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Dissolution of iron hydroxides by marine bacterial siderophore

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Abstract

A series of laboratory experiments was conducted to investigate the dissolution behavior of goethite and poorly crystalline iron hydroxide (PCIH) in the presence of siderophore produced by a marine bacterium, *Alteromonas haloplanktis*. The amounts of siderophore in the experimental solutions are expressed by the iron complexing capacity (ICC). The experimental data indicate that both the dissolution rates and solubilities of ferric hydroxides increase with increasing contents of siderophore and $[H^+]$. For example, in a solution of pH = 8 and ICC = 12 μM, the iron content of solution increased from < 0.1 to 1.3 μM by reaction with PCIH for 80 h. This Fe content is more than 50 times the solubility value of amorphous $Fe(OH)_3$ in pure water at pH = 8. At pH = 4, with increasing ICC value from 0 to 12.9 μM, the dissolution rate of goethite increased from 1.5 to 9.5 (nM/h/m^2) and that of PCIH increased from 0.4 to 3.4 (nM/h/m^2). At ICC = 12.9 μM, with increasing pH from 4 to 6.8, the dissolution rate of goethite decreased from 9.5 to 1.9 nM/h/m^2, whereas that of PCIH decreased from 3.4 to 0.7 nM/h/m^2. Our study suggests that most of the iron utilized by phytoplankton in the oceans may be liberated from ferric hydroxides in aeolian particles by siderophores generated by marine bacteria.

Keywords: Dissolution; Siderophore; Iron hydroxide; Marine bacterium

1. Introduction

Phytoplankton growth in the oceans that influences key aspects of the global carbon cycle, such as the primary productivity, the atmospheric CO_2 content, and the burial of organic carbon, appears to be limited by the availability of iron. For example, in several large areas of the modern open oceans with high nitrate but low chlorophyll (HNLC) contents, deficiency of dissolved iron appears to limit phytoplankton growth (e.g. Martin and Fitzwater 1988; Martin and Gordon, 1988; Martin et al., 1989, 1990, 1991). In the equatorial Pacific Ocean, Coale et al. (1996b) showed that seeding of small amounts of dissolved iron in surface seawaters triggered a massive phytoplankton bloom that resulted in consuming large quantities of carbon dioxide and nitrate.

Iron is an essential element for all microorganisms. In oxygenated seawater, however, concentrations of dissolved iron are extremely low. According to the vertical profiles of dissolved iron at a central part of the North Pacific (Bruland et al., 1994), the highest con-

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tent of dissolved iron in the surface mixed layer was only 0.37 nmol/kg, and the average content of dissolved iron in the intermediate and deep waters (500–4000 m) was 0.38 nmol/kg. In the Northeast Pacific, the dissolved iron contents in surface seawaters were below 0.1 nmol/kg (Martin and Gordon, 1988).

Recently, it has been suggested that the iron in surface seawater may be mostly supplied by iron-bearing aeolian particles (Zhuang et al., 1990; Duce and Tindale, 1991). Upwelling of subsurface waters containing regenerated iron may also be an important process to supply iron to surface seawater (Coale et al., 1996a). An important question following these suggestions is the mechanism for liberating iron from iron hydroxides in aeolian particles for biological use. Iron hydroxides are stable in seawater because they have extremely low solubility at neutral pH and aerobic conditions (Stumm and Morgan, 1996). Whether or not iron colloids may be utilized by phytoplankton is an important unresolved question in oceanography. Rich and Morel (1990) suggest that crystalline β-FeOOH (akaganeite) is not utilized by coastal diatom *Thalassiosira weissflogii*. They further suggest that dissolution of hydrous ferric-oxide colloids may occur by thermal or photochemical reactions. However, it has been recently suggested that the dissolved ferric iron in seawater is primarily chelated with strong Fe(III)-binding organic ligands (Rue and Bruland, 1995; van den Berg, 1995). It has also been suggested that organic ligands, produced by biological activity, promote the dissolution of minerals (Banfield and Nealson, 1997).

Aerobic and facultative anaerobic microorganisms, which live in oxic aqueous environments, excrete chelators, known as siderophore, which form very stable iron complexes. These siderophores are characterized as follows (Neilands, 1981):

(a) the molecular weights are low (500–1000 Da)
(b) they are ferric-specific ligands
(c) the biosynthesis of siderophore is regulated by iron levels in solution
(d) the formation constants for the following type of reactions are around 10^{30} or higher

$$Fe^{3+} + L^{k-} \rightarrow FeL^{3-k}, \quad (1)$$

where L is a ligand.

