Adsorption of DNA on clay minerals and various colloidal particles from an Alfisol

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Abstract

Adsorption and desorption of salmon sperm DNA on four different colloidal fractions from Brown Soil and clay minerals were studied. The adsorption isotherms of DNA on the examined soil colloids and minerals conformed to the Langmuir equation. The amount of DNA adsorbed followed the order: montmorillonite ≫ fine inorganic clay > fine organic clay > kaolinite > coarse inorganic clay > coarse organic clay. A marked decrease in the adsorption of DNA on organic clays and montmorillonite was observed with the increase of pH from 2.0 to 5.0. Negligible DNA was adsorbed by organic clays above pH 5.0. As for inorganic clays and kaolinite, a slow decrease in DNA adsorption was found with increasing pH from 2.0 to 9.0. The results implied that electrostatic interactions played a more important role in DNA adsorption on organic clays and montmorillonite. Magnesium ion was more efficient than sodium ion in promoting DNA adsorption on soil colloids and minerals. DNA molecules on soil colloids and minerals were desorbed by sequential washing with 10 mM Tris, 100 mM NaCl and 100 mM phosphate at pH 7.0. A percentage of 53.7–64.4% of adsorbed DNA on organic clays and montmorillonite was released, while only 10.7–15.2% of DNA on inorganic clays and kaolinite was desorbed by Tris and NaCl. The percent desorption of DNA from inorganic clays, organic clays, montmorillonite and kaolinite by phosphate was 39.7–42.2, 23.6–28.8, 29.7 and 11.4%, respectively. Data from this work indicated that fine clays dominate the amount of DNA adsorption and coarse clays play a more important role in the binding affinity of DNA in soil. Organic matter may not favor DNA adsorption in permanent-charge soil. The information obtained is of fundamental significance for the understanding of the ultimate fate of extracellular DNA in soil.

1. Introduction

In soil environment, extracellular DNA was excreted actively by living cells or released from lysed dead cells (Reanney et al., 1983). DNA from various organisms has been shown to be adsorbed by clay minerals, sand, humic acids and soil, which alters its activity and susceptibility to biodegradation (Recorbet et al., 1993; Widmer et al., 1996; Paget et al., 1998). Moreover, these adsorbed DNA molecules have also been shown to be capable of transforming competent bacterial cells (Khanna and Stotzky, 1992; Gallori et al., 1994; Blum et al., 1997; Crecchio and Stotzky, 1998; Stotzky, 2000). Therefore, the adsorption of DNA on particles is one of the key processes affecting its persistence and availability for uptake by cells for genetic transformation in soil.

DNA adsorption on well-characterized adsorbents and soil was affected by the ionic strength, pH of the medium, type and content of clay as well as characteristics and configurations of DNA molecules. The adsorption of DNA from *Bacillus subtilis*, calf thymus and salmon sperm on montmorillonite, kaolinite and silica increased with an increase of ionic strength or a decrease of pH (Greaves and Wilson, 1969; Melzak et al., 1996; Khanna et al., 1998). Montmorillonite clay played an important role in herring sperm DNA adsorption in soil (Ogram et al., 1988). The amount of supercoiled plasmid DNA adsorbed by sand was slightly less than that of linearized or open circular plasmid DNA (Romanowski et al., 1991). The lower molecular mass DNA from *B. subtilis* showed higher adsorption capacity by

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Ca–montmorillonite and Ca–kaolinite than the higher molecular mass DNA (Pietramellara et al., 2001). Desorption experiments were also carried out to study the extent of DNA association with minerals, sand and humic acids as well as the binding forces between DNA and adsorbents (Lorenz and Wackernagel, 1987; Khanna and Stotzky, 1992; Crecchio and Stotzky, 1998). For example, the fact that detergents did not release calf thymus DNA from EDTA-eluted sand indicates that hydrophobic interaction between DNA and sand plays only a minor role (Lorenz and Wackernagel, 1987).

Because of the complexity of soil environment, predicting the extent of adsorption and desorption is difficult. In fact, very little is known about the types of interaction between DNA and soil components as well as the effects of mineral constituents, organic matter and particle size of soil colloids on DNA adsorption. The objective of the current work was to elucidate the influence of different soil colloidal fractions on the adsorption of salmon sperm DNA. Three solutions were used to consecutively desorb DNA–adsorbents complexes with the aim of determining the percentage of various binding forces involved in adsorption reaction. The effects of pH and electrolyte on DNA adsorption were also investigated.

