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Uptake Rate of Iron by Macroalgae from the Sea of Japan (*Laminaria religiosa* Miyabe and *Undaria pinnatifida*)

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Abstract

The uptake rates of Fe by adult macroalgae were investigated by ^{59}Fe radiotracer technique. The adsorbed Fe on the blades was removed by washing with ascorbic acid solution. The uptake rates of both species depend on the initial iron concentration in seawater. The maximum rates were observed at an initial iron concentration of 200 nM. The observed maximum rates and Ks values by *L. religiosa* and *U. pinnatifida* were 2.7 and 6.4 p moles Fe cm^{-2} hr^{-1} and 54 and 98 nM, respectively. On the other hand, the uptake rate by juvenile *L. religiosa* was one order of magnitude higher than that by the adult one. The uptake rates were compared with the dissolution rate of particulate iron. An new method for obtaining their good quality for food was recommended.

1. Introduction

It is well known that iron is a very important element for photosynthetic macroalgae and phytoplankton. Dissolved iron, $Fe(OH)_2^+$, or Fe^{2+} (KUMA *et al.*, 1992) which can be taken up by them, exists in seawater, but the dissolution rate of particulate iron is very slow (KUMA *et al.*, 1992). It is important to know the uptake rates of macroalgae and to compare them with the dissolution rate because macroalgae with high iron content are thick and considered as good quality for food. It is assumed that iron would enhance concentrations of photosynthetic pigments in macroalgae.

Rocks and rock beds in coastal areas in Japan, especially in Japan Sea, are covered with calcium carbonate (Coralline alga), which means Isoyake in Japanese (seaweeds withering phenomenon on beach). Isoyake is observed to extend every year resulting to a decrease in seaweed beds. The reason for its extension is not clearly understood. Thus, in these areas it is very important to improve the environment for the growth of macroalgae. Seaweed beds are important as nursery grounds for the growth of juvenile fish and as food for abalones and sea-urchins.

Although some investigations on the mechanism of iron uptake or the iron uptake rate by phytoplankton have been reported (MURPHY *et al.*, 1976 ; HUNTSMAN and SUNDA, 1980 ; ANDERSON and MOREL, 1982 ; SCHENCK *et, al.*, 1988), the uptake rate by macroalga has been reported only for *Macrocystis pyrifera* (MANLEY, 1981). It is very difficult, however, to measure the real uptake rate into the plant because of iron adsorption. This occurs even if Fe is added as EDTA-Fe. Adsorbed Fe on macroalga and phytoplankton can be removed by washing with ethylenediamine-di(o-hydroxyphenylacetic acid), EDDHA (MANLEY, 1981), and ascorbic acid (ANDERSON and MOREL, 1982) respectively.

Initially, we investigated how to remove the adsorbed Fe on macroalgae and then the iron uptake rates by two species of macroalga. By comparing the iron uptake rate with the dissolution rate of particulate iron, a profitable method to grow the macroalgae is recommended.

2. Experimental

Initially, desorption experiment of adsorbed EDTA-Fe on ground glass plates was carried

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out using chelating and reducing reagents. Four plates were soaked separately in 1 l of seawater containing 0.18 μ mole Fe (include ^{59}Fe) and 0.36 μ mole EDTA. After 5 hr, each plate was transferred into separate beaker containing 200 ml of 0.1 mM EDTA (pH 8.2), EDDHA (pH 8.2), 0.1 M ascorbic acid (pH 2.5) or hydroxylamine (pH 2.5) in seawater solutions. Four ml of each solution was taken at a suitable time and γ-radiation in each aliquot was measured. After 3 hr, each plate was soaked in 100 ml of 6 M HCl, with which adsorbed Fe is completely removed. Elimination and uptake experiments utilized ^{59}Fe as $FeCl_3$ in 0.5 M HCl, 20 mCi mg^{-1} Fe^{-1} and the radioactivity used in each experiment was 1 μ Ci.

Adult macroalgae were collected just before the experiment from Japan Sea region. Each blade measuring 10cm from the stem was hanged in 1 l of filtered seawater (0.45 μ m) of 34 ‰ salinity and pH 8.2. Nitrate, phosphate and Fe (III) as EDTA-Fe containing ^{59}Fe were added to each beaker to final concentrations of 2, 0.2 mM and 18-360 nM, respectively. The concentration of EDTA was twice mole of Fe.

The uptake experiment was done by white fluoresent lamps under 60 μ E m^{-2} s^{-1} at 10℃. After 3, 6 and 10 hr, the blades were rinsed in 100 ml of 0.1 M ascorbic acid for 30 min to remove adsorbed EDTA-Fe and then they were digested with 35 ml of conc. HNO_3-$HClO_4$ (1 : 1) on a hot plate. The acid solution was diluted to 50 ml and γ-radiation in 4 ml aliquots was measured by a scintillation spectrometer.

