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Temperature sensitivity of mineral-enzyme interactions on the hydrolysis of cellobiose and indican by β-glucosidase

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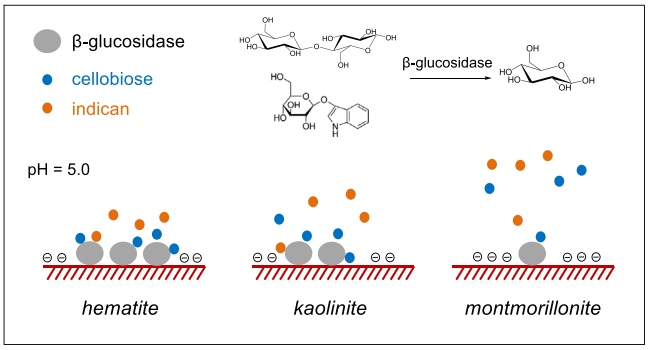
HIGHLIGHTS

- Adsorption of β-glucosidase was highly dependent on the mineral surface properties.
- Mineral-bound β-glucosidase showed a decreased enzyme activity in degrading SOM cellobiose and indican.
- Strong temperature dependence was observed for β-glucosidase hydrolysis on mineral surfaces.
- Temperature sensitivity (Q_{10}) was acquired for β-glucosidase hydrolysis of cellobiose and indican with minerals.

GRAPHICAL ABSTRACT



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ABSTRACT

Extracellular enzymes are mainly responsible for depolymerizing soil organic matter (SOM) in terrestrial ecosystems, and soil minerals are known to affect enzyme activity. However, the mechanisms and the effects of mineral-enzyme interactions on enzymatic degradation of organic matter remain poorly understood. In this study, we examined the adsorption of fungal β-glucosidase enzyme on minerals and time-dependent changes of enzymatic reactivity, measured by the degradation of two organic substrates (i.e., cellobiose and indican) under both cold (4 °C) and warm (20 and 30 °C) conditions. Hematite, kaolinite, and montmorillonite were used, to represent three common soil minerals with distinctly different surface charges and characteristics. β-glucosidase was found to sorb more strongly onto hematite and kaolinite than montmorillonite. All three minerals inhibited enzyme degradation of cellobiose and indican, likely due to the inactivation or hindrance of enzyme active sites. The mineral-bound β-glucosidase retained its specificity for organic substrate degradation, and increasing temperature from 4 to 30 °C enhanced the degradation rates by 2–4 fold for indican and 5–9 fold for cellobiose. These results indicate that enzyme adsorption, mineral type, temperature, and organic substrate specificity are important factors influencing enzymatic reactivity and thus have important implications in further understanding and modeling complex enzyme-facilitated SOM transformations in terrestrial ecosystems.

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1. Introduction

Extracellular enzymes, produced primarily by fungi and bacteria, play essential roles in soil organic matter (SOM) degradation and cycling of carbon, nitrogen, and nutrients in soil systems (Sinsabaugh et al., 2005; Quiquampoix and Burns, 2007; Kotroczó et al., 2014; Stone et al., 2014). Extracellular enzymes are sensitive to changes in environmental conditions, such as soil temperature, moisture, pH, nutrient availability, and other soil physico-chemical properties (Dick, 1997; García-Ruiz et al., 2008; Baldrian et al., 2013). Among these various factors, soil minerals can significantly influence the activity of extracellular enzymes due to enzyme-mineral interactions (Burns, 1982; Marx et al., 2005; Lammirato et al., 2010). For example, soil clays and iron oxides are usually described as having a large surface area that can attract or adsorb enzyme molecules. Previous studies suggest that, when adsorbed to mineral surfaces, soil extracellular enzymes likely lose their mobility and catalytic activity for SOM degradation (Naidja et al., 2000; Servagent-Noinville et al., 2000; Quiquampoix and Burns, 2007). In contrast, other studies reported that soil minerals could stabilize and enhance enzyme activity, possibly due to favorable alteration of enzyme conformation (Tietjen and Wetzel, 2003; Allison, 2006). Hence, there remains a significant knowledge gap in mechanistic understanding how minerals control the enzymatic activities and SOM degradation. This lack of mechanistic understanding limits our ability to develop efficient enzyme-mineral interaction models to predict climate warming effects on SOM transformation and carbon cycling in terrestrial ecosystems (Allison et al., 2010; Tang and Riley, 2014).

Interactions between soil minerals and SOM have been well documented in the past decades. Minerals are known to influence soil organic carbon storage and turnover (Torn et al., 1997), stabilize SOM by organic-mineral interactions (Eusterhues et al., 2003; Mikutta et al., 2006), and protect SOM from degradation through adsorption mechanisms (Gu et al., 1994; Kaiser and Guggenberger, 2000; Feng et al., 2014). However, how soil minerals preserve SOM from enzymatic degradation, and in which ways minerals, SOM, and enzymes interact, are not fully understood. β-glucosidase, for example, is one of the most common extracellular soil enzymes and has been widely studied due to its universal distribution and well-known characteristics (Lammirato et al., 2010; Pathan et al., 2017). Because of the critical role that β-glucosidase plays in decomposing polysaccharides to monosaccharides that microorganisms can mineralize, its activity level has been suggested as an important indicator of soil quality (Gil-Sotres et al., 2005; García-Ruiz et al., 2008). The activity of β-glucosidase can be influenced by surrounding soil minerals. Previous studies showed that the adsorption of β-glucosidase on montmorillonite could decrease its hydrolysis activity (Quiquampoix et al., 1989), and a decreased enzymatic activity was also observed for β-glucosidase when adsorbed on kaolinite and goethite (Lammirato et al., 2010). However, our knowledge of interactions between β-glucosidase and soil minerals is still quite limited, and in particular, how temperature controls the influence of minerals on β-glucosidase activity and its SOM degrading efficiency was previously unknown.

