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Bioavailability of natural colloid-bound iron to marine plankton: Influences of colloidal size and aging

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Abstract

Iron (Fe) is mostly complexed with the organic ligands or colloids that are very abundant in natural seawater. In this study, natural colloids were isolated by ultrafiltration and radiolabeled with ^{59}Fe. The biological uptake of radiolabeled colloid-bound Fe of different sizes (1–10 kDa and 10 kDa–0.2 μm) and ages (1 and 15 d) by diatoms (*Thalassiosira pseudonana*) and copepods (*Acartia spinicauda*) was then determined. The uptake of radiolabeled colloid-bound Fe was compared with the uptake of low molecular weight (LMW) complexed Fe (<1 kDa) or that of EDTA-Fe (at a ratio of 1 : 2 for Fe : EDTA). Our study demonstrates that the colloid-bound Fe of different sizes and ages was bioavailable to the diatoms. The uptake of colloidal bound Fe was, however, 6–31 times lower than the uptake of LMW Fe, suggesting that colloidal binding reduced Fe bioavailability to diatoms. Fe bound with small colloids (1–10 kDa) was taken up at a higher rate than Fe bound with large colloids (10 kDa–0.2 μm) at typical colloidal organic carbon concentrations. The uptake of colloid-bound Fe was also much higher when the Fe had been bound with the colloids for 1 d rather than for 15 d. Differences in the colloidal organic carbon concentration did not appreciably affect the uptake of colloid-bound Fe by the diatoms. Similarly, copepods accumulated colloid-bound Fe at a much higher rate when the Fe was associated with small colloids rather than with large colloids. Direct ingestion of colloidal particles by the copepods appeared to be negligible. In both diatoms and copepods, Fe uptake may involve its dissociation from the colloids before being accumulated by the organisms. The study therefore demonstrates that colloid-bound Fe is less available for marine phytoplankton and that colloidal size and thus the colloidal speciation may be critical in controlling colloid-bound Fe uptake by aquatic organisms.

Iron is an important trace metal essential for the biological requirements of marine phytoplankton. It is mainly involved in electron transport, photosynthesis, and nitrate and nitrite metabolism (e.g., nitrate and nitrite reductase) (Geider and LaRoche 1994). Over the past decade, strong evidence has accumulated that Fe is the limiting micronutrient for phytoplankton productivity in several major oceanic (e.g., Southern Ocean, Equatorial Pacific, and Subarctic Pacific) and coastal upwelling regions (Martin and Fitzwater 1988; de Baar et al. 1990; Martin et al. 1994; Hutchins and Bruland 1998; Hutchins et al. 1998). These studies have pointed to the significance of understanding the biological requirements for Fe by marine phytoplankton, which has long been an issue in biological and chemical oceanography. It is generally accepted that Fe uptake by phytoplankton is a function of inorganic Fe concentration (Anderson and Morel 1982; Sunda and Huntsman 1997). In natural seawater, however, Fe is mostly (>98%) complexed with organic ligands, resulting in an extremely low inorganic Fe concentration (Wu and Luther 1995; Rue and Bruland 1995, 1997). In Fe-limited oceanic regions, inorganic Fe concentrations are at the subpicomolar levels, much lower than the biological requirements of primary producers (Sunda and Hunstman 1997).

Marine colloids are operationally defined as those submicron particles or macromolecules within the size range 1 kDa to 0.2 μm. They are very abundant in natural seawater (Buffle and Leppard 1995; Guo and Santschi 1997). Over the past 10 yr, extensive studies have quantified the abundance of colloids and their association with trace metals and carbon in estuarine, coastal, and oceanic waters (Martin et al. 1995; Guo and Santschi 1997; Santschi et al. 1999). The quantitative significance of metal partitioning in the colloidal phase has been shown to be greatly dependent on the metals and geochemical properties such as the level of dissolved organic carbon and salinity. For several metals (e.g., Cd), a negligible fraction was detected in the colloidal phase; whereas for other metals (e.g., Fe), almost all is associated with the colloidal phase (Santschi et al. 1999). Fe has also been shown to partition in a different size spectrum of colloidal particles (particularly in the large size of colloids, Wen et al. 1999; Wells et al. 2000). Nishioka and Takeda (2000) showed that the addition of diatom *Chaetoceros* sp. considerably affected the dynamics of colloid-bound Fe (200 kD to 0.2 μm), implying that the small colloidal particles are the most dynamic fraction during the growth of diatoms. Previous studies on metal uptake, however, almost all were concerned with metals in the dissolved phase, which typically includes metals in the colloidal phase. Few studies have specifically quantified the availability of natural colloid-bound metals to aquatic organisms, including marine phytoplankton (Carvello et al. 1999; Wang and Guo 2000).

Among the limited studies on the biological uptake of colloid-bound Fe by marine phytoplankton, almost all used

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inorganically synthesized colloids (Wells et al. 1983; Rich and Morel 1990; Kuma and Matsunaga 1995; Nodwell and Price 2001). These earlier studies suggest that the bioavailability of colloid-bound Fe is related to the thermodynamic stability and kinetic lability of the colloid-bound ferric oxide phases. Thus, a few inorganic colloids with low solubility (such as beta and gamma Fe oxides) did not support the growth of phytoplankton, whereas other inorganic colloids (such as a hexanuclear complex and a polynuclear oxidation product) did support the growth of algae due to their greater lability. More recent studies have focused on the availability of organically complexed Fe to marine phytoplankton, typically using well characterized organically synthesized compounds such as siderophore-like compounds (Hutchins et al. 1999; Maldonado and Price 1999, 2000; Boye and van den Berg 2000; Kuma et al. 2000*b*). Although a few studies demonstrated that some of the organically complexed Fe was not bioavailable to phytoplankton (Wells 1999; Hutchins et al. 1999; Boye and van den Berg 2000), other studies have shown that a limited amount of organically complexed Fe can indeed be bioavailable to phytoplankton (Soria-Deng and Horstmann 1995; Maldonado and Price 1999, 2000; Kuma et al. 2000*b*). Hutchins et al. (1999) further showed the specificity of organic Fe uptake by prokaryotes and eukaryotes.

The biological acquisition of colloid-bound Fe from natural seawater by marine phytoplankton remains mostly speculative. Various mechanisms for the uptake of colloidal or organic Fe have been proposed, including photolysis (Waite and Morel 1984; Wells et al. 1991; Johnson et al. 1994), biological reduction (Price and Morel 1998; Maldonado and Price 1999, 2000), and grazing regeneration (Barbeau et al. 1996; Barbeau and Moffett 2000). All these mechanisms require that the inorganic or free ion species [Fe(II) and Fe(III)] are the ultimate Fe species taken up by marine phytoplankton. Recently, we have quantified the biological uptake of Fe bound with natural colloids isolated from different locations (coastal waters, harbor waters, and estuarine waters) by marine diatoms and natural phytoplankton assemblages (Chen and Wang, pers. comm.). The uptake of Fe bound with estuarine colloids was much lower than the uptake of Fe bound with coastal colloids, indicating that the origin of the colloids considerably affected the biological uptake. This study further examines the influence of colloid size and aging on Fe uptake by marine diatoms and copepods. Controlled experiments were conducted to ensure that the radiolabeled colloid-bound Fe remained mostly in the colloidal phase; thus measurements of colloid-bound Fe uptake using a radiotracer technique truly represent the uptake of colloid-bound Fe. The biological uptake of colloid-bound Fe was quantified as the intracellular accumulation by the diatoms. In copepods, the distribution of colloid-bound Fe in the exoskeleton and soft tissues was also determined, to investigate whether colloid-bound Fe can indeed be accumulated by copepod tissues.

