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CORRELATIONS BETWEEN DISSOLVED ALKALINE PHOSPHATASE AND ORTHOPHOSPHATE IN LAKE WATER

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Distinct negative correlation was found between the dissolved alkaline phosphatase activity and the orthophosphate concentration in Lake Kasumigaura (surface area, 171 km^2) in the phosphorus-limited season. The decrease of the alkaline phosphatase activity induced by orthophosphate ions was observed at the level of 1 ng/mL of phosphorus, which is 3 orders of magnitude smaller than that in the laboratory scale experiment.

Alkaline phosphatase is the first enzyme to be discovered in the free dissolved state while preserving the activity in natural water.[1-10] The dissolved phosphatase contributes to the hydrolysis of organic phosphate esters. The dissolved enzymes in natural water are excreted from the parent bio-organisms. Jansson characterized the various phosphatases dissolved in lake water, which are attributable to those of algae and zooplankton.[11] Since alkaline phosphatases participate in the production of orthophosphate ions and their transportation into the cytoplasm, a negative correlation has been found between intracellular alkaline phosphatase activity and orthophosphate concentration in the growing media of bacteria or algae.[12-15] Starvation of phosphorus induces an increase in the intracellular alkaline phosphatase production. Several reports have implied that dissolved phosphatase activity decreases when concentration of orthophosphate increases in lake water.[16,17] However, correlation in the seasonal variation between dissolved alkaline phosphatase activity and orthophosphate concentration has not been clearly found, because of the high number of variables in the ecological system, such as the variation in species growing in situ. To inquire into the value of the phosphatase activity as an indicator of the orthophosphate demand in the environmental system, one should examine the correlation between dissolved phosphatase activity and orthophosphate concentration.

The present report shows that the negative correlation is clearly found between the activity of the dissolved alkaline phosphatase and orthophosphate at appreciably low phosphate concentrations.

Lake Kasumigaura is the second largest lake in Japan, with a surface area of

171 km^2, with a mean depth of 4 m and maximum depth of 7.4 m. The location and sampling stations in Lale Kasumigaura are shown in Fig. 1. Lake water was collected with a Van Dorn bottle at 10 different sampling stations once a month, starting in April 1980. The depth of the sampling point was 1.0 m from the surface. The collected lake water was stored at 4 °C. A portion of the collected water was filtrated with a membrane filter of 0.2 μm pore size under mild suction (< 250 mm Hg) for the exclusion of bio-organisms. Filtration was performed within 2-4 hours after sampling. The alkaline phosphatase activity was measured at 25 °C by using p-nitrophenyl phosphate as a substrate.[19] The concentration of orthophosphate ion was determined by molybdenum-blue colorimetry[18] with a Technicon Autoanalyzer. Here, reactive phosphorus is referred to as the orthophosphate. Total phosphorus and total dissolved phosphorus were determined after the persulfate digestion of unfiltrated and filtrated waters, respectively, in an autoclave at 120 °C for 45 min. The mass concentration of the particulate phosphorus, which is proportional to the amount of biomass in Lake Kasumigaura,[20] was calculated by subtracting the mass concentration of the total dissolved phosphorus from the mass concentration of the total phosphorus.

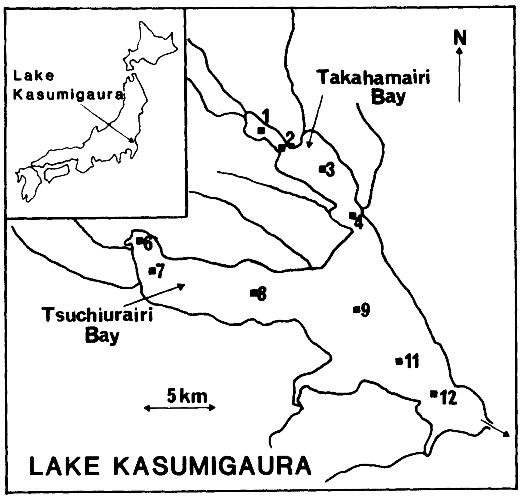

Fig. 1.
Locations of sampling staions in Lake Kasumigaura.

