References and Notes

1. R. J. H. Voorhoeve, J. P. Remeika, P. E. Freeland, B. T. Matthias, *Science* **177**, 353 (1972).
2. R. J. H. Voorhoeve, J. P. Remeika, D. W. Johnson, Jr., *ibid.* **180**, 62 (1973).
3. S. Katz, J. J. Croat, J. V. Laukonis, *Ind. Eng. Chem. Prod. Res. Dev.* **14**, 274 (1975).
4. Y. F. Y. Yao, *J. Catal.* **36**, 266 (1975).
5. C. R. Brundle, *Surf. Sci.* **48**, 99 (1975).
6. P. K. Gallagher, D. W. Johnson, Jr., E. M. Vogel, F. Schrey, *Mat. Res. Bull.* **10**, 623 (1975).

7. We thank J. C. Tracy for advice and useful suggestions during the course of this study. We also thank F. L. Williams for helpful discussions; D. J. Mitman, D. W. Gorkeiwicz, and N. J. Bradley for their skillful assistance with the experiments; and T. P. Schreiber and R. A. Rogers for microprobe and scanning electron microscope analysis.

9 February 1976; revised 8 July 1976

Phosphatases in Lake Water: Characterization of Enzymes from Phytoplankton and Zooplankton by Gel Filtration

Abstract. *Sephadex gel filtration was used to characterize phosphomonoesterases in two small lakes in northern Sweden. Two fractions, here termed phosphatase A and phosphatase B, were found both as free enzymes and associated with seston. The activity of phosphatase A was correlated with the presence of algal biomass. Phosphatase B, on the other hand, was derived from zooplankton. Phosphate served as an effective inhibitor of phosphatase A but had no such effect on phosphatase B. Both fractions had* pH *optima between 6.5 and 7.0.*

After Steiner (*1*) found an increase of inorganic phosphorus in lake water incubated with dead plankton organisms, several investigations were made of enzymatic hydrolysis of different phosphorous compounds in lakes and oceans. The phosphomonoesterases are the most studied enzymes of the many different phosphatases.

Phosphomonoesterases in the aquatic environment are produced by bacteria (*2*), algae (*3, 4*), and zooplankton (*1, 5, 6*). Besides being associated with living cells or particulate cell debris, the phosphomonoesterases are also found as free dissolved enzymes (*7–9*).

Phosphatases are often classified as either alkaline or acid, with *p*H optima well above or well below *p*H 7. Different phosphatases produced by aquatic organisms have been separated by fractionation monitored with assays of *p*H-dependent phosphatase activity (*4, 7*).

Production of phosphatases in different species of algae increases when phosphorus is a limiting factor (*4, 10*); phosphatases have been suggested to be ecologically essential because they release phosphate. Synthesis of phosphatases, on the other hand, often seems to be repressed by high concentrations of phosphate in water or in cells (*4, 8, 11, 12*).

The study reported here provides additional information on the different fractions of phosphomonoesterases occurring in the natural aquatic environment, their origin, and some of their characteristics. All specimens were taken from two adjacent lakes, Lake Magnusjaure and Lake Hymenjaure, situated at 68°27′N and 18°27′E in the subarctic region of northern Sweden. The lakes are small, each with an area of about 2 ha and with maximum depths between 4.5 and 5.5 m. Summer mean water temperatures are 9.5° to 10.5°C and conductivity is 1.6 to 1.8 millisiemens per meter. The lakes were originally oligotrophic, but a program of artificial enrichment with phosphorus and nitrogen (*13*) has brought them toward more eutrophic conditions. The work was done during the ice-free season (15 June to 10 October) in 1975.

Molecular weight was chosen as the criterion for characterization of different phosphomonoesterases. The enzymes were separated by Sephadex gel filtration, a technique that has been applied to phosphatases from bovine liver (*14*) and human placenta (*15*).

Sephadex of different grades (G-75 and G-200) and Sepharose 4B were packed in tubes of Plexiglas 2 cm in diameter and 50 cm long. The flow rate was 0.5 ml/min, with tris buffer used as eluent; the void volume was determined with blue dextran.

For separation of free dissolved phosphatases, 300 ml of lake water was filtered through membrane (Sartorius SM 11107, 0.2 μm) and the enzymes in the filtrate were concentrated 40-fold by ultrafiltration (Diaflo filter PM10; Amicon ultrafiltration cell model 50); 2 ml of the concentrated enzyme solution was refiltered before being applied to Sephadex columns.

For analysis of seston-associated enzymes, 1500 ml of lake water was passed through a glass fiber filter (Whatman GF/C); the filter was placed in tris buffer and homogenized. After homogenization the sample was centrifuged, and 2 ml of the supernatant was placed on the Sephadex column. In both analyses, phosphatase activity was determined in each 5-ml fraction collected from the column.

Phosphomonoesterase activity was assayed by the method of Perry (*11*), with 3-*O*-methylfluoresceinphosphate as substrate. The samples were incubated at 20°C in tris-HCl, *p*H 6.5. The buffer solution and all glassware were autoclaved before analysis to prevent microbial growth. Enzyme activity was measured at *p*H 6.5 because the phosphatases of the two lakes had their only *p*H optimum at 6.5 to 7.0, which is also the *p*H of the lakes.

