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DNA adsorption on synthetic and natural allophanes

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ABSTRACT

The adsorption of DNA on natural and synthetic samples of allophane, which is a primary clay mineral of andosols, was investigated with respect to the DNA concentration, pH, ionic strength in the sample solution, and competition between DNA molecules and phosphate ions for adsorption to understand the behavior of extracellular DNA molecules in andosols. The relationships between DNA adsorption and the final concentrations were significantly fitted to a simple linear Langmuir equation. DNA adsorption decreased considerably with increasing suspension pH in the range between 3 and 9. The adsorption was less affected by the ionic strength of the suspension from 0.1 to 0.5 mol L^{-1}. Under the same experimental conditions, DNA adsorption on allophanes was relatively higher than that on montmorillonite and silica but relatively lower than that on gibbsite and goethite. DNA adsorption on allophanes decreased on the addition of phosphate, indicating that there was a competition between DNA molecules and phosphate ions for adsorption on the minerals. These results suggested that DNA adsorption on allophanes occurred via two mechanisms: direct bonding of the phosphate group at the end of the DNA molecule to the OH groups of the Al-oxide layer on allophane, and the association of DNA molecules with the surface of negatively charged allophane via a bridging of inorganic cations.

1. Introduction

With the rapid developments in the commercial production of genetically modified microorganisms and plants, great emphasis has been laid on the security of genetically modified organisms (GMOs). Soil DNA plays an important role in the biological activity and diversity in the soil as well as in the transfer of genetic information among bacteria (Khanna and Stotzky, 1992; Stotzky, 2000). Therefore, knowledge of the binding of extracellular DNA to soil constituents is essential to understand the horizontal translocation and transformation of extracellular DNA in soil environments. Understanding the adsorption of extracellular DNA on soil particles is also helpful for the study of soil biodiversity and in assessing the risk of the release of GMOs into the soil.

For the last few decades, it has been believed that nucleases rapidly degrade DNA released from dead or metabolizing microorganisms (Lorenz and Wackernagel, 1994). However, recently, it has been observed that the adsorption of nucleic acids released from microorganisms and plant tissue on soil particles alters their reactivity and susceptibility to nucleases and makes them resistant to biodegradation. Moreover, DNA molecules have a persistent ability to transform competent cells when bound to clay minerals and other particles (Khanna and Stotzky, 1992; Gallori et al., 1994; Crecchio and Stotzky, 1998; Stotzky, 2000).

Most bacteria in soil microbial communities cannot be cultivated in artificial media. Therefore, for the last few decades, the presence of these nonculturable bacteria has been detected by methods based on the hybridization or PCR amplification of DNA sequences extracted directly from soil. However, it is more difficult to extract DNA from volcanic ash soils (andosols), which are found abundant in Japan, than from other types of soil (e.g., Takada-Hoshino and Matsumoto, 2004). This has been a major problem in the analysis of soil microbial communities using culture-independent methods. Therefore, it is important to understand the mechanism of DNA adsorption by soil particles for developing efficient extraction methods.

Many researchers have used 2:1 phyllosilicates such as montmorillonite as adsorbents to study DNA adsorption in soils (Greaves and Wilson, 1969, 1970; Khanna and Stotzky, 1992; Khanna et al., 1998; Pietramellara et al., 2001). However, the results of these researches cannot be applied to study the behavior of DNA molecules in variable-charge soils such as andosol. There have been few studies on DNA adsorption in natural soils (Ogram et al., 1988,1994; Saeki et al., 2008; Saeki and Sakai, 2009). To better understand the contribution of soil components to DNA adsorption in andosol, the effects of hydrogen peroxide and oxalate treatments on DNA adsorption were examined (Saeki et al., 2008; Saeki and Sakai, 2009). These studies indicated that oxide minerals in soils are one of the most important adsorbents of DNA molecules. However, DNA adsorption by oxide minerals other

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than goethite (Cai et al., 2006) and silica (Melzak et al., 1996) has not been studied.

Therefore, in the present study, we investigated DNA adsorption on natural and synthetic allophanes with respect to the DNA concentration, pH, ionic strength in the sample solution, and competition between DNA molecules and phosphate ions.

2. Materials and methods

2.1. Allophane samples

Two allophane samples, one natural and the other synthetic, were used in this study. The synthetic allophane was synthesized according to a method described by Ohashi et al. (2002), which can be briefly described as follows. 100 mmol L^{-1} of Na_2SiO_4 and $AlCl_3 \cdot 6H_2O$ solutions prepared separately were mixed together instantaneously and stirred for 1 h at room temperature to synthesize the allophane precursor. NaCl as a by-product in the suspension was removed by centrifugation. The obtained precursor was autoclaved for 2 days at 100 °C for performing the hydrothermal reaction. The obtained solid was repeatedly washed with DW until its pH was neutral then freeze-dried. The synthetic allophane consists of 56.08% SiO_2 and 43.92% Al_2O_3 with a SiO_2/Al_2O_3 molar ratio of 1.28. The N_2-BET-specific surface area of the synthetic allophane was reported to be 552 $m^2\ g^{-1}$ (Ohashi et al., 2002).

The natural allophane collected from Mouka, Tochigi, Japan (Matsumoto et al., 2004) was supplied by Shinagawa Chemicals Ltd. Its data shows that the allophane consists of 50.0% SiO_2 and 43.2% Al_2O_3 and has a N_2-BET-specific surface area of approximately 300 $m^2\ g^{-1}$.