3. Results and discussion

As shown in Fig. 1 the chelating reagents could not remove adsorbed Fe from the plates, but the reducing reagents (pH 2.5) could remove all of the adsorbed iron. Although EDDHA was used for removing iron on the macroalga by MANLEY (1981), based on our results, ascorbic acid is a better washing solution. However, it is important to know whether assimilated Fe in the cells of *L. religiosa* can be redissolved in the acid or not.

After the uptake and adsorption of EDTA-Fe by living blades of *L. religiosa* were done, the desorption experiment of adsorbed Fe was carried out. The blades at a suitable time were transferred into 100 ml of 0.1 M ascorbic acid solution and γ-radiation in the solution was measured. ^{59}Fe concentration in the solution gradually increased in the first 20 min and remained constant even after 6 hr. From this result, it is assumed that the cells were not destroyed by the acid because the iron in the cells was not redissolved. The chelating reagents could not eliminate the adsorbed EDTA-Fe, but 100 ml of 0.1 M ascorbic acid could eliminate all of the adsorbed iron even in living *L. religiosa* without destroying the cells.

About 10-20% of the EDTA-Fe in seawater was adsorbed on the walls of beakers within 10 hr, after which no adsorption was observed. The variation of 10-20% may depend on the quality of each beaker used, but the correction of initial concentration of iron added in the beakers was not done. Fig. 2 shows the uptake of iron as a function of time by the blades of *L. religiosa* when the initial iron concentrations are 54 and 180 nM. Fig. 3 shows the uptake rate as a function of initial iron concentration. The maximum uptake rate was 2.7 p moles cm^{-2} hr^{-1} and the weight of the blades used here was about 0.06 g cm^{-2}. The uptake rate in the dark was about 70 % to that in the light.

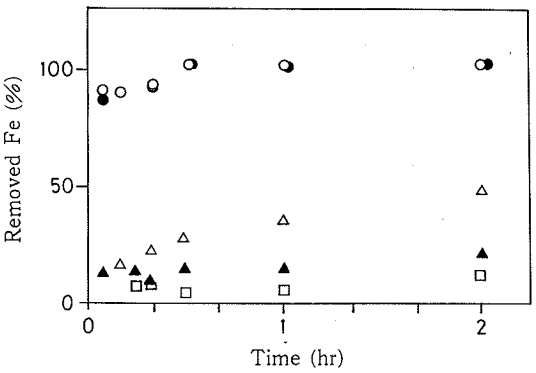

Fig. 1. Removal of adsorbed iron on grorund glass plates with chelating and reducing reagents.
○ : ascorbic acid (pH 2.5) ;
● : hydroxylamine (pH 2.5) ;
△ : ascorbic acid (pH 7.0) ;
▲ : EDDHA (pH 8.2) ;
□ : EDTA (pH 8.2)

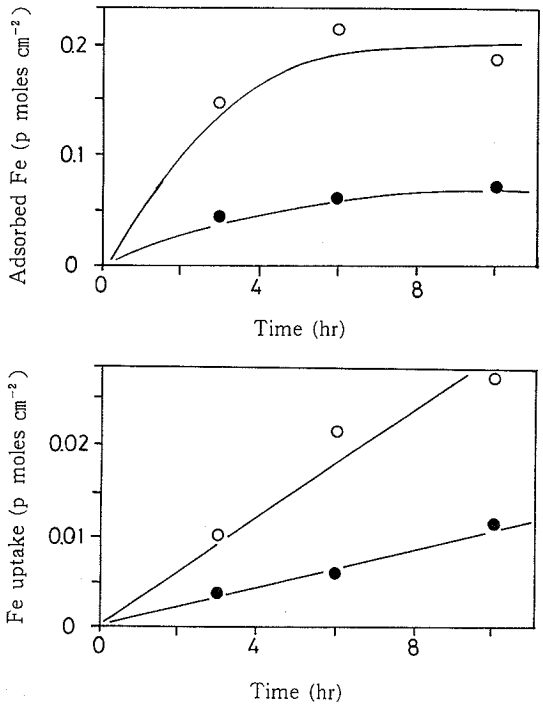

Fig. 2. Adsorption and uptake of iron by adult *Laminaria religiosa*.
○ : 180 nM Fe ; ● : 54 nM Fe

By using juvenile *L. religiosa*, the Fe uptake rate increased slowly even at an Fe concentration of 900 nM as shown in Fig. 4. Considering that the rate is about 10 times higher than that by the adult one, it is assumed that juvenile macroalga is more active than the adult one.

