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Author(s)	Nigi, Goroh; Kuma, Kenshi; Matsunaga, Katsuhiko
Description	Short paper
Citation	Fisheries science : FS, 66(5), 986-988 https://doi.org/10.1046/j.1444-2906.2000.00156.x
Issue Date	2000-10
Doc URL	https://hdl.handle.net/2115/53274
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Type	journal article
File Information	FS66_986.pdf



Short Paper

Effect of natural organic-Fe(III) complex on iron uptake and growth of a brown alga *Laminaria religiosa* Miyabe

GOROH NIGI, KENSHI KUMA* AND KATSUHIKO MATSUNAGA

Department of Fisheries Oceanography and Marine Science, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

KEYWORDS: growth, iron uptake rate, *Laminaria religiosa*, natural organic-iron complex.

Iron is an essential micronutrient for algal growth and an important element of such biological processes as synthesis of DNA, RNA and chlorophyll, electron transport, oxygen metabolism and nitrogen utilization.¹ In coastal waters, enhanced riverine discharge and regenerative decomposition contribute to significantly higher Fe concentrations, probably due to the higher concentrations of organic ligands which were possibly released by riverine input and from coastal marine organisms. Such natural organic ligands control the speciation of Fe and the dissolved Fe concentration and thus the bioavailability of Fe in seawater.^{2,3}

The short-term (3h) radioactive ⁵⁹Fe uptake rates by adult sporophytes of a brown alga *Laminaria religiosa* were measured and compared in the presence of different premixed organic-⁵⁹Fe(III) complexes [EDTA-⁵⁹Fe(III) (2:1), citric-⁵⁹Fe(III) (100:1 and 1000:1) and fulvic-⁵⁹Fe(III) (1 p.p.m. C)] and solid amorphous hydrous ferric oxide [am-⁵⁹Fe(III)] media with 200 nM Fe, containing 500 μM nitrate and 25 μM phosphate, at 10°C under 4000 lx fluorescent light. Each medium was prepared by aging for 1 day at 10°C after adding premixed organic-⁵⁹Fe(III) complex solution for organic-⁵⁹Fe(III) medium and dissolved ⁵⁹Fe(III) solution for am-⁵⁹Fe(III) medium to filtered seawater at 10°C. The addition of dissolved ⁵⁹Fe(III) without organic ligands into seawater results in rapid hydrolytic precipitation of am-⁵⁹Fe(III) (Fig. 1). Blades of adult sporophytes (~6 cm in width, 1 m in length) of a brown alga *L. religiosa* collected at a coastal site were cut to about 20 cm from the basal growth zone. At the start of iron uptake experiments, each cut blade was suspended in a medium which was stirred with a

magnetic stirrer at 10°C. After 3 h cultivation, each blade was transferred to a glass beaker containing 300 mL of 0.02 M Ti(III)-citrate EDTA solution to rapidly dissolve am-⁵⁹Fe(III) precipitates and extracellularly adsorbed iron on the blade surface by means of reductive dissolution of ⁵⁹Fe(III) without cellular damage.^{4–8} After allowing to stand for 10 min, each blade was digested with concentrated HNO₃: concentrated HClO₄ (1:1). The γ-activity of the Ti(III) solution containing the rinsed iron (extracellularly adsorbed iron) and the digested solution (intracellular iron) in counting vials was measured using a scintillation counter. Organic carbon concentration (p.p.m. C) in the fulvic acid stock solution, obtained by soil fulvic acid extraction method,⁹ was measured by a TOC analyzer (Yanaco TOC-8L, Tokyo, Japan). The ⁵⁹Fe(III) dissociative precipitation rates of premixed organic-⁵⁹Fe(III) complexes in seawater at 10°C were measured by a simple filtration (0.025 μm) and were determined from the decrease in the dissolved ⁵⁹Fe(III) concentrations with time after aging for 1 day at 10°C.⁸ In addition, the effects of iron on the development of zoospores (oogonium formation of female gametophytes) and growth of young sporophytes of *L. religiosa* were investigated in the fulvic-Fe(III) complex (1 p.p.m. C), EDTA-Fe(III) (2:1), solid am-Fe(III) and/or control (without Fe) media using non-radioactive Fe(III) at 10°C under 4000 lx fluorescent light (12:12 LD).¹⁰ The adult and mature sporophytes were collected from a coastal region in the northern Sea of Japan, Hokkaido, Japan in June and November 1996.