The main goal of this study was to examine soil mineral effects on activity changes of β-glucosidase in degrading cellobiose (fragment product from cellulose) and indican, as analogs of SOM (Turner et al., 2002; Kotroczó et al., 2014), at different soil temperatures. We chose three common soil minerals, including hematite (iron oxide), kaolinite and montmorillonite (clay minerals), because of their distinct surface charges and characteristics, and three experimental temperatures (i.e., 4, 20 and 30 °C) were chosen to mimic both cold and warm soil conditions such as Arctic tundra and tropical areas (Supramaniam et al., 2016; Yang et al., 2017; Chen et al., 2018). We hypothesized that different types of minerals would influence the enzyme activity to various extents due to their unique surface properties, and the sorbed enzymes would have different temperature sensitivity compared to the unadsorbed in degrading the organic substrates. Therefore, our experiments were designed to address the following specific questions: (1) how do soil minerals affect the adsorption and activity of β-glucosidase at different temperatures? (2) what is the temperature sensitivity of the sorbed β-glucosidase, and is this sensitivity dependent on the types of minerals? and (3) to what extent does β-glucosidase degrade different types of organic substrates on mineral surfaces? Here we describe influences of hematite, kaolinite, and montmorillonite on the β-glucosidase hydrolysis of cellobiose and indican, and we highlight the temperature sensitivity of the sorbed β-glucosidase activity in both cold and warm soil environments.

2. Materials and methods

2.1. Substrates and minerals

D-(+)-Cellobiose (>99%) and indoxyl β-D-glucoside (indican, >97%) were used as substrates for β-glucosidase. β-glucosidase was originated from a filamentous fungus *Aspergillus niger* (Yan and Lin, 1997), and both substrates and enzyme were obtained from Sigma Aldrich (St. Louis, USA). D-(+)-Glucose (>99%), a degradation product of the substrates, was also purchased from Sigma Aldrich.

Three soil minerals including hematite-coated quartz sand, kaolinite, and Na-montmorillonite were used in this study. The hematite-coated quartz was prepared using a previously established method (Gu et al., 1996). The iron content in the sample was 0.33% (*w*/w), with iron oxide particles ~200 nm in diameter on the quartz surfaces. Kaolinite and Na-montmorillonite (SWy-2) were obtained from the Clay Mineral Society (USA). All minerals were pre-washed once with 67 mM sodium phosphate buffer (pH 5.0) at room temperature before use; samples were separated by centrifugation for 15 min at 6500*g* using a Clinical 100 Lab Centrifuge (VWR, USA). The enzyme-mineral experiments were performed in the same phosphate buffer solution, at which the adsorption and activity of β-glucosidase were reported to be optimal in the presence of minerals such as montmorillonite and goethite (Quiquampoix et al., 1989; Geiger et al., 1998; Lammirato et al., 2010). An acidic pH (5.0) was used to mimic the pH found in the active layers of polygonal tundra in the Arctic (Herndon et al., 2015; Taş et al., 2018). The specific surface area of the minerals was measured by N_2 adsorption using the Brunauer, Emmett and Teller (BET) method on a Micromeritics ASAP 2020 instrument. The zeta-potential was measured using a ZetaPlus Zeta Potential Analyzer (Brookhaven Instruments, NY, USA).

2.2. Measurements of enzyme and substrate adsorption on minerals

Enzyme adsorption experiments were performed by adding a β-glucosidase solution (at concentrations from 0.01 to 0.5 mg/mL) to the mineral suspension (20 mg/mL in the phosphate buffer), and the mixtures were shaken gently at 4, 20, and 30 °C for 1 h. The 1-h equilibrium time was shown to be sufficient for β-glucosidase to be adsorbed on minerals under similar conditions (Lammirato et al., 2010). After the equilibration, samples were centrifuged for 10 min at 6500*g* and the supernatant was collected. The amount of β-glucosidase in the supernatant was measured using an enzyme assay kit (Sigma Aldrich) through the hydrolysis reaction of *p*-nitrophenyl-β-D-glucopyranoside that produces a yellow product. The absorbance of the product was measured at 405 nm in an Agilent Cary 100 UV–Vis spectrophotometer. The adsorption of β-glucosidase was then quantified by subtracting the supernatant enzyme concentration from the total enzyme concentration.

Similarly, adsorption of organic substrate (i.e., cellobiose or indican) on minerals was conducted by adding the substrate (from 1 to 10 mg/mL) to the mineral suspension (20 mg/mL). The amount of sorbed substrates was calculated by the difference between the initial substrate concentration and the concentration in the supernatant after centrifugation. Concentrations of cellobiose and indican were quantified

with a refractive index detector on a Breeze 2 high-performance liquid chromatography (HPLC) system (Waters, Milford, MA) using an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA). The system was operated at 60 °C at a flow rate of 0.7 mL/min using 5 mM H_2SO_4 as an eluent. In addition, the substrate adsorption was confirmed by measuring the dissolved organic carbon contents before and after the adsorption using a TOC-L analyzer (Shimadzu, Japan). Three replicates per temperature per mineral were performed in both enzyme and substrate adsorption experiments.

