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Binding of the Protoxin and Toxin Proteins of *Bacillus thuringiensis* subsp. *kurstaki* on Clay Minerals

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Abstract. The equilibrium adsorption and binding of the delta-endotoxin proteins, i.e., the protoxins (M_r = 132 kDa) and toxins (M_r = 66 kDa), from *Bacillus thuringiensis* subsp. *kurstaki* were greater on montmorillonite than on kaolinite (five-fold more protoxin and three-fold more toxin were adsorbed on montmorillonite). Approximately two- to three-fold more toxin than protoxin was adsorbed on these clay minerals. Maximum adsorption occurred within 30 min (the shortest interval measured), and adsorption was not significantly affected by temperatures between 7° and 50°C. The proteins were more easily desorbed from kaolinite than from montmorillonite; they could not be desorbed from montmorillonite with water or 0.2% Na_2CO_3, but they could be removed with Tris-SDS (sodium dodecyl sulfate) buffer. Adsorption was higher at low pH and decreased as the pH increased. Adsorption on kaolinite was also dependent on the ionic nature of the buffers. The molecular mass of the proteins was unaltered after adsorption on montmorillonite, as shown by SDS–PAGE (polyacrylamide gel electrophoresis) of the desorbed proteins; no significant modifications occurred in their structure as the result of binding on the clay, as indicated by infrared analysis; and there was no significant expansion of the clay by the proteins, as shown by x-ray diffraction analysis. The bound proteins appeared to retain their insecticidal activity against the third instar larvae of *Trichoplusia ni*.

Bacillus thuringiensis forms crystalline protein parasporal inclusions that exhibit insecticidal activity [17]. The inclusions (protoxins) are not toxic and require solubilization and enzymatic cleavage to yield the toxins. Although it is generally assumed that the toxicity of the parasporal proteins (sometimes referred to as insecticidal crystal proteins) of different subspecies of *B. thuringiensis* is restricted to specific target species of insects, these proteins (especially the *cytA*-specified proteins) may also be toxic to other orders of insects, as well as to mammals [1, 6, 8]. Preparations of *B. thuringiensis,* usually as a mixture of cells, spores, and parasporal crystals, have been used as microbial insecticides for more than 30 years, and apparently no unexpected toxicities have been noticed, probably because *B. thuringiensis* does not survive or grow well in natural habitats, such as soil [26, 28, 29, 38–41], and the toxins are rapidly inactivated by ultraviolet radiation [14, 18]. Consequently, there is probably little or no production of toxins in natural habitats, and the persistence of the introduced toxins is a function primarily of (a) the concentration added; (b) the rates of consumption and inactivation by insect larvae; and (c) the rates of degradation by the indigenous microbiota.

However, when the genes that code for the production of these toxins are genetically engineered into organisms that are indigenous or adapted to a specific habitat and, therefore, can persist and proliferate, the toxins may continue to be synthesized in that habitat. If production exceeds consumption, inactivation, and degradation, the toxins could accumulate to concentrations that may constitute a hazard to nontarget organisms, such as beneficial insects (e.g., pollinators, parasites, and predators of insect pests) and other animal classes, and that could result in the selection and enrichment of toxin-resistant target insects [10, 12, 13, 24, 27]. This accumulation would be enhanced if the toxins are bound on particulates in the environment (e.g., clay minerals) and, thereby, are rendered less accessible to microbial degradation but still retain their toxic

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activity. The decrease in susceptibility to microbial degradation and the retention, and even enhancement, of catalytic activity and infectivity of proteinaceous materials, including enzymes and viruses, bound on clay minerals have been demonstrated [7, 30, 32, 33].

This potential accumulation could occur not only from the release to the environment of transgenic bacteria [e.g., rhizosphere-colonizing or killed strains of *Pseudomonas fluorescens* (3, 20, 25) and endophytic strains of *Clavibacter xyli* (21)], but also from the release of transgenic plants [4, 9, 17, 19, 34]. The genetically modified strains of *P. fluorescens* and *C. xyli* are inhabitants primarily of the rhizosphere and internal plant tissues, respectively, wherein adequate nutrient and energy sources for growth of the bacteria and synthesis of the toxins are continuously provided in the form of root exudates and sloughings or xylem fluids, respectively, during most of the life of the plant. In the case of transgenic plants, only the usable portions of the plants will be harvested, and the remainder of the plant biomass containing the toxins will be incorporated into soil. Hence, the levels of toxins in soil will be vastly greater than those introduced with commercial preparations of *B. thuringiensis* and could exceed consumption, inactivation, and degradation, resulting in concentrations that could constitute a hazard to nontarget organisms, especially if some of the toxins are bound on soil constituents.

