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The protective effect of clay minerals against damage to adsorbed DNA induced by cadmium and mercury

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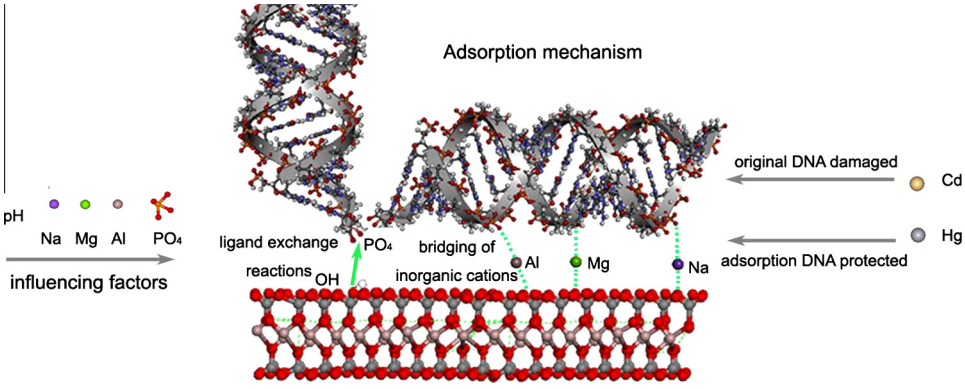
HIGHLIGHTS

- The DNA adsorption was reduced in the order: rectorite > montmorillonite > sericite.
- DNA was adsorbed on clay can be influenced by the surface charges of the samples.
- The three mechanisms for DNA adsorption on clay are concluded.
- Rectorite is important for protecting DNA against damage induced by Cd^{2+} and Hg^{2+}.

GRAPHICAL ABSTRACT



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ABSTRACT

The adsorption of Salmon Sperm DNA on three kinds of raw clay (rectorite, montmorillonite and sericite) was investigated as a function of pH, ionic strength and the concentrations of DNA and phosphate ions in solution. The DNA adsorption was reduced in the following order: rectorite > montmorillonite > sericite. Based on these findings, there is a strong evidence that the mechanisms for DNA adsorption on clay involve electrostatic forces, cation bridging and ligand exchange. Cyclic voltammetry (CV) and UV–vis absorption and fluorescence spectroscopy were used to compare the properties of unbound DNA and the absorbed DNA on rectorite, both in the absence and presence of Cd^{2+} and Hg^{2+} inaqueous solutions. The interaction of heavy metals with the unbound DNA was evidenced by the disappearance of reduction peaks in CV, a small bathochromic shift in UV–vis spectroscopy and an incomplete quenching in the emission spectra. Such changes were not observed in the DNA-rectorite hybrids, which is evidence that adsorption on the clay can reduce the extent of the DNA damage caused by heavy metals. Therefore, in these experience the rectorite played an important role in protecting DNA against Cd^{2+} and Hg^{2+} induced damage.

1. Introduction

Recently, a number of studies have suggested that extracellular DNA released from various organisms can be adsorbed on solid particles in environment (Levy-Booth et al., 2007) and resist degradation by nucleases (Crecchio and Stotzky, 1998). This DNA can exist in the natural environment for a long time (Pietramellara et al.,

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2008) and retain its biological function (Stotzky, 2000). In fact, it has been hypothesized that clay minerals could have played a role in the creation of primitive life. (Lorenz and Wackernagel, 1987; Ertem, 2004).

Therefore, increasing number of scientists are investigating the interaction between DNA and clay minerals, such as montmorillonite, mica and kaolinite which are well known to adsorb DNA (Sun et al., 2011), due to their special layered structure and good intercalation properties. Many factors such as the size and molecular characteristics of DNA (Pietramellara et al., 2001; Beall et al., 2009; Cleaves et al., 2011), the pH value (Saeki et al., 2010a), the solution ionic strength (Cai et al., 2006a) and the concentration of buffer solution (Saeki et al., 2011) affect the DNA molecule adsorption on clay minerals.

In the recent years, the biological DNA damage caused by heavy metals in the environment has become a worldwide concern. The Cd^{2+} and Hg^{2+} are common heavy metals in the ocean, which can cause DNA lesions (Habeebu et al., 1998). Unfortunately, few methods have been developed to protect DNA from damage induced by heavy metals. The clay minerals such as montmorillonite have been proven of efficiency in removal of heavy metals (Abollino et al., 2003; Zhang et al., 2012), because of their high surface area, chemical and mechanical stabilities, and various surface and structural properties (Anirudhan et al., 2012). Therefore, clay is widely used as an adsorbent for removing heavy metals. These results suggest the possibility that clay minerals could be used as a repository for adsorption and protecting DNA against Cd^{2+} and Hg^{2+} are effective and significant.

Rectorite (REC) is a regularly interstratified clay mineral with alternating pairs of dioctahedral mica-like layer (inexpansible) and dioctahedral montmorillonite-like layer (expansible) in the ratio of 1:1 (Wang et al., 2010). However, there is only limited information on the interaction of the DNA with REC available and it is not totally understood how REC protects DNA from damage by metal ions. The aim of this study is to investigate the factors that influence DNA adsorption on different clay and to probe the mechanisms by which REC protects DNA from damage by Cd^{2+} and Hg^{2+} heavy metal ions, in order to approve that clay can be used as a gene repository.

