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Bacterial utilization of phosphorus pools after nitrogen and carbon amendment and its relation to alkaline phosphatase activity

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ABSTRACT: Changes in phosphorus (P) pools after the addition of nitrogen + organic carbon to Baltic Sea bacteria, as well as the relationship of this supply to the stimulation of bacterial alkaline phosphatase activity (APA), were investigated. Furthermore, the utilization of dissolved organic phosphorus (DOP) without and after stimulation, and if DOP was sufficient to meet the P demand, was studied. The simultaneous amendment of both substrates provoked a decrease of phosphate in water and an increase of P in the bacterial fraction. During the course of the experiments, the changes occurred in parallel with the stimulation of the APA. The increase in P in the bacterial fraction was predominantly influenced by the phosphate concentration. If the phosphate concentration was below the P demand, then DOP was used. DOP usage by bacteria proved dependent on the DOP concentration in the water. At concentrations of about 0.20 µM, the DOP consisted of material not usable by bacteria. At high concentrations of 0.44 µM, 0.15 µM of DOP was used. The maximum stimulation of bacterial APA correlated inversely with DOP changes, and high APA could not be associated with the amount of DOP degradation. Without exogenous nitrogen and carbon supply, phosphate and the available DOP in Baltic Sea water samples were sufficient to satisfy the bacterial need of P. However, after the addition of nitrogen and carbon, the available P pools were not capable of covering the P demand.

KEY WORDS: Alkaline phosphatase activity · Phosphorus pools · DOP · Bioavailable DOP · Baltic Sea

INTRODUCTION

In marine ecosystems, the carbon (C), nitrogen (N), and phosphorus (P) cycles do not exist independently of each other (Karl & Björkman 2002). One interconnection in the nutrient cycles occurs with phytoplankton and bacterial alkaline phosphatase, which is an important enzyme for degrading organic P compounds, especially under phosphate-limiting conditions (Stihl et al. 2001, Hernandez et al. 2002, Mulholland et al. 2002, Hoppe 2003). This relationship functions in 2 different ways: (1) During the degradation of organic P compounds by alkaline phosphatase activity (APA), C and N are released in addition to phosphate. Thus, the elevated APA present at high phosphate concentrations in deep ocean waters (Hoppe & Ullrich 1999) is believed to satisfy the C demand of bacteria rather than the phosphate demand. (2) The availability of C and N can enhance the APA, and consequently, the degradation of organic P. Guerrini et al. (1971), Myklestad & Sakshaug (1983) and Sakshaug et al. (1984) first observed that C, and the N:P and C:P ratios of algal cells influenced the APA. Stewart & Wetzel (1982) and Carlsson & Graneli (1993) measured an increase in the APA of natural phytoplankton and bacterial communities after addition of humic substances. A clear stimulation of bacterial APA after amendment with N and C was observed by Nausch (2000) and Neddermann & Nausch (2004) in the Baltic Sea. In these experiments, the simultaneous addition of both N and C compounds caused an increase in the APA. N (Neddermann & Nausch 2004) or glucose (Chrost 1991) alone did not, or if so only weakly, enhance the APA. Therefore, a

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certain balance in the availability of N and C seems to be necessary for the effect on the APA.

In contrast to bacterial APA, phytoplankton APA may be enhanced by N alone. This is because phytoplankton can gain C through photosynthesis. Thus, ammonium alone stimulated the APA in red-tide dinoflagellates (Vargo & Shanley 1985). The high APA measured in diazotrophic cyanobacteria (Graneli et al. 1990, Nausch 1996, Stihl et al. 2001, Hernandez et al. 2002, Mulholland et al. 2002) is attributed to N fixation under P-deficient conditions. Therefore, the activity of alkaline phosphatase is often associated with phosphate usage from organic matter. The organisms produce alkaline phosphatase to overcome their imbalance of nutrients, and to satisfy their P demand from organic P compounds (Neddermann & Nausch 2004). This hypothesis is supported by Li et al. (2000), who observed a low APA during N-limitation in algae. According to all these observations, the addition of N and C should have an effect on the dissolved organic phosphorus (DOP) pool due to the stimulating effect on the APA.

