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Extracellular enzyme-clay mineral complexes: Enzyme adsorption, alteration of enzyme activity, and protection from photodegradation

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Abstract

Enzymes released extracellularly by micro-organisms have major functions in nutrient acquisition and organic matter degradation. Clay particles, common in many surface waters, can modify enzyme activity. Clay minerals are known to form aggregates with organic molecules, and the formation of enzyme-clay complexes could alter the level of activity. Montmorillonite clay and clay extracted from Elledge Lake (Tuscaloosa, Alabama) basin soil were combined with alkaline phosphatase, glucosidase, protease, and xylosidase solutions to assess adsorption and the effect of this adsorption on enzyme activity. Adsorption to Elledge Lake basin clay decreased alkaline phosphatase activity, and adsorption to montmorillonite was observed for all four enzymes with reductions in enzyme activities. Adsorption of substrate onto clay surfaces resulted in a concentration effect and increased enzyme activity associated with the particles. When enzyme-clay complexes were exposed to natural sunlight there was a decrease in enzyme activity, but this decrease was usually not significantly different from the adsorption only treatment. The formation of enzyme-clay complexes may serve to protect the enzymes from natural in situ photodegradation. The results indicate the complex interactive effects adsorption of enzymes to clay particles can have on the availability and capability of hydrolysis – reduction of enzyme reactivity, storage attached to clay particles with changes in transport and distribution, and protection from photodegradation.

Introduction

The importance of extracellular enzymes has been recognized in the entire range of aquatic habitats: planktonic, littoral, and sediment. These enzymes are produced by a variety of organisms in response to nutrient limitation (Sala et al. 2001) and to facilitate acquisition of carbon and energy by heterotrophs. Past studies of extracellular enzyme activity regulation have focused on the induction and repression of enzyme production and release (Jones and Lock 1989; Romaní and Sabater 2000), water chemistry and the biological community composition (Chappell and Goulder 1994a; Chappell and Goulder 1994b; Romaní and Sabater 1999; Romaní and Sabater 2000), the supply of organic matter (Meyer-Reil 1987; Vrba 1992; Mallet and Debroas 2001), and the complexation of enzymes with humic/polyphenolic substances (Wetzel 1991; Boavida and Wetzel 1998; Rao and Gianfreda 2000). Less attention has been given to the potential interaction between these dissolved enzymes and inorganic particles, specifically to clay minerals.

Clay–dissolved organic carbon interactions have been studied as a means of immobilizing heavy metals (Celis et al. 2000) and organic pollutants (Matthes and Kahr 2000), facilitating the utililization of polychlorinated biphenyls by bacteria (Lünsdorf et al. 2000), and the formation of larger, silt-sized, soil particles by dissolved organic carbon–clay aggregation

(Clapp and Hayes 1999). Complexes of clays and enzymes have been examined in animal feed studies, which have demonstrated that complexes of clays and digestive enzymes were less susceptible to changes in pH associated with digestion than was the enzyme alone (Cabezas et al. 1991). Adsorption of enzymes onto clay surfaces is determined by the isoelectric point (Quiquampoix et al.1993). These complex clay-enzyme interactions could combine to produce profound effects in natural systems by influencing the regulation, distribution, and activity of extracellular enzymes.

If enzymes were sorbed into the clay mineral structure in such a way as to block the active site of the enzyme or in a way that acted to deform the tertiary structure of the enzyme, activity would be expected to decrease markedly. Conversely adsorption onto a clay mineral could act to enhance or maintain enzyme activity if the sorption served to stabilize the enzyme structure. With a maximum particle size of 2 μm, clay minerals can represent a significant portion of the inorganic turbidity of aquatic systems. The phylosilicate structure of clays has the potential to provide > 100 m^2 of surface area per gram of clay, with adsorption processes capable of incorporating high molecular weight and complex aromatic compounds (Namjesnik-Dejanovic et al. 2000).

