Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7844–7848, November 1988
Biochemistry

Specificity of *Bacillus thuringiensis* δ-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts

(receptor/mode of action)

CHRISTINA HOFMANN*, HILDE VANDERBRUGGEN, HERMAN HÖFTE, JEROEN VAN RIE, STEFAN JANSENS, AND HERMAN VAN MELLAERT†

Plant Genetic Systems, J. Plateaustraat, 22, B9000 Gent, Belgium

Communicated by Marc Van Montagu, June 10, 1988

ABSTRACT **Binding studies were performed with two ^{125}I-labeled *Bacillus thuringiensis* δ-endotoxins on brush border membrane vesicles prepared from the larval midgut of the tobacco hornworm *Manduca sexta* or the cabbage butterfly *Pieris brassicae*. One δ-endotoxin, Bt2-protoxin, is a 130-kDa recombinant crystalline protein from *B. thuringiensis* subsp. *berliner*. It kills larvae of both insect species. The active Bt2-toxin is a 60-kDa proteolytic fragment of the Bt2-protoxin. It binds saturably and with high affinity to brush border membrane vesicles from the midgut of both species. The other δ-endotoxin, Bt4412-protoxin, is a 136-kDa crystalline protein from *B. thuringiensis* subsp. *thuringiensis*, which is highly toxic for *P. brassicae*, but not for *M. sexta* larvae. Bt4412-toxin, obtained after proteolytic activation of Bt4412-protoxin, shows high-affinity saturable binding to *P. brassicae* vesicles but not to *M. sexta* vesicles. The correlation between toxicity and specific binding is further strengthened by competition studies. Other *B. thuringiensis* δ-endotoxins active against *M. sexta* compete for binding of ^{125}I-labeled Bt2-toxin to *M. sexta* vesicles, whereas toxins active against dipteran or coleopteran larvae do not compete. Bt2-toxin and Bt4412-toxin bind to different sites on *P. brassicae* vesicles.**

Bacillus thuringiensis produces crystalline parasporal inclusions containing insecticidal proteins called δ-endotoxins. Most δ-endotoxins are protoxins, which are proteolytically activated in the insect midgut to smaller active toxins (1). δ-Endotoxins produced by different *B. thuringiensis* strains may exhibit different insecticidal spectra. Toxins active toward lepidopteran, dipteran, or coleopteran larvae have been described (2–4). Among δ-endotoxins specific for Lepidoptera, marked differences exist in the relative levels of toxicity toward different species of this order (5–7).

Several factors such as the solubilization and proteolytic activation of the crystals in the insect midgut (5, 8) and the presence of specific cell membrane receptors for different δ-endotoxins (9, 10) have been proposed to account for this remarkable specificity.

Recently, Hofmann *et al.* (11) demonstrated the presence of a saturable high-affinity binding site for a ^{125}I-labeled δ-endotoxin in brush border membrane vesicles of *Pieris brassicae* larval midgut. Such saturable binding was not observed in vesicles derived from rat intestinal brush border membrane. To assess the role of such specific binding in determining the insecticidal spectrum of *B. thuringiensis* δ-endotoxins, we report here on the binding of two types of toxins specific for lepidopteran larvae, which differ in their insecticidal activity toward the tobacco hornworm *Manduca sexta* and the cabbage butterfly *P. brassicae*. Furthermore, we report on the competition between Bt2-toxin and several other *B. thuringiensis* δ-endotoxins for specific binding to larval *M. sexta* midgut brush border membrane vesicles.

MATERIALS AND METHODS

Insect Toxicity Assays. Insect toxicity assays on *M. sexta* and *P. brassicae* have been described (12). *M. sexta* was reared on an artificial diet (13); *P. brassicae* was reared on fresh cabbage leaves (*Brassica oleracea* var. *gemnifera* D.C.).