Fig. 5 shows the uptake rate by *U. pinnatifida*. The rate was about twice higher than that by *L. religiosa*. These rates, however, are two or three orders of magnitude lower than that by *Macrocystis pyrifera* (MANLEY, 1981). At this point, we can not explain the reason for the discrepancy.

From the experiment of ammonium and nitrate uptake by marine macroalgae, HAINES and WHEELER (1978) reported that Vmax (maximal uptake rate) is an indicator of uptake ability at high nutrient concentrations, whereas Ks (half-saturation constant) is an indicator of uptake ability at low nutrient concentrations compared to that at high concentrations. The Ks values by *L. religiosa* and *U. pinnatifida* were 54 and 98 nM Fe, respectively. These values were also two orders of magnitude higher than that by *M. pyrifera* (MANLEY, 1981). At this point, we can not explain the reason for the discrepancy.

The growth rate of phytoplankton in the presence of Fe and some chelating agents,

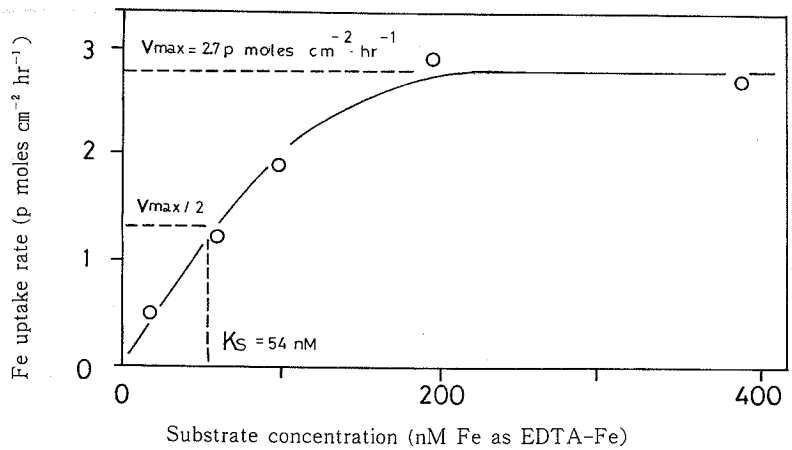

Fig. 3. Uptake rate of iron by adult *Laminaria religiosa* vs. substrate concentration of iron.

which have different stability constans, was done by ANDERSON and MOREL. From these results, it was assumed that Fe assimilated by phytoplankton is not chelated Fe but Fe ion. Fe assimilated by macroalgae would be Fe ion as well as phytoplankton.

If 100 g of *L. religiosa* or *U. pinnatifida* is alive in 1 *l* of seawater, they take up 5.4-10.7 n moles Fe hr^{-1}. Considering an average daylight period of 10 hr, these macroalgae can remove more than 50-100 n moles Fe from the seawater in a day because they take up iron even in the dark.

Based on the dissolution rate, $Fe(OH)_2^+$, which will be taken up by macroalgae or phytoplankton, is produced at a rate of 0.08 n moles l^{-1} day^{-1} when the particulate iron concentration is 20 μ mole (Japan Sea). Considering the maximum uptake rate and the dissolution rate, $Fe(OH)_2^+$ dissoluted from particulate iron is below 1 % of their maximum uptake under calm conditions. To obtain good quality of *L. religiosa* and *U. pinnatifida* for eating, desirable chemical conditions, such as an ample supply of dissolved iron, are necessary.

To increase the amount of dissolved iron during the propagation of macroalgae, a propagation cage made of iron is recommended. The iron is slowly oxidiged to Fe(Ⅱ) by dissolved oxygen. Fe(Ⅱ) is a soluble form until its concentration of about 10 mM and with a half life (1/2 of initial concentration) of about 10 min at 15℃, we observed that the half life of Fe(Ⅱ) was about 30 min. It seems therefore that Fe(Ⅱ) in low water temperature is not so unstable and macroalgae can take up this Fe(Ⅱ).

Experimental results of Fe(Ⅱ) diffusion in seawater from the cage will be reported elsewhere.

