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Effects of nitrate supply on ammonium assimilations in the blade of *Laminaria japonica* (Phaeophyceae)*

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Abstract

The effects of nitrate supply to ammonium uptake and the assimilations in *Laminaria japonica* Areschoug were investigated by culture experiments in four combinations ($^{15}NH_4^+$ only, $^{15}NH_4^+ + NO_3^-$, $^{15}NO_3^-$ only and $^{15}NO_3^- + NH_4^+$). Ammonium and nitrate were simultaneously uptaken from the medium by the blade tissue segments. The NO_3^- supply resulted in the increase of ^{15}N allocations of $^{15}NH_4^+$ to insoluble and soluble organic nitrogen pools. This indicates that nitrate supply promotes the assimilation and incorporation activities of ammonium in the blade of *L. japonica*. Further, the importance of nitrate within the blade of seaweeds was discussed.

Introduction

Ammonium and nitrate are major nitrogen sources of seaweeds in the field and are used as nitrogen fertilizer in the effective management of seaweed cultivation systems (Tseng, et al., 1955; Yamada and Iwasaki, 1964). The utilization of these inorganic nitrogen sources by seaweeds varies with species and their populations (reviewed by DeBoer, 1981). The nitrogen status of seaweed also influences nitrogen uptake (D'Elia and DeBoer, 1978; Ryther et al., 1981). Some experiments have been conducted for several seaweeds on the interactions of ammonium and nitrate uptake. The suppression of nitrate uptake by ammonium (Hanisak and Harlin, 1978) and the increase of ammonium uptake by nitrate (Thomas et al., 1987) were observed. Moreover, simultaneous uptake of ammonium and nitrate was reported in red (Bird, 1976) and brown seaweeds (Topinka, 1978; Harlin and Craigie, 1978). However, these interactions were only discussed on nitrogen transport through the blade membrane from the medium. The mechanisms for these interactions between ammonium and nitrate uptake are still not elucidated.

In the present study, the interactive effects of ammonium and nitrate supply on nitrogen assimilation by blades of *Laminaria japonica* Areschoug were investigated using ^{15}N tracer technique. Furthermore, the importance of nitrate on nitrogen metabolism within the blade of *L. japonica* were discussed.

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Materials and Methods

The sporophytes of *Laminaria japonica* (total blade length, ca. 50 cm) were collected from the intertidal zone of Hakodate Bay, Hokkaido, in April 1990. These sporophytes, kept in a plastic bag, were brought to the laboratory and maintained in a 5-l bottle filled with nitrogen poor seawater ($NH_4^+ < 1\ \mu M$, $NO_2^- + NO_3^- < 1\ \mu M$) at 10°C under 2,000 lux. Before the experiments began, tissue segments (5×5 cm) were cut from portions located 20-25 cm from the stipe-blade transition. Microorganisms on the segment surface were removed by brushing, wiping and washing with filtered seawater. The segments were precultured for 24 hours at the same condition mentioned above. Mucus from the segments was removed by wiping with a sterilized gauze prior to use in the nitrogen uptake and ^{15}N assimilation experiments. Seawater used in the culture was filtered with a Whatman GF/F glass fiber filter and autoclaved at 120°C for 20 min.

Uptake experiments were conducted in 500 ml bottles filled with seawater enriched with NH_4Cl or $NaNO_3$ under agitating (120 rpm) by using a magnetic stirring bar (0.5×2.0 cm). Disappearances of ammonium and nitrate from the seawater medium were calculated followed by sampling every 30 min. for about 2 hr. Ammonium uptake was estimated from the disappearance occurring over a 30 min. period in five sets of the mediums in which different nitrate and ammonium concentrations were combined. For ^{15}N assimilation experiments, the sample segments were incubated in 500 ml seawater enriched with $^{15}NH_4Cl$ and $Na^{15}NO_3$ (99.0 atom%). The incubation was done in four combinations of $^{15}NH_4^+$ and $^{15}NO_3^-$ ($^{15}NH_4^+$ only, $^{15}NH_4^+ + NO_3^-$, $^{15}NO_3^- + NH_4^+$ and $^{15}NO_3^-$ only) for 3 hr. The ^{15}N-labelled nitrogen and non-labelled nitrogen were supplied at 60 μM, respectively.

