Microb Ecol (1987) 13:229–248

MICROBIAL ECOLOGY

Kinetics of Alkaline Phosphatase Activity and Phosphorus Availability for Phytoplankton and Bacterioplankton in Lake Plußsee (North German Eutrophic Lake)

Ryszard J. Chróst* and Jürgen Overbeck

Max-Planck Institut of Limnology, Department of Microbial Ecology, D-2320 Plön, FRG

Abstract. Annual studies of kinetics of alkaline phosphatase (APA) activity and phosphorus availability for microplankton in the photic zone of an eutrophic lake are reported. The total APA activity of microplankton varied strongly. V_{max} was highest during summer P depletion, and in autumn and winter total APA activity was low. The total APA specific activity of the microplankton was also highest (average 3.55 pmole PO_4^{3-} ng ATP^{-1} min^{-1}) when ambient orthophosphate concentrations were very low. Both V_{max} and specific APA activity were not dependent on the biomass of microplankton; they were strongly affected by P available for microplankton. A differential filtration technique was used for separation of microplankton into two size classes, i.e., algal, larger than 3 μm, and bacterial fraction with size 0.2–3.0 μm. The algal size fraction had lower specific APA activity (average 1.224 pmole PO_4^{3-} ng ATP^{-1} min^{-1}) and higher K_M values (38.8 μmole × $liter^{-1}$) than microorganisms which were smaller than 3 μm (2.011 pmole PO_4^{3-} ng ATP^{-1} min^{-1} and 25.4 μmole $liter^{-1}$, respectively). The K_M values of free, dissolved APA (36.8 μmole $liter^{-1}$) indicated that free APA was probably released by algae. Phytoplankton were major APA activity producers in the photic zone of the lake from March to November, and their activity constituted, on the average, 48.6% of the total APA activity in the water. Bacteria were the dominant APA activity producers in winter (41.3–44.9%); however, during other periods they contributed significantly (average 21.7%) to total APA activity. When surplus constituted less than 10% of particulate P in seston, phytoplankton produced high specific APA activity, and when surplus P was higher than 15%, the specific APA activity of phytoplankton size fraction rapidly decreased. APA of the bacterial size fraction of the seston was not affected by P concentrations. Orthophosphate was a competitive inhibitor of APA produced by microorganisms of the size fraction larger than 3.0 μm, and increasing concentrations of inorganic phosphate caused an increase in K_M values. The hypothetical metabolic-coupling between phytoplankton and bacterioplankton in the phosphorus cycle in conjunction with carbon metabolism in the lake is discussed.

* *Permanent address:* Institute of Microbiology, University of Warsaw, Department of Environmental Microbiology, Warsaw, Poland.

Introduction

The major form in which microorganisms acquire phosphorus is dissolved inorganic phosphate (DIP), the ions $H_2PO^{4-} + HPO_4^{2-}$. In nonpolluted freshwater habitats DIP availability is often regarded as the nutritional factor that limits or controls the rate of primary production [36], and its content in the water can be used to predict the total biomass of phytoplankton that will develop in a lake [14]. Although DIP is the form of phosphorus that is biologically mobile and the principal state in which phosphorus is interchanged among various biological components in water systems, a major share of the phosphorus resources of the surface waters is in an organic form. 80–100% of the phosphorus in freshwaters in the summer epilimnion is in an organic form, either dissolved or contained within particulate matter [8, 42]. Demonstration that the stores of DIP measured in the open water of the photosynthetic zones of lakes were inadequate to provide the necessary phosphorus for production of phytoplankton for any extended period [23] made it necessary to hypothesize that major stores of phosphorus were regenerated from the particulate phosphorus (P_p) of the phytoplankton and zooplankton and the dissolved organic phosphorus (DOP). It is now clear that recycling of phosphorus may be rapid and that regeneration accounts for a major share of the needs of the system. In many unpolluted freshwater systems, rapid regeneration within the water column is believed to take place because the DIP concentration is frequently below 1 μg P liter^{-1}, or nondetectable, and variations in DIP concentrations may be small despite large changes in plankton biomass. Since the concentration of directly assimilable P in the photic zone often does not provide the phosphorus requirement for microplankton metabolism, phytoplankton and bacteria must assimilate it from the DOP pool. It appears that the ability of algae and bacteria to take up phosphorus from DOP requires extracellular enzymatic hydrolysis of organic phosphorus with subsequent uptake of the resulting PO_4^{3-} [27, 43]. This process involves the hydrolysis of phosphoric esters, mainly phosphomonoesters, by a phosphoric monoester hydrolases (E.C. 3.1.3.) usually called phosphatases.

The majority of data in the literature indicate that alkaline phosphatase (APA) is produced in copious amounts when DIP becomes limiting in lakes [21, 22], suggesting derepression of this enzyme. Measurement of APA activity in algae has been suggested as a procedure for detecting P deficiency or restricted P availability in aquatic ecosystems [41]. Moreover, it has been suggested also that the level of APA indicates the degree of eutrophication of the lakes [8, 24]. The presence of APA has been demonstrated in filtrates of lake water, as a free, dissolved enzyme [9], and in phytoplankton [39], bacteria [20], and zooplankton [45].

Most of the published reports on alkaline phosphatase activity in natural waters are descriptive and have shown the different relationships between APA and the distribution in plankton biomass, phosphorus content, etc. Only a few of them have attempted to study the biochemical mechanisms of the regulation of synthesis and APA activity produced by the natural plankton communities. Moreover, data on the kinetics of APA activity of various components of the plankton are almost lacking.

Here we report on seasonal variation in the kinetics of alkaline phosphatase activity (as expressed by the Michaelis constant, K_M, and the maximum enzyme reaction velocity, V_{max}) in the small eutrophic lake Plußsee. We consider the kinetics of alkaline phosphatase activity of the total (unfiltered water samples), dissolved (filtered through 0.2 μm), and algal and bacterial size fractions of microplankton, and its relation to phosphorus availability for phytoplankton and bacteria in the photic zone of the lake.

Materials and Methods

Description of the Study Area

The Plußsee, located in East Holstein (North Germany), is a small (area 14.3 ha, max. depth 29 m, average depth 9.4 m), naturally eutrophic, Baltic type, wind-shaded lake, largely surrounded by deciduous woodland. In addition to the low influence of wind on the hydrography of the lake, the water exchange rate is low. Lake Plußsee is dimictic, has marked temperature stratification from May to October, and a shallow epilimnion of 3–4 m depth. The lake is ice covered during winter, usually from January to the beginning of April.

Sampling

The sampling site (max. depth 29 m) was located in the pelagic zone of the lake. From August 1983–September 1984 integrated water samples, taken at 0.5 m intervals, were collected weekly with a 2.5 liter Ruttner sampler from the photic zone, which was established by Secchi disc visibility. Large zooplankton was immediately removed by filtration through 55 μm plankton net. Water samples from each depth of the photic zone were mixed together (v/v) and the obtained integrated sample was used as a representative for the whole photic zone.

Pigment Analysis

Chlorophyll *a* and phaeophytin content in the seston were measured by filtering a 1 liter sample through Whatman GF/F glass fiber filters (0.7 μm nominal pore size). Pigment concentration was determined spectrophotometrically according to Golterman and Clymo [19].

