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Adsorption of Cry1Ab Protein Isolated from *Bt* Transgenic Rice on Bentone, Kaolin, Humic Acids, and Soils

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Adsorption on a soil matrix of the insecticidal protein from *Bacillus thuringiensis* (*Bt*) transgenic plants affects their accumulation and release and, hence, bioavailability in soil. Cry1Ab protein isolated from *Bt* transgenic rice was used to evaluate the adsorption and desorption on bentone, kaolin, and humic acids (HAs). The adsorption equilibrium of Cry1Ab protein was reached within 1−2 h for bentone and kaolin and within 4−8 h for HAs. The adsorption isotherms were better described by linear expressions ($R^2 \geq 0.973$) rather than by the Freundlich model. No saturation was observed, even at the maximum concentration used (3.71 μg mL^{-1}). The adsorbed protein was not easily desorbed at the used protein concentrations (0.18−3.71 μg mL^{-1}); more than 50−70%, 70−80%, and 90% of the adsorbed protein remained on HAs, kaolin, and bentone, respectively, after washing with water. Adsorption and desorption of the Cry1Ab protein were further studied using five soils, and the isotherms were also well-described by linear equations ($p < 0.05$). Adsorption of the Cry1Ab protein on soils was positively related to the soil organic matter content.

KEYWORDS: Cry1Ab protein; *Bt* transgenic rice; bentone; kaolin; humic acids; soil

INTRODUCTION

Over the past decade, genetically modified (GM) crops have been adopted in 23 countries covering an area of more than 114.3 million hectares with an annual growth rate of over 10% (*1*). A recent survey in China demonstrated the agricultural benefits of planting pest-resistant transgenic plants, including reduction of pesticide use and human poisonings and increase of net incomes for farmers (*2*). However, the ecological effects of GM crops remain poorly understood and, hence, are often controversial.

Bacillus thuringiensis (*Bt*) is the major source of genes for the expression of lepidopteran insect resistance in transgenic plants. Truncated forms of the genes that code for Bt toxins have been genetically engineered into plants. The release of *Bt* toxin from *Bt* transgenic plants into the environment has drawn wide attention due to the potential environmental effects (*3–6*). If the toxins accumulate significantly in agroecosystems, two potential effects may occur. The accumulation may enhance the selection and enrichment of toxin-resistant target insects. The accumulated toxin may pose possible risks to nontarget insects and organisms in higher and lower trophic levels. Accumulation of *Bt* toxins in the environment depends closely on processes such as adsorption to soil or soil components and bioavailability. The environmental behavior and fate of the insecticidal toxin produced by *B. thuringiensis* ssp. *kurstaki* and from *Bt* corn or *Bt* cotton, and the bioavailability of soil-bound toxin residues, have been studied by various researchers (*7–11*). Stotzky and co-workers verified that the toxin from *Bt* ssp. *kurstaki* was easily adsorbed and bound tightly to montmorillonite, kaolinite, and humic acids (*12–14*). The bound toxin retained its insecticidal properties but was protected against biodegradation in various soils for at least 180 d (*15–18*). Zhou et al. (*19*) reported that adsorption of the protein from *B. thuringiensis* ssp. *kurstaki* on montmorillonite, kaolinite, silica, goethite, and a red soil followed the order montmorillonite > soil > goethite > kaolinite > silica.

To improve expression of toxin genes, the genes are often artificially modified, which may result in *Bt* proteins with different chemical structures and properties. Most studies to date have used plant materials or the Cry1Ab protein produced by *B. thuringiensis* instead of free proteins isolated from *Bt* transgenic plants. If differences exist between the expression of the *cry1Ab* gene and crystallized proteins from *B. thuringiensis*, these studies might not reflect the actual environmental

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behavior and fate of Cry1Ab protein from transgenic plants. Due to the low expression of *Bt* proteins and high difficulty in extraction of toxin proteins (*7–9, 11–13, 20–23*), till now, there have been few studies on the environmental behavior and fate of free Cry1Ab protein produced by *Bt* transgenic plants.

