



Interaction of alkaline phosphatase with minerals and sediments: Activities, kinetics and hydrolysis of organic phosphorus



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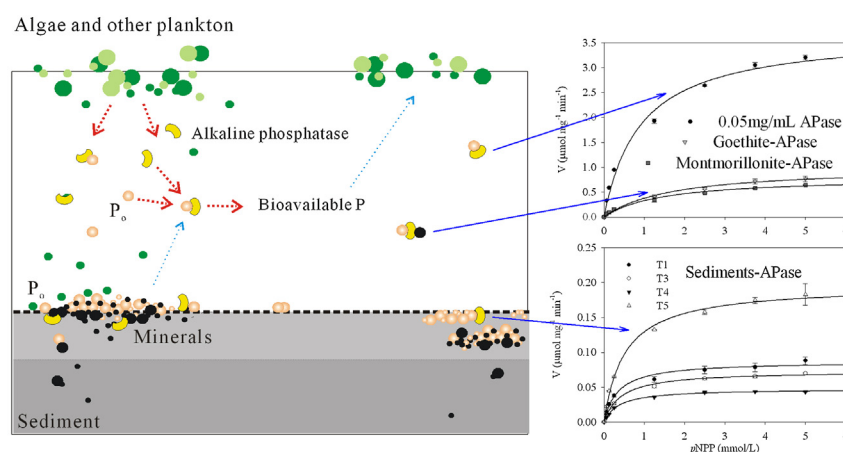
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HIGHLIGHTS

- Adsorption of alkaline phosphatase by sediments of lakes and minerals were investigated.
- Activities of the alkaline phosphatase immobilized by minerals and sediments would be reduced.
- Enzymatic hydrolysis of organic phosphorus in the suspensions of sediments was investigated by ³¹P-NMR.
- Organic phosphorus would be enzymatically hydrolyzed when they released from sediments.
- Organic phosphorus could not be hydrolyzed directly at the interface of sediments-water by enzymes

GRAPHICAL ABSTRACT



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ABSTRACT

Alkaline phosphatase (APase) plays an important role in phosphorus (P) cycling in water and sediments of lakes. Interaction of APase with minerals including goethite and montmorillonite, and sediments from Lake Tai (Ch: *Taihu*) and Lake Dianchi were investigated. Degradation and bioavailability of organic P (P_o) in sediments were further investigated by APase hydrolysis and solution ³¹P-nuclear magnetic resonance (NMR) spectroscopy. Little APase is adsorbed by sediments, but APase could be strongly adsorbed by goethite and montmorillonite. Some adsorptive sites could be occupied by organic matter or ions in sediments from lakes. Activities of APase immobilized by sediments could be reduced to 1.3% to 5.3% of that of free APase. However, APase immobilized by goethite and montmorillonite could retain 27.3% and 21.6% of the activity of free APase. Thus, the majority of APase is likely dissolved in overlying water or loosely adsorbed by sediments in lakes. Enzymatic hydrolysis and liberation of orthophosphate from suspensions of sediments were 0.26–4.25 mg kg⁻¹ that was readily bioavailable to algae or other organisms. After APase hydrolysis, ³¹P NMR analysis showed that no detectable changes in P_o or condensed P in sediments extracted by NaOH–EDTA. Thus, P_o immobilized in sediments couldn't be directly hydrolyzed by APase, but more P_o immobilized in the sediments could be hydrolyzed by APase when they were released

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into the overlying water under appropriate conditions. Treatments, such as additions of Al hydroxides dosing, would not adsorb or immobilized P, but also immobilized phosphatase, thus decreasing activity of phosphatase and bioavailability of P_o in a eutrophic lake.

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1. Introduction

Sediment–water interfaces play key roles in biogeochemical cycling of nutrients in lakes [1]. A series of physical, chemical and biological reactions can occur at sediment–water interfaces [1,2]. These reactions include dissolution and deposition, sorption and desorption, migration and transformation, oxidation and reduction, enzymatic hydrolysis and bacterial biochemical process. Therefore, the sediment–water interface is an important site where cycling of elements between sediments and overlying water is regulated and controlled. Organic matter and minerals (e.g., Si, Fe, Al, Ca, and Mg oxides) are primary constituents of sediments. Sorption and desorption of nutrients and organic matter by Fe and Al oxides can influence bioavailability of nutrients, such as organic carbon [3,4] and phosphorus (P) [5–7], and the activities of enzymes [8,9].

Alkaline phosphatase, which is important for internal cycling of P in lakes, widely exists in water and sediments [10–12]. Phosphatase activity in lakes can be due to phosphatases localized on surfaces of algal and bacterial cells, dissolved enzymes supplied by autolysis or excretion from algae, bacteria or zooplankton [10]. Both alkaline and acid phosphatases, found in lakes, can hydrolyze monoester P, except for phytate [10,12]. Acid phosphatases are often found inside algal cells, which are likely produced to modulate internal metabolism of P [10]. However, alkaline phosphatases can also have external functions, which would be synthesized and excreted into the surrounding medium depending on the ambient phosphorus nutrition, especially when bioavailable P is deficient [10]. Thus, alkaline phosphatase would be more likely adsorbed by minerals or sediments in the lakes. The activity and stability of enzymes associated with minerals in the environment can vary [8,13]. Activity of alkaline phosphatase was less when adsorbed on minerals, such as clay and montmorillonite [13]. Thus, abilities of immobilized enzymes, such as alkaline phosphatase, to hydrolyze the substrates, such as labile monoester P [12], could be affected [8,13,14]. However, few studies have investigated interactions of alkaline phosphatase with minerals and sediments [13].

