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The Isotope Effect on the Nitrogen in Biochemical, Oxidation-Reduction Reactions

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Abstract

The nitrogen isotope effect was studied using marine nitrifying and denitrifying bacteria.

In the oxidation of ammonia to nitrite, $\delta^{15}N‰$ in nitrite produced ranged from −21 to −5 with respect to that in ammonia (0‰) used as a substrate.

The lighter isotope was enriched in the molecular nitrogen produced through the denitrification process. Assuming a first order reaction, the ratio of the rate constants, k_{14}/k_{15}, was calculated to be 1.02.

The variation of ^{15}N content in the decomposition of *Scenedesmus* sp. was studied.

Introduction

The relative abundance of nitrogen isotopes in nitrogenous compounds in the marine environment has been investigated by Miyake and Wada (1967). Their results show that ^{15}N value of the total nitrogen in the sea is 7‰ on an average with respect to the atmospheric nitrogen and ^{15}N content tends to increase along the link in the food chain, low in the organisms at lower trophic level and high in those at higher trophic level.

According to Hoering (1955) and Cheng *et al.* (1964), ^{15}N contents in natural nitrogenous organic compounds varied between −6 and 21‰. The variation might be attributed to the isotope fractionation in the biochemical reactions in the nitrogen cycle.

Hoering and Ford (1960), however, failed to find out any isotope effect of nitrogen in the fixation of nitrogen gas by azotobacter species beyond the experimental error. No study has been done yet on other reactions partaining to the nitrogen cycle.

The present experiment was undertaken to estimate the extent of isotope effect in the following three, biochemical oxidation-reduction reactions involving nitrogen.

1. Oxidation of ammonia to nitrite by marine nitrifying bacteria.
2. Reduction of nitrate to nitrogen gas by marine denitrifying bacteria.
3. Aerobic decomposition of *Scenedesmus* sp.

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Materials and methods

The marine nitrifying and denitrifying bacteria used were supplied by Dr. A. Kawai, Kyoto University. The following culture media were adopted.

For nitrifying bacterium: 32 g NaCl, 189 mg $(NH_4)_2SO_4$, 2 mg KH_2PO_4, 0.2 mg EDTA-Fe, 10 g SiO_2 and 1 *l* distilled water. pH was adjusted to 8.2 with saturated K_2CO_3 solution.

For denitrifying bacterium: 1.53 g yeast extract, 2.36 g peptone, 19.78 g $NaNO_3$ and 500 m*l* sea water.

The media were sterilized by autoclaving at 120°C for 20 minutes.

Nitrification

A small portion of a preculture of the nitrifying bacterium was inoculated into 500 m*l* of the culture medium in 500 m*l* glass bottle. The nitrogen content in the bacteria transferred into the new medium was so small that the amount of organic nitrogen was negligible. After the incubation at 25°C for 10 to 30 days, bacterial cells were removed by filtration through HA-Millipore filters (0.45 μ). Ammonia and the sum of nitrite and nitrate were separated by the method as described in the previous paper (Miyake *et al.*, 1967). Since the relative amount of nitrate formed during the period was found very small, we call, hereafter, the sum of nitrite and nitrate simply "nitrite".

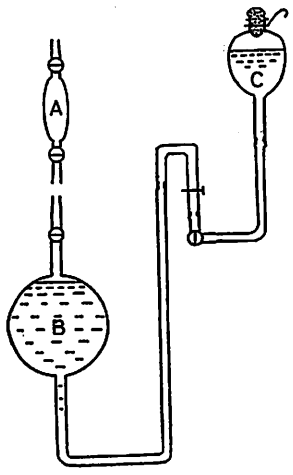

Fig. 1. Incubation bottle used for denitrification.

A: Gas sampling reservoir.
B: Incubation bottle filled with the medium.
C: Reservoir for ajusting a level, filled with 3.2% NaCl solution.