Recently, several research groups have been conducting laboratory experiments to investigate the role of siderophore in promoting dissolution of iron-bearing minerals. For example, Hersman et al. (1995, 1996) explored the dissolution of hematite at pH = 3 by siderophore produced by a soil bacterium, *Pseudomonas* sp. Dissolution of goethite by commercial hydroxamate siderophores has been examined by Kraemer et al. (1999). The Brantley group (Liermann et al., 2000; Kalinowski et al., 2000a,b; Brantley et al., 2000) has conducted dissolution experiments of hornblende by commercial hydroxamate siderophores and by two types of bacteria, both of which produce catecholate siderophores.

In contrast to the siderophores produced by terrestrial microorganisms, knowledge about the siderophores produced by marine microorganisms is scarce. Only a few types of siderophores produced by marine microorganisms have been studied. They are Anguibactin produced by *Vibrio anguillarum* (Jalal et al., 1989), Biscaberin produced by *Alteromonas haloplanktis* (Takahashi et al., 1987), Alterobactin A and Alterobactin B produced by *A. luteoviolacea* (Reid and Butler, 1991; Reid et al., 1993), Aerobactin from *Vibrio* sp. (Haygood et al., 1993), and Vulnibactin from *V. vulnificus* (Okujo et al., 1994). Recently, it has been reported that Aquachelin and Marinobactin are produced from *Halomonas aquamarina* and *Marinobacter* sp., respectively (Martinez et al., 2000).

The main purpose of this study is to examine the extent to which siderophores produced by *marine* microorganisms promote the dissolution of iron hydroxides, which are mostly transported in the oceans as aeolian particles, and to increase the availability of utilizable iron for phytoplankton. To our knowledge, this is the first laboratory study on the dissolution kinetics of iron hydroxides using the siderophore (Biscaberin) produced by a marine bacterium. Biscaberin is known to have three hydroxamate functional groups (Takahashi et al., 1987).

2. Experimental methods

Siderophores produced by marine organisms are not commercially available. Therefore, we produced the large quantity of siderophore needed in our experiments by culturing *A. haloplanktis* marine bacterium.

We also synthesized the iron hydroxides (goethite and poorly crystalline iron hydroxide) used in the dissolution experiment.

2.1. Production of siderophore

A. haloplanktis (IAM 12918), characterized by Takahashi et al. (1987), was obtained from the Institute of Molecular and Cellular Biosciences, University of Tokyo. *A. haloplanktis* was aerobically cultured for 5 days at room temperature in 500 ml of medium containing 5.0 g/l bactopeptone, 1.0 g/l yeast extract, and 0.01 g/l Na_2HPO_4 in an artificial seawater (ASW). The ASW was prepared following the method of Samuelsson and Kirchman (1990), by mixing in distilled deionized water 17.6 g/l NaCl, 1.5 g/l Na_2SO_4, 0.08 g/l $NaHCO_3$, 0.2 g/l KCl, 0.04 g/l KBr, 2.9 g/l $MgCl_2{\cdot}6H_2O$, 0.4 g/l $CaCl_2{\cdot}2H_2O$, 0.008 g/l $SrCl_2{\cdot}6H_2O$, and 0.008 g/l H_3BO_3. After 5 days, the solution was filtered through 0.1-μm filter paper to remove bacteria. The amount of siderophore in the filtrate was measured by the liquid chrome azurol S (CAS) assay (Schwyn and Neilands, 1987). Siderophore in the culture solution reacts with the ferric iron in CAS/Fe^{3+}/HDTMA [hexadechyl-trimethylammonium, $CH_3(CH_2)_{15}N(CH_3)^{3+}$] to form a stable complex:

$$\mathrm{Fe-dye}^{3-k} + \mathrm{L}^{n-} \rightarrow \mathrm{FeL}^{3-n} + \mathrm{dye}^{k-}. \quad (2)$$

When a strong ligand, such as siderophore, is added to a bright blue CAS solution, the ligand forms a complex with ferric iron and the color of the solution changes to orange because free dye is released. A decrease in the absorbance at 630 nm is due to the removal of ferric iron from CAS/Fe^{3+}/HDTMA complex. The amount of ferric iron forming a stable complex with siderophore, i.e. FeL^{3-n} in reaction (2), is defined here as the iron complexing capacity (ICC). Because absolute value of siderophore concentration is difficult to determine, the ICC value is used as a measure of the relative amount of siderophore in the experimental solutions.

2.2. Synthesis of ferric hydroxides

The ferric hydroxides used in the experiments were synthetic goethite and poorly crystalline iron hydroxides (PCIH). Goethite was synthesized following the procedures by Atkinson et al. (1967). Solutions of 0.1 M $Fe(NO_3)_3{\cdot}9H_2O$ and 0.2 M KOH were mixed at pH = 12 and the precipitates were aged for 24 h at 60°C. PCIH was synthesized by mixing of 0.02 M $Fe(NO_3)_3{\cdot}9H_2O$ and 1 M NaOH at pH = 7, and the precipitates aged for 24 h at room temperature. Goethite and PCIH were confirmed by powder X-ray diffraction; no sharp peaks were observed from PCIH. The specific surface area of the goethite and PCIH, measured by the BET N_2 method, were 43.8 and 259.0 m^2/g, respectively.