In the present study, the effects of N and organic C supplementation on inorganic and organic P pools in Baltic Sea water, as well as the relationship between changes in P pools and the APA stimulation were investigated. Furthermore, we examined whether dissolved organic phosphorus (DOP) is the limiting factor for bacterial growth when N and organic C are abundantly available.

MATERIALS AND METHODS

Experimental layout. From May 2002 until August 2003, 10 experiments were conducted in the manner described by Neddermann & Nausch (2004). Water was sampled from the same near-shore station in front of Heiligendamm (54°09′N, 11°51′E; Mecklenburg-Vorpommern, Germany) in winter, spring, and summer, covering the development of the growth season and the concurrent changes in nutrient conditions. The water depth of the station is shallow (2.5 m). At times of strong winds, when the water column is completely mixed, phosphate is introduced from the sediment into the water column. Therefore, the annual nutrient cycle is influenced by such temporal events, and high phosphate concentrations can also be measured in summer.

For the experiments, water samples were prefiltered within 1 h after sampling through 0.8 µm cellulose nitrate filters using a vacuum of –200 mbar. All filters (Sartorius, Schleicher & Schuell) were tested for intrinsic phosphate release. Five litres of the filtrate were collected in pre-cleaned (1 N HCl) and sterilized Duran bottles. In Expts 1 to 4, the dominating question was the change in P pools and their relationship with APA after addition of N and organic C. Therefore, 1 mg l^{-1} bovine serum albumin (Merck 112018), or 7 µM ammonium chloride (Merck 10145) + 1 mg l^{-1} D-(+)-glucose (Merck 4074) were added (Table 1). Control bottles contained no additions. The question was extended in the subsequent experiments. In addition, in Expts 6 to 10, it was tested whether the available DOP was acting as a limiting factor. DOP, in the form of 0.30 µM DL-α-glycerophosphate (Sigma G-6014), was added alone or together with ammonium + glucose (Table 1). The incubations with albumin were omitted because the results were similar to those of ammonium + glucose addition in the first experiments. Incubations were done at 15°C in the dark, and aerated with 0.2 µm filtered air. Expts 1 to 5 were conducted as single experiments, and Expts 6 to 10 were done in duplicate. Subsamples (350 ml) were taken for the measurement of phosphate, DOP, bacterial P, APA, and bacterial counts immediately after the respective additions, and each day during the following 7 to 10 d period.

Analytical methods. Phosphate concentrations were determined manually using a standard colorimetric method (Rohde & Nehring 1979, Grasshoff et al. 1983) immediately after subsampling. For the estimation of bacterial P and DOP, 35 ml samples were stored deep frozen (–20°C) before and after filtration through 0.2 µm cellulose nitrate filters (Schleicher & Schuell) until determination. The defrosted samples were oxidized with peroxydisulfate in an acidic medium (Grasshoff et al. 1983). The subsequent phosphate determination was done using a 10 cm cuvette. The detection limit was 0.01 µM. Determinations were performed in duplicate from each incubation bottle. The bacterial P was determined as the difference between the P concentrations in the 0.8 µm pre-filtered and the 0.2 µm filtered water, whereas the DOP was calculated as the difference between the total dissolved P (TDP)

Table 1. Experimental design and substrate additions (x) of the experiments (AG = ammonium + glucose; AGG = ammonium + glucose + glycerophosphate; G = glycerophosphate). In each experiment, controls without any substrate addition were done

Expt	Substrate additions			
	Albumin	AG	AGG	G
1	x			
2	x	x		
3	x	x		
4	x	x		
5		x		
6		x	x	x
7		x	x	x
8		x	x	x
9		x	x	x
10		x	x	x

concentration in the 0.2 µm filtered water and the phosphate concentration.

For the quantification of bacterial cells, 20 ml samples were preserved with 0.5% v/v formaldehyde. Subsamples of 1 to 5 ml were filtered onto 0.2 µm black Nuclepore filters, stained with a 4′,6-diaminidino-2-phenylindole (10 mg l^{-1} DAPI) (Hoff 1993) solution for 5 min, and mounted on a slide with fluorescence-free immersion oil (Cargille oil). Bacteria were counted with an epifluorescence microscope (Zeiss-Axioskop) combined with an image analysis system (Photometrix), and the computer software, 'IP Lab Spectrum' (Signal Analytic).

