Mineral Surfaces As Agents of Environmental Proteolysis: Mechanisms and Controls

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ABSTRACT

We investigated the extent to which contact with mineral surfaces affected the molecular integrity of a model protein, with an emphasis on identifying the mechanisms (hydrolysis, oxidation) and conditions leading to protein alteration. To this end, we studied the ability of four mineral surface archetypes (negatively charged, positively charged, neutral, redox-active) to abiotically fragment a well-characterized protein (GB1) as a function of pH and contact time. GB1 was exposed to the soil minerals montmorillonite, goethite, kaolinite, and birnessite at pH 5 and pH 7 for 1, 8, 24, and 168 hours and the supernatant was screened for peptide fragments using Tandem Mass Spectrometry. To distinguish between products of oxidative and hydrolytic cleavage, we combined results from the SEQUEST algorithm, which identifies protein fragments that were cleaved hydrolytically, with the output of a deconvolution algorithm (DECON-Routine) designed to identify oxidation fragments. All four minerals were able to induce protein cleavage. Manganese oxide was effective at both hydrolytic and oxidative cleavage. The fact that phyllosilicates – which are not redox active - induced oxidative cleavage indicates that surfaces acted as catalysts and not as reactants. Our results extend previous observations of proteolytic capabilities in soil minerals to the groups of phyllosilicates and Fe-oxides. We identified structural regions of the protein with particularly high susceptibility to cleavage (loops and beta strands) as well as regions that were entirely unaffected (alpha helix).

INTRODUCTION

Proteins are the tools, engines, and catalysts among biomolecules and hence are indispensable elements of a thriving biosphere. For this reason, a mechanistic understanding of the constraints on the functional lifespan of protein and peptides is highly desirable. Since protein functionality is tied to the three-dimensional structure and the accessibility of the active site, catalytic proteins in soils and sediments often lose a fraction of their maximum activity as a result of adsorption to mineral surfaces [1,2]. However, this negative effect may be offset by the protective nature of the mineral-protein association, i.e., a loss in efficiency may be balanced or even overcome by a gain in lifespan. The persistence of a protein involved in a mineral association, and, by extension, its functional lifespan, are thought to be constrained by eventual microbial degradation. However, previous research [3-5] demonstrated abiotic fragmentation of protein by manganese oxides, but little is known about the propensity of other relevant surface archetypes (such as negatively charged, positively charged, and predominantly neutral surfaces) to fragment a protein.

Pedogenic oxides can act as oxidants towards reduced organic compounds, i.e., they may accept electrons and become chemically modified in the process. However, phyllosilicates typically contribute most of the reactive surface area in soils and sediments. These minerals vary in specific surface area and surface site density (surface sites = single coordinated hydroxyls [6]) providing three major options for sorptive interactions: (i) zero charge surfaces; (ii) permanently charged surfaces, and (iii) hydroxylated surfaces. Cleavage of protein by a phyllosilicate has been reported at least once when Johnson, et al. [7] adsorbed prion protein to montmorillonite, encouraging us to investigate the following research questions:

1. How does the susceptibility of a protein to disintegration vary as a function of exposure to the four major mineral surface archetypes in soil (zero-charge, permanent charge, hydroxylated, and redox active) ?
2. Are fragmentation patterns random or do they show signs of regularity that may help to constrain mechanisms of mineral induced protein fragmentation?
3. How do the mechanisms of protein fragmentation and disintegration, such as oxidation and hydrolysis, depend on the proton concentration in the solvent?
4. What are the kinetics of protein disintegration by mineral surfaces - how long do proteins need to be in contact with mineral surfaces for disintegration reactions to occur?

Our conceptual approach involved exposing a well-characterized and structurally stable model protein to the four mineral surface archetypes mentioned above at two pH levels in an aqueous system while monitoring the eventual production of fragmentation products in the supernatant for one week. Kaolinite is a 1:1 phyllosilicate with little isomorphic substitution, rendering basal planes mostly hydrophobic. Montmorillonite is a 2:1 phyllosilicate with isomorphic substitution predominantly in the inner octahedral sheet, creating basal planes where hydrophobic microsites alternate with areas of weak permanent charge [8, 9]. The montmorillonite interlayer space can expand and intercalate small organic molecules. It has been posited that unfolded peptide fragments may preferentially associate with broken edges of montmorillonite particles, with amino acid side chains pointing into expanded interlayer spaces [10]. All phyllosilicates may possess some reactive hydroxyls at the edges of octahedral sheets. Kaolinite and montmorillonite contain similar adsorption sites (zero charge and permanent charge basal surfaces combined with hydroxylated edges), yet at different proportions. Goethite is a pedogenic iron oxide that exhibits positively

charged hydroxylated surfaces below a pH of 9. Birnessite is a redox active phyllomanganate known to participate in oxidation reactions with organic matter and to fragment proteins in acidic solutions[11]. The model protein (GB1) is the B-1 domain of Protein G from Streptococcal bacteria. It is an extracellular domain of Protein G that binds human immunoglobulin protein IgG. We chose GB1 for our studies because it is highly soluble, thermally stable, exhibits beta-strand and alpha helix secondary structure, reversibly refolds and has been the subject of extensive structural and biophysical analysis using many techniques. These characteristics provide confidence that the results will be representative of many stable, globular proteins, including extracellular enzymes. GB1 has an alpha helix, beta strand, and loop regions, the latter being somewhat flexible connections between alpha helices and beta strands. To detect any minute chemical modifications on GB1 after interacting with minerals even at low concentrations, we used liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). We expected this data-rich technique to provide us with a comprehensive overview of the fragmentation products.