After incubation for ^{15}N assimilation experiments, sample segments were collected, washed with distilled water, wiped and then ground. These segments were placed in 10 ml of chilled 80% ethanol (4°C) for 24 hr to separate insoluble and soluble nitrogen. After extraction, the ethanol extracts were diluted to 25 ml with distilled water. A 10 ml aliguot of this solution was loaded on a Sephadex G-10 column (1.7×75 cm) which had been equilibrated with 0.05 M formic acid. The same buffer was used for elutions at a flow rate of 15 ml • hr^{-1} and 10 ml fractions were collected. The fractionated samples were submitted to nitrogen content (total nitrogen, NH_4^+ and $NO_2^- + NO_2^-$) and ^{15}N enrichment analysis.

Total soluble nitrogen was analyzed using a nitrogen analyzer (Sumigraph N-200, Sumitomo Chemical Industry Co.). Inorganic nitrogen was measured using an Autoanalyzer (Technicon Autoanalyzer II) according to Strickland and Parsons (1972). Insoluble nitrogen content was measured by the Kjeldahl method. Isotopic enrichment (^{15}N atom %) was determined using a ^{15}N analyzer (NOI-5, Statron) after Kjeldahl digestion and distillation as described by Fieldler and Proksch (1975). The ^{15}N content in each nitrogen fraction was calculated as;

$$N_{(allocation)} = \frac{\text{Excess atom \%}}{R} \cdot \frac{N_p}{W}$$

where, $N_{(allocaotion)}$ denotes ^{15}N allocated to each fraction ($\mu g\ N \cdot g^{-1}$ wet wt.); Excess atom %, (final atom % ^{15}N) − (measured natural abundance ^{15}N); W, wet weight of sample segment (g); R, ^{15}N enrichment of the dissolved nutrients (99.0 atom %);

N_p, nitrogen content of each nitrogen fraction (μgN), respectively. The ^{15}N content in NH_4^+ fraction was calculated by subtracting the ^{15}N in the organic nitrogen and $NO_2^- + NO_3^-$ fraction from that in the soluble nitrogen pool.

The apparent rates ($\mu gN \cdot g^{-1}$ wet wt. $\cdot h^{-1}$) of nitrogen incorporation (V_i), assimilation (V_a) and nitrate reduction (V_r) were calculated by the equation:

$$V_i = \frac{N_{(\text{insol. N})}}{T}$$

$$V_a = \frac{N_{(\text{insol. N})} + N_{(\text{sol. org. N})}}{T}$$

$$V_r = \frac{N_{(\text{insol. N})} + N_{(\text{sol. org. N.})} + N_{(\text{ammonium})}}{T}$$

where $N_{(\text{insol. N})}$ was the nitrogen allocated into insoluble nitrogen pool, $N_{(\text{sol. org. N})}$ was the nitrogen allocated into soluble organic nitrogen and $N_{(\text{ammonium})}$ was the sitrogen allocated into ammonium fraction of incubation rate (T).

Results

The typical time course of decrease for NH_4^+ or NO_3^- concentrations in the medium is shown in Fig. 1. This indicates the simultaneous uptake of NH_4^+ and NO_3^- by the blade of *L. japonica*. The effects of NO_3^- supply to NH_4^+ uptake is shown in the disappearance of NH_4^+ concentration in the medium (Fig. 1B) and is summarized in Table 1. The uptake rate of NH_4^+ was enhanced to 105-142% by

