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Research paper

Exfoliation and intercalation of montmorillonite by small peptides

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ABSTRACT

Understanding structural changes in clay minerals induced by complexation with organic matter is relevant to soil science and agricultural applications. In this study, the effect of peptide storage in montmorillonite and the thermal stability of peptide–clay complexes were examined through characterization by X-ray diffraction (XRD), electron microscopy, UV absorption, and thermogravimetric analysis (TGA). XRD analysis of small peptide–montmorillonite clay complexes produced profiles consisting of reflections associated with the smectite 001 reflection and related peaks similar to that produced by a mixed layer clay mineral structure. Shifts in higher order diffraction maxima were attributed to disorder caused by the intercalation with the peptides. Increasing peptide concentrations resulted in greater shifts toward smaller 2θ from 6.37° (1.39 nm) to 5.45° (1.62 nm) as the interlayer space expanded. The expansion was accompanied by broadening of the 001 reflection (FWHM increases from 0.51 to 1.22° 2θ). The XRD line broadening was interpreted as caused by poorer crystallinity resulting from intercalation and tactoid exfoliation. SEM images revealed montmorillonite platelets with upwardly rolled edges that tend toward cylindrical structures with the production of tubules. High-resolution TEM images revealed bending of montmorillonite platelets, confirming exfoliation. The distribution of basal spacings in the micrographs was determined from the spatial frequencies obtained by Fourier analysis of density profiles. The distribution indicated the presence of discrete coherent crystallite domains. XRD and TGA results indicated that higher peptide concentrations resulted in a greater fraction of intercalated peptides and that surface adsorption of peptides mediated intercalation. Therefore, higher peptide concentration led to more stable organoclay complexes. However, UV absorption and TGA found that peptide adsorption onto montmorillonite had a finite limit at approximately 16% by weight.

1. Introduction

Poorly crystalline minerals, i.e., clays, protect soil organic matter (OM) produced by leaf and root litter from microbial degradation (Kleber et al., 2005). The persistence of smaller peptides in association with clays has been acknowledged as possibly leading to longer residence times for soil carbon and nitrogen (Kleber et al., 2007).

The mineral fraction in soils has been shown to enhance chemical recalcitrance in the degradation of OM (Eusterhues et al., 2003), lending stability to the carbon reservoir in old soils. This has implications for the global carbon cycle and for clay-mediated storage of nutrients in soils at extended time scales. However, the role of peptides in association with minerals and their relative contribution to the stabilization of carbon in soils are poorly understood (Kleber et al., 2007). The structure of clays, and its role in stabilizing carbon and nitrogen from peptides may be important in estimating turnover time of soil OM (Rillig et al., 2007).

Clays are able to react with amino acids and other organic molecules forming organoclays (Greenland et al., 1965a,b; Harter and Stotzky, 1971). Organoclays mediate the relationship of microbes to soils and sediments and may have played a role in the development of prebiotic life (Bouchoucha et al., 2011; Carneiro et al., 2011a,b; Fraser et al., 2011; Georgelin et al., 2013). Conversely, organic molecules may modify the structure of clays. For example, microbes are known to play a role in the smectite to illite transition (Kim et al., 2004; Zhang et al., 2007a,b; Jaisi et al., 2011).

Smectites are highly reactive and possess an overall negative charge under ordinary critical zone conditions. The interlayer space cations in smectites can be readily exchanged with positively charged or polar molecules, including organics, to create intercalated and pillared structures (Bergaya and Lagaly, 2013). Montmorillonite (Mt), a common smectite clay, can form organoclay complexes with proteins, enhancing the retention of organic matter in soil (Mayer, 1994; Sollins et al., 1996; Sheng et al., 2001). At near neutral pH, smectite platelets have negatively charged faces and positively charged edges, thus, smectites may potentially interact electrostatically with charged amino acids (Hedges and Hare, 1987; Senwo and Tabatabai, 1998; Benincasa et al., 2000; Laird et al., 2001; Benetoli et al., 2007; Carneiro et al., 2011a,b).