The present study was undertaken to investigate adsorption and desorption of Cry1Ab protein isolated from *Bt* transgenic plants on the soil matrix (bentone, kaolin, and humic acids) and well-characterized soils under laboratory conditions. Results from this study are expected to improve the understanding of the environmental behavior of toxin protein released from *Bt* transgenic plants and the risks derived from such practices as returning *Bt* rice straw to the field as a soil amendment after harvest.

MATERIALS AND METHODS

Purification of Cry1Ab Protein from KMD Rice. Straws of *Bt* transgenic rice KMD and nontransgenic rice (wild-type XS11) were harvested at the booting stage when the content of expressed *Bt* toxin proteins in KMD was expected to reach its highest level. KMD straw was treated with liquid nitrogen and ground in a Udy cyclone mill (0.3 mm; Udy Corp., Fort Collins, CO), and the resulting straw powder was stored at 4 °C. Cry1Ab protein from *Bt* transgenic rice was purified as described by Wu and Ye (*24*) and determined by ELISA (*25, 26*). Crude extracts were obtained after freeze-drying, ammonium sulfate precipitation, desalinization, ultrafiltration, and again freeze-drying, followed by further separation on DEAE Sephadex A-50 and Sephadex G-150 columns. The purity (greater than 80%) and insecticidal activity of the isolated protein were analyzed according to Wu et al. (*24*). The Cry1Ab protein has a molecular weight of 66 000 as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and consists of 618 amino acid residues (*27*).

Preparation of Adsorbents. *Extraction, Purification, and Characterization of Humic Acids.* The HA was obtained from the topsoil of fluvio-marine yellow loamy soil (S_3) at the Agricultural Experiment Station next to the Huajiachi Campus, Zhejiang University, in Hangzhou, China. The HA was extracted from the soil and purified as described by Schnitzer and Preston (*28*) and Crecchio and Stotzky (*13*). The HA was washed for 1 h with 0.003 mol L^{-1} HCl (pH 2.5) to remove soluble potassium that may have remained from the clay removal step. The HA was centrifuged, dried, and ground with a mortar and pestle.

The elemental composition of HA was determined with a Carlo Erba EA 1110 elemental analyzer (CE Instruments, Carloerba, Milan, Italy) and ICP-MS spectrometer (Agilent 7500a, Agilent Technologies, Palo Alto, CA). The total acidity and the content of carboxyl groups were determined by titration with $Ba(OH)_2$ and the calcium acetate exchange method, respectively (*29*). The content of phenolic hydroxyl groups was calculated as the difference between the total acidity and the content of carboxyl groups. The degree of polymerization was evaluated by the ratio of the absorbances at 464 and 665 nm (E4/E6) with a UV-300 spectrophotometer (Thermo Electron, Cambridge, U.K.). The water content was determined by drying at 105 °C. and the ash content was determined by combustion at 550 °C (*30*). The cation exchange capacity (CEC) was determined by the ammonium acetate exchange method (*31*). The organic matter (OM) content was determined with a TOC analyzer (TOC-500, Shimadzu, Kyoto, Japan). The specific surface area (SSA) and basal spacing were determined with an autochemisorb system (Coulter Omnisorp 100CX, Florida). Some characteristics of the HAs are listed in **Table 1**.

Bentone, Kaolin, and Soils. Bentone is composed mainly of montmorillonite, a clay mineral of the smectite group. Bentone and kaolin were obtained from Shanghai Wusi Chemical Co. (Shanghai, China) and Advanced Technology and Industrial Co. (Hong Kong, China), respectively. The five soils used were collected from the surface layer (0−15 cm) in fields in Zhejiang province where transgenic rice had never been planted. The soils were air-dried, crushed, passed through a 1 mm sieve, and homogenized before use. Some basic physicochemical characteristics of these adsorbents are shown in **Tables 2** and **3**.