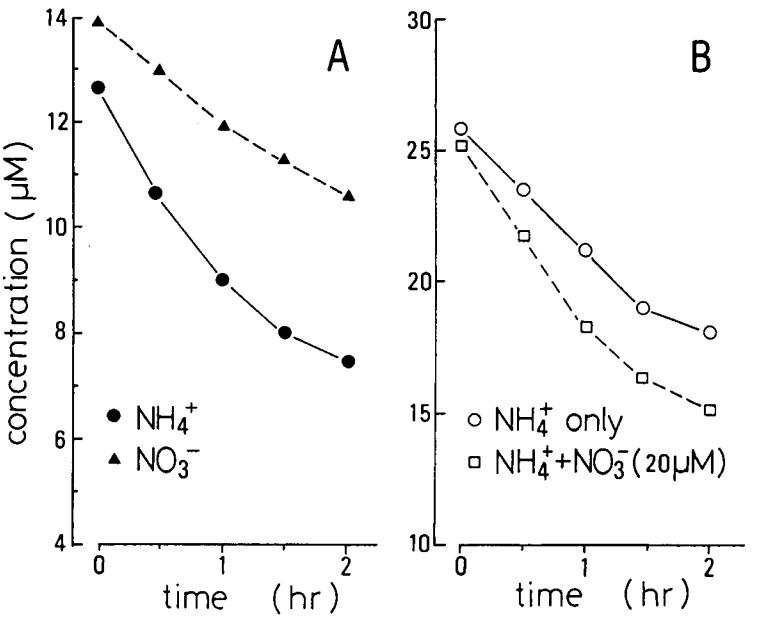

Fig. 1. Simultaneous uptake of NH_4^+ and NO_3^- (A), and the effect of NO_3^- supply on NH_4^+ uptake (B) by *Laminaria japonica* at 10°C.

Table 1. Effects of NO_3^- supply on NH_4^+ uptake rate by *Laminaria japonica*.

concentration (μM)		NH_4^+ uptake rate (μmol·g^{-1} wet wt.·h^{-1})	
NH_4^+	NO_3^-		
3.0	0.5	a	0.072
3.0	13.0	b	0.097
		b/a %	127.6
7.3	0.5	a	0.173
7.3	13.0	b	0.182
		b/a %	105.2
16.0	2.0	a	0.469
16.0	22.0	b	0.498
		b/a %	106.2
25.0	1.0	a	0.706
25.0	20.0	b	0.820
		b/a %	115.4
35.0	1.0	a	0.820
35.0	20.0	b %	1.162
		b/a %	141.7

the nitrate supply. Hovever, the increase of NH_4^+ uptake did not correlated with the ambient NH_4^+ and NO_3^- concentrations.

Fig. 2 shows ^{15}N allocation to insoluble and soluble nitrogen pools in tissue segments in cultures of four combinations. In the cultures with ^{15}N-labelled ammonium, the ^{15}N allocation to inoluble nitrogen pools significantly increased by

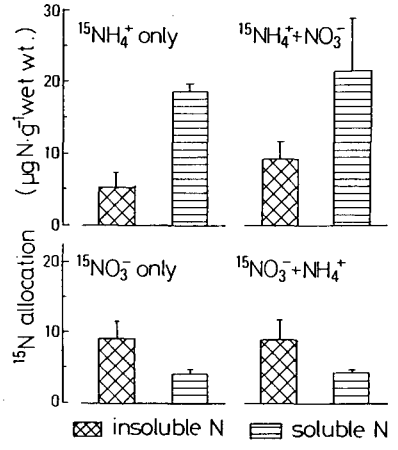

Fig. 2. Allocation of ^{15}N ($\bar{x} \pm$S.D.) to soluble and insoluble nitrogen pools in the tissue segments (n=3-4) of *Laminaria japonica* in the culture medium of four combinations of NH_4^+ and NO_3^-.

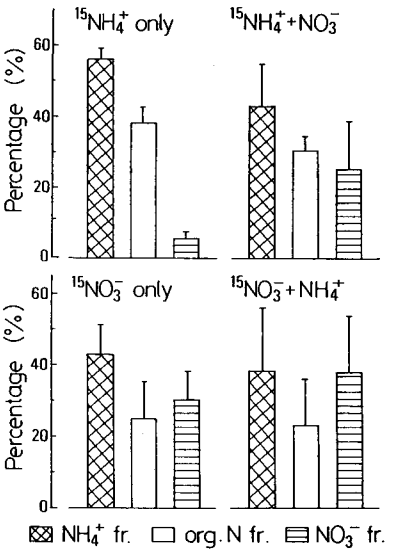

Fig. 3. ^{15}N distribution ($\bar{x} \pm$S.D.) in soluble nitrogen pool of the tissue segments (n= 3-4) of *Laminaria japonica* in the culture mediums of four combinations of NO_3^- and NH_4^+.