Denitrification

Denitrification was conducted at 25°C in the apparatus (200 m*l*) shown in Fig. 1. The gases produced were transferred into the reservoir (A) at intervals by ajusting the level of the reservoir (C) which was filled with sterile 3.2% NaCl solution.

The isotopic composition of nitrogen gas and N_2/Ar ratio were measured by a mass-spectrometer (Hitachi RMU-6) fitted with double collecting system. Atmospheric nitrogen was used as a reference.

Results and discussions

Nitrification

As seen in Table 1, a significant isotope fractionation between ammonia and nitrite was observed. The fraction of nitrite produced to the initial amount of ammonia was from 0.2 to

Table 1. Nitrogen isotope fractionation in the oxidation of ammonia to nitrite by marine nitrifying bacterium.

Exp. No.	Period of incubation (days)	$f_{NO_2^-}$	$f_{NH_4^+}$	δNO_2^-	δNH_4^+	α
1	20	0.50	—	−7.2	8.9	0.984
2	30	0.51	0.26	−5.4	10.1	0.985
3	10	0.25	—	—	5.7	—
4	10	0.22	0.50	−21.1	4.0	0.975

f: Mole fraction of ammonia or nitrite produced to the initial amount of ammonia. $\delta^{15}N$ value in ammonia used as substrate was 0.0±0.3‰.

α: Fractionation factor between nitrite and ammonia at the end of the reaction.

0.5, while the observed $\delta^{15}N$ value of nitrite was from −21.1 to −5.4 and that of ammonia at the end of the reaction was from +4 to +10.

There are two factors affecting the isotope ratios of nitrite produced. One is the kinetic effect in the oxidation reaction. The rate constant of the reaction involving lighter molecules is ordinarily larger than that for heavier ones.

The other is the fractionation of nitrogen in the bacterial growth. According to Watson (1965), the generation time of a marine nitrifier (*Nitrosocystis oceanus*) is around 24 hours. In the present experiment the incubation time was so long that the growth of bacterium would have occurred and affect the isotopic ratios of nitrite produced.

Denitrification

In the gas sample collected, small amounts of the atmospheric nitrogen and other gases initially dissolved in the medium were contained. The isotopic ratios in the produced gases, therefore, should be corrected by using the observed values of N_2/Ar ratios in the sample gases. $\delta^{15}N$ values in nitrate, yeast extract and peptone used in this experiment were 1.3, 6.0 and 30‰, respectively. $\delta^{15}N$ values of nitrogen gas produced were from −20.7 to −14.4‰, increasing with the progress of denitrification (Table 2). The most probable source of gaseous nitrogen produced might be nitrate-N, since the reduction of nitrate is the only known metabolic process of biochemical denitrification (Fry, 1955).

Assuming the first order reaction for the reduction of nitrate to molecular nitrogen, the ratio of the rate constants, k_{14}/k_{15} were calculated by the following equations;

$$\frac{k_{14}}{k_{15}} = \frac{ln(1-f)}{ln(1-rf)} \quad \text{or} \quad r=-\frac{1-F^{\frac{k_{15}}{k_{14}}}}{1-F}, \quad F+f=1 \qquad (1)$$

Table 2. Nitrogen isotope fractionation in the reaction of nitrate to nitrogen gas by marine denitrifying bacterium.

Exp. No.	Incubation hours	N_2 m*l* produced	δN_2 ‰
1	0–16	10	−19.7
2	16–19	6	−20.7
3	19–24	8	—
4	24–26	6	−17.6
5	26–32	16	−14.4
6	32–35	8	−15.8
7	35–39	7	−12.8
8	39–42	7	−18.8

$\delta^{15}N$ value in nitrate used as a substrate was 1.3±0.3‰.

where $r=(^{15}N/^{14}N)_{N_2}/^{15}(N/^{14}N)_{NO_3,\ t=0}$ and f, the molar fraction of the produced nitrogen gas to the initial amount of nitrate-N. In Fig. 2 the variation of δ values of the produced nitrogen gas is shown as a function of f_{N_2}. As shown in Fig. 2, the observed variation is fairly in good agreement with the calculation using the value of k_{14}/k_{15} of 1.02 (solid line in Fig. 2).