It was hypothesized that dissolved enzymes can be removed from solution by adsorption onto clay minerals. This finding would support the idea that dissolved enzymes can be physically transported from sites of release and potentially introduced into different ecosystem areas, e.g., from drainage basin to stream or lake or from epilimnion to the hypolimnion or sediments. These interactions also have the potential to affect the activity, longevity, and distribution of dissolved enzymes in nature (Wetzel 1991). If clay minerals change enzymatic activity, the benefit of producing and releasing these enzymes will be influenced in systems subject to high turbidity or turbid inflows.

The presence of inorganic turbidity has the potential to impact enzyme-irradiance interactions in a variety of ways. Particulate concentration can be the most useful factor in predicting light attenuation (Smith et al. 1999). Dissolved enzymes are negatively impacted by irradiance to a greater extent than are particulate forms (Garde and Gustavson 1999). Humic acid-enzyme complexes can release active enzymes following mild exposure to ultraviolet irradiance (Boavida and Wetzel 1998). Increased clay turbidity could have duel, competing effects on light absorption and scattering. If light were absorbed by clay particles, this light would not be available to degrade the enzymes in solution, though adsorbed enzymes might be subject to increased light exposure. If light is scattered rather than absorbed, the light path traveled by photons is increased, and thereby increasing the probability for encountering an enzyme molecule. These competing processes have the potential to either enhance or diminish enzyme activity. Sunlight also has the potential to enhance enzyme activity from enzymes attached to clays, whether these enzyme-clay complexes are formed in the soils of the drainage basin or in the water column of a turbid lake, because they could be transported and then released by exposure to sunlight.

The objectives of the present study were to evaluate (1) the effects of adsorption relationships between clay minerals and dissolved enzymes, and (2) how photolytic processes of sunlight can alter these relationships.

Materials and methods

Enzyme solutions and clay suspensions

Enzyme solutions were prepared in a modified Moss medium (Moss 1972) without trace elements and vitamins, which had been sterilized and adjusted to pH 7. Modified Moss medium was used in all of experiments as past studies have demonstrated that both enzyme activity (Münster et al. 1989) and the adsorption and desorption of organic compounds onto clay minerals (Arnarson and Keil 2000) can be affected by the ionic strength and pH of solution. The purified enzymes utilized were selected to illustrate the potential interactions of enzymes with clay particles as would occur in natural inland waters. It is known that the enzymes released by natural microbiota function similarly to those isolated from other microbes (e.g., Boavida and Wetzel 1998). Alkaline phosphatase (Sigma P-5931) from *Eschericha coli* was used at 0.125 mg L^{-1} (3.5 enzyme units L^{-1}) for both the clay adsorption and the light exposure experiments. β-glucosidase (Sigma G-0395) was used at 0.110 mg L^{-1} for the adsorption experiment and 0.055 mg L^{-1} for the light exposure experiment (0.44 and 0.22 enzyme units L^{-1}, respectively). Protease (Sigma P-5147) extracted from *Strepomyces griseus* was used at 0.138 mg L^{-1} for both experiments (0.58 enzyme

units L^{-1}). β-xylosidase extracted from *Aspergillus niger* (Sigma X-3501) was administered at a level of 1.92 enzyme units L^{-1}. All enzyme solutions were prepared immediately prior to the initiation of the experiment.

The stock montmorillonite clay suspension was prepared at 52 g L^{-1} using commercial montmorillonite (Acros 27603-0010) that was added to modified Moss media and suspended by sonication for 30 seconds. The Elledge Lake Basin clay was separated from riparian soil of Elledge Lake, a small impoundment in west Alabama (Tietjen and Wetzel 2003). Initial removal of plant material and larger particles was accomplished by wet sieving the whole soil to 45 μm. This fraction was then sonicated, to disperse clay particles and de-aggregate larger particles for 30 seconds in ultrapure water (Millipore Milli-Q). The resulting suspension was then allowed to settle for 8 hours at room 21 °C. After settling, the upper 10 cm of the suspension was removed by gentle siphoning from the surface. This fraction, containing particles $\leq$ 2 μm or less, was then concentrated by lyophilization. The stock, 52 g L^{-1}, Elledge Lake basin clay suspension was then prepared in modified Moss media.