Materials and methods

Plankton—A coastal diatom *Thalassiosira pseudonana* (Clone 3H) was obtained from the Provasoli-Guillard Phytoplankton Collection Center and maintained in f/2 medium (Guillard and Ryther 1962) in axenic culture at 18°C. Cells in the exponentially growing phase were collected and resuspended in nutrient-depleted low molecular weight seawater (<1 kD, LMW) before the uptake experiments. The LMW water was obtained following ultrafiltration (described below). The copepod *Acartia spinicauda* was collected by net towing from Clear Water Bay, Hong Kong, and maintained in the laboratory for 1 d before the uptake experiments (temperature of 18°C and salinity of 30 psu). During the acclimation period, the copepods were fed with the nonradioactive diatom *T. pseudonana.* One hour before the uptake experiments, the copepods were removed from their feeding suspension and placed in LMW seawater to defecate their feces to minimize the potential influence of fecal production on colloidal uptake by the copepods.

Table 1. Summary of experiments examining the biological uptake of colloid-bound Fe by the marine diatom *Thalassiosira pseudonana* and copepods *Acartia spinicauda.* DOC: dissolved organic carbon. ^{59}Fe labeling efficiency was calculated as the amount of ^{59}Fe labeled onto the colloids after the dialysis divided by the amount of ^{59}Fe spiked into the colloids.

Experiment	Colloids	^{59}Fe labeling efficiency (%)	Background DOC concentration (μM)
Diatoms			
Colloidal aging	1-d labeling colloids	29.1	123
	15-d labeling colloids	44.2	123
Colloidal size	1–10 kDa colloids	68.9	93
	>10 kDa colloids	88.0	93
Copepods			
Colloidal size	1–10 kDa colloids	50.3	93
	>10 kDa colloids	85.3	93

Isolation of colloids—Natural seawater (salinity of 27 psu) was collected from Tolo Harbor, Hong Kong, and stored in the laboratory overnight before the ultrafiltration. The background dissolved organic carbon concentration in each experiment is shown in Table 1. The seawater was first passed through the glass fiber filter and then through a 0.22-μm Poretics cartridge. The filtered seawater was then ultrafiltered by a spiral-wound cross-flow ultrafiltration cartridge (Amicon S10Y10) with a 10 kDa cutoff (Guo and Santschi 1996; Guo et al. 2000; Wang and Guo 2000) and then further ultrafiltered by ultrafiltration cartridge (Amicon S10Y1) with a 1 kDa cutoff. All ultrafiltration was performed in enclosed system to avoid contamination of the seawater sample. The concentration factor in each ultrafiltration step was about 40. The collected colloids were immediately radiolabeled with ^{59}Fe (obtained from New England Nuclear, in 0.1 N HCl). ^{59}Fe (296 kBq, corresponding to 122 nM) was spiked into 70 ml of colloids in a Telfon bottle. After radiolabeling (described below), the colloids were placed in a dialysis bag (1 kD or 10 kD) suspended in

Table 2. Summary of experimental treatments used in different experiments to examine the biological uptake of Fe by marine diatoms and marine copepods. COC: colloidal organic carbon. Radiolabeled colloids were added to result in different COC concentrations (and thus spiked Fe concentrations).

Experiments	Treatment	COC concentration (μM)	Fe spiked concentration (nM)
Diatoms			
LMW Fe uptake	LMW Fe	0	4.1
^{59}Fe-EDTA (1:2) uptake	EDTA-Fe	0	2.1
Colloidal size	1–10 kDa (LC)	24	2.8
	1–10 kDa (HC)	72	8.4
	>10 kDa (LC)	13	2.8
	>10 kDa (HC)	39	8.4
Colloidal aging	1-d labeling colloids (LC)	24	3.0
	1-d labeling colloids (HC)	72	9.0
	15-d labeling colloids (LC)	24	3.0
	15-d labeling colloids (HC)	72	9.0
Copepods			
Colloidal size	1–10 kDa (LC)	24	2.3
	1–10 kDa (HC)	72	11.5
	>10 kDa (LC)	13	2.3
	>10 kDa (HC)	39	11.5

LMW seawater for 2–3 d. Any unlabeled Fe was removed by replacing the LMW seawater (2.2 L) every 12 h. Preliminary experiments demonstrated that after 2–3 d there was a negligible amount of ^{59}Fe released into the LMW seawater (corresponding to <0.34 pM), indicating that the majority of remaining Fe was in fact labeling the colloids. The radiolabeling efficiency of ^{59}Fe, calculated as the amount of ^{59}Fe labeled onto the colloids after the dialysis divided by the amount of ^{59}Fe spiked into the colloids, in each experiment is shown in Table 1. The colloids were then filtered again through a 0.2-μm polycarbonate membrane and immediately used for the experiments.

The influences of colloidal size and aging on Fe uptake by marine phytoplankton were tested. Two different colloid sizes were considered in this study: 1–10 kD and 10 kD–0.2 μm. In the colloidal size experiments, both colloids were radiolabeled for 2 d. In the colloid aging experiments, the colloids (>1 kD–0.2 μm) were radiolabeled for 1 and 15 d, respectively, and the unbound Fe was removed with a dialysis bag, as described above.

Diatom uptake of colloid-bound Fe—Diatom cells in the exponential growing phase were filtered from the f/2 growth medium and resuspended in LMW seawater without the addition of macronutrient (i.e., nutrient depleted) for 1 d before the uptake experiments. The diatom cells were subsequently filtered onto a polycarbonate membrane (3 μm), rinsed with LMW seawater, and distributed into 150 ml LMW seawater held in a polycarbonate bottle. Trace metal clean technique was employed to minimize potential Fe contamination of the samples, although uptake of the radiolabeled colloid-bound Fe was the primary concern. The cells were added at a density of 2×10^5 cells ml^{-1} (corresponding to 3.2 mg C L^{-1}). The radiolabeled colloids were then added to the polycarbonate bottle at two colloidal organic carbon (COC) concentrations (Table 2). The lower COC concentration was similar to natural COC concentrations in the field (calculated from the concentration factor), whereas the higher COC concentration was three times higher than the COC concentration in natural seawater. In the control treatment, no diatom was added, and radiolabeled colloids were added at the natural background concentration. The controlled treatment was used for monitoring the partitioning of Fe and the possible coagulation of colloids. There were two replicated bottles for each experimental treatment.

The colloid-bound Fe uptake was compared with the uptake of Fe complexed with EDTA or LMW Fe. ^{59}Fe was complexed with EDTA at a ratio of 1:2 (with a ^{59}Fe concentration of 2.1 nM) before spiking into the LMW water. Previous studies have indicated that the speciation of Fe(III) was minimally affected by complexing with EDTA at nanomolar concentrations under equilibrium (Gerringa et al. 2000). In a separate experiment, the uptake of LMW Fe by the diatoms was also measured. To obtain the LMW complexed ^{59}Fe, the colloids were labeled as described above, and the LMW complexed ^{59}Fe was separated by the dialysis bag. The LMW complexed ^{59}Fe was then added into LMW seawater containing the diatoms, and its uptake was subsequently measured. In this study, whether Fe in the LMW water was inorganically or organically complexed was not clear.