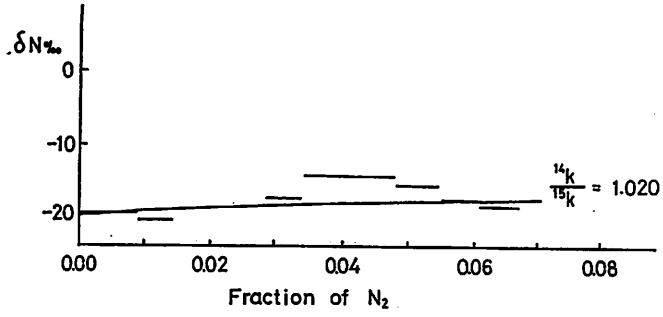

Fig. 2. Relation between δ value and the mole fraction of the produced nitrogen gas.

According to Brown *et al.* (1967) the isotope effect for the reduction of nitrate to ammonia was 1.075 at 25°C in an alkali solution using $FeSO_4$ as a reducing agent. This value agrees well with the calculated value on the assumption that the cleavage of N–O bond is the rate determining step.

The discrepancy in the isotope fractionation between the biochemical denitrification and the purely chemical reduction suggests that there is difference in reaction mechanism between them.

The biochemical denitrification is a major pathway through which molecular nitrogen is produced and also plays an important role in the geochemical processes in the nitrogen cycle. According to Hoering (1955) and Miyake *et al.* (1967), ^{15}N content in the biogenic nitrogenous compounds is higher than that in the atmospheric nitrogen. On the other hand, Hoering *et al.* (1960) have reported that the isotope fractionation factor in the fixation of atmospheric nitrogen by several kinds of azotobacter is 1.000±0.001. Thus, it may be said that the isotope fractionation in the denitrification contributes to a greater extent to the relative abundance of nitrogen isotopes in nature than nitrogen fixation.

The decomposition of Scenedesmus sp.

The decomposition or mineralization processes may be also regarded as one of the factors effecting the distribution of ^{15}N in nature.

The nitrogen isotope ratio in the residual matter of *Scenedesmus* sp. was measured through its aerobic decomposition process. The samples were offered by Dr. A. Ohtsuki of Faculty of Science, Tokyo Metropolitan University. The details of the experiment were reported by Ohtsuki (1968).

In Fig. 3, the changes in $\delta^{15}N‰$ and C/N in the residual matter are presented. The variation of ^{15}N content of the residual matter was about 3‰ and δ value at the end of decomposition process was a little higher than that in intact cells of *Scenedesmus* sp. During the first 20 days of decomposition, a marked decrease in ^{15}N content in the residual matter was observed. This might be account for that the decomposition took place successively from easily utilizable material to harder ones.

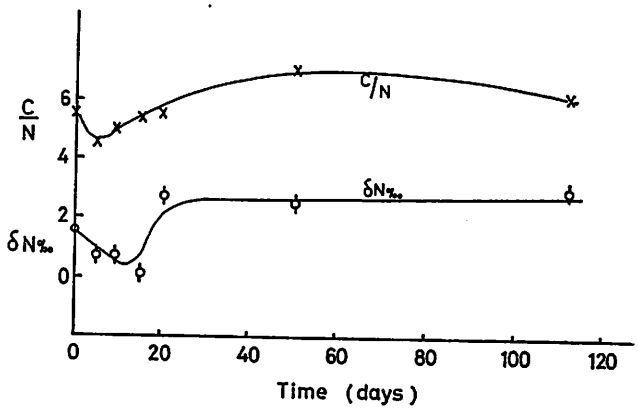

Fig. 3. Variation of C/N and δ values of the residual particulate matter during the decomposition of *Scenedesmus* sp.

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