***B. thuringiensis* δ-Endotoxins.** Cloning of *bt2*, the gene encoding the 130-kDa δ-endotoxin (Bt2-protoxin) from *B. thuringiensis* subsp. *berliner* strain 1715, purification of recombinant Bt2-protoxin from *Escherichia coli*, and generation of the toxic 60-kDa tryptic fragment (Bt2-toxin) has been described by Höfte *et al.* (12). For further purification Bt2-toxin was precipitated in $(NH_4)_2SO_4$ (70%) and was then dissolved in Tris buffer (20 mM Tris·HCl/200 mM NaCl, pH 8.65) with 5% (vol/vol) glycerol. Streptomycin sulfate was added to a concentration of 0.2% to remove contaminating nucleic acids. The solution was allowed to stand for 60 min at 4°C and the precipitate was spun down in a Sorvall SS34 rotor at 4°C (20 min at 16,000 rpm). The supernatant was dialyzed against Tris buffer for 16 hr at 4°C. After dialysis, the Bt2-toxin solution (≈1 mg/ml) was filter sterilized and loaded onto a Mono Q HR 10/10 anion-exchange column (Pharmacia FPLC system) previously equilibrated with Tris buffer. Bt2-toxin eluted from the column in a linear gradient (20 mM NaCl per ml) at ≈0.3 M NaCl.

Bt3-protoxin is a recombinant 130-kDa δ-endotoxin encoded on a gene cloned from *B. thuringiensis* subsp. *aizawai* strain HD68 (H. T. Dulmage, U.S. Department of Agriculture, Brownsville, TX) (H.H., unpublished data). Bt8-protoxin is a recombinant mosquito-active δ-endotoxin of 130 kDa. The gene has been cloned from *B. thuringiensis* subsp. *israelensis* (14). Bt13-protoxin is a 73-kDa recombinant δ-endotoxin toxic against coleopteran insects. The gene has been cloned from *B. thuringiensis* subsp. *tenebrionis* (15). The recombinant δ-endotoxins were produced in *E. coli*, purified, and eventually activated according to ref. 12.

Crystals of *B. thuringiensis* subsp. *kurstaki* strain HD73 (H. T. Dulmage) were produced and purified as described (16). The δ-endotoxin was solubilized and activated according to ref. 12. Crystals of *B. thuringiensis* subsp. *thuringiensis* strain 4412 (Culture Collection of the Institute of Microbiology, Swiss Federal Institute of Microbiology, Zürich) were produced, purified, solubilized, and activated according to ref. 17. The crystals of both strains contain a single δ-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Permanent address: Institute of Microbiology, Swiss Federal Institute of Technology, Zürich, Switzerland.
†To whom reprint requests should be addressed.

endotoxin designated, respectively, as Bt73-protoxin and Bt4412-protoxin. Activated toxins are referred to, respectively, as Bt73-toxin and Bt4412-toxin.

Preparation of Brush Border Membrane Vesicles. Brush border membrane vesicles were prepared from the larval midgut of *P. brassicae* and of *M. sexta* as described by Wolfersberger *et al.* (18). Alkaline phosphatase, a brush border marker enzyme, was enriched 12 times (activity per mg of total protein) in the *M. sexta* vesicle preparation compared to the primary homogenate. Eight times enrichment of this enzyme was reported for *P. brassicae* vesicles (18).

Iodination Procedures. Iodination of Bt2-toxin was done by the chloramine-T method (19). $Na^{125}I$ (2 mCi; 1 Ci = 37 GBq) or an equivalent amount of $Na^{127}I$, 50 μg of purified Bt2-toxin (in 20 mM Tris·HCl/300 mM NaCl, pH 8.65) and 20 μg of chloramine T [2.5 mg/ml in phosphate-buffered saline (PBS); 10 mM P_i/150 mM NaCl, pH 7.4] were gently shaken (15 sec at room temperature). The reaction was stopped with potassium metabisulfite in H_2O (11 μl of a solution containing 4.4 mg/ml). Free iodine was removed by gel filtration on Sephadex G-50 (20). A ^{125}I-labeled Bt2-toxin preparation with specific activity of 6 mCi/mg was obtained. Iodination of Bt4412-toxin using the Iodo-Gen method was described (11). The specific activity of ^{125}I-labeled Bt4412-toxin was 3.2 mCi/mg.

Binding Assay. Brush border membrane vesicles (10 μg of protein) were incubated with ^{125}I-labeled toxin in 100 μl of PBS containing 0.1% bovine serum albumin. After 30 min incubation at room temperature (21°C ± 1°C), the sample was centrifuged in a Heraeus microcentrifuge (5 min at 13,000 rpm) to separate bound from free toxin. The pellet containing the bound toxin was washed twice with 0.5 ml of PBS/bovine serum albumin and the resulting pellet was counted immediately (^{125}I-labeled Bt2-toxin) or after resuspension in 50 μl of PBS/bovine serum albumin (^{125}I-labeled Bt4412-toxin) in a γ-counter. For the competition experiment, the indicated amounts of unlabeled toxin were always mixed with the labeled toxin in PBS/bovine serum albumin before addition of the vesicles. Data were analyzed according to Scatchard (22).