The diatoms were incubated at a light illumination of 70 μmol m^{-2} s^{-1} and at 18°C. At different times, the cells were taken for cell density measurements using a Coulter counter. A 1-ml water sample was removed for measurement of ^{59}Fe radioactivity. A 10-ml water sample was filtered onto a 1-μm polycarbonate membrane, rinsed with Ti-EDTA-citrate regents (Hudson and Morel 1989) for 2 min, and then further

rinsed with LMW seawater. The filter (representing the intracellular ^{59}Fe) was then measured for radioactivity. At the end of the exposure, the total radioactivity of ^{59}Fe in the diatom cells was also measured by filtering a 10-ml sample onto the polycarbonate membrane (0.2-μm or 3-μm pore size) and rinsing with LMW seawater. The distribution of ^{59}Fe in the diatom cytoplasm was quantified using a method described by Fisher et al. (1983) and Reinfelder and Fisher (1991). Briefly, a 30-ml sample was filtered onto the polycarbonate membrane and the loosely surface bound Fe was removed by washing with 0.1 mM EDTA. The cells were then resuspended in 5 ml of Nanopure distilled water and frozen. The thawed samples were centrifuged sequentially at 2,000 g (15 min) and 20,000 g (20 min). The supernatant was defined at the cytosolic fraction.

We used the percentage of intracellular Fe uptake to quantify the colloid-bound Fe uptake by the diatoms. The percentage of intracellular Fe uptake was calculated as the radioactivity detected in the intracellular compartment of diatoms (measured after the Ti washing) divided by the total ^{59}Fe radioactivity in the aqueous phase. Consequently, such calculation did not require measurement of total ambient Fe concentration in the water. The percentage of intracellular Fe uptake was further normalized to cell concentration to calculate the concentration factor (CF, L kg^{-1}), according to the following equation:

$$\mathrm{CF} = C_f/C_w \quad (1)$$

where C_f is the radioactivity in the intracellular diatoms (ccpm kg^{-1} dry weight) and C_w is the radioactivity in seawater (ccpm L^{-1}). Thus, the uptake rate constant can be calculated from the slope of the linear regression between the CF against time of exposure (4–48 h) and the uptake rate can be calculated as the uptake rate constant times metal concentration in the aqueous phase. In this study, we used the uptake rate constant to compare the Fe uptake among different treatments. It was assumed that the uptake rate constant was independent of the Fe concentration within 2–8 nM used in our study.

The percentage of total cellular Fe in diatom's intracellular compartment was calculated as the radioactivity in diatom after Ti washing divided by the radioactivity in diatom without Ti washing. The percentage of total cellular Fe in diatom's cytoplasm was calculated as the radioactivity in diatom's cytoplasm (after fractionation) divided by the radioactivity in diatoms without Ti washing.

The partitioning of radiolabeled colloid-bound Fe after the exposure to the diatoms was also quantified. To quantify the distribution of ^{59}Fe into the truly dissolved phase and the colloidal phase (>1 kDa–0.2 μm), a stirrer cell with a 1 kDa ultrafiltration membrane (Amicon YM1, regenerated cellulose) was used. The permeate was squeezed by N_2 gas at a pressure of 30 psi until the membrane was close to dry. The radioactivity in the membrane and permeate was counted. To quantify the distribution of ^{59}Fe in the >10 kD fraction, a centrifugal ultrafilter with a molecular weight cutoff of 10 kDa was used. The filter was centrifuged at 15,000 g for 40 min until the membrane was nearly dry. The percentage partitioning of total dissolved ^{59}Fe in the colloidal phase (1 or 10 kDa to 0.2 μm) or truly dissolved phase was then calculated. In addition, in one experiment (uptake of LMW ^{59}Fe), we also measured the distribution of total ambient Fe in different size fractions (>3 μm, 0.2–3 μm, 1 kDa–0.2 μm, and truly dissolved phase).

Copepod uptake of colloid-bound Fe—We compared the uptake by the copepods of ^{59}Fe bound with colloids of different sizes (1–10 kDa, and 10 kDa–0.2 μm). The copepods were placed in 50 ml of LMW seawater spiked with radiolabeled colloids at two COC concentrations (Table 1). The low COC concentration was similar to the natural background concentration. The high COC concentration was three times higher than the background COC concentration. The actively swimming copepods were added at a density of 1 copepod ml^{-1} and kept in the dark. No diatom was provided to the copepods during the uptake experiments. There were three replicates for each experimental treatment. At 2, 4, and 8 h of exposure, copepods were removed with a nylon mesh, rinsed with LMW seawater, and placed in a counting tube containing 3 ml LMW seawater; then their radioactivity was counted. Short-term exposure was employed to minimize the starvation effects on the physiology of copepods. After the radioactivity measurement, the copepods were then returned to a new batch of seawater containing the same concentration of colloids. In general, the decrease of ^{59}Fe radioactivity in the water due to copepod uptake and surface sorption onto the glassware was <10% during the exposure period.

The accumulation of ^{59}Fe by copepods was similarly quantified by the concentration factor using Eq. 1, where C_f is the radioactivity in the copepods (ccpm kg^{-1} tissue dry weight), and C_w is the radioactivity in seawater (ccpm L^{-1}). Because there was about a 10% decrease in the radioactivity of the water during the exposure period, C_w was calculated as the mean of the radioactivity measured in the seawater before and after the exposure. The copepod dry weight, measured after drying the copepods at 80°C overnight, was 13.4 μg for each individual. Similarly, the uptake rate constant was calculated from the slope of the linear regression between CF against time of exposure (h).

At the end of 8 h exposure, the distribution of ^{59}Fe in the soft tissue and exoskeletons of the copepods was determined as described by Wang and Fisher (1998). The copepods were filtered onto an 8-μm polycarbonate membrane. The surface weakly bound ^{59}Fe was removed with a 0.1-mM EDTA rinse. The copepods were then placed in 3 ml of 0.2 N NaOH at 70°C for 3 h. The extracted soft tissues were filtered through an 8-μm polycarbonate membrane and radioassayed. The exoskeleton was retained by the membrane and radioassayed.

Radioactivity and organic carbon measurements—The radioactivity of ^{59}Fe in the sample was measured by a Wallac 1480 gamma detector at 1092 keV. Counting time was adjusted to result in a counting propagated error <5%. The dissolved organic carbon concentration of the seawater sample was measured by a Shimadzu-5000A TOC analyzer as described in Guo et al. (1994).

