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Differential capacity of kaolinite and birnessite to protect surface associated proteins against thermal degradation

Stephany S. Chacon[a,*], Manuel García-Jaramillo[a,b], Suet Yi Liu[c], Musahid Ahmed[c], Markus Kleber[a]

[a] Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, United States
[b] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), P.O. Box 1052, 41080 Seville, Spain
[c] Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, United States

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ABSTRACT

It is widely accepted that soil organic carbon cycling depends on the presence and catalytic functionality of extracellular enzymes. Recent reports suggest that combusted and autoclaved soils may have the capacity to degrade organic test substrates to a larger extent than the living, enzyme-bearing soils. In search of the underlying mechanisms, we adsorbed Beta-Glucosidase (BG) and Bovine Serum Albumin (BSA) on the phyllosilicate kaolinite and the manganese oxide birnessite at pH 5 and pH 7. The protein-mineral samples were then subjected to gradual energy inputs of a magnitude equivalent to naturally occurring wildfire events. The abundance and molecular masses of desorbed organic compounds were recorded after ionization with tunable synchrotron vacuum ultraviolet radiation (VUV). The mechanisms controlling the fate of proteins varied with mineralogy. Kaolinite adsorbed protein largely through hydrophobic interactions and, even at large energy inputs, produced negligible amounts of desorption fragments compared to birnessite. Acid birnessite adsorbed protein through coulombic forces at low energy levels, became a hydrolyzing catalyst at low energies and low pH, and eventually turned into a reactant involving disintegration of both mineral and protein at higher energy inputs. Fragmentation of proteins was energy dependent and did not occur below an energy threshold of 0.20 MW cm^{-2}. Neither signal abundance nor signal intensity were a function of protein size. Above the energy threshold value, BG that had been adsorbed to birnessite at pH 7 showed an increase in signal abundance with increasing energy applications. Signal intensities differed with adsorption pH for BSA but only at the highest energy level applied. Our results indicate that proteins adsorbed to kaolinite may remain intact after exposure to such energy inputs as can be expected to occur in natural ecosystems. Protein fragmentation and concomitant loss of functionality must be expected in surface soils replete with pedogenic manganese oxides. We conclude that minerals can do both: protect enzymes at high energy intensities in the case of kaolinite and, in the case of birnessite, substitute for and even exceed the oxidative functionality that may have been lost when unprotected oxidative enzymes were denatured at high energy inputs.

1. Introduction

The paradigm of "mineral control" (Torn et al., 1997) posits that the mineral matrix protects soil organic matter (SOM) against microbial decomposition by regulating accessibility and bioavailability of organic substrates through the processes of aggregation and adsorption. Past research into the phenomenon has concentrated on the stabilizing effects of the mineral matrix (Baldock and Skjemstad, 2000; Rasmussen et al., 2006; Basile-Doelsch et al., 2007; Kemmitt et al., 2008; Marin-Spiotta et al., 2008; Schmidt et al., 2011; Dungait et al., 2012; Torn et al., 2013; Doetterl et al., 2015), i.e. the ability of minerals to retard the decomposition of organic substrates. But this research focus is contrasted by long standing evidence for the ability of certain soil minerals to do the exact opposite: promote organic matter degradation by effectively oxidizing (Stone, 1987) and hydrolyzing (Torrents and Stone, 1993) a plethora of organic compounds. Apparently, the mineral matrix has a fundamental capacity to do both: protect organic substrates from decomposition as well as facilitate their disintegration.

Extracellular enzymes depolymerize soil organic matter (SOM) for transport through the cell membrane for full mineralization. To successfully complete their task, extracellular enzymes must retain activity in soil over reasonable time scales. This means they must survive

* Corresponding author.
E-mail address: Stephany.chacon@oregonstate.edu (S.S. Chacon).

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mineral interactions with minimal impediments to their functionality. In fact, attachment to mineral surfaces may prove beneficial regarding functionality. Upon adsorption, enzyme activity decreases (Quiquampoix, 2008), but this negative effect can be balanced by some degree of protection from microbial predation. An extension of functional life span may result but at a lesser degree of catalytic efficiency than for the free enzyme (Yan et al., 2010). Some noteworthy exceptions where extracellular enzymes have greater reaction rates when adsorbed than free have been observed (Allison, 2006; Fiorito et al., 2008). Given that the mineral phase contributes approximately half the volume of an average surface soil, attachment to mineral surfaces appears to be inevitable for many if not all extracellular enzymes. But what if an enzyme encounters one of those minerals that have the demonstrated ability (Sunda and Kieber, 1994; Miltner and Zech, 1999) to either oxidize or hydrolyze organic substrates?

Reports of the fate of proteins at oxidizing/hydrolyzing mineral surfaces are scarce but particularly revealing. A prion protein was fully fragmented in soil upon interacting with birnessite in solution at pH 5 (Russo et al., 2009). Protein disintegration after contact with birnessite surfaces was recently confirmed by Reardon et al. (2016) and the mechanism of fragmentation identified as mineral-catalyzed hydrolysis. The reports of Russo et al. (2009) and Reardon et al. (2016) are in contrast to the work of Naidja et al. (2002), who identified birnessite as a strong adsorbent for protein. If we assume both types of observations to be valid, i.e. when birnessite can act towards protein as both, protective sorbent and fragmenting catalyst, then there is a need to identify mechanisms and circumstances that determine when a mineral surface changes its role.