2.3. Measurements of β-glucosidase activity

The hydrolytic activity of β-glucosidase was monitored by measuring the concentration changes of the organic substrates over time. Experiments were carried out either in the presence or absence of minerals. In the non-mineral experiments, 2 mL of β-glucosidase solution (2.5 mg/mL) was added to 8 mL of cellobiose or indican solution (10 mg/mL) to reach a final concentration of 0.5 mg/mL and 8 mg/mL for the enzyme and the substrate, respectively. Small aliquots of samples (0.5 mL) were taken at various reaction times (i.e., 1, 2, 3, 4, and 6 h) and acidified immediately with sulfuric acid (1 M) to stop enzyme activity before HPLC analysis. In the mineral experiments, 2 mL of β-glucosidase was first equilibrated with 8 mL of the mineral suspension (25 mg/mL in phosphate buffer) for 1 h to allow sufficient β-glucosidase sorption on the mineral. The unadsorbed enzyme in supernatant was then removed by centrifugation and decantation, and the hydrolysis reactions were initiated by adding the organic substrate solution (8 mg/mL as starting concentration) to the minerals with the adsorbed enzyme. During reaction intervals of 1, 2, 3, 4, and 6 h, samples were collected and filtered through a 0.45 μm nylon syringe filter to remove minerals. The filtrates were also acidified with sulfuric acid before HPLC analysis, and no acid-catalyzed hydrolysis was observed during the analysis.

2.4. Statistical analysis

ANOVA was used to evaluate the effect of minerals on both enzyme and organic substrate adsorption. The differences in the enzyme degradation rates and temperature sensitivity (Q_{10}) were also analyzed using one-way ANOVA. The errors of rate constants in Table 1 are ±1 S.D. of the mean of triplicate experiments. Q_{10} values were calculated based on the quotient of substrate degradation rates either at 30 vs. 20 °C or at 20 vs. 4 °C. The tests were conducted using the R statistical software (http://cran.r-project.org/).

3. Results

3.1. Adsorption of β-glucosidase and substrates on different minerals

The characteristics of minerals used in our experiments were summarized in terms of specific surface area (SSA) and zeta potential, along with the literature data on the point of zero charge (PZC) and cation exchange capacity (CEC) (Table S1). As expected, montmorillonite had the highest SSA among the three minerals. The measured zeta-potentials for hematite, kaolinite, and montmorillonite were all negative in the phosphate buffer (pH 5.0), indicating negatively charged mineral surfaces under the experimental conditions.

The adsorption behavior of β-glucosidase was measured on hematite, kaolinite, and montmorillonite at pH 5.0. As shown in the adsorption isotherms (Fig. 1a), the amount of enzyme adsorbed on all minerals increased with increasing equilibrium enzyme concentration in solution but plateaued at relatively high concentrations (e.g., >0.05 mg/mL). The adsorption isotherms of β-glucosidase also showed a strong dependence on the type of mineral. Significantly higher amounts of β-glucosidase were adsorbed on hematite and kaolinite than on montmorillonite (Fig. 1a, $P < 0.01$), indicating stronger interactions between β-glucosidase and hematite/kaolinite. In addition, the enzyme adsorption was greatly influenced by the solution temperature. At an initial enzyme concentration of 0.5 mg/mL, the adsorption fractions of β-glucosidase at 30 °C were 89.7%, 70.3%, and 14.1% on hematite, kaolinite, and montmorillonite, whereas the adsorption at 4 °C decreased to 48.4%, 36.2%, and 6.9%, respectively. To examine the enzyme adsorption efficiency and stability on minerals, the enzyme-adsorbed minerals were further rinsed by DI water and centrifuged. Negligible amounts of enzyme (<0.5 μg/mg) were detected in the supernatant by the TOC analyzer, suggesting β-glucosidase was immobilized or irreversibly adsorbed on the minerals.

Adsorption of substrates (i.e., cellobiose and indican) was also observed on the surface of minerals but lower than that of β-glucosidase. Compared to the enzyme adsorption, a substantially lower amount of cellobiose or indican was adsorbed on hematite, kaolinite, and montmorillonite (Fig. 1b and c), suggesting a much lower adsorption affinity of the organic substrates. The adsorption isotherms of cellobiose and indican also showed similar mineral effect patterns to the enzyme, in which hematite and kaolinite exhibited a significantly higher adsorption than montmorillonite (Fig. 1b and c, $P < 0.01$). In addition, a slightly lower adsorption affinity of indican than cellobiose was observed on each of the studied minerals ($P < 0.05$).

3.2. Hydrolysis of cellobiose by β-glucosidase on minerals

To determine potential changes of enzymatic reactivity due to its sorption on minerals, we systematically compared degradation rates of cellobiose and indican between the sorbed β-glucosidase enzyme and the free enzyme at three different temperatures (Figs. 2 and 3). Compared to mineral-free systems, all studied minerals (hematite, kaolinite, and montmorillonite) exhibited a negative effect on the reactivity of β-glucosidase. For cellobiose degradation, the calculated pseudo first-order rate constants in mineral-free enzyme solutions were 0.27 ± 0.03, 0.56 ± 0.02 and 1.02 ± 0.1 h^{-1} at 4, 20 and 30 °C, respectively, which were approximately 2–5 times greater than those observed in the mineral experiments (Table 1, $P < 0.01$).

In the presence of the sorbed β-glucosidase on hematite, kaolinite, and montmorillonite, cellobiose concentrations all decreased over time (Fig. 2), demonstrating that the mineral adsorbed β-glucosidase is still effective in hydrolyzing cellobiose, albeit lower than that observed in the mineral-free solution. The impacts of minerals on β-glucosidase hydrolysis were evident when comparing the changes of the normalized cellobiose concentrations (mol/g), which were based on the amount of β-glucosidase adsorbed per gram of mineral at each

Table 1
Estimated initial first-order rate constants of cellobiose and indican degradation by normalized free β-glucosidase and the sorbed β-glucosidase on minerals within the first 6 h. Errors represent ±1 S.D. of the mean of triplicate experiments. Significance levels for rate constant differences are $P < 0.01$.

