



Marine diatom uptake of iron bound with natural colloids of different origins

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Abstract

Natural colloids are abundant in seawater and are an intermediary in the fate, transport and bioavailability of many trace elements. Knowledge of the pathways and mechanisms of the biological uptake of colloidal Fe and other Fe species is of paramount importance in understanding Fe limitation on marine phytoplankton and thus carbon sequestration in the ocean. Whether the natural colloids serve as a source for the biological Fe requirements of marine phytoplankton, or just as a sink for particle-reactive metals in the oceans remains largely unknown. This study examined the bioavailability of Fe bound with colloids from different regions to a coastal diatom (*Thalassiosira pseudonana*). Natural colloids were isolated by cross-flow ultrafiltration and radiolabeled with ⁵⁹Fe before being exposed to phytoplankton. Control experiments were conducted to ensure that ⁵⁹Fe radiolabeled onto the colloids remained mostly in the colloidal phase. Both the natural oceanic and coastal colloidal organic matter complexed Fe (1 nm–0.2 μm) can be biologically available to the marine diatom even though its uptake was lower than the low molecular weight counterparts. By comparing the measured Fe internalization fluxes and the calculated maximum diffusive uptake fluxes, it is evident that ligand exchange kinetics on the cell surface may control the internalization of macromolecular Fe. The calculated concentration factors under dark and light conditions were generally comparable. Colloidal Fe, as an important intermediary phase, can be actively involved in the planktonic food web transfer through biological uptake and regeneration processes. The bioavailable fraction of Fe may be substantially underestimated by only considering the truly dissolved Fe or overestimated when using the external fluxes, such as aerosol Fe, as the bioavailable fraction.

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1. Introduction

The importance of iron in marine ecology and oceanography has received substantial attention because Fe limits primary production in several parts of the ocean (Martin and Fitzwater, 1988; Martin et al., 1994; Coale et al., 1996; Behrenfeld and Kolber, 1999;

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Boyd et al., 2000). Iron is essential for nitrogen utilization and metabolism, chlorophyll biosynthesis, and numerous cellular respiratory functions in phytoplankton and, therefore, plays a critical role in the ocean 'biological pump'. The biological acquisition of various forms of Fe is important for assessing the actual degree to which Fe limits primary production in the ocean. Previously, the bioavailability of Fe was thought to be a function of the concentration of free or inorganic Fe species (Anderson and Morel, 1982; Harrison and Morel, 1986; Campbell, 1995; Sunda and Huntsman, 1997). However, cathodic stripping voltammetric studies have indicated the presence of Fe complexing ligands in natural seawater (Gledhill and van den Berg, 1994; Rue and Bruland, 1995, 1997; Wu and Luther, 1995; van den Berg, 1995; Witter and Luther, 1998). Binding with organic ligands increases Fe solubility in seawater and may play an important role in Fe bioavailability and its geochemical cycling (Sunda et al., 1991; Kuma et al., 2000; Wu et al., 2001). The organic ligands may have included siderophores produced by microorganisms in response to Fe stress (Trick et al., 1983; Granger and Price, 1999), or the release of other intracellular materials (e.g., porphyrin complexes, Hutchins et al., 1999a).

Iron may also be complexed by macromolecular organic ligands, such as natural organic colloids, operationally defined as size range between 1 nm and 0.2 μm (Buffle and Leppard, 1995). Marine colloids are very abundant in the surface oceans (Wells and Goldberg, 1991; Benner et al., 1992; Guo et al., 1995), and are mostly organic in nature. Recent measurement showed that >90% of Fe in the traditionally defined dissolved phase (<0.2 μm) was in fact in the colloidal phase (>1 kDa), particularly in the larger colloidal size spectrum (8 kDa–0.2 μm) (Wen et al., 1999; Wells et al., 2000; Wu et al., 2001). Fe in the colloidal fraction has been shown to be the most dynamic size fraction during the growth of marine diatoms (Nishioka and Takeda, 2000).

There has been a longstanding interest in the biological availability of colloidal Fe by marine phytoplankton. Although several studies have shown that Fe bound with synthetic inorganic colloids was related to the thermodynamic stability and kinetic liability of colloids as sources for uptake by marine phytoplankton (Wells et al., 1983; Rich and Morel, 1990; Kuma and Matsunaga, 1995), the applicability of these studies to

natural organic colloids has yet to be explored. Recent evidence revealed that artificially synthetic organic Fe (such as porphyrin, ferrioxamines B and E) are biologically assessable for marine phytoplankton (Soria-Dengg and Horstmann, 1995; Maldonado and Price, 1999; Kuma et al., 1999, 2000). Several studies demonstrated that Fe bound with fungal siderophore desferrioxime B (DFB) can indeed be utilized by marine diatoms and natural phytoplankton communities in the subarctic Pacific (Soria-Dengg and Horstmann, 1995; Maldonado and Price, 1999), whereas other studies have indicated that the addition of DFB reduced the biological uptake of Fe and the growth rate of phytoplankton (Hutchins et al., 1999a,b; Wells, 1999). Hutchins et al. (1999a) further suggested the specificity of Fe uptake, e.g., Fe bound with siderophore was more bioavailable to prokaryotic picoplankton, whereas porphyrin-complexed Fe was more bioavailable to eukaryotic phytoplankton. Both photo-reduction and biological reduction (such as plasma membrane ferrireductase) have been proposed to convert the organic-complexed Fe to inorganic bioavailable Fe species (Jones et al., 1987; Rich and Morel, 1990; Johnson et al., 1994; Weger, 1999; Gerringa et al., 2000; Maldonado and Price, 2000).