This potential hazard is exacerbated by modifications of the introduced toxin genes to code only for the synthesis of the toxins, or of a portion of the toxins, rather than of the nontoxic crystalline protoxins [17]. Consequently, it will not be necessary for an organism that ingests the toxins to have a high midgut pH (~ pH 10.5), for solubilization of the protoxins, and specific proteolytic enzymes, to cleave the protoxins into toxic subunits. Therefore, nontarget insects, earthworms, and higher trophic levels could be susceptible to the toxins, even though they do not have an alkaline gut pH and the appropriate proteolytic enzymes. Although specific receptors for the toxic proteins on midgut epithelium appear to be necessary for toxicity, and these are apparently present in large numbers in susceptible larvae [35], their absence in nontarget organisms has not been definitively established [17].

Despite the rapid progress being made in the genetics and molecular biology of these insecticidal proteins, not much is known about their persistence in and potential ecological effects on the environment. The present studies were conducted to determine whether the protoxins and toxins produced by *B. thuringiensis* subsp. *kurstaki*, which have antilepidopteran activity, adsorb and bind on the clay minerals, montmorillonite and kaolinite, which are present in soils and sediments, and whether adsorption and binding affect their insecticidal activity.

Materials and Methods

Bacterium and cultural conditions. *B. thuringiensis* subsp. *kurstaki* HD-1 (BGSC #4D1), obtained from Abbott Laboratories, was grown in 1-L flasks containing 300 ml of medium for 5 days with shaking (250 rpm) at 28°C until sporulation. The medium contained (per L) 3 g beef extract, 10 g tryptose (both from Difco), 5 g NaCl, and filter-sterilized (0.45 μm) 0.2 ml of 0.1 M $MnCl_2$ and 5.0 ml of 1 M $MgSO_4 \cdot 7\ H_2O$, which were aseptically added after autoclaving. The bacterium was maintained on agar (2%) slants of this medium and subcultured weekly. Dipel, a commercial mixture of cells, spores, and parasporal crystals, supplied by Abbott Laboratories, was used in some studies.

Isolation of protoxin and toxin. Three hundred milliliters of 5-day-old cultures was centrifuged at 7970 *g* (Sorvall RC2-B); GSA-rotor), and the mixture of cells, spores, and parasporal crystals was washed twice with 1 M NaCl and twice with double-distilled water (ddH_2O). The protoxin (M_r = 132 kDa) was isolated by gradient centrifugation on Renografin-76 [31]. The toxin (M_r = 66 kDa) was isolated by a method developed in this laboratory [36]: the bacterial mixture, after being washed with NaCl and ddH_2O, was extracted overnight with MOPS buffer (0.1 M 3-*N* morpholinopropanesulfonic acid, pH 7.8) containing 0.5 M dithiothreitol and 1 M KSCN; the resultant extract was dialyzed against ddH_2O, and the precipitated protein was lyophilized. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that these preparations contained primarily protoxin and toxin, respectively. Both proteins were active against the third instar larvae of *Trichoplusia ni* [36].

Adsorption studies. The lyophilized proteins were dissolved in 0.2% Na_2CO_3, and any insoluble material was discarded after centrifugation at 17,300 *g* for 20 min (Sorvall RC2-B; SS-34 rotor). The absorbance of the solutions was measured at 280 nm (A_{280}; Varian Techtron Model-635), and the protein content (based on a standard curve prepared with bovine serum albumin) was adjusted with 0.2% Na_2CO_3 to the desired concentrations for the adsorption studies. Protoxin or toxin (ranging from 0–1000 μg protein) was added to suspensions of clay (2–5 μm) containing 100–500 μg of montmorillonite or kaolinite with a mixed cation complement (Fisher Scientific Company) [15, 16] in a total volume of 1 ml. The clay–protein mixtures were rotated in text tubes (13 × 100 mm) at 40 rpm on a motorized wheel at room temperature (24° ± 2°C). The contact time between the clays and proteins, the concentrations of proteins and clays, the pH, and the temperature were varied. After adsorption, the mixtures were centrifuged at 27,000 *g*, and the A_{280} of the supernatants was measured. The difference in A_{280} of the initial protein solutions and of the supernatants after being mixed with the clays was used to calculate the amount of protein adsorbed at equilibrium on the clays [15, 16].

