in such a way that their binding interferes with Cry1Ab or Cry1Ac binding but not with each other. The latter possibility could be tested in competition experiments performed with Cry1Aa and labeled Cry1F.

Resistant strain PHI apparently has an alteration in the common binding site that greatly reduces or eliminates binding of Cry1Ab without affecting binding of Cry1Aa or Cry1Ac. Binding is likely to occur through multiple interactions between the binding sites and several loops of domain II of ICPs (18, 26). Binding of Cry1Aa and Cry1Ac to the common binding site in PHI suggests that binding of Cry1Ab involves a distinct binding determinant that can change without affecting binding of the other ICPs. This same phenotype has been reported in a resistant diamondback moth population from Malaysia, in which binding was reduced for Cry1Ab but not for Cry1Aa, Cry1Ac, or Cry1C (48). This very specific change in the binding site is also consistent with the observation that in one set of bioassays conducted soon after PHI was established in the laboratory, this strain showed extremely high resistance to Cry1Ab but apparently not to Cry1Aa or Cry1Ac (1). Genetic analyses also have shown that in PHI, resistance to Cry1Aa is not controlled by the same gene that confers resistance to Cry1Ab (41).

The binding changes in NO-QA are the same as those observed in PEN. Both strains show very little or no binding of Cry1A proteins to the putative common binding site. These
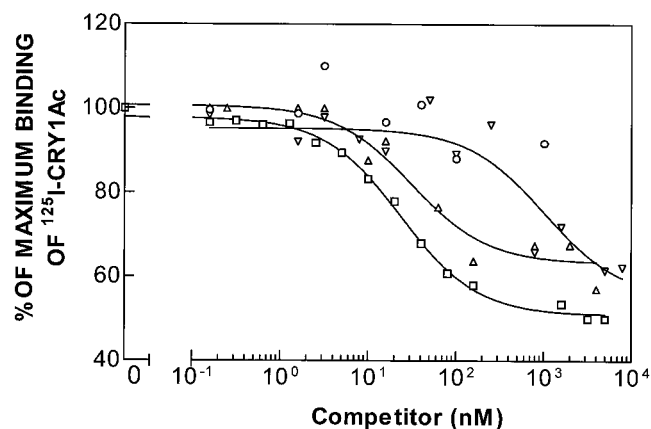

FIG. 5. Binding of ^{125}I-labeled Cry1Ac to BBMV of resistant strain PHI at different concentrations of unlabeled competitor. Symbols: △, Cry1Aa; ○, Cry1Ab; □, Cry1Ac; ▽, Cry1F.

TABLE 3. Equilibrium K_d and R_t values for *B. thuringiensis* crystal proteins for BBMV from resistant strain PHI of *P. xylostella* obtained with different ^{125}I-labeled ICPs

[125]I-labeled ICP	Binding characteristics of ICPs[a]									
	Cry1Aa				Cry1Ab		Cry1Ac		Cry1F	
	K_{d1} (nM)	R_{t1} (pmol/mg of protein)[b]	K_{d2} (nM)	R_{t2} (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]	K_d (nM)	R_t (pmol/mg of protein)[b]
Cry1Aa	0.3 ± 0.1	0.2 ± 0.2	20.3 ± 4.4	5.1 ± 1.0	NB[c]	NB	0.7 ± 0.2	3.9 ± 2.2		
Cry1Ab	NB	NB	NB	NB	NB	NB	NB	NB		
Cry1Ac	21.4 ± 0.7	1.2 ± 0.3			NB	NB	19.2 ± 0.3	1.0 ± 0.1	14.0 ± 1.0	1.6 ± 0.1

[a] Values are means ± standard deviations for four experiments performed with two independently prepared batches of BBMV for labeled Cry1Aa and for two experiments performed with the same batch of BBMV for the other labeled ICPs.
[b] Picomoles per milligram of vesicle protein.
[c] NB, no binding.

two strains also have virtually identical values of K_d and R_t for binding of Cry1Aa to the site that is not shared by the other ICPs. According to our model, NO-QA and PEN have an alteration in the common binding site that impedes binding of any ICP. The model nicely explains the resistance phenotype; both strains are resistant to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (and Cry1J), although they are susceptible to Cry1C and Cry1B (39, 41). We observed specific binding of Cry1C in both of these resistant strains (41). A previous analysis also detected no reduction in Cry1C binding in NO-QA (38). One gene can confer resistance to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F in NO-QA (40), whereas in a related diamondback moth strain from Hawaii, resistance to Cry1C segregates independently from resistance to Cry1Ab (27). The genetics of resistance in NO-QA and PEN supports our model, because it appears that one gene is responsible for the major alteration in the common binding site and one or more other genes confer resistance to Cry1C.

Quantitative differences between K_d and R_t values obtained by using homologous competition data and K_d and R_t values obtained by using heterologous competition data are common (4, 45), even though both sets of data should give the same result. Differences may arise because the labeled proteins are not the same as the unlabeled proteins used as homologous competitors, since the labeling procedure changes the proteins at least by adding the ^{125}I residue. Values of K_d and R_t usually are calculated from the results of homologous competition experiments, which are regarded as more indicative of the binding of the ICPs to their target sites.