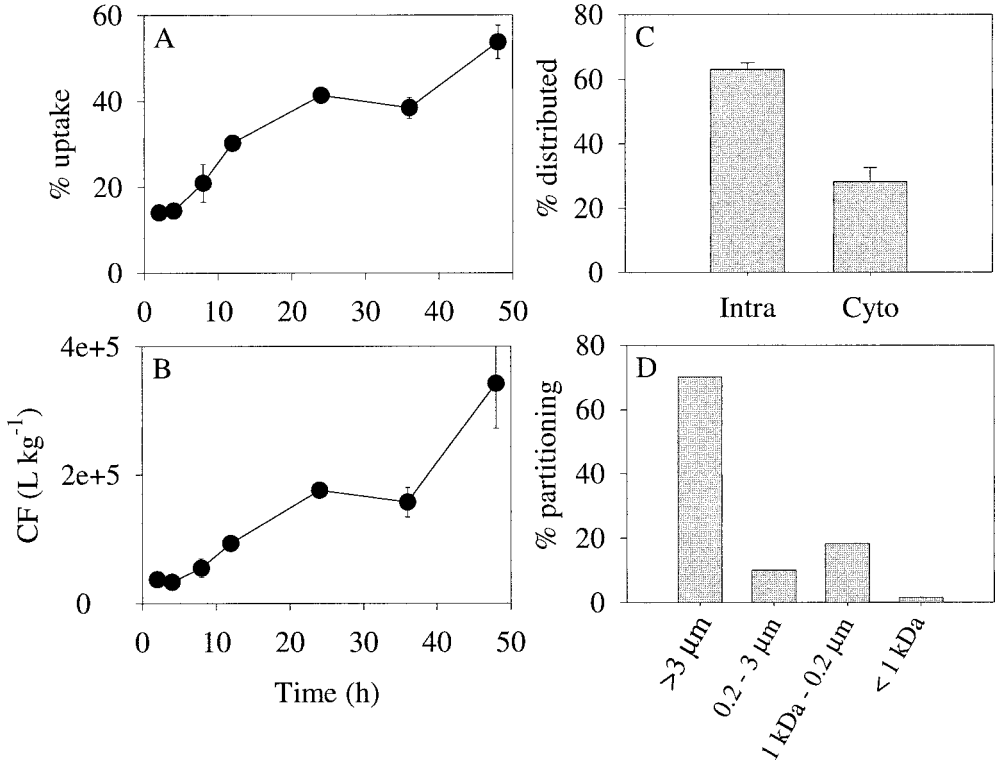

Fig. 1. *Thalassiosira pseudonana.* The uptake of low molecular weight ^{59}Fe (<1 kDa) by the diatom during the 2-d exposure period. (A) percentage of intracellular ^{59}Fe uptake by the diatoms; (B) concentration factor (CF) of ^{59}Fe in diatoms; (C) distribution of total cellular ^{59}Fe in the intracellular compartments (Intra) and cytosolic compartment (Cyto) of diatoms by the end of 2 d exposure; (D) partitioning of total ambient ^{59}Fe in different size fractions by the end of 2 d exposure. Mean ± semirange (n = 2).

Results

Diatom uptake of colloid-bound Fe—In general, there was no major growth of the diatoms during the experiments because the cells had previously been exposed to LMW water without macronutrient addition for 1 d before the colloid-bound Fe uptake measurements. When the diatoms were exposed to ^{59}Fe in the low molecular weight fraction (LMW), there was an approximately linear intracellular uptake of ^{59}Fe (radioactivity in intracellular compartment divided by radioactivity in water) over the 2-d exposure period (Fig. 1). The calculated concentration factor (CF) of ^{59}Fe was also approximately linear within the 2-d exposure period (Fig. 1B). The uptake rate constant, calculated from the linear regression between the CF against time of exposure (4–48 h), was 6,112 ± 1,194 L kg^{-1} h^{-1}. By the end of the 48 h exposure, 63% of total cellular ^{59}Fe was associated with the intracellular compartment, whereas 28% of total cellular ^{59}Fe was associated with the cytoplasmic component (Fig. 1C). The majority of the total ^{59}Fe (77%) was detected in the >3-μm fraction (diatoms), and about 20% was in the dissolved phase (<0.2 μm) (Fig. 1D). When the ^{59}Fe in the total dissolved phase was further partitioned into the colloidal (1 kDa −0.2 μm) and truly dissolved phase, about 92% of total dissolved Fe was present in the colloidal phase (1 kDa–0.2 μm), and about 8% of dissolved Fe was in the truly dissolved phase (or LMW fraction, <1 kDa).

Among the four experiments carried out to determine colloid-bound Fe uptake by the diatoms, a very small fraction of colloid-bound Fe was detected in the >1-μm fraction (e.g., colloidal coagulation) in the control treatment without diatoms (<4% in all experiments) (Fig. 2). Among these four different radiolabeled colloids, it appeared that the >10 kDa colloids were the most stable, with the smallest fraction being detected in the >1-μm fraction. Partitioning of ^{59}Fe in the colloidal phase in the control treatment was also measured by the end of 48 h exposure (Fig. 2). The majority of ^{59}Fe (80–93%) remained in the colloidal phase after 2-d suspension in LMW water.

The uptake of colloid-bound Fe by diatoms followed a linear pattern over the 2-d exposure period, with a few exceptions noted (Figs. 3 and 4). Fe complexed with EDTA had the highest uptake among the different treatments. A significant difference in Fe uptake was found among different colloidal treatments in experiments examining the influence of colloid size on Fe uptake (Fig. 3). In this experiment, about 21% of total ambient small colloid-bound Fe (1–10 kDa) was biologically taken up (i.e., in the intracellular compartment) by diatoms after 2 d exposure at the low colloidal organic carbon (COC) concentration, whereas only 5% of

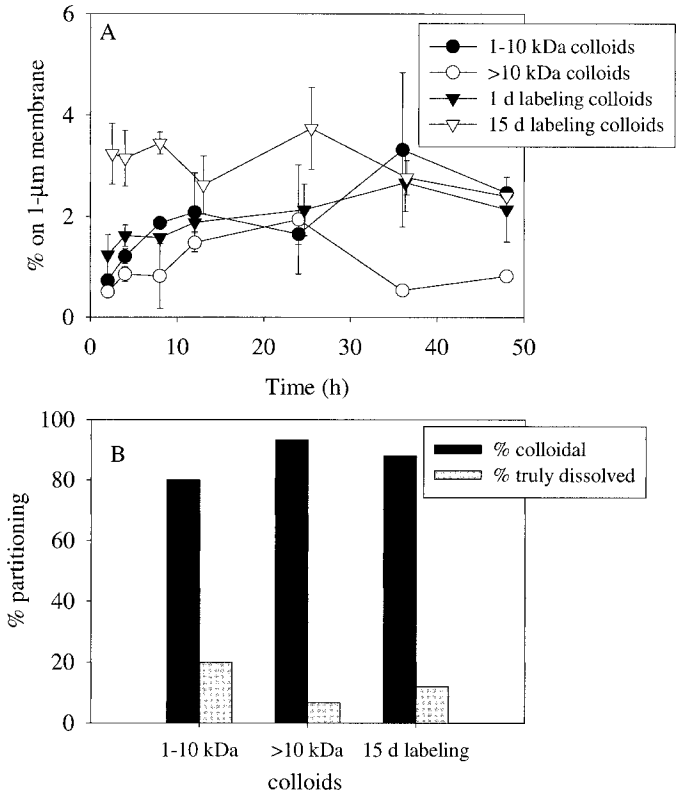

Fig. 2. (A) The fractions of ^{59}Fe detected in the >1-μm fraction following the addition of radiolabeled colloids to low molecular weight seawater without diatoms, and (B) the partitioning of ^{59}Fe in the colloidal phase and truly dissolved phase after 2 d without diatom. Mean ± semirange (n = 2).

total ambient large colloid-bound Fe (>10 kDa) was biologically taken up by the diatoms (Fig. 3A). At a higher COC concentration, 8% of small colloid-bound Fe was biologically taken up by the diatoms, comparable to the accumulation of larger colloid-bound Fe. The uptake rate constant, calculated from a linear regression between the CF against time of exposure (4–48 h, Fig. 3B), was significantly higher for small colloids than large colloids at low COC concentration ($p < 0.001$, t-test), but was comparable between these two treatments at high COC concentration (Fig. 5). The uptake of small colloids at the low COC concentration was also comparable to the uptake of Fe complexed with EDTA. In this experiment, a high percentage of total cellular ^{59}Fe was found in the intracellular compartment with the small colloidal treatment at the low COC concentration (57%), whereas only 28–31% of total cellular Fe was in the intracellular compartment in the other colloidal treatments (Fig. 3C). There was no major difference in the distribution of ^{59}Fe in the cytoplasm of the diatoms among the different treatments, except for ^{59}Fe bound with large colloids (>10 kDa) at a low COC concentration (Fig. 3C). By the end of the exposure, the majority of ambient total dissolved ^{59}Fe (>90%) remained in the colloidal phase (Fig. 3D). For EDTA-Fe, about 71% of total dissolved ^{59}Fe was detected in the colloidal phase (and 29% in the truly dissolved phase, <1 kDa).