Desorption of the proteins from clays. After equilibrium adsorption, the clay-protein complexes were washed twice with 1 ml of

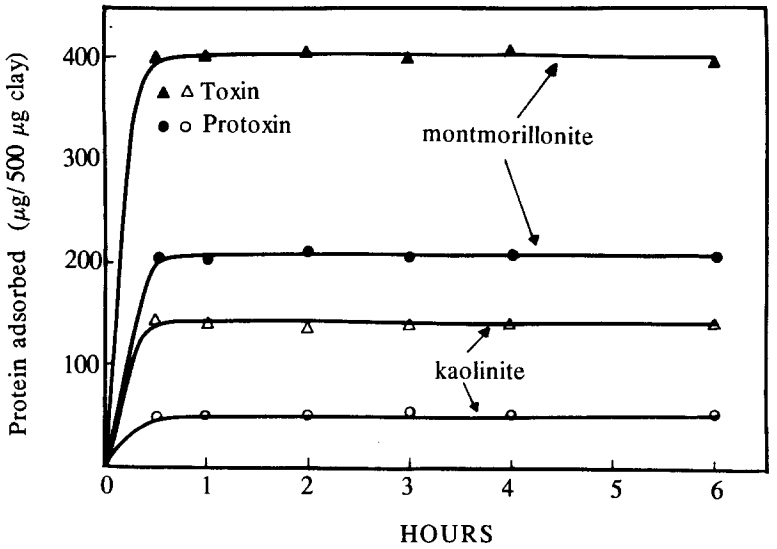

Fig. 1. Rate of adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite and kaolinite. The adsorption mixture contained 500 μg clay and approximately 1000 μg protein in a total volume of 1 ml. Means ± SEM, which are within the dimensions of the symbols.

ddH_2O and then with 1 ml of 0.2% Na_2CO_3. The supernatants were analyzed (A_{280}) after each wash for the presence of protein. In some studies, the clay pellets were extracted, after the washes with ddH_2O and Na_2CO_3, with 1 ml of Tris-HCl (0.1 M, pH 8.0) buffer containing 2% SDS, 2% β-mercaptoethanol, and 20% glycerol, and the supernatants were analyzed by SDS–PAGE [37].

Fourier-transform infrared (FT-IR) and x-ray diffraction (X-RD) analyses of the montmorillonite-protein complexes. About 30 mg of the protoxin or toxin, in 0.2% Na_2CO_3, was mixed with 20 mg of montmorillonite in a total volume of 30 ml for 30 min at room temperature on a rotating wheel (40 rpm). After adsorption, the complexes were centrifuged at 27,000 *g* for 20 min, and the pellet was washed, with centrifugation, once with 0.2% Na_2CO_3 and then twice with ddH_2O. The pellet was suspended in 2–3 ml of ddH_2O, lyophilized, and subjected to FT-IR and X-RD analyses [11].

FT-IR spectra of the montmorillonite–protein complexes were obtained with a Perkin-Elmer 1710 spectrophotometer interfaced with a microcomputer and digital plotter. The spectra of KBr pellets of the proteins and clay–protein complexes were recorded in the range of 2000–1400 cm^{-1}, with particular reference to the Amide I and II vibrations of the peptide bond.

Oriented samples for X-RD analysis were prepared by drying the montmorillonite–protein complexes, as well as the clay and proteins individually, on plastic slides. Analysis at intervals of 2θ, from 3° to 50°, was conducted at room temperature with a Philips PW 1410 diffractometer, using Co K_α radiation.

Bioassays. The insecticidal activity of the protoxin and toxin and their complexes with the clays was evaluated by Abbott Laboratories with third instar larvae of *T. ni*, as described earlier [36].

Statistics. All experiments were conducted in duplicate, and experiments were repeated several times. The data are presented as the means ± the standard error of the means ($\bar{x}$ ± SEM).

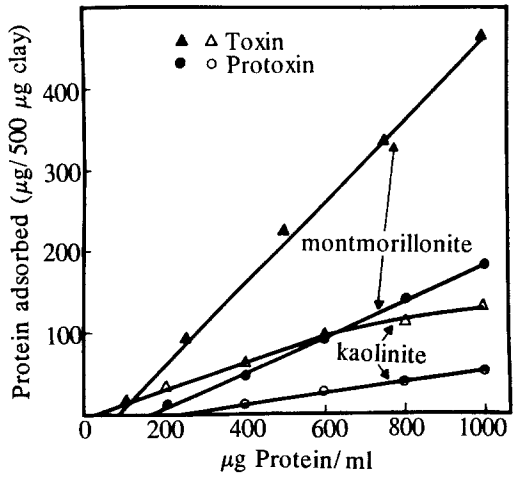

Fig. 2. Adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite and kaolinite as a function of protein concentration. The concentration of the clays was 500 μg/ml of adsorption mixture, and the contact time was 30 min. Means ± SEM, which are within the dimensions of the symbols.