Sephadex G-200 elution diagrams showing the composition of free and seston-associated enzymes (Fig. 1, a and b) indicated similar patterns for free and seston-associated phosphatases. One fraction, called phosphatase A, was not separated by the gel and eluted with the void volume. Another fraction, phosphatase B, was well separated and, after calibration with proteins of known molecular weight, was calculated to have a molecular weight of about 80,000. Elution from Sephadex G-75 gave only one peak at the void volume.

To determine whether the phosphatase A peak could be fractionated, the experiment was repeated with Sepharose 4B, which has an upper separation limit of molecular weight 3×10^6 for dextrans. However, phosphatase A still eluted with the void volume and can therefore be assumed to consist either of very large molecules, molecules associated with colloidal material, or fragments small enough to pass through a 0.2-μm filter. The question of whether phosphatase A consists of more than one fraction was not answered by this experiment.

A decrease in phosphatase activity often follows the addition of phosphate to a medium containing phosphatase-producing organisms. In many cases repression of the enzyme synthesis rather than inhibition of the enzyme itself causes such a decrease (*4, 12*). To test how the two phosphatase fractions found in Lake Hymenjaure and Lake Magnusjaure reacted to additions of phosphate, the following experiment was performed. Free phosphatases were concentrated and filtered on Sephadex G-200 as described above. A parallel sample was concentrated and NaH_2PO_4 was added immediately before gel filtration. Elution diagrams for the two samples (Fig. 1c) show an almost total inhibition of phosphatase A by added phosphate but no effect on phosphatase B. The fact that phosphate was added to a sterile filtered sample shows that the decrease in enzyme activity was a result of inhibition and not repression. The con-

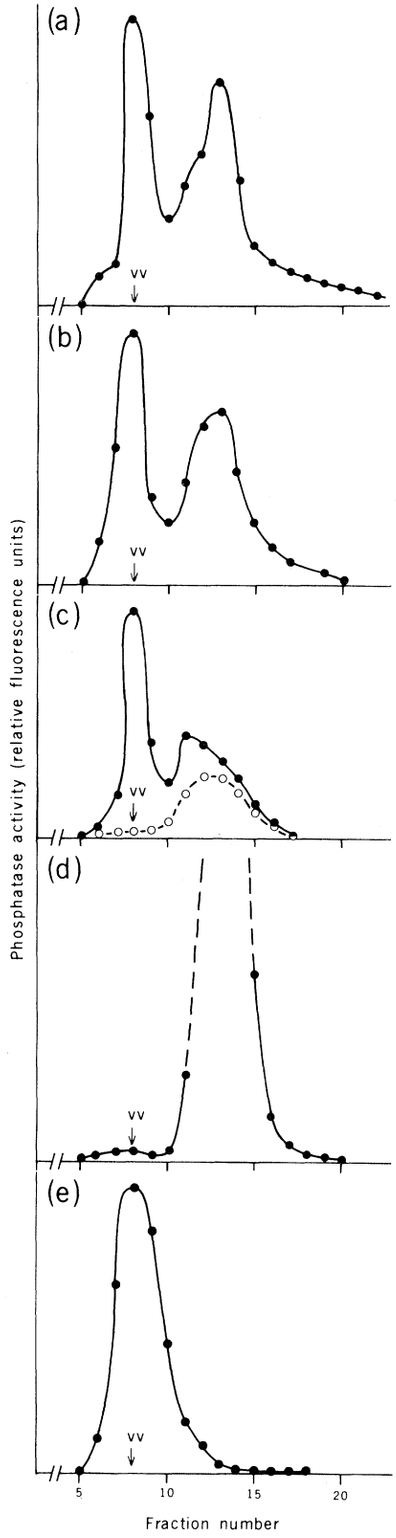

Fig. 1. Elution diagrams from Sephadex G-200; *vv*, void volume. (a) Free dissolved phosphatases, Lake Magnusjaure, 17 June 1975. (b) Seston-associated phosphatases, Lake Magnusjaure, 19 June 1975. (c) Free dissolved phosphatases, Lake Magnusjaure, 14 July 1975, before (closed circles) and after (open circles) addition of phosphate. (d) Phosphatases in zooplankton extract, Lake Hymenjaure, 3 October 1975. (e) Free dissolved phosphatases, Lake Magnusjaure, 30 September 1975.

centration of phosphate P was about 200 μg/liter. However, since the addition was made to a sample that had been concentrated about 40-fold, inhibition might also be effective at concentrations occurring under natural conditions.