Despite the intensive interest in the biological availability of colloidal Fe to aquatic organisms (particularly marine phytoplankton), the biological uptake of Fe bound with colloids isolated from natural waters remains largely undetermined (Chen and Wang, 2001). It remains less certain whether the colloidal Fe (between 1 kDa and 0.2 μm) is available for phytoplankton. In this study, we examined the bioavailability of Fe bound with natural colloids isolated by cross-flow ultrafiltration from different regions, including estuarine, coastal and oceanic waters. Previously, we had compared the bioavailability of Fe bound with different sized and aged colloids to the marine diatom (Chen and Wang, 2001). Here, we quantified the intracellular biological uptake by phytoplankton using the established Ti-citrate–EDTA washing technique (Hudson and Morel, 1989). Control laboratory experiments were first carried out to monitor the physico-chemical behavior of radiolabeled colloids in our experimental system. Partitioning of Fe in different phases was monitored after the exposure experiment. The cellular distribution of Fe following exposure to natural colloids was also quantified.

2. Materials and methods

2.1. Isolation of natural colloids

Seawater was collected from different regions representing estuarine, coastal and oceanic environments. The Yuan Long (salinity of 10, Hong Kong) is under heavy influence from the Pearl River Estuary Plume and can be considered as a representative of estuarine waters. The Tolo Harbor (with a salinity of 30, Hong Kong), on the other hand, is a coastal environment. Oceanic water was collected from Equatorial Pacific, 00°00.6'S and 145°00.0'E. Seawater was immediately processed to isolate colloids from the truly dissolved phases upon arrival in the laboratory. For estuarine and coastal waters, it took 1–2 h to transport the seawater samples from the field to the laboratory and about 4 h for the ultrafiltration of 20 l of seawater. The oceanic sample was prefiltered onboard ship but ultrafiltered in the laboratory.

Natural colloids were collected using methods described in Guo and Santschi (1996). The seawater was first passed through a 0.2- μm Poretics cartridge, and then ultrafiltered using a spiral-wound cross-flow ultrafiltration cartridge (Amicon S10Y1) with a 1-kDa molecular weight cutoff (Guo and Santschi, 1996). The ultrafiltration cartridge was sequentially cleaned with 2% Microdetergent, 0.05 N NaOH and 0.05 N HCl before the ultrafiltration. The cartridge was also cleaned with a large volume of Nanopure water during and after each step of cleaning. The concentration factor used in the ultrafiltration was approximately 40. Concentrations of dissolved organic carbon (DOC) and colloidal organic carbon (COC) were quantified by a Shimadzu TOC-5000A total organic carbon analyzer

(Guo et al., 1994). The isolated colloids were stored at 4 °C for 2 days before the radiolabeling and uptake experiments.

2.2. Radiolabeling natural colloids

The natural colloids were radiolabeled with ^{59}Fe (in 0.1 N HCl, from NEN, Boston, MA, USA) for 2 days in a Teflon bottle. Isotopic addition of ^{59}Fe corresponded to a Fe concentration of 92 nM in the labeling bottle. The colloids were concentrated about 40 folds from the natural seawaters, thus the isotopic addition of ^{59}Fe only resulted in a maximum additional Fe concentration of 2 nM in the uptake experiments. Microliter amount of 0.5 N NaOH was also added to the colloids to maintain the pH at 8 after the addition of radioactive Fe. The water was gently stirred several times a day. After 2 days of radiolabeling, about 100 ml of radiolabeled colloids were added into a 1-kDa dialysis bag (Spectra) suspended in 2.2 l low molecular weight (LMW, <1 kDa) seawater. The unlabeled or free ionic ^{59}Fe was removed through the dialysis by frequently changing the LMW seawater ($5\times$ over a period of 3 days). The radioactivity in the LMW solution outside the dialysis bag was monitored at different time intervals. Results indicated that the dialysis process reached an equilibrium approximately 12 h. The LMW seawater was therefore renewed every 12 h. After 3 days, ^{59}Fe activity in the LMW water was undetectable ($<3.4\times 10^{-12}$ M), indicating that ^{59}Fe was in fact mostly complexed with the colloids within the dialysis bag. The radiolabeling efficiencies of ^{59}Fe , calculated as the radioactivity of ^{59}Fe in colloids following the 3-day dialysis to radioactivity of ^{59}Fe spiked into the colloids, are shown in Table 1. All radiolabeled colloids, including oceanic,

Table 1

Summary of experiments to examine the biological uptake of colloid-bound Fe by marine diatom *T. pseudonana* and natural phytoplankton assemblage

Treatment	Phytoplankton	^{59}Fe labeling efficiency (%)	Experimental DOC concentration (μM)	Experimental COC concentration (μM)
Low molecular weight Fe uptake	<i>T. pseudonana</i>		80	0
Oceanic colloids (Pacific, $S=35$)	<i>T. pseudonana</i>	48.7	80	28
Coastal colloids (Tolo Harbor, $S=30$)	<i>T. pseudonana</i>	53.2	201	28
Estuarine colloids (Yuan Long, $S=10$)	<i>T. pseudonana</i>	64.4	126	28

DOC: dissolved organic carbon; COC: colloidal organic carbon.

coastal and estuarine, were again passed through the 0.2- μm polycarbonate membrane immediately before the uptake experiments to ensure that only the $<0.2\text{-}\mu\text{m}$ colloids were used in the uptake experiments. Since the bioavailability of Fe complexed with different colloids to a specific phytoplankton species can only be compared at the similar experimental conditions (e.g., salinity, Fe and DOC concentrations), the labeling of the estuarine colloids was also carried out by using coastal seawaters with a salinity of 30. Even though this may potentially cause estuarine colloids to coagulate when exposing to higher salinity seawaters, this possible effect was minimized by filtering the radiolabeled estuarine colloids through a 0.2- μm membrane before the uptake experiments.