Table 1. Some Characteristics of the Humic Acids Used

water content (%)	13.1
ash content (%)	15.7
total acidity (cmol kg^{-1})	540
acidic group content (cmol kg^{-1})	154
phenolic group content (cmol kg^{-1})	386
CEC (cmol kg^{-1})	87.8
E4/E6[a]	5.7
basal spacing (nm)	3−10
SSA (m^2 g^{-1})	23.2
[C] (%)	39.8
[H] (%)	4.0
[N] (%)	3.6
[S] (%)	0.3
[O] (%)	52.0

[a] Ratio of absorbances at wavelengths of 465 and 665 nm.

Adsorption and Desorption on bentone, kaolin, or HAs. *Equilibrium Experiment.* An aliquot of 1.0 mL of Cry1Ab protein solution (2.56 μg mL^{-1}) in phosphate buffer (pH 6.0) was mixed with 50 mg of bentone, kaolin, or HAs and rotated for 24 h at 100 rpm at room temperature. Three replicates were used for each treatment. Samples were removed after 1, 2, 4, 8, and 12 h, and the suspensions were centrifuged for 10 min at 2417*g*. The concentration of Cry1Ab protein in the supernatant was determined by ELISA with a Quantiplate kit (AP 003, Envirologix, Portland, ME) for Cry1Ab/Cry1Ac at 450 nm (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Equilibrium adsorption was reached when the amount of the Cry1Ab protein in the supernatant remained constant with time. The amount of protein adsorbed at equilibrium was calculated from the difference between the initial and equilibrated solutions.

Adsorption Experiment. Sample tubes with a final volume of 1.0 mL containing the Cry1Ab protein at 0.18, 0.30, 0.60, 1.22, 2.58, and 3.71 μg mL^{-1} were prepared. Three replicates of each treatment were mixed with 50 mg of bentone, kaolin, or HAs and shaken at 100 rpm for 1 h at 25 ± 1 °C, followed by centrifugation at 9668*g* for 5 min. The amount of Cry1Ab protein in the supernatants was determined by ELISA. The difference between the amounts of protein added and detected in the supernatants was used to calculate the amount of Cry1Ab protein adsorbed on bentone, kaolin, or HAs.

Desorption Experiment. The protein−adsorbent complexes were transferred to clean 5 mL centrifuge tubes with lids, and 1.0 mL of doubly distilled water (ddH_2O) was added. The sample tubes were shaken for 1 h at 25 ± 1 °C. After centrifugation at 9668*g* for 5 min, the amount of protein in the supernatants was analyzed and the amounts desorbed were calculated as above.

Adsorption and Desorption on Soils. An aliquot of 10 g of each soil was placed in 250 mL conical flasks with lids, and 50 mL portions of protein solutions with concentrations of 0.13, 0.21, 0.25, 0.52, and 0.78 μg mL^{-1} were added. The soil−protein solution mixtures were rotated for 24 h at 100 rpm at 25 ± 1 °C, and the suspensions were centrifuged for 10 min at 2417*g*. The concentration of Cry1Ab protein in the supernatants was similarly determined by ELISA. The amounts of the protein adsorbed on the soils at equilibrium were calculated from the amounts of protein detected and added initially, and equilibrium adsorption isotherms were constructed.

The soil pellet was transferred to clean conical flasks with lids, and 50 mL of ddH_2O was added. The mixture was resuspended and shaken for 24 h at 25 ± 1 °C, and after centrifugation, the concentration of Cry1Ab protein in the supernatant was determined and the remaining fraction was calculated from the initially adsorbed and the desorbed fractions.

Statistical Analysis. All measurements included three replicates, and the data were expressed as geometric mean values and standard errors of the means (means ± SEM). Significance between treatments was determined by Student's t-test, and one-way analysis of variance (ANOVA) was carried out for regression analysis using Origin 6.0 (Microcal Software, Northampton, MA).