Working clay suspensions were prepared by adding an aliquot of the stock suspension to the appropriate enzyme solution. The final clay concentration was 0.26 g L^{-1} which simulated. concentrations of clay at the highest turbidity levels encountered under natural conditions in Elledge Lake (Tietjen and Wetzel 2003).

Clay adsorption experiments

The clay adsorption experiments were carried out by amending the enzyme solutions with the appropriate clay suspension (or modified Moss media in the enzyme only treatments), mixing the resulting suspension and allowing the enzyme to adsorb to the clays for 1 hour in the dark at 21 °C. Prior experiments demonstrated that although organic matter continued to sorb to clays for more than 5 hours, most ($>90\%$) of the adsorption occurred during the first hour. After adsorption, samples in the clay removal treatment were filtered through a pre-combusted (500 ºC) glass fiber filter (Whatman GF/F. 0.6–0.7-μm pore size) to remove the clay and adsorbed material. The appropriate fluorescently labeled substrate (see below) was then added to the enzyme, enzyme and clay (Elledge Lake basin or montmorillonite), or filtered enzyme and clay (Elledge Lake basin or montmorillonite) treatments and then mixed. A 5-ml subsample was immediately removed, added to a cuvette, and fluorescence measured for specific time intervals (Turner Model 112, 364 nm excitation, 445 nm emission for 4-methylumbelliferyl (MUF) substrates, 380 nm excitation, 440 nm emission for 4-methylcoumarinyl-7-amide (AMC) substrates). Replicated samples (n = 3) with MUF labeled substrates were added to cuvettes that contained 1 ml of inorganic pH 10 buffer in order to maximize fluorescence. This combination of sample and buffer was adequate to overcome the buffering capacity of the modified Moss media. AMC-labeled substrates were measured at pH 7. Subsamples (n = 3) were taken 3–5 times from the treatments at appropriate intervals: ~20 minutes for alkaline phosphatase and glucosidase, 30–40 minutes for the xylosidase, and 2–4 minutes for the protease. Fluorescence values were converted to MUF or AMC concentrations with standard curves prepared for both MUF and AMC in modified moss media alone and with Elledge Lake basin or montmorillonite clays at 0.26 g L^{-1}. The standards with clay were used to compensate for adsorption of the fluorescent molecules onto the clay and quenching by the increased turbidity. These values varied but were usually less than 20% of the total. Enzyme activities, as velocities in μmol substrate hydrolyzed per liter per hour of incubation, were calculated as the slope of the sampling time versus AMC or MUF concentration. Only measurements from the linear portion (minimally 3 to 4 points) of the curve were used in these slope calculations. Sampling continued for up to 2 hours or until a fluorescence plateau was reached when substrate concentrations were no longer at saturating levels. This plateau was only observed in the protease experiments.

Clay-sunlight experiments

Experiments examining the combined effects of clay and exposure to sunlight were performed with the enzyme and clay concentrations described above. Quartz sample containers were filled with enzyme solutions or enzyme-clay suspensions and allowed to adsorb for 1 hour, and then exposed to sunlight in a constant 20 °C for 1 (alkaline phosphatase, glucosidase, and xylosidase) or 4 hours (protease). Separate quartz tubes wrapped in aluminum foil were incubated with the light exposure treatment to serve as a dark control. Results were normalized to equivalent

amounts of light (measured with a calibrated LiCor quantum sensor). Following sun exposure, the enzyme-clay suspension from 5 of the tubes was filtered through pre-combusted Whatman GF/F filters for the clay-removed treatment.