Materials and Methods

GB1 was produced using the method of Reardon, et al. [3]. In short, *Escherichia coli* cells with a plasmid encoding the GB1 gene were grown at 37°C in Luria broth until optical densities (OD600 nm) reached ~0.6. Protein expression was induced by the addition of IPTG at a final concentration of 1 mM for 6 hours. Cell pellets were lysed, and GB1 was purified using affinity chromatography. GB1 was buffer exchanged into MilliQ water and stored frozen until use.

We obtained montmorillonite (STx-1b) and kaolinite (KGa-1) from the Clay Resource Repository (Purdue University, West Lafayette). Charge balancing cations were exchanged for Na following the procedure of Soukup, et al. [12]. Birnessite was synthesized using the acid birnessite

protocol in Villalobos [13]. Goethite was synthesized following the protocol of Atkinson, et al. [14]. The synthesized minerals were dialyzed against Milli-Q water using a membrane rated at 1000 MWCO. All minerals were washed or dialyzed with MilliQ water until the electric conductivity of the filtrate was less than 40 $\mu S\ cm^{-1}$. Minerals were freeze-dried and stored in amber bottles until further use. Mineral properties are reported in Table S2.

General details on the preparation of protein-mineral samples and analysis of the supernatant are provided in the supplemental information. We combined GB1 at 0.4 mg protein mL^{-1} with 20 mg of mineral at a total volume of 1 mL. The pH of the dispersion was adjusted to the desired value (5 or 7) and the sample allowed to react for up to one week without further adjustment of pH. Sample aliquots were removed at 1, 8, 24, and 168 hours. Our strategy to identify the products of hydrolytic and oxidative protein cleavage consisted of three steps (Figure 1). We first analyzed the supernatants using a reverse phase liquid chromatography separation coupled to a Tandem Mass Spectrometer; details are provided in the SI. The resulting data were then processed using the SEQUEST algorithm [15]. Because the SEQUEST algorithm makes the inherent assumption of hydrolytic cleavage, we needed an additional step to identify potential oxidation fragments. This was done with the help of a procedure that we developed for the purpose and that we call the DECON - Routine (Figure 1). The details of our analysis are described in the SI.

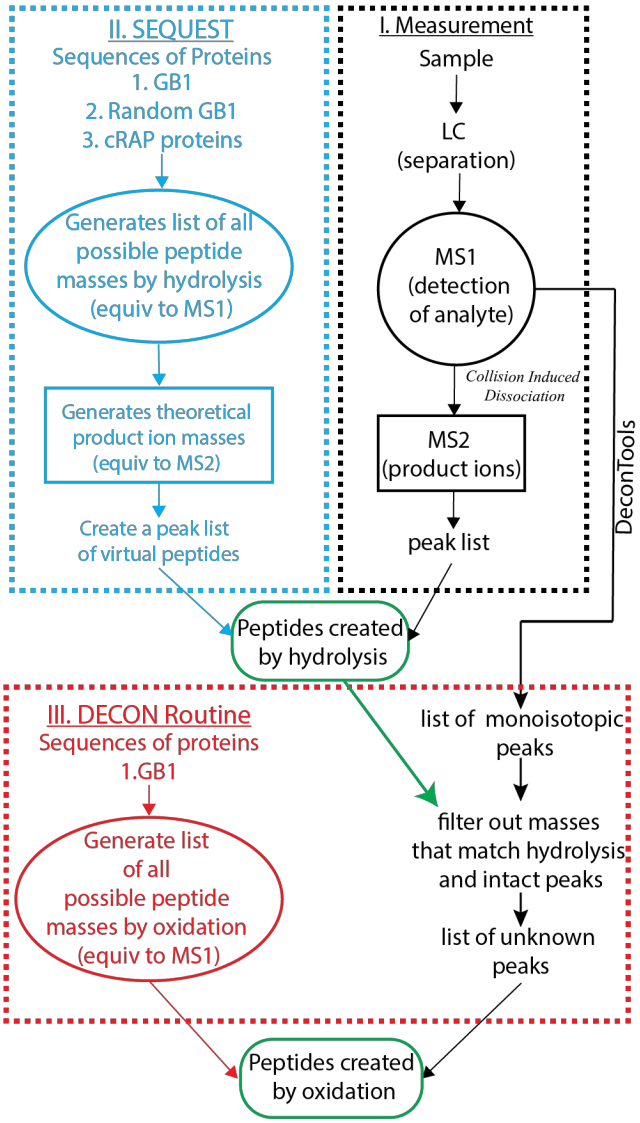

Figure 1. The sequence of events leading to the identification of protein fragments generated by hydrolysis and oxidation reactions. Experimental measurements are represented in black, the SEQUEST analysis for the identification of hydrolysis products is represented in blue and the DECON-Routine for the identification of oxidation products in red. Note that the DECON-Routine is applied to MS1 data after filtering out any hydrolysis products identified by the SEQUEST algorithm. Because it does not process MS2 data, the DECON - Routine for the identification of oxidation products does not take full advantage of the MS/MS approach and may return false positives.

