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The interaction between inorganic iron and cadmium uptake in the marine diatom *Thalassiosira oceanica*

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Abstract

We examined substrate-saturated Fe(II) vs. Fe(III) uptake rates of *Thalassiosira oceanica* preconditioned to varying degrees of Fe limitation. Inorganic Fe(III) uptake rates by Fe-sufficient *T. oceanica* were 2.4-fold faster than the corresponding inorganic Fe(II) uptake rates. However, when cultures were severely Fe limited, the rate of Fe(II) uptake was upregulated 15-fold, while that of Fe(III) uptake increased only fivefold. The interactions between substrate-saturated uptake rates of inorganic Cd(II) and either Fe(II) or Fe(III) by Fe-limited *T. oceanica* were also investigated. The addition of equimolar Cd(II) concentration to the Fe(II) uptake media resulted in a ~50% reduction of inorganic Fe(II) uptake rates compared with those in Cd-free media. In turn, Cd uptake rates were inhibited ~36% in the presence of an equimolar Fe(II) concentration. In contrast to Fe(II), Fe(III) transport exhibited no interaction with Cd(II). *T. oceanica* thus has separate transporters for inorganic Fe(III) and Fe(II). Cadmium(II) and Fe(II) appear to enter the cell through a common putative divalent metal transporter that is upregulated under Fe deficiency. The interaction of Fe(II) and Cd(II) transport under Fe deficiency provides a plausible mechanism to explain some laboratory and field observations of higher Cd quotas in Fe-limited phytoplankton.

The seawater distributions of dissolved cadmium (Cd) and phosphate (PO_4^{3-}) throughout the water column are closely correlated (Bruland et al. 1978; de Baar et al. 1994). Under the assumption of a constant spatial and temporal relationship between Cd and PO_4^{3-} in the ocean, Cd : Ca ratios of fossil benthic foraminifera tests have been used to reconstruct past oceanic PO_4^{3-} concentrations in deep waters (Boyle 1988). This approach has also been applied to surface waters using planktonic foraminifera Cd : Ca ratios, providing new insights into nutrient use and primary productivity in the Southern Ocean during the last glacial maximum (Elderfield and Rickaby 2000). However, a caveat associated with extending the Cd : Ca paleonutrient proxy to surface waters is that the dissolved Cd : PO_4^{3-} relationship is not constant in the surface ocean (de Baar et al. 1994), especially in Fe-limited regions where dissolved Cd : PO_4^{3-} ratios are significantly lower.

Several factors have been suggested to account for surface dissolved Cd : PO_4^{3-} variability. Uptake rates of Cd by phytoplankton cultures are directly related to free Cd(II) concentrations and inversely related to free Zn(II) and Mn(II) concentrations, likely because of competitive inhibition at cellular uptake sites (Sunda and Huntsman 1996, 1998, 2000). Aqueous CO_2 concentrations control phytoplankton Cd quotas in culture, which is attributed to the role Cd plays in carbonic anhydrase (Lane and Morel 2000). Finkel et al. (2007) investigated the effect of growth irradiance on phytoplankton Cd : P ratios and found large taxonomic differences but no consistent trend with respect to changes in irradiance. In addition, shipboard incubations have demonstrated that the Cd content of phytoplankton is sensitive to changes in dissolved Cd, Zn, Mn, and aqueous CO_2 concentrations (Cullen et al. 1999; Cullen and Sherrell 2005). However, in oceanic surface waters, low dissolved Fe concentrations are not always correlated with low dissolved Zn and Mn concentrations (Löscher 1999; Fitzwater et al. 2000), and low CO_2 is uncommon in Fe-limited regions (Takahashi et al. 2002). Therefore, these factors cannot explain the significant deviations in dissolved Cd : PO_4^{3-} ratios in Fe-limited surface waters.