Results

Contact time. Maximum adsorption of both the protoxin and toxin from *B. thuringiensis* subsp. *kurstaki* on the clay minerals occurred within 30 min (the shortest interval measured), and no significant increase in adsorption was observed to 6 h (Fig. 1). The maximum amount of protoxin adsorbed on montmorillonite and kaolinite was equivalent to 22% and 4.5% of the protein added, respectively, whereas the respective values for toxin were 38%

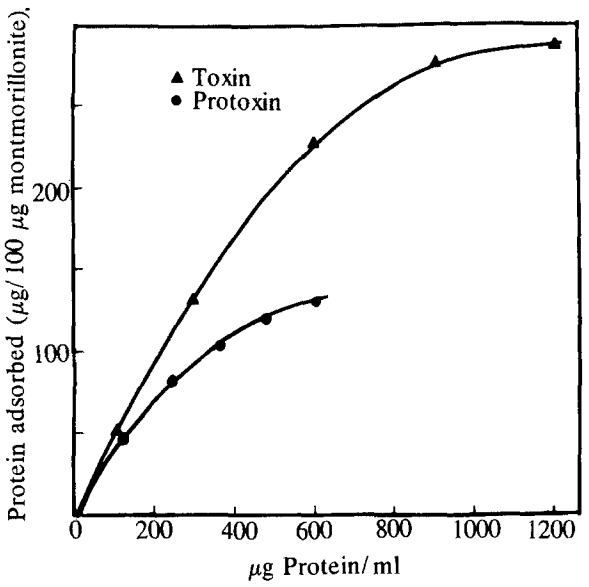

Fig. 3. Adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite as a function of protein concentration. The concentration of clay was 100 μg/ml of adsorption mixture, and the contact time was 30 min. Means ± SEM, which are within the dimensions of the symbols.

and 13.5%. Montmorillonite was a more efficient adsorbent of these proteins than was kaolinite (by a factor of ~5 for protoxin and 3 for toxin). More toxin than protoxin was adsorbed on these clay minerals (~ two-fold more on montmorillonite and three-fold more on kaolinite). When the data were expressed in terms of mol protein adsorbed/mg clay, the differences were even more pronounced: 2.99 nmol (395 μg) protoxin and 11.97 nmol (790 μg) toxin/mg montmorillonite; 0.72 nmol (95 μg) protoxin and 4.24 nmol (280 μg) toxin/mg kaolinite.

Effect of protein concentration. Adsorption of the proteins was dependent on their concentration and was essentially linear, especially on montmorillonite (Fig. 2). At the highest protein concentration used (1000 μg protein/ml), approximately 180 μg protoxin and 460 μg toxin were adsorbed on 500 μg montmorillonite, and approximately 50 μg protoxin and 130 μg toxin were adsorbed on 500 μg kaolinite. With kaolinite, adsorption, especially of the toxin, tended towards saturation, and only about 5% of the protoxin and 13% of the toxin was adsorbed at a kaolinite : protein ratio of 1 : 2.

As there was no saturation of 500 μg montmorillonite with either protoxin or toxin, the adsorption of the proteins on 100 μg montmorillonite was determined (Fig. 3). Adsorption reached saturation at approximately 600 μg protoxin and 1200 μg toxin/ml of incubation mixture, and the amount of adsorption at saturation was approximately 130 μg protoxin and 287 μg toxin/100 μg montmorillonite, which was equivalent to the adsorption of 22% of protoxin added at a clay : protein ratio of 1 : 6 and of 24% of toxin added at a ratio of 1 : 12.

Effect of clay concentration. When the protein concentration was maintained constant at 1000 μg/ml and the concentration of the clays was varied, the relative adsorption of both the protoxin and toxin decreased as the clay concentration was increased (Fig. 4). Significantly more protein was again adsorbed on montmorillonite than on kaolinite (Fig. 5).

Effect of pH. Adsorption of the protoxin and toxin on montmorillonite decreased as the pH increased from 4.4–10.0 (Fig. 6). The decrease was linear for the adsorption of the toxin, but the decrease for the adsorption of the protoxin was gradual up to pH 8.0 and then decreased sharply. When compared with the amounts adsorbed at pH 4.4, only 56% of the protoxin and 50% of the toxin were adsorbed at pH 10.0.