Table 2. Some Physicochemical Properties of the Soils Used

property	S_1 (paddy field on quaternary red soil)	S_2 (paddy field on red sandstone soil)	S_3 (fluvio-marine yellow loamy soil)	S_4 (paddy field on pale muddy soil)	S_5 (coastal saline soil)
pH (H_2O)	4.16	4.55	7.02	5.81	8.84
OM content (g kg^{-1})	8.40	6.53	30.50	25.11	9.50
total [N] (%)	0.34	0.28	2.90	2.70	1.80
CEC (cmol kg^{-1})	6.62	4.53	10.83	8.15	10.17
clay content (%)	39.0	17.2	8.0	21.7	24.3
silt content (%)	41.1	7.4	71.2	60.1	71.1
sand content (%)	19.9	75.4	20.8	18.2	4.6
[P] (mg kg^{-1})	3.21	2.71	25.20	8.80	10.80
[K] (mg kg^{-1})	4650	8823	8122	8563	9768
[Mg] (mg kg^{-1})	65.43	110.59	572.47	215.95	903.08
[Ca] (mg kg^{-1})	54.45	36.60	4338	267.83	10332
[Fe] (mg kg^{-1})	20895	23604	12837	17726	23604
[Al] (mg kg^{-1})	3345	2998	7420	3766	11920
[Mn] (mg kg^{-1})	461.61	197.72	270.20	218.9	539.82
[Zn] (mg kg^{-1})	54.79	50.54	64.50	90.83	72.84
[Cu] (mg kg^{-1})	24.33	27.62	18.18	37.31	25.48

Table 3. Basic Characteristics of Clay Minerals Used in This Study

	elemental composition (%)											
clay mineral	C	H	N	Al	Na	Mg	K	Fe	Zn	SSA[a] (m^2 g^{-1})	basal spacing (nm)	CEC (cmol kg^{-1})
bentone	28.13	7.33	1.38	8.39	0.02	0.23	0	0.91	0	26.77	3−20	41.2
kaolin	0.60	0.63	0	4.27	0.05	0	0.14	0.10	0.04	19.66	2−10	39.3

[a] SSA = specific surface area.

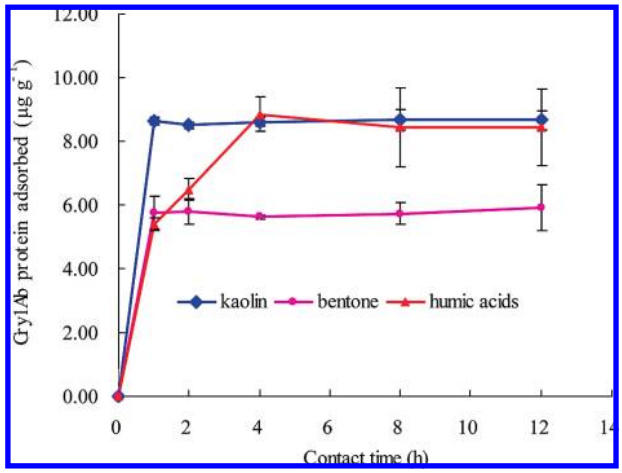

Figure 1. Effect of the contact time on adsorption of Cry1Ab protein isolated from *Bt* transgenic rice on bentone, kaolin, and humic acids.

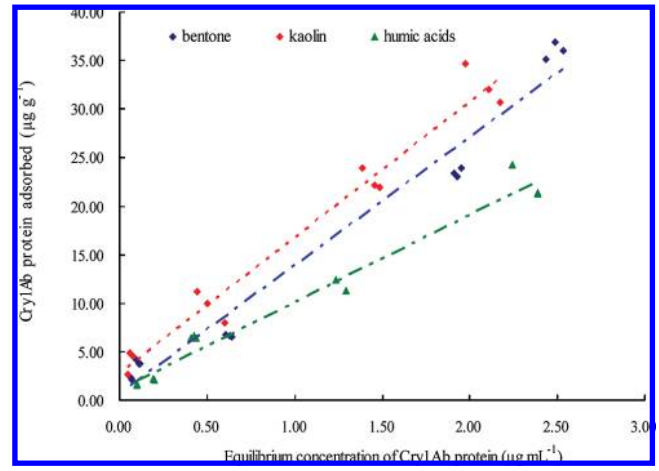

Figure 2. Adsorption of Cry1Ab protein on bentone, kaolin, and humic acids.