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Smectites may also potentially interact with either the amide or carboxyl terminus of non-charged amino acids. Adsorption of different combinations of amino acids by smectites is known to be influenced by interlayer space cations (Arfaioli et al., 1999; Benincasa et al., 2000).

While large proteins can be adsorbed on the surface of clays where they are protected from enzymatic degradation (Theng, 1982), the products of degradation, such as peptides and amino acids, favor the possibility of absorption into the smectite interlayer space due to their smaller size. Kalra et al. (2003) utilized UV spectroscopy to investigate the adsorption of simple peptides onto divalent cation exchanged Mt. They determined that peptide adsorption is maximal at neutral pH and 23 °C and was greatest for glycine tetramers followed by glycine trimers and glycine–alanine dimers. The glycine dimers showed the lowest adsorbability.

Effenberger et al. (2009) found that the intercalation of Mt with alanine, leucine or phenylalanine produced a modified Mt with an increased d_{001}-spacing. Kollár et al. (2003) ion-exchanged several amino acids into Na-Mt and verified by Fourier transform infrared spectroscopy and XRD that the amino acids were successfully intercalated. Parbhakar et al. (2007) examined adsorption of L-lysine onto smectites and discussed the potential role of clays in promoting catalytic reactions in organic compounds. Naidja and Huang (1994) observed that aspartic acid absorption into Ca-Mt is a fast reaction process with 84% of the aspartic acid absorbed (56.2 μmol/g clay); the d_{001} spacing of the aspartic acid–Mt complex decreased at temperatures above 150 °C. They found that the aspartic acid appeared weakly bound to the surface indicating that it intercalated in the Mt through a water bridge.

The majority of the studies on smectite–amino acid interactions have focused on the effects that smectites have on amino acids and proteins (Bujdák et al., 1996; Lagaly, 2006; Lambert, 2008; Yu et al., 2013; Jaber et al., 2014). There have been fewer investigations into the changes that occur in the smectite structure as a result of amino acid, peptide or protein intercalation. Nonetheless, it is known that adsorption onto the surface or into the interlayer space plays a significant role in forming clay–protein complexes (Theng, 2012). To examine the role of mineral structure in the formation of peptide–clay complexes tryptone was utilized as a peptide source. Tryptone is a casein pancreatic digest which contains all twenty amino acids in peptide form. Tryptone oligomers consist mostly of monomers, dimers, trimers, and tetramers; therefore it is an excellent source of small peptides of multiple amino acids that makes it suitable for experiments with clay.

In this paper, the incorporation of suites of small oligopeptides, which are the products of OM decomposition, into Mg-Mt is examined by measuring the effect of peptides on clay structure, and determining the thermal stability of organo-clay complexes. The results of X-ray diffraction (XRD), high-resolution transmission electron microscopy (HRTEM) and thermogravimetric analysis (TGA) experiments that demonstrate the nature of the interactions are presented. XRD is compared to HRTEM data to determine the effect of adsorption on clay crystallinity at the bulk and local scales. TGA was utilized to ascertain the effect of peptide concentration on adsorption. As a conclusion, the results are placed in the context of the existing models for OM adsorption.

