Colloids and Surfaces A: Physicochem. Eng. Aspects 495 (2016) 46–53

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

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

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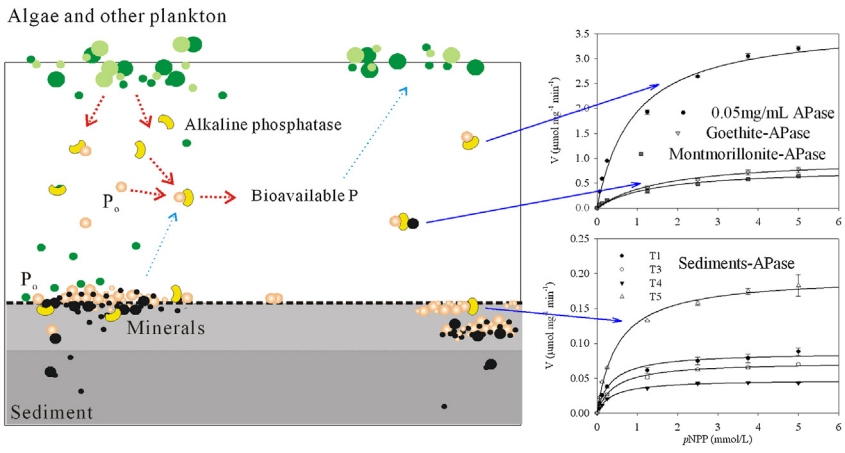
HIGHLIGHTS

- 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 ^{31}P-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



ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 24 January 2016
Accepted 29 January 2016
Available online 1 February 2016

Keywords:
Enzymatic hydrolysis
Goethite
Montmorillonite
Organic phosphorus
^{31}P NMR
Eutrophication

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 ^{31}P-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, ^{31}P 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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http://dx.doi.org/10.1016/j.colsurfa.2016.01.056
0927-7757/

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.

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 phyate), 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 montmorillonite) 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.

Table 1
Surface areas ($m^2\ g^{-1}$) 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

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, $Fe(NO_3)_3{\cdot}9H_2O$ 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 × *g* for 15 min, and then filtered with a 0.45 μm membrane filter. Concentrations