APA was measured in kinetic assays using the substrate analogous, 4-methylumbelliferryl-phosphate (MUF-P) (Hoppe 1993), at a final concentration of 240 µM. For each incubation bottle, the APA was estimated in triplicate cuvettes, each containing 2.5 ml sample, over a period of 2 h at 15°C in the dark. During that time, fluorescence readings were carried out every 30 min with a spectrofluorimeter (Hitachi, F-2000) at an excitation wavelength of 364 nm and an emission wavelength of 445 nm. The fluorescence intensity increase over time was used for the calculation of the APA. Controls were done with boiled water. Fluorescence units were calibrated with 4-methylumbelliferone standard solutions over the range 0.3 to 1 µM. The maximum level of bacterial APA measured was used to compare the experiments.

RESULTS

General course of the experiments

The addition of N and C in the form of albumin or ammonium + glucose initiated a stimulation of bacterial APA (Table 2). The maximum APA level was reached at an incubation time of 3 to 4 d. In all experiments, the stimulation of APA was accompanied by decreasing phosphate concentrations in water, and an increase in P in the bacterial fraction (Fig. 1). The maximum APA was observed when phosphate in the water was at the lowest concentration, and reached the detection limit. At that time, the bacterial fraction

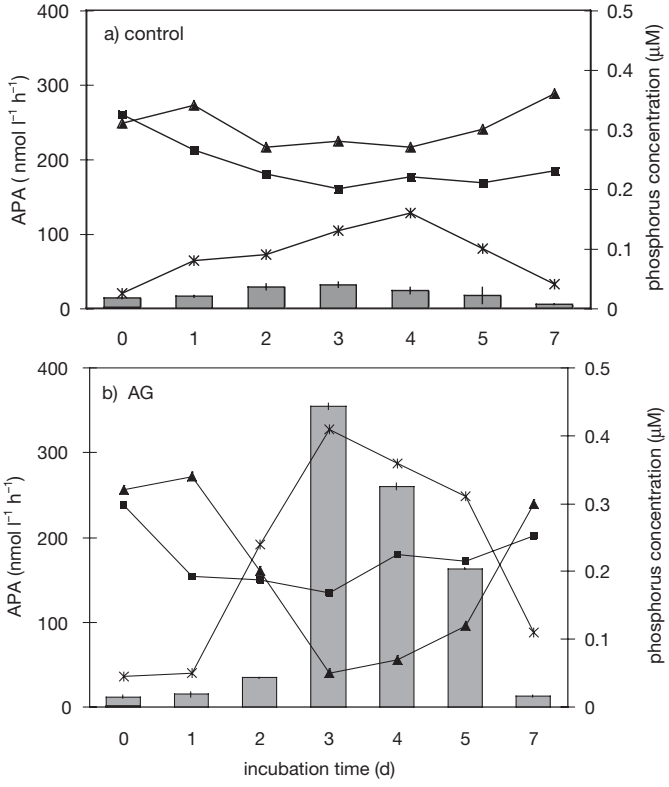

Fig. 1. Development of alkaline phosphatase activity (APA), phosphate concentrations and P in the bacterial fraction during the course of the experiments (a) in the control and (b) after ammonium + glucose (AG) addition. Grey bars: APA; (▲) PO_4, (⚹) bacterial P, (■) DOP

Table 2. Changes in the APA, bacterial counts and calculated phosphorus per bacterial cell from the beginning of the experiments to the time when maximum APA stimulation occurred (AG = ammonium + glucose; nd = not determined)

Expt	APA ($nmol^{-1}$ l^{-1} h^{-1})			Bacterial numbers ($\times 10^6$ ml^{-1})			Phosphorus per bacterial cell ($amol$ $cell^{-1}$)		
	Control	AG	Albumin	Control	AG	Albumin	Control	AG	Albumin
1	–7.3		11.1	4.2		5.2	nd		nd
2	8.8	1036	103.8	2.1	3.1	2.2	4.4	26.7	25.8
3	3.4	47	41.4	2.0	4.0	5.4	16.0	46.2	23.9
4	164.3	1133	133.1	0.8	1.4	5.3	20.3	31.9	22.5
5	–22.1	348		1.4	3.3		12.5	21.7	
6	115.6	838		2.8	3.3		30.3	41.3	
7	9.9	248		3.4	4.6		10.3	39.4	
8	1.9	1007		1.5	2.0		7.8	19.1	
9	27.9	1035		1.0	0.9		5.8	16.4	
10	39.1	1068		nd	nd		nd	nd	

