Distribution of Alkaline Phosphatase-Hydrolyzable Phosphorus in 15 Lakes in Japan

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Abstract

Distribution of total phosphorus (T-P), orthophosphate (Ort-P) and alkaline phosphatase-hydrolyzable phosphorus (ALP-P) were examined in the water of 15 lakes. The ratio of Ort-P to T-P was low in most lakes studied. A considerable proportion of soluble organic phosphorus (62% on average) was ALP-P. The sum of Ort-P and ALP-P, which was thought to be utilized by plankton in lake water, readily accounted for 71% of total soluble phosphorus.

Gel chromatography analyses (Sephacryl S-200) revealed that two fractions of phosphorus compounds (high and low molecular weight) existed in the water of L. Inbanuma and L. Kawaguchiko. A portion of the high molecular weight phosphorus of each sample was hydrolyzed by alkaline phosphatase, which indicated that it was composed of a phosphomonoester bound to a high molecular substance such as humic acid.

Key words: lake, alkaline phosphatase-hydrolyzable phosphorus, total phosphorus, orthophosphate

1. Introduction

Phosphorus has been found to be the most important factor in regulating primary production by phytoplankton and its standing crop in natural water. Various forms of phosphorus, in addition to orthophosphate, are present in natural waters (HOOPER, 1973). Attempts have been made to classify phosphorus in water (TAKIMOTO *et al.*, 1979; 1981). A phosphatase-hydrolyzable phosphorous compound has also been found in natural water (HERBES *et al.*, 1975; FRANCKO and HEATH, 1979; CHROST *et al.*, 1988).

Phosphatase activity of lake or sea waters has been determined, and the role of the enzyme in mineralization of dissolved organic phosphorus in the water has been assessed (BERMAN, 1970; KOBORI and TAGA, 1979; MAEDA, 1988).

It has thus been concluded that phosphatase-hydrolyzable phosphorus is significant as a bioavailable phosphorus in natural water in addition to orthophosphate. Distribution of and

variation in alkaline phosphatase-hydrolyzable phosphorus (ALP-P) in lakes, however, has not been thoroughly studied. The present study was carried out to analyse the distribution of ALP-P concentrations and their correlation with chlorophyll *a* in 15 lakes in Japan.

2. Materials and methods

2-1. Samples

Water samples were collected from the surface layer of the 15 lakes listed in Table 3 from May to September, 1987. Samples were brought back to the laboratory, kept at 4°C and analysed as soon as possible.

2-2. Chemical analyses

Lake water was filtrated using a membrane filter (TOYO, 0.45µm). Total phosphorus (T-P) and ALP-P concentrations of whole lake water as well as the filtrate (soluble total phosphorus; Solb. T-P, soluble ALP-P; Solb. ALP-P) and orthophosphate (Ort-P) of the filtrate were measured according to the following method:

Suspended total phosphorus (Susp. T-P) concentration was obtained by subtracting Solb. T-P from T-P. Suspended ALP-P (Susp. ALP-P) was estimated by subtracting Solb. ALP-P from ALP-P.

Ort-P and T-P were measured by the molybdenum-blue method (PHARMACEUTICAL SOCIETY JAPAN, 1980). ALP-P was measured as follows: 100ml of sample containing 9ml of 0.15M Tris-HCl buffer (pH10.0) and 1ml of alkaline phosphatase solution (Sigma Type I, $0.67\text{mg} \cdot \text{ml}^{-1}$ Tris-HCl buffer solution) was mixed and incubated at 37°C . After 1 hour and again after 2 hours, Ort-P concentrations of a portion of the mixture were measured as described above.

2-3. Alkaline phosphatase activity and bacterial number determination

Alkaline phosphatase activity was measured by the fluorometric method using 4-methylumbelliferyl phosphate as a substrate after HASHIMOTO *et al.* (1983).

Phosphatase producing bacteria in the lake water were counted by the most probable number method (PHARMACEUTICAL SOCIETY JAPAN, 1980) using a medium of 0.25g peptone, 0.25g yeast extract, 0.25g phenolphthalein diphosphate sodium salt, and 0.01g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 1000ml of water and adjusted to pH7.6. The diluted lake water was incubated in this medium for 5 days at 25°C .

2-4. Gel chromatography

Filtrate of lake water (using a $0.45\mu\text{m}$ membrane filter) was concentrated by ultrafiltration (TOYO UH-1, fractionated molecular weight 1000). The concentrate was then applied to a gel column (Sephacryl S-200, Pharmacia). The sample was eluted with 0.2M borate buffer (pH8.6), and T-P concentration of each fraction (5ml) was measured by the method described above.