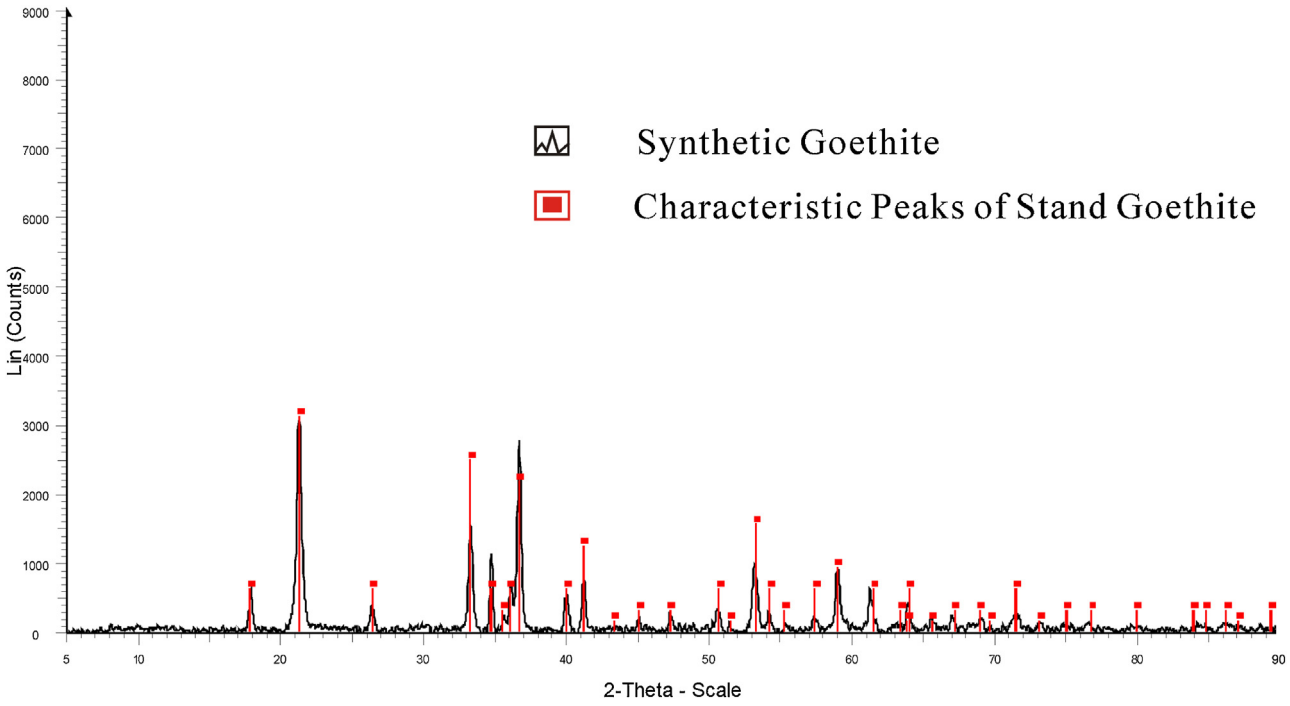

Fig. 1. X-ray diffraction analysis of synthesized goethite.

of the enzyme in the filtrate was measured by the absorption peak (252–305 nm) integration method of UV spectroscopy with alkaline phosphatase as the standard, which had been studied previously [26]. The amount of enzyme adsorbed was calculated from the difference between the amount of enzyme added and that remaining in the filtrate.

Based on adsorption equilibrium time, 30 mg of adsorbent including goethite, montmorillonite, sediments from T1 and T3 of Lake Tai was mixed with 5 mL of alkaline phosphatase. These alkaline phosphatase solutions included a series of concentrations from 0 mg mL^{-1} to 2.0 mg mL^{-1} prepared in Tris–HCl buffer (pH 9.0). Mixtures were gently shaken at 37 °C during adsorption equilibrium time. The mixture was then centrifuged and filtered, enzyme remaining on the filter was determined. The amount of enzyme adsorbed was calculated, and the isothermal adsorption curve was analyzed.

2.3. Kinetic study of immobilized enzyme complex

Based on results of adsorption experiments, the immobilized enzyme complex was prepared. 50 mg of minerals or sediments was equilibrated with 8 mL of 1 mg mL^{-1} APase solution in Tris-HCl buffer (pH 9.0) at 37 °C for 1 h. The suspension was centrifuged at 15,000 × *g* for 15 min. The residue was washed with 8 mL of the same buffer twice each to remove free and loosely bound enzyme, and then suspended in 30 mL buffer. Concentrations of enzyme in solutions, including supernatant fraction and washings, were determined by UV spectroscopy after filtering, as described previously. The amount of enzyme immobilized on the minerals or sediments was calculated from the difference between the amount of enzyme added and that which remained in the solutions. Suspensions with enzyme free buffer were also prepared as the blanks.

In 10 mL reaction tubes, 1 mL of model minerals- or sediments-enzyme complex suspensions prepared as above were mixed with 2 mL of 0.01 M Tris–HCl buffer (pH 9.0). Then, 1 mL of substrate (*p*NPP, *para*-nitrophenyl phosphate disodium, Amresco Chemicals, USA) ranging from 0 to 20 mM in 0.01 M Tris–HCl buffer solution (pH 9.0) was added. The mixture was incubated at 37 °C with slight agitation for 30 min. The enzyme reaction was stopped by the addition of 2 mL of 1 M NaOH solution [8]. After filtration, the amount of the product, *p*-nitrophenol, in the filtrate was determined spectrophotometrically at 405 nm. The specific activities of the free or immobilized enzyme were expressed as μM of *p*-nitrophenol hydrolyzed per mg of enzyme per min (μM mg^{-1} min^{-1}). Kinetics of free and immobilized enzymes were described by use of the Michaelis–Menten function.

2.4. APase hydrolysis of organic phosphorus in suspensions of sediment

Based on results of the investigation of adsorption, activities and kinetics of alkaline phosphatase, release of P from suspensions of sediments directly hydrolyzed by this enzyme was further investigated. In those studies, 3.0 g sediments from T1, T3, T5 or S20, were mixed with 200 mL 2 U/mL APase buffer solution (pH 9.0, 0.01 M Tris–HCl) in 250 mL polyethylene bottles. Mixtures were incubated for 24 h at 37 °C with shaking. Suspensions were centrifuged and filtered after incubation. Molybdate reactive phosphorus (MRP) in supernatants was analyzed by use of the molybdenum blue method [27]. APase free buffer suspensions were also done simultaneously. Release of MRP in the suspensions by APase was calculated by the difference between these incubations of APase and APase free buffer suspensions.