Zooplankton produces (*1*, *5*) and excretes (*6*) phosphatases. Rigler (*6*), however, did not consider phosphatases from *Daphnia magna* to be of ecological importance. In the present study, *Bosmina obtusirostris* from Lake Hymenjaure excreted considerable amounts of phosphatases when incubated in test tubes with autoclaved water and 3-*O*-methylfluoresceinphosphate (Fig. 2). Similar results were obtained with other crustaceans, such as *Holopedium gibberum* and *Cyclops scutifer*. *Eudiaptomus graciloides*, however, excreted only small amounts. No decrease in phosphatase activity was detected after filtration of the water in which the animals had been incubated; this confirms that the activity was due to excreted enzyme. In an attempt to identify phosphatases produced by zooplankton as phosphatase A or B (or both), zooplankton, mainly *Bosmina*, was sampled from Lake Hymenjaure with a 63-μm plankton net. The animals were starved for about 5 hours, filtered, placed in tris buffer, and homogenized. After the homogenate was centrifuged, 2 ml of the supernatant was filtered on a G-200 column. Phosphatase B was totally predominant in the zooplankton extract (Fig. 1d). The enzyme activity in fractions 12 to 14 in Fig. 1d was too high for measurement.

An opportunity to characterize phosphatases from algae occurred in Lake Magnusjaure in September. Because of the artificial enrichment with nitrogen and phosphorus there was a "bloom" of the green alga *Schroederia setigera* (Schröd.) Lemn. which gave chlorophyll concentrations of more than 100 μg/liter in the lake. During the intensive growth period a remarkable increase in the activity of free phosphatases was noticed. When free enzymes from Lake Magnusjaure were filtered on Sephadex G-200, the elution diagram (Fig. 1e) showed the presence of phosphatase A only; this indicated that the algae produced and actually excreted phosphatase A during a period of active algal growth.

The results reported here show at least two fractions of phosphomonoesterases in the lakes investigated. Both fractions were detected as free dissolved phosphatases and were associated with seston. One of the fractions (phosphatase A) was found together with large amounts of green algae and the other was identified

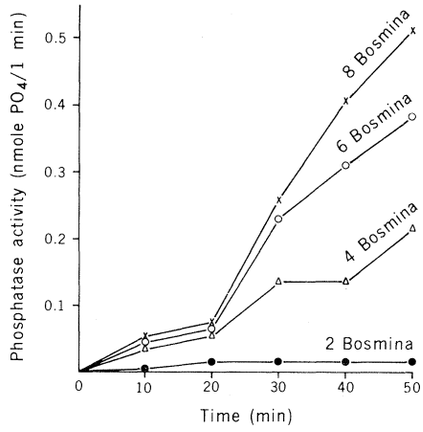

Fig. 2. Activity of free dissolved phosphatases in 5 ml of autoclaved water containing different numbers of *Bosmina obtusirostris*.

in extracts from zooplankton. The fact that zooplankton was shown to excrete phosphatases and that the specific zooplankton phosphatase was traced in lake water in amounts that equaled the other phosphatase fraction indicates that phosphatases produced and excreted by zooplankton are as important as those produced by other organisms. The ecological impact of zooplankton phosphatases should be high since they are not inhibited by phosphate. Furthermore, these results suggest a biological feedback mechanism whereby zooplankton feeding on algae also supports the growth of algae by excreting enzymes capable of releasing phosphate.

MATS JANSSON

Institute of Limnology,
University of Uppsala,
S-751 22 Uppsala, Sweden

References and Notes

1. M. Steiner, *Naturwissenschaften* **27**, 723 (1938).
2. M. Pavlascyk-Scpilowa, A. Filc-Bauer, E. D. Ziendziel, *Pol. Arch. Hydrobiol.* **21**, 335 (1974).
3. J. Overbeck, *Verh. Int. Ver. Limnol.* **14**, 226 (1961); F. P. Healey, *J. Phycol.* **9**, 383 (1973).
4. E. J. Kuenzler and J. P. Perras, *Biol. Bull.* **123**, 134 (1965).
5. R. Margalef, *Trav. Assoc. Int. Limnol. Appl.* **11**, 246 (1951).
6. F. H. Rigler, *Limnol. Oceanogr.* **6**, 165 (1961).
7. J. Overbeck and H. D. Babenzien, *Arch. Hydrobiol.* **60**, 107 (1964).
8. W. Reichardt, J. Overbeck, L. Steubing, *Nature (London)* **216**, 1345 (1967).
9. T. Berman, *Limnol. Oceanogr.* **15**, 663 (1970); J. G. Jones, *J. Ecol.* **60**, 777 (1972).
10. G. P. Fitzgerald and T. C. Nelson, *J. Phycol.* **2**, 32 (1966).
11. M. J. Perry, *Mar. Biol.* **15**, 113 (1972).
12. A. Torriani, *Biochim. Biophys. Acta* **38**, 460 (1960); M. Møller, S. Myklestad, A. Haug, *J. Exp. Mar. Biol.* **19**, 217 (1975).
13. G. Persson, S. K. Holmgren, M. Jansson, A. Lundgren, B. Nyman, C. Ånell, *Proceedings, Circumpolar Conference on Northern Ecology*, Ottawa, Ont., Canada, 1976 (in press).
14. L. Heinrikson, *J. Biol. Chem.* **244**, 299 (1969).
15. D. L. Di Pietro and F. S. Zengerle, *ibid.* **242**, 3391 (1967).
16. A contribution from the Kuokkel project, supported by the National (Swedish) Environment Protection Board and the J. C. Kempe Memorial Foundation.

16 March 1976