We used the following metrics to test our hypotheses. The number of cleavage sites (discrete number) informs about how often and where the protein has been cleaved. The signal intensity per fragment (ion count based on the monoisotopic mass of peptide fragment) is used to

assess the relative abundance of a given fragment, and the number of unique peptide fragments detected (discrete number) is used to compare the effects of variations in pH and exposure time. To reduce the possibility of false positives, we removed peptides that were observed in the control data from subsequent analysis.

Results

All minerals induce protein fragmentation

The susceptibility of our protein GB1 to disintegration was sensitive to the type of mineral surface encountered, with the number of protein cleavage sites declining in the order: birnessite >> goethite ≅ kaolinite ≅ montmorillonite. When oxidation and hydrolysis mechanisms were considered separately, the trend remained with the qualification that oxidative cleavage occurred about 2.5 times more frequently than hydrolytic cleavage. There were a total of 46 sites on the protein where oxidative cleavage occurred with any of the minerals, while there were only 17 sites that showed hydrolytic cleavage.(Table 1). Goethite did not induce hydrolytic cleavage of GB1 (Figure 2; Table 1). All four minerals induced oxidative cleavage between residues 38/39 (amino acids G and V, Figure 2).

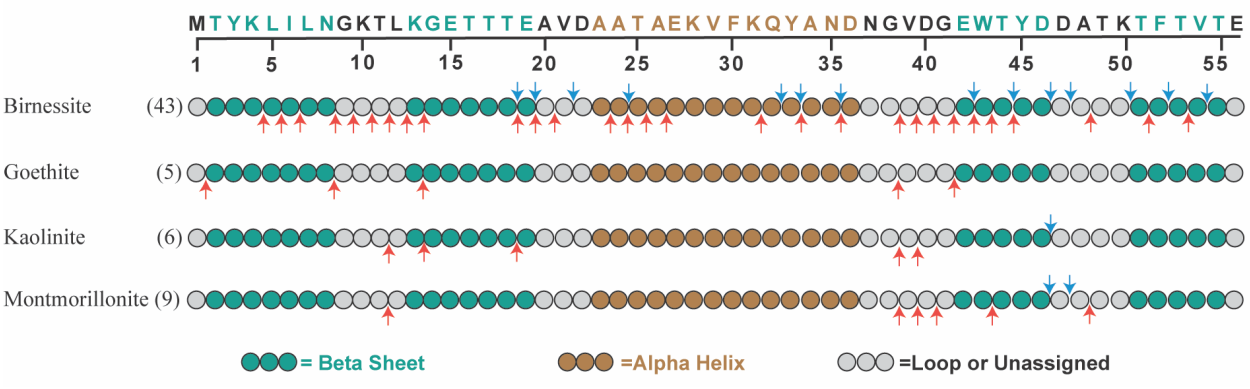

Figure 2. The cleavage sites on GB1 as a function of mineral exposure. The minerals tested are identified on the left, next to a schematic of the GB1. The total number of cleavage sites are noted within parentheses. The top row has the one letter amino acid sequence of GB1. Below the sequence is the amino acid residue number. Letters and circles colored green designate amino acids within the beta sheets. The brown colored letters and circles designate amino acids within the alpha helix. Letters and circles in black and grey designate amino acids that are unassigned or in a loop region. Blue arrows indicate the location of hydrolytic cleavage sites, red arrows indicate the location of oxidative cleavage.

Table 1. The abundance of cleavage sites per structural region of the protein and mineral surface type.

Oxidative Cleavage

Cleavage site	Residue #	Birn	Goe	Kao	Mont	SUM
inside alpha helix	24-36	7	0	0	0	7
between helix and loop	22-23;36-37	0	0	0	0	0
inside beta strand	2-8; 13-19;42-46;51-55	10	1	2	2	5
between beta strand and loop	1-2; 8-9; 12-13; 19-20; 41-42; 46-47; 51-52; 55-57	4	2	0	1	7
inside loop	9-12; 20-21; 37-41; 47-50;	8	2	3	4	17
	SUM:	29	5	5	7	46

Hydrolytic Cleavage

Cleavage site	Residue #	Birn	Goe	Kao	Mont	SUM
inside alpha helix	24-36	4	0	0	0	4

between helix and loop	23-24;36-37	0	0	0	0	0
inside beta strand	2-8; 13-19;42-46;51-55	5	0	0	0	5
between beta strand and loop	1-2; 8-9; 12-13; 19-20; 41-42; 46-47; 51-52; 55-57	3	0	1	1	5
inside loop	9-12; 20-21; 37-41; 47-50;	2	0	0	1	3
	SUM:	14	0	1	2	17
	TOTAL	43	5	6	9	

Figure 2 illustrates that exposure to birnessite generated a greater variety of fragments than exposure to phyllosilicate surfaces. We observed variations in the total ion counts for each fragment as indicated by the color code used in Figure 3. The peptides generated after interaction with birnessite tend to have higher ion counts than the ones resulting from interactions with phyllosilicates and goethite indicating more efficient fragmentation. Although this observation is in line with previous reports[3], the non-quantitative nature of our analytical method prevents us from drawing quantitative inference. We did not recognize an obvious correlation between fragment length and ion count.