3. Results and discussion

In this experiment, alkaline phosphatase from bovine intestine (EC. 3.1.3.1, Sigma Type I) was used to determine ALP-P concentration of lake waters. Table 1 shows the rate of activity of the alkaline phosphatase against several compounds as measured by the release of ortho-

Table 1. Activity of alkaline phosphatase against different compounds

Compound tested as substrate	Relative reaction velocity (%)
β -glycerophosphate	100
ADP	84
Glucose-6-phosphate	68
ATP	18
Phosphocholine	17
Pyrophosphate	1
cyclic-AMP	1
DNA	0
Lecithin	0
Tripolyphosphate	0
Phytin	0
Phytic acid sodium salt	0

Each substrate was incubated with 0.67mg of Alkaline phosphatase (Sigma Type I) at pH10, 37°C , 1 hour.

Ortho-phosphate of the mixture was measured by molybdenum blue method.

Table 2. Effect of orthophosphate on alkaline phosphatase activity.

Orthophosphate ($\mu\text{g} \cdot \text{l}^{-1}$)	ALP activity (%)
0	100
0.01	98
0.1	95
1	93
10	55
100	0

phosphate. Activity was observed against all the phosphomonoesters tested, but the enzyme did not liberate orthophosphate from nucleic acid, phytin, tripolyphosphate or lecithin.

The effect of orthophosphate concentrations on the activity of the enzyme was examined (Table 2). The activity was inhibited by orthophosphate of over $1\mu\text{g} \cdot \text{ml}^{-1}$. Since orthophosphate concentrations from lake water samples were below $1\mu\text{g} \cdot \text{ml}^{-1}$, hydrolyzation of ALP-P in lake water to orthophosphate by the enzyme would be thoroughly complete.

Orthophosphate liberation from mixtures of each sample and alkaline phosphatase was measured after 1 and 2 hour incubations. All the ALP-P of each sample was considered to

Table 3. Distribution of phosphorous compounds, chlorophyll *a* and phosphatase-producing bacteria in lake water.

Lake	Date	pH	ALP	Chl- <i>a</i>	Org-P bacteria	Phosphorous compounds					Ort-P
						T-P	Susp. T-P	Susp. ALP-P	Solb. T-P	Solb. ALP-P	
Teganuma	10 June	8.7	34.1	348.0	—	870	444	24	426	33	319
Ushigomebori	5 June	8.3	25.9	181.0	320	334	302	2	32	19	5
Koaidame	15 July	9.1	16.6	98.4	1100	270	250	15	20	8	9
Kitaura	24 June	9.3	35.3	89.7	930	134	99	10	35	13	4
Inbanuma	10 June	7.5	18.7	76.0	—	268	206	3	62	34	23
Beshonuma	15 July	8.4	58.3	74.5	17200	171	155	0	16	8	4
Shakujiiike	15 July	9.3	13.3	42.2	1700	117	96	7	21	17	2
Shinseiko	3 July	7.6	57.8	29.3	2400	43	28	0	15	10	2
Kasumigaura	24 June	8.5	17.9	19.5	9300	124	91	0	33	12	6
Akagi-Kono	9 July	6.3	309.9	14.7	790	31	23	2	8	4	4
Shojiko	26 May	7.1	4.9	6.2	—	66	13	0	53	5	2
Nakanuma	10 June	7.3	8.5	5.2	—	26	7	5	19	6	4
Kawaguchiko	26 May	7.6	14.8	5.2	—	33	6	0	27	10	2
Akagi-Ono	9 July	8.2	10.5	2.0	110	16	8	0	8	5	2
Kakumanbuchi	9 July	6.4	16.6	1.8	7900	18	8	3	10	6	2

ALP-activity : alkaline phosphatase activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$); Chl-*a* : Chlorophyll *a* ($\mu\text{g} \cdot \text{l}^{-1}$); Org. — P bacteria : number of phosphatase-producing bacteria ($\text{N} \cdot \text{ml}^{-1}$); T-P : total phosphorus ($\mu\text{g} \cdot \text{l}^{-1}$); Susp. T-P : suspended total phosphorus ($\mu\text{g} \cdot \text{l}^{-1}$); Susp. ALP-P : suspended phosphorus decomposed with alkaline phosphatase ($\mu\text{g} \cdot \text{l}^{-1}$); Solb. T-P : soluble total phosphorus ($\mu\text{g} \cdot \text{l}^{-1}$); Solb. ALP-P : soluble phosphorus decomposed with alkaline phosphatase ($\mu\text{g} \cdot \text{l}^{-1}$); Ort-P : orthophosphate ($\mu\text{g} \cdot \text{l}^{-1}$); — : not determined.

decompose to orthophosphate after 1 hour incubation, since no increase in orthophosphate liberation was observed after 2 hours.