Adsorption of the proteins on kaolinite also decreased with an increase in pH (Fig. 7). However, in contrast to the results obtained with montmorillonite, adsorption on kaolinite was also dependent on the ionic nature of the buffers used to obtain the desired pH. Maximum adsorption of toxin on kaolinite occurred in acetate buffer at pH 4.4 and was significantly reduced at pH 5.6. However, in Tris buffer, adsorption of the toxin was significantly higher at pH 7.2 than at pH 5.6 in acetate buffer and then decreased at higher pH. Adsorption was also higher at pH 5.6 and 6.6 in phosphate buffer, at pH 8.4 in Tris buffer, and at pH 10 in HCO_3^-/CO_2^{2-} than at pH 5.6 in acetate buffer. Similar patterns were obtained with the protoxin. The adsorption of these proteins on kaolinite was apparently not only dependent on pH but also on the ionic nature and strength of the suspending medium. Nevertheless, with any one type of buffer, adsorption of the proteins on kaolinite decreased with increased pH, similar to what occurred with montmorillonite.

Effect of temperature. The adsorption of the protoxin and toxin on montmorillonite and kaolinite was not significantly affected by temperatures between 7° and 50°C (Table 1).

Desorption of adsorbed proteins. More than 90% of

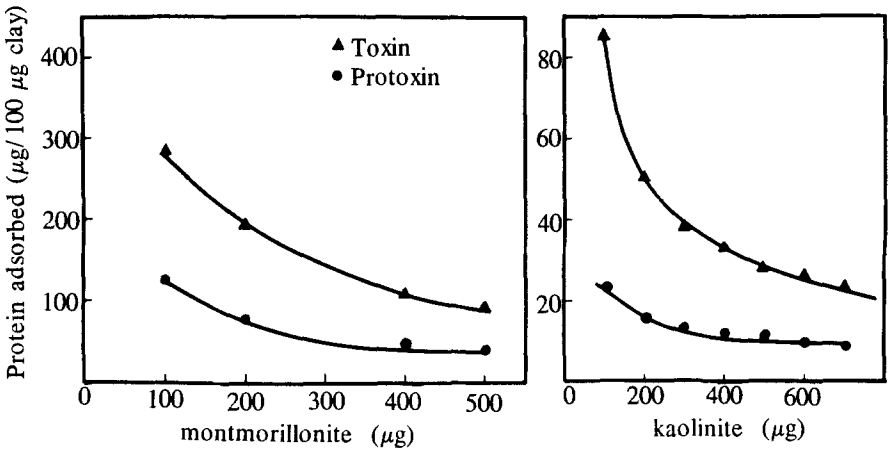

Fig. 4. Adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite and kaolinite as a function of clay concentration and expressed on the basis of the amount of protein adsorbed/100 μg clay (see Fig. 5). Means ± SEM, which are within the dimensions of the symbols.

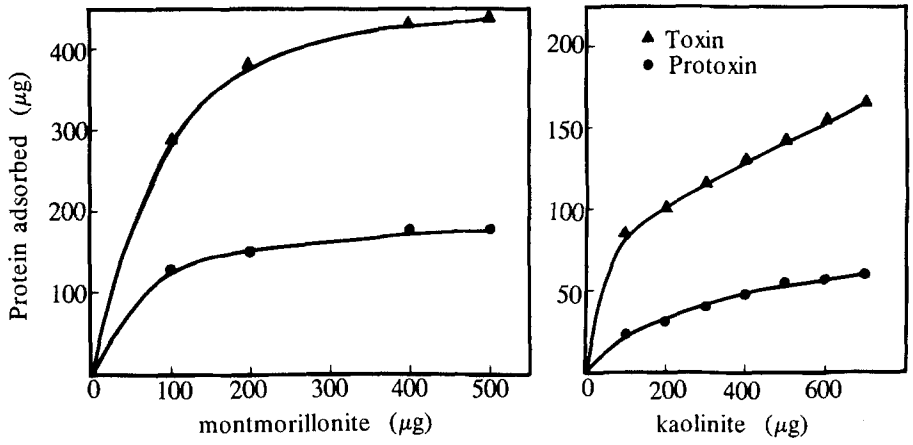

Fig. 5. Adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite and kaolinite as a function of clay concentration. The concentration of the proteins was constant at approximately 1000 μg/ml of adsorption mixture, and the contact time was 30 min. Means ± SEM, which are within the dimensions of the symbols.

the protoxin and toxin adsorbed on kaolinite was desorbed by two to three washings with ddH_2O and 0.2% Na_2CO_3 (Table 2). However, the proteins adsorbed on montmorillonite could not be desorbed with either water or 0.2% Na_2CO_3, but they could be removed with 0.1 M Tris buffer (pH 8.0) containing 2% SDS, 2% β-mercaptoethanol, and 20% glycerol. The amounts of the proteins desorbed from montmorillonite could not be determined spectrophotometrically, as the result of the presence of interfering substances in the extracts and of the small amounts of proteins desorbed from the small quantities of complexes used in the adsorption/desorption studies. However, the molecular mass of the protoxin and the toxin was not altered by adsorption on montmorillonite, as shown by SDS-PAGE performed on the washings of the clay-protein complexes (Fig. 8).