2.2. DNA sample

In this study, we used a salmon sperm DNA (average size of 1000 bp) supplied by Invitrogen Co. (California, USA). The DNA solution, which was diluted to a suitable concentration with UPW, was used for the adsorption experiments.

2.3. DNA adsorption experiments

2.3.1. Isotherm

Ten mg of autoclaved allophane was added to 50 μL of 2.0 mol L^{-1} (M) NaCl and the diluted DNA solution, then made up to 1.0 mL. The initial DNA concentration ranged from 0 to 100 mg L^{-1}. The suspension was shaken for 2 h in an air-conditioned room at 298 ± 1 K and centrifuged at 16,000 × *g* for 20 min. The DNA, pH, and electric conductivity (EC) of the collected supernatant was analyzed. The amounts of DNA adsorbed were calculated from the differences between the amounts of DNA added and those remaining in the solutions.

The adsorptive affinity of DNA to the allophanes was analyzed using the Langmuir equation given below:

$$A = \frac{k \cdot A_{max} \cdot C}{1 + k \cdot C} \quad (1)$$

where A represents the adsorption of each DNA, A_{max} is the maximum adsorption, k is an adsorptive equilibrium constant, and C is the equilibrium concentration of each DNA.

2.3.2. Effects of pH and ionic strength

2.3.2.1. Basic procedure. Ten mg of autoclaved mineral sample was added to 100 μL of 1.0 M NaCl and 100 μL of 1 mg mL^{-1} DNA solution and adjusted by dilute NaOH addition to pH 7.0. Distilled water was then added to make up the solution to 1.0 mL. The DNA concentration of the working solution was 100 μg mL^{-1}. The suspension was shaken for 2 h in an air-conditioned room at 298 ± 1 K and then centrifuged at 20,000 × *g* for 20 min. The DNA, pH, and EC of the supernatant were analyzed.

In the experiment to investigate the effect of the pH, the basic procedure consisted of adjusting the suspension to the desired pH (between 3 and 9) using dilute HCl or NaOH.

In the experiment to analyze the effect of the ionic strength, the basic procedure consisted of changing the ionic strength of the suspension by adding different volumes of 1.0 M NaCl to it. The pH of the suspension was adjusted to 7.0 using a dilute NaOH solution. The ionic strength of the solution was estimated from the EC value using the following equation (Marion and Babcock, 1976):

$$\log(Ie) = -1.841 + 1.009 \times \log(EC) \quad (2)$$

where Ie is the ionic strength (mol L^{-1}) and EC, the electrical conductivity of the solution (mS cm^{-1}). The ionic strength of the solution ranged from 0.1 to 0.5 mol L^{-1}.

2.3.3. Comparison among minerals

DNA adsorption by allophanes was compared to those by other clay minerals under the same experimental conditions. For the comparison, we used goethite, gibbsite, silica, montmorillonite (K10), and kaolinite. Goethite was synthesized by the method described by Atkinson et al. (1967) and Saeki et al. (1995). Gibbsite, silica, and montmorillonite (K10) were supplied by Showa Denko Ltd., Malinkrodt Ltd., and Aldrich Ltd., respectively (Saeki, 2004). Kaolinite was collected from Iriki, Kagoshima, Japan (Saeki et al., 2004). The procedure used to analyze DNA adsorption on different minerals was the same as the basic procedure.

2.3.4. Competition between DNA molecules and phosphate ions

Since it is assumed that the phosphate groups at the end of a DNA molecule play an important role as adsorption sites, the effect of phosphate addition on DNA adsorption on allophanes was investigated. Autoclaved samples, each containing 10 mg of allophane, were added to 50 μL of 2.0 M NaCl and different volumes (0, 25, 50, 75, and 100 μL) of 1 M NaH_2PO_4. Distilled water was then added to make up the solution to 0.8 mL. The suspension was shaken for 24 h in an air-conditioned room at 298 ± 1 K. Then, 200 μL of 1 mg mL^{-1} DNA solution was added to the suspension and shaken for 2 h. For comparison, we performed an experiment in which the suspensions were added concomitantly to both the phosphate and the DNA solutions. Thereafter, the suspension was centrifuged at 20,000 × *g* for 20 min and then its DNA, pH, and EC were analyzed.

2.4. Analysis of DNA solutions

The collected supernatant was analyzed by UV spectrophotometry at 260 nm using a 1-cm quartz cell to determine the amount of DNA. This method has conventionally been used by many researchers (e.g. Greaves and Wilson, 1970; Cai et al., 2006). Standard DNA calibration solutions were made in similar backgrounds to the sample solutions obtained in each experimental section. The amount of DNA adsorbed was calculated from the difference between the amount added and that remaining in the solution.

3. Results and discussion

3.1. DNA adsorption isotherms

Fig. 1 shows the DNA adsorption isotherms for natural and synthetic allophanes. The solution pH was regulated from 5.6 to 6.0 for the synthetic allophane sample and from 4.9 to 5.3 for the natural one. The relation between adsorption and the final concentration of DNA significantly fitted a simple linear Langmuir equation [$r = 0.9958$ ($p < 0.001$, $n = 19$) for the natural allophane and $r = 0.9914$ ($p < 0.001$,