Immunoblotting. Bt2-protoxin, Bt8-protoxin, or Bt13-toxin (4 μg) was incubated with vesicles (10 μg of vesicle protein in 100 μl of PBS/bovine serum albumin) for 30 min at room temperature. Proteins were transferred from a $NaDodSO_4$/polyacrylamide gel (10% acrylamide) (21) to nitrocellulose with an LKB NovaBlot transfer unit. Antigens were detected with the appropriate polyclonal rabbit antisera and an anti-rabbit IgG alkaline phosphatase conjugate as described in Bio-rad's Alkaline Phosphatase Immunoblot Assay Kit.

Determination of Protein Content. Protein concentrations were determined according to Bradford (23) using bovine serum albumin as a standard.

RESULTS

Bt2-protoxin was toxic both to *M. sexta* and *P. brassicae* larvae, whereas Bt4412-protoxin only killed *P. brassicae* larvae (Table 1). Bt73-protoxin and Bt3-protoxin were also toxic to *M. sexta*. In contrast, the mosquitocidal Bt8-protoxin (14), and Bt13-protoxin, active against coleopteran larvae (15), were nontoxic to *M. sexta* at the highest concentration tested (Table 1).

$NaDodSO_4$/PAGE followed by autoradiography demonstrated that the ^{125}I-labeled Bt2- and Bt4412-toxin were not degraded upon a 30-min incubation with membrane vesicles from *M. sexta* or *P. brassicae* (data not shown). Iodination with ^{127}I did not reduce the toxicity of the Bt2-toxin against *M. sexta* (data not shown). The toxicity of ^{127}I-labeled Bt4412-toxin toward *P. brassicae* was reduced relative to noniodinated Bt4412-toxin (11).

Table 1. Toxicity of various δ-endotoxins to *M. sexta* and *P. brassicae*

δ-Endotoxin	*M. sexta*		*P. brassicae*	
	LD_{50}	FL_{95}	LD_{50}	FL_{95}
Bt2-protoxin	6.8	3.7–12.6	0.9	0.6–1.4
Bt4412-protoxin	>625		2.8	1.3–5.9
Bt73-protoxin	5.3	4.1–7.0	NT	
Bt3-protoxin	10.1	6.6–15.5	NT	
Bt8-protoxin	>400		NT	
Bt13-protoxin	>400		NT	

Fifty percent lethal doses (LD_{50}) and 95% fiducial limits (FL_{95}) were calculated from probit analysis (24). Data for *M. sexta* are expressed in ng of protoxin per cm^2 applied on artificial medium. Data for *P. brassicae* are expressed in μg of protoxin per ml; 5-μl samples were applied on leaf discs fed to *P. brassicae*. First-instar larvae were used (12). NT, not tested.

Binding of both labeled toxins to vesicle preparations from *M. sexta* and *P. brassicae* was investigated and the two types of unlabeled toxins were used as competitors (Fig. 1). A 30-min incubation time was sufficient for equilibrium. Data from two experiments are given below; data for experiments not shown in the figures are in parentheses.

In ^{125}I-labeled Bt2-toxin binding experiments to *M. sexta* vesicles, 52% (47%) of the labeled toxin was bound to the vesicles. Binding was saturable with unlabeled Bt2-toxin in the range of 0.1 to 10 nM (Fig. 1*A*). The Scatchard plot indicates a single high-affinity binding site with an equilibrium dissociation constant, K_d, of 0.43 nM (0.23 nM) and a binding-site concentration of 4.3 pmol/(3.3 pmol) per mg of vesicle protein. Remarkably, at ≈150 nM toxin, we observed an increase in binding, which could again be saturated with concentrations of unlabeled Bt2-toxin increasing further to 1 μM. Bt4412-toxin did not compete for binding of labeled Bt2-toxin; on the contrary, a slight increase in ^{125}I-labeled Bt2-toxin binding was also observed in the presence of high concentrations of Bt4412-toxin.