In order to evaluate variation of forms of P in sediments or residues after incubation with APase solution, phosphorus in residues were extracted with a solution of 60 mL 0.25 M NaOH-50 mM EDTA solution for 16 h. Suspensions were then centrifuged and filtered. Concentrations of total phosphorus (TP) in extractants were analyzed by ICP-OES (Optima 5300DV, Perkin Elmer™). The remaining volumes of extractants were freeze-dried, and then redissolved in 3 mL Milli-Q water with ultrasonic vibration. Excess Na_2S was then added to precipitate paramagnetic ions (e.g., Fe and Mn) and to maintain reducing conditions [28]. Aliquots of

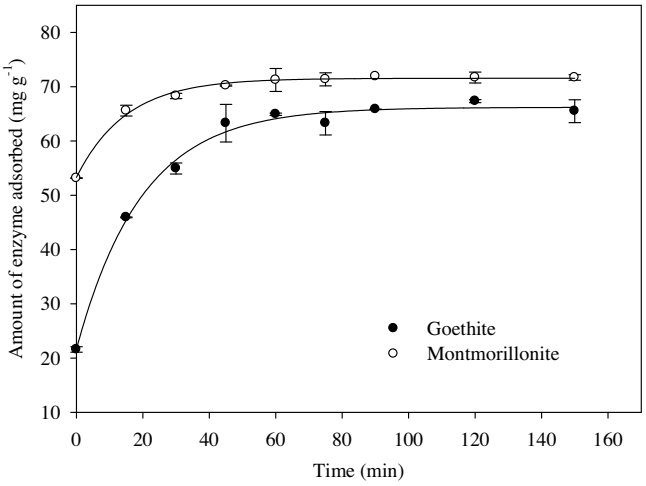

Fig. 2. Amount of enzyme adsorbed by goethite or montmorillonite as a function of duration of incubation. The error bars are based on the standard deviation from two individual experiments.

0.5 mL were transferred to NMR tubes, and then 50 μL of D_2O was added for use as a signal lock. ^{31}P NMR spectra were measured at 161.98 MHz by use of a Bruker AV 400 MHz spectrometer equipped with a 5 mm broadband observe probe. Chemical shifts were referenced to 85% H_3PO_4 via the signal lock. Peaks were assigned based on literature values [29,30], integrated to obtain peak areas, and concerted to concentrations of P relative to concentrations of TP in the extracts.

3. Results and discussion

3.1. Adsorption of alkaline phosphatase by minerals and sediments

When goethite or montmorillonite were added to solution of APase, a large of proportion of the APase was adsorbed immediately (Fig. 2). This indicated that some minerals could adsorb enzymes quickly and efficiently. Time to equilibrium for adsorption of APase by goethite or montmorillonite was approximately 60 min. Therefore, 60 min was used as the adsorption equilibrium time duration to develop adsorption isotherms of APase on minerals and sediments (Fig. 3). Adsorption isotherms for goethite and montmorillonite, which exhibited *L*-type curves, were fitted with the Langmuir function (Fig. 3a). Specific surface area of montmorillonite was smaller than that of goethite (Table 1). However, the total amount of APase adsorbed by montmorillonite was greater than that adsorbed by goethite (Figs. 2 and 3a). Results of studies on other enzymes such as urease, invertase, and acid phosphatase have also shown no relationships between adsorptive capacity of these enzymes by minerals and their respective surface areas [8,31]. SiO_2 and Al_2O_3 are the main constituents of Montmorillonite KSF, with 4.76% of Fe_2O_3 and other chemical composition [32]. Montmorillonite KSF is a non-porous solid, with an isoelectric points of 5 [32]. Except for particle size and surface area, mechanisms of adsorption of proteins such as APase, onto surfaces of minerals could include hydrophobic interaction, electrostatic interaction and hydrogen bonding [8]. The isoelectric point for APase utilized in this investigation was approximately 5.7 [33]. Therefore, APase would have had a net negative charge when in Tris–HCl buffer (pH 9.0) used in this study. Thus, the zeta potential could be approximately −25 mV for goethite, and about −3 mV for montmorillonite KSF in Tris–HCl buffer (pH 9.0). Therefore, the adsorptive capacity of montmorillonite KSF for APase due to electrostatic interaction at pH 9.0 was relatively great. Differences in surface structures of goethite and montmorillonite KSF and the arrangements of APase on their surfaces would also result in different capacities of minerals to adsorb APase [31].