Enzyme activity

Enzyme activity was determined by measuring the hydrolysis of fluorescently labeled substrates (Hoppe 1993) in modified Moss media appropriate for the individual enzymes. 4-methylumbelliferyl phosphate (MUF-phosphate) solution was prepared to yield a final concentration of 500 μM when added to the alkaline phosphatase solutions and alkaline phosphatase-clay suspension. Glucosidase activity was measured using 4-methylumbelliferyl β-D-glucoside (MUF-glucoside) at a final concentration of 400 μM. Leucine 7-amido-4-methylcoumarin (L-AMC) was used to measure protease activity at a final concentration of 300 μM, and xylosidase activity was determined using 4-methylumbelliferyl 7-β-D-xyloside (MUF-xyloside) at a final concentration of 500 μM.

Statistical analyses

Velocities were compared using the general linear model procedure in the DataDesk statistical package. Bonferroni pairwise comparisons were carried out after the ANOVA analyses to compare treatments pairs. Data from the alkaline phosphatase experiments was $\log_{10}$ transformed prior to statistical analysis to correct for heteroscedasticity. The data from the glucosidase and xylosidase experiments were square root transformed to correct for heteroscedasticity and departures from normality. The protease data did not require transformation.

Results

Enzyme adsorption

Alkaline phosphatase activity was reduced in the presence of both Elledge Lake basin and montmorillonite clays (Figure 1A). With Elledge Lake clay present in suspension, enzyme activity was reduced to 23% of that in the unamended enzyme treatment ($p \leq 0.001$). In the montmorillonite clay treatment, activity was similarly reduced to 29% of the control

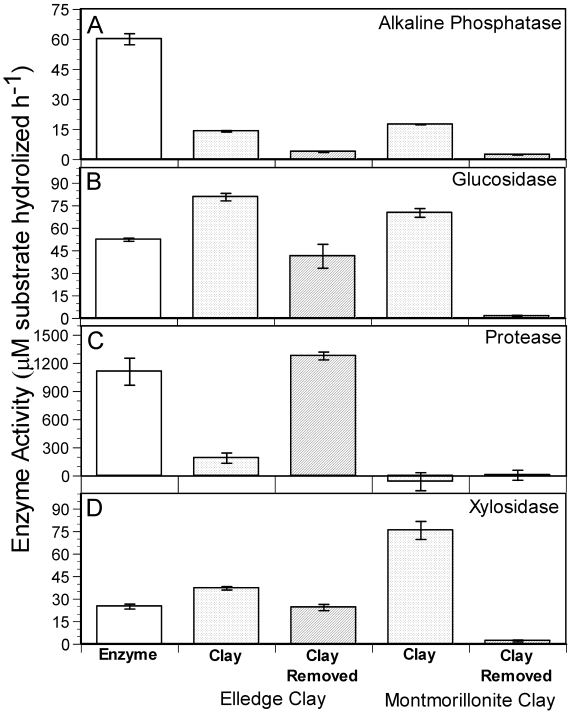

Figure 1. The effect of enzyme adsorption onto clay minerals on enzyme activity. Error bars = standard error of the mean (n = 5). The negative mean value is effectively zero within variance.

($p \leq 0.001$). The removal of either clay from the suspension further reduced enzyme activity, to less than 10% of the control in both treatments ($p \leq 0.001$ for both treatments).

The addition of clay minerals to a glucosidase solution resulted in increased enzyme activity, 154% of control for Elledge Lake basin clay ($p \leq 0.001$) and 134% for montmorillonite clay ($p = 0.05$) (Figure 1B). The removal of the clay reduced enzyme activity by 21% for the Elledge Lake basin clay ($p = 0.49$). The removal of the montmorillonite clay decreased enzyme activity to 3% of the control ($p \leq 0.001$).

