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Availability of colloidal ferric oxides to coastal marine phytoplankton

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Abstract Cell growth of a coastal marine diatom, *Phaeodactylum tricornutum* (stock cultures), and two red tide marine flagellates, *Heterosigma akashiwo* and *Gymnodinium mikimotoi* (stock cultures), in the presence of soluble chelated Fe(III)-EDTA (1:2) and of four different phases of ferric oxide colloids were experimentally measured in culture experiments at 20°C under 3000 lux fluorescent light. Soluble Fe(III)-EDTA induced the maximal growth rates and cell yields. The short-term uptake rate of iron by *H. akashiwo* in Fe(III)-EDTA medium was about eight times faster than that in solid amorphous hydrous ferric oxide (Fe₂O₃·xH₂O) medium. In culture experiments supplied with four different ferric oxide forms, the orders of cell yields are amorphous hydrous ferric oxide >γ-FeOOH (lepidocrocite) >γ-FeOOH (hydrated ferric oxyhydroxide polymer) >α-FeOOH (goethite). The specific growth rates (μ) at logarithmic growth phase in Fe(III)-EDTA, amorphous hydrous ferric oxide and γ-FeOOH media were significantly greater than those in Fe₇O₇(OH)·4H₂O and α-FeOOH media. The thermodynamically stable forms such as Fe₇O₇(OH)·4H₂O and α-FeOOH supported a little or no phytoplankton growth. The iron solubilities and/or proton-promoted iron dissolution rates of these colloidal ferric oxides in seawater at 20°C were determined by simple filtration techniques involving γ-activity measurements of ⁵⁹Fe. The orders of solubilities and estimated dissolution rate constants of these ferric oxides in seawater were consistent with that of cell yields in the culture experiments. These results suggest that the availability of colloidal iron to provide a source of iron for phytoplankton is related to the thermodynamic stability and kinetic lability of the colloidal ferric oxide phases, which probably control the uptake rate of iron by phytoplankton.

Introduction

Iron is an essential micronutrient for phytoplankton growth (Finden et al. 1984; Martin and Fitzwater 1988; Martin et al. 1989; Price et al. 1991; DiTullio et al. 1993) and an important component of such processes as synthesis of DNA, RNA and chlorophyll, electron transport, oxygen metabolism and nitrogen utilization (Weinberg 1989). Iron has often been implicated in the initiation of red tides (Doucette and Harrison 1990, 1991 a, b). Recently, a number of studies have documented the effect of iron on the uptake of NO₃⁻ by *Scenedesmus quadricauda* (Chlorophyceae) (Reuter and Ades 1987), red tide dinoflagellate *Gymnodinium sanguineum* Hirasaka (Doucette and Harrison 1991 b) and phytoplankton in iron-deficient natural seawater (Price et al. 1991), indicating the increase in NO₃⁻ uptake rate by addition of iron. The stable oxidation state of iron in oxic seawater is Fe(III), which has an extremely low solubility, and the dissolved inorganic species of Fe(III) in seawater are predominantly the hydrolysis products Fe(OH)²⁺, Fe(OH)³⁺ and/or Fe(OH)⁴⁻ (Byrne and Kester 1976; Stumm and Morgan 1981; Hudson et al. 1992; Kuma et al. 1992, 1993). Therefore, phytoplankton growth might be controlled by the solubility and the dissolution rate of particulate Fe(III) since it has generally been assumed that phytoplankton assimilate soluble iron species (Anderson and Morel 1982). In oxic seawater, iron colloids may exist in a number of noncrystalline and crystalline ferric oxide forms and may have widely varying origins, chemical compositions and sizes. The principal phases commonly found in soils and natural waters are amorphous hydrous ferric oxide (ferrihydrite), α-Fe₂O₃ (hematite), γ-Fe₂O₃ (maghemite), α-FeOOH and γ-FeOOH (Murray 1979; Schneider 1988; Lindsay 1991). Since the internal structure of iron colloid affects its solubility and dissolution rate, it should also influence the uptake rate of iron by phytoplankton at which the colloid is able to provide biologically available dissolved inorganic species of Fe(III). The order of solubilites of crystalline ferric oxides is γ-Fe₂O₃ >γ-FeOOH >α-Fe₂O₃ >α-FeOOH. The metastable amorphous
phase is the least stable and is approximately three orders of magnitude more soluble than \( \alpha \)-FeOOH (Lindsay 1991). The amorphous phase induced the maximal growth rates and cell yields because of the faster thermal dissolution rate, while the crystalline ferric oxides, such as \( \alpha \)-FeOOH, \( \beta \)-FeOOH (akaganite) and \( \alpha \)-Fe\textsubscript{2}O\textsubscript{3}, did not support phytoplankton growth at all (Wells et al. 1983; Rich and Morel 1990). Therefore, the thermodynamic stability of iron colloids may be important in controlling the supply of biologically available inorganic species of iron. In addition, the biologically available species of iron with phytoplankton appear to include some dissolved organic complexes (Lewin and Chen 1971) as well as dissolved ionic species (Anderson and Morel 1982). In flagellate cultures, the addition of soluble chelated Fe(III)-EDTA to culture media induced the phytoplankton to grow (Lewin and Chen 1971; Iwasaki and Iwasa 1982; Yamochi 1983). These biologically available species exist in equilibrium with the more dominant colloidal ferric oxides in seawater. The ferric oxide forms may be important in the coastal waters because of the much higher input of iron colloids from atmosphere and rivers.