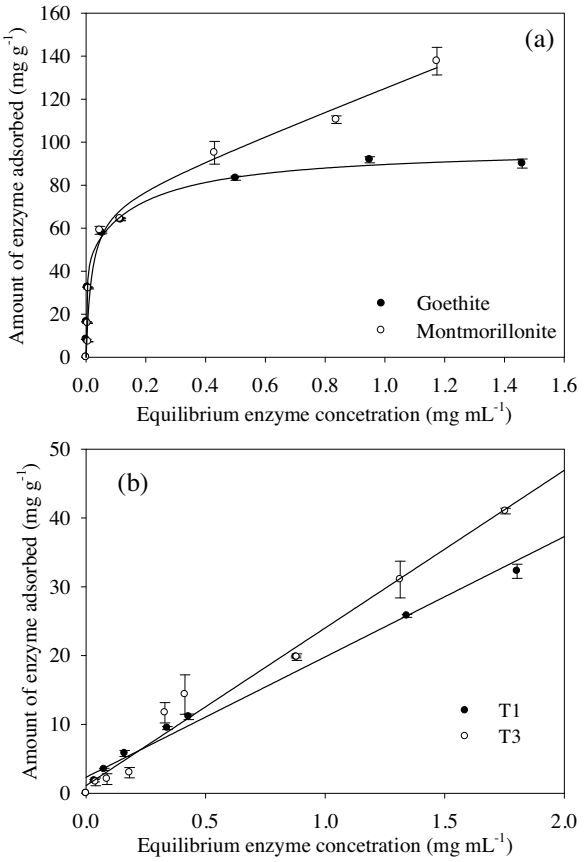

Fig. 3. Adsorption isotherms of APase on minerals or sediments. (a), minerals (Geothite and montmorillonite); (b), sediments (T1 and T3). The error bars are based on the standard deviation from two individual experiments.

Adsorption isotherms of APase on sediments were different from those for goethite or montmorillonite KSF (Fig. 3b). Adsorption isotherms for sediments were of a linear pattern, which are classified as *C*-type curves. Affinities of *C*-type of adsorption isotherms is less than those of *L*-type of adsorption isotherms [34]. This is likely because sediments of Lake Tai included large amounts of organic matter, such as humic acid, and ions, such as PO_4^{3-}, from overlying water, such that there were few sites on the surface of sediments to which enzymes could be adsorbed. Therefore, for adsorption of enzymes by these sediments, greater concentrations and more energy would be needed to exchange the organic matter or ions adsorbed to surfaces of sediments [34,35].

3.2. Kinetics of immobilized alkaline phosphatase

Compared with free APase, kinetics of immobilized APase were significantly less (Fig. 4). Adsorption of the substrate (*p*NPP) on inorganic components studied could be negligible for the kinetic parameters of immobilized enzymes [8]. Kinetics of free and immobilized APase could be described by use of the Michaelis–Menten function with kinetic parameters, K_m and V_{max}, calculated by fitting the equation by Sigmaplot 10.0 (Table 2). The Values of V_{max} and K_m represent maximum reaction velocity and the affinity of the enzyme for substrate, respectively. The K_m value of free APase was 0.907 mmol L^{-1}. When APase was immobilized by minerals or sediments, K_m values were altered. Values of K_m for APase when it was adsorbed onto goethite or montmorillonite were 1.666 mM