Diatoms appeared to have a much higher uptake of EDTA-Fe than of colloid-bound Fe radiolabeled for 1 and 15 d (Fig. 4). By the end of 48 h exposure, about 35% of EDTA-Fe was biologically taken up by the diatoms, whereas 19–22% of 1-d radiolabeled colloid-bound Fe and 9–15% of 15-d radiolabeled colloid-bound Fe was taken up by the diatoms (Fig. 4A). The calculated uptake rate constant of Fe is shown in Fig. 5. In this experiment (Fig. 5B), the uptake rate constant of EDTA-Fe was comparable to the uptake of EDTA-Fe in the experiment involving different sizes of colloids (Fig. 5A). Different COC concentrations did not affect the rate of uptake of colloid-bound Fe by the diatoms, whereas the colloid-bound Fe radiolabeled for 1 d had a much greater uptake rate constant (2.7–3.4 times) than colloid-bound Fe radiolabeled for 15 d. There was no notable difference in the distribution of total cellular Fe between the intracellular and cytoplasmic compartments among the different treatments, except for colloids radiolabeled for 15 d at a high COC concentration (Fig. 4C). After 48 h of exposure, the majority of ambient dissolved ^{59}Fe (>85%) remained in the colloidal phase (Fig. 4D). For EDTA-Fe, about 68% of total dissolved ^{59}Fe was in the colloidal phase.

Colloid-bound Fe uptake by copepods—The uptake of radiolabeled colloid-bound Fe by marine copepods is shown in Fig. 6. There was a linear pattern of uptake over the 8-h exposure period. The calculated concentration factor of colloid-bound Fe was much higher for the small colloids than for the large colloidal treatment (Fig. 6A). For example, by the end of the 8-h exposure period, the calculated dry weight concentration factor (CF) of Fe in the copepods was 6.9–8.0 times higher with the 1–10 kDa colloids than with the >10 kDa colloids. The uptake rate constant was calculated from the slope of the regression between CF against time of exposure and was 7.4–10.9 times higher for the small colloidal treatment (335–375 L kg^{-1} h^{-1}) than for the large colloidal treatment (31–50 L kg^{-1} h^{-1}) (Fig. 6B). There was no significant difference in the calculated CF or uptake rate constant between the two COC concentrations for each colloidal size. After 8 h of exposure, the percentage of total copepod ^{59}Fe distributed in the tissue of the copepods was 62–63% for the >10 kDa colloids and 73–77% for the smaller colloids (Fig. 6C). The majority of radiolabeled colloid-bound Fe also remained in the colloidal phase (>93%) after the 8-h exposure (Fig. 6D).

Discussion

The majority of colloid-bound Fe remained in the colloidal phase during the uptake period, thus the measured uptake of radiolabeled Fe reflected predominantly uptake from the colloidal phase. Both Fe in the LMW fraction (or truly dissolved, <1 kDa) and Fe complexed with EDTA at nanomolar concentrations were used to compare with the measurements of colloid-bound Fe uptake by diatoms. In these LMW and EDTA-Fe treatments, a large fraction of the dissolved Fe was in fact detected in the colloidal phase by the end of the 48-h exposure period. One possibility is that colloidal particles may have been produced by diatom exudation, which then complex with the LMW Fe in the water.

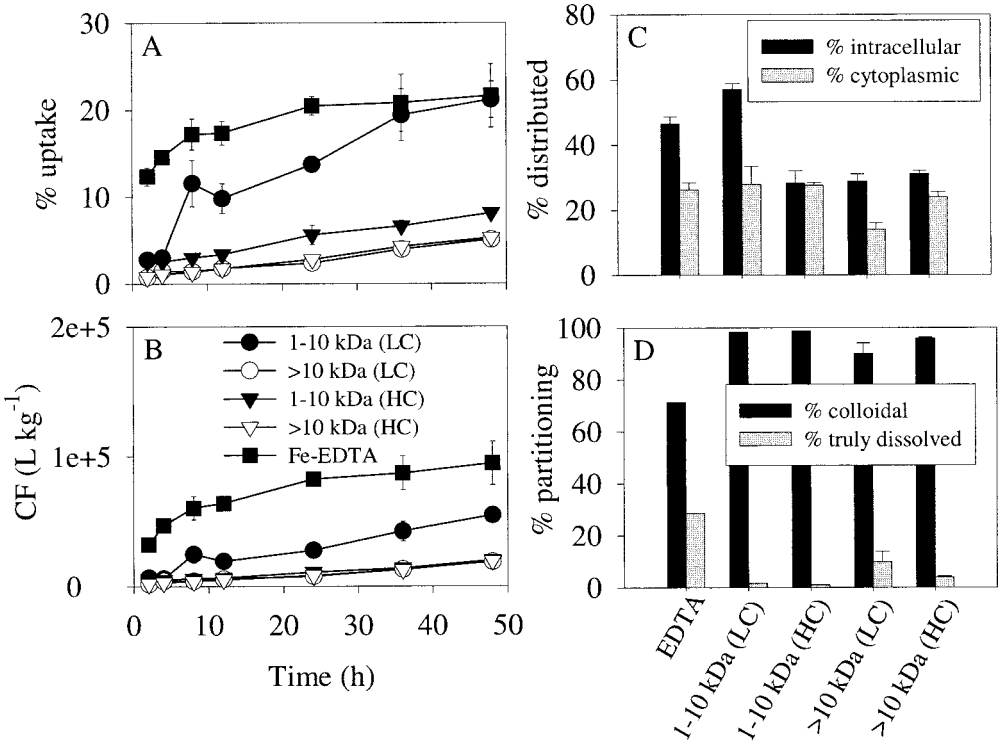

Fig. 3. The uptake of ^{59}Fe bound with different sizes of natural colloids by the diatom *Thalassiosira pseudonana* during the 2-d exposure period. (A) percentage intracellular ^{59}Fe uptake by the diatoms; (B) concentration factor (CF) of ^{59}Fe in diatoms; LC, low colloidal organic carbon concentration, HC, high colloidal organic carbon concentration; (C) distribution of the total cellular ^{59}Fe in the intracellular and cytosolic compartments of diatoms by the end of 2-d exposure; (D) partitioning of total dissolved ^{59}Fe in the colloidal (1 kD–0.2 μm or 10 kDa–0.2 μm) and truly dissolved phases ($<$1 kD or $<$10 kDa) at the end of 2-d exposure. Mean $\pm$ semirange ($n = 2$).