Intact protein was detected in the supernatants from birnessite, montmorillonite, and kaolinite samples (Figure 3, row 1). The supernatants of kaolinite and montmorillonite contained two modified versions of GB1 (second and third rows of Panel A, Figure 3, Table S2). The fragment "InH2" is GB1 with an oxidized methionine. The fragment "InH3" is GB1 with the first methionine cleaved off. Because these modifications were detected in the mineral-free control and are known to be common post-translational modifications of protein expression in *E. coli* cells [16,17], we do not consider these fragments as products of mineral-induced alteration. The fragment "InO1" (first row of Panel B, Figure 3, Table S3) is a GB1 missing the four terminal amino acids.

Because it is not detected in control, we consider this fragment as resulting from oxidative cleavage by birnessite.

The SEQUEST algorithm revealed evidence for alteration of aromatic side chains among hydrolytic fragments, affecting the amino acids phenylalanine (F), tyrosine (Y), and tryptophan (W). The alteration of the side chains involved addition of oxygen or hydrolxyls to the aromatic side chains. This is simply modifying the side chains of the peptides but not resulting in the oxidation of the protein backbone. Modification of amino acid side chains were only seen after exposure to birnessite (Tables S2, Figure 3) and not for the other minerals. Fragments with oxidized amino acid sides chains are H8, H9, H13, H15, H22 (Table S3).

Fragmentation patterns are not entirely random

The propensity of our model protein for cleavage was not constant across minerals, and the positions of the cleavage sites were not random (Figure 2 and Table 1). For instance, the immediate ends of the alpha helix (residues 22-23 and residues 36-27) were not cleaved by any mineral (Table 1). We also point out that the amino acid combination DA occurs twice in the chain, at residues 23-24 at the beginning of the alpha helix, and again at residues 47-48 inside one of the loop regions. Although the latter is a cleavage site for both hydrolysis and oxidation at two surface types (birnessite and montmorillonite), the former is not cleaved by any of the minerals.

Some commonality seems to exist between kaolinite and montmorillonite because they share four oxidative cleavage sites (out of 5 and 7 total, respectively) whereas goethite and kaolinite share three oxidative cleavage sites (out of 5 total, Figure 2). Between goethite and montmorillonite, only one common oxidative cleavage site was observed.