Dissolved Fe concentrations have also been suggested to control dissolved Cd : PO_4^{3-} variability in surface waters (Cullen 2006). Cultures of Fe-limited *Thalassiosira oceanica* (Sunda and Huntsman 2000), as well as other phytoplankton taxa (Lane et al. unpubl.), have significantly elevated cellular Cd content relative to Fe-replete cultures. Moreover, Cullen et al. (2003) observed a significant reduction in particulate Cd : P ratios with Fe supplementation in an on-

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deck incubation of a phytoplankton assemblage from an Fe-limited site in the Southern Ocean.

While observations of enhanced Cd accumulation in Fe-limited phytoplankton are numerous, the physiological mechanism underlying these findings is unknown. We hypothesized that Cd(II) and Fe(II) enter the cell via a divalent cation transporter that is upregulated under Fe limitation. We investigated the interactions between inorganic Fe(II), Fe(III), and Cd(II) uptake by Fe-limited phytoplankton, using *T. oceanica* as a model organism.

Materials and methods

Culturing—T. oceanica (clone 1003), a small 6-μm diameter centric diatom isolated from the Sargasso Sea, was obtained from the Center for Culture of Marine Phytoplankton. *T. oceanica* was grown in Aquil at ~19°C under a continuous photon flux density of 175 μmol quanta m^{-2} s^{-1}. Aquil medium, with 100 μmol L^{-1} ethylenediaminetetraacetic acid (EDTA), was prepared and enriched with macronutrients and trace elements (Cu, Mn, Co, Mo, and Se) as previously described (Maldonado et al. 2006). Premixed Fe-EDTA (1 : 1.05) was added separately to achieve a total Fe concentration of 1.37 μmol L^{-1} (pFe 19; pFe = $-\log$[Fe(III)]) or 4.2 nmol L^{-1} (pFe 21.5) for the Fe-sufficient or Fe-limiting media, respectively. A total concentration ($[Cd_T]$) of 35 nmol L^{-1} Cd (pCd 12) was added as $CdCl_2$, to obtain at equilibrium a free Cd(II) ion activity akin to typical high-nutrient low-chlorophyll (HNLC) waters. Approximately 70% of $[Cd]_T$ in the open ocean is present as organic complexes, resulting in 30% of $[Cd]_T$ as inorganic species ([Cd′]) (Bruland 1992). Approximately 3% of the inorganic Cd species in seawater is present as free Cd(II) (Byrne et al. 1988). Therefore, the surface [Cd(II)] typical of HNLC surface waters (where $[Cd_T]$ = 0.25 nmol L^{-1}; Cullen 2006) is ~2.2 pmol L^{-1}, corresponding to a pCd = 11.7. Absolute growth rates reported ($n \geq 19$) were monitored by in vivo fluorescence as described (Maldonado and Price 2001) and are expressed as doublings per day (dd^{-1}). To convert dd^{-1} to specific growth rates (d^{-1}), divide dd^{-1} by 1.443.

Short-term inorganic Fe and Cd uptake rates—All uptake rates by *T. oceanica* were determined using a basal uptake media (sterile synthetic ocean water [SOW] with 10 μmol L^{-1} PO_4^{3-}, 100 μmol L^{-1} SiO_3^{2-}, and no trace metals or vitamins). For the inorganic Fe(II) uptake kinetic experiments, ^{55}Fe (3.83 MBq μg^{-1}, PerkinElmer) was added to the basal uptake media to achieve concentrations of 0, 5, 10, 15, 25, 35, and 50 nmol L^{-1}. These media were used to resuspend severely Fe-limited (pFe 21.5, μ/μ_{max} = 0.50) *T. oceanica* to determine Fe(II) uptake rate as a function of Fe(II) concentration. The half-saturation constant for Fe uptake (K_m) and the maximum rate of Fe uptake (V_{max}) were derived by fitting the hyperbolic Michaelis–Menten equation to the uptake data (Fig. 1A). The curve approached an asymptote at an Fe(II) concentration of ~13 nmol L^{-1} (Fig. 1A). Thus, a saturating concentration of 20 nmol L^{-1} was selected for the following Fe(II) uptake experiments.