Alternatively, the fact that there was conversion from the truly dissolved Fe to the colloidal Fe suggests that there was no strong organic complexation of the Fe or that significant concentrations of Fe may already present in excess of the organic complexing capacity of the seawater. In EDTA-Fe(III) (2 : 1) medium, about 70% of the added Fe(III) may be present in the dissolved phase after 24 d because of the slow dissociative precipitation rate of EDTA-Fe (2 : 1) when added into the seawater (Kuma et al. 1999). Because the addition of a nanomolar amount of EDTA will not change the equilibrium speciation in ambient seawater, uptake of EDTA-Fe may reflect the uptake of inorganic Fe as it was dissociated from the EDTA. The Fe uptake was quantified as the biological intracellular accumulation, thus Fe sorption onto the algal surface was not considered when comparing the uptake of colloid-bound Fe. The relative uptake of colloid-bound Fe (i.e., the ratio of the radioactivity of the intracellular Fe in the diatoms to the radioactivity in the ambient water) was used to quantify the Fe uptake. The uptake rate constant, which has been used extensively in quantifying metal uptake in aquatic organisms (e.g., Wang et al. 1996; Wang 2001), can be calculated from the regression between the concentration factor of Fe and the time of exposure.

The calculated biological uptake rate constant of LMW Fe by diatoms was about 6–31 times higher than the uptake rate of colloid-bound Fe. The uptake rate of EDTA-Fe was somewhat comparable to the uptake rate of colloid-bound Fe, although the total accumulation of EDTA-Fe was also much higher than that of the colloid-bound Fe. Because of the exchange reaction between the premixed EDTA-Fe(III) and the major alkaline earth metals (such as Ca^{2+} and Mg^{2+}), the dissociation of Fe(III) from the EDTA-Fe(III) complex is generally slow but is an important process in supplying the biologically available Fe by increasing the dissolved Fe(III)′ concentration. It should be noted that the uptake rate of ^{59}Fe was calculated based on the measurements between 4 and 48 h of exposure, thus the initial uptake (the first 4 h) was not considered. For inorganic colloids, Kuma and Matsunaga (1995) indicated that the short-term uptake rate of iron by the dinoflagellate *H. akashiwo* in Fe(III)-EDTA medium was about eight times faster than that in solid amorphous hydrous ferric oxide ($Fe_2O_3 \cdot H_2O$). Kuma et al. (1999) further found that short-term Fe uptake by *Chaeoceros sociale* was about five to six times faster for fulvic-Fe(III) and citric-Fe(III) than for EDTA-Fe(III) (100 : 1) and solid amorphous Fe(III).

The much lower biological Fe uptake from the colloidal phases compared to Fe in the LMW fractions strongly suggests that the biological uptake of Fe was inhibited by its complexation with colloids. This is consistent with the general notion that the biological uptake of Fe is a function of the concentration of free or inorganic iron species (Anderson

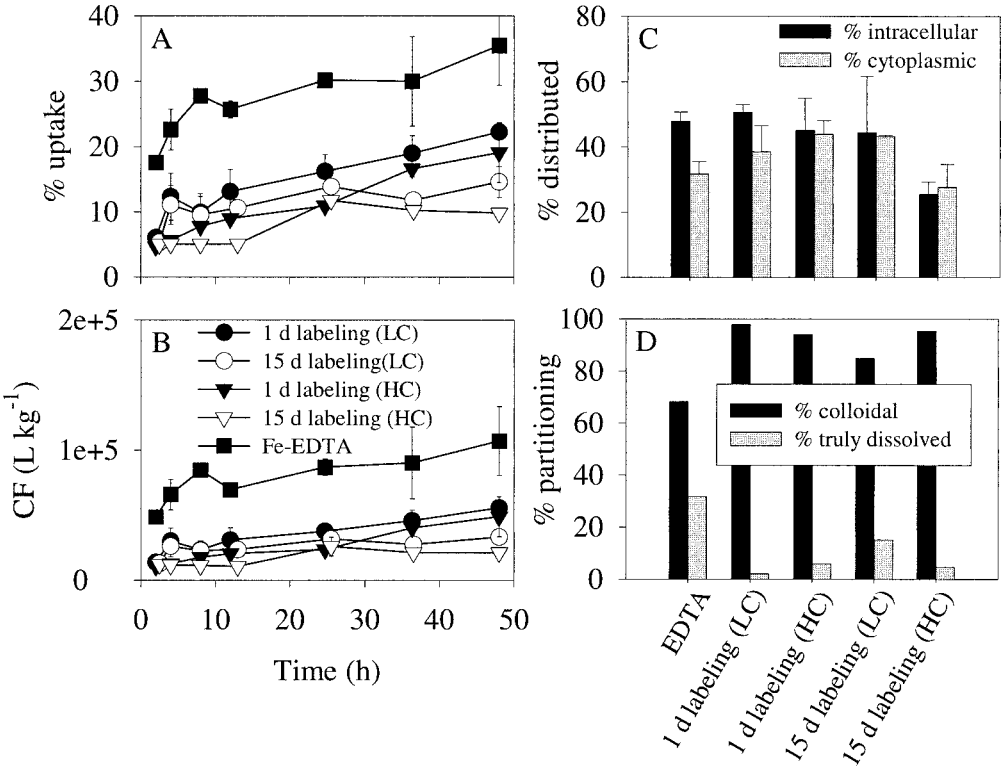

Fig. 4. The uptake of ^{59}Fe bound with natural colloids for different periods by the diatom *Thalassiosira pseudonana* during the 2-d exposure period. (A) percentage intracellular ^{59}Fe uptake by the diatoms; (B) concentration factor (CF) of ^{59}Fe in diatoms; LC, low colloidal organic carbon concentration, HC, high colloidal organic carbon concentration; (C) distribution of total cellular ^{59}Fe in the intracellular and cytoplasmic compartments of diatoms by the end of 2-d exposure; (D) partitioning of total dissolved ^{59}Fe in the colloidal (1 kD–0.2 μm) and truly dissolved phases (<1 kD) at the end of 2 d exposure. Mean ± semirange (n = 2).

and Morel 1982; Harrison and Morel 1986; Campbell 1995; Sunda and Huntsman 1997). Nevertheless, these results do indicate that natural colloid-bound Fe may be a source for Fe uptake by marine phytoplankton and may have significant implications for the prediction of Fe uptake in natural seawater, in which most of the traditionally defined dissolved Fe is in fact in the colloidal phase or organically complexed (Wu and Luther 1995; Rue and Bruland 1995, 1997). In Fe-limited oceanic regions, the concentrations of inorganic Fe species are extraordinarily low as a result of organic complexation (e.g., 0.1–0.01 pM in the subarctic Pacific, Rue and Bruland 1995, which was much lower than the minimum Fe requirement by oceanic phytoplankton, Sunda et al. 1991). Colloid-bound Fe uptake, albeit at a much lower rate than the uptake of LMW Fe, must have provided a source for Fe in these regions given its much higher concentrations (e.g., more than two orders of magnitude) than the inorganic Fe concentrations.

It is generally accepted that the metal must be first dissociated from its complexing ligands, and the free ion is subsequently complexed with a membrane transporting ligand before it can be taken up intracellularly (Campbell 1995; Kuma et al. 1999). For Fe, recent studies have suggested that transplasmalemma bound redox enzymes may play an important role in the uptake of organic bound Fe (Guerinot 1994; Maldonado and Price 1999, 2000). For example, ferrireductase activity has been detected in several diatoms and green algae (Price and Morel 1998). In our study, biological reduction at the cell surface is presumably important for the acquisition of colloid-bound Fe. For most metal species, direct transport of metal ions (e.g., passive diffusion) is thought to be less important (except for a few lipophilic metal ions that may diffuse easily across the biological membrane, Simkiss and Taylor 1989). Direct transport of colloids across the cell membrane is also unlikely (Rich and Morel 1990; Sunda and Huntsman 1997; Price and Morel 1998; Gerringa et al. 2000).