Table 3 shows the data of pH, phosphorous compounds, activity of alkaline phosphatase and number of phosphatase producing bacteria in the water of 15 lakes. Chlorophyll *a* concentration was not in proportion to numbers of phosphatase producing bacteria. pH of lake waters was almost neutral or weakly alkaline. Phosphorous concentration in L. Teganuma was remarkably high. Suspended and soluble ALP-P values were 0–24 and 4–34 $\mu\text{g} \cdot \text{l}^{-1}$, respectively, and were slightly higher than those of sea and river water noted by CHROST *et al.* (1986).

Table 4 shows the ratio of the various forms of phosphorus in samples. The ratio of Ort-P to T-P was low (average 8.5%). ALP-P accounted for 62% of soluble organic phosphorus. The greater proportion of Solb. T-P was concluded

to be in phosphorus compounds which are readily utilized by plankton in natural water, since the sum of Ort-P and Solb. ALP-P accounted for 71% to Solb. T-P on average. TAKIMOTO *et al.* (1979) measured concentrations of phosphorus decomposed by UV radiation as organic monophosphate in sea and river water, and also reported a high ratio of the organic monophosphate to T-P.

The correlation coefficients of chlorophyll *a* and the various forms of phosphorus (except the data of L. Teganuma) are shown in Table 5. Chlorophyll *a* correlated very well with total phosphate, but not with the others. A weak relationship was observed between chlorophyll *a* and the sum of Solb. ALP-P and Susp. ALP-P, and between chlorophyll *a* and the sum of Ort-P and Solb. ALP-P and Susp. ALP-P. The ratios of Ort-P to T-P and of Susp. ALP-P plus Solb. ALP-P to T-P have a tendency to be inversely proportional to chlorophyll *a* con-

Table 4. Ratio of various forms of phosphorous compounds in lake water.

Lake	Ort-P/T-P (%)	Solb.T-P /T-P (%)	Solb.ALP-P /Solb. Org-P* (%)	Ort-P+Solb. ALP-P /Solb.T-P (%)
Teganuma	36.7	49.0	30.8	82.6
Ushigomebori	1.5	9.6	70.4	75.0
Koaidame	3.3	7.4	72.7	85.0
Kitaura	3.0	26.1	41.9	48.6
Inbanuma	8.6	23.1	87.2	91.9
Beshonuma	2.3	9.4	66.7	75.0
Shakujiiike	1.7	17.9	89.5	90.5
Shinseiko	4.7	34.9	76.9	80.0
Kasumigaura	4.8	26.6	44.4	54.5
Akagi-Kono	12.9	25.8	100.0	100.0
Shojiko	3.0	80.3	9.8	13.2
Nakanuma	15.4	73.1	40.0	52.6
Kawaguchiko	6.1	81.8	40.0	44.4
Akagi-Ono	12.5	50.0	83.3	87.5
Kakumanbuchi	11.1	55.6	75.0	80.0
Average	8.5	38.0	61.9	70.7

*: Solb. Org-P = Solb. T-P - Ort-P

Table 5. Correlation coefficients between various form of phosphorous compound and chlorophyll *a*.

	Chlorophyll <i>a</i>
T-P	0.9122***
Solb. T-P	0.2660
Ort-P	0.3436
Solb.ALP-P	0.5129*
Susp. ALP-P	0.3745
Ort-P + Solb. ALP-P	0.4675*
Solb. ALP-P + Susp. ALP-P	0.6102**
Ort-P + Solb. ALP-P + Susp. ALP-P	0.5431**
Ort-P/T-P	-0.5746**
Solb. ALP-P+ Susp. ALP-P/T-P	-0.5990**

n=14, ***: P 0.01; **: P 0.05; *: P 0.1

tents. It is supposed that plankton immediately consumed Ort-P or ALP-P in the lake water, and then stored it in their bodies. MINEAR (1972) found that phytoplankton consume and release

organic phosphorus simultaneously. After phytoplankton die, decomposition of the debris and release of orthophosphate occurs. Thus, the total amount of phosphorus and the decomposition rate of organo phosphorus to orthophosphate in the lake water are thought to be significant factors to regulating phytoplankton growth.