Addition of Elledge Lake basin clay to a protease solution resulted in an 83% decrease in enzyme activity ($p \leq 0.001$), whereas the addition of montmorillonite clay decreased enzyme activity to 0 ($p \leq 0.001$) (Figure 1C). The removal of Elledge Lake basin clay from the suspension produced a small not significant increase (15%) in enzyme activity over the enzyme alone ($p = 0.87$). The removal of montmorillonite reduced activity to less than 1% of the enzyme alone ($p \leq 0.001$).

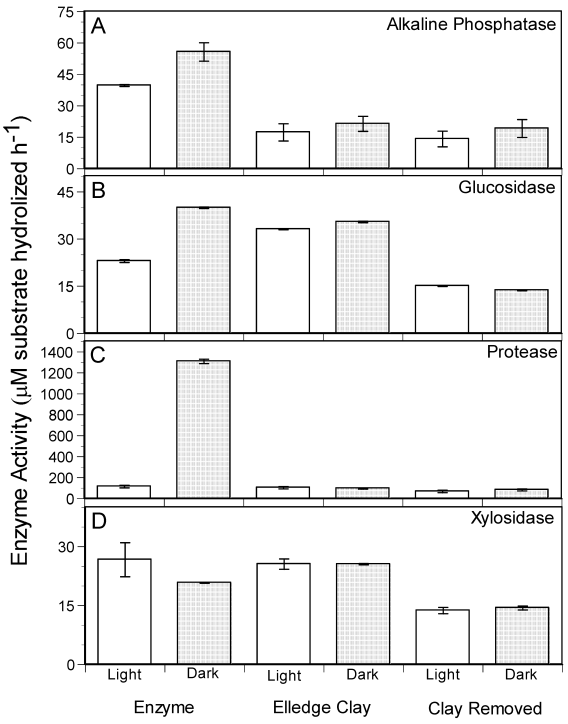

Figure 2. The effect of enzyme adsorption onto Elledge Lake basin clay and sunlight on enzyme activity. Error bars = standard error of the mean.

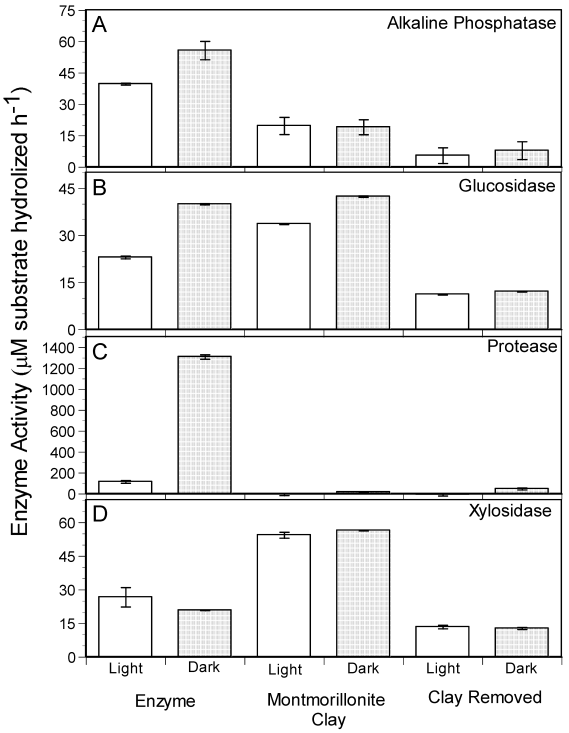

Figure 3. The effect of enzyme adsorption onto montmorillonite clay and sunlight on enzyme activity. Error bars = standard error of the mean.

Xylosidase activity increased slightly with the addition of the Elledge Lake basin clay, (48%; $p = 0.09$) and markedly with montmorillonite clay (202%; $p \leq 0.001$) (Figure 1D). When the Elledge Lake basin clay was removed from the suspension, activity decreased to 97% of the control ($p = 1$), though there was not a significant difference between the Elledge Lake basin clay treatment with or without removal ($p = 0.06$). Removal of the montmorillonite clay resulted in enzyme activity being reduced to 9% of the activity in the control treatment ($p \leq 0.001$) and to 2% with montmorillonite present ($p \leq 0.001$).