Organic P (P_o) can comprise a large proportion of the total P in the aquatic systems, such as overlying water, suspension particulate and sediments of lakes [15–17]. However, P_o remains poorly understood and currently represents the greatest gap in understanding cycling of P in lakes [18,19]. Thus, P_o in lakes, especially sediments from lakes, has been widely investigated by sequential extraction, ^{31}P NMR, and enzymatic hydrolysis [12,16,17,20,21]. Organic P compounds and condensed P, characterized by ^{31}P NMR, contain phosphonates, monoester P (e.g., glucose-6-phosphate, α - or β -glycerophosphate, mononucleotides, choline phosphate and phytate), diester P (e.g., RNA, DNA and phospholipids) and condensed P (e.g., pyrophosphate and polyphosphate) in sediments of lakes [21,22]. Alkaline phosphatase could hydrolyze the substrate of labile monoester P (e.g., glucose-6-phosphate, α - or β -glycerophosphate, mononucleotides, choline phosphate) and condensed P [12,23]. Though the P_o in the sediments was extracted then hydrolyzed by enzymes [12,21], little information on degradation and release of P_o in the sediments by enzymatic hydrolysis directly is available [24].

Thus, the objective of this study was to investigate interactions of alkaline phosphatase with minerals (e.g., goethite and mont-

Table 1

Surface areas ($\text{m}^2 \text{g}^{-1}$) of goethite, montmorillonite and sediments.

Materials	Minerals		Sediments				
	Goethite	montmorillonite	T1	T3	T4	T5	S20
Surface area	41.0	2.8	18.2	18.2	26.3	6.1	22.9

morillonite) and sediments. Activities and kinetics of adsorption alkaline phosphatase was analyzed. And then release and hydrolysis of P_o in the sediments was investigated by enzymatic hydrolysis directly followed by use of ^{31}P NMR. Finally, biogeochemical cycling of P_o in lakes was discussed based on the interaction of alkaline phosphatase with sediments and hydrolysis of P_o in sediments.

2. Materials and methods

2.1. Preparation of minerals and sampling of sediments

Goethite was prepared by the method of Atkinson et al. [25]. Briefly, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dissolved in water in a polyethylene beaker, and then 2.5 M NaOH was added dropwise until the pH reached 11.9. The resulting suspension was aged for 48 h at 60 °C. The precipitate (Goethite) was rinsed several times with Milli-Q grade water until the pH approached 7.0, and was then lyophilized. The synthetic goethite was confirmed by powder X-ray diffraction analysis (Bruker D8 Advance, Germany) (Fig. 1). Montmorillonite KSF purchased from Alfa Aesar (A Johnson Matthey Company).

Surface sediments (0–3 cm) were collected from Lake Tai (Ch: Taihu) (33°55′–31°32′N, 119°52′–120°36′ E) and Lake Dianchi (24°40′–25°02′N, 102°36′–102°47′E). Lake Tai, a large shallow, eutrophic lake, is located in Jiangsu Province, China. Lake Dianchi, a eutrophic lake, is situated in Kunming City, Southwest China. For Lake Tai, sampling site of T1 (31°28′0.95″N, 120°10′37.86″E) was located in Meiliang Bay, T3 (31°24′50.64″N, 120°21′5.86″E) was located in Gonghu Bay, T4 (31°26′34.80″N, 120°02′39.03″E) was located in Zhushan Bay, T5 (31°05′51.65″N, 120°32′54.97″E) was located in the East Lake Tai. For Lake Dianchi, the site of S20 (24°48′43.56″N, 102°42′31.05″E) was located in central area. Sediments were transported to the laboratory in air-tight plastic bags and placed in cold storage on dry ice. Sediments were lyophilized and ground to powder and stored at –20 °C until analysis.

Specific surface areas of the minerals studied, goethite and montmorillonite, and sediments were determined by use of BET nitrogen adsorption (Nova 4200e, Quantachrome Instruments, USA) (Table 1).

2.2. Adsorption experiment

Alkaline phosphatase (EC 3.1.3.1) was purchased from Sigma–Aldrich Chemicals (A China branch, Shanghai). A solution containing 0.5 mg mL^{-1} alkaline phosphatase was prepared by dissolution in 0.01 M Tris–HCl buffer (pH 9.0). 30 mg of goethite or montmorillonite were mixed with 5 mL of alkaline phosphatase solution. Mixtures were gently shaken at 37 °C for 0, 15, 30, 45, 60, 75, 90, 120, or 150 min to study kinetics of adsorption. Studies were replicated. Suspensions were centrifuged at 15,000 $\times g$ for 15 min, and then filtered with a 0.45 μm membrane filter. Concentrations

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