2. Materials and methods

2.1. Sample preparation

2.1.1. Montmorillonite

A high purity dioctahedral Mt ($(Na,Ca)_{0.33}(Al_{1.67}Mg_{0.33})Si_4O_{10}(OH)_2 \cdot nH_2O$; source: Belle Fourche, South Dakota, USA) — commercial name Volclay Accofloc 350 (American Colloid Company, Arlington Heights, IL) was made homoionic with magnesium through the cation substitution technique of Moore and Reynolds (1997). Accofloc is known to have a cation exchange capacity (CEC) of 79 meq/100 g (Sterte and Shabtai, 1987). The clay was initially washed in 5% sodium hypochlorite to remove organic contaminants, followed by a triple wash in distilled water to remove the sodium hypochlorite. Large clay particles and non-clay minerals were removed by centrifugation at 1200 rpm (235 *g*) for 5 min. The supernatant comprising the fraction smaller than 2 μm was collected. The purified clay was suspended in 0.1 M $MgCl_2$ overnight and centrifuged at 3600 rpm (2100 *g*) for 30 min. The pellet was vortexed, rinsed in distilled water 8–10 times, and then resuspended in distilled water. Two drops of $AgNO_3$ were added to the suspension after the rinses to verify that all chloride was removed. The suspension was then autoclaved at 122 °C and 0.103 MPa for 15 min to ensure sterility. The concentration (w/v) of the stock Mt suspension is 6.0 mg/ml. Visual inspection of the Mt suspension indicated no noticeable settling or aggregation during storage.

2.1.2. Tryptone Mt aggregates

Tryptone consists of oligomers, 50% of which have a molecular mass less than 500 Da; 33% are monomers, and only 10% of the peptides have a molecular mass greater than 2 kDa. A complete compositional breakdown of tryptone was obtained from the vendor website (Bactotryptone, Becton Dickinson; San Jose, CA; URL: http://www.bd.com/ds/technicalCenter/). Typical certificate of analysis values reported by the vendor are less than 8% ash, 13.8% nitrogen (Kjeldahl), and 6.6% amino nitrogen (modified Sorensen). Tryptone stock solutions were prepared by dissolving tryptone powder in distilled water at a concentration of 0.1 g/ml followed by autoclaving at 122 °C and 0.103 MPa for 15 min. Tryptone Mt aggregates (Mt_{TP}) were prepared by mixing 9 ml of Mt suspension with 1 ml of dilutions of the tryptone stock to give tryptone concentrations ranging from 0.5 mg/ml to 10 mg/ml mixed with 5.4 mg/ml of Mt in 10 ml suspensions. Aggregates were allowed to stand for up to 72 h prior to acquiring the XRD pattern. The aggregates were centrifuged at 3600 rpm (2700 *g*) for 10 min. The supernatant components were analyzed by optical absorption and the pellets collected for further analysis. For XRD analysis, the pellets were smeared on glass slides. Samples for SEM were fixed and mounted on aluminum stubs, coated with palladium–gold alloy. For HRTEM, pellets were vortexed and fixed in plastic resin (Leser et al., 2009). The resin samples were microtomed (UltraCut6 Ultramicrotome, Leica, Buffalo Grove, IL) to produce ~70 nm slices for HRTEM. The microtomed slices were placed on carbon-coated grids for TEM analysis.

2.2. X-ray diffraction

XRD patterns were acquired with a PANalytical X'Pert Pro equipped with a chromium cathode tube producing Cu Kα radiation at 1.5418 Å; a Ni foil filter to attenuate Cu Kβ emission; and a PixCel 1-D detector array. A divergence slit of 1/16th of a degree and an anti-scatter slit of 5.7 mm were used to minimize background scatter at low 2θ. Scans were run in the range of 4° to 35° 2θ at a step size of 0.007° and a scan rate of 0.35 steps/s.

2.3. UV absorption

UV absorption spectroscopy (Cary 500 UV–Vis absorption spectrophotometer, Agilent Technologies Inc., Foster City, CA) in the range of 250 to 300 nm was used to estimate the fraction of tryptone sorbed (intercalated or surface adsorbed) onto the Mt based on the relative optical absorption of the Mt_{TP} supernatant compared to a stock tryptone solution. The supernatant extinction spectra in the 400 to 700 nm range, a non-absorbing region for both Mt and tryptone, was used to estimate the scattering losses from (non-aggregated) Mt tactoids that remained in suspension. To keep the transmission above the noise, the supernatant was diluted 5×. Spectra were collected in 1 cm path length quartz cuvettes.