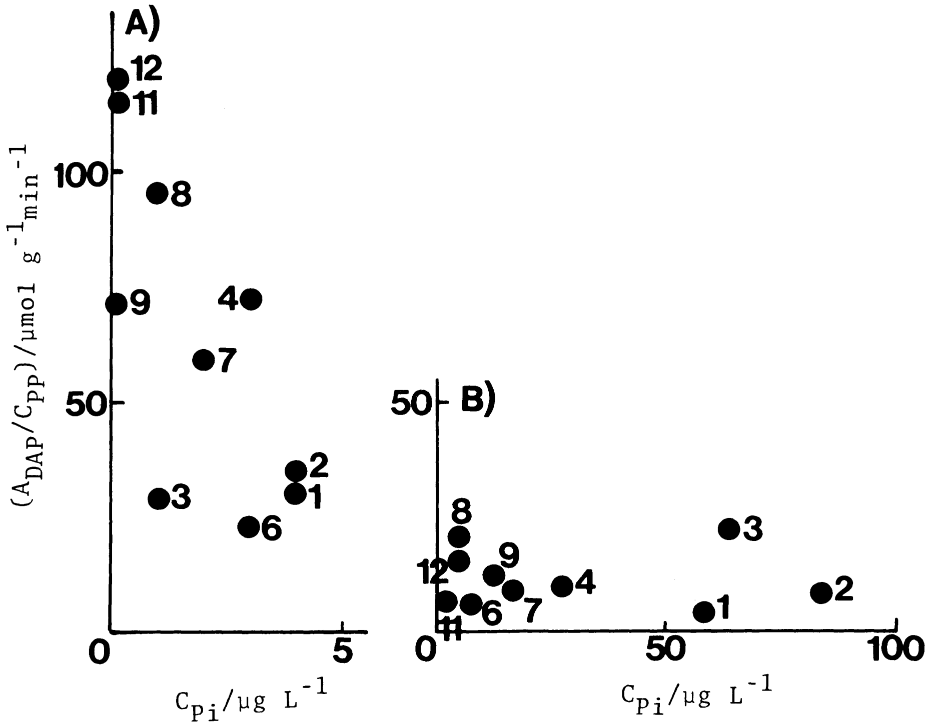

Fig. 2.

Correlation between dissolved alkaline phosphatase activity and orthophosphate concentration. A): May, 1981, B): August, 1981. A_{DAP}/C_{PP}: dissolved alkaline phosphatase activity per mass concentration of particulate phosphorus. C_{Pi}: mass concentration of phosphorus as orthophosphorus. The numbers in the figure show the sampling stations in Fig. 1.

Ultrafiltration of filtrated lake water was done with a Toyo Ultrafilter UK-10 for concentrating large molecular species (M.W. > 10 000), followed by a high performance liquid chromatography.

Figure 2 shows the correlation between orthophosphate and dissolved phosphatase activity for the various sampling stations in May and August, 1981. In both months, Microcystis aeruginosa was the dominant species which excretes the dissolved enzyme. In August, when the maximum orthophosphate concentration was observed in the lake water, the dissolved alkaline phosphatase activity at each sampling station was low and not correlated to the concentration of orthophosphate. On the other hand, distinctive negative correlation between the activity and orthophosphate concentration appeared in May (correlation coefficient = -0.72), when the concentration of orthophosphate was less than 5 ng/mL at all the sampling stations.

Table 1 lists the seasonal variation in the correlation coefficients between the dissolved phosphatase activity and orthophosphate; it also lists the phosphatase activity, and the orthophosphate and particulate phosphorus concentrations at Station 2. In the table, distinctive negative correlation appears in spring and winter, indicating phosphorus starvation in the ecosystem of Lake Kasumigaura in these seasons. On the other hand, rather positive correlation appears from late spring to early fall. Alternation in appearance of negative and positive (or low) correlation was found in Table 1 although some deviations in its timing exist. Dissolved phosphatase activity itself increases twice a year : one peak was found in early summer and the other in winter. From the chromatographic separation of the dissolved alkaline phosphatase in the lake water, it was found that the blue-green algae (Microcystis aeruginosa) was responsible for the alkaline phosphatase released from May to September, and diatoms and flagellates were responsible for the release in the winter. In Table 1, it can be seen that the orthophosphate concentration increased very much in middle summer. This was due to the summer appearance of orthophosphate from the sediment at the bottom of the lake, a characteristic of shallow lakes such as Lake Kasumigaura.[21] Although the biomass (or particulate phosphorus) increased in this season, the

Table 1.
Seasonal variation in the relationship between phosphorus concentration and phosphatase activity