Size Fractionation of Seston

In our studies we have applied a differential filtration technique [13] using gravitional filtration through 3.0 μm and vacuum filtration (less than 90 mm Hg) through 0.2 μm pore size polycarbonate membrane filters (Nuclepore). The contribution of photosynthetic picoplankton to the 0.2–3.0 μm size fraction of seston was examined by chlorophyll *a*, primary production [8], and fluorescence microscopy [2, 46]. Bacterial activity in both separated fractions of seston was measured by ^{14}C-glucose uptake [43] and ^{3}H-thymidine incorporation [17].

Phosphorus

Dissolved inorganic phosphate (DIP) and dissolved organic phosphorus compounds (DOP) in the water samples filtered through prewashed 0.2 μm pore size polycarbonate membrane filters (Nu-

clepore), and total phosphorus (P_t) of unfiltered sample were determined according to Koroleff [26]. The method used here is a modification of the Murphy and Riley [29] procedure.

Enzymatically hydrolyzable phosphate (EHP), i.e., amount of PO_4^{3-} released from DOP after enzymatic hydrolysis by natural free phosphohydrolases (mainly APA), was determined in 0.2 μm (Nuclepore) prefiltered water samples according to Chróst et al. [9].

The amount of surplus P ($P_{surpl.}$) stored in phytoplankton cells was determined by a modification of the method of Wynne and Berman [44]. Water samples (0.1–0.5 liter) were filtered through 3.0 μm pore size, 50 mm diameter, membrane filters (Nuclepore). The filters retaining the seston were placed in screw-cap 50 ml erlenmeyer flasks and 20 ml of double-distilled water was added. Flasks were tightly closed and autoclaved at 121°C for 60 min. The samples, after cooling to the room temperature, were analyzed for DIP concentration [26].

ATP Extraction and Determination

Samples of lake water (0.10–0.25 liter) were filtered through 3.0 μm filters to measure ATP in the algal size fraction, while samples of lake water (0.5–1.0 liter) filtered through 0.2 μm polycarbonate membrane filters (Nuclepore Corp., USA) were used to measure ATP in the bacterial size fraction (0.2–3.0 μm). Filters were then immediately plunged into boiling 0.025 M Tris buffer pH 7.75 (Trizma Base + Trizma HCL, Sigma) and extracted twice with 5 ml of buffer in a boiling water bath for 5 min. The extracts were stored at −25°C. For ATP determination we applied a highly sensitive method of using highly purified luciferin-luciferase in 0.025 M HEPES buffer, pH 7.75, obtained from Lumac Medical Products Division (USA). Bioluminescence was detected in ATP-photometer Biolumat LB 9500 (Berthold, FRG) and measured by time-integration (60 sec) of light emission. The amount of ATP was calculated from the standard curve, which was prepared with the use of ATP standard preweighed vials (Sigma, FRG).

Kinetics of Alkaline Phosphatase

APA activity was measured as an increase in fluorescence as the nonfluorescent substrate MUFP (methylumbelliferyl phosphate, Sigma, FRG) was enzymatically hydrolyzed with a subsequent release of the highly fluorescent product methylumbelliferon (MUF). A stock solution of MUFP was prepared to a concentration of 2 mM in double-distilled water and stored at a temperature of −25°C. The stock MUFP solution was thawed at room temperature and diluted to 0.10, 0.25, 0.50, and 1.0 mM immediately before assay. For all enzyme assays, 0.5 ml of substrate solution and 0.5 ml of 0.1 M Tris buffer pH 7.0–9.5 (pH corresponding to lake water) were added to 4.0 ml of unfiltered water sample (total APA activity) or to water filtered through 0.2 μm Nuclepore membrane filter (free, dissolved APA activity). The final MUFP concentrations in the assays were 10, 25, 50, 100, and 200 μM. Autoclaved lake water was used as a reagent control. The incubation of samples was performed at 20°C for 30–90 min, depending on the enzyme activity. MUF fluorescence was measured at 460 nm under 365 nm excitation with a Kontron SFM-25 (Switzerland) spectrofluorometer with a temperature-stabilized sample holder, and recorded on a Kontron Printer-Plotter 800 over incubation time. The amount of released $P\text{-}PO_4^{3-}$ was calculated according to Chróst and Krambeck [7].

APA activity of the phytoplankton size fraction of seston, i.e., that larger than 3 μm, was determined as follows: 16 ml of lake water was filtered through a 3.0 μm pore size, 25 mm diameter, membrane Nuclepore filter and placed in a glass tube with 16 ml of filtered (0.2 μm), autoclaved lake water. The filtered material was incubated in sterile lake water with 2 ml of 0.1 M Tris buffer (pH of lake water) and 2 ml of substrate solution (final MUFP concentrations in assay: 10, 25, 50, 100, and 200 μM), and the fluorescence of released MUF was measured, usually three times during the incubation time.

APA activity of the bacterial size fraction (0.2–3.0 μm) was measured as described above, except that prefiltered 3.0 μm water samples were passed through 0.2 μm Nuclepore filters, and the filters holding 0.2–3.0 μm pore size seston fraction were used for assay.

Varying amounts of MUFP substrate were added to the samples in order to establish enzyme-substrate saturation and to enable the calculation of the kinetic parameters (V_{max} and K_M) of enzyme activity.

P-PO_4^{3-} Uptake Kinetics

Determinations of P-PO_4^{3-} uptake kinetic were performed on samples taken from the photic zone during summer stratification. A series of 100 ml samples were used, and a mixture of a known amount of orthophosphate (0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, and 125.0 μg P-PO_4^{3-} liter^{-1}) and 10 μCi of ^{32}P-PO_4^{3-} (New England Nuclear) was added to each sample, with duplicate flasks at each concentration. The samples were gently rotated on a shaker table, in light (ca. 150 μE m^{-2} s^{-1}) at room temperature (20°C). Aliquots were filtered through 3.0 μm and 0.2 μm pore size Nuclepore filters after incubation times of 5, 15, 30, 60, or 100 min, and activity was assayed by liquid scintillation counting. Total activity of added isotope in the analyzed samples was measured by placing 0.5 ml of labeled lake water in a scintillation vial containing 10 ml of Insta-Gel fluid (Packard). The ratio between ^{31}P-orthophosphate present in water and ^{32}P-added was included in the calculation of P-assimilation rates.

Kinetic Analysis

Computer system Digital VAX 11/750 and terminal monitor Digital VT 100 were used for the calculation of the data. The computer transformations of the Michaelis-Menten equation for the reaction velocity in terms of a constant and the substrate concentration were used for calculation of the V_{max} and K_M values of APA and V_{max} and K_t of ^{32}P uptake [3]. We calculated V_{max}, K_M and K_t from Lineweaver-Burke, Eddie-Hofstee, and Woolf transformations and from a direct plot of reaction velocity versus substrate concentration. For all of the kinetic assays the best fit was found using the direct plot; therefore the values of kinetics reported in the paper were calculated with this method.