In binding studies with ^{125}I-labeled Bt4412-toxin on *M. sexta* vesicles, 5% (7%) of the labeled toxin was bound in the absence of competitor. However, no saturation was observed. Instead, concentrations of both toxins of 0.1–1 μM caused a >2-fold increase in binding of ^{125}I-labeled Bt4412-toxin. Binding again decreased, but not below its initial level, when concentrations of competitor were further increased (Fig. 1*b*).

As with *M. sexta* vesicles, a large fraction of ^{125}I-labeled Bt2-toxin (33%, 31%) was bound to the *P. brassicae* vesicles and the binding was saturable with concentrations of unlabeled analogue in the nanomolar range (Fig. 1*C*). A K_d value of 3.2 nM (2.6 nM) was obtained and a concentration of binding sites of 13.6 pmol (11.0 pmol) per mg of vesicle protein was calculated from the Scatchard plot. Bt4412-toxin at high concentrations (>1 μM) competed only marginally for the binding of labeled Bt2-toxin.

Three to four percent of ^{125}I-labeled Bt4412-toxin was bound to the *P. brassicae* vesicles. In contrast with *M. sexta*, ^{125}I-labeled Bt4412-toxin binding to *P. brassicae* vesicles was saturable in the range of 1 to 100 nM (Fig. 1*D*) as demonstrated (11). The Scatchard plot indicated that a high-affinity binding site with a K_d of 47.6 nM (18.0 nM) was present and that the concentration of binding sites was 18.8 pmol (7.6 pmol) per mg of vesicle protein. No significant competition was observed with Bt2-toxin up to 1.5 μM.

Competition studies for several other toxins were performed on *M. sexta* vesicles (Fig. 2). Both Bt73-toxin and Bt3-toxin did compete for the binding of ^{125}I-labeled Bt2-toxin. Compared to the competition curve for Bt2-toxin, the