2.3. Coagulation and release of Fe from radiolabeled colloids

Colloidal coagulation and ^{59}Fe release from the radiolabeled colloids into the truly dissolved phase are critical for the interpretation of the bioavailability results (Wang and Guo, 2000). Experiments were therefore first carried out to measure the coagulation of colloidal particles and possible release of ^{59}Fe from the radiolabeled colloids into the truly dissolved phase following the suspension of radiolabeled colloids into the LMW water. Radiolabeled colloids were distributed into a 1-kDa dialysis bag suspended in LMW seawater. At time intervals, the ^{59}Fe released into the LMW water was quantified by taking a 20-ml water sample for radioactivity measurements (representing the total radioactivity), and the percentage of ^{59}Fe released into the LMW water was then calculated. ^{59}Fe was measured by a Wallac 1480 gamma detector at 1092 keV.

To quantify the colloidal coagulation, the radiolabeled colloids were dispersed into the $<1\text{-kDa}$ LMW seawater. At time intervals, a 20-ml sample was filtered onto a 0.2- μm polycarbonate membrane, rinsed twice with $<1\text{ kDa}$ seawater, and the ^{59}Fe radioactivity was counted. The percentage of colloidal ^{59}Fe detected in the particulate phase ($>0.2\text{ }\mu\text{m}$) was then calculated. In addition to these experiments, the coagulation and partitioning of colloidal ^{59}Fe was also determined in each biological uptake experiment (see below).

2.4. Diatom uptake of radiolabeled colloidal ^{59}Fe

The diatom *Thalassiosira pseudonana* was obtained from Provasoli-Guillard Phytoplankton Collection Center, Maine, USA, and maintained in axenic culture in a f/2 medium (Guillard and Ryther, 1962). Cells in the exponential growth phase were filtered through a 3- μm polycarbonate membrane, rinsed with LMW seawater, and resuspended in LMW seawater before the uptake experiments. The colloidal Fe uptake was compared with Fe uptake in the LMW fraction collected outside the dialysis bag during the colloidal labeling. We also determined the uptake of Fe by the dead cells via heat killing the diatoms (for 10 min at 50 °C). Microscopic inspection indicated that the cells remained intact after the heat treatment. The uptake of colloidal Fe was also compared under dark and under light conditions (white fluorescence tubes, 70 $\mu\text{mol m}^{-2}\text{ s}^{-1}$). A control treatment without the addition of diatoms was used to monitor the partitioning and coagulation of radiolabeled colloidal Fe.

To measure the colloidal Fe uptake, the exponentially growing diatom cells were filtered from the f/2 medium and added into 150 ml LMW water containing radiolabeled colloids, in an acid-rinsed polycarbonate bottle. Colloids were added to result in a COC concentration representative of the natural seawater (Table 1). Total Fe concentrations in all treatments due to isotopic addition of ^{59}Fe were below 2 nM, which was much lower than those found in Hong Kong coastal seawaters. The Fe background concentrations were not measured in our experiments. Our recent measurements indicated that the dissolved Fe concentrations from Tolo Harbor and Yuan Long seawaters were 59 and 50 nM, respectively, as determined using ICP-MS with Dynamic Reaction Cell (DRC) technique and clean procedures. If we assume that the dissolved Fe concentrations in our experiments were similar to these values, isotopic addition of ^{59}Fe should contributed $<4\%$ of the original seawater Fe concentration. However, the addition of ^{59}Fe may increase the Fe concentration in the oceanic treatment. Because we were primarily interested in the bioavailability of colloidal Fe to phytoplankton, we did not consider the influence of physiological changes of phytoplankton, such as the transfer of phytoplankton from a high nutrient

medium to a low nutrient medium, on the biological uptake of colloidal Fe. The initial cell concentration was about 2×10^5 cells ml^{-1} . The cells were then placed in an incubator with a 24-h light illumination (white fluorescence tubes, $70 \mu\text{mol m}^{-2} \text{s}^{-1}$, except in the dark treatment) at 18°C . At different time intervals (over a period of 48 h), a 1-ml water sample was removed and measured for its radioactivity (representing the total ^{59}Fe radioactivity). A 10-ml sample was filtered onto a 1- μm polycarbonate membrane, and rinsed with Ti-citrate–EDTA reagent, as described in Hudson and Morel (1989). The radioactivity (representing the intracellular ^{59}Fe) was then measured. The percentage of intracellular Fe uptake was defined as the ratio of intracellular ^{59}Fe radioactivity to total ^{59}Fe radioactivity in the water sample. Biological Fe concentration factor (CF) was calculated as the ratio of the Fe activity in diatoms (ccpm kg^{-1}) to the Fe activity in ambient seawater (ccpm l^{-1}). In this study, we did not consider the extracellular uptake of ^{59}Fe (e.g., surface sorption) during the course of experiments. This fraction was quantified at the end of 48-h exposure. The change in cell density during the course of experiment was monitored by a Coulter counter.

Trace metal clean technique was applied in all experiments to avoid Fe contamination of the samples and bottles. All polycarbonate bottles (Nalgene™), carboys and Teflonwares were cleaned by a series of soaking steps over 1–2 days consisting of detergent followed by 2% Microdetergent, $18 \text{ M}\Omega \text{ cm}^{-1}$ Nanopure water, 2 N HCl (trace metal grade) and $18 \text{ M}\Omega \text{ cm}^{-1}$ Nanopure water. All transfers and filtration were conducted in a class 100 laminar flow hood.

2.5. Metal cellular distribution and ^{59}Fe partitioning

The cellular distribution of ^{59}Fe in the intracellular and cytosolic compartments was measured at the end of exposure (48 h) in each uptake experiment. A 10-ml sample was filtered onto a 3- μm polycarbonate membrane, and rinsed with LMW water (without Ti-washing), and ^{59}Fe radioactivity was measured. The percentage of cellular ^{59}Fe in the intracellular compartment was then calculated by comparing the radioactivity detected in the intracellular pool with the radioactivity in whole diatom cells. The distribution

of ^{59}Fe in the cytoplasm was measured as described in Fisher et al. (1983) and Wang and Fisher (1996). Briefly, a 30-ml sample was filtered onto a 3- μm polycarbonate membrane, and rinsed with LMW water. The surface-bound metals were removed by 0.1 mM EDTA (which removed only a small fraction of surface-bound ^{59}Fe). The cells were then suspended in distilled water and frozen. The samples were subsequently thawed and centrifuged sequentially at $8000 \times g$ (for 15 min) and $20,000 \times g$ (for 20 min). The distribution of ^{59}Fe in the cytoplasm (represented by supernatant fraction after $20,000 \times g$ centrifugation) was then calculated as the percentage of the radioactivity in the cytoplasm to that in the whole cells. It should be noted that the %cytoplasmic distribution has no relation with the ambient Fe concentration.