Results

Size Fractionation of Microplankton

A major problem of lake water sampling is the difficulty in separating the seston into biologically distinct groups, i.e., to separate zooplankton, phytoplankton, bacteria, and detritus. Table 1 presents the results of phytoplankton and bacterioplankton separation using differential filtration of water samples through 3.0 and 0.2 μm pore size Nuclepore filters. In a natural microplankton community in Lake Plußsee, the bulk of the photosynthetic microorganisms was retained by a 3.0 μm filter, i.e., from March to September on the average 95.0% (SE 3.8) of total chlorophyll *a* and 94.7% (SE 4.3) of particulate primary production were found in this seston fraction. Heterotrophic uptake of ^{14}C-glucose (mean 8.4%, SE 3.1) and ^{3}H-thymidine incorporation (mean 8.1%, SE 3.1) in the fraction larger than 3.0 μm were low. Photosynthetic picoplankton (small green and blue-green algae) in the 0.2–3.0 μm size fraction contributed on the average 5.0% (SE 3.8) to the total chlorophyll *a*, 5.3% (SE 4.3) to primary production, and 9.2% (SE 3.5) to the total number of microbial cells. The activity of the 0.2–3.0 μm size fraction was mainly associated with the bacterial cells, i.e., 91.6% (SE 3.1) of glucose and 91.9% (SE 3.1) of thymidine was taken

Table 1. Partitioning of microplankton from the photic zone of Lake Plußsee by differential filtration of water samples through 3.0 μm and 0.2 μm pore size Nuclepore filters

	Chlorophyll *a*[a]		Primary production[a]		Thymidine uptake[a]		Glucose uptake[a]		FD-C[b]
	>3	<3	>3	<3	>3	<3	>3	<3	<3
	Microplankton size fraction (μm)								
Sample (1984)									
March 26	99.0	1.0	99.7	0.3	13.2	86.8	—	—	7.7
April 4	99.2	0.8	99.7	0.3	8.7	91.3	13.4	86.6	12.3
April 12	92.0	8.0	90.8	9.2	6.8	93.2	9.8	90.2	10.4
May 14	97.5	2.5	96.1	3.9	3.2	96.8	5.6	94.4	6.2
June 5	96.3	3.7	—	—	7.2	92.8	4.9	95.1	4.8
June 19	89.4	10.6	90.7	9.3	—	—	—	—	15.6
July 17	98.7	2.3	94.6	5.4	8.0	92.0	8.2	91.8	8.1
Aug. 7	93.6	6.4	91.2	8.8	10.1	89.9	7.7	92.3	6.5
Sept. 4	90.7	9.3	—	—	7.8	92.2	9.3	90.7	11.8
Mean	95.0	5.0	94.7	5.3	8.1	91.9	8.4	91.6	9.2
SE	3.8		4.3		3.1		3.1		3.5

[a] % of total, unfiltered water sample
[b] Fluorescence direct counts, picoplankton algal cells contribution to the total number of microbial cells in fraction 0.2–3.0 μm

up by the microorganisms of size less than 3.0 μm. Thus algae and bacteria were differentially collected on 3.0 μm pore size filters and in 0.2–3.0 μm size fraction, respectively. Because both the bacterial contribution to the fraction larger than 3.0 μm and the photosynthetic picoplankton participation in fraction 0.2–3.0 μm were low, we use an operational definition of fraction larger than 3.0 μm (algal) and 0.2–3.0 μm size fraction (bacterial).

Kinetics of Alkaline Phosphatase Activity

We studied APA activities of the total microplankton and in algal and bacterial size fractions, and the activity of free, dissolved enzymes in the water. The total APA activity of microplankton varied strongly over time, and V_{max} was highest from May until the middle of September (Fig. 1A). The rate at which PO_4^{3-} could be potentially released into the water varied from 0.835 to 7.141 (mean 2.432, SE 1.891, n 13) nmole liter^{-1} min^{-1}. During autumn and winter, total APA activity was relatively low, and V_{max} varied between 0.119 and 1.078 (mean 0.496, SE 0.291, n 10) nmole PO_4^{3-} liter^{-1} min^{-1}.

The measured value of total APA activity in water samples with a mixed microbial population is a function of the amount of enzyme produced, differences in APA production between algal and bacterial species, environmental factors that may affect the enzyme activity, and the enzyme assay conditions. To evaluate the relationship between APA activity and the biomass of microorganisms, we expressed V_{max} per unit of ATP which is a biomass estimator,

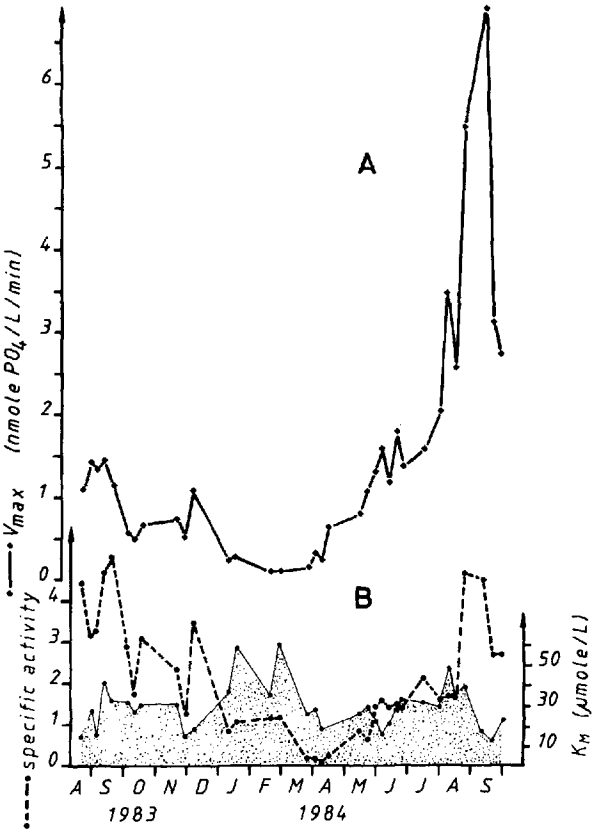

Fig. 1. V_{max} of the alkaline phosphatase activity (**A**), the specific APA activity (pmole PO_4^{3-} ng ATP^{-1} min^{-1}), and Michaelis constants (**B**, shaded area) of enzyme from unfiltered water samples from the photic zone of Lake Plußsee.

i.e., we calculated the specific enzyme activities (V^s_{max}). Figure 1B shows the specific APA activity of unfiltered water samples. The highest V^s_{max} values of total APA (2.113–5.850, mean 3.553, SE 1.240, n 14, pmole PO_4^{3-} ng ATP^{-1} min^{-1}) were noted during summer stratification, when the ambient DIP concentration was below 5 µg P-PO_4^{3-} $liter^{-1}$. The microplankton had the lowest V^s_{max} of total enzyme activity during the spring bloom (0.070–0.239, mean 0.150, SE 0.064, n 5, pmole PO_4^{3-} ng ATP^{-1} min^{-1}), when DIP content varied between 87 and 143 µg P $liter^{-1}$. APA activities, total V_{max}, and specific total APA activity did not covary with microplankton biomass in the photic zone. This fact indicates that APA activity produced by microplankton was not a function of biomass size, and thus other factors must regulate enzyme production and activity.

The Michaelis constant values (K_M) of the total APA varied strongly from 11.8–59.6 (mean 30.5, SE 12.0, n 35) µmole $liter^{-1}$, and increased with an increase of DIP content in the water (Fig. 1B).