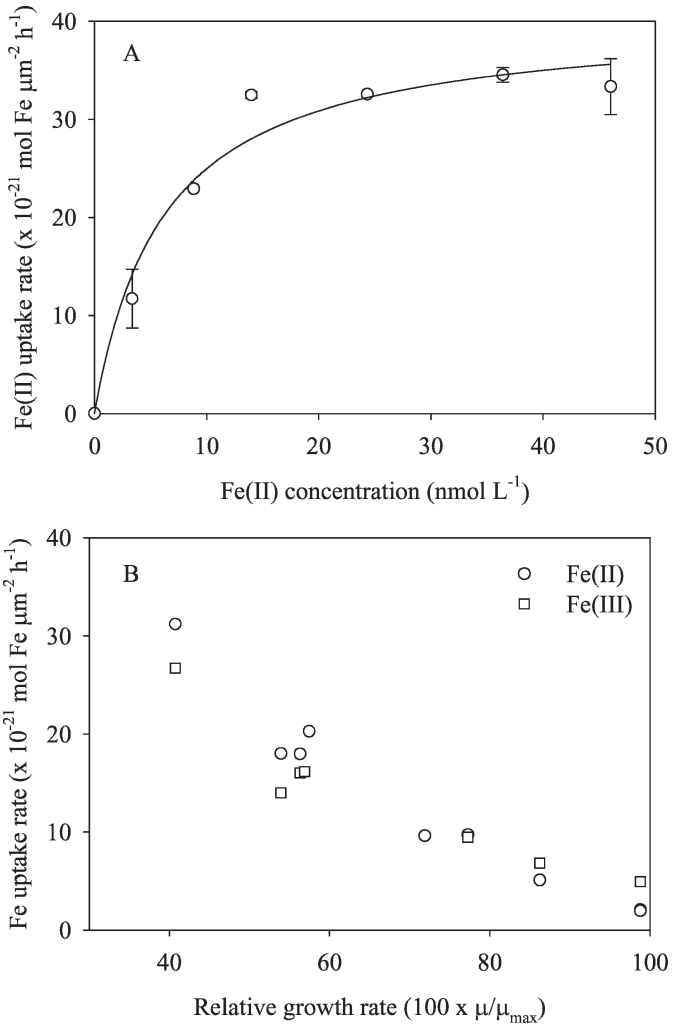

Fig. 1. (A) Fe(II) uptake rates as a function of inorganic Fe(II) concentration by Fe-limited (pFe 21.5, μ/μ_{max} = 0.50) *T. oceanica* at pH 6.6. The uptake rate was determined at seven different Fe(II) concentrations (0, 5, 10, 15, 25, 35, and 50 nmol L^{-1}). Points represent the mean ± range of duplicate analyses of a single culture. The half-saturation constant for Fe uptake (K_m) and the maximum rate of Fe uptake (V_{max}) were derived by fitting the hyperbolic Michaelis–Menten equation to the uptake data. The curve fit the data with a r^2 = 0.97 and a calculated K_m of 6.19 ± 1.72 nmol L^{-1} and a V_{max} of 40.4 ± 2.91 × 10^{-21} mol Fe μm^{-2} h^{-1}. (B) Inorganic Fe(II) and Fe(III) uptake rates by *T. oceanica* as a function of Fe-limited relative growth rate (100 × μ/μ_{max}). Experiments were conducted at pH 6.6 in the presence of 20 nmol L^{-1} inorganic Fe(II) or Fe(III).