Photolytic effects of sunlight on enzyme-clay complexes

In sunlight, an overall significant reduction ($p = 0.047$) in alkaline phosphatase activity occurred after 1 hour of exposure to either clay type (Figure 2A, Figure 3A). In the enzyme only treatment a 29% decrease in activity occurred, though this reduction was not statistically significant ($p = 1$). Although there was a 62% decrease with the addition of Elledge Lake basin clay and a 66% decrease with the addition of montmorillonite clay, neither of these differences was statistically significant ($p = 0.988$ and $p = 0.762$, respectively).

When exposed to sunlight the addition of clay minerals to the enzyme suspension did not have a significant effect on phosphatase activity (Figure 2A, Figure 3A). The Elledge Lake basin clay treatment exposed to sunlight was 19% lower than the corresponding dark treatment ($p = 0.147$). In the montmorillonite treatment, there was a negligible (3%; $p = 1$) increase in activity when exposed to sunlight. When the clay was removed from suspension prior to the addition of substrate, there was not a significant difference between light and dark for either clay type ($p = 1$ for both).

Exposure to sunlight for one hour produced a significant response in glucosidase activity both in overall affect and as a 62% decrease in the enzyme only treatment ($p < 0.001$ for both comparisons) (Figure 2B, Figure 3B). With Elledge Lake basin clay present there was a small (17%) non-significant decrease in enzyme activity ($p = 0.142$). Montmorillonite clay

increased enzyme activity only 6% ($p = 0.972$). When the clays were removed from the suspension, the glucosidase activity was significantly lower, 66% for Elledge Lake basin clay and 70% for montmorillonite, than the dark control ($p \leq 0.001$ for both). No significant difference was found between the Elledge Lake basin clay treatments exposed to sunlight as compared to that held in the dark (7% decrease, $p = 0.951$). Glucosidase activity was significantly higher in the montmorillonite treatment held in the dark (21%, $p = 0.001$). In the treatments with clay removed before the addition of substrate, there was no significant difference between the light and dark treatments ($p = 1$ for both clays).

Protease activity was significantly lower overall in treatments exposed to light ($p \leq 0.001$) and there was a 92% decrease in enzyme activity in the protease only treatment when exposed to light ($p \leq 0.001$) (Figure 2C, Figure 3C). Elledge Lake basin clay significantly lowered enzyme activity in both the clay present and the clay removed treatments, as did montmorillonite ($p \leq 0.001$) and less than 10% of the activity in the dark control for all combinations. There was no significant difference within the clay addition treatments between the light and dark groups ($p = 1$ for both Elledge Lake basin clay and montmorillonite). Similarly when the clay was filtered out of suspension, there was no effect of light exposure ($p = 1$ for Elledge Lake basin clay and 0.96 for montmorillonite).

Xylosidase activity in the light exposure treatment without clay present was not significantly different in the light exposure treatment ($p = 0.485$), nor was light exposure significant as a factor overall ($p = 0.266$) (Figure 2D, Figure 3D). There was no significant difference between the treatments held in the dark when comparing the enzyme alone to the Elledge Lake basin clay addition. Also in the dark treatments, when montmorillonite clay was present with the enzyme solution, or when either Elledge Lake basin clay or montmorillonite clay was removed from the suspension prior to the addition of substrate, there was a significant change in enzyme activity. In the montmorillonite treatment there was a 171% increase in enzyme activity ($p \leq 0.001$). In the clay removal treatments there was a 31% decrease with the Elledge Lake basin clay ($p = 0.029$) and a 39% decrease with montmorillonite ($p = 0.002$). No significant differences were found between the light and dark groups in the treatments with clay removed ($p = 1$).