RESULTS AND DISCUSSION

Adsorption Kinetics. The Cry1Ab protein was rapidly adsorbed on HAs, bentone, and kaolin. Equilibrium adsorption was reached within 1−2 h for bentone and kaolin and within 4−8 h for HAs (**Figure 1**). At equilibrium, the average amounts of Cry1Ab protein adsorbed were 8.62 μg g^{-1} for kaolin, 8.65 μg g^{-1} for HA, and 5.77 μg g^{-1} for bentone, respectively. These results were consistent in general with those of Crecchio and Stotzky (*13*), who showed that adsorption to HAs extracted from soils was reached after 1−2 h, while somewhat inconsistent with those of Venkateswerlu and Stotzky (*12*), who found that maximum adsorption of *Bt* toxin on the clay minerals occurred within 30 min.

Adsorption Isotherms. The adsorption of Cry1Ab protein on bentone, kaolin, and HAs increased as a function of the equilibrium concentrations of protein over the range of 0.18−3.71 μg mL^{-1} (**Figure 2**). The linear and Freundlich regression results were utilized for fitting the adsorption data (**Table 4**). Adsorption of the protein on the clay minerals and HAs was expressed much better by linear relationships than by the Freundlich model, with the adsorption coefficient, $K_{d,ads}$, ranging from 8.99 to 13.94, $R^2 \geq 0.973$, and F values from 466.1 to 484.6 ($p < 0.001$) for the linear relationships. Adsorption at equilibrium of Cry1Ab protein was consistently greater on the two clay minerals than on HAs. No saturation plateau was reached even at the highest Cry1Ab protein concentration (3.71 μg mL^{-1}) used. These results suggest that in soils with high contents of organic matter and clay, Cry1Ab protein may be strongly adsorbed and is highly immobile. Stotzky and coworkers first showed that *Bt* protein from *B. thuringiensis* ssp. *kurstaki* and ssp. *tenebrionis* was adsorbed rapidly to soil components including montmorillonite, kaolinite, and humic acids (*12–14, 20*). Zhou et al. (*19*) reported that adsorption of the protein from *B. thuringiensis* ssp. *kurstaki* on montmoril-

Table 4. Adsorption and Desorption Isotherm Parameters of Cry1Ab Protein on the Tested Adsorbents

	adsorption					desorption				
	linear model		Freundlich model			linear model		Freundlich model		
adsorbent	$K_{d,ads}$	R^2	log K_F	n	R^2	$K_{d,des}$	R^2	log K_F	n	R^2 [a]
HAs	8.99	0.973	3.43	0.847	0.975	16.33	0.953	2.13	0.642	0.905
bentone	13.16	0.973	4.85	0.688	0.933	288.6	0.950	2.97	0.711	0.883
kaolin	13.94	0.974	5.77	0.585	0.95	42.25	0.963	2.20	0.826	0.917
S_1	2.47	0.937	1.75	0.498	0.836	48.99	0.924	2.12	0.770	0.921
S_2	4.84	0.922	1.41	0.713	0.803	32.30	0.940	2.08	0.732	0.955
S_3	1.37	0.935	1.21	0.620	0.86	80.31	0.939	2.40	0.723	0.950
S_4	1.94	0.939	1.41	0.589	0.877	63.51	0.924	2.39	0.660	0.913
S_5	3.44	0.937	1.89	0.505	0.953	61.91	0.986	2.31	0.721	0.953

[a] R^2 is the correlation coefficient of the model.