2.3. Dissolution of ferric hydroxides by siderophores

The stock solution prepared above (Section 2.1) contained the culture media and siderophore with the ICC value of 12.9 μM. $HgCl_2$ was added to change the concentration level of Hg in the stock solution to 10 ppm to inhibit biological activity in the experimental solutions. A 20-ml stock solution was placed, together with 2 mg of goethite in a 50-ml volume polypropylene bottle. The bottles containing siderophore and iron hydroxides were kept at 20°C in a dark room to prevent any photochemical reactions. Aliquots of solution were extracted using a syringe at several time intervals during an experiment, which typically lasted 80 h. The solutions from the syringe were filtered through 0.45-μm cellulose acetate filters. The iron concentrations in the filtrates were measured by flameless atomic absorption spectroscopy (AAS) with a detection limit of 5×10^{-7} M (30 ppb). The background Fe concentration in the stock solution was below the detection limit.

The pH value of the stock solution was 8. In a preliminary series of experiments at pH = 8, we found that the dissolution rates of goethite and PCIH were too slow to yield measurable amounts of iron. The iron content of the solution after several days of reaction with goethite was less than the detection limit of AAS (i.e. 30 ppb). Subsequently, we carried out the dissolution experiments at acidic pH conditions as well as at neutral pH. The initial pH values were adjusted by HCl to 4.0, 5.3, or 6.8. Dissolution experiments were also carried out to investigate the dependence of siderophore concentration on the reaction kinetics. These experiments were conducted at pH = 4 because dissolution rates were greater at lower

Table 1
Experimental conditions and results on the dissolution of iron hydroxides (iron hydroxide 0.1 g/l, 20°C)

Material	Initial pH	ICC (μM)	Dissolution rate (nM/h/m^2)
Goethite	8.02	12.9	[a]
Goethite	6.78	12.9	1.9
Goethite	5.27	12.9	3.7
Goethite	4.11	12.9	9.5
Goethite	3.94	6.4	6.9
Goethite	4.00	4.3	5.8
Goethite	4.09	3.2	4.3
Goethite	3.95	0	1.5
PCIH	8.02	12.9	0.6
PCIH	6.84	12.9	0.7
PCIH	5.26	12.9	1.3
PCIH	4.11	12.9	3.4
PCIH	3.94	6.4	2.1
PCIH	4.10	3.2	1.6
PCIH	3.90	0	0.4

[a] The Fe content of solution was less than the detection limit of AAS, i.e. $<5\times10^{-7}$ M. From this, the maximum rate of Goethite dissolution after 80 h is calculated to be 1.4 nM/h/m^2.

pH conditions and it is easier to obtain the dissolution rates. Solutions with the ICC values of 3.2, 4.3, 6.4, or 12.9 μM were prepared from the stock solution by dilution with distilled and deionized water. For comparison, dissolution experiments were also conducted in siderophore-free (i.e. ICC = 0 μM) culture medium. A total of 15 series of experiments were conducted. Their experimental conditions are summarized in Table 1.

3. Results

Because the pH values of solutions were not buffered, the solution pH changed during the dissolution experiments. In most cases, the solution pH gradually increased. The pH increases were between 0.3 and 1.2 after 80 h of reaction in the solutions of pH > 5.26, but less than 0.1 when the initial pH was 4. The pH increases may have been due to the adsorption of protons on the surface of Fe-hydroxide, or to the release of hydroxyl ions from the iron hydroxides.

Dilution of the stock solution by distilled water causes to decrease the ionic strength and to change the solubility products of iron hydroxides, but their effects are much smaller than those of siderophore. For example, the ionic strength (I) of the most diluted solution (ICC = 3.2 μM) is 0.091 compared to 0.365 for the undiluted artificial seawater (ASW). Using the data on the solubility product of goethite by Hsu and Marion (1985) and Schindler et al. (1963), we can calculated the log K_{sp} value to be -40.60 and -40.99 at $I=0.091$ and 0.365, respectively. Then, the concentration of [Fe^{3+}] in solution in equilibrium with goethite at pH = 4 can be calculated to be 2.5×10^{-11} and 1.0×10^{-11} M at $I=0.091$ and 0.365, respectively. In contrast, the contents of dissolved Fe in the siderophore-bearing solutions were greater than 1 μM ($=10^{-6}$ M). Therefore, we do not need to consider the effects of dilution in interpreting our experimental data.