Year	Month	r	Sta.2 A_{DAP}	C_{PP}	A_{DAP}/C_{PP}	C_{Pi}
1980	April	-0.80	2.6	144	0.018	7
	May	-0.15	2.1	116	0.018	5
	June	+0.23	1.1	96	0.011	11
	July	+0.38	9.0	127	0.071	2
	August	+0.70	2.9	67	0.043	13
	September	-0.10	2.4	172	0.014	64
	October	+0.83	0.9	96	0.009	9
	November	+0.62	1.2	97	0.012	2
	December	-0.69	1.3	68	0.019	3
1981	January	-0.71	4.7	53	0.089	2
	February	-0.78	2.6	35	0.074	<2
	March	-0.59	1.3	92	0.014	2
	April	-0.31	1.5	61	0.025	6
	May	-0.72	3.2	92	0.035	4
	June	+0.32	3.4	179	0.019	6
	July	+0.51	4.3	60	0.072	78
	August	0.00	1.0	105	0.010	85
	September	+0.26	1.7	123	0.014	3
	October	+0.14	0.4	84	0.005	3
	November	-0.48	2.3	72	0.032	<2
	December	-0.57	1.0	82	0.012	3
1982	January	-0.66	1.3	37	0.035	3
	February	-0.73	1.0	58	0.017	4
	March	-0.80	2.2	91	0.024	11

r: correlation coefficient between A_{DAP}/C_{PP} and C_{Pi} for all the stations.
A_{DAP}: dissolved phosphatase activity at Sta. 2 (nmol L^{-1} min^{-1}).
C_{PP}: particulate phosphorus concentration at Sta. 2 (ng mL^{-1}).
C_{Pi}: orthophosphate concentration at Sta. 2 (ng mL^{-1}).
The enzymatic activity was provided as the decomposition rate of substrate (1 mM in 0.6 M Tris-HCl at pH 8.0) at 25 °C.

dissolved alkaline phosphatase activity in the lake water decreased. These phenomena were found in all the sampling stations. No seasonal correlations were observed between the dissolved alkaline phosphatase activity and other factors such as total phosphorus, total dissolved phosphorus, and dissolved organic phosphorus.

In the laboratory incubation experiments of Escherichia coli,[12] green algae (including Microcystis aeruginosa), and diatoms,[13] it was found that increase of orthophosphate concentration in their growing media suppressed intracellular phosphatase production. However, the repression of the enzyme production can be also observed under orthophosphate concentrations of 7-10 μg/mL (as P). On the other hand, the decrease in the dissolved alkaline phosphatase in the lake water appears at ca. 1 ng/mL (as P) (Fig. 2), which means a three-order difference between the lake and experimental laboratory systems. Furthermore, when considering that the surface area of Lake Kasumigaura is 171 km^2, it is important to note that such negative feedback is also maintained between orthophosphate and the release of dissolved phosphatase on the scale of a large lake.

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References

1) W. Reichardt, J. Overbeck, and L. Steubing, Nature, 216, 1374 (1964).
2) T. Berman, Nature, 224, 1231 (1969).
3) T. Berman and G. Moses, Hydrobiologia, 40, 487 (1972).
4) S. E. Herbes, H. E. Allen, and K. H. Mancy, Science, 187, 432 (1975).
5) J. Olâh and E. O. Tóth, Aquaculture Hungarica, 1, 15 (1978).
6) N. Taga and H. Kobori, Mar. Biol., 49, 223 (1978).
7) H. Kobori and N. Taga, Deep-sea Res., 26A, 779 (1979).
8) K. Pettersson and M. Jansson, Verh. Internat. Verein. Limnol., 20, 1226 (1978).
9) H. Haraguchi, K. Kobayashi, M. Matui, and K. Fuwa, Chem. Lett., 1982, 175.
10) K. Kobayashi, M. Matui, H. Haraguchi, and K. Fuwa, J. Inorg. Biochem., 18, 41 (1983).
11) M. Jansson, Science, 194, 320 (1976).
12) A. Torriani, Biochim. Biophys. Acta, 38, 460 (1960).
13) G. P. Fizgerald and T. C. Nelson, J. Phycol., 2, 32 (1966).
14) W. Reichardt and J. Overbeck, Ber. Deut. Bot. Ges., 81, 391 (1968).
15) F. P. Healey, J. Phycol., 9, 383 (1973).
16) R. J. Stevens and M. P. Parr, Freshwater Biol., 7, 351 (1977).
17) K. Petterson, Arch. Hydrobiol., 89, 54 (1980).
18) J. Murphy and J. P. Riley, Anal. Chim. Acta, 27, 31 (1962).
19) K. Kobayashi, K. Iwase, M. Matsui, M. Watanabe, H. Ueda, K. Fujiwara, H. Haraguchi, and K. Fuwa, Bull. Chem. Soc. Jpn., 55, 3459 (1982).
20) A. Otsuki, S. Kasuga, and T. Kawai, Verh. Internat. Verein. Limnol., 21, 634 (1981).
21) T. Kawai, A. Otsuki, M. Aizaki, and M. Nishikawa, Res. Rep. Natl. Inst. Environ. Study, 22, 23 (1981).

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