demonstrated maximum P concentrations as well. The elevated P in the bacterial fraction after substrate additions was due to both bacterial growth and an elevated P content per bacterial cell, as can be shown by the calculations of P content per bacterial cell (Table 2). Thus, the P per bacterial cell was between 1.3 and 6 times higher after ammonium + glucose addition than in the control. The decline of APA after its peak is caused by the collapsing bacterial population along with another increase in phosphate and, in some experiments, an increase of DOP (Fig. 1).

Origin of P in the bacterial fraction

The transfer of phosphate into the bacterial fraction after stimulation was dependent on the phosphate concentration in the water. The bacteria took up more phosphate at higher ambient phosphate concentrations. This is demonstrated by comparing the phosphate decrease with P changes in the bacterial fraction from the start of the experiments to times of maximum APA stimulation (Fig. 2) because phosphate was taken up completely. A correlation coefficient of $r = -0.82$ ($n = 13$, $p < 0.01$) was determined.

The decrease of phosphate in the water was less than the observed gain of P in the bacterial fraction. Therefore, additional P must have originated from the DOP pool. We defined this difference between P increase in the bacterial fraction and the phosphate decline as 'phosphate shortage' of bacteria. We found a correlation between the 'phosphate shortage' and the DOP changes after ammonium + carbon stimulation ($r = -0.73$, $n = 12$, $p < 0.01$), as well as in the control experiments ($r = -0.83$, $n = 9$, $p < 0.01$) (Fig. 3). The correlation indicated that the bacteria used P from DOP if the phosphate concentration was apparently not sufficient to fulfill their P demand.

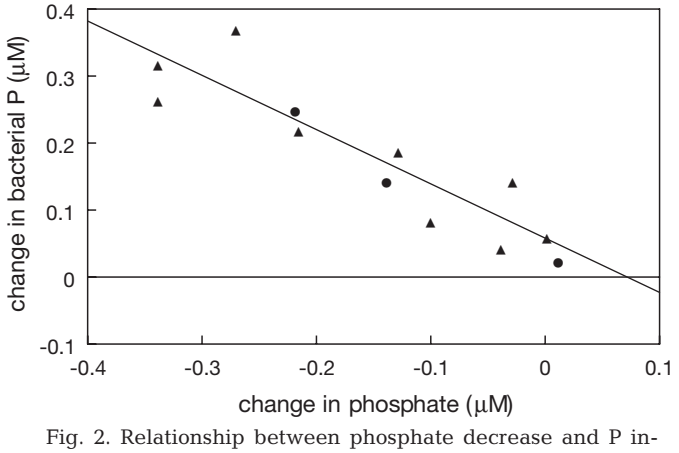

Fig. 2. Relationship between phosphate decrease and P increase in the bacterial fraction ($r = -0.82$, $r = 13$, $p < 0.01$) from the start of the experiments until the time of maximum APA stimulation. Results from albumin (●) and ammonium + glucose (▲) additions are included in the calculation

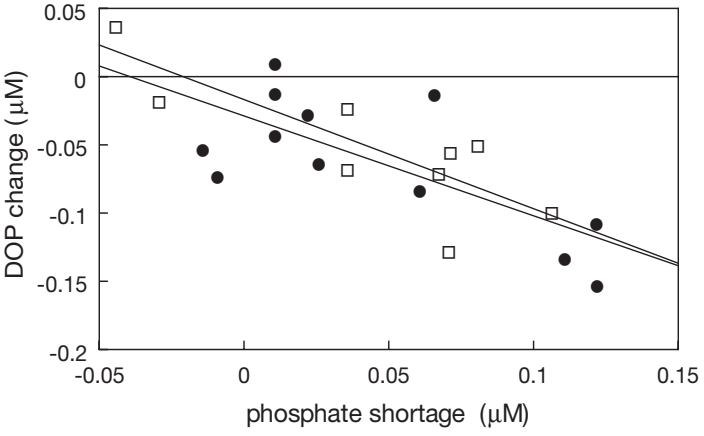

Fig. 3. Changes in DOP concentrations after substrate stimulation (●) and in the controls (□) in relation to the 'phosphate shortage' (= the difference of the increase of bacterial P and phosphate decrease)