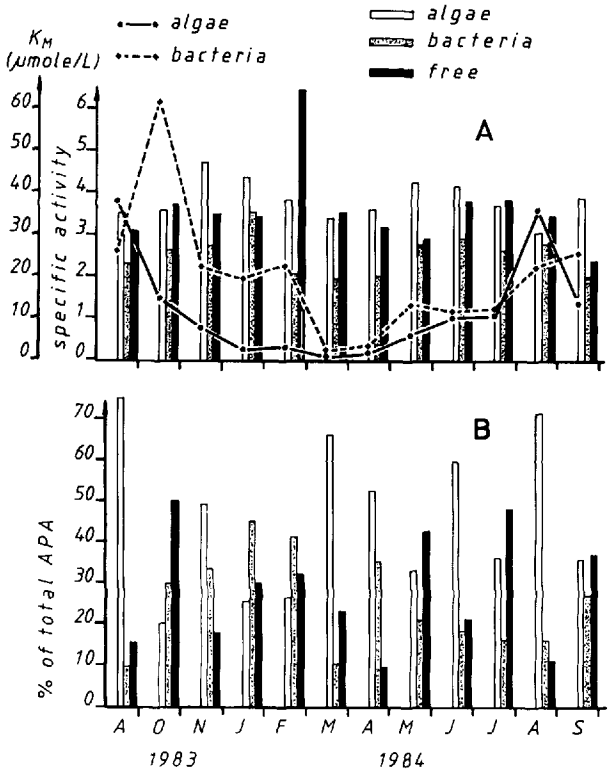

Fig. 2. A. Specific APA activity (pmole PO_4^{3-} ng ATP^{-1} min^{-1}) of algal and bacterial size fractions (curves), the Michaelis constants (bars), and the contribution of algal and bacterial size fraction and free APA activity to the total APA activity (**B**) in the photic zone of Lake Plußsee.

Using differential filtration, we roughly separated the microplankton assemblages into the algal and bacterial size fractions, and measured their APA kinetics. Figure 2 presents the results of these APA activity separations (A) and the contributions of algal and bacterial size fractions and dissolved free APA activity to the total activity of phosphatases in lake water (B). The specific APA activity of algal fraction displayed a pattern similar to the total activity, and had minimum values from January to April, i.e., when the ambient DIP concentration in the lake was high. The bacterial specific APA activity was higher, with two exceptions, than the algal size fraction. In late August 1983 and at the end of September 1984, specific APA activity was higher in the algal fraction. The lowest V^s_{max} of APA activity of the bacterial size fraction was observed during the spring phytoplankton bloom. The specific APA activity of phytoplankton and bacterial size fraction varied from 0.090–3.870 (mean 1.224, SE 1.271, n 12) and from 0.120–6.131 (mean 2.011, SE 1.548, n 12) pmole PO_4^{3-} ng ATP^{-1} min^{-1}, respectively. The Michaelis constants (Fig. 2A) estimated for algal size fraction were in all cases higher (31.1–47.7, mean 38.8, SE 4.7, n 12, μmole $liter^{-1}$) than the bacterial K_M (19.5–35.7, mean 25.4, SE 4.9, n 12, μmole

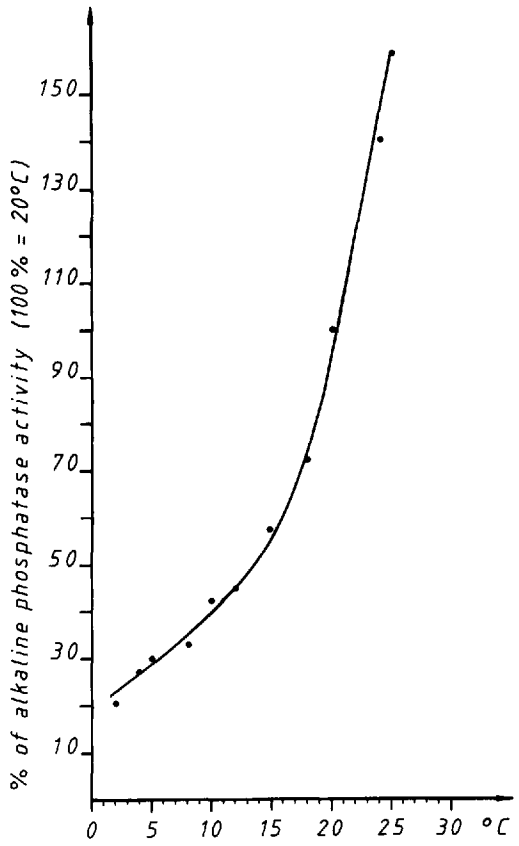

Fig. 3. Dependence of the V_{max} of the total APA activity on the temperature (points represent the mean values of triplicate samples, SE 0.7–2.5).

liter^{-1}). The K_M values of free dissolved APA varied from 24.5–65.2 (mean 36.8, SE 9.8, n 12) μmole liter^{-1}.

Phytoplankton were the major producer of APA in the photic zone of Lake Plußsee from March to November, except for one occasion in October, and during this period their activity constituted from 25.6–75.0% (mean 53.6, SE 16.1, n 9) of the total APA in the water (Fig. 2B). Bacteria were the major APA activity producers in winter (41.3–44.9%, mean 42.2, SE 2.3, n 6), but even during other seasons they contributed significant activity (9.4–35.5%, mean 21.7, SE 9.1, n 10) to total APA. Activity of free enzymes in the photic zone varied from 9.2–50% (mean 28.4, SE 14.3, n 12) of the total APA activity, and probably originated mostly from phytoplankton because the K_M values of the free enzymes were in the same range as the algal K_M.

To evaluate the actual APA activity in the photic zone, which is a function of V_{max}, K_M, the natural substrate concentration and water temperature, we substituted the measured values of V_{max}, K_M and substrate (EHP) concentration in the Michaelis-Menten equation. The calculated values of velocity, i.e., APA

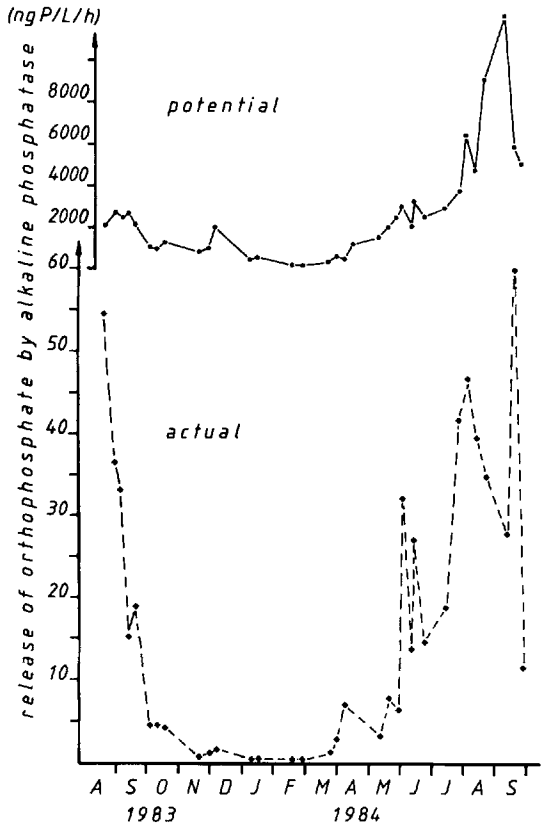

Fig. 4. Potential (V_{max}) and the actual amount of orthophosphate released by the total APA hydrolytic activity in the photic zone of Lake Plußsee (points represent mean values of triplicate samples).

activity attained with the enzyme-substrate complex at a given EHP concentration, must be corrected for temperature of lake water, because all enzyme assays were run at 20°C. The dependence of APA velocity on enzyme reaction temperature is presented in Fig. 3. From this relationship we calculated the temperature correction factor, which was substituted into the Michaelis-Menten equation. The values of the actual and potential (V_{max}) total APA activity of microplankton are shown in Fig. 4. The release of PO_4^{3-} by APA hydrolysis increased with decreases in the ambient concentration of DIP in the photic zone. Similar maximum rates of orthophosphate release from 14.84–59.85 ng P liter^{-1} hour^{-1} (mean 34.06, SE 16.77, n 10) were observed at the end of summer stratification in August–September 1983 and 1984.