In the present study, we tested the hypothesis that differences in the solubilities and the dissolution rates of ferric oxide colloids will affect their ability to supply iron for the growth of marine diatom Phaeodactylum tricornutum and red tide flagellates Heterosigma akashiwo and Gymnodinium mikimotoi. The ferric oxide forms used in our study are amorphous hydrous ferric oxide (ferrihydrite), \( \gamma \)-FeOOH, FesO\textsubscript{4}(OH)-4H\textsubscript{2}O and \( \alpha \)-FeOOH. The cell numbers as a function of time in cultures supplied with four different ferric oxide forms were observed during the culture experiments. Furthermore, the solubilities and/or proton-promoted dissolution rates of these ferric oxide forms in seawater at 20°C were determined by simple filtration techniques involving \( \gamma \)-activity measurement of \(^{55}\)Fe. The solubilities and dissolution rates were compared to those of amorphous phase and \( \gamma \)-FeOOH in seawater within the pH range 5.5 to 8 at 20°C determined by the dialysis technique (Kuma et al. 1992, 1993).

**Materials and methods**

In the present study, seawater collected from Funka Bay in Japan (salinity=33.6%) was used after being filtered through an acid-cleaned 0.45-\( \mu \)m Millipore membrane filter. The iron concentration in the filtered seawater was determined by a graphite furnace-atomic absorption spectrophotometer after preconcentration by APDC/DDDC-chloroform organic extraction (Landing and Bruland 1987). The iron concentration was 1.5 \( \mu \)M.

Culture experiment of phytoplankton in the presence of different ferric oxide forms

The filtered seawater was autoclaved for 20 min at 121°C (1.1 kg cm\textsuperscript{-2} pressure), and the culture media were prepared by adding \( f/2 \) nutrients (Guillard and Ryther 1962) without trace metals and EDTA to the autoclaved filtered seawater. The \( f/2 \) media contain 880 \( \mu \)M nitrate, 38 \( \mu \)M phosphate, 35 \( \mu \)M silicate and small amounts of vitamins. The marine diatom Phaeodactylum tricornutum (stock culture) was grown in 1 liter of the \( f/2 \) media (without any added iron) at 20°C under 3000 lux fluorescent light (12 h light:12 h dark). The red tide flagellates Heterosigma akashiwo and Gymnodinium mikimotoi (stock cultures) were grown in the \( f/2 \) media containing 0.2 \( \mu \)M iron, in which ferric iron stock solution (FeCl\textsubscript{3} · 6H\textsubscript{2}O in 0.005 M HCl) was added, in order to obtain cell concentration expected at the start of following culture and iron uptake experiments.