The amount of DOP degraded after stimulation showed a relationship with the ambient DOP concentrations in water samples, which ranged from 0.18 to 0.44 µM (Table 3). This can be shown most clearly for the stimulation by ammonium + glucose, where DOP changes and DOP concentrations correlated with $r = 0.81$ ($n = 9$, $p < 0.01$). At low ambient DOP concentrations of about 0.20 µM, only marginal DOP changes occurred. DOP changes increased up to 0.15 µM at ambient concentrations of 0.44 µM (Table 3). Based on the assumption that bioavailable DOP is equal to the amount taken up after substrate addition, bioavailable DOP ranged from 4 to 43% of total DOP, with the highest values measured in March during a spring bloom.

Table 3. DOP concentrations at the beginning of the experiments and at times of maximum APA stimulation, as well as the calculated proportion of bioavailable DOP (AG = ammonium + glucose) (values in parentheses are uncertain because DOP changes were within the detection limit; nd = not determined)

Expt	DOP (µM)	DOP (µM) after stimulation		Proportion of bioavailable DOP (%)	
	Start of expt	AG	Albumin	AG	Albumin
1	nd		nd		nd
2	0.26	0.25	0.27	(4)	(0)
3	0.30	0.25	0.24	17	20
4	0.31	0.23	0.27	26	10
5	0.44	0.29		34	
6	0.31	0.20		35	
7	0.30	0.17		43	
8	0.24	0.17		29	
9	0.18	0.17		(6)	
10	0.20	0.16		20	

Effect of glycerophosphate on P enrichment in the bacterial fraction

The addition of glycerophosphate in parallel to ammonium + glucose yielded an increased amount of P in the bacterial fraction in 4 out of 5 experiments in comparison to the addition of ammonium + glucose alone (Table 4), mainly due to an increase of P per bacterial cell because bacterial growth was not increased substantially. In Expts 5 and 10, phosphate was released from glycerophosphate. This is an indication that the bioavailable DOP concentrations in sampled Baltic Sea water were not sufficient to satisfy the P demand after an additional C and N supply.

In seawater without ammonium + glucose amendment, glycerophosphate addition mostly induced a weak increase in P in the bacterial fraction compared with the control. Additional glycerophosphate was not incorporated into the bacterial biomass, but was mineralized, as indicated by increasing phosphate concentrations (Table 4). It follows that without additional N and C, the available P compounds in water samples were capable of fulfilling the P demand for bacteria.

Relationship between APA stimulation and P pools

The relationship between the level of maximum APA stimulation after substrate addition and the changes in P in the bacterial fraction is shown in Fig. 4. Using only the results from the stimulation by ammonium + glucose, a clear inverse relationship was found between the APA and the increase in P in the bacterial fraction ($r = -0.82$, $n = 9$, $p < 0.01$). Thus, the increase of APA depended on P in the bacterial fraction or intracellular P. The relationship was not quite clear after albumin addition.

The relationship between the increase of APA and the DOP changes is shown in Fig. 5. When low or no APA was measured, DOP increased compared with the control, due to DOP release by bacteria. Here, phosphate seemed to be transferred into DOP and is the case in 3 out of 4 experiments with albumin addition and could explain the missing relationship in Fig. 4. If APA was 100 nmol l^{-1} h^{-1} or higher, DOP was used (with some degree of variation), as indicated by the decrease of DOP compared with the control. DOP degradation increased with increasing APA up to