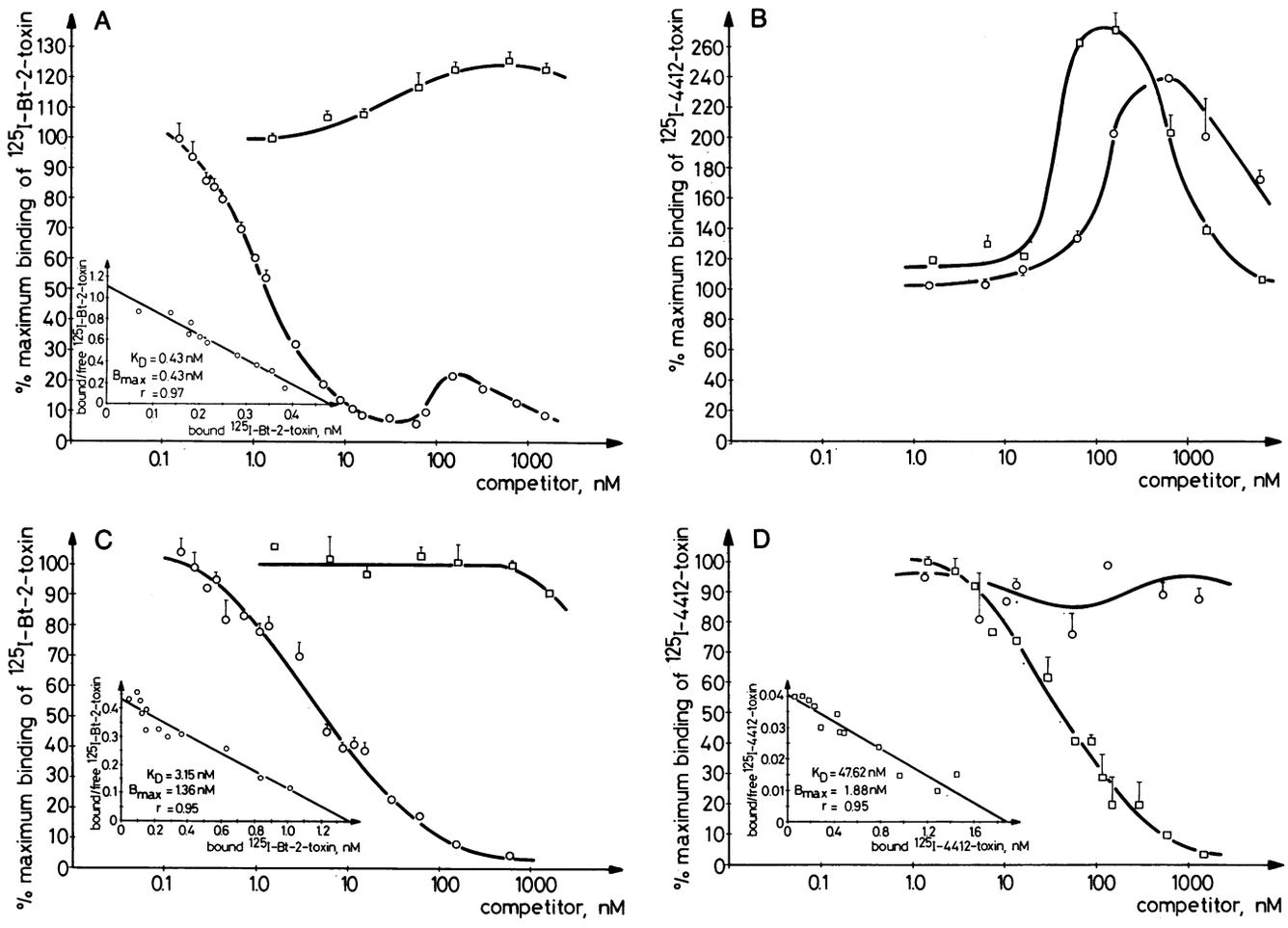

FIG. 1. (*A*) Binding of ^{125}I-labeled Bt2-toxin to *M. sexta* vesicles. (*B*) Binding of ^{125}I-labeled Bt4412-toxin to *M. sexta* vesicles. (*C*) Binding of ^{125}I-labeled Bt2-toxin to *P. brassicae* vesicles. (*D*) Binding of ^{125}I-labeled Bt4412-toxin to *P. brassicae* vesicles. Brush border membrane vesicles (100 μg of protein per ml) were incubated with labeled toxin (*A* and *C*, 0.15 nM ^{125}I-labeled Bt2-toxin; *B* and *D*, 1.67 nM ^{125}I-labeled Bt4412-toxin) in the presence of the indicated concentrations of Bt2-toxin (○) or Bt4412-toxin (□). Binding is expressed as percentage of the amount bound upon incubation with labeled toxin alone. Each point represents the mean of a duplicate sample; bars indicate deviation from the mean. When no bar is indicated, the deviation was smaller than the symbol. (*Insets*) Presentation of the same data (unlabeled competitor corresponding to labeled toxin) in Scatchard coordinates. Data were corrected for nonspecific binding. The dissociation constant (K_d) and the concentration of binding sites (B_{max}) were calculated by least-squares linear regression. The correlation coefficient (r) of the line is indicated.

competition curves were shifted to the right by factors of ≈3 and ≈10, respectively. The coleopteran-specific Bt13-toxin did not compete for binding of ^{125}I-labeled Bt2-toxin. The mosquitocidal δ-endotoxin, Bt8-protoxin, is subject to rapid degradation with trypsin (14). The competition experiment was therefore performed with Bt8-protoxin. No competition was observed. Immunoblotting demonstrated that both Bt13-toxin and Bt8-protoxin remained intact after incubation with vesicles (Fig. 3). Bt2-protoxin competed for the binding of ^{125}I-labeled Bt2-toxin in a concentration range ≈25 times higher than for Bt2-toxin itself. By means of immunoblot analysis, we observed that Bt2-protoxin was proteolytically processed by the vesicles. Major bands were around 66–70 kDa, and a faint band was also visible at 60 kDa, probably corresponding to fully activated Bt2-toxin (Fig. 3).

DISCUSSION

We have investigated whether the difference in the insecticidal spectrum of two *B. thuringiensis* δ-endotoxins, Bt2 and Bt4412, is correlated with the presence of specific binding sites or receptors in the midgut of the target insects. We used binding assays of ^{125}I-labeled δ-endotoxins (11).