Summary of results

1. Alkaline phosphatase enzyme activity decreased more than 90% when the Elledge Lake clay was removed from suspension, confirming that the removal of clay also resulted in the removal of enzyme. When the natural Elledge clay was left in suspension, enzyme activity decreased 70% versus the unamended control. Glucosidase activity remained unchanged when the clay was removed from suspension; no significant adsorption of the enzyme occurred.
2. Enzyme adsorption to montmorillonite was observed for all four enzymes, and removal of the clay also resulted in the removal of enzymes attached to the clay. When the clay and enzymes were together in suspension, the results were more complex. Only in the protease experiment was the enzyme completely inactivated by adsorption onto the montmorillonite.
3. Xylosidase adsorption produced enhanced enzymatic activity which approximately doubled when the enzyme-clay complex was present, though activity was near zero when the clay was removed. This combined effect was similar to that encountered in the glucosidase treatments.
4. Light exposure across all treatments and within the enzyme only treatment resulted in a significant reduction ($p \leq 0.05$) in enzyme activity for all enzymes, except xylosidase which was unchanged by exposure to light. Glucosidase in the presence of montmorillonite decreased significantly upon exposure to sunlight; all other clay-enzme combinations showed no significant difference between light and dark conditions.

Discussion

These results indicated that the adsorption of the some enzymes, such as alkaline phosphatase, onto clay acted to inactivate or limit the activity of the adsorbed enzyme. Other processes appeared to be operating in the cases of the other enzymes examined in the presence of Elledge Lake basin clay. In the treatment with clay present, glucosidase activity was significantly higher than the control. Adsorption onto the clay is not limited to the enzyme; the substrate can also become attached to the clay potentially resulting in a concentration effect. As substrate becomes con-

centrated onto the clay particles, enzyme activity could increase as the glucosidase encounters these concentrated substrate particles.

L-AMC similarly appeared to have attached to clay particles while the protease did not. In this case, however, the adsorption of the substrate appeared to have prevented the protease from hydrolyzing the substrate. In this case, removal of the clay did not remove the protease. This combination of results suggests that the attachment of L-AMC occurs in a way that prevents hydrolysis by protease. Adsorption to montmorillonite removed the protease from solution in such a way as to prevent the enzyme and substrate from coming into contact, or deforming the enzyme so that it was no longer functional.

Elledge Lake basin clay, largely a mixture of kaolinite and smectite, had lower adsorption values than to montmorillonite. These lower adsorption values suggest that the natural clay retained adsorbed soil organic matter from long-term environmental exposure. In addition kaolinite is comprised of a single Si-O layer bound to a single Al-OH layer of hydrogen bonds (Cairns-Smith 1986). In contrast, montmorillonite clay consists of multiple alumna-silicate layers and a phyllosilicate structure adjoined by interlayer cations and van der Waals forces that can expand to incorporate adsorbed compounds into the internal structure as well as on external surfaces. As a result, the capacity for adsorption within the Elledge Lake basin clay matrix, where sites may have been partially occupied, is appreciably reduced in comparison to that by montmorillonite.

The same general pattern was observed for alkaline phosphatase and glucosidase activity following adsorption onto montmorillonite clay as was observed following adsorption onto Elledge Lake basin clay. The phosphatase was partially inactivated by adsorption to clay while the glucosidase had higher activity in the enzyme clay-suspension than in the enzyme only treatment. It is likely that the same processes were occurring in the presence of montmorillonite clay as in Elledge Lake basin clay. Adsorption of alkaline phosphatase to montmorillonite partially inactivates or obscures the enzyme, resulting in decreased activity. In the case of the glucosidase, the effect of substrate concentration onto clay, as proposed for Elledge Lake basin clay, may be confounded by the simultaneous adsorption of the enzyme. If this is the case, the adsorbed enzyme continued to hydrolyze the substrate, which had also been concentrated on the surface of the clay, resulting in the activity measurement being greater than that with the enzyme alone. The adsorption of enzyme was presumably accompanied by the adsorption of substrate onto the clay surface, resulting in a greatly enhanced hydrolysis as the effective concentrations were increased.