the supply of NO_3^-. The ^{15}N allocation to soluble nitrogen pool also tended to increase with the supply of NO_3^-. On the other hand, there was no effect on $^{15}NO_3^-$ uptake or the ^{15}N allocation by the NH_4^+ supply. For the ^{15}N allocation into three fractions (organic N, NH_4^+ and $NO_2^- + NO_3^-$) in the soluble nitrogen pool, the ^{15}N allocation to each fraction was expressed as percentage to ^{15}N in soluble pool and is shown in Fig. 3. The NO_3^- supply resulted in the decrease of ^{15}N allocation of $^{15}NH_4^+$ to NH_4^+ fraction and the increase of ^{15}N allocation to $NO_2^- + NO_3^-$ fraction. The allocation to the $NO_2^- + NO_3^-$ fraction in the blade suggests intercellular nitrification in the blade (Mizuta and Maita, in preparation). However, NH_4^+ supply did not affect the ^{15}N allocation of $^{15}NO_3^-$ to each fraction. Based on these results, the ^{15}N incorporation rate to insoluble nitrogen, and both rates of $^{15}NH_4^+$ assimilation and $^{15}NO_3^-$ reduction were calculated (Table 2) In the case of culture medium supplied with ^{15}N-labelled ammonium only, the ^{15}N incorporation and $^{15}NH_4^+$ assimilation rates were 1.60 and 3.98 μgN $\cdot$ g^{-1} wet wt. $\cdot$ h^{-1}, respectively.

Table 2. Effects of NH_4^+ and NO_3^- on ^{15}N incorporation rate to insoluble nitrogen, $^{15}NH_4^+$ assimilation rate and $^{15}NO_3^-$ reduction rate (μgN$\cdot g^{-1}$ wet wt.$\cdot h^{-1}$: $\bar{x} \pm$ S.D.) in the tissue segments (n=3-4) of *Laminaria japonica* in the culture mediums of four combinations of NH_4^+ and NO_3^-.

medium	incorporation rate	$^{15}NH_4^+$ assimilation rate	$^{15}NO_3^-$ reduction rate
$^{15}NH_4^+$ only	1.60±0.55	3.98±0.90	—
$^{15}NH_4^+ + NO_3^-$	2.63±0.57	4.48±0.89	—
$^{15}NO_3^-$ only	3.06±0.84	3.43±0.87	3.97±0.81
$^{15}NO_3^- + NH_4^+$	2.97±0.84	3.31±1.02	3.89±1.25

Particularly, the ^{15}N incorporation rate when only $^{15}NH_4^+$ was supplied was significantly lower in comparison with that of other combinations ($^{15}NH_4^+ + NO_3^-$, $^{15}NO_3^-$ only and $^{15}NO_3^- + NH_4^+$). However, both rates of incorporation and assimilation of $^{15}NH_4^+$ were promoted by the NO_3^- supply. Noteworthily, the incorporation rate increased to about 1.6 times the rate when only ^{15}N-labelled ammonium was added. On the contrary, the ^{15}N incorporation rate, ^{15}N assimilation rate and $^{15}NO_3^-$ reduction rate after only $^{15}NO_3^-$was supplied were 3.06, 3.43 and 3.97, respectively. These three process rates did not change with the supply of ammonium.