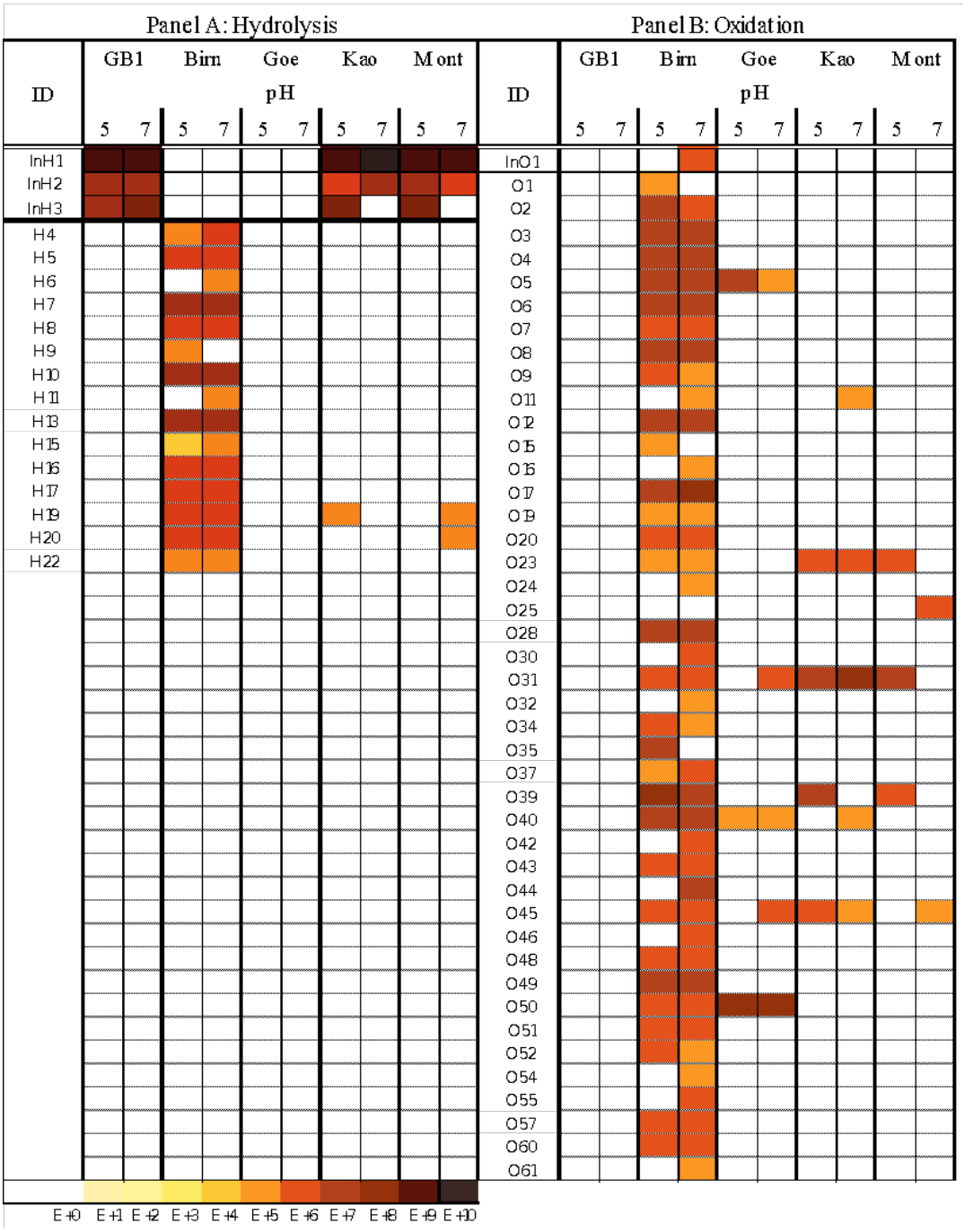

Figure 3. Heat map indicating the magnitude of the ion counts for the observed fragments. Panel A represents fragments generated by hydrolytic cleavage; Panel B has fragments generated by oxidative cleavage. Ion counts were summed over four time points and comprised three replicate mineral-protein exposure samples per time point and treatment. The signals plotted are those that

remained after application of the rigorous SEQUEST and DECON-Routine filtering processes. The color gradient spans 10 orders of magnitude.

Looking only at oxidative cleavage, we note that out of the eight peptides that produced intense ion counts in goethite, kaolinite, and montmorillonite samples, five were from cleavage of the 3rd beta-sheet and the 4th loop. The same regions of the protein were susceptible to hydrolytic cleavage in the kaolinite and montmorillonite samples. The other three oxidation-derived peptides found in the phyllosilicate and goethite samples were within the 2nd beta sheet and 1st loop regions. The complete list of peptides that include the sequences of the excluded peptides are provided in Tables S2 and S3.

Cleavage is not restricted to acidic pH

Previous studies [3-5] indicated that the propensity of proteins to become cleaved increased with decreasing pH, suggesting the process might be particularly efficient in, and possibly restricted to, acidic soils and environments. When we cumulated the number of unique peptide fragments over all four time points (Figure 4), the previously noted pH dependence of protein fragmentation by birnessite disappeared. The phyllosilicates, however, exhibited opposing trends: kaolinite hydrolytically cleaved the protein at pH 5, whereas montmorillonite did so only at pH 7. The number of unique oxidative fragments detected by mass spectrometry did not show an obvious dependency within the pH range considered here.

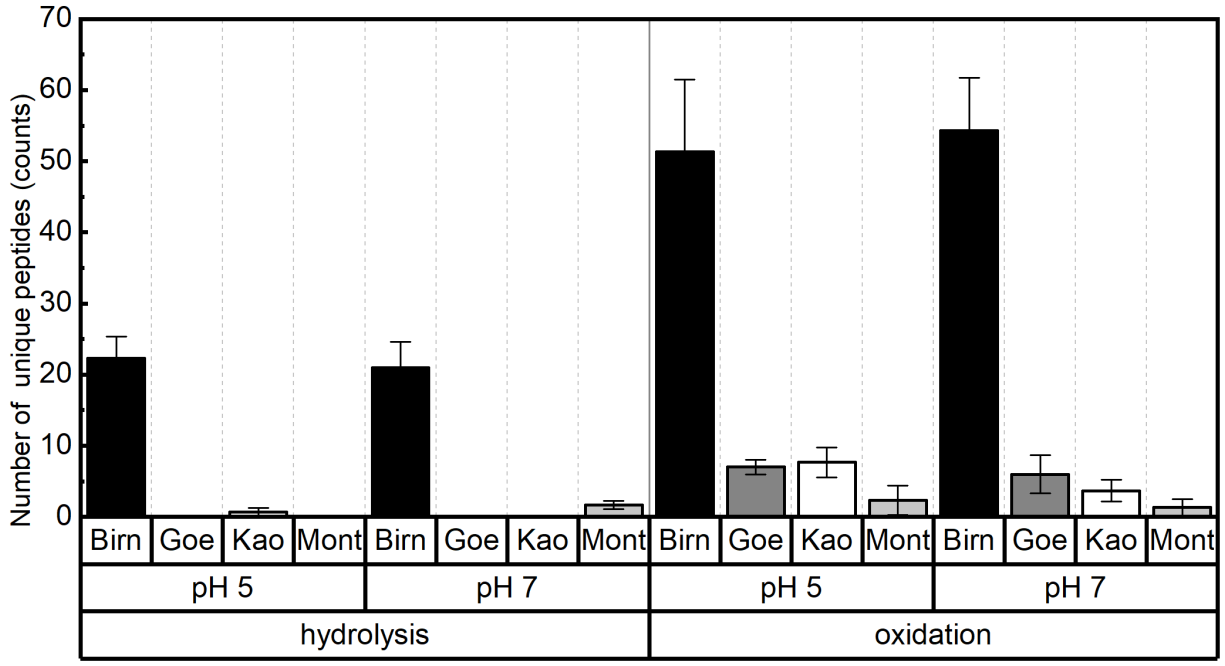

Figure 4. The number of unique peptides observed, accumulated over four time points. Error bars indicate variability expressed as standard deviations across replicates and time points (n = 12). Data are organized by cleavage mechanism, pH and mineral surface type.