Relationship Between APA and P Uptake

Measurements of P-PO_4^{3-} uptake kinetics in the lake showed that summer microplankton had P-uptake rates in the same range as actual release of P-PO_4^{3-}

Table 2. Range of the actual APA hydrolytic release of $P\text{-}PO_4^{3-}$ and orthophosphate uptake rates (ng $P\text{-}PO_4^{3-}$ liter^{-1} hour^{-1}) and the kinetics of P uptake by microplankton in the photic zone of Lake Plußsee during summer stratification

Sample	Actual rate		V^s_{max}[a]	K_t[b]
	APA	P uptake		
Total microplankton	27–124 (50) (SE 31, n 9)	21–130 (56) (SE 17, n 9)	0.57–3.46 (1.98) (SE 1.13, n 8)	22–55 (41) (SE 12, n 8)
Phytoplankton	15–105 (42) (SE 35, n 5)	45–110 (51) (SE 14, n 9)	0.60–4.24 (2.06) (SE 1.29, n 8)	30–60 (46) (SE 10, n 8)
Bacteria	10–90 (24) (SE 18, n 5)	15–65 (19) (SE 8, n 9)	0.93–6.58 (4.57) (SE 2.03, n 8)	2–6 (2) (SE 1, n 8)

[a] Maximal specific rate orthophosphate uptake (ng P ng ATP^{-1} hour^{-1})
[b] μg P liter^{-1}
In parentheses, mean value, standard error, and number of samples, respectively

from EHP pool by APA (Table 2). The microplankton P-uptake kinetics conformed to the Michaelis-Menten model over the orthophosphate range investigated. The maximal specific P-uptake rates of microorganisms in bacterial size fraction were higher than phytoplankton and had transport affinities (K_t) more than an order of magnitude greater than the microplankton cells larger than 3.0 μm.

Relationship Between APA and Phosphorus

The specific activity of total APA significantly increased when the ambient DIP concentration was below 10 μg P liter^{-1}, (Fig. 5). A similar response of the specific APA activity to orthophosphate was also found in the algal size fraction, i.e., with decreasing amounts of DIP, specific APA increased, slowly at DIP range 20–168 μg P liter^{-1} and rapidly at inorganic phosphate range 0–6 μg P liter^{-1}. Thus phytoplankton compensated for the lack of orthophosphate by an increase in enzyme production and specific activity. Specific APA of the bacterial size fraction of seston was not affected by DIP in water (Fig. 5). Both low and high specific activities occurred in low and high DIP concentrations.

The amount of $P_{surpl.}$ stored in algal cells, which is an indicator of P status of the cells, was inversely proportional to the specific activity of APA of the algal size fraction (Fig. 6A). When $P_{surpl.}$ constituted less than 10% of the particulate phosphorus in seston, the microorganisms larger than 3.0 μm produced APA with a high specific activity, and when $P_{surpl.}$ was higher than 15% of P_p, this activity rapidly decreased (Fig. 6B). The Michaelis constants, which indicate the affinity of APA for its substrate (a lower K_M indicating a higher affinity) showed the direct positive relationship to DIP in the water. Higher K_M values of APA of total microplankton occurred in higher DIP concentration (Fig. 7A). We found a better relationship by plotting K_M values against the ratio DIP:EHP (Fig. 7B). This relationship suggested that in many situations DIP was a competitive inhibitor of APA activity in the lake.

To prove this observation we examined the effect of adding inorganic phos-

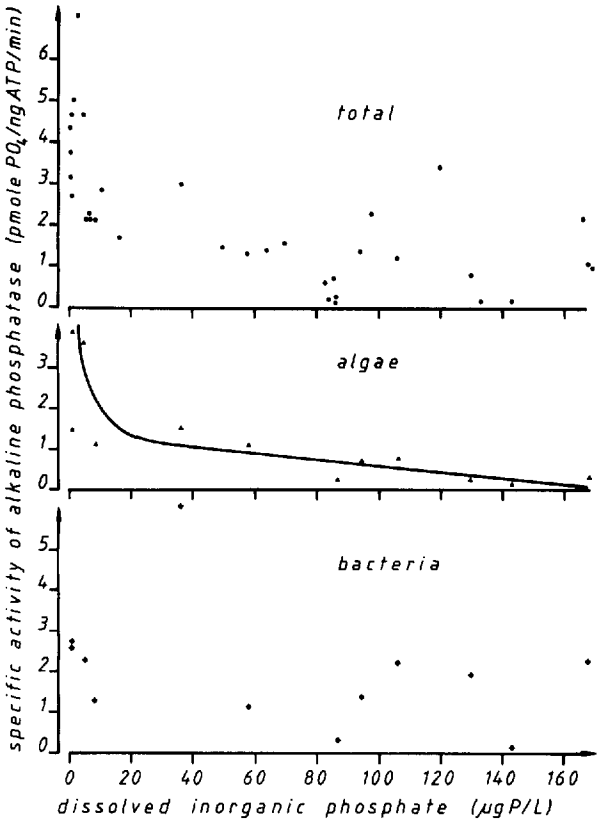

Fig. 5. The relationship between specific APA activity of the total microplankton, algal, or bacterial size fractions and the concentration of dissolved inorganic phosphate (DIP) in the photic zone of Lake Plußsee.

phate to lake water on the kinetics of APA activity of the algal and bacterial size fractions, as well as of the total, unfiltered water samples from Lake Plußsee. Figure 8 presents an example of Lineweaver-Burke plots of competitive inhibition of APA by the orthophosphate. After addition of 100 and 150 µg P liter^{-1} to the water (original concentration 1 µg P liter^{-1}), V_{max} of APA was almost unchanged, but K_M values of the total APA and APA of the algal size fraction were increased significantly in comparison to control samples. K_M values of total and algal APA increased from 23.4 (SE 1.7, n 3) to 57.5 (SE 2.6, n 3) µmole liter^{-1}, and from 24.7 (SE 1.2, n 3) to 65.5 (SE 2.2, n 3) µmole liter^{-1}, respectively. APA of the bacterial size fraction was not strongly inhibited by orthophosphate, and K_M values increased only slightly from 20.3 (SE 1.4, n 3) to 25.6 (SE 1.6, n 3) µmole liter^{-1}.

Discussion

The ability of phytoplankton to utilize phosphorus from dissolved organic phosphorus compounds has been intensively studied recently in both marine

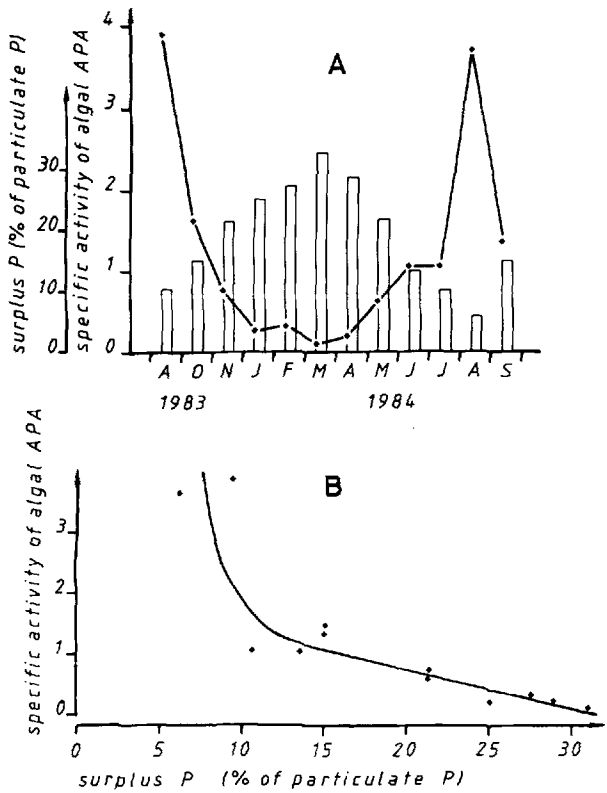

Fig. 6. Surplus-P content (bars) and specific APA activity (pmole PO_4^{3-} ng ATP^{-1} $hour^{-1}$) of algal size fraction (**A**), and the relationship between both parameters (**B**) in the photic zone of Lake Plußsee.