Discussion

Simultaneous uptake mechanisms of ammonium and nitrate were reported in several Laminariales (Harlin and Craigie, 1978; Machiguchi et al., 1985). In this study, *Laminaria japonica* simultaneously uptook ammonium and nitrate (Fig. 1A) and promoted the ammonium uptake by nitrate supply (Fig. 1B, Table 1). The suppression of nitrate uptake was not observed by ammonium supply. According to Blasco and Conway (1982), NH_4^+ inhibited NO_3^- assimilation in two steps; first nitrate uptake and second nitrate reduction on phytoplankton. In *L. japonica*, the three processes of nitrate assimilation were not influenced by ammonium supply (Table 2). These facts indicate that the simultaneous uptake of NH_4^+ and NO_3^- are caused by the promotion of assimilation activities with nitrate supply (Table 2). Moreover, it was considered that this promotion of assimilation activities suppressed accumulation of ammonium in the tissue. Fujita et al. (1988) reported that membrane transport of ammonium appeared to be inhibited when ammonium accumulated in the tissue of *Ulva rigida*. Therefore, it seems likely that the increase of ammonium uptake by nitrate supply was based on this promotion of assimilation activities. Thomas et al. (1987) reported the simultaneous uptake kinetics of ammonium and nitrate varied with populations; that is the age and stage of the blade influenced the interaction of ammonium and nitrate uptake (Thomas et al., 1985; Harrison et al., 1986). These facts suggest that the interaction of ammonium and nitrate uptake seems to be complex and may be changed by the physiological status of the blade as well as environmental factors. However, the simultaneous uptake of ammonium and nitrate is advantageous for acquiring greater amounts of nitrogen, particularly under conditions of nitrogen limitation (Thomas and Harrison, 1987).

The uptake rate of ammonium was higher than that of nitrate (Fig. 1A). However, the rate of incorporation to insoluble nitrogen was very low (Table 2). This phenomenon seems to support the fact (Ito et al., 1960) that ammonium was easily absorbed but did not increase the protein-nitrogen in the blade. However, the low activity of the incorporation was promoted by nitrate supplied to the medium. On the contrary, the rate of $^{15}NO_3$ reduction, assimilation and incorporation was not influenced by the supply of ammonium (Table 2). From the relationship of rates on three processes of nitrate assimilation, it was also shown that the nitrate-nitrogen was rapidly assimilated after nitrate was reduced. Sato et al. (1959) reported that the supply of nitrate brought a considerable increase of asparatic acid and glutamic acid which was not recognized from the supply of ammosium. These amino acids accounted for the large proportion in total amino acid pool in

Laminaria japonica (Takagi and Kuriyama, 1956). It was then considered that NH_4NO_3 was better than NH_4Cl or $NaNO_3$ as a useful nitrogen for seaweeds (Yamada, 1961 ; Sato et al., 1959). Furthermore, Ammonium is often toxic for the blade of seaweeds (Waite and Mitchell, 1972). However, nitrate supply is likely to lead to the suppression of toxicity of ammonium (Fig. 3). These results show that the existence of nitrate in the blade plays a very important role for nitrogen metabolism of seaweeds. The promotion of activities in ammonium uptake, assimilation and incorporation are considered among of the important roles of nitrate.

Nitrogen is often very poor from late spring to summer in the natural environment and the production of seaweeds is limited (Chapman and Craigie, 1977, 78). Therefore, fertilization containing nitrogen compounds for seaweeds has been supplied during these nitrogen deficient periods (Tseng, 1955 ; Yamada and Iwasaki, 1964). From this point of view, it is suggented the nitrate is a more effective fertilizer in promoting seaweed growth.

It is concluded from these results that the simultaneous uptake was due to the promotion of ammonium assimilations in the blade of *L. japonica* and this promotion was carried out by the supply of nitrate. These results suggest the significance of nitrate in the blade of *L. japonica*, particularly during periods or in places where ammonium is the main nitrogen source.

Aknowlegement

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References

Bird, K.T. (1976) Simultaneous assimilation of ammonium and nitrate by *Gelidium nudifrons* (Gelidiales : Rhodophyta) *J. Phycol.*, **12**, 238-241.

Brasco, D. and Conway, H.L. (1982) Effect of ammonium on the regulation of nitrate assimilation in natural phytoplankton popluations. *J. Exp. Mar. Biol. Ecol.*, **61**, 157-168.

Chapman, A.R.O. and Craigie, J.S. (1977) Seasonal growth in *Laminaria longicruris* : relations with dissolved inorganic nutrients and intertidal reserves of nitrogen. *Mar. Biol.*, **40**, 197-205.

Chapman, A.R.O. and Craigie, J.S. (1978) Seasonal growth in *Laminaria longicruris* : relations with reserve carbohydrate storage and production. *Mar. Biol.*, **46**, 209-213.