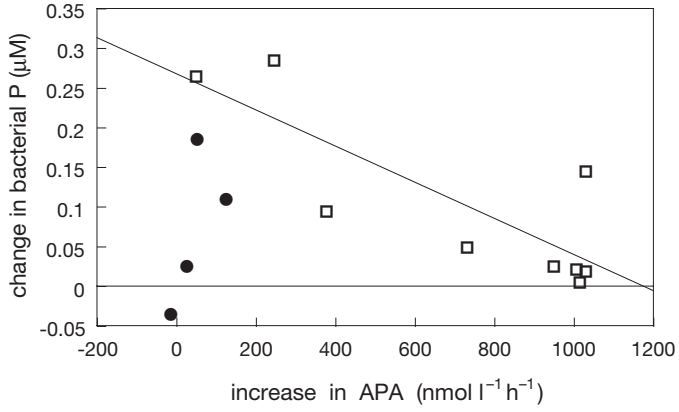

Fig. 4. Relationship between increase in APA after substrate addition and changes in P in the bacterial fraction; stimulation by albumin (●), ammonium + glucose (□) above the control

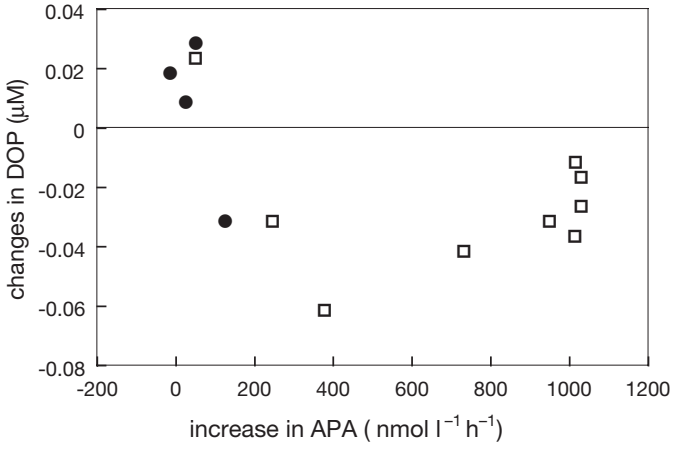

Fig. 5. Relationship between the increase in APA after substrate addition and changes in DOP; stimulation by albumin (●), ammonium + glucose (□) above the control

Table 4. Influence of glycerophosphate on P in the bacterial fraction, phosphate concentrations and bacterial counts without and after stimulation (AG = ammonium + glucose; AGG = ammonium + glucose + glycerophosphate; G = glycerophosphate; nd = not determined). Values refer to the time when maximum stimulation by AG additions were reached

Expt	Bacterial P (µM)				Phosphate concentrations (µM)				Bacterial counts (×10^6 cells ml^{-1})			
	Control	AG	AGG	G	Control	AG	AGG	G	Control	AG	AGG	G
5	0.12	0.22	0.44	0.30	0.09	0.01	0.49	0.46	1.4	3.3	1.6	2.4
6	0.11	0.15	0.42	0.17	0.00	0.00	0.00	0.02	2.8	3.3	4.8	3.0
7	0.13	0.41	0.38	0.11	0.28	0.05	0.07	0.12	3.4	4.6	4.7	1.3
8	0.08	0.11	nd	0.11	0.08	0.00	nd	0.25	1.5	1.9	nd	2.1
9	0.11	0.12	0.34	0.15	0.00	0.00	0.03	0.22	1.0	0.9	1.1	0.7
10	0.26	0.29	0.41	0.18	0.02	0.02	0.17	0.4	1.0	1.3	1.2	0.6

400 nmol l^{-1} h^{-1} ($r = 0.91$, $n = 7$, $p < 0.01$). Thereafter, the relationship became inverse, and DOP degradation declined with further increasing APA ($r = 0.89$, $n = 7$, $p < 0.01$).

The addition of glycerophosphate decreased the APA when it was added together with ammonium + glucose (Fig. 6). Furthermore, the addition of glycerophosphate alone reduced the APA compared with the control. The decline was quite variable, i.e. the ammonium + glucose-stimulated APA was reduced by 11.6 to 84.4% by glycerophosphate, the APA in controls was inhibited by 15.1 to 91.0%. No relationship between the degree of APA reduction and bacterial P, and changes in DOP (glycerophosphate enriched) could be determined.