Amorphous hydrous ferric oxide medium was directly prepared by aging for 1 d at 20°C after adding ferric iron acidic stock solution to 50 ml of autoclaved filtered seawater in each glass culture tube at 20°C. The amorphous phase tested here is different from freshly precipitated phase in Wells et al. (1983) because aging 1 d leads to substantial decrease in its reactivity. Similarly, \( \gamma \)-FeOOH medium was prepared by adding ferrous iron stock solution [FeSO\textsubscript{4}·(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}·6H\textsubscript{2}O in 0.005 M HCl] to autoclaved filtered seawater in each culture tube. Iron oxyhydroxide precipitates derived from ferric iron and ferrous iron, by using conditions very similar to those encountered in natural waters, were identified as amorphous ferric oxide forms in the initial phases (but containing 10 to 20% \( \alpha \)-FeOOH after aging for 12 d) and as poorly crystalline \( \gamma \)-FeOOH, respectively. Both were analyzed using X-ray diffraction, infrared and Mössbauer spectroscopies (Crospy et al. 1983). Fe\textsubscript{6}O\textsubscript{7}(OH)\textsubscript{4}·4H\textsubscript{2}O medium was prepared by autoclaving for 1 h at 120°C after adding ferric stock solution to autoclaved filtered seawater in each culture tube. \( \alpha \)-FeOOH medium was prepared by adding a small amount of prepreepared colloidal \( \alpha \)-FeOOH suspended water solution, as described below, to autoclaved filtered seawater. Total iron concentration in the \( \alpha \)-FeOOH suspended water solution was determined spectrophotometrically by the ferrozine method (Stookey 1970) after strong acid digestion (2N HCl). In addition, soluble chelated Fe(III)-EDTA (1:2) and control (without any added iron) media were prepared to compare the growth rates and cell yields with those containing iron colloids.

To confirm the ferric oxide forms produced in the above procedures, they were produced by adding ferric or ferrous iron solutions to seawater. Amorphous hydrous ferric oxide and \( \gamma \)-FeOOH were produced by adding ferric and ferrous iron solutions, respectively, to the filtered seawater at 20°C, adjusting to pH 8.0 with NaOH, additional bubbling air (aerial oxidation of ferrous iron at pH 8) for \( \gamma \)-FeOOH and then aging for 1 wk at 20°C. Fe\textsubscript{6}O\textsubscript{7}(OH)\textsubscript{4}·4H\textsubscript{2}O was produced by autoclaving (for 1 h at 120°C) amorphous hydrous ferric oxide produced in seawater. \( \alpha \)-FeOOH was obtained by aerial oxidation of ferrous iron [FeSO\textsubscript{4}·(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}·6H\textsubscript{2}O] in strong alkaline solution (1N NaOH) at 45°C (Hamada and Kuma 1977). Washed and air-dried products were analyzed by X-ray diffraction with filtered Cu and/or Co K\alpha radiation (40 kv, 20 mA) and confirmed the amorphous solid phase, which was identified as colloidal hydrous ferric oxide (ferrihydrite), poorly crystalline \( \gamma \)-FeOOH, poorly crystalline Fe\textsubscript{6}O\textsubscript{7}(OH)·4H\textsubscript{2}O and crystalline \( \alpha \)-FeOOH. The \( \alpha \)-FeOOH suspended water solution used in the culture experiments was prepared by dilution after washing several times with distilled water.

At the start of culture experiments, an \( f/2 \) stock solution and then a small amount of each culture (ca. 500 \( \mu \)l) in the stationary growth phase were added to 50 ml of the new growth media in glass culture tubes. The iron concentrations in the growth media were 0.02 \( \mu \)M for the diatom and 0.2 \( \mu \)M for the flagellates. Diatom and flagellates cell concentrations at the start of culture experiments were approximately 5000 and 300 cells ml\textsuperscript{-1}, respectively. The light and temperature conditions and nutrient concentrations were the same as those of the stock cultures, as described above. During experiments the cell concentrations were optically counted by using an optical microscope on a daily or weekly basis.

Each specific growth rate (\( \mu \), d\textsuperscript{-1}) at the logarithmic growth phase was determined from the slope of the linear portion of the logarithmic cell concentration vs time by using a least-squares linear regression of the data, as follows

\[
\ln N_t = \mu t + \ln N_0
\]

where \( N_t \) and \( N_0 \) are the cell concentrations at \( t \) and time 0, respectively.