In this study, the orders of short-term Fe uptake rates and amounts of extracellularly adsorbed iron on the blade surface by adult sporophytes of *L. religiosa* were: fulvic-⁵⁹Fe(III) (1 p.p.m. C) > citric-⁵⁹Fe(III) (100:1) > citric-⁵⁹Fe(III) (1000:1) > EDTA-⁵⁹Fe(III) (2:1) > solid am-⁵⁹Fe(III) (Table 1). The lowest Fe uptake rate (0.129 pmol/cm² per h) in solid am-⁵⁹Fe(III) medium is probably due to the low solubility and slow dissolution

*Corresponding author: Tel: 81-138-40-8823. Fax: 81-138-40-8823. Email: kuma@fish.hokudai.ac.jp

Received 18 August 1999. Accepted 11 May 2000.

rate of solid hydrous ferric oxide in seawater.^{6,8} The Fe uptake rate (0.392 ± 0.011 pmol/cm² per h) in EDTA-⁵⁹Fe(III) (2:1) medium was about one-seventh lower than that in same medium in a previous study.¹¹ This is probably due to the use of Ti(III)-citrate EDTA solution with the stronger reductive dissolution of adsorbed iron on the blade surface than the ascorbic acid solution used in previous studies.^{4,12} The Fe uptake rate (2.75 ± 0.57 pmol/cm² per h) in fulvic-⁵⁹Fe(III) (1 p.p.m. C) medium was about seven times faster than that in EDTA-⁵⁹Fe(III) (2:1) and 20 times faster than that in solid am-⁵⁹Fe(III) media. In addition, the largest amount of iron adsorbed on the blade surface was observed in fulvic-⁵⁹Fe(III) medium (Table 1). The order of ⁵⁹Fe(III) dissociative precipitation rates of premixed organic-⁵⁹Fe(III) complexes was: citric-⁵⁹Fe(III) (100:1) >> citric-⁵⁹Fe(III) (1000:1) > EDTA-⁵⁹Fe(III) (2:1) > fulvic-⁵⁹Fe(III) (1 p.p.m. C) (Table 1; Fig. 1). The Fe uptake rate by *L. religiosa* in fulvic-⁵⁹Fe(III) (1 p.p.m. C) medium

and the development of zoospores (Table 1) in fulvic-Fe(III) (1 p.p.m. C) medium were the highest of those in all media in this study although the ⁵⁹Fe(III) dissociative precipitation in the fulvic-⁵⁹Fe(III) (1 p.p.m. C) complex system was not observed during the ⁵⁹Fe(III) dissociative precipitation rate measurements for 1–36 days (Fig. 1). In addition, the growth of young sporophytes after the development of zoospores in fulvic-⁵⁹Fe(III) (1 p.p.m. C) medium was higher than that in am-Fe(III) medium (Fig. 2), consistent with the result for the growth rate of young sporophytes of *L. japonica*.¹⁰

We have recently found that the order of Fe uptake rates by a coastal marine diatom, *Chaetoceros sociale*, was nearly consistent with those of estimated initial ⁵⁹Fe(III) dissociative precipitation rates of premixed organic-⁵⁹Fe(III) complexes in seawater at 10°C and cell yields in the culture experiments.⁸ The order of Fe uptake rates was: fulvic-⁵⁹Fe(III) (0.1 p.p.m. C) $\approx$ fulvic-⁵⁹Fe(III) (0.2 p.p.m. C) $\geq$ citric-⁵⁹Fe(III) (100:1) > EDTA-⁵⁹Fe(III) (2:1) $\geq$ fulvic-⁵⁹Fe(III) (1 p.p.m. C) > EDTA-⁵⁹Fe(III) (100:1) $\approx$ solid am-⁵⁹Fe(III). The metal-exchange reaction of premixed organic-Fe(III) complexes by major alkaline-earth metals (such as Ca²⁺ and Mg²⁺) in seawater possibly results in slow dissociation of organic-Fe(III) complexes and subsequent Fe(III) hydrolytic precipitation.^{8,13} The dissociation of supersaturated organic-Fe(III) complexes in seawater enhanced the concentration of bioavailable inorganic Fe(III) species [predominantly the hydrolysis products such as Fe(OH)₂], which could be a factor determining the iron uptake rate.^{8,12,14} Therefore, the higher Fe(III) dissociative precipitation rate of premixed organic-Fe(III) complexes in media probably results in a higher concentration of inorganic Fe(III) species in media and thus the higher iron uptake rate by phytoplankton.

The high Fe uptake rate by *L. religiosa* in fulvic-⁵⁹Fe(III) (1 p.p.m. C) medium resulted in the high growth. However, the Fe uptake and growth of *C. sociale* in fulvic-Fe(III) (1 p.p.m. C) medium were markedly limited because of an extremely low supply of biologi-

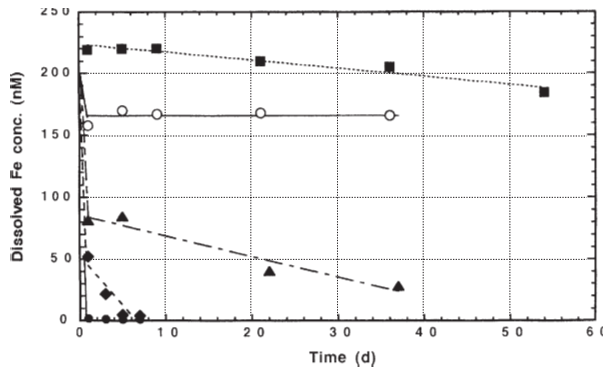


Fig. 1 Time-course of dissociative precipitation of premixed synthetic and natural organic-⁵⁹Fe(III) complexes [■, EDTA-⁵⁹Fe(III) (2:1), citric-⁵⁹Fe(III) (◆, 100:1 and ▲, 1000:1) and ○, fulvic-⁵⁹Fe(III) (1 p.p.m. C)] and hydrolytic precipitation of Fe³⁺ [●, am-⁵⁹Fe(III)] with 200 nM Fe in seawater at 10°C.