Another possibility for the uptake of colloidal Fe is by photoreduction, which reduces colloidal Fe to Fe(II) before the rapid uptake by phytoplankton across the biological membrane (Rich and Morel 1990; Wells et al. 1991; Johnson et al. 1994). We have recently demonstrated that a considerable fraction of colloidal Fe was accumulated by the diatom *T. pseudonana* under the dark conditions, further implying that the biological reduction is presumably critical for the acquisition of colloidal Fe (Chen and Wang, pers. comm.). In our study, photoreduction was probably not important in converting the colloidal Fe to the bioavailable species. The white fluorescence tubes used in our experiments may not be sufficient to generate strong UV lights (e.g.,

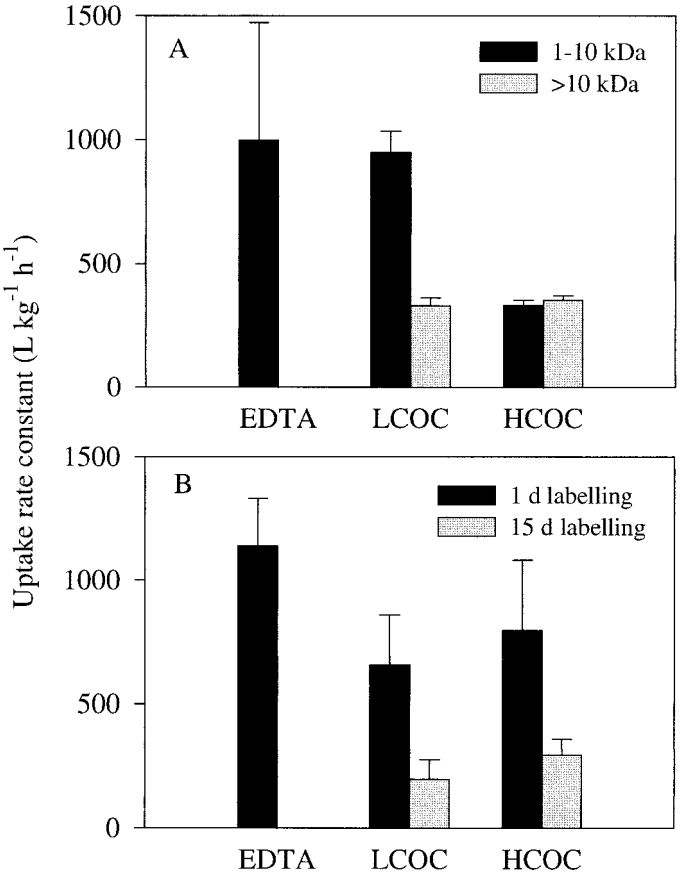

Fig. 5. The calculated uptake rate constant of ^{59}Fe by the diatom *Thalassiosira pseudonana.* (A) ^{59}Fe bound with different sizes of colloids; (B) ^{59}Fe bound with colloids for different periods of time. EDTA, Fe complexed with EDTA; LCOC, low colloidal organic carbon (COC) concentration; HCOC, high COC concentration. For comparison, the uptake rate constant of LMW ^{59}Fe by diatoms was 6112 ± 1194 L kg^{-1} h^{-1}. Mean ± semirange (n = 2).

<300–400 nm) that result in a strong photoreductive dissolution of colloidal Fe. However, there could be rapid photoredox reaction of colloidal Fe under natural conditions, thus increasing the 'transient' availability of colloidal Fe to marine phytoplankton.

The uptake rate constant of colloid-bound Fe was 2.7–3.4 times higher when the colloids were radiolabeled with Fe for 1 d than when the colloids were radiolabeled with Fe for 15 d. These results imply that Fe dissociation from the colloidal particles may control Fe uptake from natural colloids. Aging of the colloids may have reduced the chemical lability of the metal. Wells et al. (1983) demonstrated that the aging or heating of prepared ferric hydroxide stock caused a reduction in cell yield of diatoms, which may be related to increased thermodynamic stability of the colloid. Wells et al. (1991) further examined the biological availability of colloid-bound ferrihydrites (amorphous FeOOH) to support the growth of three species of neritic phytoplankton. In that study, early-phase (freshly precipitated) ferrihydrite proved to be an excellent source of available Fe, but low-temperature aging processes caused sharp decreases in algal growth response.

In our study, it remains unclear whether the radioisotope was in full equilibrium with the stable metals in the colloids. The colloids were radiolabeled with ^{59}Fe for 1–15 d before the radioactive uptake experiments. If the radiolabeled iron were not in true equilibrium (e.g., uniformly radiolabeling), this would lead to it being in a more labile form than the natural iron and would overestimate the bioavailability. The radiotracer may be initially more associated with the surface of the colloids, while the bulk of the natural iron is bound internally in the colloidal matrix. Thus, if the radiotracer and stable Fe were in different types of complexes or in different crystal lattice forms, the labeling would not be considered uniform. This can be further complicated by the aging process where the colloid becomes less labile (in essence more crystalline) and thus the iron less exchangeable. Under such circumstances, the experiments may only measure the availability of the outside iron to the plankton, rather than the total colloidal iron. Further studies are thus required to examine the time series of the exchange taking place between iron bound inside the colloidal matrix and the newly added radiotracer.

The uptake rate constant of small colloid-bound Fe was much higher (2.9 times) than the uptake of large colloids (>10 kDa) at typical colloidal organic carbon concentration, indicating that the uptake generally decreased with increasing size of the ligands binding with the Fe. Although comparison of the uptake of different sizes of colloids cannot unambiguously reveal the mechanisms of colloid-bound Fe uptake by phytoplankton, these results suggested that dissociation kinetics may be critical in controlling the rate of colloid-bound Fe uptake. Given the lability of the complexes, it may be possible that ^{59}Fe bound with the large colloids was dissociated more slowly to dissolved Fe than the small colloidal ^{59}Fe, resulting in less bioavailability to the diatoms. Consequently, the biological uptake of colloid-bound Fe in natural seawater may have been overestimated if only small colloids are considered.

One possible artifact in interpreting the difference between small and large colloidal Fe uptake may be the concentration effect. For example, the background colloidal Fe concentration in the large colloidal treatment may have been greater than the background colloidal Fe concentration in the small colloidal treatment, given the recent discovery that colloidal Fe was largely associated with the large colloids (Wen et al. 1999; Kuma et al. 2000*a;* Wells et al. 2000). If the Fe background concentration was very high in the large colloidal Fe treatment, it may be possible that the uptake rate may have been lower than that measured in the small colloidal Fe treatment. In our study it was assumed that the calculated relative uptake rate constant was independent of the ambient colloidal Fe concentration. The influence of colloidal Fe concentration on colloidal Fe uptake by marine phytoplankton clearly needs to be further studied.