The partitioning of dissolved ^{59}Fe after the exposure experiments was also determined. The water sample was first filtered through a 0.2- μm polycarbonate membrane and then ultrafiltered on a 1-kDa stirrer cell ultrafiltration (Amicon, with YM1 regenerated cellulose membrane). The partitioning of ^{59}Fe between the colloidal phase (1 kD–0.2 μm) and the truly dissolved phase (<1 kDa) was determined by the ^{59}Fe activity concentrations in the 1-kDa filtrate, 1-kDa retentate and 0.2- μm filtrate.

3. Results

3.1. Colloidal ^{59}Fe radiolabeling

Dialysis technique was employed to remove the uncomplexed Fe from the colloiddally complexed Fe to ensure that there was minimum uncomplexed Fe added into the experimental system. Our results showed that the radiolabeling efficiency varied among natural colloids of different origins and salinities (Table 1). ^{59}Fe was radiolabeled at the highest efficiency to the estuarine colloids (64.4%). The radiolabeling efficiencies were rather comparable for colloids isolated from coastal and oceanic waters.

Re-partitioning experiments were carried out to examine the possibility of colloidal ^{59}Fe release into the <1-kDa seawater and of colloidal coagulation. Overall, release of colloidal ^{59}Fe into the <1-kDa seawater was <6% throughout the 60-h period, sug-

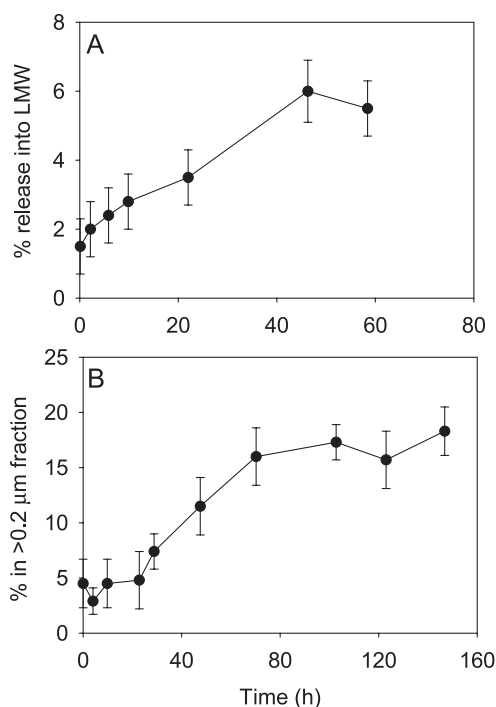


Fig. 1. The release of ^{59}Fe from radiolabeled colloids into the low molecular weight water (LMW) (A) and the percentage of ^{59}Fe bound with natural colloids detected in the particulate phase ($>0.2\ \mu\text{m}$) (B) over time of exposure. Mean \pm S.D. ($n=3$).

gesting that the majority of the radiolabeled colloidal ^{59}Fe ($>94\%$) indeed remained in the colloidal phase (Fig. 1A). Colloidal coagulation, quantified as the fraction of ^{59}Fe detected in the $>0.2\text{-}\mu\text{m}$ fraction following the addition of radiolabeled colloidal ^{59}Fe into the LMW waters, was less than 18% during the 6.2-day exposure period (Fig. 1B). Within the first and second day (the time course of our phytoplankton uptake experiments), $<5\%$ and 12% of colloidal Fe was detected in the $>0.2\text{-}\mu\text{m}$ fraction. We did not specifically quantify the coagulation of colloidal Fe in the presence of diatoms, as it was difficult to distinguish colloidal coagulation and biological uptake with the addition of algae. Regardless, colloidal ^{59}Fe appeared to be relatively stable in terms of re-partitioning and coagulation during the short-term uptake period. In our study, the colloidal ^{59}Fe uptake was quantified as the intracellular uptake, thus colloidal coagulation, if any, should have minimal effect on the interpretation of the uptake results.

3.2. Diatom uptake of LMW ^{59}Fe and natural colloidal ^{59}Fe

Very little intracellular ^{59}Fe ($<4\%$) was detected in the heat-killed dead diatoms during the 48-h exposure to colloidal ^{59}Fe (time series data not shown), indicating that the Ti-citrate–EDTA washing was extremely efficient in removing the extracellular Fe from the cells. In the control treatment (without the addition of diatoms) in each experiment, 2–4% of the colloidal ^{59}Fe was found in the $>0.2\text{-}\mu\text{m}$ fraction after rinsing with Ti-citrate–EDTA reagent, presumably due to the sorption of ^{59}Fe onto the polycarbonate membrane or negligible colloidal coagulation.