The values of K_d and R_t obtained in the present work agree well with those previously published for *P. xylostella* (Table 2). Note that the values reported previously for Cry1Aa were obtained in heterologous competition with labeled Cry1Ab (Table 2) and therefore represent the low-affinity binding site of Cry1Aa observed in the present study (Table 1). The values obtained here for Cry1Ab in homologous competition experiments (Table 1) are not much different from the values reported previously (Table 2). For Cry1Ac, two different sets of values have been obtained previously for K_d (Table 2). Values of 22.4 and 27.3 nM (48) were obtained with the same batch of labeled Cry1Ac that was used in the present work; experiments with strains LAB-V (Table 1), ROTH (48), and SERD (48) were performed simultaneously in the same laboratory. The value obtained in heterologous competition with labeled Cry1Ab, 3.7 nM (Table 2), agrees well with the value obtained in the present work with labeled Cry1Ab (5.5 nM) and is close to the value obtained for homologous competition in a different laboratory (2.0 nM). Therefore, for now, we think that there are not enough independent replicates to choose a K_d value for

homologous competition of Cry1Ac. However, the differences in the values for the binding parameters of Cry1Ac do not invalidate the model which we propose and do not affect the comparisons among the strains of diamondback moth studied here. Finally, the K_d values obtained for Cry1F (Tables 1 and 3) are also close to the value shown in Table 2.

Binding of Cry1A proteins to strain NO-QA has been determined by using techniques different from the technique used in our study. Binding of Cry1Aa, Cry1Ab, and Cry1Ac to tissue sections of the midguts of NO-QA larvae was observed by using histochemical detection (6), as well as binding of Cry1Ac to BBMV with surface plamon resonance (31). Finally, a Cry1Ac binding protein with aminopeptidase activity was purified from both susceptible insects and NO-QA (29). Several explanations have been proposed for these discrepancies (6,
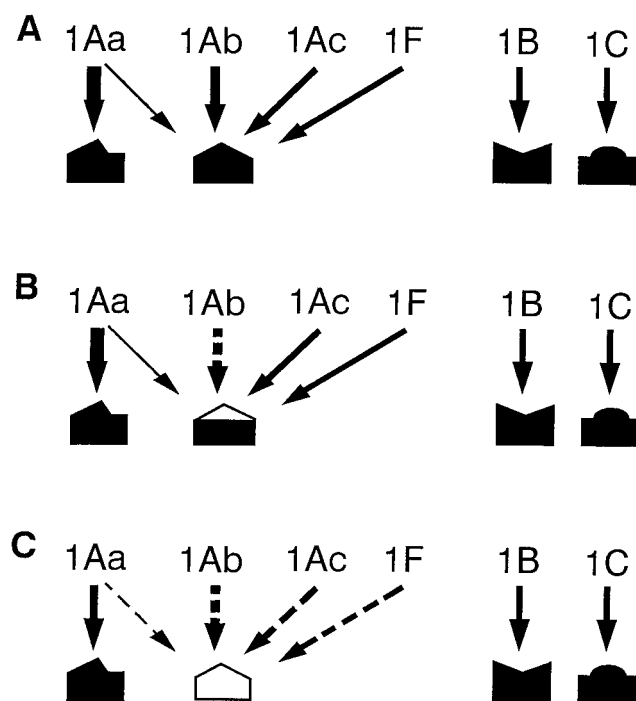

FIG. 6. Model proposed for binding of *B. thuringiensis* ICPs to binding sites in the *P. xylostella* epithelial midgut membrane in susceptible insects (A), in strain PHI (B), and in strains NO-QA and PEN (C). The wider arrows indicate greater binding affinity. Dashed arrows indicate that no binding or extremely reduced binding occurs.

29, 31). The hypothesis underlying all of them is that strain NO-QA has binding sites for the ICPs that have been altered in such a way that they do not bind toxins in vivo but in vitro bind ICPs under some circumstances but not others.

The results presented in this paper have important implications for resistance management. On the one hand, they show that binding site models obtained by using susceptible insects may correctly predict some aspects of the patterns of resistance. For example, based on the model derived from our competitive binding assays performed with susceptible strain LAB-V, we would have predicted that a single mutation could confer resistance to Cry1Ab, Cry1Ac, and Cry1F because these ICPs each bind only to a shared target site. However, because Cry1Aa binds to two sites in LAB-V, we might have made the incorrect prediction that two mutations were necessary for resistance to Cry1Aa. The finding that binding to the high-affinity binding site for Cry1Aa occurs in NO-QA and PEN without toxicity agrees with the results of other reports showing that binding is not sufficient for toxicity (7, 13, 21, 24, 47). The receptor alteration observed in PHI that confers resistance to Cry1Ab but not Cry1Ac would not have been predicted from the binding model. Furthermore, the results also show that a binding site model does not provide any information concerning the mechanisms of resistance that are not related to binding site alteration. This is true for strain PHI, and the model does not predict that this strain is resistant to Cry1Aa and Cry1Ac. However, in cases like this, the fact that the model cannot explain the resistance pattern clearly shows that there is more than one mechanism of resistance.

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