and freshwater ecosystems [27, 40]. Chu [11] was one of the first to demonstrate experimentally that some sources of DOP (phytin, glycerophosphate) support fairly good growth of *Phaeodactylum tricornutum*. Subsequently, others showed that many species of algae are capable of such growth in the absence of DIP but in the presence of phosphoesters [32, 35]. It is generally believed that the ability to take up phosphorus from organic P compounds is restricted to those microorganisms producing a phosphatase that hydrolyzes the phosphoesters in, or on, the cell membrane, releasing PO_4^{3-} ions.

DOP may be the major soluble P form in the epilimnion during summer stratification of eutrophic lakes [8], but all forms of organic phosphorus are not equally available for enzymatic hydrolysis. Data from freshwaters show that two general types of DOP exist [16]. The high molecular weight compounds are slowly hydrolyzable by APA and are supposed to be mainly nucleic acids. The second type of DOP, which is easily degraded and hydrolyzed by the phosphatases, includes all the phosphomonoesters. The latter is often found at low concentrations relative to total DOP [8, 38]. During DIP depletion in Lake Plußsee in summer, DOP constituted ca. 82% of the total soluble P in the photic zone, and on average 63% of the DOP was enzymatically hydrolyzable.

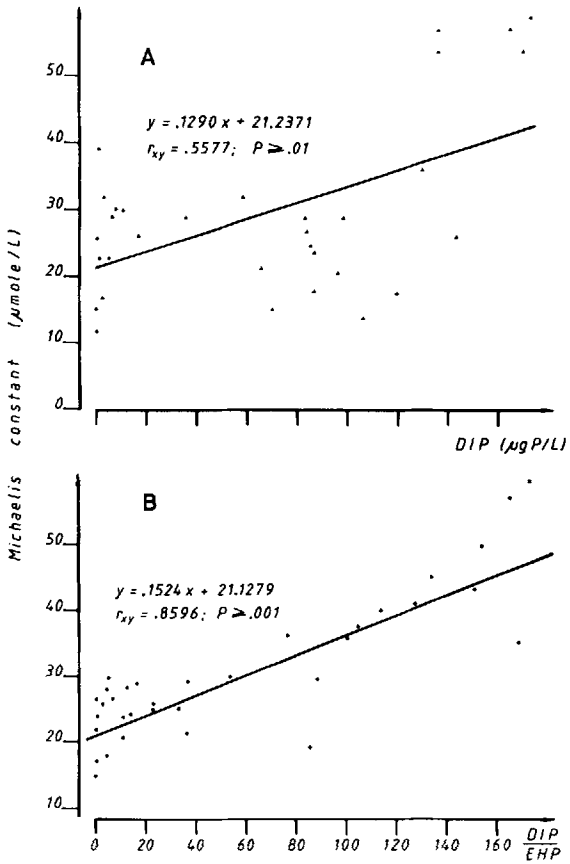

Fig. 7. Relationship between Michaelis constants of the total APA and the concentration of dissolved inorganic phosphate (**A**), and between K_M and the ratio of enzymatically hydrolyzable phosphate (EHP) to dissolved inorganic phosphate (**B**) in the photic zone of Lake Plußsee.

The high EHP contribution to the DOP pool in Lake Plußsee is not unusual for freshwaters, since Francko [15] found that EHP comprised from 0–65% of DOP in eutrophic Sangre Isle Lake, Oklahoma. Obviously, the EHP concentration effectively released to the water is much higher than that expected from concentrations usually found in the lake, because EHP release and hydrolysis occur simultaneously.

Enzymatic phosphorus release from EHP does not occur immediately at the beginning of DIP depletion in the lake because of the intracellular pools of orthophosphates and polyphosphates ($P_{surpl.}$) stored in the microbial cells. Effectively, the algal capacity to store internal P is usually great. Also in cultures phosphorus starvation is followed by an increase in P uptake after the replenishment of the medium. In cells newly enriched with DIP, the proportion of polyphosphates in cells can reach very high values, and this form of phosphorus can contribute as much as 50% to total cell-P [1, 30]. Obviously, this reservoir dramatically enhances the capability of the phytoplankton to survive during

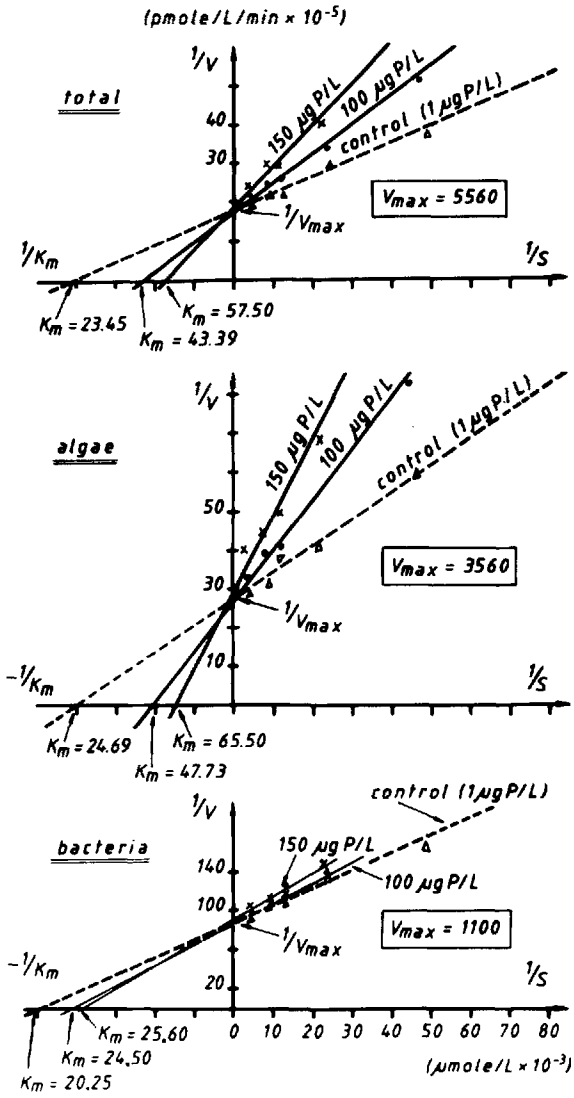

Fig. 8. Lineweaver-Burke plots of competitive inhibition of APA activity by orthophosphate of total unfiltered water sample and of bacterial and algal size fraction (total APA: control, y = 0.42x + 17.13; 100 µg-P, y = 0.77x + 16.60; 150 µg-P, y = 1.14x + 16.76. Algae: control, y = 0.68x + 26.68; 100 µg-P, y = 1.40x + 26.44; 150 µg-P, y = 1.63x + 27.57. Bacteria: control, y = 2.0x + 89.50; 100 µg-P, y = 1.72x + 92.24; 150 µg-P, y = 2.0x + 90.84 (all linear regressions at 95% confidence interval).