The remaining short-term Fe uptake experiments were conducted using the basal uptake media with a saturating concentration of 20 nmol L^{-1} total inorganic Fe(II) or Fe(III) (50% as ^{55}Fe) in the presence or absence of 20 nmol L^{-1} Cd. Equimolar Cd and Fe concentrations were selected to achieve the most environmentally relevant conditions possible. The saturating Fe concentrations were chosen based on Michaelis–Menten saturation kinetics for Fe(II) (*see* above) and on published K_m for Fe(III) uptake (K_m = 1.18 nmol L^{-1}, Maldonado and Price 2001). Experiments of Cd uptake were conducted with 20 nmol L^{-1} $[Cd_T]$, 50%

as ^{109}Cd (14.53 MBq μg^{-1}, Amersham) with or without 20 nmol L^{-1} cold inorganic Fe(II) or Fe(III). This Cd concentration was saturating based on the K_m of Cd uptake for *Thalassiosira weissflogii* (2.9 nmol L^{-1}, Lee et al. 1995).

To avoid the rapid oxidation of Fe(II) and precipitation of Fe(III), all experiments were performed in pH 6.6 basal uptake medium (Anderson and Morel 1982; Maldonado et al. 2006), which was prepared by first buffering it with 1 mmol L^{-1} of chelexed Piperazine1,4 bis-(2-ethanesulfonic acid) (PIPES, pKa 6.8). The pH was then adjusted using 10% trace metal grade HCl (Seastar Chemicals) while bubbling with 0.2-μm filtered air. The seawater was bubbled until it reached equilibrium with the air and remained stable for months at pH 6.6. Iron(II) was prepared by reducing Fe(III) as described (Maldonado et al. 2006). The half-life of Fe(II) in the pH 6.6 basal uptake media was 15 h (Maldonado et al. 2006); thus, the uptake experiments were conducted for 1.5 h. A single set of experiments was conducted in pH 7.5 basal uptake media (*see* above), containing N-(2-hydroxyethyl)-piperazine-N′-2-ethanesulfonic acid (HEPES, pKa 7.55) as the buffer. The pH 7.5, Fe(II) uptake experiments were conducted for 0.5 h to account for the shorter Fe(II) half-life ($\sim$1 h).

Batch cultures were grown in Fe-replete or Fe-limited media. During midexponential phase, cells were harvested onto acid-washed 3-μm polycarbonate filters (Poretics) and immediately resuspended in the basal uptake media. Live cell density and size were determined using a Coulter Counter (model Z2). All experiments were conducted under ambient temperature and light in a laminar flow hood using acid-washed polycarbonate bottles. Experiments were initiated by the addition of ^{55}Fe or ^{109}Cd. Each resuspended culture was sampled in duplicate $\sim$ every 15 min by filtering 4-mL aliquots onto a 3-μm polycarbonate filter. The pH was monitored throughout the experiments using a Corning 350 pH meter and varied $<$1%. Before running dry, the cells were washed with either a 5 mL Ti(III) EDTA–citrate solution (Hudson and Morel 1989) or a 1 mmol L^{-1} diethylenetriaminepentaacetic acid (DTPA) rinse (Lee et al. 1995, dissolved in SOW, pH adjusted to $\sim$8.1) to remove extracellularly bound Fe or Cd, respectively. Measurements of Fe and Cd uptake rates were replicated with independent cultures. The radioactivity on the filters was measured using Beckman–Coulter LS 6500 liquid scintillation counter. Uptake rates were calculated from linear regression of particulate metal concentration as a function of incubation time (h) and were normalized to total cellular surface area (mol metal μm^{-2} h^{-1}).

Effects of ammonium tetrathiomolybdate (TTM) additions on Fe(II) uptake—To test whether Fe(II) was entering the cell via the multicopper oxidase (MCO)–permease complex or a separate Fe(II) transporter, MCO activity was inhibited with TTM [$(NH_4)_2MoS_4$; Alfa Aesar]. During midexponential phase, half of the Fe-limited *T. oceanica* culture (pFe 21.5) was exposed to 25 μmol L^{-1} TTM for 3 h prior to the Fe uptake experiment (Maldonado et al. 2006). The subsequent Fe(II) uptake experiments were conducted with 20 nmol L^{-1} (50% ^{55}Fe) inorganic Fe(II) at pH 6.6.