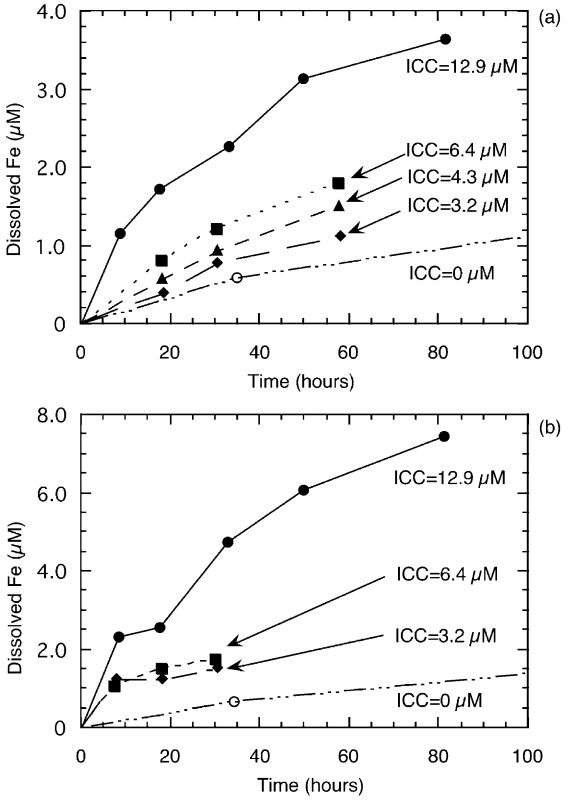

Fig. 1. Dissolution of iron hydroxides in siderophore-bearing solutions; (a) goethite, (b) poorly crystalline iron hydroxide (PCIH). ICC is the iron complexing capacity of siderophore. Each experimental system was 20 ml in volume and contained 2 mg of fine-grained iron hydroxide. The initial pH values of all experiments were 4.

3.1. Dissolution of goethite and PCIH at various ICC values

Goethite and PCIH dissolved faster in siderophore-bearing solutions compared to siderophore-free solutions. In the solutions of pH = 4, the dissolution rates and solubilities of both goethite and PCIH increased with increasing content of siderophore (Fig. 1). The iron contents of solutions continuously increased but the dissolution rates decreased with time (Fig. 1). Such phenomena were also observed during the dissolution experiments of iron (hydr)oxides using other organic ligands (Borggaard, 1991; Hersman et al., 1995; Nowack and Sigg, 1997), and may be due to the selective dissolution of surface iron atoms at the corners and edges of crystals (Borggaard, 1991).

Steady-state Fe concentrations were not achieved in 80 h of reaction. For example, after 60 h of reaction with goethite, the dissolved Fe content increased from 0 to ~ 3.3 μM in solution with ICC = 12.9 μM, to 1.8 μM in solution with ICC = 6.4 μM, to 1.5 μM in solution with ICC = 4.3 μM, and to 1.2 μM in solution with ICC = 3.2 μM (Fig. 1a). These data indicate that only about 30% of the iron complexing capacity of siderophore in each solution was used in 60 h of reaction with goethite. Under the same pH and ICC conditions, the observed rates of increase in Fe contents of solutions were higher in the PCIH experiments compared to the goethite experiments. For example, at pH = 4, 30% of the ICC values were reached in about 30 h of reaction with PCIH, rather than in ~ 60 h of reaction with goethite (Fig. 1b). After 80 h of reaction at pH = 4 and ICC = 12.9 μM, the dissolved Fe content increased to 7.5 μM by reaction with PCIH; in comparison, the Fe increase was 3.6 μM by reaction with goethite. These data give an impression that the dissolution rates of PCIH are faster than those of goethite in siderophore-bearing solutions. However, the specific surface area of the PCIH was about six times higher than that of the goethite (259 vs. 43.8 m^2/g). In fact, it turns out that the specific rates of reaction for PCIH are lower than those for goethite (see below).

By assuming that the total surface area of iron hydroxides remained constant during the experiments, we have calculated the specific rates of dissolution ($nM/h/m^2$) from the slopes of the lines representing

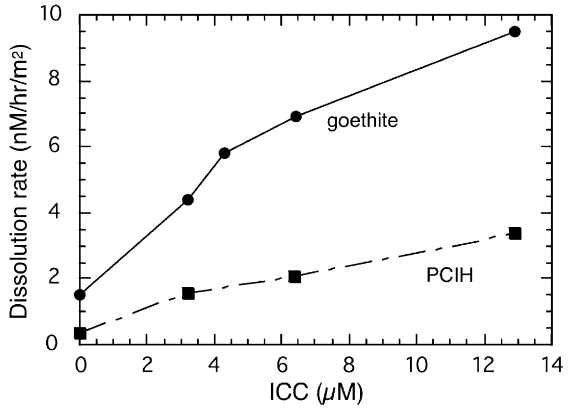

Fig. 2. Dissolution rates of goethite and poorly crystalline iron hydroxide (PCIH) in siderophore-bearing solutions as a function of the ICC values. The initial pH values of all experiments were 4.