DeBoer, J.A. (1981) Nutrients. *In* : Lobban, C.S. and Wynne, M.J. (Ed.) *Biology of Seaweeds*. Blackwell Scientific Publications, Oxford, 356-391.

D'Elia, C.F. and DeBoer, J.A. (1978) Nutritional studies of two red algae 2. Kinetics of ammonium and nitrate uptake. *J. Phycol.*, **14**, 266-272.

Fieldler, R. and Proksch, G. (1975) The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis : A review. *Anal. Chemi. Acta*, **78**, 1-62.

Fujita, R.M., Wheeler, P.A. and Edwards, R.L. (1988) Metabolic regulation of ammonium uptake by *Ulva rigida* (Chlorophyta) : A commpartmental analysis of the rate-limiting step for uptake. *J. Phycol.*, **24**, 560-566.

Hanisak, M. and Harlin, M.M. (1978) Uptake of inorganic nitrogen by *Codium fragile* subsp. *tomentosoides* (Chorophyta). *J. Phycol.*, **14**, 450-454.

Harlin, M.M. and Craigie, J.S. (1978) Nitrate uptake by *Laminaria longicruris* (phaeophyceae). *J. Phycol.*, **14**, 464-467.

Harrison P.J., Druehl, L.D., Lloyed, K.E. and Thompson, P.A. (1986) Nitrogen uptake kinetics in three year-classes of *Laminaria groenlanidca* (Laminariales: Phaeophyceae). *Mar. Biol.*, **93**, 29-35.

Ito, K., Sato, S., Sato, Y. and Matsumoto, F. (1960) On the utilization of various nitrogen compounds. *Bull. Jap. Soc. Sci. Fish.*, **26**, 938-941. (In Japanese)

Machiguchi, Y., Sanbonsuga, Y. and Oakda, Y. (1985) Nitrogen uptake and growth of *Laminaria* renewal stage. *Bull. Hokkaido Reg. Fish. Res. Lab.*, **50**, 45-61. (In Japanese)

Ryther, J.H., Corwin, N., DeBusk, T.A. and Williams, L.D. (1981) Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). *Aquaculture*, **26**, 107-115.

Sato. S., Sato, Y., Ito, K., and Matsumoto, F. (1959) Biochemical studies on the edible seaweed, *Porphyra tenera*-1. Contents of various nitrogenous compositions in the frond as affected by the type of the nitrogen source. *Bull. Jap. Soc. Sci. Fish.*, **25**, 953-957. (In Japanese)

Strickland J.D.H. ad Persons, T.R. (1972) Apractical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can.*, **167**, 1-311.

Takagi, M. and Kuriyama, K. (1956) Chemical studies of marine algae. l. The free amino acids in several species of marine algae. *Bull. Fac. Fish. Hokkaido Univ.*, **10**, 72-76.

Thomas, T.E., Harrison, P.J. and Talylor, E.B. (1985) Nitrogen uptake and growth of germlings and mature thalli of *Fucus distichus*. *Mar. Biol.*, **84**, 267-274.

Thomas, T.E. and Harrison, P.J. (1987) Rapid ammonium uptake and nitrogen interactions in five intertidal seaweeds grown under field condition. *J. Exp. Mar. Biol. Ecol.*, **107**, 1-8.

Thomas, T.E., Harrison, P.J. and Turpin, D.H. (1987) Adaptations of *Glacilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. *Mar. Biol.*, **93**, 569-580.

Topinka, J.A. (1978) Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). *J. Phycol.*, **14**, 241-247.

Tseng, C.K., Sun, K.Y. and Wu, C.U. (1955) Studies on fertilization application in the cultivation of Haitai (*Laminaria japonica* Aresch.). *Acta Botanica Sinica*, **4**, 375-392.

Waite, T. and Mitchell, R. (1972) The effect of nutrient fertilization on the benthic alga. *Bot. Mar.*, **15**, 151-156.

Yamada, N. and Iwasaki, Y. (1964) Studies on the mature for seaweeds-4. On the maturing for yellow *Gelidium* bed. *Bull. Jap. Soc. Sci. Fish.*, **30**, 986-992. (In Japanese)