Protein fragmentation changes with exposure time

Hydrolysis products did not appear in the supernatant from phyllosilicate samples until 24 hours had elapsed. The time course of fragment appearance (number of unique peptides) in phyllosilicate samples differed with pH, with oxidation fragments becoming more prevalent through time in kaolinite samples at pH 5, wherease hydrolytic fragments in the montmorillonite samples increased with time at pH 7. The number of unique peptides generated by oxidation after exposure to goethite did not vary significantly through time or with pH. Slightly higher numbers of unique peptides from oxidative cleavage were detected in kaolinite samples at pH 7 than at pH 5 after 8 hours,

whereas the abundance of oxidation derived peptides found at pH 7 in montmorillonite diminished after 24 hours of exposure.

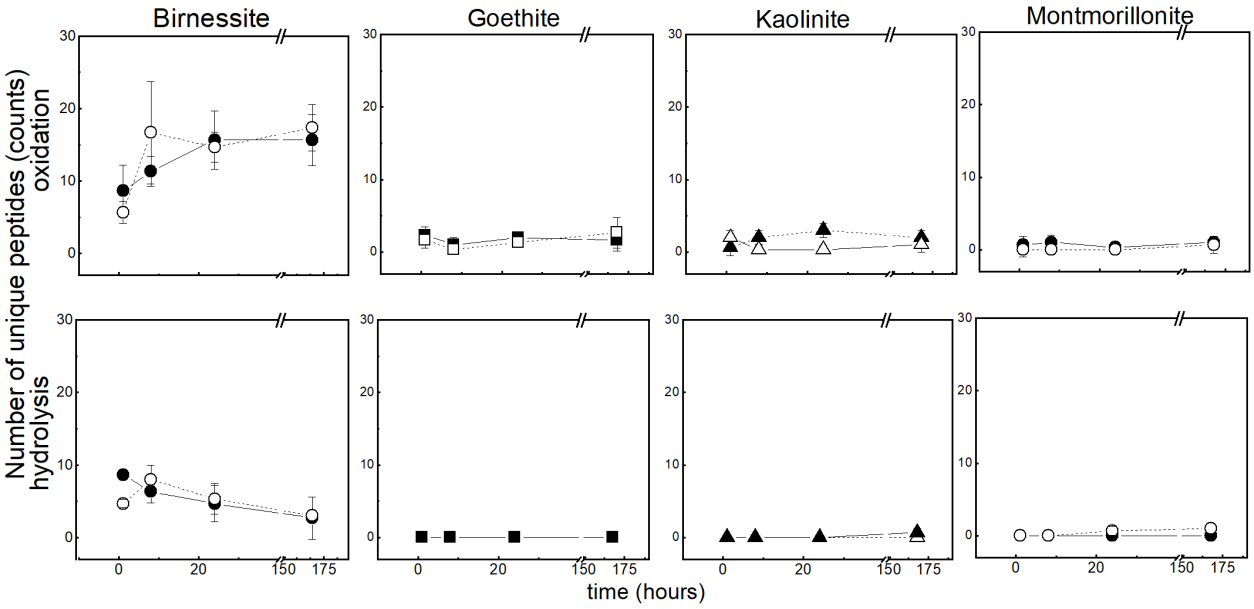

Figure 5. The effect of exposure time on the number of unique peptides generated by hydrolysis and oxidation at two pH values. Symbols represent mean values among three replicate samples, with error bars indicating the corresponding standard deviation. Closed symbols and straight lines are pH 5, open signals and dotted lines are respective values at pH 7.

Discussion

Minerals may have both a destructive and a protective role in cycling organic matter through soils and sediments. Redox active minerals such as manganese oxides are long known as agents of organic matter degradation[18,19]. By contrast, poorly crystalline minerals[6, 20] and phyllosilicate clay minerals[21] are generally viewed as protective towards organic matter. Although there has been conflicting evidence regarding the protective capacity of phyllosilicate clay minerals for soil organic matter (strong correlation with r = 0.86; n = 65 between clay content and