Table 1 Estimated ⁵⁹Fe(III) dissociative precipitation rates of premixed EDTA-⁵⁹Fe(III) (2 : 1), citric-⁵⁹Fe(III) (1000 : 1 and 100 : 1) and fulvic-⁵⁹Fe(III) (1 p.p.m. C) in seawater (10°C) after aging for 1 day at 10°C (see Fig. 1), iron uptake rates and extracellularly adsorbed iron by adult sporophytes of *Laminaria religiosa* Miyabe in media with organic-⁵⁹Fe(III) complexes and solid am-⁵⁹Fe(III) and the percentages of oogonium formation bearing female gametophytes in non-radioactive Fe(III) media

Organic-Fe(III) complexes (ratio or p.p.m. C)	Estimated Fe(III) dissociative precipitation rate (nM/d $\pm$ 1 SE, n = 3–6)	Iron uptake rate (pmol/cm ² per h)	Adsorbed iron (pmol/cm ²)	Oogonium formation bearing female gametophyte for 30 days (%)
Control (without Fe)	—	—	—	5
Solid am- ⁵⁹ Fe(III)	—	0.129	16.9	25
EDTA- ⁵⁹ Fe(III) (2 : 1)	0.648 ± 0.086 ($r^2 = 0.93$)	0.392 ± 0.011	24.9 ± 2.3	54
Citric- ⁵⁹ Fe(III) (1000 : 1)	1.64 ± 0.25 ($r^2 = 0.96$)	1.03 ± 0.17	41.9 ± 6.2	no data
Citric- ⁵⁹ Fe(III) (100 : 1)	11.9 ± 1.9 ($r^2 = 0.97$)	1.53 ± 0.30	186 ± 21	no data
Fulvic- ⁵⁹ Fe(III) (1 p.p.m. C)	0	2.75 ± 0.53	430 ± 45	70

Mean $\pm$ SE of duplicate or triplicate measurements for iron uptake rate and adsorbed iron.

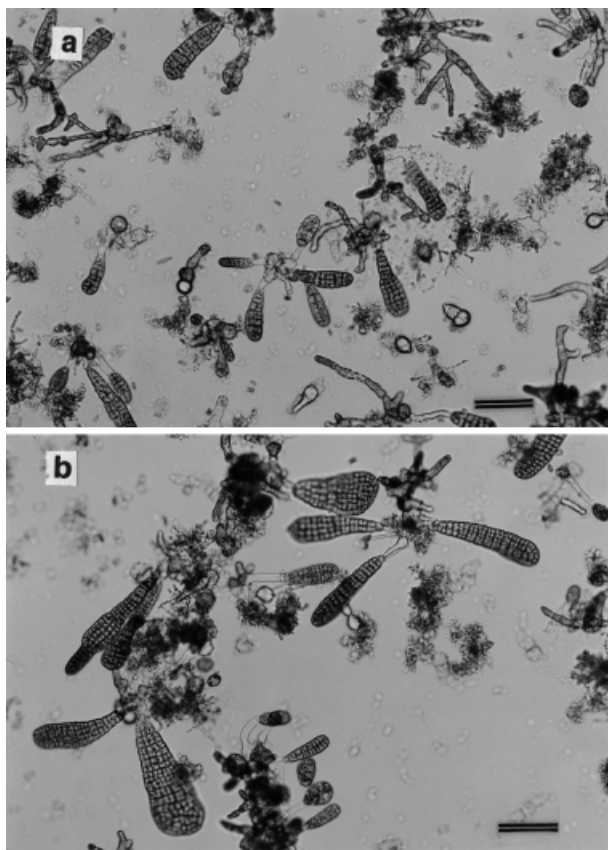


Fig. 2 Growth of young sporophytes after the development of zoospores of *Laminaria religiosa* cultured in (a) solid am-Fe(III) medium and (b) dissolved fulvic-Fe(III) (1 p.p.m. C) medium using non-radioactive Fe(III) for 50 days at 10°C. Bar = 100 μ m.

cally available inorganic Fe(III) species through the dissociation of fulvic- $^{59}\text{Fe(III)}$ (1 p.p.m. C) complex in seawater.⁸ These results may suggest that *L. religiosa* has a biochemical function to take up Fe bound to fulvate, which cannot be utilized by *C. sociale*, in addition to bioavailable inorganic Fe(III) species.

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