Results and discussion

Inorganic Fe(II) vs. Fe(III) uptake rates—The mean growth rate (μ_{max}) for *T. oceanica* was 1.67 $\pm$ 0.047 dd^{-1} (μ_{max}) in Fe-sufficient media (pFe 19). Rates of growth averaged 1.33 $\pm$ 0.042 dd^{-1} (mean $\pm$ standard error, μ/μ_{max} = 0.80) in pFe 21 media and 0.893 $\pm$ 0.029 dd^{-1} (mean $\pm$ standard error, μ/μ_{max} = 0.53) in pFe 21.5 media. The growth rates of *T. oceanica* in Fe-replete and Fe-limiting media in the presence of 35 nmol L^{-1} Cd (pCd 12) were identical to those in its absence (unpaired *t*-test, p = 0.952, 0.973, and 0.917 for pFe 19, pFe 21, and pFe 21.5, respectively), suggesting that Cd was not toxic and that the cells were able to fulfill their Fe requirements in the presence of Cd.

We investigated the relative upregulation of substrate-saturated (20 nmol L^{-1}) Fe(II) and Fe(III) uptake rates by Fe-limited *T. oceanica*. Substrate-saturated uptake rates are a direct function of the number of Fe transporters (Hudson and Morel 1990) and an inverse function of intracellular Fe (Harrison and Morel 1986). Substrate-saturated Fe uptake rates by *T. oceanica* were dependent on the cellular Fe status (Fig. 1B). When *T. oceanica* was not Fe limited, the Fe(II) uptake rates were slower than those of Fe(III) uptake (Fig. 1B, $\mu/\mu_{max} > 0.98$; 2.02 vs. 4.93 $\times$ 10^{-21} mol Fe μm^{-2} h^{-1}, respectively). Slower uptake of inorganic Fe(II) than Fe(III) by mildly Fe-limited *T. oceanica* (pFe 20.5) has been previously shown (3.51 $\times$ 10^{-21} and 29.8 $\times$ 10^{-21} mol Fe μm^{-2} h^{-1}, respectively, at 70 nmol L^{-1} Fe; Maldonado et al. 2006). However, in our study, severely Fe-limited *T. oceanica* (pFe 21.5) had similar rates of inorganic Fe(II) and Fe(III) uptake (Fig. 1B, $\mu/\mu_{max} < 0.60$; (23.14 $\pm$ 4.09) $\times$ 10^{-21} vs. (18.22 $\pm$ 3.4) $\times$ 10^{-21} mol Fe μm^{-2} h^{-1}, respectively). Iron-limited *T. weissflogii* also achieved comparable inorganic Fe(II) and Fe(III) uptake rates at pH 6.3 (Anderson and Morel 1982). Overall, under Fe-limiting conditions, Fe(II) uptake is upregulated more (approximately 15-fold) than Fe(III) uptake (approximately fivefold). To provide more conclusive findings, future quantitative studies are required on the relative degree of upregulation of Fe(II) vs. Fe(III) transport under Fe limitation.

The physiological data presented here for *T. oceanica* complement genomic data for *Thalassiosira pseudonana* (Kustka et al. 2007), which showed that the transcript abundance of genes for a putative divalent metal transporter belonging to the natural resistance-associated macrophage protein (NRAMP) family, as well as the components of the high-affinity Fe(III) transport system (HAFeT; putative reductases, MCOs, and permease complexes) were upregulated by Fe-limited *T. pseudonana* (Kustka et al. 2007). Although the specific physiological role of *Tp*NRAMP remains elusive, the expression of *Tp*NRAMP was upregulated by Fe limitation to a much greater extent than some of the genes involved in the putative HAFeT (Kustka et al. 2007). Under Fe sufficiency, *Tp*NRAMP gene transcript levels in *T. pseudonana* were virtually undetectable, whereas those involved in the putative HAFeT showed significant constitutive expression.