Our data demonstrate that Bt2-toxin, toxic to both *M. sexta* and *P. brassicae* larvae, binds saturably and with high affinity

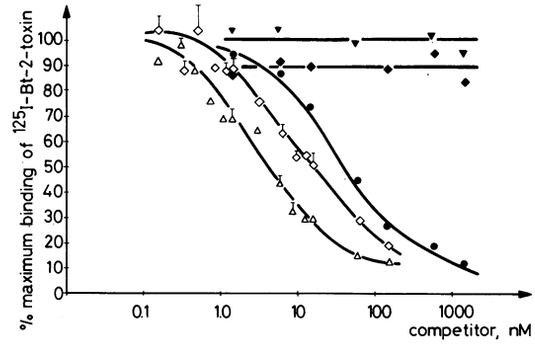

FIG. 2. Binding of ^{125}I-labeled Bt2-toxin to *M. sexta* vesicles as a function of the concentration of unlabeled competitor. Vesicles (100 μg of protein per ml) were incubated with ^{125}I-labeled Bt2-toxin (0.15 nM) in the presence of the indicated amounts of Bt73-toxin (△), Bt3-toxin (◇), Bt13-toxin (◆), Bt8-protoxin (▼), and Bt2-protoxin (●). Binding is expressed as percentage of the amount bound upon incubation with ^{125}I-labeled Bt2-toxin alone. Each point represents the mean of duplicate samples. Bars indicate deviation from the mean. When no bar is indicated, the deviation was smaller than the symbol.

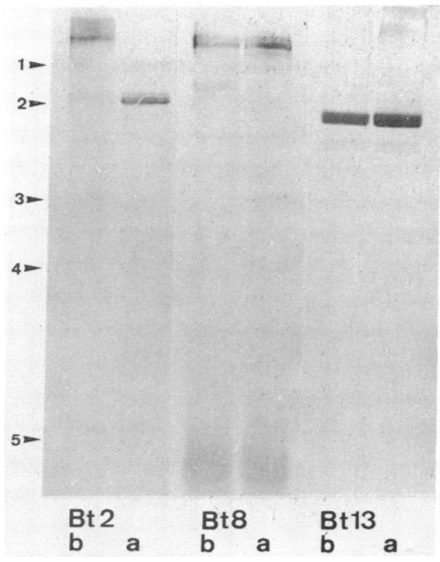

FIG. 3. Immunoblot of Bt2-protoxin, Bt8-protoxin, and Bt13-toxin before (lanes b) and after (lanes a) incubation with vesicles. Each toxin (40 μg/ml) was added to vesicles (100 μg of protein per ml) in PBS/bovine serum albumin. Samples (containing 2 μg of protoxin or toxin) were prepared for $NaDodSO_4$/PAGE immediately or after a 30-min incubation at room temperature. Polyclonal rabbit antisera used for detection on immunoblots were directed against solubilized crystal proteins of *B. thuringiensis* subsp. *berliner* (for Bt2-protoxin), Bt8-protoxin and against solubilized crystal proteins of *B. thuringiensis* subsp. *tenebrionis* (for Bt13-toxin). Standards: 1, 97.4 kDa; 2, 66.2 kDa; 3, 42.7 kDa; 4, 31.0 kDa; 5, 14.4 kDa.

to vesicles from both insect species. Scatchard plots are apparently linear and dissociation constants are $\approx 10^{-9}$ M with concentrations of binding sites in the range of 10 pmol per mg of vesicle protein. On the other hand, Bt4412-toxin binds saturably only to vesicles of the sensitive species *P. brassicae*, whereas only nonsaturable binding is found with membranes from the nonsensitive species *M. sexta*. The dissociation constant for binding of Bt4412-toxin to *P. brassicae* vesicles is 20–50 nM. This is considerably below the affinity of Bt2-toxin for its binding site in *P. brassicae* midgut. However, one has to take into account that the ^{125}I-labeled Bt4412-toxin preparation consists of a mixture of 70- and 55-kDa bands, of which only the 55-kDa band binds to the receptor (11, 25). Thus, with only a part of the toxin in the active conformation, the calculated K_d values underestimate the real affinity, and binding site concentrations are overestimated.

The data demonstrate that *P. brassicae* has two distinct binding sites for Bt4412-toxin and Bt2-toxin, respectively, since the toxins compete only negligibly for each others' binding site.