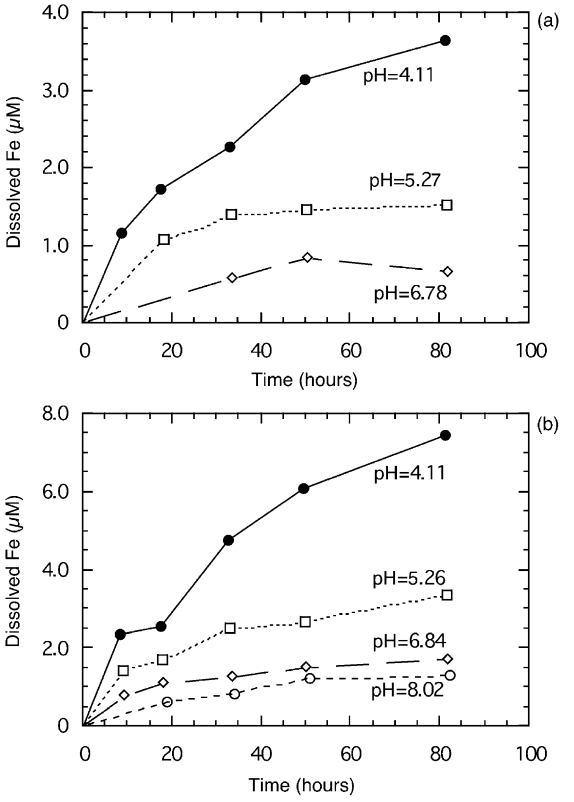

Fig. 3. Dissolution of goethite (a) and PCIH (b) in siderophore-bearing solutions as a function of the initial pH values. The suspension contained 0.1 g/l of iron hydroxide. The ICC values of all experiments were 12.9 μM.

the least square fits of the experimental data points. The calculated dissolution rates of goethite and PCIH are presented in Table 1 and Fig. 2. The dissolution rates of goethite and PCIH increased from 1.5 to 9.5 and from 0.4 to 3.4 (nM/h/m^2), respectively, as the ICC value increased from 0 (siderophore-free) to 12.9 μM. The dissolution rates of PCIH were, therefore, always lower than those of well-crystalline goethite under the same ICC condition (Fig. 2).

3.2. Dissolution of goethite and PCIH at various pH values

The results of dissolution experiments of goethite and PCIH by siderophore with ICC = 12.9 μM, but at different initial pH conditions, are presented in Figs. 3 and 4. The dissolution rates of both goethite and PCIH decreased with increasing pH, and the dissolution rates of PCIH were systematically lower than those of well-crystalline goethite in the pH range below 7. For example, with increasing pH from 4.1 to 6.8, the dissolution rate of goethite decreased from 9.5 to 1.9 nM/h/m^2, whereas the dissolution rate of PCIH decreased from 3.4 to 0.7 nM/h/m^2. The iron content of siderophore-bearing solution (ICC = 12.9 μM) after reaction with goethite at pH = 8 for 80 h was below the detection limit of AAS (30 ppb), indicating that the dissolution rate was less than 1.4 nM/h/m^2. This value may still be higher than the dissolution rate of 0.6 nM/h/m^2 for PCIH at pH = 8. If so, the dissolution rates of PCIH in siderophore-bearing solutions would become lower than those of goethite even at pH > 7.

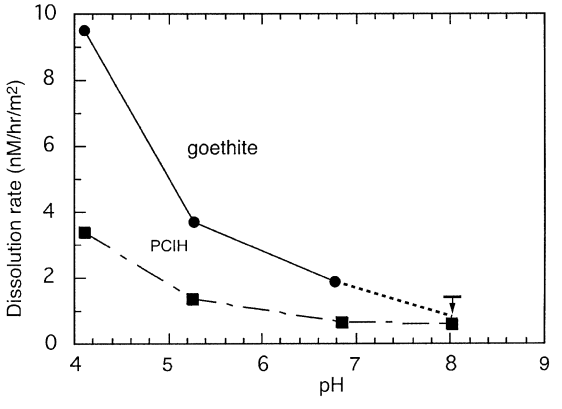

Fig. 4. The rates of siderophore-promoted dissolution of goethite and PCIH as a function of the initial pH values. The dissolution rate of goethite at pH = 8 is a maximum value. The ICC values of all experiments were 12.9 μM.

