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Soil Biology & Biochemistry 39 (2007) 3034–3042

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Effects of physical and chemical properties of soils on adsorption of the insecticidal protein (Cry1Ab) from *Bacillus thuringiensis* at Cry1Ab protein concentrations relevant for experimental field sites

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Received 7 December 2006; received in revised form 13 June 2007; accepted 18 June 2007
Available online 20 July 2007

Abstract

The adsorption of the insecticidal Cry1Ab protein of *Bacillus thuringiensis* (*Bt*) on Na-montmorillonite (M-Na) and soil clay fractions was studied. The aim of this study was not to find the adsorption capacity of the soils from the experimental field site, where *Bt* corn (MON810) was cultivated, but rather to characterize the adsorption behavior of the Cry1Ab protein at concentrations typically found at experimental field sites. In kinetic experiments, the Cry1Ab protein adsorbed rapidly (<60 min) on M-Na. As the concentration of M-Na was varied and the added Cry1Ab protein concentration was kept constant (20 and 45 ng ml^{-1}), the adsorption per unit weight of Cry1Ab protein decreased with increasing concentrations of M-Na. Adsorption of Cry1Ab protein on M-Na decreased as the pH value of the suspension increased. All adsorption isotherms could be described mathematically by a linear regression with the parameter k, the distribution coefficient, being the slope of the regression line. Although their mineralogical composition was nearly identical, the soil clay fractions showed different k values. The different k values were correlated with the physical and chemical properties of the soil clay fractions, such as the organic carbon content, the specific external surface area, and the electrokinetic charge of the external surfaces of the clays, as well as with the external surface charge density. An increase in the amount of soil organic matter, as well as an increase in the electrokinetic external surface charge of the soil clays, decreased the distribution coefficient k. An increase of the specific external surface areas of the soil clays resulted in a higher distribution coefficient k.

Less than 10% of adsorbed Cry1Ab protein was reversibly adsorbed on the soil clays and, thus, desorbed. The desorption efficiency of distilled water was higher than that of a solution of $CaCl_2$ (2.25 mmol) and of dissolved organic carbon (50 mg C l^{-1}).

Keywords: Insecticidal Cry1Ab protein; *Bacillus thuringiensis*; Na-montmorillonite; Soil clay fraction; Adsorption; Desorption; Organic carbon; Specific external surface area; Electrokinetic external surface charge; Surface charge density

1. Introduction

The area cultivated with genetically modified plants (GMP) has increased within the last years: e.g., in 2005, the global area of GMP cultivation was 90 million hectares, up from 81 million hectares in 2004 (James, 2005). One of the genetic modifications enables plants, such as corn, to synthesize a toxic protein formed by *Bacillus thuringiensis* subsp. *kurstaki*, which is directed against the larvae of the European corn borer (*Ostrinia nubilalis* Hübner), so that these plants (here referred to as *Bt* plants) are protected against this pest.

Insecticidal proteins synthesized by *Bt* plants enter the soil by different pathways, e.g., in root exudates during the vegetative period (Saxena et al., 1999; Saxena and Stotzky, 2000), after transgenic plants have been harvested and the biomass is incorporated into soil (Tapp and Stotzky, 1998; Stotzky, 2000), and some input from pollen (Losey et al., 1999). Under field conditions, the highest amount of *B. thuringiensis* endotoxin in soil samples was present in

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0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.06.015

living root fragments. Smaller, but significant concentrations of *Bt* toxin could also be detected in decomposing corn residues from previous years of cultivation (Hopkins and Gregorich, 2003). The concentration of Cry1Ab protein in the bulk soil of *Bt* corn (MON810) in field plots was lower than that found in the rhizosphere (Baumgarte and Tebbe, 2005). The insecticidal proteins produced by *B. thuringiensis* become partially resistant to microbial degradation, as a result of the interaction of the *Bt* toxins with soil constituents (e.g., Koskella and Stotzky, 1997; Saxena and Stotzky, 2000; Stotzky, 2000, 2004). The toxins bound on soil constituents had a decreased bioavailability and were less susceptible to degradation. Therefore, the toxins could accumulate in the soil environment (Crecchio and Stotzky, 1998, 2001; Tapp and Stotzky, 1998; Vettori et al., 2003; Muchaonyerwa et al., 2004). Moreover, the insecticidal activity of the *Bt* toxins were retained and, sometimes, even enhanced when bound on soil particles (Tapp and Stotzky, 1995; Crecchio and Stotzky, 1998; Zhou et al., 2005). Nevertheless, the persistence of *Bt* toxins in soil, as well as their microbial degradation, is also a function of soil type, environmental conditions, source of the proteins (plant produced versus purified), and the particular Cry protein studied (Clark et al., 2005).