DIP depletion and helps them to compete successfully with bacteria in which this property is not as well developed. Rhee [34] demonstrated this experimentally. Finally, when all external and internal phosphorus reservoirs are depleted, microplankton must produce phosphatases and hydrolyze phosphoesters as an alternative P source to compensate for the lack of easily available

DIP. This observation is based on our studies in Lake Plußsee. When DIP concentrations were high, e.g., during spring homothermy, phytoplankton accumulated phosphorus in the intracellular pool, and $P_{surpl.}$ content of algal cells constituted from 20–33% (mean 29, SE 5) of the total particulate P. In parallel with the decrease in DIP concentration in the water, we observed a decrease of $P_{surpl.}$ in phytoplankton, i.e., part of the stored P was used to support P metabolism. In parallel to the gradual decrease in DIP and $P_{surpl.}$ concentrations, the specific APA activity in algal size fraction also increased slowly. However, when the $P_{surpl.}$ amount decreased to below ca. 15% of the particulate P, and DIP concentrations were very low, the phytoplankton reacted rapidly and produced APA with high specific activity (Fig. 6).

The activity of APA in the microplankton has been reported to be directly related to inorganic phosphorus limitation [21, 37]. Several authors have concluded that high phosphatase activity can be derepressed or activated by low DIP concentrations. However, it is not quite correct to conclude that APA production is derepressed directly by low DIP concentration. The mechanism of APA derepression is regulated by the intracellular P level in microorganisms, which obviously depends on ambient inorganic phosphate concentrations. Moreover, in many freshwaters there are time lags between the beginning of DIP depletion and high APA activity production, because of intracellular P. Recently published data [18] support the above observation. Consequently, the synthesis of APA by phytoplankton in Lake Plußsee was repressed by high intracellular levels of $P_{surpl.}$. On the other hand, orthophosphate was a strong competitive inhibitor of APA activity of the phytoplankton size fraction (Fig. 8), but inhibition of APA activity of microorganisms smaller than 3.0 μm was only slight. We observed both direct inhibition of APA activity by DIP after orthophosphate addition to lake water and rapid increase of $P_{surpl.}$ content in microbial cells. In addition, this inhibition is evident from the relationship between K_M values of the total APA and DIP (Fig. 7). The hallmark of competitive inhibition of APA is that the orthophosphate combines with the enzyme in such a way that it competes with a substrate for binding at the active site, thus increasing the apparent K_M of the APA for substrate. Therefore, when we plotted DIP : EHP ratios versus K_M values, we found that this relationship gave a better fit than a plot of DIP versus K_M (Fig. 7). Thus the K_M values of total APA in the photic zone were affected by both DIP concentrations and by the quantity and quality of EHP. This phenomenon has an ecological significance in the regulation of APA activity.

The capacity of phytoplankton to assimilate phosphorus from low EHP concentrations is another problem. Taft et al. [40] observed that in the Chesapeake Bay natural assemblages had uptake characteristics that permitted utilization of EHP at low concentrations. K_M values of APA hydrolysis were lower than, or in the same range as, K_t values of PO_4^{3-} uptake from glucose-6-phosphate. Our studies support this observation. Measurements of $P\text{-}PO_4^{3-}$ uptake in Lake Plußsee showed that summer microplankton had P uptake rates in the same range as actual release of $P\text{-}PO_4^{3-}$ from EHP by APA (Table 2).

We compared the efficiency of microplankton biomass production per 1 μg of biologically available P in the water during spring, when high P concentration was not the limiting factor (but silicon was), and during summer P deficiency

Table 3. The ratio between chlorophyll *a*, ATP in microplankton, and biologically available phosphorus concentrations during spring and summer microplankton mass development

Period	μg chlorophyll *a* μg P^{-1}	ng ATP μg P^{-1}
Spring	0.289	18.966
Summer	1.030	62.548
Summer : spring	3.564	3.297

when DIP concentrations were low and other sources of P must be utilized (Table 3). The microplankton biomass production during summer was ca. 3-fold higher per unit of available P than during spring, although the concentration of directly assimilable DIP was much lower in summer than in spring. One explanation is that orthophosphate was turned over rapidly by microplankton in summer. Alternatively, microplankton may have utilized phosphorus very efficiently from the EHP pool. Utilization of the EHP pool probably was important since the specific APA activity was highest during this period. However, both orthophosphate and EHP probably were utilized simultaneously.

One difficulty in interpreting the significance of APA for phytoplankton P metabolism is that bacteria are also able to produce APA and use phosphomonoesters in natural waters [20, 25]. The potential role of bacteria in measurements of total APA activity makes using APA activity as an index of P limiation of the phytoplankton more difficult. During our studies we found that ATP in the bacterial size fraction contributed from 11–30% (mean 22, SE 7, n 12) of the total microplankton ATP in the photic zone of the lake, and the APA activity of this fraction constituted ca. 25% (SE 9, n 12) of the total activity (Fig. 2). We must also emphasize that bacteria produced higher specific APA activity and had lower K_M values (higher affinity to EHP) than phytoplankton.

We can also conclude that, excluding only a short period during summer stratification, bacteria probably were not P-limited, due to their high affinity P-uptake system, which was efficient at very low ambient DIP concentrations. We found that bacterial K_t values for orthophosphate uptake were ca. 15 to 20-fold lower than phytoplankton K_t (Table 2). The question arises, "Why did bacteria produce highly efficient APA when DIP concentrations were sufficient or exceeded their P requirement for uptake and metabolism?" It is known that in lab cultures, when APA was produced, the microbial cells also switched to a very active P-uptake mode from the usually slow mode due to the production of a highly active phosphate-binding protein [33]. However, this phenomenon was not observed during our studies. Thus there are two possible answers. First, bacteria were not P limited, but their activity and growth was limited by supply of easily assimilable carbon. Many authors have described the strong dependence of the growth and activity of heterotrophic bacteria on the amount of easily available carbon [10, 12]. Therefore bacteria produced APA to hydrolyze organophosphoric compounds but probably utilized the organic moiety as an

organic carbon source. Second, bacteria hydrolyzed EHP and increased the DIP pool in the water, which affected the primary production of phytoplankton. Consequently, more photosynthetic organic carbon was released [4] and served as carbon substrates for bacteria. The organic carbon released by algae is known to be a good carbon source for aquatic bacteria [5, 6, 12], and the metabolic coupling of algae and bacteria in the carbon cycle in natural waters has been well described [28].

This hypothetical situation could occur in Lake Plußsee when bacteria might be carbon limited at the beginning of summer stratification (end of June–July). During this period DIP concentrations varied between 10 and 20 μg P liter^{-1} and were sufficient for the bacterial P requirement. Nevertheless, bacterial APA had high specific activities and low K_M values. The amount of orthophosphate actually released by algal APA plus DIP present in the water did not meet the total phosphorus requirement for phytoplankton. It was possible that a significant portion of orthophosphate utilized by phytoplankton was provided by bacterial APA activity.

Bacterial heterotrophy and phytoplankton production may be coupled via phosphatases, which may be important in some freshwater ecosystems. However, further studies are needed to prove the existence of this coupling.

Acknowledgments. The grant of research postdoctoral fellowship to R. J. Chróst by Max-Planck Society, FRG is gratefully acknowledged. We are grateful Dr. S. Craig for her picoplankton analysis. We thank Dr. D. Kirchmann (University of Delaware, USA) for his helpful discussion and Dr. H. J. Krambeck (Max-Planck Institut of Limnology, Plön) for help with computer calculations.