261 OM content reported by Nichols [22] for warm grassland soils; very weak correlation r = 0.21; n = 83
262 reported by McDaniel and Munn [23] for cool grassland soils), there is widespread consensus that
263 proteins have a particular affinity for phyllosilicates [24]. However, *close association with minerals*
264 *often reduces the functionality of adsorbed protein* [25]*. The main reasons for such an impediment in*
265 *functionality, particularly regarding adsorbed enzymes, have so far been attributed to a)*
266 *conformational changes to an adsorbed protein and b) to concealment of the active site*[26, 27]*"* (Figure
267 1 therein 27). Our discovery that even kaolinite can cause protein fragmentation adds to this picture
268 by suggesting that adsorbed protein will undergo eventual, albeit slow, fragmentation. The
269 resulting peptide fragments may become readsorbed (consistent with some of the time series data
270 presented in Figure 5) and hence remain protected against microbial decomposition, which would
271 render fragmented proteinaceous matter still protected but no longer functional.
272 Both kaolinitic and smectitic minerals are marketed as catalysts in industrial applications[28], thus
273 it is no surprise that the mechanisms through which they fragment protein involve catalytic
274 hydrolysis and catalytic oxidation. The fact that the potentially redox-active manganese oxide
275 behaves as a catalyst towards protein – at least under the conditions chosen for this experiment, is
276 more difficult to rationalize. When carbohydrate and the GB1 protein were exposed together to a
277 manganese oxide in an aqueous system[11], oxidation of carbohydrate was observed with concomitant
278 reduction of MnO_2 and production of Mn^{2+}. Interestingly, oxidation of carbohydrates and the
279 concomitant production of Mn(II) decreased when the proportion of GB1 in the system increased.
280 These observations support our view that in the systems investigated in the work presented here,
281 MnO_2 acts as a catalyst in both protein hydrolysis and protein oxidation, i.e., it fundamentally acts
282 in the same manner as the phyllosilicates and the Fe-oxide. We posit that, unlike standard MnO_2 –
283 organic matter reactions, where MnO_2 serves as a reactant that is consumed in the reaction, the

MnO_2 – protein reactions investigated here follow fundamentally different, namely catalytic reaction schemes. This latter finding seems to be generalizable across the four mineral archetypes investigated.

When comparing abiotic and biotic cleavage patterns we found mineral catalyzed proteolysis to share striking similarities with protease-mediated disassembly of proteins. In biochemistry research, limited proteolysis catalyzed by proteases has been used to determine protein structural domains[29,30,31]. Proteases preferentially cleave exposed and flexible loop regions of proteins [32]. Additional sites of cleavage also occur in areas susceptible to large conformational changes (local unfolding).[33] An evaluation of proteolytic events recorded in the CutDB[34] database showed cleavage to occur in helices and beta sheets as well, but to a lesser degree than in loop regions [35] Specifically, secondary structures were likely to break apart in such parts of the helices that tended to unfold and in the periphery of the beta strands[36]. Computational models indicated the appearance of a loop within the alpha helix of the protein when GB1 interacted with birnessite but not when it was paired with kaolinite, montmorillonite, or goethite[37]. This may explain why we detected fragmentation sites within the alpha helix when it interacted with birnessite but not when it interacted with the other soil minerals.

Previous research by Russo, et al. [4] saw a strong pH dependence of protein degradation by birnessite. Our previous NMR-based work [3] also showed faster fragmentation of GB1 at pH 5 than at pH 7, with the number and intensity of signals from fragmentation products increasing over time. However, in the mass spectrometry based data presented here, the number of unique hydrolytic peptides decreased over time. When reconciling this apparent contradiction it is important to keep in mind that mass spectrometry measures the number of unique peptides produced by the mineral interaction and is not a quantitative measure of the concentration of

reaction products or reaction efficiency. The mass spectrometry data shows that the number of unique peptides is similar between the two pH values tested, suggesting that the hydrolytic cleavage sites do not change based on the pH range that was used in this study. This again is consistent with our previous NMR data, which indicated that the reaction between birnessite and GB1 was slowed at pH 7, but that similar reaction products were produced at both pH values. When looking at the peptides resulting from catalytic oxidation by birnessite, we found that the number of unique peptide fragments tended to increase over time, even with our conservative filtering of oxidative fragments to reduce the number of false positives. However, the number of unique peptides remained similar between the two pH conditions for oxidation, suggesting that pH does not significantly alter the abundance of oxidation reaction products. A change in pH will affect the speciation of adsorption sites at hydroxylated surfaces. Low pH conditions below the point of zero charge (pzc) of a mineral surface would generate greater amounts of protonated surface hydroxyls ($M\text{-}OH_2^+$) than unprotonated surface hydroxyls ($M\text{-}O^{+1}$), which would yield a net positive surface charge. When pH rises above the pzc, a greater proportion of unprotonated hydroxyls will generate a net negative charge. Goethite (pzc 8.43; Table S2) is positively charged at both pH conditions investigated here. But birnessite has a very acidic pzc of pH 1.9. Adsorption sites are mostly unprotonated $Mn\text{-}O^-$, which may explain why the number of unique peptides is not different between pH 5 and pH 7. The only pH dependent surface charges in phyllosilicates are located at the edges. Deprotonation of the hydroxylated surfaces of kaolinite and montmorillonite only becomes relevant as pH conditions go above pH 5.0 (Liu et al 2013). Although the specific mechanism that catalyzes hydrolysis by kaolinite or montmorillonite is yet unknown, we posit that the sites that catalyze the hydrolysis of GB1 may be different between these two phyllosilicates given the appearance of peptides at different pH.