For the copepod *Acartia spinicauda,* uptake was significantly lower from large colloidal particles (10 kDa–0.2 μm) than from small colloids (1–10 kDa), whereas COC concentration did not affect the uptake of Fe by the animals. In this experiment, the majority of Fe remained in the colloidal phase; thus measurements of the Fe uptake mainly reflected the uptake from the colloidal phase. The lower uptake from

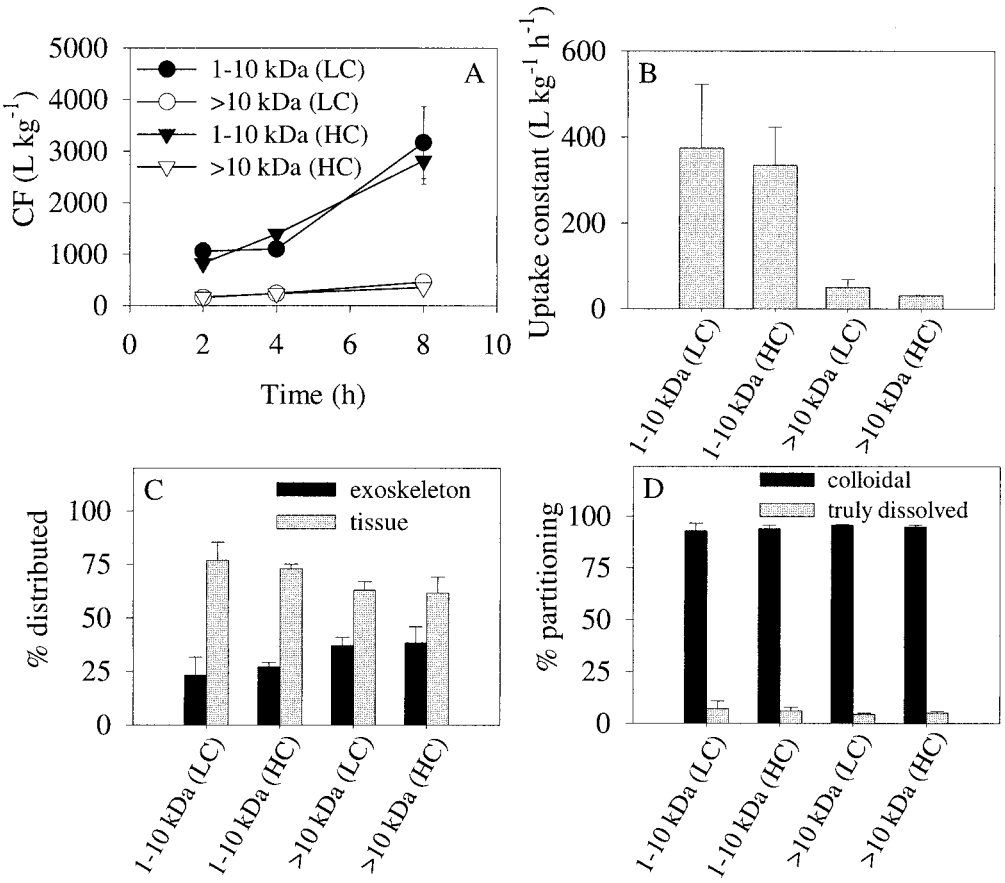

Fig. 6. The uptake of ^{59}Fe bound with different sizes of natural colloids by the marine copepod *Acartia spinicauda* during the 8-h exposure period. (A) the calculated dry weight concentration factor (CF) of Fe in the copepods as a function of exposure time; (B) the calculated uptake rate constant of Fe in the copepods; (C) the distribution of total copepod Fe in the exoskeleton and soft tissues; (D) partitioning of dissolved ^{59}Fe in the colloidal (1–10 kD or 10 kDa–0.2 μm) and truly dissolved phases (<1 kD or <10 kDa) by the end of 8-h exposure. LC, low colloidal organic carbon (COC) concentration; HC, high COC concentration. Mean ± semirange or SD (n = 2 or 3).

larger colloids implies that colloid ingestion by the copepods was probably not important in the acquisition of colloid-bound Fe by the animals. Similarly, Wang and Guo (2000) recently have also concluded that direct ingestion of natural colloids by marine copepods is probably minimal. In that study, the bioavailability of colloidal Cr to copepods was significantly enhanced, whereas the bioavailability of colloidal Zn was depressed compared with their uptake of LMW metals. This study did not compare colloid-bound Fe uptake by copepods with LMW Fe uptake. As with marine phytoplankton, accumulation of colloid-bound Fe by marine copepods may involve sorption followed by metal dissociation from the colloids before being accumulated by the animals. It is likely that dissociation of Fe from the large colloids may be slower than dissociation of Fe from the small colloids. Furthermore, Fe may also be trapped inside large colloids and not easily exchangeable, as well as being in a more crystalline matrix; this may further explain the differences in uptake between the large and small sizes of colloid-bound Fe. In a recent study on the bioavailability of colloid-bound metals to the marine shrimp *Penaeus aztecus*, Carvalho et al. (1999) found that uptake rates were comparable between the LWM and colloidal complexed Fe. They further suggested that colloid complexed metals may have first accumulated in the shrimp gills and were subsequently dissociated from the complex before entering the organisms as free ions.

The measurements of the distribution of Fe in the exoskeleton and soft tissues further demonstrated that colloid-bound Fe can indeed be accumulated in the soft tissues of copepods. The percentage of colloid-bound Fe penetration into the soft tissues decreased with increasing colloid size; thus, metal dissociation may play an important role in the uptake of colloid-bound Fe by the animals. Up to 77% of the total copepod Fe was eventually found in the soft tissues of the copepods. No previous study measured the distribution of Fe in the exoskeleton and soft tissues of copepods to compare with our data, although the distribution of other metals in the copepod's soft tissues is also generally high when the metals had been accumulated from the dissolved phase (e.g., >80% for Cd and >30% for Zn in copepod *Temora longicornis*, Wang and Fisher 1998). In this study, the initial surface sorption of colloid-bound Fe was probably insignificant or minimal, because the intercept of the regres-

sion between the dry weight concentration factor and the time of exposure was close to zero. This is consistent with a previous study on metal accumulation in marine copepods from the aqueous phase (Wang and Fisher 1998).

The uptake rate constants for colloid-bound Fe (0.7–1.2 L g^{-1} d^{-1} for the large colloid-bound Fe and 8–9 L g^{-1} d^{-1} for the small colloid-bound Fe) measured for the copepod *A. spinicauda* are somewhat comparable to the uptake rate constants measured for other metals such as Ag (10.4 L g^{-1} d^{-1}), Cd (0.7 L g^{-1} d^{-1}), and Zn (3.3 L g^{-1} d^{-1}) from the dissolved phase with the copepod *T. longicornis* (Wang and Fisher 1998). In modeling metal bioaccumulation in marine copepods, Wang and Fisher (1998) demonstrated that uptake from both the aqueous and dietary phases can contribute to metal accumulation by marine copepods. Whether dissolved Fe or dietary Fe contributes more to its overall accumulation by marine copepods is unknown. However, based on these measurements of the uptake rate constant from colloid-bound Fe, it is reasonable to speculate that zooplankton may greatly affect the colloid dynamics in natural seawater. The relatively higher uptake of colloid-bound Fe, coupled with the low assimilation of Fe from ingested particles (<10%, Hutchins et al. 1995; Wang and Dei 2001), indicates that colloid-bound Fe may become a dominant source for Fe accumulation in the animals. Because of the high regeneration rate of metals by the copepods (Wang and Fisher 1998; Xu et al. 2001), it is likely that the colloidally origined Fe may be further excreted by the animals into the ambient environment and subsequently taken up by phytoplankton.

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