To evaluate the effects of sun exposure on enzyme activity, it was expected that exposure to sunlight would result in photodegradation of the enzymes, resulting in inactivation of the enzyme, and the concurrent decrease in enzyme activity. This hypothesis was tested for each enzyme as response across all treatments; enzyme only, enzyme and clay (Elledge Lake basin and montmorillonite), and with the enzyme-clay complex removed by filtration. The susceptibility of these enzymes to photodegradation was confirmed without the potential confounding effects of the clay treatments using the enzyme only treatment.

Both clays were effective in decreasing enzyme activity, but the effect of Elledge Lake basin clay was eliminated when the clay was removed. Removal of the Elledge Lake basin clay resulted in the removal of enzyme activity. The likely explanation for this difference is the longer exposure of the enzyme to the clay. In the light exposure experiment the suspension with clay and protease were exposed for 4 hours, whereas in the adsorption experiment, the clay and enzyme were only together in solution for 1 hour. While previous experiments with leaf leachate had indicated that adsorption continued for extended periods of time, the majority (~90%) of that adsorption occurred within the first hour. The combination of Elledge Lake basin clay and alkaline phosphatase may be an exception to this rapid adsorption; longer periods of exposure, as would be common in nature, would increase the inactivation and removal of the enzyme from suspension.

These results indicate that enzyme stabilization by clay adsorption was occurring in these experiments. The protease experiments were excluded from this discussion as the presence of clay of either type resulted in nearly complete reductions in activity, obscuring any light induced effects that might have occurred. Stabilization of enzymes by adsorption has previously been observed to prevent the denaturing of enzyme by pH extremes (Cabezas et al. 1991) and similar clay-enzyme interactions may be capable of providing protection from light.

The final hypothesis considered in these experiments sought to test the potential for adsorbed enzymes to be released from clays in a functional form. This type of sunlight induced, reactivation has

been demonstrated for alkaline phosphatase adsorbed to humic substances (Boavida and Wetzel 1998). The release of enzymes could also have an important impact in the transport of enzymatic activity across ecosystem boundaries. Clay particles in the soils could potentially adsorb enzymes in terrestrial environments and then transport the enzyme-clay complex to an aquatic system where it could be released from the clay in a functional form.

Sunlight may be unable to induce the release of the adsorbed enzymes. In this case, the transport of enzymes from one part of the lake ecosystem to another will only increase or expand the enzymatic capabilities of the recipient site if these enzymes are functional in their adsorbed state. This suggestion was best illustrated by the glucosidase and xylosidase adsorption experiments where enzyme activity of both enzymes was significantly increased in the montmorillonite and Elledge Lake basin clay treatments. It is unclear from these experiments if the increased enzyme activity is a result of enzyme, substrate, or enzyme and substrate concentration on the surface of the clays or if some other factor acted to influence substrate hydrolysis.

An additional explanation for the failure to detect a change in enzyme activity following the removal of clay is that re-adsorption of released enzymes back onto the clay surface may have occurred. It was assumed that the exposure to sunlight would shift the equilibrium of dissolved and adsorbed enzymes, and that promptly filtering the clay out of suspension following light exposure would be sufficient to capture this change in equilibrium. If the re-adsorption were to occur rapidly, as would be anticipated, this procedure may not have captured the change brought about by the treatment.

Clearly adsorption to clay minerals is possible over a range of clay-enzyme combinations. These aggregates have been shown to have the full range of effects by increasing, decreasing, and maintaining enzyme activity. This range of responses presents a variety of complex clay-enzyme scenarios: simple enzymatic activity enhancement/reduction, the transport of adsorption-stabilized enzymes between or within systems, enzymes storage attached to clay particles, and protection from the photodegradative effects of sunlight.

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