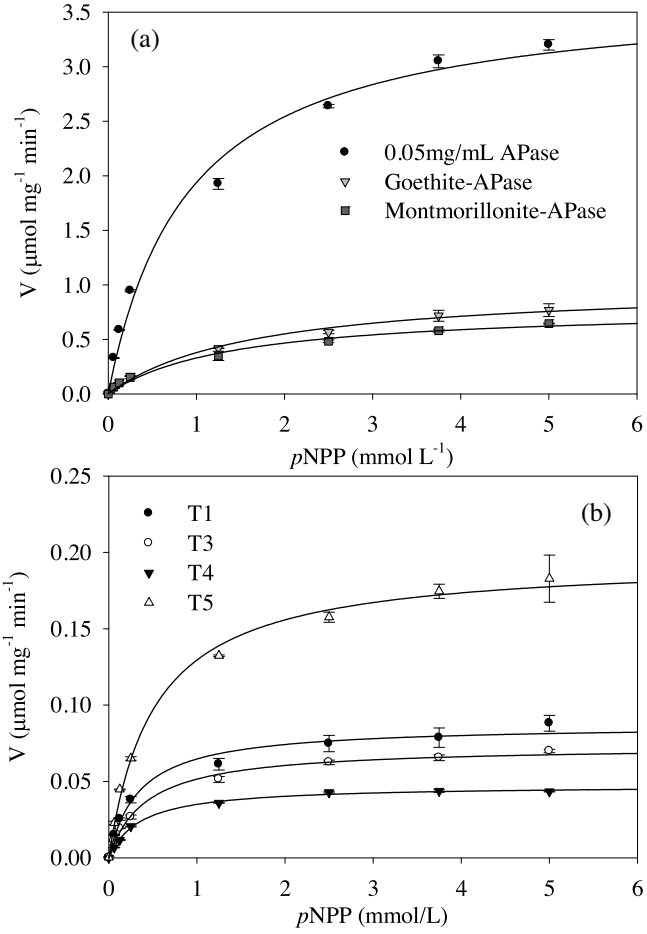

Fig. 4. Variations of activities and kinetics of free and immobilized APase. (a), free APase and APase immobilized by goethite and montmorillonite; (b), APase immobilized by sediments from Lake Tai. The error bars are based on the standard deviation from two individual experiments.

Table 2
Kinetic parameters of free and immobilized alkaline phosphatase.

Free and immobilized enzyme	Km (mM)	Vmax		Vmax/Km
		μmol mg-1 min-1	%	
APase	0.907	3.693	100	4.072
Goethite-APase	1.666	1.008	27.3	0.605
Montmorillonite-APase	1.430	0.799	21.6	0.559
T1-APase	0.345	0.087	2.4	0.252
T3-APase	0.419	0.073	2.0	0.174
T4-APase	0.358	0.048	1.3	0.134
T5-APase	0.511	0.196	5.3	0.384

and 1.430 mM, respectively. These results indicated that affinities of APase for the substrate were less when adsorbed to goethite or montmorillonite than when APase was free in solution. Compared with free enzymes, the lesser affinity also observed when acid phosphatase [8] or invertase [31] were adsorbed by minerals. However, K_m values of APase adsorbed to sediments were less than that of free APase (Table 2), which indicated the affinity of APase was enhanced when adsorbed to sediments. Interactions of an enzyme (e.g., acid phosphatase) with clay (e.g., montmorillonite), organic molecules (tannic acid) or organo-mineral complexes have been investigated previously [36], which showed that value for K_m of acid phosphatase in acid phosphatase–tannic acid–minerals complexes were less than those for free acid phosphatase. This is likely because the structure of such complexes could have a reticulated three-dimensional organization with large pores, which favor diffusion

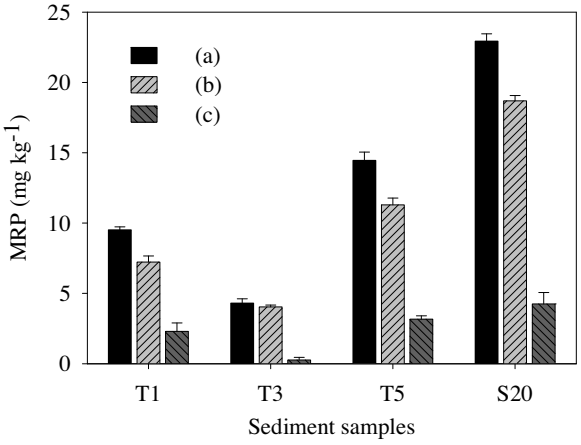

Fig. 5. Release of MRP from the sediment suspensions by APase. (a), sediment suspensions with APase incubation; (b), sediment suspensions with buffer controls; (c), MRP released by APase directly. The error bars are based on the standard deviation from three individual experiments.

of substrates [36]. Thus, it could be speculated that organic matter adsorbed on surfaces of particles in sediment would be organized in a similar way, which would favor diffusion of the substrate.