There was an approximately linear pattern of increase in the percentage of biological ^{59}Fe uptake

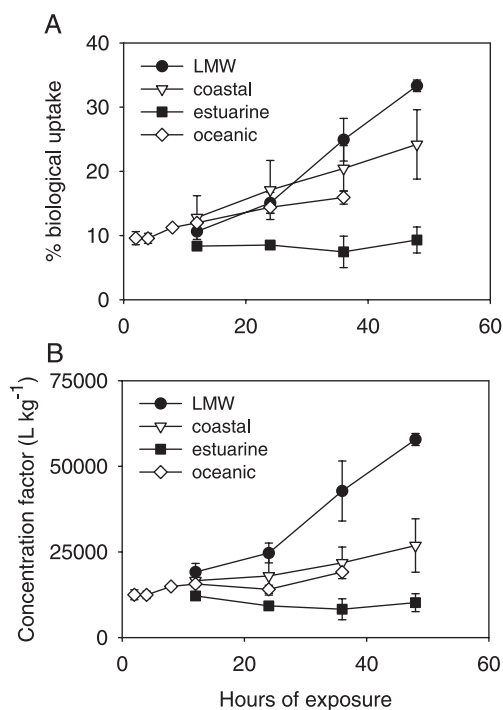


Fig. 2. Biological uptake of ^{59}Fe bound with natural colloids isolated from oceanic (Equatorial Pacific), coastal (Tolo Harbor) and estuarine (Yuan Long) waters by the diatom *T. pseudonana* during the 2-day exposure period under light conditions ($70\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$). (A) The intracellular biological ^{59}Fe uptake; (B) the concentration factor calculated as the ^{59}Fe accumulated in the diatoms divided by ^{59}Fe in the solution phase. The uptake of colloidal Fe was compared with the uptake of low molecular weight (LMW) Fe. Mean \pm semi-range ($n=2$).

(calculated as the ratio of radioactivity in the intracellular compartment of diatom to radioactivity in the aqueous phase) during the 2–48-h exposure, except for the estuarine colloids (Fig. 2). Over the 48-h exposure period, up to 33% of total LMW ^{59}Fe was biologically accumulated in the intracellular diatoms in the LMW treatment. Up to 16%, 24%, and 9% of total colloidal ^{59}Fe was accumulated in the intracellular pools of diatoms in the oceanic (Equatorial Pacific), coastal (Tolo Harbor) and estuarine (Yuan Long) treatment, respectively. Furthermore, about 88%, 74%, and 72% of the total colloidal Fe was associated with the total cellular diatoms (including both extracellular and intracellular Fe) by the end of exposure, in the oceanic, coastal and estuarine colloidal treatment, respectively. The biological concentration factor (CF) exhibited an approximate linear increase over time, except for the estuarine colloidal treatment. The uptake rate constant (k_u), calculated from the slope of the linear regression between the CF and the time of exposure, was 158–288 $\text{l kg}^{-1} \text{h}^{-1}$ in the coastal and oceanic colloidal treatments under light conditions, compared to 1137 $\text{l kg}^{-1} \text{h}^{-1}$ in the LMW treatment (Fig. 3). The uptake

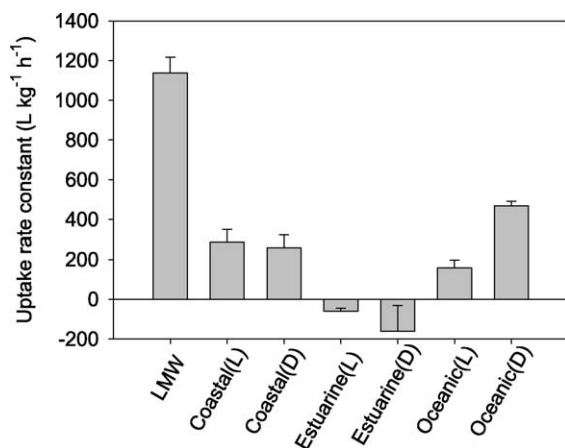


Fig. 3. Calculated uptake rate constant (k_u) of Fe in the diatom *T. pseudonana*. LMW: low molecular weight complexed Fe (<1 kDa); Oceanic (L): Fe bound with oceanic colloids (Equatorial Pacific) and exposed under light; Oceanic (D): Fe bound with oceanic colloids and exposed under dark; Coastal (L): Fe bound with coastal colloids (Tolo Harbor) and exposed under light; Coastal (D): Fe bound with coastal colloids (Tolo Harbor) and exposed under dark; Estuarine (L): Fe bound with estuarine colloids (Yuan Long) and exposed under light; Estuarine (D): Fe bound with estuarine colloids (Yuan Long) and exposed under dark. Mean + semi-range ($n=2$).

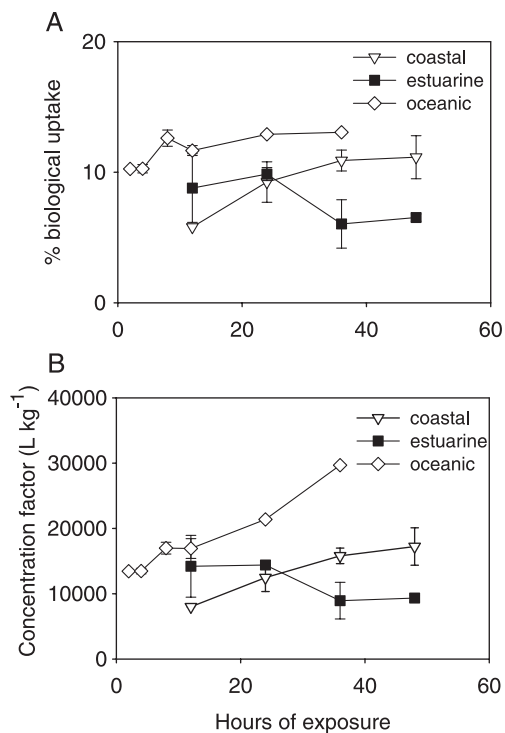


Fig. 4. Biological uptake of ^{59}Fe bound with natural colloids isolated from oceanic (Equatorial Pacific), coastal (Tolo Harbor) and estuarine (Yuan Long) waters by the diatom *T. pseudonana* during the 2-day exposure period under dark condition. (A) The intracellular biological ^{59}Fe uptake; (B) the concentration factor calculated as the ^{59}Fe accumulated in the diatoms divided by ^{59}Fe in the solution phase. Mean \pm semi-range ($n=2$).

rate constants of the colloidal Fe were significantly higher in the oceanic and coastal treatments than the estuarine colloidal treatments, demonstrating that oceanic and coastal colloidal Fe was utilized by the marine diatoms. There was no apparent uptake of colloidal Fe in the estuarine colloidal treatment.