DISCUSSION

The APA is often used to describe the P status and P-stress of algal and bacterial cells in natural ecosystems, as well as in experiments with algal cultures (Myklestad & Sakshaug 1983, Gage & Gorham 1985, Paasche & Erga 1988, Rose & Axler 1998, Rengefors et al. 2001, Stihl et al. 2001, Dyhrman et al. 2002). Furthermore, APA is supposed to be an indicator for the degree of DOP utilization by phytoplankton and bacteria (Hong et al. 1992, Hantke et al. 1996, Jamet et al. 1997, Reynolds & Davies 2001, Mulholland et al. 2002). The quantity of usable phosphomonoesters is estimated via phosphate release after the addition of alkaline phosphatase to natural and predominantly fresh water samples (Hernandez et al. 1996, 2000, Suzumura et al. 1998). It is generally accepted that DOP is the main P source if phosphate is exhausted, and that cleavage by APA is an important mechanism in DOP decomposition. However, there is little information available about the relationship between APA and DOP degradation. Our investigations address this issue and extended it by including P changes in the bacterial fraction and raising the question of whether DOP in Baltic Sea water is sufficient to fulfill the P demand. In a manner similar to Stepanauskas et al. (2002), who estimated the bioavailable DOP fraction in rivers entering the Baltic Sea, we measured the bacterial decomposition of DOP. Stepanauskas et al. (2002) added bacteria from laboratory cultures to natural water samples. We applied the natural bacterial population and induced a P demand by ammonium and glucose addition.

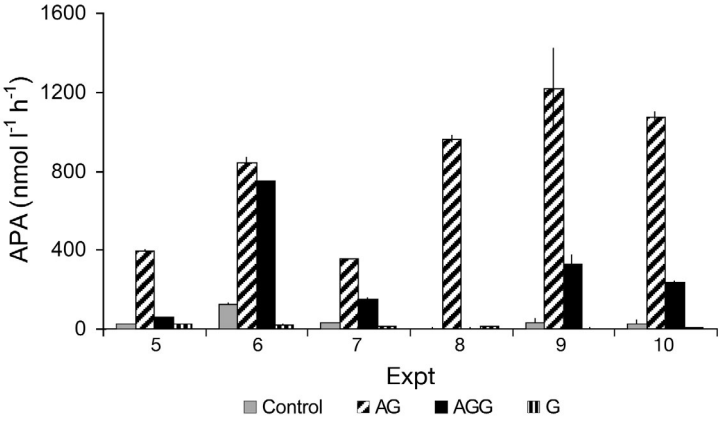

Fig. 6. Mean values and standard deviation of the maximum APA in the control, after the addition of ammonium + glucose (AG), ammonium + glucose + glycerophosphate (AGG), and glycerophosphate (G)

In the present experiments, the development of the APA after substrate addition was similar to that observed by Neddermann & Nausch (2004), where the stimulation of APA by albumin and ammonium + glucose was observed during the growth season. Changes in P pools showed that the lowest phosphate concentration coincided with the highest P concentrations in the bacterial fraction, predominantly based on an increase in P per bacterial cell. During the course of experiments, highest changes in P pools occurred when APA stimulation reached maximum values. Thus, it seems that the bacterial cells are not P impoverished at the time of maximum APA stimulation. This development is different from that observed in phytoplankton, which has the highest APA at the lowest internal P content (Myklestad & Sakshaug 1983). The synchronous development of the P pools (phosphate and bacterial P) and APA can be explained by its molecular regulation, as shown, e.g., for *Escherichia coli*, *Bacillus subtilis* (Torriani-Gorini 1987, Rao et al. 1994, Wanner 1994, Hulett 1996) and *Synechocystis* sp. (Hirani et al. 2001). The gene for the alkaline phosphatase (PhoA) is a component of a complex of 31 or more genes (Wanner 1993) which constitute the Pho regulon encoding for proteins related to transport and assimilation of phosphate and P compounds as well as alkaline phosphatase (Shinagawa et al. 1987, Makino et al. 1994). All the genes of the Pho regulon are induced during phosphate starvation and are controlled by the same 2-component regulatory system PhoB and PhoR. PhoR acts as a sensor for external phosphate. Thus, the Pho regulon is regulated by external phosphate and not by cytoplasmatic Pi (inorganic phosphate) (Rao et al. 1994). However, this manner of regulation does not explain the different height of APA stimulation reached in the experiments, because phosphate was exhausted in all incubations. One possible explanation is based upon the composition of the bacterial population which can have a different competency to express alkaline phosphatase or can be different in its response to N and C addition. On