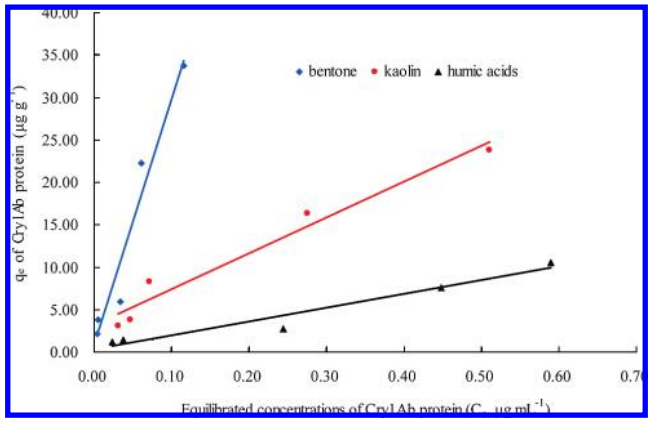

Figure 3. Desorption isotherms of Cry1Ab protein on bentone, kaolin, and humic acids. q_e is the equilibrium concentration of the protein remaining on the adsorbent after desorption. C_e is the desorption-equilibrated concentration of the protein in the aqueous phase.

lonite, kaolinite, silica, goethite, and a red soil followed the order montmorillonite > red soil > goethite > kaolinite > silica.

In the present study, adsorption of Cry1Ab protein was greater on kaolin than on bentone, which was inconsistent with the results of Stotzky's and Zhou's studies. Stotzky and co-workers (*8, 12, 14, 15, 20*) found that the protoxin and toxin protein of *B. thuringiensis* ssp. *kurstaki* toxin were readily adsorbed and bound on clay minerals and significantly more protein was adsorbed and bound on montmorillionite than on kaolinite. The specific surface areas (**Tables 1** and **3**) suggested that the bentone used in this study should be a more active clay mineral than kaolin and, thus, have a greater potential for adsorbing Cry1Ab protein (*32*). The relatively greater adsorption on kaolin than on bentone may, therefore, be attributed to the difference in the CEC and the fact that there are polar locations in the Cry1Ab protein. This observation was opposite the earlier results reported for *Bt* proteins from *B. thuringiensis* (*12, 19*).

Desorption of Cry1Ab Protein from Clay Minerals and Humic Acids. Figure 3 shows the desorption isotherms of Cry1Ab protein from bentone, kaolin, and HAs as a function of adsorption-equilibrated concentrations (C_e) of the protein in the aqueous phase. Desorption of Cry1Ab from HAs was more rapid than that from bentone or kaolin. However, more than 50−70%, 70−80%, and >90% of the adsorbed protein, compared with applied concentrations of the protein (0.18−3.71 μg mL^{-1}), were bound on HAs, kaolin, and bentone, respectively, after washing with dd H_2O. Desorption isotherms of the protein as a function of desorption-equilibrated concentrations in the aqueous phase (C_e) were well described with linear equations, with the desorption coefficients, $K_{d,des}$, ranging from 16.33 to 288.6 and F values from 55.2 to 79.3 ($p < 0.005$, $R^2 \geq 0.950$) (**Table 4**). On the basis of the $K_{d,des}$ values, desorption of the protein from the various adsorbents followed the order HA > kaolin ≫ bentone. The apparent adsorption−desorption hysteresis was quantified for each adsorbent−solute system using the hysteresis index (HI) defined by Huang et al. (*33*). The HI was calculated from the model HI = $(q_e^d - q_e^s)/q_e^s||_{T,C_e}$, where q_e^s and q_e^d are solid-phase solute concentrations for the single-cycle sorption and desorption experiments, respectively, and the subscripts T and C_e specify conditions of constant temperature and residual solution phase concentration (*33*). The HI was 1.62 for humic acids, 0.30 for kaolin, and 0.06 for bentone, respectively. Therefore, no hysteresis was found in desorption of the protein from HA. However, there was significant hysteresis in the desorption of Cry1Ab protein from bentone and kaolin. The results of the desorption studies further demonstrated that the Cry1Ab protein isolated from *Bt* transgenic rice was adsorbed more strongly on clay minerals, especially on bentone than on HAs, which was in agreement with the results of Stotzky and co-workers. Tapp et al. (*20*) reported that only 10% of the adsorbed protein was desorbed by ddH_2O and that clay minerals irreversibly adsorbed *Bt* protein. Crecchio and Stotzky (*13*) found that, after two washings, no additional toxin from *B. thuringiensis* ssp. *kurstaki* was desorbed from HAs derived from four soils. They also reported that the toxin from *B. thuringiensis* ssp. *kurstaki* bound rapidly and strongly on complexes of montmorillonite−humic acid−aluminum hydroxypolymers and was resistant to degradation by soil microbes, although some insecticidal activity was retained (*14*). Wu (*27*) found that Cry1Ab toxin isolated from *Bt* transgenic corn could form tight complexes with HA and decreased the bioactivity of the toxin.