2. Materials and methods

2.1. DNA

Salmon sperm DNA was purchased from Sigma Chemical Co., St. Louis, Mo.

2.2. Soil colloids and minerals

A clay loamy Brown Soil (Alfisol) was sampled from the 0–17 cm layer of a forest land in Tianwai village, Taishan, Shandong province, China. Pertinent characteristics of the soil include: pH (H_2O) 6.3, organic matter 42.4 g kg^{-1} and a cation exchange capacity of 15.3 cmol kg^{-1}. After removal of organic residue the soil was rinsed in deionized distilled water (ddH_2O) and dispersed by adding 0.01 M NaOH solution dropwise to pH 7–8 together with

sonication. Two soil colloidal components i.e. fine clay (<0.2 μm) and coarse clay (0.2–2 μm) were separated by sedimentation. Part of fine and coarse clays was oxidized by H_2O_2 to remove organic matter. After flocculation by the addition of $CaCl_2$ solution, the colloidal suspension was washed to be free of Cl^- ions by ddH_2O and ethanol, and then air-dried. Some properties of the clay fractions are listed in Table 1.

Kaolinite and montmorillonite were purchased from Shanghai Wusi Chemical Reagent Company and Henan Xinyang Montmorillonite Company. The clays were suspended in ddH_2O and adjusted to pH 10 with 0.01 M NaOH and dispersed by sonication occasionally. The <2 μm fraction was separated by sedimentation. The clays were washed with ddH_2O and ethanol until free of Cl^- ions and air-dried. All the soil colloids and minerals prepared were ground to pass a 100 mesh sieve.

2.3. Adsorption of DNA

Ten milligram of soil colloid or kaolinite was mixed with 2.5 ml of 10 mM Tris buffer (pH 7.0) containing 25–350 μg of DNA. As for montmorillonite, 1 mg of sample was used to construct adsorption isotherm. The mixture was gently shaken at 25 °C for 2 h and centrifuged at 20,000g for 20 min. DNA in the supernatant was determined directly at 260 nm spectrophotometrically. The amount of DNA adsorbed was calculated by the difference between the amount of DNA added and that remaining in the supernatant. Adsorption was also conducted in the range of pH from 2.0 to 9.0 in which 10 mg of soil colloid or mineral and 400 μl of Tris buffer (pH 7.0) containing 200 μg of DNA were employed. The similar experiment was carried out in the presence of 0–200 mM of Na^+ or 0–60 mM of Mg^{2+}.

2.4. Desorption of DNA

Ten milligram of soil colloid or mineral and 400 μl of Tris buffer (pH 7.0) containing 200 μg of DNA were mixed in the centrifuge tube. The mixture was shaken at 25 °C for 2 h and centrifuged at 20,000g for 20 min. The residue was washed with 1 ml of 10 mM Tris, 100 mM NaCl and 100 mM phosphate at pH 7.0 sequentially. Each washing

Table 1
Selected properties of soil colloids and minerals[a]

Soil colloid or mineral	O.M. (g kg^{-1})	SESA (m^2 g^{-1})	PZC	CEC (cmol kg^{-1})	Clay mineral composition
Coarse organic clay	67.6	31.7	2.4	46.2	Hydromica (45%), vermiculite (35%), kaolinite (20%)
Coarse inorganic clay	5.9	37.5	3.5	41.2	Hydromica (45%), vermiculite (35%), kaolinite (20%)
Fine organic clay	74.4	97.1	1.8	68.9	Hydromica (60%), vermiculite (30%), kaolinite (10%)
Fine inorganic clay	13.3	116.5	3.0	54.5	Hydromica (60%), vermiculite (30%), kaolinite (10%)
Montmorillonite	–	73.6	2.5	90.2	Montmorillonite
Kaolinite	–	22.9	3.6	7.1	Kaolinite

[a] Clay mineral was determined by X-ray diffraction analysis. Organic matter (O.M.), point of zero charge (PZC), cation exchange capacity (CEC) and specific external surface area (SESA) were analyzed by $K_2Cr_2O_7$ digestion, Mehlich, NH_4AcO method (Xiong, 1985) and N_2 adsorption method (Beijing Analytical Instrument Company), respectively.