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ALKALINE PHOSPHATASE DISSOLVED IN NATURAL WATER

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Alkaline phosphatase activity dissolved in sea and lake water was determined, and alkaline phosphatase was identified as dissolved chemical species of trace metals (zinc) in natural water using high performance liquid chromatography (HPLC).

In recent years, the determination of the chemical forms of trace metals in natural waters have become of more interest. Among them, organic-formed metal ions have been noted by some workers.[1] However, the identification of the chemical forms of trace elements is still difficult because of lack of proper analytical or experimental methods, since total concentrations of most trace metals are extremely low, i. e., at the ppb (μg dm^{-3}) level or less.

The present authors remarked "metalloenzymes" as one of the chemical species of this kind, and alkaline phosphatase was selected. The latter is one of the principal zinc-containing metalloenzymes, that distributes widely in vivo, and catalyzes the hydrolysis of phosphate monoesters. We have determined dissolved alkaline phosphatase activity (DAPA) in sea and lake water instead of in vivo, and tried to identify the dissolved alkaline phosphatase by using high performance liquid chromatography (HPLC).

Sea water (surface) off the coast of Misaki in the Sagami Bay, and lake water of Lake Kasumigaura (Ibaraki) were examined. All the samples were filtered through membrane filter (pore size: 0.45 μm) right after the sampling. The filtrates were concentrated to 1000—5000 fold with ultrafiltration technique using an Amicon Diaflo cell and ultrafiltration membrane (type UM-10), when necessary. DAPA was determined by using p-nitrophenyl phosphate as the substrate (in 0.6 M Tris buffer; pH 8.0).[2] (1 M = 1 mol dm^{-3}.)

In sea water sampled in December in 1979, 0.6 nmol dm^{-3} min^{-1} of DAPA was found, and 0.4—9.0 nmol dm^{-3} min^{-1} of DAPA were also found in lake water of Lake Kasumigaura in July in 1980, that were almost the same as those determined in Lake Kinneret.[3] The enzymatic characteristics of alkaline phosphatase in natural waters has been examined. At first, the phosphatase activity was inhibited by adding a chelating agent (2, 6-pyricinedicarboxylic acid or EDTA), and re-activated by adding zinc(II) or cobalt(II) ion into the solution. The activity was also decreased, when heated over 80 °C. Furthermore, the HPLC chromatograms of concen-

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trated sea water sample were measured as are shown in Fig. 1. The peak of phosphatase activity in the concentrated water coincided with the position for the elution volume of E. coli alkaline phosphatase, and zinc was also detected in this fraction. From the results described above, it was concluded that the zinc-metalloenzyme which gave the DAPA in the present experiment was alkaline phosphatase, and that the molecular weight was close to that of E. coli alkaline phosphatase (89,000). Calibrating from the activity-value, about 60 pg dm^{-3} of zinc was dissolved in the form of alkaline phosphatase in sea water (off Misaki). This amount of zinc is corresponding to 0.005 % of total dissolved zinc.

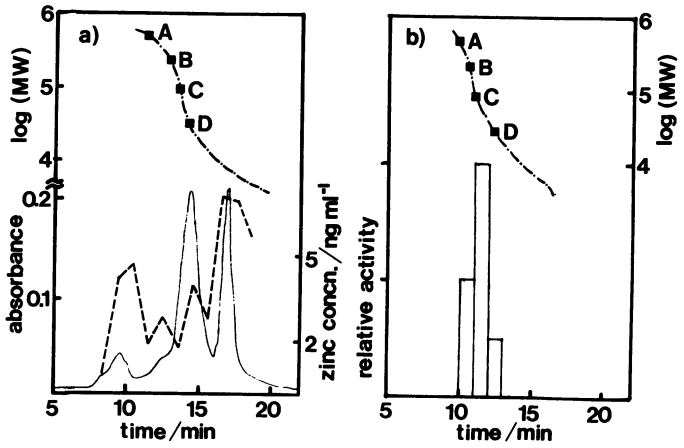

Fig. 1. HPLC chromatograms of concentrated sea waters.
Instrument: Shimadzu LC-3A high performance liquid chromatography, column: Shimadzu W-71 aqueous porous gel column (7.9 mm i. d. x 50 cm), sample: concentrated (x 5000) sea water sampled off Misaki, carrier: (a) 0.2 % KH_2PO_4-KOH buffer (pH 7.3); (b) 0.025 M Tris-HCl buffer (pH 8.0), flow rate: 1.0 cm^3 min^{-1}, at room temperature.
(a) ——: UV absorbance at 210 nm, ----: zinc concentration, —·—·—: calibration curve for molecular weight; (b) ▭: phosphatase activity, —·—·—: calibration curve for molecular weight. A: urease (Jack bean; molecular weight: 480,000), B: catalase(Bovine liver; 230,000), C: alkaline phosphatase (E. coli; 89,000), D: carbonic anhydrase (Bovine erythrocytes; 31,000).

Alkaline phosphatase in natural water are to be considered from the environmental point of view, such as eutrophication, because alkaline phosphatase is an enzyme performing the hydrolysis of phosphate monoesters. It has been well known in vivo that inorganic phosphates are mainly supplied by the hydrolysis of phosphate monoesters due to alkaline phosphatase, which determine the biological availability and kinetics of phosphorus in the phosphorus cycle. Therefore, it may be of interest to elucidate the biological activity of dissolved alkaline phosphatase in natural waters.[4] Towards this end we found that DAPA showed some seasonal corelation with the phosphorus concentration in Lake Kasumigaura. The details will be reported elsewhere.

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