As shown in Fig. 4A, the intracellular uptake of colloidal Fe under dark conditions was much lower than the uptake under light conditions. For estuarine colloids, there was no apparent increase in the intracellular uptake within the 2-day exposure period under the dark conditions. However, because the cell density decreased under the dark condition whereas it increased under the light condition, the calculated concentration factors under dark and light conditions were comparable (Figs. 2 and 4). The uptake rate constant of oceanic colloidal Fe under dark conditions was even $3 \times$ higher than that under light conditions

(Fig. 3). In addition, the biological concentration factor (CF) exhibited an approximate linear increase over time for the coastal and oceanic colloidal treatments under the dark conditions (Fig. 4B). Apparently, there was also a substantial uptake of colloidal Fe under the dark conditions, especially for coastal and oceanic colloidal treatments.

By the end of the 2-day exposure, about 8–32% and 8–19% of the total cellular ^{59}Fe were detected in the intracellular and cytoplasmic compartments, respectively (Fig. 5). In the LMW treatment, a much higher fraction of cellular Fe was in the cytoplasm (34%) and in the intracellular compartment (56%).

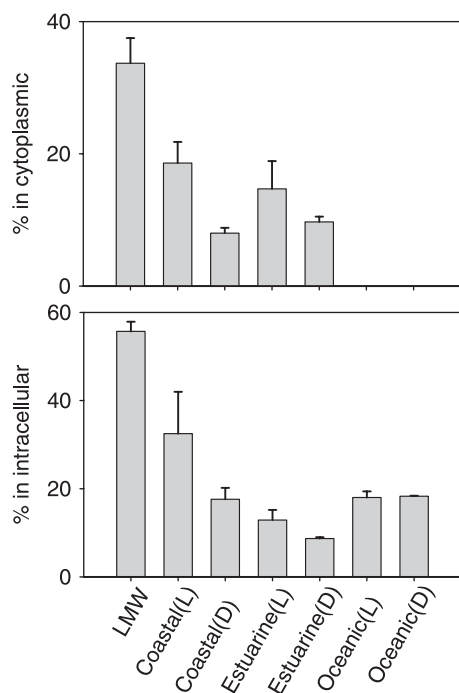


Fig. 5. Percentage of total cellular diatom ^{59}Fe in the intracellular and cytoplasmic pools of the diatom *T. pseudonana*, following a 2-day exposure to different complexed Fe. LMW: low molecular weight complexed Fe (< 1 kDa); Oceanic (L): Fe bound with oceanic colloids (Equatorial Pacific) and exposed under light; Oceanic (D): Fe bound with oceanic colloids and exposed under dark; Coastal (L): Fe bound with coastal colloids (Tolo Harbor) and exposed under light; Coastal (D): Fe bound with coastal colloids (Tolo Harbor) and exposed under dark; Estuarine (L): Fe bound with estuarine colloids (Yuan Long) and exposed under light; Estuarine (D): Fe bound with estuarine colloids (Yuan Long) and exposed under dark. The cytoplasmic distribution in the oceanic colloidal treatments (both light and dark) was not determined. Mean + semi-range ($n = 2$).

These fractions were higher than those in the colloidal treatments. The total cellular fraction of Fe associated with the intracellular and cytoplasmic compartment was the lowest for estuarine colloidal treatment. Diatoms maintained under dark conditions had a lower percentage of ^{59}Fe in the cytoplasm and intracellular pool than those maintained under light, except for the oceanic colloidal treatment in which Fe distributions in the intracellular pool were comparable between the light and the dark treatments. A slightly lower or comparable fraction of total cellular Fe in the cytoplasm was found for the estuarine colloids than for the coastal colloids. There was no apparent uptake of colloidal Fe in the estuarine colloidal Fe during the experimental period (the slope or the uptake rate constant was zero or below zero). However, there was an initial uptake (or sorption) when the diatoms were first in contact with the colloidal Fe and some of these Fe penetrated into the diatom cytoplasm.

After the 2-day exposure, about 26% of ^{59}Fe was found in the colloidal phase, whereas 74–77% of ^{59}Fe remained in the LMW fraction in the LMW treatment. In the control treatments (without addition of diatoms), 92–94% of originally radiolabeled Fe remained in the colloidal phase after 2 days. In the colloidal treatments, most of the colloidal ^{59}Fe (>85%) were associated with the colloidal phase after the 2-day exposure to diatoms.

4. Discussion

Our results suggested that the biological uptake of colloidal Fe were significant regardless of light or dark conditions, except for the estuarine colloidal treatments. Natural colloidal Fe can indeed be accumulated in the algal cytoplasm, in addition to its accumulation in the intracellular pool. These experimental results provide strong evidence that phytoplankton is able to acquire macromolecular complexed Fe species from the coastal and oceanic environments, and can utilize the largest reservoir of colloidal Fe in the ocean as a Fe source. Several recent studies have shown that the well-defined, organically complexed Fe may be biologically accessible for phytoplankton growth (e.g., porphyrin, ferrioxamines B and E) (Hutchins et al., 1999a; Maldonado and Price, 1999, 2000). Recently,

Kuma et al. (2000) demonstrated that a terrestrial fungal hydroxamate ferrisiderophore ferrichrome (DFC) at a ratio of DFC–Fe(III) 2:1 induced the highest Fe uptake rates among different organic–Fe(III) complexes [DFC–Fe(III), EDTA–Fe(III), citric–Fe(III) and fulvic–Fe(III)]. They concluded that DFC complexes of Fe in seawater may be able to supply the biologically available Fe(III) species to phytoplankton. However, Boye and van den Berg (2000) showed that the organically complexed Fe presented in culture was not immediately available to the marine coccolithophore *Emiliania huxleyi*. Our results suggested that besides the low molecular weight organically complexed Fe, macromolecular complexed Fe can also be a source for phytoplankton growth. Given that the MW of siderophores is <1 kDa, and that most of the Fe in the dissolved phase is in the colloidal phase (Wen et al., 1999; Wells et al., 2000; Wu et al., 2001), it is unlikely that siderophores are the major Fe-binding organic ligands in natural seawater but could be important in the Fe uptake pathways.