	Cellobiose (h^{-1})			Indican (h^{-1})		
	4 °C	20 °C	30 °C	4 °C	20 °C	30 °C
No mineral	0.27 ± 0.03	0.56 ± 0.02	1.02 ± 0.1	0.13 ± 0.03	0.26 ± 0.04	0.60 ± 0.03
Hematite	0.11 ± 0.02	0.36 ± 0.03	0.56 ± 0.04	0.074 ± 0.01	0.14 ± 0.01	0.17 ± 0.01
Kaolinite	0.081 ± 0.01	0.31 ± 0.03	0.49 ± 0.05	0.076 ± 0.01	0.13 ± 0.01	0.16 ± 0.01
Montmorillonite	0.022 ± 0.002	0.13 ± 0.02	0.20 ± 0.03	0.046 ± 0.01	0.079 ± 0.01	0.10 ± 0.01

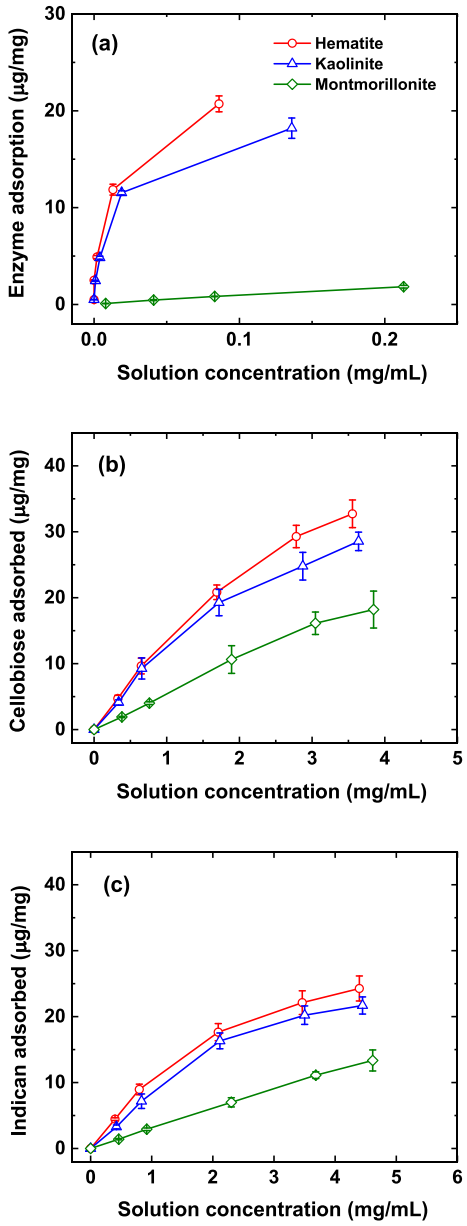

Fig. 1. Adsorption isotherms of β-glucosidase (a), cellobiose (b), and indican (c) on hematite (red circles), kaolinite (blue triangles), and montmorillonite (green diamonds) at pH 5.0 at 20 °C. Y axis represents adsorbed enzyme or substrate on the mineral, and X-axis represents equilibrium concentrations in solution. Each data point represents an average of three independent sample analyses, and error bars represent one standard deviation. Significance levels for data differences are $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperature. The measured and normalized concentrations over time allowed for calculating hydrolysis rates. Data points of cellobiose were best fitted with an exponential decay (Fig. 2), which suggest a pseudo first-order kinetics with respect to cellobiose concentration under our experimental conditions. Specifically, the estimated rates for cellobiose degradation by the adsorbed β-glucosidase at 20 °C were 0.36 ± 0.03, 0.31 ± 0.03, and 0.13 ± 0.02 h^{-1} on hematite, kaolinite, and montmorillonite, respectively (Table 1). The fact that cellobiose degradation was 2–3 times faster with hematite/kaolinite than montmorillonite indicates a higher inhibitory effect of montmorillonite on β-glucosidase hydrolytic activities. At 4 and 30 °C, similarly, lower hydrolysis rates were observed for montmorillonite than for kaolinite and hematite (Table 1, $P < 0.01$).

Strong temperature dependence of the cellobiose hydrolysis by adsorbed β-glucosidase is also observed. Consistent with mineral-free conditions, the hydrolysis rates in the presence of hematite or kaolinite increased by a factor of 5–6 when temperature increased from 4 to 30 °C (Table 1). Although with low hydrolysis rates, temperature appeared to have the most dramatic effect on β-glucosidase hydrolysis on montmorillonite, as the rate increased by nearly an order of magnitude from 4 to 30 °C (Table 1, $P < 0.01$). These results demonstrate the cellobiose hydrolysis rates could be strongly influenced by the type of minerals that β-glucosidase adsorbed on.