FT-IR and X-RD. Inasmuch as the protoxin and toxin were bound on montmorillonite but not on kaolinite, the montmorillonite–protein complexes were subjected to FT-IR and X-RD analyses. Only minor shifts occurred in the relative frequencies of the Amide I and II bands of the proteins as the result of their binding on montmorillonite (Table 3). The results of X-RD analysis indicated that no significant intercalation of the clay by the proteins occurred. For example, binding of the protoxin caused an increase in the d_{001} spacing from 1.30–1.58 nm, and binding of the toxin resulted in only a broadening of the 1.30 nm *d* spacing.

Insecticidal activity. Although the quantities of clay–protein complexes available were insufficient to obtain LC_{50} values, the complexes were toxic to the third instar larvae of *T. ni*.

Discussion

To evaluate any potential adverse impacts on the environment of the release of organisms that have

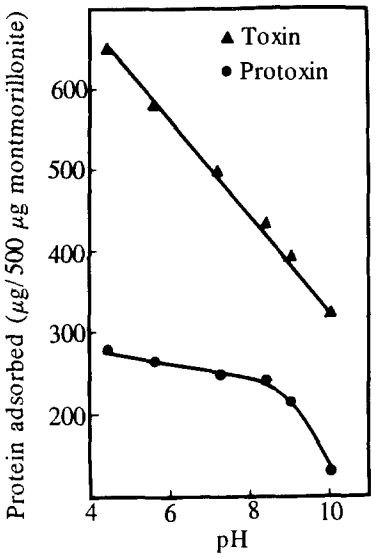

Fig. 6. Effect of pH on the adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite. The adsorption mixture contained 500 μg clay and approximately 1000 μg protein in a total volume of 1 ml, and the contact time was 30 min. Acetate and Tris buffers were used to obtain the required pH. Means ± SEM, which are within the dimensions of the symbols.

Table 1. Effect of temperature on the adsorption of the delta-endotoxins of *Bacillus thuringiensis* subsp. *kurstaki* on montmorillonite and kaolinite

Clay[a]	Temperature (°C)	Protoxin (μg protein adsorbed/ 500 μg clay)[b]	Toxin
Montmorillonite	7	177.5 ± 2.5	395.0 ± 5.0
	25	181.5 ± 1.0	372.5 ± 7.5
	37	177.5 ± 7.5	392.5 ± 2.5
	50	179.0 ± 1.0	383.5 ± 3.5
Kaolinite	7	52.0 ± 2.0	153.5 ± 4.0
	25	56.0 ± 1.5	150.0 ± 3.5
	37	55.5 ± 1.0	157.0 ± 2.0
	50	51.5 ± 2.5	155.5 ± 2.5

[a] The incubation system contained 500 μg clay and 1000 μg protein in a total volume of 1 ml. The contact time was 30 min.
[b] $\bar{x}$ ± SEM.

been genetically modified to contain the protoxin or toxin genes of subspecies of *B. thuringiensis* (e.g., transgenic species of *Pseudomonas* and *Clavibacter* and of plants), the adsorption and binding of these toxins on clay minerals and other soil particulates and the effects of such surface interactions on the activity and persistence of the toxins must be established. The adsorption and binding of enzymes and other proteins, as well as of viruses, on clays and the effects of such surface interactions on the resistance to biodegradation and on the activity of the proteinaceous materials have been studied [15, 30, 32, 33]. There have been no comparable studies with the toxins of *B. thuringiensis*.