Although colloidal Fe can be utilized by marine phytoplankton, bioavailability of organically complexed Fe could be affected by the ligands types. In our experiment, the uptake rate constant (k_u) of Fe was about $3.9\text{--}7.2 \times$ higher for the LMW-complexed Fe [including the free Fe(III) or other inorganic or small organic complexed Fe(III)] than the uptake rate of colloid-bound Fe. Thus, complexation with colloidal ligands inhibited the biological uptake rate of Fe to marine phytoplankton. In addition, the uptake rate constants of the colloidal Fe were significantly higher in oceanic and coastal treatments than estuarine colloidal treatments. The much lower bioavailability of Fe bound with estuarine colloids is likely a result of the difference in Fe-binding organic ligands between estuarine and oceanic waters. Estuarine, coastal and oceanic waters may have contrasting organic composition. For example, estuarine colloids could contain largely humic substances and other terrestrial components (Sholkovitz et al., 1978), whereas coastal and oceanic colloids are mostly freshly photosynthesized organic matter, such as exopolymeric polysaccharides and other biopolymers (Benner et al., 1992; Hung et al., in press).

Assuming that the dissolved Fe background concentration in the coastal treatment (Tolo Harbor)

was 59 nM and 90% of the dissolved Fe was in the colloidal phase (see Materials and methods), the absolute uptake rate of coastal colloidal Fe was estimated to be $0.024 \text{ amol cell}^{-1} \text{ h}^{-1}$. For comparison, Kuma et al. (1999) showed that the uptake rates of fulvic–Fe(III) and EDTA–Fe(III) by *Chaetoceros sociale* were 12 and $2 \text{ amol cell}^{-1} \text{ h}^{-1}$, respectively. Volker and Wolf-Gladrow (1999) estimated that the uptake rate of Fe-siderophore was $0.6 \text{ amol cell}^{-1} \text{ h}^{-1}$ by the dinoflagellate *Prorocentrum minimum*. The maximum uptake rates of ferrioxamines B-Fe and ferrioxamines E-Fe by *Phaeodactylum tricorutum* were estimated as 0.001 and 0.002 $\text{amol cell}^{-1} \text{ h}^{-1}$ (Soria-Dengg and Horstmann, 1995). Maldonado and Price (1999) determined the Fe-siderophore uptake rates of field phytoplankton in the subarctic Pacific Ocean as $0.07 \text{ amol Fe cell}^{-1} \text{ h}^{-1}$ (by assuming $1 \text{ cell} = 10^{-12} \text{ g C}$). Even though the colloidal Fe uptake rates were $3.9\text{--}7.2 \times$ lower than those of LMW treatments, the overall biological uptake of colloidal Fe is higher than or comparable to that predicted from the LMW-complexed Fe which may possibly be dissociated from the colloidal Fe, if we assume that only LMW Fe is bioavailable (Guo et al., 2002). Such an inhibition on colloidal Fe uptake rates likely causes Fe limitation in the HNLC oceanic environment because the majority of dissolved Fe is associated with the colloidal particles (e.g., Wen et al., 1999; Wu et al., 2001).

Metal uptake by phytoplankton is generally believed to involve an initial rapid surface sorption, followed by a slower step of internalization, e.g., transport across the plasma (Hudson and Morel, 1990; Volker and Wolf-Gladrow, 1999). For the internalization of organic-complexed Fe, the key question is whether the organically complexed Fe(III) needs to be reduced to Fe(II) before the transport into the intracellular compartment or only by physical diffusion. By comparing the observed Fe internalization fluxes with the calculated maximum Fe diffusion fluxes, one can estimate the relative importance of these two mechanisms. For a fixed uptake rate and spherical cells, the maximum diffusive uptake fluxes ($Q_{\text{diff}}^{\text{max}}$) can be expressed as (Volker and Wolf-Gladrow, 1999):

$$Q_{\text{diff}}^{\text{max}} = k_{\text{diff}} C_{\text{bulk}} = 4\pi R D C_{\text{bulk}}$$

where k_{diff} is the diffusion-limited uptake rate constant ($\text{cm}^3 \text{ cell}^{-1} \text{ h}^{-1}$), R is the radius of the diatom cell ($2 \times 10^{-4} \text{ cm}$ for *T. pseudonana*), C_{bulk} is the Fe concentration in the solution, and D is the diffusion coefficient of the colloids. According to the Stokes–Einstein relationship, the diffusion coefficient (D) of a solute can be defined as: $D = kT/6\pi\eta r$, where kT is the average thermal energy of the medium, η is the temperature-dependent viscosity of the solvent. From this equation, one will know that the diffusion coefficient is inversely related to particle radius (r). Volker and Wolf-Gladrow (1999) adopted a value of $0.9 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for the diffusion coefficient of Fe(III)' in seawater at 20 °C. Assuming that the colloids are rigid spheres and the average size of the colloids is 5 nm and the size of Fe(III)' is 0.25 nm, the diffusion coefficient for colloids was estimated to be $4.5 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ from the Stokes–Einstein relationship. In our study, Fe internalization fluxes (Q_{m} , $\text{cm}^3 \text{ cell}^{-1} \text{ h}^{-1}$) can be calculated as:

$$Q_{\text{m}} = k_{\text{u}}WC_{\text{bulk}} \times 1000$$

where k_{u} is the measured uptake rate constant for colloidal complexed Fe ($1 \text{ kg}^{-1} \text{ h}^{-1}$), W is the dry weight per cell ($2.2 \times 10^{-11} \text{ g cell}^{-1}$). A relative index I , calculated as the measured internalization fluxes divided by the maximum diffusive fluxes, can be used to examine the importance of physical diffusion:

$$I = \frac{Q_{\text{m}}}{Q_{\text{diff}}^{\text{max}}} = \frac{k_{\text{u}}W \times 1000}{4\pi RD}$$