Note Added in Proof. James W. Ammerman and Farooq Azam (Science 1985, 227:1338–1340) have found that a bacterial periplasmic or membrane bound enzyme, 5′-nucleotidase, may account for a large fraction of the organic phosphorus hydrolysis in natural waters. As in the case of exoproteases, most of the nucleotidase activity is also associated with the cells rather than free in water. Importantly, the PO_4^{3-} molecules released from 5′-nucleotides are 10–40 times more likely to be taken up by the microbial populations than the PO_4^{3-} molecules present in the bulk-phase water. This preference for uptake of hydrolysis products is strong evidence for a tight metabolic coupling between the utilizable DOM pruduction and utilization. They predict also that bacteria may take up the organic moiety of the organic phosphorus compound hydrolyzed in preference to the same molecular species in the bulk-phase.

References

1. Aitchison PA, Butt VS (1973) The relation between the synthesis of inorganic polyphosphate and phosphate uptake by *Chlorella vulgaris*. J Exp Bot 24:497–510
2. Brock TD (1978) Use of fluorescence microscopy for quantifying phytoplankton, especially filamentous blue-green algae. Limnol Oceanogr 23:158–160
3. Button DK (1985) Kinetics of nutrient-limited transport and microbial growth. Microbiol Rev 49:270–297
4. Chróst RJ (1978) Extracellular release in *Chlorella vulgaris* culture and the role of bacteria-accompanying algae in this process. Acta Microbiol Polon 27:55–62
5. Chróst RJ (1984) Use of ^{14}C-dissolved organic carbon (RDOC) released by algae as a realistic

tracer of heterotrophic activity measurements for aquatic bacteria. Arch Hydrobiol Beih Ergebn Limnol 19:207–214
6. Chróst RJ, Faust MA (1983) Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. J Plankton Res 5:477–493
7. Chróst RJ, Krambeck HJ (1986) Fluorescence correction for measurements of enzyme activity in natural waters using methylumbelliferyl-substrates. Arch Hydrobiol 106:70–90
8. Chróst RJ, Siuda W, Halemejko GZ (1984) Long-term studies on alkaline phosphatase activity (APA) in a lake with fish-aqua-culture in relation to lake eutrophication and phosphorus cycle. Arch Hydrobiol (Suppl) 70:1–32
9. Chróst RJ, Siuda W, Albrecht D, Overbeck J (1986) A method for determination of enzymatically hydrolyzable phosphate (EHP) in natural waters. Limnol Oceanogr 32:662–667
10. Chróst RJ, Wcislo R, Halemejko GZ (1986) Enzymatic decomposition of organic matter by bacteria in eutrophic lake. Arch Hydrobiol 107:145–165
11. Chu SP (1946) The utilization of organic phosphorus by phytoplankton. J Mar Biol Assoc UK 26:285–295
12. Cole JJ, Likens GE, Strayer DL (1982) Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnol Oceanogr 27:1080–1090
13. Derenbach JB, Williams PJLeB (1974) Autotrophic and bacterial production: fractionation of plankton populations by differential filtration of samples from the English Channel. Mar Biol 25:263–269
14. Dillon PJ, Rigler FH (1974) The phosphorus-chlorophyll relationship in lakes. Limnol Oceanogr 19:767–773
15. Francko DA (1984) Relationship between phosphorus functional classes and alkaline phosphatase activity in reservoir lakes. J Freshwat Ecol 2:541–547
16. Francko DA, Heath RT (1979) Functionally distinct classes of complex phosphorus compounds in lake water. Limnol Oceanogr 24:463–473
17. Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters; evaluation and field results. Mar Biol 66: 109–120
18. Gage MA, Gorham E (1985) Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. Freshwater Biology 15: 227–233
19. Golterman HL, Clymo RS (1969) Methods for chemical analysis of freshwaters. IBP Handbook No 8, Blackwell Sci Publishers, Oxford
20. Halemejko GZ, Chróst RJ (1984) The role of phosphatases in phosphorus mineralization during decomposition of lake phytoplankton blooms. Arch Hydrobiol 101:489–502
21. Healey FP, Hendzel LL (1980) Physiological indicators of nutrient deficiency in lake phytoplankton. Can J Fish Aqua Sci 37:442–453
22. Heath RT, Cooke GD (1975) The significance of alkaline phosphatase in a eutrophic lake. Int Ver Theor Angew Limnol Verh 19:959–965
23. Hutchinson GE (1941) Limnological studies in Connecticut. IV. The mechanism of intermediary metabolism in stratified lakes. Ecol Monogr 11:21–60
24. Jones JG (1972) Studies of freshwater microorganisms: phosphatase activity in lakes of differing degrees of eutrophication. J Ecol 60:777–791
25. Kobori H, Taga N (1979) Phosphatase activity and its role in the mineralization of organic phosphorus in coastal seawater. J Exp Mar Biol Ecol 36:23–39
26. Koroleff F (1976) Determination of phosphorus. In: Grasshoff K (ed) Methods of seawater analysis. Verlag Chemie, New York, pp 117–126
27. Kuenzler EJ (1965) Glucose-6-phosphate utilization by marine algae. J Phycol 1:156–164
28. Meffert ME, Overbeck J (1979) Regulation of bacterial growth by algal release products. Arch Hydrobiol 87:118–121
29. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36
30. Perry MJ (1976) Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the Central North Pacific. Limnol Oceanogr 21:88–107

31. Pettersson K (1980) Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. Arch Hydrobiol 89:54–87
32. Provasoli L (1958) Nutrition and ecology of protozoa and algae. Annu Rev Microbiol 12: 279–308
33. Raven JA (1980) Nutrient transport in microalgae. In: Rose AH, Morris JG (eds) Advances in microbial physiology, vol. 21. Academic Press, San Francisco, pp 48–227
34. Rhee GY (1972) Competition between an alga and an aquatic bacterium for phosphate. Limnol Oceanogr 17:505–514
35. Rivkin RB, Swift E (1980) Characterization of alkaline phosphatase and organic phosphorus utilization in the oceanic dinoflagellate *Pyrocystis noctiluca*. Mar Biol 61:1–8
36. Schindler DW (1977) Evolution of phosphorus limitation in lakes. Science 195:260–262
37. Smith RE, Kalff J (1981) The effect of phosphorus limitation on algal growth rates: evidence from alkaline phosphatase. Can J Fish Aqua Sci 38:1421–1427
38. Solorzano L (1978) Soluble fractions of phosphorus compounds and alkaline phosphatase activity in Loch Creran and Loch Etire, Scotland. J Exp Mar Biol Ecol 34:227–232
39. Stewart AJ, Wetzel RG (1982) Phytoplankton contribution to alkaline phosphatase activity. Arch Hydrobiol 93:265–271
40. Taft JL, Loftus ME, Taylor WR (1977) Phosphate uptake from phosphomonoesters by phytoplankton in the Chesapeake Bay. Limnol Oceanogr 22:1012–1021
41. Vincent WV (1981) Rapid physiological assays for nutrient demand by the plankton. II. Phosphorus. J Plankton Res 3:699–710
42. Wetzel RG (1983) Limnology. Saunders College Publishing, Philadelphia
43. Wright RT, Hobbie JE (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447–464
44. Wynne D, Berman T (1980) Hot water extractable phosphorus: an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret? J Phycol 16:40–46
45. Wynne D, Gophen M (1981) Phosphatase activity in freshwater zooplankton. Oikos 37:369–376
46. Zimmermann R, Meyer-Reil LA (1974) A new method for fluorescence staining of bacterial population on membrane filters. Kiel Meeresforsch 30:24–28