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Short Paper

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

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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 ^{59}Fe uptake rates by adult sporophytes of a brown alga *Laminaria religiosa* were measured and compared in the presence of different premixed organic-^{59}Fe(III) complexes [EDTA-^{59}Fe(III) (2:1), citric-^{59}Fe(III) (100:1 and 1000:1) and fulvic-^{59}Fe(III) (1 p.p.m. C)] and solid amorphous hydrous ferric oxide [am-^{59}Fe(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-^{59}Fe(III) complex solution for organic-^{59}Fe(III) medium and dissolved ^{59}Fe(III) solution for am-^{59}Fe(III) medium to filtered seawater at 10°C. The addition of dissolved ^{59}Fe(III) without organic ligands into seawater results in rapid hydrolytic precipitation of am-^{59}Fe(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-^{59}Fe(III) precipitates and extracellularly adsorbed iron on the blade surface by means of reductive dissolution of ^{59}Fe(III) without cellular damage.[4–8] After allowing to stand for 10 min, each blade was digested with concentrated HNO_3: concentrated $HClO_4$ (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 ^{59}Fe(III) dissociative precipitation rates of premixed organic-^{59}Fe(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 ^{59}Fe(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-^{59}Fe(III) (1 p.p.m. C) > citric-^{59}Fe(III) (100:1) > citric-^{59}Fe(III) (1000:1) > EDTA-^{59}Fe(III) (2:1) > solid am-^{59}Fe(III) (Table 1). The lowest Fe uptake rate (0.129 pmol/cm^2 per h) in solid am-^{59}Fe(III) medium is probably due to the low solubility and slow dissolution

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rate of solid hydrous ferric oxide in seawater.[6,8] The Fe uptake rate (0.392 ± 0.011 pmol/cm^2 per h) in EDTA-^{59}Fe(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/cm^2 per h) in fulvic-^{59}Fe(III) (1 p.p.m. C) medium was about seven times faster than that in EDTA-^{59}Fe(III) (2:1) and 20 times faster than that in solid am-^{59}Fe(III) media. In addition, the largest amount of iron adsorbed on the blade surface was observed in fulvic-^{59}Fe(III) medium (Table 1). The order of ^{59}Fe(III) dissociative precipitation rates of premixed organic-^{59}Fe(III) complexes was: citric-^{59}Fe(III) (100:1) >> citric-^{59}Fe(III) (1000:1) > EDTA-^{59}Fe(III) (2:1) > fulvic-^{59}Fe(III) (1 p.p.m. C) (Table 1; Fig. 1). The Fe uptake rate by *L. religiosa* in fulvic-^{59}Fe(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 ^{59}Fe(III) dissociative precipitation in the fulvic-^{59}Fe(III) (1 p.p.m. C) complex system was not observed during the ^{59}Fe(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-Fe(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]

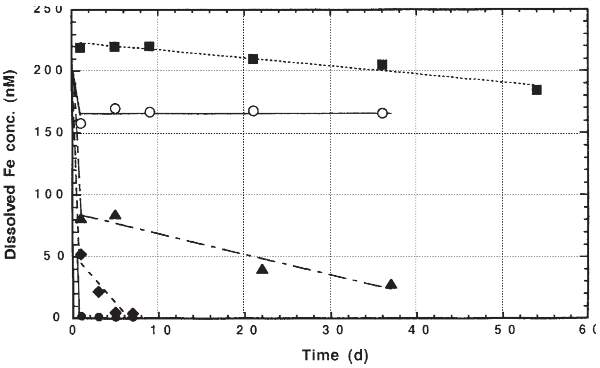

Fig. 1 Time-course of dissociative precipitation of premixed synthetic and natural organic-^{59}Fe(III) complexes [■, EDTA-^{59}Fe(III) (2:1), citric-^{59}Fe(III) (◆, 100:1 and ▲, 1000:1) and ○, fulvic-^{59}Fe(III) (1 p.p.m. C)] and hydrolytic precipitation of Fe^{3+} [●, am-^{59}Fe(III)] with 200 nM Fe in seawater at 10°C.

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 ^{59}Fe(III) dissociative precipitation rates of premixed organic-^{59}Fe(III) complexes in seawater at 10°C and cell yields in the culture experiments.[8] The order of Fe uptake rates was: fulvic-^{59}Fe(III) (0.1 p.p.m. C) ≈ fulvic-^{59}Fe(III) (0.2 p.p.m. C) ≥ citric-^{59}Fe(III) (100:1) > EDTA-^{59}Fe(III) (2:1) ≥ fulvic-^{59}Fe(III) (1 p.p.m. C) > EDTA-^{59}Fe(III) (100:1) ≈ solid am-^{59}Fe(III). The metal-exchange reaction of premixed organic-Fe(III) complexes by major alkaline-earth metals (such as Ca^{2+} and Mg^{2+}) 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-^{59}Fe(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-

Table 1 Estimated ^{59}Fe(III) dissociative precipitation rates of premixed EDTA-^{59}Fe(III) (2 : 1), citric-^{59}Fe(III) (1000 : 1 and 100 : 1) and fulvic-^{59}Fe(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-^{59}Fe(III) complexes and solid am-^{59}Fe(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 ± 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 ± SE of duplicate or triplicate measurements for iron uptake rate and adsorbed iron.

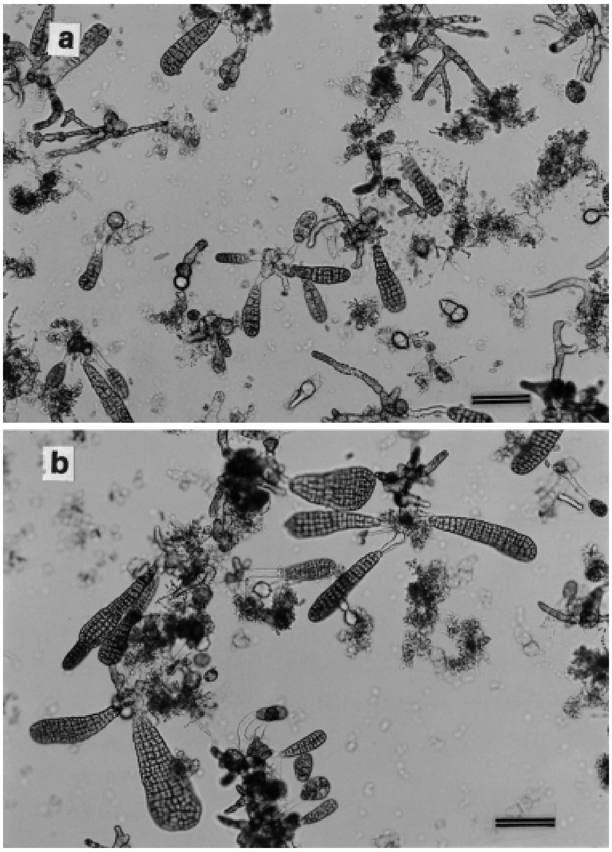

Fig. 2 Growth of young sporophytes after the development of zoospores of *Laminaria religiosa* cultured in (a) solid am-Fe(III) medium and (b) dissolved fulvic-Fe(III) (1 p.p.m. C) medium using non-radioactive Fe(III) for 50 days at 10°C. Bar = 100 μm.

cally available inorganic Fe(III) species through the dissociation of fulvic-^{59}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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