An unexpected phenomenon in the binding experiments is observed with *M. sexta* membrane vesicles: binding increases at ≈ 0.1 μM and can again be saturated with concentrations ≈ 1 μM. This phenomenon occurs with both Bt2-toxin and Bt4412-toxin on *M. sexta* vesicles, even when one labeled ligand is combined with the other unlabeled competitor, but it does not occur with *P. brassicae* vesicles. Possibly, aggregation of toxin molecules occurs at high toxin concentrations in the presence of a factor from *M. sexta* membrane vesicles. We believe this to be a nonspecific effect of the toxin since the concentration range in which the increase in binding occurs is substantially higher than the concentrations needed for saturation of the high-affinity binding site. Furthermore, *in vitro* physiological effects on K^+ gradient-dependent amino acid transport of brush border membrane vesicles (26) as well as *in vivo* toxicity (5) both occur with concentrations of δ-endotoxin in the nanomolar range.

The relationship between toxicity and "receptor" binding is further strengthened by the results of competition experiments with various ligands on the binding between *M. sexta* vesicles and labeled Bt2-toxin. Bt73-protoxin (for sequence information, see ref. 27) and Bt3-protoxin (a δ-endotoxin highly homologous to the one reported in ref. 28) both yield a Bt2-toxin-related 60-kDa fragment after trypsin activation. Both compete for Bt2-toxin binding with an affinity, respectively, ≈ 3 and ≈ 10 times less than that of Bt2-toxin. The *in vivo* toxicity of these δ-endotoxins toward *M. sexta* is not significantly different from Bt2-protoxin.

On the other hand, Bt8-protoxin and Bt13-toxin, δ-endotoxins specific for Diptera and Coleoptera, respectively, did not compete for the Bt2-toxin binding site. The data for Bt8, however, must be interpreted cautiously, since the protoxin was not proteolytically activated before incubation. The vesicles did not process Bt8-protoxin.

An interesting observation was made in the competition experiment with Bt2-protoxin. The competition curve was shifted to the right by a factor of 25 as compared to the curve for Bt2-toxin. This suggests that the affinity of Bt2-protoxin for the receptor is ≈ 25 times lower. However, immunoblots of Bt2-protoxin incubated with vesicles clearly demonstrated that Bt2-protoxin was partially activated. Thus, the Bt2-protoxin binds, if at all, with an affinity that is >25 times lower than that of Bt2-toxin.

Earlier work on binding of *B. thuringiensis* δ-endotoxins to sensitive cells and cell membrane vesicles has been reported (17, 29, 30). These binding experiments used fluorescence or immunological methods. These studies should be interpreted with caution since the sensitivity of the methods used is too low to discriminate clearly between specific and nonspecific binding. Knowles *et al.* (31) have observed protection of cultured *Choristoneura fumiferana* cells from *B. thuringiensis* δ-endotoxin action *in vitro* by *N*-acetylgalactosamine and suggested that a sugar moiety is involved in toxin binding. *N*-Acetylgalactosamine, however, did not interfere with specific binding of ^{125}I-labeled Bt4412-toxin to *P. brassicae* vesicles (11) or with the binding of ^{125}I-labeled Bt2-toxin to *M. sexta* vesicles (H.V.M., unpublished data). These results suggest that the action of *B. thuringiensis* δ-endotoxin on non-midgut cells *in vitro* may not be directly compared to its effect on the midgut *in vivo*.

The present results open interesting perspectives for further studies on the mode of action of *B. thuringiensis* δ-endotoxins. Using different labeled toxins and midgut preparations from other insects (including Diptera, Coleoptera, etc.), it will be possible to address the question of whether the relationship between toxicity and the presence of specific binding sites applies as a general rule. Another question that arises is which "effector" system is switched on by "receptor" binding. Recent *in vitro* studies on vesicles suggest that K^+ permeability of the brush border membrane vesicles is affected by the toxin (26). The availability of an *in vitro* system, which at the same time allows binding studies and physiological work, represents a potent tool for the further study of the mode of action of *B. thuringiensis* δ-endotoxins.

Dr. M. De Wolf (Rijks Universitair Centrum Antwerpen, Antwerp) is gratefully acknowledged for valuable advice and for the iodination of Bt2-toxin.