3.3. Hydrolysis of indican by β-glucosidase on minerals

Degradation of indican by adsorbed β-glucosidase shared similar patterns with those observed for cellobiose, albeit to a lower extent. The normalized indican concentrations were best fitted with the pseudo first-order kinetics (Fig. 3), and the calculated rate constants in pure enzyme solutions were significantly higher than those observed with mineral-bound enzymes (Table 1, $P < 0.01$), again indicating strong inhibitory effects of minerals on β-glucosidase hydrolytic activities. Different mineral effects on indican degradation were also observed (Table 1). At 20 °C, for example, the degradation rates of indican were 0.14 ± 0.01 h^{-1} on hematite and 0.13 ± 0.01 h^{-1} on kaolinite, which were approximately twice higher than that on montmorillonite (0.079 ± 0.01 h^{-1}). The results are consistent with the degradation trends of cellobiose, in which hematite/kaolinite-bound β-glucosidase exhibited higher activities than β-glucosidase adsorbed on montmorillonite. However, the extent of indican degradation was significantly lower than those of cellobiose at the same temperature (Table 1, $P < 0.01$, except for montmorillonite at 4 °C). For example, at 20 °C there was a 40–60% decrease in hydrolysis rate for indican on minerals when compared to cellobiose ($P < 0.01$), which is consistent with the hydrolysis rate difference (~50%) in solution (i.e., mineral free conditions) (Table 1). Furthermore, hydrolysis of indican was also enhanced by temperature, as the rates doubled from 4 to 30 °C (Table 1, $P < 0.01$). However, the temperature effects on indican hydrolysis were lower than those observed on cellobiose, since the cellobiose degradation rates increased >5 times for all the three minerals from 4 to 30 °C (Table 1, $P < 0.01$). The different temperature dependences between indican and cellobiose degradation further imply a high substrate selectivity for the adsorbed β-glucosidase on the mineral surface.

4. Discussion

4.1. Minerals effects on β-glucosidase hydrolysis

The present study shows a substantially higher adsorption of an extracellular enzyme, *Aspergillus niger* β-glucosidase, on hematite and kaolinite than on montmorillonite at pH 5 (Fig. 1a). This observation is consistent with the data from Lammirato et al. (Lammirato et al., 2010), which showed higher adsorption of β-glucosidase on goethite and kaolinite than montmorillonite. However, the adsorption of β-glucosidase is not dependent on the specific surface area of minerals, because montmorillonite, which has the highest SSA (Table S1), appeared to be the least effective in adsorbing β-glucosidase on its surface. Instead, enzyme adsorption is more likely controlled by the mineral surface charge density. All three minerals have negative zeta potentials in the phosphate buffer (Table S1) and should thus be negatively charged. Purified β-glucosidase from *Aspergillus niger* is a dimeric protein with an isoelectric point of 3.2 (Yan and Lin, 1997), suggesting that the enzyme should also be negatively charged at pH 5.0. However, this protein is also glycosylated that could confer neutral, hydrophilic surface properties on the enzyme (Lima et al., 2013). Since montmorillonite has a high surface charge density with a CEC more than an order of magnitude higher than that of kaolinite (Table S1), the strong electrostatic repulsion may thus be responsible for the weak interactions between

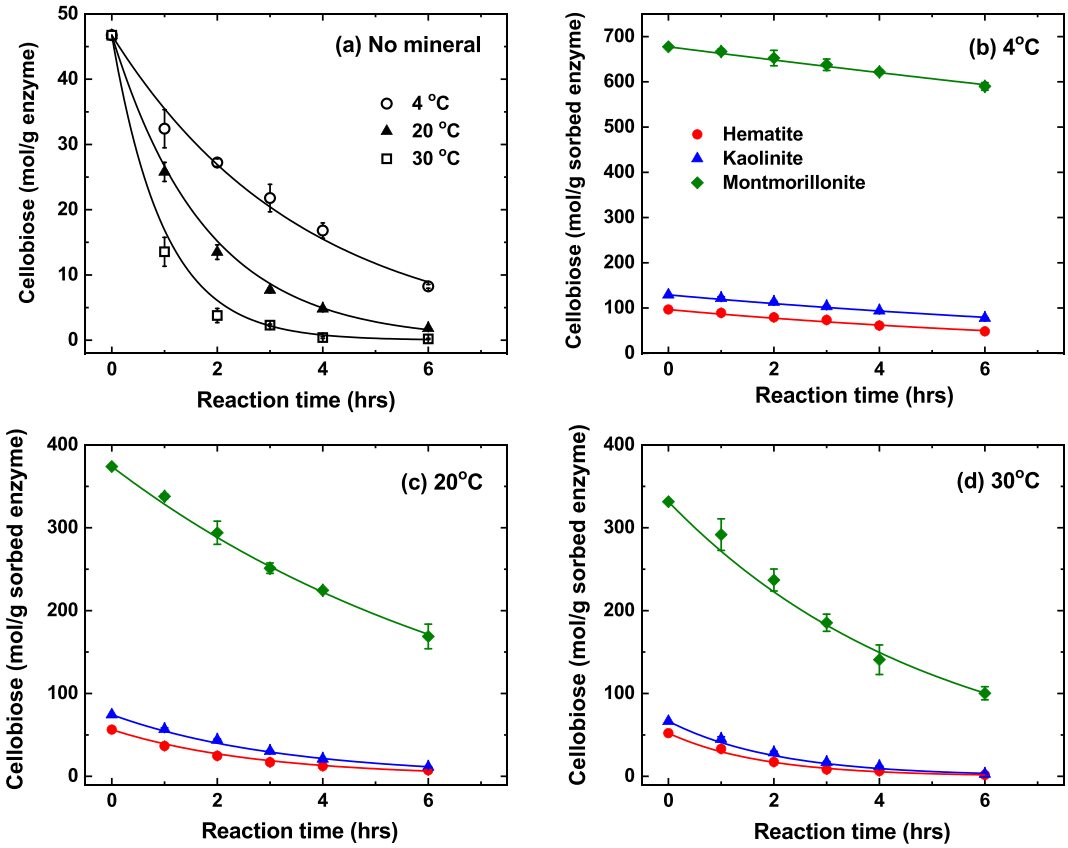

Fig. 2. Comparisons of cellobiose degradation by β-glucosidase in mineral-free solution (a), and by the adsorbed β-glucosidase on hematite (b), kaolinite (c), and montmorillonite (d) at 4, 20, and 30 °C, respectively. For (b), (c) and (d), the cellobiose degradation (mol/g sorbed enzyme) was normalized by the amount of β-glucosidase sorbed on each mineral. Data points were best fit with an exponential decay to show the first-order kinetics. Error bars represent one standard deviation of triplicate measurements. Significance levels for data differences are $P < 0.01$.

montmorillonite and β-glucosidase. This conclusion is supported by other studies, which suggested that the electrostatic repulsion between montmorillonite and the enzyme could outweigh the sum of the attractive non-coulombic forces under similar experimental conditions (Quiquampoix et al., 1989; Lammirato et al., 2010).