2. Materials and methods

2.1. Materials

Rectorite (REC) refined from clay minerals was provided by Clippe Inc. Co. (Wuhan, China). Raw calcium montmorillonite (MMT) and sericite (SER) were obtained from Nanhai Inc. Co. (Guangdong, China). Salmon sperm dsDNA (Sigma Chemical Co., St. Louis, MO.) was dissolved in 10.0 mM of Tris–HCl buffer (pH 7.0). The concentration of the DNA solution was 1000 mg L^{-1}, as determined by UV absorption at 260 nm (Teeters et al., 2004), was of satisfactory purity (A260/A280 nm > 1.8). All other reagents used in this experiment including $Cd(NO_3)_2{\cdot}H_2O$, $HgCl_2$, NaH_2PO_4, HCl and NaOH were of analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangdong, China).

The zeta potential values of the powder clay suspensions (pH = 7.0) were measured using a zeta sizer (Malvern Instruments, UK). Suspensions of 2 g L^{-1} in distilled water were used.

The BET surface area was determined by N_2 adsorption-desorption isotherms measured by a Micromeritics ASAP 2020 V3.00 E volumetric adsorption analyzer.

2.2. DNA adsorption experiments

Batch adsorption experiments were conducted at 298 ± 1 K. Fifty mg of adsorbent (REC, MMT, SER) was mixed with 5 mL DNA solution and 10 mM Tris–HCl buffers, with different initial concentrations ranging from 10 to 700 mg L^{-1} in the flask. Besides, the effects of pH (2–10), selected cations (0-200 mM, Na^+, Mg^{2+}, Al^{3+}) and phosphate ions in solution (0–10 mM, NaH_2PO_4) were conducted in the similar manner to clarify interactions between DNA and adsorbents. The solution pH was adjusted by HCl and NaOH solutions.

After the adsorption for 2 h, 20 mL of the suspension was centrifuged at 10,000 rpm for 20 min, and the resultant solids were filtered and dried in a vacuum oven at 60 °C for 12 h. The obtained samples were marked as DNA-REC, DNA-MMT and DNA-SER (Cai et al., 2008). The collected supernatant from each sample was analyzed by UV spectrophotometry at 260 nm to determine the amounts of DNA remaining in solution. The amounts of adsorbed DNA were obtained by differences:

$$Q_e = V_0 \frac{C_0 - C_e}{W_s} \quad (1)$$

where Q_e represents the adsorption of DNA (mg g^{-1}). C_0 is the initial concentration of DNA (mg L^{-1}). C_e is the equilibrium concentration of DNA (mg L^{-1}). W_s is the adsorbent dosage (g). V_0 is DNA solution (L) (Saeki et al., 2010b).

The adsorption of DNA on the clay minerals was analyzed by the Langmuir, Freundlich and Redlich–Peterson.

The DNA-clay compound were characterized by X-ray diffractometry (XRD, D/max-IIIA, Rigaku, Japan), Fourier transform infrared spectroscopy (FTIR, Vector-33, Bruker, Germany) and scanning electron microscopy (SEM, 1530VP, LEO, Germany).

2.3. DNA desorption experiments

For desorption experiments, 50 mg of dry DNA-clay compound were washed with 10 mL NaH_2PO_4 and NaOAc by shaking at room temperature (298 ± 1 K), followed by centrifugation at 10,000 rpm for 20 min. Washing was repeated until no more DNA was detected by UV in the supernatant. The percentage of DNA desorption (%) = the total amount of DNA desorbed/the amount of DNA adsorbed (Cai et al., 2008).

2.4. DNA and DNA-REC exposure to heavy metal ions

To further explore the protection of DNA provide by clay, REC was chosen because of its strong adsorption capacity for the DNA in this experiment. 10 mL heavy metal solutions (Cd^{2+} and Hg^{2+}, 10–80 mg L^{-1}) were added to 50 mg of dried DNA-REC. The suspensions were shaken for 30 min at room temperature and then centrifuged at 10,000 rpm for 20 min. The concentration of heavy metal ions in the supernatant was measured with an atomic absorption spectrophotometer (Z-500, Hitachi, Japan). In addition, precipitation was added in 10 mL Tris–HCl buffers solutions for desorption as described above. After attaining equilibrium, the aqueous phase containing the desorbed DNA was recovered by centrifugation for 10 min, and marked as protected-DNA.

In order to make a comparison, 5 mL of the DNA solution (100 mg L^{-1}) was added in 10 mL of the heavy metal solutions at different concentrations and marked as Cd^{2+}–DNA and Hg^{2+}–DNA.

Also, 50 mg of REC were added to 10 mL metal solutions (80 mg L^{-1} Cd^{2+}, 10 mg L^{-1} Hg^{2+}) to determine the extent of adsorption of heavy metal ions on the clay.

2.5. Evaluation of DNA damage

The interaction between DNA and metal ions was investigated by measuring the emission spectra in the wavelength range of 250–500 nm by fluorescence spectroscopy (F-7000, Hitachi, Japan)