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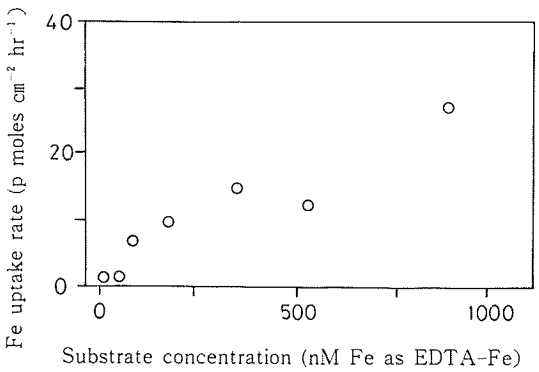
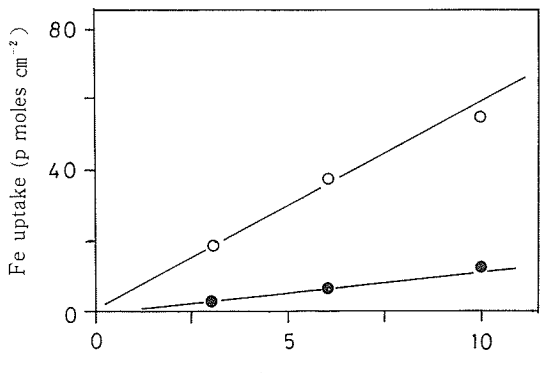


Fig. 4. Uptake rate of iron by juvenile *Laminaria religiosa* vs. substrate concentration of iron.



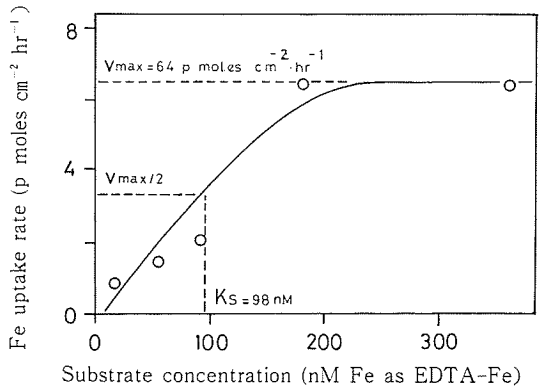


Fig. 5. Uptake and uptake rate of iron by adult *Undaria pinnatifida*.
○ : 180 μM Fe ; ● : 54 nM Fe

References

ANDERSON, M. A. and F. M. M. MOREL (1982) The influence of aqueous iron chemistry on the uptake of iron by coastal diatom *Thalassiosira weisstfogii*. Limnol. Oceanogr., **27**, 789-813.

HAINES, K. C. and P. A. WHEELER (1978) Ammonium and nitrate uptake by the marine macrophytes *Hypnea musciformis* (Phodophyta) and *Macrocystis pyrifera* (Phaeophyta). J. Phycol., **14**, 319-324.

HUNTSMAN, S. A. and W. G. SUNDA (1980) The role of trace metals in regulating phytoplankton growth with enphasis on Fe, Mn and Cu, In Morris, I. Ed. Physiological Ecology of Phytoplankton. Studies in Ecology. Vol. 7. Blackwell Sci. Pub. Oxford, 285-328.

KUMA, K., S. NAKABAYASHI, Y. SUZUKI, I. KUDO and K. MATSUNAGA (1992) Photoreduction of Fe(Ⅲ) by dissolved organic substances and existence of Fe(Ⅱ) in seawater during spring blooms. Mar. Chem., in press.

KUMA, K., S. NAKABAYASHI, Y. SUZUKI and K. MATSUNAGA (1992) Dissolution rate and solubility of colloidal hydrous ferric oxide in seawater. Mar. Chem., in press.

MANLEY, S. L. (1981) Iron uptake and translocation by *Macrocystis pyrifera*. Plant Physiol., **68**, 914-918.

MILLERO, F. J., S. SOTOLONGO and M. ZAGUIRRE (1987) The oxidant kinetics of Fe(Ⅱ) in seawater. Geochim. Cosmochim. Acta., **51**, 793-801.

MURPHY, T. P., D. R. S. LEAN and C. NALEWAJKO (1976) Blue green alge; their excretion of iron-selestive chelators enables them to dominate other algae. Science, **192**, 900-902.

SCHENCK, R. C., A. TESSIER and P. G. C. CAMPBELL (1988) The effect of pH on iron and manganese uptake by a green alga. Limnol. Oceanogr, **33**, 538-550.

日本海産ホソメコンブ，ワカメによる鉄の摂取速度

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^{59}Fe-EDTAを用いて，大型藻類による鉄の取り込み速度を求めた。真の取り込み速度を求めるために，大型藻類の表面に吸着した鉄をアスコルビン酸で除去した。ホソメコンブ（*Laminaria religiosa* Miyabe），ワカメ（*Undaria pinnatifida*）による鉄の取り込み速度ならびにKsの値はそれぞれ2.7，6.4 p moles Fe/cm^2・hrならびに54，98 nMであった。日本海での粒状鉄（20 nM）の溶解速度と実験で得られた摂取速度を比較したところ，これら大型藻類は粒状鉄から溶解する溶存鉄の100倍以上の鉄を摂取出来，人為的な鉄イオンの供給が質的に優れた大型藻類を生産出来ることを示唆した。

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