1. Lilley, M., Ruffell, R. N. & Somerville, H. J. (1980) *J. Gen. Microbiol.* **118**, 1–11.
2. Whiteley, H. R. & Schnepf, H. E. (1986) *Annu. Rev. Microbiol.* **40**, 549–576.
3. Goldberg, L. J. & Margalit, J. (1977) *Mosq. News* **37**, 355–358.

4. Krieg, A., Huger, A. M., Langenbruch, G. A. & Schnetter, W. (1983) *Z. Angew. Entomol.* **96,** 500–508.
5. Jaquet, F., Hütter, R. & Lüthy, P. (1987) *Appl. Environ. Microbiol.* **53,** 500–504.
6. Yamamoto, T., Garcia, J. A. & Dulmage, H. T. (1983) *J. Invertebr. Pathol.* **41,** 122–130.
7. Kalfon, A. R. & De Barjac, H. (1985) *Entomophaga* **30,** 177–186.
8. Haider, M. Z., Knowles, B. H. & Ellar, D. J. (1986) *Eur. J. Biochem.* **156,** 531–540.
9. Knowles, B. H. & Ellar, D. J. (1986) *J. Cell Sci.* **83,** 89–101.
10. Haider, M. Z. & Ellar, D. J. (1987) *Biochem. J.* **248,** 197–201.
11. Hofmann, C., Lüthy, P., Hütter, R. & Pliska, V. (1988) *Eur. J. Biochem.* **173,** 85–91.
12. Höfte, H., De Greve, H., Seurinck, J., Jansens, S., Mahillon, J., Ampe, C., Vandekerckhove, J., Vanderbruggen, H., Van Montagu, M., Zabeau, M. & Vaeck, M. (1986) *Eur. J. Biochem.* **161,** 273–280.
13. Bell, R. A. & Joachim, F. G. (1976) *Ann. Entomol. Soc. Am.* **69,** 365–373.
14. Chungjatupornchai, W., Höfte, H., Seurinck, J., Angsuthanasombat, C. & Vaeck, M. (1988) *Eur. J. Biochem.* **173,** 9–16.
15. Höfte, H., Seurinck, J., Van Houtven, A. & Vaeck, M. (1987) *Nucleic Acids Res.* **15,** 7183.
16. Mahillon, J. & Delcour, J. (1984) *J. Microbiol. Meth.* **3,** 69–76.
17. Hofmann, C. & Lüthy, P. (1986) *Arch. Microbiol.* **146,** 7–11.
18. Wolfersberger, M., Lüthy, P., Maurer, A., Parenti, P., Sacchi, V. F., Giordana, B. & Hanozet, G. M. (1987) *Comp. Biochem. Physiol.* **86,** 301–308.
19. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194,** 495–496.
20. Penefski, H. S. (1979) *Methods Enzymol.* **56,** 527–530.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51,** 660–672.
23. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
24. Finney, D. J. (1962) *Probit Analysis* (University Press, Cambridge), pp. 50–80.
25. Huber-Lukac, H. E. (1982) Dissertation (Federal Institute of Technology, Zurich).
26. Sacchi, V. F., Parenti, P., Hanozet, G. M., Giordana, B., Luthy, P. & Wolfersberger, M. G. (1986) *FEBS Lett.* **204,** 213–218.
27. Adang, M. J., Staver, M. J., Rocheleau, T. A., Leighton, J., Barker, R. F. & Thompson, D. V. (1985) *Gene* **36,** 289–300.
28. Schnepf, H. E., Wong, H. C. & Whiteley, H. R. (1985) *J. Biol. Chem.* **260,** 6264–6272.
29. Hofmann, C., Wolfersberger, M. G. & Lüthy, P. (1986) in *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene I. Abteiling,* eds. Falmagne, P., Alout, J. E., Fehrenbach, F. J., Jeljaszewicz, J. & Thelestam, M. (Fisher, Stuttgart and New York), Suppl. 15, pp. 235–236.
30. Wolfersberger, M. G., Hofmann, C. & Lüthy, P. (1986) in *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene I. Abteiling,* eds. Falmagne, P., Alout, J. E., Fehrenbach, F. J., Jeljaszewicz, J. & Thelestam, M. (Fisher, Stuttgart and New York), Suppl. 15, pp. 237–238.
31. Knowles, B. H., Thomas, W. E. & Ellar, D. J. (1984) *FEBS Lett.* **168,** 197–202.