Compared with free APase, V_{max} values of APase immobilized on surfaces of minerals and sediments were all decreased significant (Table 2) as was the activity of acid phosphatase [8,31,36]. The reasons for this might be the active point being affected due to changes in the structure of enzymes. Complexes of APase with goethite or montmorillonite had 27.3% and 21.6% of the activity of free APase, respectively. However, complexes of APase with sediments were only 1.3–5.3% of that of free APase. After washing twice, there was 53 and 103 mg g^{-1} of APase adsorbed by goethite or montmorillonite, respectively. There were only 13, 15, 12 and 3 mg g^{-1} of APase adsorption by sediments of T1, T3, T4 and T5, respectively. A large proportion of APase adsorbed by goethite or montmorillonite, even if after washing twice, some APase was likely loosely adsorbed. However, APase is likely to compete for adsorptive sites with other organic matter and ions. As a result, it is predicted that APase would be strongly bound onto surfaces of sediments and thus immobilized, such that activities would be strongly inhibited. Therefore, activities of APase adsorbed onto goethite or montmorillonite were greater than those for APase adsorbed by sediments. These binding capacities are indicated by ratios of V_{max}/K_m (Table 2), which the ratios would be lesser when binding capacities less [8].

3.3. *Phosphatase immobilized by sediments and biogeochemical cycling of organic P in lakes*

Hydrolysis of organic phosphorus is in common a necessary step for growth of plants, including algae in lakes [12,14,15]. Enzymatic hydrolysis, as a biotic pathway, plays a key role in liberation of phosphate from the P_o compounds in water, sediments and soils [12,15,37]. APase is present in water and sediments of lakes and could be important for internal cycling of P from sediments to overlying water column [11,12,15,38]. There was more MRP released when hydrolysis of suspensions of sediment by APase which resulted in greater amounts of P becoming bioavailable (Fig. 5). Contents of MPR released due to hydrolysis of P_o by APase ranged from 0.26 to 4.25 mg kg^{-1} in sediments of Lake Tai and Lake Dianchi (Fig. 5). These results are consistent with previously reported enzymatic hydrolysis of suspensions of sediment from Lake Leman, France [24]. Enzymatic liberation of MPR from suspensions of sediments from Lake Tai and Lake Dianchi were related to contents of TOC accumulated in sediments (Fig. 5 and Table 3).

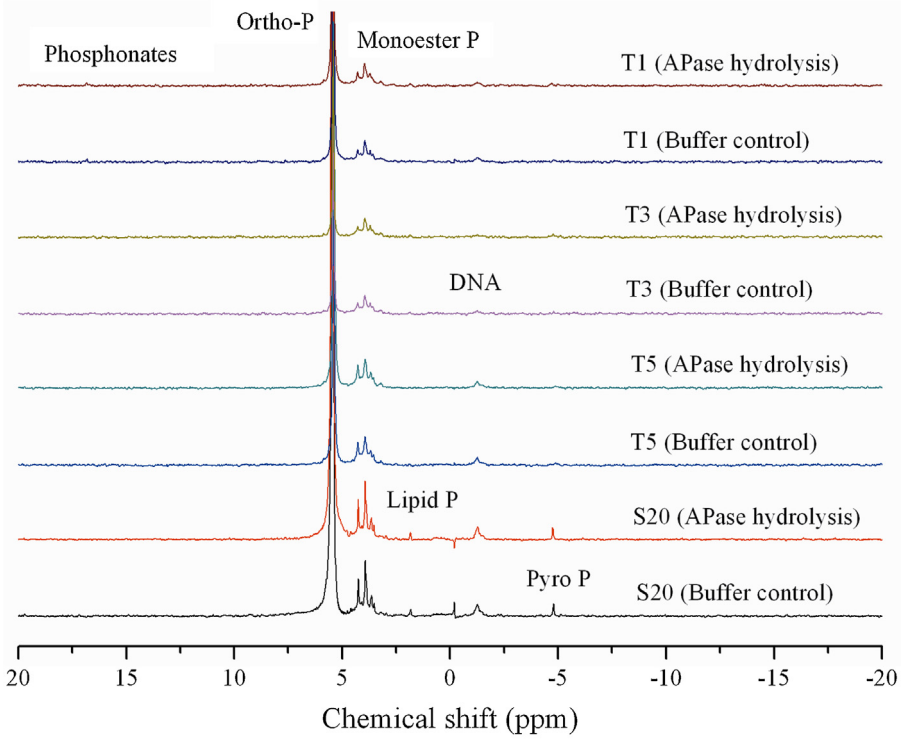

Fig. 6. ^{31}P NMR spectroscopy of NaOH–EDTA extractable P in the sediments after incubation.