Adsorption of Cry1Ab Protein in Soils. The amount of Cry1Ab protein adsorbed by five soils increased with an increase in the equilibrium concentration of the protein (**Figure 4**). Regression analysis showed that the adsorption isotherms of the Cry1Ab protein for these soils were, in general, well described with a linear relationship ($R^2 \geq 0.922$, $p < 0.05$), although the fit was better for some soils than for the others. The linear adsorption coefficient, $K_{d,ads}$, ranged from 1.37 to 4.84, and the adsorption of the protein on the selected soils followed the order $S_2 > S_5 > S_1 > S_4 > S_3$. Further regression analysis demonstrated that adsorption of the protein on the soils was positively related to the soil OM content ($p < 0.05$). The most adsorption occurred on soils S_1, S_2, and S_4, which had a pH < 6.0 and relatively high OM levels. However, other factors could have also contributed to the differences in adsorption, but their respective importance was difficult to discern due to the relatively small number of soils used. Saxena et al. (*17*) indicated that desorption of *Bt* protein from *B. thuringiensis* in

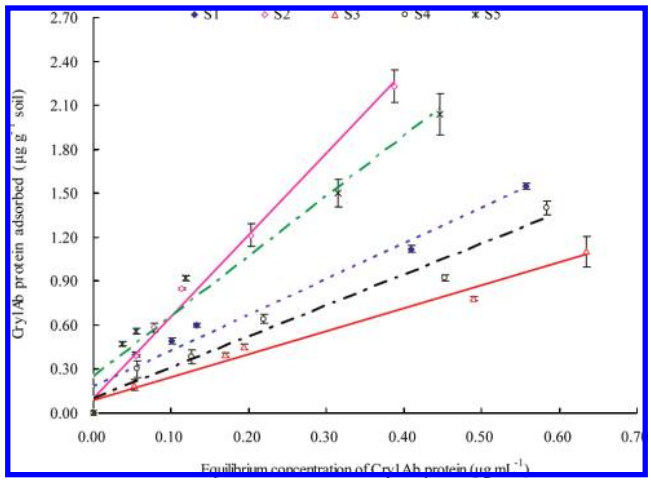

Figure 4. Adsorption of Cry1Ab protein on soils.

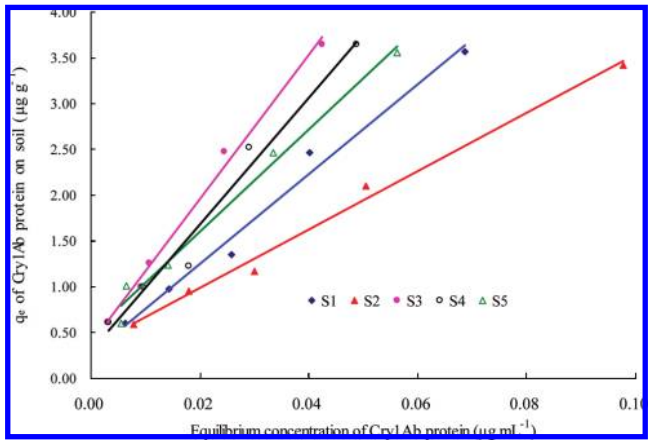

Figure 5. Desorption of adsorbed Cry1Ab protein on soils. q_e is the equilibrium concentration remaining on soil.