The protoxin and toxin proteins from *B. thuringiensis* subsp. *kurstaki* were readily adsorbed on montmorillonite and kaolinite, similar to the adsorption of many other proteins. Adsorption was complete in less than 30 min, was independent of temperature, and was greater on montmorillonite than on kaolinite. The rapid adsorption of these proteins on the clays suggests that toxins released from transgenic plant or microbial biomass would be free and available for microbial degradation in soil for only a short time. Studies with other proteins have indicated that proteins bound on clay minerals become resistant to such degradation [32, 33]. The microbial utilization of these protoxin and toxin proteins, both free and bound, is under investigation. Maximum adsorption (i.e., a plateau) of the protoxin and toxin on 500 μg kaolinite occurred at a clay: protein ratio of 1 : 2, but no such plateau occurred with montmorillonite at this ratio. However, when the concentration of montmorillonite was reduced from 500 μg to 100 μg, a plateau in adsorption occurred at a montmorillonite : protein ratio of 1 : 6 and 1 : 12 for protoxin and toxin, respectively. The larger amounts of the proteins required to saturate montmorillonite than kaolinite was probably a reflection of the significantly higher specific surface area and cation-exchange capacity of montmorillonite [33]. The maximum amount of added protein adsorbed was approximately 22% for protoxin and 24% for toxin, indicating that only a relatively small portion of the proteins was capable of adsorbing on the clays. No adsorption of either protein on the clays was observed at low concentrations (50 μg/ml) of the proteins.

The amount of proteins bound on clays generally appears to increase as the M_r of the proteins increases [15, 33]. In contrast, the toxin, with an M_r smaller than that of the protoxin, bound more on the clays. This may have resulted from more binding sites on the toxin than on the protoxin, and fewer molecules of the toxin were necessary to saturate binding sites on the clay, as suggested for other proteins [15, 33]. The toxin of *B. thuringiensis* subsp. *kurstaki* is derived from the N-terminal amino end of the protoxin [5, 17], and the enzymatic cleavage of the larger unit into the smaller toxin may have resulted in the exposure of additional binding sites.

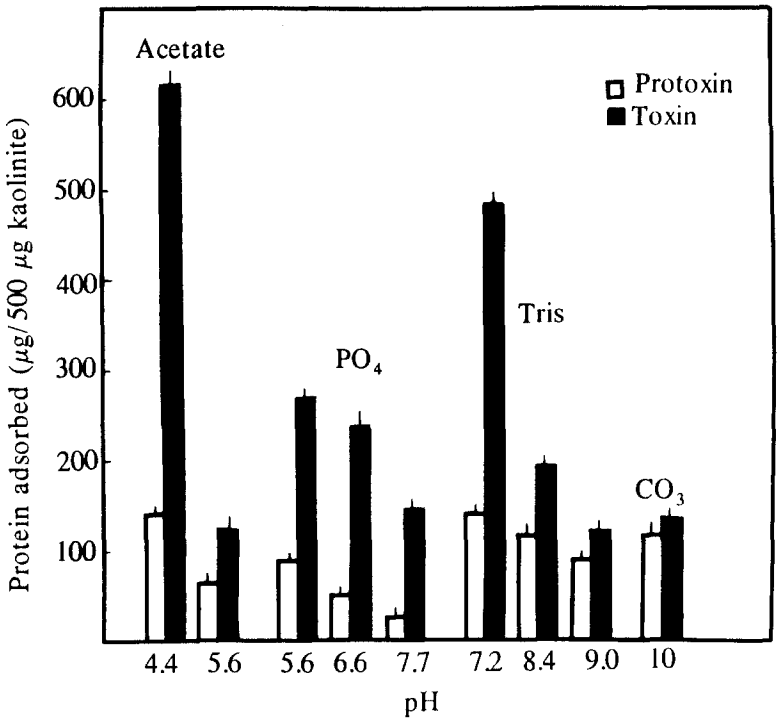

Fig. 7. Effect of pH on the adsorption of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* on kaolinite. The adsorption mixture contained 500 μg clay and approximately 1000 μg protein in a total volume of 1 ml, and the contact time was 30 min. Acetate, phosphate, Tris, or HCO_3^-/CO_3^{2-} buffers were used, as indicated. Means ± SEM.

Table 2. Desorption of the delta-endotoxins of *Bacillus thuringiensis* subsp. *kurstaki* adsorbed on kaolinite

Washings[a] (number)	Protoxin (μg protein desorbed/500 μg clay)[b]	Toxin
1	34.5 ± 1.8	117.6 ± 3.2
2	9.0 ± 0.4	18.5 ± 1.6
3	Traces	Traces

[a] After the proteins were adsorbed on the clay, the supernatant was removed by centrifugation, and the clay pellet was washed with 1 ml of ddH_2O and 1 ml of 0.2% Na_2CO_3.
[b] $\bar{x}$ ± SEM.

