

DISSOLVED NITRATE REDUCTASE IN NATURAL WATER

Kazumi KISHIBE, Kitao FUJIWARA,* Kensei KOBAYASHI, Hiroki HARAGUCHI
and Keiichiro FUWA

Department of Chemistry, Faculty of Science, University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113

Nitrate reductase activity dissolved in natural water was detected for the first time. The characteristics of this enzymatic activity were compared with the commercial nitrate reductase in terms of thermal denaturation, inhibitor spectrum, and gel permeation chromatography.

Free enzyme molecules (not bound to bio-organisms) which are dissolved in natural water have been detected with respect to alkaline phosphatase.¹⁻³⁾ For the purpose of providing further evidence that enzyme proteins exist in natural water while preserving their structures and functions, we report herein on the dissolved nitrate reductase activity in lake and sea water: Nitrate reductase is an iron-molybdenum enzyme found in various organisms which catalyzes the reduction of nitrate ion to nitrite ion.^{4,5)}

The natural water samples were collected from Lake Kasumigaura (Ibaraki prefecture), the Shinobazu and the Sanshiro ponds (Tokyo), and the North Pacific Ocean. The samples were filtered through a 0.2 μm membrane filter under mild suction conditions (over 250 mmHg) for exclusion of bio-organisms, which was performed within 2-4 hours after sampling. The activity was measured after storing the filtered sample at -4°C for sea water, or immediately after filtration for lake and pond water. A portion of the filtered sample was condensed with reverse osmosis-ultrafiltration technique. All the containers used were sterilized by soaking in ethanol or heating at 110°C . The nitrate reductase activity of the sample was measured according to the modified method by Lowe and Evans⁶⁾ (substrate: 14 mM KNO_3 ; electron donor: 0.003% methyl viologen; buffer: 20 mM phosphate buffer (pH 7.0); incubation time: 110 min).

Table 1 shows the dissolved nitrate reductase activity in the lake and pond water. Fig.1 shows the inhibition spectra of nitrate reductase activity in the sample from the Shinobazu pond and in the commercial *E. coli* enzyme purchased from Sigma

Table 1. Dissolved Nitrate Reductase Activity in Freshwaters.

Sampling Location	Dissolved Nitrate Reductase Activity munit/l*
Lake Kasumigaura**	
Station 1	4.0 \pm 0.4
2	3.0 \pm 0.4
3	3.2 \pm 0.5
4	2.0 \pm 0.5
6	4.5 \pm 0.5
7	1.6 \pm 0.3
8	1.4 \pm 0.5
9	2.6 \pm 1.4
11	3.0 \pm 0.5
12	1.9 \pm 0.5
The Shinobazu pond [†]	0.5 \pm 0.2
The Sanshiro pond ^{††}	1.5 \pm 0.6

* 1 munit means the enzyme amount which catalyzes 1 nmol of substrate per minute.

** Sampled on June 24, 1981.

† Sampled on June 17, 1981.

†† Sampled on June 2, 1981.

Chem. Co. The spectral patterns of the enzymatic activity in the pond water is consistent with that of the *E. coli*'s nitrate reductase activity. Both enzymatic activities disappeared by means of heat treatment at 100 °C for 5 min. The chromatographic separation by Sepharose CL-4B of the concentrated lake and pond water showed that this nitrate reductase activity appeared in the fractions equal to those of commercial *E. coli* nitrate reductase (molecular weight > 500000).

Fig. 2 shows the vertical distribution of dissolved nitrate reductase activity in the North Pacific Ocean; sea water sampled were 1000 times concentrated by ultrafiltration (membrane: Toyo Kagaku Type UK-10).

The obtained results provide the evidence that the enzyme proteins of molecular weight more than 500000 containing several subunits can exist in natural water (lake and sea water), while preserving their activity. On the standpoint of chemical speciation of molybdenum dissolved in natural water, it can be estimated that 10^{-2} — 10^{-3} % of molybdenum takes the form as nitrate reductase.

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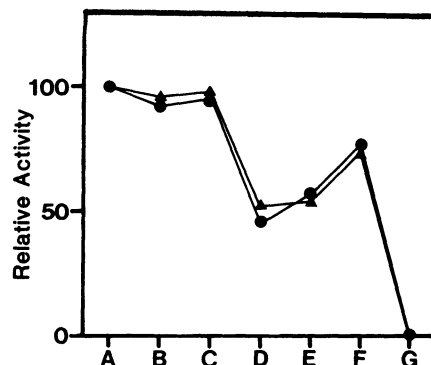


Fig. 1. Inhibition Spectra of Nitrate Reductase Activity in Pond Water and Commercial *E. coli* Enzyme.

▲: The Sanshiro pond water; ●: commercial enzyme. A: none; B: ethylenediaminetetraacetic acid; D: 1,10-phenanthroline; E: 8-hydroxyquinoline; F: sodium diethyldithiocarbamate; G: sodium cyanide. These reagents were added to incubation media at a concentration of 1 mM.

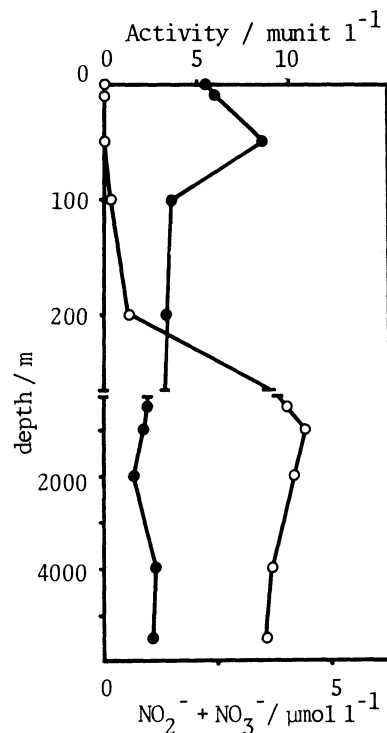


Fig. 2. Vertical Distribution of Dissolved Nitrate Reductase Activity.

The Sampling point was the North-West Pacific Ocean (30°00'N, 170°00'W), which was reached on May 16, '81. ●: Activity; ○: NO₂⁻ + NO₃⁻ concentration.

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