soils has a potential for causing contamination of surface water and groundwater resources. Given the intensive water management used in rice production, it is important to characterize desorption of Cry1Ab protein from *Bt* transgenic rice. In the present study, the desorption of Cry1Ab protein from the soils generally followed a linear relationship as a function of the equilibrium concentrations of the protein (**Figure 5**). The linear desorption coefficient, $K_{d,des}$, ranged from 32.30 to 80.31 ($R^2 \geq 0.924$, $p < 0.01$, **Table 4**). The desorption of Cry1Ab protein from the soils was positively correlated with the soil OM content. The HI values ranged from only 0.10 to 0.20, calculated by the model of Huang et al. (*33*). The fact that the HI values were significantly smaller than 1 suggested that the Cry1Ab protein was irreversibly adsorbed on the soils. The significant hysteresis in Cry1Ab protein desorption will likely decrease the potential for the protein to move from rice fields to off-site surface water and groundwater systems. On the other hand, the strong hysteresis of desorption of the Cry1Ab protein may imply a prolonged persistence of the protein in agroecosystems. Accumulation of Cry1Ab protein may have potential risks, such as toxicity to nontarget insects and soil microorganisms (*18*).

In our previous studies, degradation of Cry1Ab protein in KMD straw and that of free Cry1Ab protein isolated from *Bt* transgenic rice were examined in five paddy soils under aerobic conditions (*25, 26*). The half-lives of the Cry1Ab protein in KMD straw ranged from 11.5 to 34.3 d in the same five soils amended with KMD straw (4%, w/w), whereas degradation of isolated Cry1Ab protein in the same soils was slower than that in KMD straw under comparable conditions with half-lives of 19.6−41.3 d. The free Cry1Ab protein persisted in the soils for 345 d, whereas the Cry1Ab protein from KMD straw persisted in the soils for only 146 d. Stotzky et al. reported that the adsorbed toxin protein retained some of its insecticidal activity but was protected against biotic degradation in various soils for at least 180 d (*16, 17*) or in some cases for over 234 d (*15*). In addition, Accinelli et al. (*34*) applied carbon-14-labeled Cry1Ac endotoxin and found that 59% of Cry1Ac degraded to $^{14}CO_2$ after 20 d. All these results indicated that the toxins adsorbed or bound to the soil matrix were resistant to biodegradation and that there may be differences in persistence due to the use of the free protein or protein embedded in plant materials.

In summary, adsorption of free Cry1Ab toxin protein isolated from *Bt* transgenic rice rapidly reached equilibrium on bentone, kaolin, and humic acids, with adsorption occurring more rapidly on bentone and kaolin than on humic acids. Moreover, adsorption and desorption of Cry1Ab protein on these soil components were, in general, well described by linear relationships. The adsorption of the Cry1Ab protein followed the order kaolin > bentone > humic acids (**Figure 2**), while the desorbed amounts followed the order humic acids > kaolin ≥ bentone. Adsorption and desorption of the same Cry1Ab protein in soils were also better described by linear equations rather than by the Freundlich model. In addition, adsorption of Cry1Ab protein in these soils was positively correlated with the soil organic matter content. Significant hysteresis was observed in the desorption process of the adsorbed protein, and the hysteresis may limit off-site movement of the toxin protein from rice fields, but it may contribute to a prolonged persistence of the protein. The off-site transport and accumulation of Cry1Ab protein from transgenic rice should be studied under field conditions, including consideration of practices such as returning *Bt* rice straw to the field as a soil amendment after harvest, when a large pulse of protein release into soil is expected.

ABBREVIATIONS USED

GM, genetically modified; *Bt*, *Bacillus thuringiensis*; HA, humic acid; ELISA, enzyme-linked immunosorbent assay; KMD, Ke−Ming−Dao (*Bt* transgenic rice); XS11, Xiushui11; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; CEC, cation exchange capacity; HI, hysteresis index.

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