Table 3
Chemical properties and NaOH–EDTA extractable P characterized by ^{31}P-NMR in sediments after incubation with or without APase.

Sediments	Chemical properties		Incubation	NaOH–EDTA TP (mg kg^{-1})	NaOH–EDTA extractable P characterized by ^{31}P NMR (mg kg^{-1})					
	TOC (%)	TP (mg kg^{-1})			Phosphonates	Ortho-P	Monoester P	Lipid P	DNA	Pyro P
T1	1.1	595	APase	239.1	3.5(1.5) [a]	177.0(74.0)	49.4(20.7)	–	5.5(2.3)	3.7(1.6)
			Buffer	243.0	2.0(0.8)	180.8(74.4)	48.8(20.1)	–	9.2(3.8)	2.2(0.9)
T3	1.0	267	APase	94.9	–[b]	56.5(59.5)	32.5(34.3)	–	3.8(4.1)	2.0(2.1)
			Buffer	116.1	–	77.4(66.7)	34.1(29.4)	–	3.3(2.9)	1.2(1.1)
T5	3.0	366	APase	283.6	–	192.9(68.0)	69.3(24.4)	–	16.2(5.7)	5.2(1.8)
			Buffer	279.8	–	198.1(70.8)	69.2(24.7)	–	10.3(3.7)	2.2(0.8)
S20	8.3	2623	APase	1168.8	–	1047.3(89.6)	91.1(7.8)	3.1(0.3)	19.9(1.7)	7.3(0.6)
			Buffer	1164.8	–	1045.6(89.8)	91.0(7.8)	6.3(0.5)	16.7(1.4)	5.2(0.5)

[a] Numbers in the parentheses are percentages (%).
[b] "–", data not detected.

There was more organic matter accumulated in sediments from the East Lake Tai (T5) and Lake Dianchi (S20). These results indicated that accumulation of organic matter due to greater primary production during eutrophication of lakes would enhance release of MRP by enzymatic hydrolysis. These P_o from sediments directly dissolved in the water could be hydrolyzed by the enzyme dissolved in the water or enzyme immobilized by sediments, which could maintain the eutrophic status of lakes for long periods [12,39].

MRP released by APase accounted for only 0.4%, 0.1%, 0.9% and 0.2% of TP in sediments of T1, T3, T5, and S20, respectively. Organic P remaining in sediments, which was extracted by NaOH–EDTA and analyzed by ^{31}P NMR further (Fig. 6 and Table 3), would be important for understanding biogeochemical cycling of P in lakes. NaOH–EDTA extractable P, which includes phosphonates, orthophosphate (ortho-P), orthophosphate monoesters (monoester P), phospholipids (lipid P), DNA, and pyrophosphate (pyro P) were characterized by ^{31}P NMR in the sediments from Lake Tai and Lake Dianchi (Fig. 6). Ortho-P, the main constituent of inorganic P, represents the largest proportion of P in NaOH–EDTA extracts (Table 3). Monoester P was the major form of P_o in extracts of NaOH–EDTA. Monoester P includes a range of P_o compounds, such as sugar bound phosphates (e.g., glucose-1-phosphate, glucose-6-phosphate), diester degradation products (e.g., mononucleotides, α- and β-glycerophosphate), choline phosphate, and inositol phosphates [21,39]. Diester phosphates including lipid P and DNA was detected in these samples (Table 3). Lipid P, which would be expected to quickly degrade to α- and β-glycerophosphate [21,40], occurred in only trace amounts in sediments from Lake Dianchi. DNA, which originated primarily from bacterial DNA, decomposing phytoplankton and macrophytes, was widely observed in sediments (Table 3). Pyro P, which can be synthesized by bacteria, fungi and bacteria as a response to oxic conditions, is a labile group of P compounds [41,42]. Pyro P was found in all sediments from lakes (Table 3). Trace amounts of phosphonates, which might originate from agricultural chemicals such as glyphosate [43], were observed in sediments from location T1 in Lake Tai.