The protoxin and toxin bound on montmorillonite could be removed with Tris buffer containing SDS but not with H_2O and Na_2CO_3, indicating that the proteins were bound very tightly on this clay, whereas more than 90% of the proteins adsorbed on kaolinite were desorbed with 0.2% Na_2CO_3 and ddH_2O. The greater and stronger adsorption of the protoxin and toxin on montmorillonite than on kaolinite was in agreement with observations made with other proteins [7, 30]; for example, phenol oxidases were bound more strongly on montmorillonite than on kaolinite, and their activity was inhibited by binding on the former but not on the latter clay [7]. The sorption of some enzymes on soil colloids is irreversible, and they can not be extracted in an active form [22]. It is not known whether the protoxin and toxin desorbed with Tris–SDS retained their antilepidopteran activity, as insufficient protein was recovered for bioassay. However, they appeared to be structurally unaltered, as shown by SDS–PAGE after desorption from the clay, and the minor shifts observed in the Amide I and II bands indicated that no significant modifications occurred in the structure of the proteins when bound on montmorillonite.

The pH had a significant effect on the adsorption of the toxins on both clays, as adsorption was highest at lower pH and decreased as the pH was increased. The isoelectric points (pI) of the protoxin and toxin have been reported to be approximately pH 4.4 and 5.5, respectively [2, 5], and adsorption was maximum near these pH values. At pH values near the pI, a net neutral protein will encounter minimal repulsive forces, which can result in maximum collisions with charged clay surfaces and, hence, in increased adsorption [23, 33]. The adsorption of phenol oxidases was also highest at pH values near their pI, and ionic bonds between the net neutral or positively charged proteins and the net negatively charged surfaces of the clay minerals may have been involved [7], at least in the initial phases of adsorption [33].

Although adsorption of the proteins on kaolinite

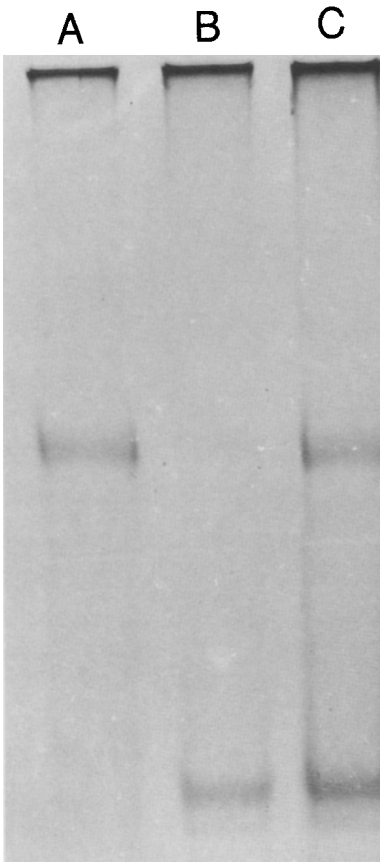

Fig. 8. SDS–PAGE (7.5% gel) of the protoxins and toxins of *Bacillus thuringiensis* subsp. *kurstaki* desorbed by 0.1 M Tris–SDS buffer (pH 8.0) from montmorillonite. Lanes: (A) Tris–SDS wash of montmorillonite after adsorption of protoxin; (B) Tris–SDS wash of montmorillonite after adsorption of toxin; and (C) reference protoxin and toxin. See text for details.

Table 3. Frequencies (cm^{-1}) of the infrared bands (Amide I and II) of the delta-endotoxins of *Bacillus thuringiensis* subsp. *kurstaki* alone and bound on montmorillonite[a]

Sample	Amide I	Amide II
Protoxin	1657	1546
Protoxin–montmorillonite	1652	1541
Toxin	1651	1537
Toxin–montmorillonite	1658	1537

[a] Difference spectra obtained by FT-IR spectrometry.

was also greatest at low pH, the amounts of the proteins adsorbed differed with the buffer used to obtain the desired pH. Consequently, not only the charges on the proteins and the clay but also the ionic nature of the buffers appeared to be important in the adsorption of these proteins on kaolinite. No such effect of buffers was observed with montmorillonite.

Although many proteins when bound on clays expand the clays, especially 2:1 Si:Al swelling clays, such as montmorillonite [16, 33], the protoxin and toxin caused no significant expansion. This apparent lack of significant intercalation may have been the result of the large size of the proteins, as suggested with catalase (M_r = 250 kDa), the binding of which caused no expansion, even though large quantities were bound on montmorillonite [15, 16, 33].

The results of these studies indicated that the antilepidopteran protoxins and toxins of *B. thuringiensis* subsp. *kurstaki* adsorb on clay minerals and that they bind more tightly on montmorillonite than on kaolinite. This binding, however, did not appear to alter the structure of the proteins or to eliminate their insecticidal activity. The resistance of the proteins adsorbed or bound on clay minerals to microbial degradation is under investigation. These results have relevance to the potential risks associated with the release to the environment of transgenic bacteria and plants containing toxin genes, especially truncated genes, from subspecies of *B. thuringiensis*.

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