Alkaline phosphatase activity and phosphatase producing bacteria which participated in the decomposition of ALP-P were detected, but no correlation was found between the phosphatase activity and the concentrations of phosphorous compounds in the lakes. It has been reported that, under certain circumstances, some algae or bacteria release extracellular alkaline phosphatase when orthophosphate disappears (WYNNE, 1981). In this experiment, an inverse proportion between alkaline phosphatase activity and orthophosphate concentrations in lake waters was not observed.

Gel filtration chromatography was conducted on filtrated water of L. Inbanuma and L. Kawaguchiko (Figure 1). On both chromatograms, two peaks of phosphorus were obser-

Fig. 1. Elution profile of phosphorous compound of (A) Inbanuma and (B) Kawaguchiko lake water on Sephacryl S-200.

---○--- ALP treated

—●— ALP no treated

(C) Sephacryl S-200 calibration curve

△ Blue dextran (molecular weight : 2,000,000)

□ Alcohol dehydrogenase (molecular weight : 150,000)

○ Cytochrome C (molecular weight : 12,400)

Table 6. Elution rate of phosphorus compound on Sephacryl S-200.

Sample	phosphorus (%)			
	High molecular fraction ¹⁾		Low molecular fraction ²⁾	
	—ALP	+ALP ³⁾	—ALP	+ALP ³⁾
Inbanuma	45	29	20	34
Kawaguchiko	31	24	18	33

1) $V_e \cdot V_0^{-1} = 0.94 - 1.19$ on chromatogram of Sephacryl S-200 (Fig. 1)

2) $V_e \cdot V_0^{-1} = 2.21 - 2.38$ on chromatogram of Sephacryl S-200 (Fig. 1)

3) Sample was treated with 0.67mg of ALP (Sigma Type I) at pH10 37°C, 1hour.

ved: one at high molecular weight fraction ($V_e \cdot V_0^{-1} = 0.94 - 1.19$); and another at a low molecular weight fraction ($V_e \cdot V_0^{-1} = 2.21 - 2.38$). Standard compounds such as Ort-P, adenosine-5'-monophosphate and DNA were eluted at $V_e \cdot V_0^{-1} = 2.3, 2.2, 1.1$, respectively. After the samples were incubated with alkaline phosphatase (Sigma Type I), phosphorus concentration of high molecular weight fractions decreased and those of low molecular weight fractions increased in both samples. The rates

of phosphorus in each fraction to total eluted phosphorus are shown in Table 6. Recovery rate of phosphorus eluted in each chromatography was 88-98%. After alkaline phosphatase treatment, phosphorus of the high molecular weight fraction was thought to have been hydrolyzed to orthophosphate. In addition to Ort-P, phosphomonoesters hydrolyzed by alkaline phosphatase may also be present in the low molecular weight fraction. Nucleic acids such as DNA are presumed to be components of the

high molecular weight fraction. MINEAR (1972) reported that the concentrations of soluble DNA ranged from 4 to $30\mu\text{g} \cdot \text{l}^{-1}$ for the lake samples, and that 40-60% of the high-molecular weight ($>250,000$) phosphorus was attributable to DNA. The alkaline phosphatase used in this experiment did not decompose DNA. ALP-P of the high molecular weight fraction may be composed of phosphomonoesters bound to a high molecular weight substance such as humic acid.

摘 要

日本の15湖沼におけるアルカリホスファターゼ分解性リン量の分布

日本の富栄養化度の異なる15湖沼水の、総リン、オルトリンおよびアルカリホスファターゼ分解性リン(ALP-P)含量を評価した。総リンに対するオルトリンの割合はいずれも低かった。溶存している有機リンのうち、平均62%はALP-Pであった。また植物プランクトンに利用され易いリンと考えられるオルトリンとALP-Pの総和量は、溶存の総リンの平均71%を占めていた。

印旛沼と河口湖水のリン化合物をゲルクロマトグラフィー(Sephacryl S-200)により分析したところ、分子量150,000以上の高分子画分と13,000以下の低分子画分に分画された。高分子画分のリンの一部はアルカリホスファターゼで分解されるが、それらはリン酸モノエステル類が、フミン酸等のような高分子成分に付着したものであると考えられた。

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