When enzymes, such as APase are adsorbed onto surfaces of model minerals or sediments, their activities for hydrolyzing P_o or condensed P were less (Fig. 4 and Table 2). Adsorption of organic P on minerals or sediments have been shown to protect some adsorbed organic P (e.g., inositol phosphates) from enzymatic

hydrolysis [6,44]. Whereas, surfaces of goethite can also effectively concentrate substrates (glucose-1-phosphate) and enzymes (acid phosphatase). Therefore reduction of enzymatic activity is almost balanced by this concentration, thus the adsorbed glucose-1-phosphate was hydrolyzed by acid phosphatase on the surface of goethite [14]. Process that occur on surfaces tend to release carbon to the solution whereas ortho-P remains adsorbed on goethite [14]. Though some labile monoester P, such as sugar bound phosphates and mononucleotides, as well as pyro P could be hydrolyzed by APase when dissolved in the solution [12], there were no significant differences between hydrolysis of P_o adsorbed onto sediment (NaOH–EDTA extractable P_o) by APase and that of buffer controls (Table 3). These results suggested that some labile monoester P_o including pyro P adsorbed by sediments could not be hydrolyzed by APase adsorbed to surfaces of sediments or free in solution. Therefore, enzymatic hydrolysis of P_o adsorbed on surfaces of goethite reported by Olsson et al. [14] was not consistent with the results of enzymatic hydrolysis of sediments studied here. Some P_o in sediments could be extracted by H_2O, $NaHCO_3$, or NaOH, and then hydrolyzed by enzymes such as APase, phosphodiesterase, or phytase [12]. These P_o extracted by various extractants could be released into overlying water under certain conditions. For example, under anoxic conditions, NaOH extractable P_o would be released into overlying water [5,12]. This indicated that some NaOH–EDTA extractable P_o would likely dissolve into overlying water then hydrolyzed by enzymes or enzymes immobilized by the sediments rather than hydrolyzed on the surface of sediments directly in lakes. Also, some organic P is adsorbed by minerals or sediments, and then diffuses into the interior of the minerals [7]. This portion of organic P would be less likely to be released and accessible to enzymes and thus couldn't be hydrolyzed. Further studies of enzymatic hydrolysis of P_o in suspended sediment under various conditions, such as various temperature, oxygen level and pH, are needed for a better understanding the bioavailability and preservation of organic P in sediments of lakes [6].

4. Conclusion

Phosphatase (e.g., APase) can be strongly adsorbed by minerals including goethite and montmorillonite. However, adsorption of APase on surfaces of sediments was less than that on surface of the two model minerals. It is likely in lakes some adsorptive sites occupied by organic matter or ions in lakes will complete with enzymes. Thus, the majority of APase is likely dissolved in overlying water or loosely adsorbed to surfaces of sediments in lakes. Activity of APase immobilized by sediments was small, averaging between 1.3 to 5.3% of free APase, though APase immobilized by goethite and montmorillonite retained 27.3% and 21.6%, respectively of activity of free APase.

There was 0.26 to 4.25 mg kg^{-1} of readily dissolved P_o in sediments from Lake Dainchi and Lake Tai, such as dissolution of P_o or condensed P when suspension of sediments, could be hydrolyzed immediately by free APase in water or APase immobilized on surfaces of particles. Hydrolyzed P_o would then become more bioavailable for algae or other organism. More accumulation of organic matter with eutrophication of lakes would result in more bioavailable P_o releasing and hydrolysis by phosphatase. However, there was no degradation of P_o immobilized in sediments due to hydrolysis by APase. More P_o immobilized in the sediments would be hydrolyzed by APase, and thus become bioavailable only when they were released into the overlying water under appropriate conditions. Also, treatments, such as additions of Al hydroxides dosing, would not adsorb and immobilized only P, but would also immobilize phosphatase, thus decreasing activity of phosphatase and bioavailability of P_o in eutrophic lakes.

Acknowledgements

This research was jointly supported by China's the National Natural Science Foundation of China (No. 41403094, 41130743, 41261140337) and by the open funds of State Key Laboratory of Environment Criteria and Risk Assessment (SKLECRA2014OFP02). Prof. Giesy was supported by the program of 2012 "High Level Foreign Experts" (#GDT20143200016) funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences. He was also supported by the Canada Research Chair program.

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