Several studies have investigated the behavior of *Bt* toxins in soils, as well as the adsorption of *Bt* toxins on soil particles (e.g., Stotzky, 2004). Because of their large specific surface area and high cation-exchange capacity, clay minerals and humic substances are the most important adsorbents in soils (Sposito, 1984; Stotzky 1986). Therefore, the clay size fractions adsorbed more *Bt* toxin than the silt size fractions or bulk soils (Muchaonyerwa et al., 2006). Adsorption of *Bt* toxins on montmorillonite (M) was greater than on kaolinite (K) (Venkateswerlu and Stotzky, 1992; Tapp et al., 1994; Lee et al., 2003). Maximum adsorption occured within 30 min. Longer contact times did not result in an increase in adsorption of *Bt* toxin (Crecchio and Stotzky, 2001; Venkateswerlu and Stotzky, 1992; Lee et al., 2003; Zhou et al., 2005). Maximum adsorption of *Bt* toxin on a sandy loam soil was reached after 3 h in contrast to a clay loam soil, where maximum adsorption occurred after 4 h (Sundaram, 1996). The insecticidal protein from *B. thuringiensis* subsp. *kurstaki* bound rapidly and strongly on complexes of M-humic acids-Al hydroxypolymers (Crecchio and Stotzky, 2001). The adsorption and binding of *Bt* toxin on humic acids increased with increasing amounts of humic acids (Crecchio and Stotzky, 1998). Moreover, the amount of *Bt* toxin adsorbed on clay minerals was not affected by temperatures between 7 and 50 °C (Venkateswerlu and Stotzky, 1992; Zhou et al., 2005); it was highest at pH values near the isoelectric point (IEP) of the protein (Venkateswerlu and Stotzky, 1992; Crecchio and Stotzky, 2001); less than 10% of the *Bt* toxin adsorbed could be desorbed (Chevallier et al., 2003; Lee et al., 2003); and, there was essentially no desorption of *Bt* toxin after extensive washing of toxin–organomineral complexes with double distilled water or NaCl (Crecchio and Stotzky, 2001). A summary of the interactions between *Bt* toxins and soil particles and the effects of the interactions on the persistence of *Bt* toxins and their larvicidal activity is given by Stotzky (2004).

To obtain a more detailed understanding of the role of the physical and chemical properties of soil on the adsorption of the Cry1Ab protein, adsorption experiments were performed with clay fractions obtained from soils of experimental field sites on which *Bt* corn MON810 was cultivated. The concentrations of Cry1Ab protein used in these adsorption experiments were those concentrations found on these experimental field sites by Baumgarte and Tebbe (2005), and Nguyen and Jehle (2007).

Chemical and physical properties of the fractionated clay samples, such as pH, organic carbon content, and mineralogical composition were determined. Due to their sizes (Li et al., 1991), the toxins from *B. thuringiensis* were not able to penetrate into the interlayer space between the elementary layers of expandable layer silicates (Tapp et al., 1994), so that the adsorption takes place only on the external surfaces of the clay particles. Therefore, the electrokinetic charge and the area of the external surfaces of the clay particles were measured. These physical and chemical properties were related to the adsorption of the Cry1Ab protein.

The time to reach equilibrium adsorption of Cry1Ab protein on Na-montmorillonite (M-Na) was determined. The concentration of M-Na and the pH were varied. The adsorption of the Cry1Ab protein on M-Na was mathematically described. Desorption of adsorbed Cry1Ab protein was measured by adding a solution to the protein–clay complexes obtained in the adsorption experiments. This solution, simulating a soil solution, contained $CaCl_2$ (2.25 mmol) and dissolved organic carbon (50 mg C l^{-1}).

2. Materials and methods

2.1. Cry1Ab protein

The purified trypsin-activated Cry1Ab protein was microbially produced in a genetically engineered *Escherichia coli* (Nguyen et al., 2004) and was kindly provided by Johannes Jehle (SLVA, Neustadt a. d. Weinstr., Germany). The protein had a molecular mass of 66.7 kDa and consisted of 594 amino acid residues.

2.2. Na-montmorillonite and clay fractions

The sedimentary homoionic M-Na (<2 μm) was purchased from Erbslöh & Co, Geisenheim, Germany.

The soil samples were collected from three different locations near Halle (*soil A*) and Bonn (*soils B and C*) in Germany:

Soil A: Luvic Phaeozem from sandy loess above glacial drift marl.