the other hand, the inverse relationship between APA stimulation and P content in the bacterial fraction refers to an additional mechanism including the internal P content. The bacterial APA is stimulated strongest if the internal P content is low as a result of the uptake of both phosphate and bioavailable DOP. Investigations of Wanner (1993, 1994) and Kim et al. (1996) show that, in addition to the Pi-dependent regulation mechanisms, there exist Pi-independent regulations of the Pho regulon which are cross linked to the central pathways of C and energy metabolisms. Kim et al. (2000) found that a gene of the Pho regulon is regulated by phosphate and C. However, investigations of Chrost (1991) and Neddermann & Nausch (2004), in which glucose or ammonium alone did not stimulate the APA, presume that both N and C seem to be necessary for APA stimulation in marine bacteria.

In our experiments, the APA stimulation was negatively correlated with DOP concentrations and with DOP changes. The degree of DOP cleavage indicated that at concentrations above 0.20 µM in Baltic Sea water, DOP predominantly consists of easily degradable DOP, which is termed bioavailable. Bioavailable or low molecular weight DOP can have an effect similar to phosphate, namely an inhibition of APA (Huang & Hong 1999, Huang et al. 2000, Dyhrham et al. 2002). Either easily degradable DOP needs only minimal APA for its degradation, or it can be taken up without any degradation (González-Gil et al. 1998). On the other hand, observations of Walter & Frie (1976) show that P has to be split from the organic molecule before P can be taken up by bacteria. According to Huang & Hong (1999) and Huang et al. (2000), APA degrades high molecular weight DOP. However, according to Hino (1989), APA released P more easily from low molecular weight fractions (<1500 Da) than from high molecular weight fractions (>10 000 Da). Glycerophosphate, used in our experiments, is an easily degradable compound of low molecular weight that enables very effective P release (Huang & Hong 1999). Observations of González-Gil et al. (1998) suggest that phytoplankton does not need significant amounts of APA to extract P from glycerophosphate. However, the effect of glycerophosphate on APA seems to be quite variable. In our experiments, the addition of glycerophosphate reduced the N+C-induced APA stimulation by 11 to 84%. Uchida (1992) and Huang et al. (2000) also observed an inhibition of APA by glycerophosphate in phytoplankton. In cyanobacteria of the genus *Trichodesmium*, Stihl et al. (2001) observed an inhibition. In contrast, Mulholland et al. (2002) found that a stimulation of APA occurred after the addition of glycerophosphate. Garcia Ruiz et al. (1997) explained that the inhibition of APA by glycerophosphate occurred because it is preferred over MUF-P, which is the artificial substrate for APA measurements.

In the central Baltic Sea, DOP concentrations range between 0.14 and 0.34 µM (authors' unpubl. data). Higher concentrations of DOP can occur temporally and spatially. Thus, DOP has reached concentrations of 0.98 and 0.78 µM in buoyant cyanobacterial surface blooms (Nausch et al. 2004). In seasons when phosphate is available, DOP amounts to 24–58% of the TDP. In the growth season, when phosphate concentrations decline and are exhausted in summer, the percentage of DOP rises to 95% of TDP. Even in surface blooms, DOP amounted to 98% of TDP. During the present investigations, DOP concentrations of 0.18–0.44 µM were measured in water samples, corresponding to 44–94% of TDP. Therefore, the P conditions in our experiments can be seen as representative for the central Baltic Sea. Assuming that bacteria use the bioavailable DOP completely, then between 4 and 43% of DOP was bioavailable. The relationship between changes in DOP after substrate addition, the DOP concentrations in water, and the inverse relationship with APA indicate that a DOP increase above 0.20 µM is due to bioavailable DOP. Degradable DOP occurred mostly in nanomolar concentrations (0.01–0.15 µM). Under 'normal' conditions, this amount seems to be sufficient for bacterial growth (deduced from the addition of only glycerophosphate). If N and C are introduced into Baltic Sea water, either by internal production by phytoplankton (e.g. N fixation) or by introduction from land run-off, then phosphate, and later DOP, can be a limiting factor for bacteria.

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