Immobilization or binding of enzymes on clay minerals can be attributed to electrostatic interactions, hydrogen bonding, hydrophobic interactions, van der Waals forces, and even covalent bonding (An et al., 2015). Montmorillonite (a 2:1-type smectite) is usually considered to have higher enzyme adsorption capacity than 1:1-type clay kaolinite, due to its expandable interlayers (Serefoglou et al., 2008; An et al., 2015). However, our results do not support the theory of enzyme intercalation into the interlayer space. This is probably because under our experimental conditions, β-glucosidase molecules were negatively charged and thus unable to enter into the interlayer space of montmorillonite by cation exchange reactions. The interlayer space of montmorillonite has a height of approximately 2.5 Å (Bertuoli et al., 2014), which may also prevent the bulky enzyme to be intercalated. Furthermore, when the enzyme is immobilized on the mineral surface, specific interactions (e.g., complexation, covalent bonding) between the enzyme functional groups and the mineral structures could be expected (An et al., 2015). Adsorption of β-glucosidase can be restricted to the external structures and the edges of the minerals, when the solution pH is higher than the isoelectric point of enzyme. For example, at pH > p*K*a, the –Al–OH_2OH (Al–hydroxide) groups at the edges of kaolinite and montmorillonite are deprotonated into –Al–$(OH)_2$, which can lead to complexation with carboxylic groups of the enzyme (Lagaly et al., 2006). The deprotonated edge surface groups could also form O—C or O—N covalent bonds, possibly with the amino or carboxylic groups from N-terminus or amino acid chains of β-glucosidase (Dinur et al., 1986; Yan and Lin, 1997; Abdel-Naby et al., 1999). Indeed, the p*K*a's of the –Al–OH_2OH groups of kaolinite and montmorillonite have been calculated to be 5.7 and 8.3, respectively (Liu et al., 2013), suggesting that the edge surfaces of montmorillonite are much less likely to be deprotonated than kaolinite at pH 5 used in this study. Hence, the p*K*a's of specific functional groups and their interactions at the edge surfaces of clay minerals, could play important roles in determining the enzyme adsorption.

Hematite and kaolinite exhibited a relatively high adsorption for β-glucosidase (Fig. 1a) through specific and strong interactions between enzyme functional groups and hematite surface structures. Previous studies have demonstrated a ligand exchange mechanism between carboxyl and hydroxyl groups of natural organics on iron oxide surfaces (e.g., hematite) (Gu et al., 1994; Gu et al., 1995), which support ligand-metal bonding between β-glucosidase and the –Fe–oxide groups of hematite in this study. Although hematite has a PZC value above pH 5.0, the coulombic attraction should be limited since the measured zeta potential was -30 ± 4 mV in the phosphate-buffered solution (Table S1). Similar findings have been reported for goethite, FeO(OH), in which a surface charge-reversal model was proposed (Quiquampoix, 1987; Lammirato et al., 2010). The model illustrated the phosphate anions could adsorb onto the mineral surface and thus form negatively charged goethite-phosphate complexes in solution.

4.2. Mechanism of β-glucosidase hydrolysis on minerals

The hydrolysis experiments demonstrate a decreasing activity of the sorbed β-glucosidase in degrading cellobiose or indican at all given temperatures (Figs. 2 and 3, Table 1). This result is consistent with previous findings that the enzyme decreases its reactivity by 20–90% when