Our results showed that the uptake rate constant (k_{u}) was 158 and 288 $1 \text{ kg}^{-1} \text{ h}^{-1}$ for the coastal and oceanic colloidal treatments under light conditions, respectively. The relative index I for coastal and oceanic colloids was estimated to be 8×10^{-4} and 2×10^{-3} , respectively, which were much lower than 1. For comparison, Sunda and Huntsman (1995) showed that the observed intracellular uptake rates of soluble labile Fe species were 4–6% of the maximum diffusion rate for *T. pseudonana* at low Fe concentrations. The extremely low index values suggested that under these conditions, uptake fluxes are limited and would remain limited even for colloids of much larger sizes. Furthermore, the low index suggested that the diffusive flux of colloidal iron did

not limit its biouptake. Instead, ligand exchange kinetics on the cell surfaces may control the internalization of macromolecular Fe. This situation is similar to the uptake of labile dissolved hydrolysis Fe species by marine diatoms (Hudson and Morel, 1990; Sunda and Huntsman, 1995), in which the limitation by ligand exchange kinetics is more important than diffusion limitation. This mechanism is consistent with our results that the colloidal Fe uptake rates were $3.9\text{--}7.2 \times$ lower than those of LMW treatments, which suggested that exchange kinetics of Fe from the colloids appear to be a critical step in controlling the biological availability of colloidal organic-bound Fe to phytoplankton.

Two mechanisms have been proposed for the uptake of organically complexed Fe, namely photo-reduction and biological reduction. Based on our results, it is possible to speculate the process responsible for the uptake of colloidal Fe by phytoplankton. There is mounting evidence that Fe undergoes a dynamic redox cycling in the upper water column. This cycling involves a photoreduction of Fe(III)–organic complexes either in solution or on particle surfaces, followed by dissociation of resultant Fe(II)–organic species to form Fe(II)', reoxidation of Fe(II)' by H_2O_2 and O_2 to form Fe(III)', and re-chelation of Fe(III)' (Sunda, 1994). In our experiments, considerable uptake of colloidal Fe by diatoms was also observed under dark conditions, although the fraction of intracellular uptake was much lower than that measured under light. However, the calculated concentration factor under dark and light conditions was comparable and in one treatment (oceanic), the uptake rate constant under dark conditions was even $3 \times$ higher than that under light conditions. Such a higher oceanic colloidal Fe uptake under dark condition remained unexplained, but may be due to the fact that the cells did not grow compared to the light treatment (e.g., caused by the difference in cell concentrations). The white fluorescence tubes used in our experiments were probably not sufficient to generate strong UV lights (e.g., $<300\text{--}400 \text{ nm}$), which may have resulted in strong photoreductive dissolution of colloidal Fe. Under natural conditions, therefore, we would expect that the colloidal Fe was removed at a much faster rate by marine phytoplankton than the rate measured in our study as a result of facilitating photoreduction.

Surface-associated natural colloidal Fe may have been internalized by the biological reduction (e.g., transplasma-membrane-bound redox enzymes), which is now believed to play an important role in the uptake of organic-bound Fe (Guerinot, 1994; Maldonado and Price, 1999, 2000). The reduction of organically complexed Fe by reductases located in the cell membranes was demonstrated to work efficiently by model calculations, with only a few electrons needed per atom Fe taken up (Volker and Wolf-Gladrow, 1999). Ferrireductase activity has been detected in several diatoms and green algae (Price and Morel, 1998). Colloidal-bound Fe(III) may be reduced to Fe(II), which may either be taken up directly across the cell membrane, or is re-oxidized rapidly to Fe(III) before internalization. Maldonado and Price (1999) provided evidence that large phytoplankton are capable of reducing Fe(III) bound in the organic complexes. More recently, Maldonado and Price (2000) concluded that in situ Fe(III) biological reduction rate may be comparable to the rate of phytoplankton demand for Fe in the Fe-limited regions, although biological reduction of Fe bound with organic complexes represented only a partial solution of Fe limited environment, because phytoplankton is still under Fe stress in these regions.

In summary, our bioavailability study provides the evidence that natural colloidal Fe can be biologically accessible for marine phytoplankton. Biological uptake of colloidal Fe may be mainly involved with biological transformation once the colloids are in contact with the phytoplankton. Our study indicates that natural colloids are actively linked to biogeochemical cycling in the ocean through biological uptake by phytoplankton. Colloidal Fe may provide an additional Fe source for the biological requirements of marine phytoplankton. The low rate of biological uptake of colloidal Fe is consistent with the mechanisms in which the algae use an uptake system based on Fe reduction at the cell surface, followed by ionic Fe transport (Soria-Dengg and Horstmann, 1995; Lynnes et al., 1998). Due to the slow ligand exchange kinetics, the uptake of organic Fe(III) is expected to be slow if Fe is organically complexed (Hudson and Morel, 1990). However, the bioavailability of colloidal Fe appeared to be greatly dependent on the origins and presumably the speciation of colloidal Fe in natural

seawater. Thus, the natural colloids may considerably affect our estimation of regenerated vs. new production in the oceans, especially in regions of Fe limitation. The amount of bioavailable Fe may be substantially underestimated by only considering the truly dissolved Fe or by assessing the input of 'external' (e.g., aerosol) iron. It is thus necessary to incorporate colloidal Fe uptake into the oceanic Fe biogeochemical models.

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