4. Discussion

In aquatic environments, such as oceans, rivers, and sediments, organic ligands play an important role in the dissolution of minerals. Enhanced dissolution of iron (hydr)oxides by organic ligands, such as oxalate and EDTA, have been demonstrated by many previous researchers (e.g. Sulzberger et al., 1989; Borggaard, 1991; Stumm, 1992; Stumm and Sulzberger, 1992). Siderophores produced by soil bacteria have been shown to significantly increase the dissolution of hematite and hornblende (Hersman et al., 1996; Liermann et al., 2000; Kalinowski et al., 2000a,b). The present study shows that siderophores produced by marine bacteria also significantly increase the dissolution rates and solubilities of ferric hydroxides. Important questions that follow our experimental results include the following: (1) the dissolution mechanisms of ferric hydroxides in siderophore-bearing solutions, and (2) the mechanisms for marine organisms to utilize the siderophore-bound Fe.

Generally, organic ligands dissolve iron (hydr)oxides through one of two different mechanisms (Sulzberger et al., 1989; Stumm, 1992; Stumm and Sulzberger, 1992). One is nonreductive dissolution where the ferric iron in ferric (hydr)oxides dissolve in solutions as ferric–chelate complexes. The other is reductive dissolution where the ferric iron in ferric (hydr)oxides dissolves in solutions as ferrous ion. Siderophores are not ferrous- but ferric-specific ligands and form very stable complexes with ferric iron in solution (Neilands, 1981). Therefore, the dissolution of ferric (hydr)oxides in siderophore-bearing solutions probably follows the nonreductive, ligand-promoted dissolution model and may proceed through the following two steps (c.f. Stumm and Morgan, 1996). The first step is the adsorption of organic ligands (or siderophore) on the surface of ferric hydroxides and the formation of Fe^{3+}-bearing surface complexes. The second step is the release of Fe^{3+}-bearing surface complexes into solution.

The iron content of siderophore-bearing solution (ICC = 12.9 μM) that reacted with PCIH at pH = 8 for

80 h was 1.3 μM or 73 ppb (Fig. 3b). In contrast, the concentration of aqueous ferric iron in water in equilibrium with ferric hydroxides at pH = 8 is between 2.6×10^{-8} and 3.2×10^{-10} M, according to the solubility constant of amorphous $Fe(OH)_3$ ($37.1 < -\log K_{sp} < 39$; Langmuir, 1997). Therefore, the iron content of the siderophore-bearing solution was more than 50 times higher than the solubility value of amorphous $Fe(OH)_3$ in pure water. This clearly indicates that siderophores produced by marine bacteria enhance the dissolution of amorphous ferric hydroxides in the oceans. The iron content of siderophore-bearing solution (ICC = 12.9 μM) that reacted with goethite at pH = 8 for 80 h was below the detection limit, i.e. < 30 ppb. However, the analysis of the specific dissolution rates suggests that the dissolution rates of goethite in siderophore-bearing solutions are likely to be higher than those of PCIH, even at pH = 8 (see Section 3.2). Therefore, we may suggest that marine siderophores also enhance the dissolution of goethite in ocean water.

The next question is whether the ferric–siderophore complexes are directly incorporated into the cell structures of common microorganisms or additional reactions, such as decomposition of ferric–siderophore complexes, are necessary for biological adoption of iron (Fig. 5). For example, Hersman et al. (1996) demonstrated that the siderophore produced by a soil bacterium, *Pseudomonas* sp., promoted dissolution of hematite at pH = 3, and suggested that the bacteria incorporated iron from hematite. However, it is not clear if the bacteria directly incorporated ferric–siderophore complex into their cells. Gottschalke (1986) suggests that Gram-negative microorganisms have specific receptor proteins for siderophores in their outer membranes. Marine microorganisms may also incorporate ferric–siderophore complexes using specific transport proteins. Morel et al. (1991) also suggests that iron uptake by phytoplankton occurs by binding Fe to a cellular surface ligand.

Wells et al. (1983) reported that EDTA enhanced the amount of utilizable iron for phytoplankton, but the mechanisms were not known. Hudson and Morel (1990) suggest that Fe–EDTA complexes do not react directly with transport ligands of phytoplankton; thermal or photochemical dissociation of Fe–EDTA complexes to produce Fe^{3+} ions is necessary to promote the iron uptake by phytoplankton. On the other hand, some researchers (Soria-Dengg and Horstmann, 1995; Butler, 1998) suggest that phytoplankton acquire iron–siderophore complexes through multiple transport mechanisms. For example, marine diatom *Phaeodactylum tricornutum* may take up iron from iron–siderophore complexes through both direct and indirect pathways (Soria-Dengg and Horstmann, 1995). Hutchins et al. (1999) suggest that the ability to