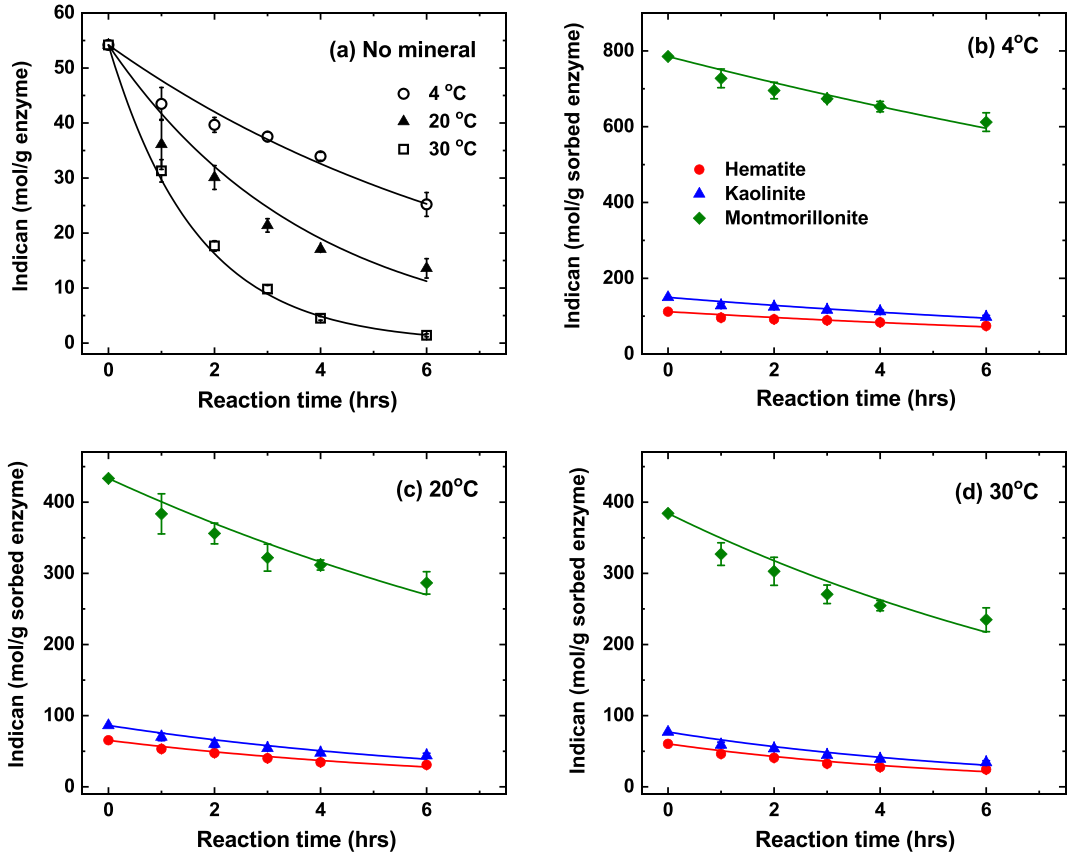

Fig. 3. Comparisons of indican degradation by β-glucosidase in mineral-free solution (a), and by the adsorbed β-glucosidase on hematite (b), kaolinite (c), and montmorillonite (d) at 4, 20, and 30 °C, respectively. For (b), (c) and (d), the indican degradation (mol/g sorbed enzyme) was normalized by the amount of β-glucosidase sorbed on each mineral. Data points were best fit with an exponential decay to show the first-order kinetics. Error bars represent one standard deviation of triplicate measurements. Significance levels for data differences are $P < 0.01$.

sorbed on montmorillonite, kaolinite or goethite mineral surfaces (e.g., Quiquampoix et al., 1989). These previous studies proposed that the enzyme binding strength could be determined by "hardness" of the protein, spatial distribution of surface charges, and spatial distribution of glycans on the protein surface. When β-glucosidase was adsorbed on the mineral surface, differences in chemical bonding (e.g., ligand or covalent bonding) between enzyme functional groups (e.g., amino acid chains or glycans) and mineral surface structures (e.g., Al–oxide or Al–hydroxide) determine the binding strength and orientation of the enzyme on the surface. Our results show a decreasing enzyme reactivity in the order: hematite ≈ kaolinite > montmorillonite (Table 1). This rate difference may thus be attributed to the deformation or orientation changes of enzyme active sites on mineral surfaces. The active sites of β-glucosidase with hematite or kaolinite are likely more orientated or exposed to the bulk solution than montmorillonite at pH 5 since higher hydrolysis rates were observed on hematite and kaolinite. It is important to note, however, that the orientation of enzyme active sites strongly depends on environmental conditions, such as pH controls on protonation/deprotonation of functional groups (with different p*K*a's).

Furthermore, our results illustrate that the hydrolysis rate was not solely governed by the mineral type, but also by the chemical property of organic substrates. At the same temperature, degradation rates of cellobiose were found to be substantially higher than the rates of indican in the presence or absence of minerals (Figs. 2 and 3, Table 1), suggesting a strong substrate specificity for hydrolysis by the sorbed β-glucosidase. Indeed, previous studies have examined the substrate affinity toward β-glucosidase by comparing various substrate compounds, including cellobiose, indican, and *p*NPG, in non-mineral solutions (Dekker, 1986; Kempton and Withers, 1992; Chauve et al., 2010; Nascimento et al., 2010). Despite different experimental conditions, the reported kinetic parameters including Michaelis-Menten constant (K_m) and maximum velocity (V_m) were generally larger for cellobiose than for indican and *p*NPG (Dekker, 1986; Maugard et al., 2002; Chauve et al., 2010; Song et al., 2010), consistent with the kinetics patterns observed in the present study (Figs. 2 and 3). Hydrolysis of cellobiose and indican by β-glucosidase breaks the glycosidic bond. Substrate specificity is generally dependent on the formation of enzyme-substrate complexes and how well the substrate fits into the enzyme active sites (e.g., with steric hindrance or not). The flat and aromatic indole group can probably make indican less competitive than disaccharide-structured cellobiose, to bind with active sites of β-glucosidase. Our results also suggest that, aside from the enzyme active sites that were "hindered" by the mineral surface, other active sites faced toward the bulk solution were not affected. Although detailed hydrolysis mechanism of *Aspergillus niger* β-glucosidase is not fully understood, hydrolysis by another β-glucosidase from *Agrobacterium faecalis* suggested a two-step reaction mechanism that involved a glucosyl-enzyme intermediate formation (Kempton and Withers, 1992). The glucosyl-enzyme intermediate formation (glycosidic bond cleavage) was proposed to be S_N2-like reaction, in which the properties (e.g., p*K*a) of the substrate leaving group (e.g., indole group on indican) could be important in determining the reaction rates. Hence, the nature/specificity of organic substrates should be taken into account for such mineral-bound enzymatic reactions in complex environmental systems.