The kinetics of protein fragmentation vary with mineral surface type

The time dependence of protein- mineral interactions will require close attention in future work aiming to further elucidate the underlying mechanisms. Given enough time, even the supposedly protective phyllosilicates eventually fragmented GB1. The observed time dependence may include the adsorption process as well as the hydrolysis and oxidation reactions eventually leading to fragmentation. Figure 5 illustrates how the number of peptides detected evolved during the 168 hours of exposure in our experiment. Time-dependent variation in the presence of fragments in the supernatant was most pronounced in the birnessite treatment, but occurred in the presence of the other minerals as well, raising the possibility that newly generated fragments may find themselves readsorbed quickly. There is also the possibility that products of hydrolytic cleavage might turn out to be particularly susceptible to further oxidative alteration, and vice versa. The extent to which such interactive scenarios contribute to the overall picture seen in Figure 5 remains to be explored in future investigations.

Significance and implications. Phyllosilicates have traditionally been viewed as sorbents for protein[24]. Numerous industrial and technical applications take advantage of this sorptive capacity[10]. This understanding has informed a prevailing view of a stabilizing role of phyllosilicate surfaces for soil protein. Our work extends this view to include the insight that contact times on the order of several days may very well induce fragmentation of a protein that is adsorbed to a phyllosilicate. The detection of peptide products generated through oxidation of the protein backbone emphasizes the need to reevaluate fundamental assumptions in proteomic analysis of soils. Current tools for analyzing LC-MS/MS data (such as SEQUEST) typically focus on hydrolytic cleavage of proteins by proteases. Our results suggest that soil protein extracts will also contain oxidatively modified

353 proteins or peptides that could be overlooked by analysis techniques that focus solely on
354 hydrolysis. Expanding our assumptions to include modifications of proteins from mineral
355 interaction could increase the detection and identification of soil proteins during proteomic
356 analysis. Our work emphasizes the necessity to augment existing databases of peptide fragments
357 with information about oxidative fragmentation products and illustrates the need to develop
358 bioinformatics tools that can identify these oxidation products of proteins from secondary ion
359 spectra data.

360 Awareness of the capacity of minerals to fragment proteins will be important for assessments of
361 the functional lifespans of extracellular enzymes in soil and should be further investigated as a
362 potential reason for the frequently observed reduction in the catalytic activity of mineral-associated
363 enzymes [25]. Hence our work not only expands knowledge about the role of mineral surfaces in
364 determining the functional lifespan of protein in the terrestrial biosphere, but it also suggests that
365 the different elements of the protein secondary structure vary in their susceptibility to abiotic
366 cleavage.

367 The similarity of cleavage products between proteases and mineral surfaces indicates the abiotic
368 matrix in the soil can supply peptides to microorganisms in the absence of enzymes. Soils
369 containing a significant proportion of reactive mineral surfaces must be considered as able to
370 contribute relevant amounts of abiotically derived peptides to the microbiota for further
371 processing. This property should be accounted for when analyzing fluxes of carbon and nitrogen
372 among soil carbon pools and particularly in work investigating the ecological significance of low
373 molecular weight compounds. Less reactive minerals should not be considered inert. This means
374 the balance between biotic and abiotic pathways for peptide production in the soil will vary as a
375 function of mineralogy.

We conclude that our work reveals a previously underappreciated proteolytic functionality of soil minerals. By recognizing this functionality across different mineral groups, such as pedogenic oxides and phyllosilicates, this observation warrants an extension of the paradigm of mineral control of soil carbon stabilization [20] to include a more active role of the mineral phase than that of a passive and protective sorbent.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures, data processing, equations for the oxidation fragments, sample injection order (Table S1), hydrolysis and oxidation reaction examples (Figure S1), GB1 adsorption as a function of potential electrostatic attraction (Figure S2), mineral properties (Table S2), list of detected peptides generated by hydrolysis or intact protein (Table S3), list of detected peptides generated by oxidation (Table S4), table of peptides detected in phyllosilicate samples and blanks to determine carryover potential (Table S5), additional references.

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ASSOCIATED CONTENT

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ABBREVIATIONS

pI, isoelectric point; NMR nuclear magnetic resonance; LC-MS/MS , liquid chromatography coupled with tandem mass spectrometry; MS1, mass spectrometry, MS2, tandem mass spectrometry; GB1, Protein G B1 domain; IPTG, isopropyl-β-D-thiogalactoside chromatography; MWCO, molecular weight cut off; PVDF, polyvinylidene difluoride; BCA, bicinchoninic acid, Birn, birnessite; Goe, goethite; Kao, kaolinite; Mont, montmorillonite; EPR, electron paramagnetic resonance; SOM, soil organic matter; CutDB, proteolytic event database

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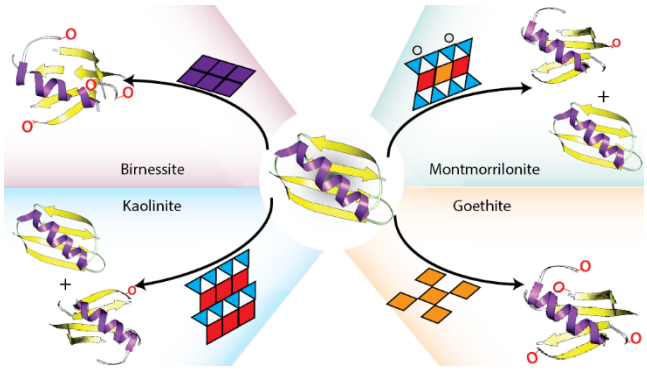

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