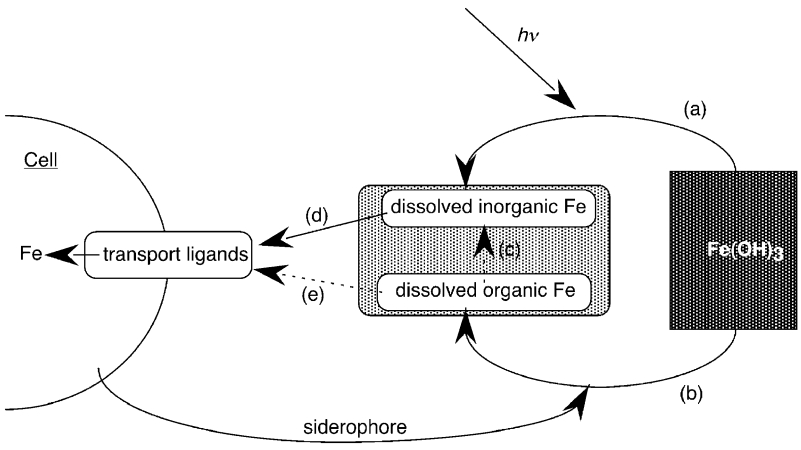

Fig. 5. The proposed model for dissolution pathways of ferric hydroxides and iron uptake by microorganisms in the oceans. The first pathway (a) is photochemical reductive dissolution (Rich and Morel, 1990), and the second pathway (b) is through formation of organically chelated Fe during interaction with siderophores (this study). The organically chelated Fe may dissociate to aqueous inorganic Fe species (c; Hudson and Morel, 1990). It is not fully understood whether the uptake of Fe by microorganisms proceeds through (d) or (e).

take up iron–siderophore complexes differs between prokaryotic and eukaryotic phytoplankton.

Although the exact pathways for incorporation of siderophore-bound iron into marine organisms are still not well understood, our study suggests that most of this iron is liberated from ferric hydroxides in aeolian particles by the siderophores generated by marine bacteria. Therefore, the production rate of marine siderophores may be an important factor influencing the production of phytoplankton and the biogeochemical cycle of iron in the oceans.

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References

Atkinson, R.J., Posner, A.M., Quirk, J.P., 1967. Adsorption of potential-determining ions at the ferric oxide–aqueous electrolyte interface. J. Phys. Chem. 71, 550–558.

Banfield, J.F., Nealson, K.H., 1997. Geomicrobiology. Mineralogical Society of America.

Borggaard, O.K., 1991. Effects of phosphate on iron oxide dissolution in ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid and oxalate. Clays Clay Miner. 39, 324–328.

Brantley, S.L., Liermann, L., Wu, S., Bau, M., 2000. Uptake of trace metals and rare earth elements from hornblende by a soil bacterium. Geomicrobiology, in press.

Bruland, K.W., Orians, K.J., Cowen, J.P., 1994. Reactive trace metals in the stratified central North Pacific. Geochim. Cosmochim. Acta 58, 3178–3182.

Butler, A., 1998. Acquisition and utilization of transition metal ions by marine organisms. Science 281, 207–210.

Coale, K.H., Fitzwater, S.E., Gordon, R.M., Johnson, K.S., Barber, R.T., 1996a. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. Nature 379, 621–624.

Coale, K.H. et al., 1996b. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383, 495–501.

Duce, R.A., Tindale, N.W., 1991. Atmospheric transport of iron and its deposition in the ocean. Limnol. Oceanogr. 36, 1715–1726.

Gottschalke, G., 1986. Bacterial Metabolism, 2nd edn. Springer.

Haygood, M.G., Holt, P.D., Butler, A., 1993. Aerobactin production by a planktonic marine *Vibrio* sp. Limnol. Oceanogr. 38, 1091–1097.

Hersman, L., Lloyd, T., Sposito, G., 1995. Siderophore-promoted dissolution of hematite. Geochim. Cosmochim. Acta 59, 3327–3330.

Hersman, L., Maurice, P., Sposito, G., 1996. Iron acquisition from hydrous Fe(III)-oxides by an aerobic *Pseudomonas* sp. Chem. Geol. 132, 25–31.

Hudson, R.K.M., Morel, F.M.M., 1990. Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions. Limnol. Oceanogr. 35, 1002–1020.

Hutchins, D.A., Witter, A.E., Butler, A., Luther III, G.W. 1999. Competition among marine phytoplankton for different chelated iron species. Nature 400, 858–861.

Jalal, M.A.F., Hossain, M.B., van der Helm, D., Sanders-Loehr, J., Actis, L.A., Crosa, J.H., 1989. Structure of Anguibactin, a unique plasmid-related bacterial siderophore from the fish pathogen *Vibrio anguillarum*. J. Am. Chem. Soc. 111, 292–296.

Kalinowski, B.E., Liermann, L.J., Givens, S., Brantley, S.L., 2000a. Rates of bacteria-promoted solubilization of Fe from minerals: a review of problems and approaches. Chem. Geol. 169, 357–370.