To constrain this issue, it is useful to recall that the main mechanisms of protein – mineral interactions include electrostatic attraction and repulsion, hydrogen bonding, hydrophobic interactions, and entropy driven conformational change (Boyd and Mortland, 1990; Craig and Collins, 2002). Among these four mechanisms, electrostatic interactions are the ones that are most susceptible to environmental controls such as soil pH and should therefore receive initial attention. The remaining three factors (hydrophobic interactions, hydrogen bonding and ability to change conformation upon adsorption) are largely determined by protein type and molecular size (Balcke et al., 2002; Sander et al., 2010). We deduced that an attempt to investigate the requirements for an abrupt change in the outcome of mineral – organic interactions should include some variation in protein size and in protein responsiveness to electrostatic forces, the former reflected in molecular mass and the latter modified by variation of the isoelectric point of the protein (Quiquampoix et al., 1995; Norde, 2008). We further decided to vary energy input to the system based on a recent observation of temperature-induced variation in the presumably abiotic reactivity of mineral surfaces. This phenomenon was reported by Bach et al. (2013) and Blankinship et al. (2014) who independently performed measurements of polyphenol oxidase (PPO) and peroxidase (PER) enzyme activities in soil samples. In their attempt to quantify any background contribution of the mineral matrix, Bach et al. (2013) and Blankinship et al. (2014) autoclaved and/or combusted their soils to sterilize and completely denature the enzymes and thus supposedly eliminated any enzymatic contribution to their assays. Yet some of the combusted and autoclaved soils degraded the aromatic test substrate (L-DOPA) to a larger extent than the living, enzyme bearing soils, with soils combusted at 500 °C showing greater efficacy of oxidation than autoclaved soils. These observations led us to speculate that external energy input, as it occurs in the topsoils of many fire–prone ecosystems, may have the potential to enhance the general capacity of the mineral matrix to fragment organic matter and may potentially act to convert “sorptive” into “reactive” mineral surfaces.

Consequently, the overarching goal of this research was to contribute to a mechanistic understanding of the dual role of mineral surfaces as both (i) stabilizing agents for soil protein and (ii) catalysts or reactants involved in their abiotic fragmentation. Previous evidence from Russo et al. (2009) and Reardon et al. (2016) looked at the supernatant in their samples, but these studies did not investigate the reactivity of minerals towards proteins in the absence of the aqueous phase, such as in periodically dry topsoils. Hence, our conceptual approach was to document the fate of protein on dry mineral surfaces of different potential surface reactivity while varying four known controls on protein-mineral interactions:

(i) protein size (measured in kDa),
(ii) mineral surface type (sorbent type versus known catalyst/reactant type mineral)
(iii) surface charge status of proteins and minerals as controlled by soil pH (varying pH as well as the isoelectric point of the proteins and the point of zero charge of the minerals),
(iv) the energy input to the protein-mineral association (subjecting the protein-mineral system to progressively higher inputs of precisely dosed laser energy)

Our experimental design consisted of reacting two types of protein with two kinds of minerals in a slurry at two pH levels bracketing the main pH region for many soils (pH 5 and pH 7). After drying on an inert silica wafer, the protein-mineral mixtures were inserted into a vacuum chamber, subjected to a defined input of laser energy and the abundance and chemical composition of desorbed organic compounds was recorded after Vacuum-Ultraviolet (VUV) photoionization, using a time of flight Mass Spectrometer. To do so, we took advantage of an experimental setup at Beamline 9.0.2 of the Advanced Light Source at Berkeley, CA. Our experimental approach allowed us to test the following hypotheses:

(1) The extent of protein adsorption at a mineral surface will be proportional to the extent of attractive electrostatic interactions.
(2) With constant protein size and pH, fragmentation is a function of mineralogy, even in the absence of an aqueous phase.
(3) The number of peptide signals in the mass spectrum is a function of
   a) protein size (constant energy and pH)
   b) pH (constant energy and protein size)
   c) energy applied (constant protein size and pH)
(4) With constant protein size and pH, the intensity of signals in the mass spectrum is a function of energy applied.

2. Materials and methods

We selected the readily available proteins Beta Glucosidase (BG) and Bovine Serum Albumin (BSA) to achieve variation in size and isoelectric point (pI) of the protein. Proteins were adsorbed to acid birnessite (catalyst/reactant type mineral) and kaolinite (sorbent type mineral). The proteins were allowed to interact with the minerals at pH 5 and pH 7 to create variation in the extent of electrostatic attraction and repulsion between constituents (Fig. 1).

2.1. Materials

Beta-glucosidase and bovine serum albumin were obtained from Sigma Aldrich and used directly from their containers. Acid birnessite was synthesized using the protocol described by Villalobos et al. (2003) and purified with a 1000 kDa dialysis tube until conductivity of supernatant was less than 40 μS cm^{-1}. The dialyzed birnessite was freeze-dried and stored at room temperature in amber glass bottles. Kaolinite (KGa-1b) was ordered from the Clay Minerals Society Source Clays Repository and exchanged with sodium chloride to standardize the cation population at the surface. The Na-kaolinite was washed until ionic conductivity was less than 40 μS cm^{-1} and freeze-dried. The point of zero charge for birnessite was measured using the Prolonged Salt Titration (PST) method (reported in SI). The general properties of the proteins and minerals are reported in Table 1.