4.3. Temperature sensitivity of β-glucosidase hydrolysis

Because of the large uncertainty in soil carbon-climate feedback predictions and the pressing needs for accurate biogeochemical models (Allison et al., 2010; Tang and Riley, 2014; Tang et al., 2016), temperature sensitivity (i.e., Q_{10}) of β-glucosidase activity was also estimated

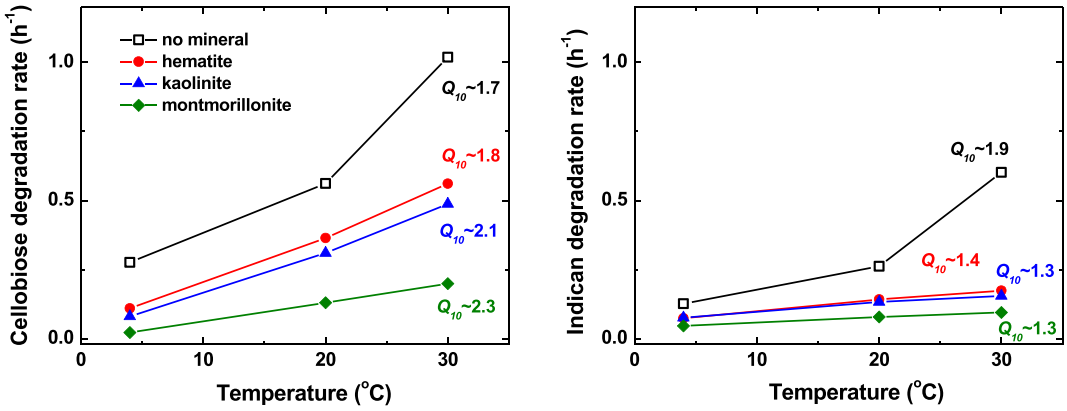

Fig. 4. Degradation rates of cellobiose and indican by β-glucosidase in the presence or absence of minerals at 4, 20, and 30 °C. Q10 are calculated average values based on the degradation rates at three temperatures. Significance levels for Q10 differences are $P < 0.02$.

in this work. Strong temperature dependence was observed for β-glucosidase hydrolysis on mineral surfaces, where degradation rates of the organic substrates increased when temperature changed from cold to warm conditions (Figs. 2, 3, Table 1). Based on Arrhenius equations and the reaction rate ratios between 4 and 30 °C, Q_{10} values in the presence and absence of minerals were calculated for cellobiose and indican degradation and summarized in Fig. 4. In cellobiose experiments, the presence of hematite, kaolinite and montmorillonite all showed higher Q_{10} values (1.8–2.3) than those without minerals (1.7) ($P < 0.02$), reflecting a greater temperature sensitivity of the sorbed β-glucosidase. The Q_{10} values for indican degradation, however, were all lower in the presence of minerals (1.3–1.4) than mineral-free controls (1.9) ($P < 0.01$), showing a decreased temperature sensitivity for the sorbed enzyme. The changes in temperature sensitivity by the type of minerals suggest: (1) the active sites of sorbed β-glucosidase may be reoriented or altered in different ways from the active sites of freely-diffused enzyme as temperature changes; and (2) the hydrolysis mechanisms of the sorbed β-glucosidase for cellobiose and indican are likely different in their transition states (e.g., oxocarbonium ion-like) that determine the rate limiting steps for rate equations (Kempton and Withers, 1992). The high variability of β-glucosidase temperature sensitivity also seems consistent with the model prediction by Tang and Riley, who considered mineral sorption capacity and substrate activation energy as important parameters for enzymatic SOM degradation (Tang and Riley, 2014). Their model predicted that increasing the activation energy of substrate due to mineral adsorption can increase SOM respiration temperature sensitivities. As observed in the cellobiose experiments, the relatively high temperature sensitivity could be due to stronger enzyme-cellobiose interaction than enzyme-indican interaction on the mineral surface. However, temperature sensitivity may also depend on many other properties of the system such as soil mineral type and microbial community, as well as enzyme thermostability at different temperatures. Additional studies are obviously needed to further evaluate the factors that control the temperature sensitivity. Our results imply that, the acquisition of temperature sensitivity (e.g., using Q_{10} values) in the presence of different minerals, could be useful in distinguishing mineral effects on enzyme reactivity, thereby providing feedback to parameterize organic-enzyme-mineral interactions in ecosystem models.

5. Conclusions

This work demonstrates the mineral effects on the hydrolysis of cellobiose and indican by β-glucosidase from *Aspergillus niger* at three environmentally relevant temperatures. Adsorption of β-glucosidase was highly dependent on the type of minerals and their surface properties as well as the p*Ka*'s and functional groups on the enzyme, which determine the formation of mineral-enzyme bonds with different binding strengths and characteristics. Sorption of β-glucosidase on minerals all decreased enzyme activity, likely due to the inactivation or hindrance of enzyme active sites, leading to decreased degradation of organic substrates. Enzymatic hydrolysis of cellobiose and indican were also greatly affected by temperature, evidenced by increasing Q_{10} values from 1.3 to 2.3 or higher degradation rates at warmer conditions. However, the adsorbed β-glucosidase retained its specificity for organic substrate degradation, similar to that observed in mineral-free conditions. The present study highlights that enzymatic degradation of SOM is an interplay of several critical factors, such as enzyme adsorption, mineral property, temperature, and organic substrate specificity. Recommendations for future research may include probing enzyme hydrolysis mechanisms on mineral surfaces using advanced analytical techniques such as vibrational and X-ray absorption spectroscopy, and measuring enzyme hydrolysis rates of SOM to fully understand mineral effects on carbon degradation in natural soil systems.

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The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data for this article can be found online at http://doi.org/10.1016/j.scitotenv.2019.05.479.

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