Kalinowski, B.E., Liermann, L.J., Brantley, S.L., Barnes, A.B., Pantano, C.G., 2000b. X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende. Geochim. Cosmochim. Acta 64, 1331–1343.

Kraemer, S.M., Cheah, S.F., Zapf, R., Xu, J., Raymond, K.N., Sposito, G., 1999. Effect of hydroxamate siderophores on Fe release and Pb(II) adsorption by goethite. Geochim. Cosmochim. Acta 63, 3003–3008.

Langmuir, D., 1997. Aqueous Environmental Geochemistry. Prentice Hall, New Jersey.

Liermann, L.J., Kalinowski, B.E., Brantley, S.L., Ferry, J.G., 2000. Role of bacterial siderophores in dissolution of hornblende. Geochim. Cosmochim. Acta 64, 587–602.

Martin, J.H., Fitzwater, S.E., 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331, 341–343.

Martin, J.H., Gordon, R.M., 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. Deep-Sea Res. 35, 177–196.

Martin, J.H., Gordon, R.M., Fitzwater, S., Broenkow, W.W., 1989. VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res. 36, 649–680.

Martin, J.H., Gordon, R.M., Fitzwater, S.E., 1990. Iron in Antarctic waters. Nature 345, 156–158.

Martin, J.H., Gordon, R.M., Fitzwater, S.E., 1991. The case of iron. Limnol. Oceanogr. 36, 1793–1802.

Martinez, J.S., Zhang, G.P., Holt, P.D., Jung, H.-T., Carrano, C.J., Haygood, M.G., Butler, A., 2000. Self-assembling amphiphilic siderophores from marine bacteria. Science 287, 1245–1247.

Neilands, J.B., 1981. Microbial iron compounds. Annu. Rev. Biochem. 50, 715–731.

Nowack, B., Sigg, L., 1997. Dissolution of Fe(III) (hydr)oxides by metal–EDTA complexes. Geochim. Cosmochim. Acta 61, 951–963.

Okujo, N., Saito, M., Yamamoto, S., Yoshida, T., Miyoshi, S., Shinoda, S., 1994. Structure of vulnibactin, a new polyamine-containing siderophore from *Vibrio vulnificus*. BioMetals 7, 109–116.

Reid, R.T., Butler, A., 1991. Investigation of the mechanism of iron acquisition by the marine bacterium *Alteromonas luteoviolaceus*: characterization of siderophore production. Limnol. Oceanogr. 36, 1783–1792.

Reid, R.T., Live, D.H., Faulkner, D.J., Butler, A., 1993. A siderophore from a marine bacterium with an exceptional ferric ion affinity constant. Nature 366, 455–458.

Rich, H.W., Morel, F.M.M., 1990. Availability of well-defined iron colloids to the marine diatom *Thalassiosira weiissflogii*. Limnol. Oceanogr. 35, 652–662.

Rue, E.L., Bruland, K.W., 1995. Complexation of iron(III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. 50, 117–138.

Samuelsson, M., Kirchman, D.L., 1990. Degradation of adsorbed protein by attached bacteria in relationship to surface hydrophobicity. Appl. Environ. Microbiol. 56, 3643–3648.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56.

Soria-Dengg, S., Horstmann, U., 1995. Ferrioxamines B and E as iron sources for the marine diatom *Phaeodactylum tricornutum*. Mar. Ecol.: Prog. Ser. 127, 269–277.

Stumm, W., 1992. Chemistry of the Solid–Water Interface. Wiley.

Stumm, W., Morgan, J.J., 1996. Aquatic Chemistry, 3rd edn. Wiley.

Stumm, W., Sulzberger, B., 1992. The cycling of iron in natural environments: considerations based on laboratory studies of heterogeneous redox processes. Geochim. Cosmochim. Acta 56, 3233–3257.

Sulzberger, B., Suter, D., Siffert, C., Banwart, S., Stumm, W., 1989. Dissolution of Fe(III) oxides in natural waters; laboratory assessment on the kinetics controlled by surface coordination. Mar. Chem. 28, 127–144.

Takahashi, A. et al., 1987. Biscaberin, a new siderophore, sensitizing tumor cells to macrophage-mediated cytolysis. J. Antibiot. 40, 1671–1676.

van den Berg, C.M.G., 1995. Evidence for organic complexation of iron in seawater. Mar. Chem. 50, 139–157.

Wells, M.L., Zorkin, N.G., Lewis, A.G., 1983. The role of colloid chemistry in providing a source of iron to phytoplankton. J. Mar. Res. 41, 731–746.

Zhuang, G., Duce, R.A., Kester, D.R., 1990. The dissolution of atmospheric iron in surface seawater of the open ocean. J. Geophys. Res. 95, 207–216.