Fe(II) uptake via a nonspecific Fe(II) transporter—Even though it is well established that inorganic Fe(II) and

Table 1. Effects of Cd on inorganic Fe(III) and Fe(II) uptake rates by Fe-limited (pFe 21.5) *T. oceanica*. Experiments were conducted with 20 nmol L^{-1} (50% ^{55}Fe) inorganic Fe(III) or Fe(II), with or without additions of 20 nmol L^{-1} Cd at pH 6.6. The *p* value between two columns for Fe(III) uptake treatments represents a two-tailed paired *t*-test analysis. The *p* value between two columns for Fe(II) uptake treatments represents a one-tailed paired *t*-test analysis.

T. oceanica Fe uptake rates ($\times 10^{-21}$ mol Fe μm^{-2} h^{-1})				
	Fe(III)		Fe(II)	
	−Cd	+Cd	−Cd	+Cd
	16.0	16.0	18.0	8.44
	16.2	16.0	9.72	5.10
	9.46	8.83	9.61	5.52
Average	13.89	13.62	12.4	6.35
SE	2.22	2.40	2.77	1.05
t-test *p* value	0.275		0.036	

Table 2. Effects of Fe additions on Cd uptake rates by Fe-limited (pFe 21.5) *T. oceanica* at pH 6.6. Experiments were conducted with 20 nmol L^{-1} Cd (50% ^{109}Cd) in the absence of Fe or with 20 nmol L^{-1} inorganic Fe(III) or Fe(II). The *p* value under the +Fe(III) column represents a two-tailed paired *t*-test analysis comparing the Cd uptake rates of the −Fe and +Fe(III) experiments. The *p* value under the +Fe(II) column represents a one-tailed paired *t*-test analysis comparing the Cd uptake rates of the −Fe and +Fe(II) experiments.

Cd uptake rates ($\times 10^{-21}$ mol Cd μm^{-2} h^{-1})			
	−Fe	+Fe(III)	+Fe(II)
	28.5	25.9	17.2
	21.8	21.6	13.8
	31.7	32.4	20.8
Average	27.3	26.6	17.3
SE	2.93	3.14	2.01
t-test *p* value		0.554	0.005

Fe(III) may be taken up by diatoms, the physiological evidence for the specific transporters used to internalize Fe(II) and Fe(III) is more controversial. Both inorganic and organically bound Fe(III) are believed to be accessible to phytoplankton via enzymatic reduction of Fe(III), followed by oxidation of Fe(II) and subsequent internalization (Shaked et al. 2005; Maldonado et al. 2006). The relative rates of Fe(III) uptake from inorganic and organic complexes are determined by the cellular Fe status (Maldonado and Price 2001). In contrast, the mechanism of Fe(II) transport is less understood, and it is unknown whether Fe(II) is exclusively transported through a nonspecific Fe(II) transporter or through the MCO–permease complex transporter as suggested by Maldonado et al. (2006). To establish the mechanism of Fe(II) transport we determined Fe(II) uptake rates by cells preincubated with TTM, a well-known inhibitor of MCOs. We found that TTM caused no inhibition of Fe(II) uptake rates by severely Fe-limited *T. oceanica* (19.1 ± 1.16) $\times$ 10^{-21} and (20.1 ± 3.18) $\times$ 10^{-21} mol Fe μm^{-2} h^{-1} for −TTM and +TTM, respectively, $n = 2$, $p = 0.714$, paired *t*-test), suggesting that the Fe(II) was transported directly into the cell through a putative divalent metal transporter. However, Maldonado et al. (2006) also using *T. oceanica* cultures found, in a single experiment, that TTM had a remarkable inhibitory effect on Fe(II) uptake rates. They argued that Fe(II) was being oxidized and subsequently internalized by the MCO–permease complex. The paired reduction and oxidation-transport steps are expected to be highly coupled to ensure the high affinity and specificity for Fe(III) of a HAFeT. Thus, it is surprising that Fe(II) can access the MCO–permease complex. Indeed, more recent physiological data suggest that only biologically reducible transition metals, such as Cu(II), are able to access the reductase–MCO–permease system (Kustka et al. 2007). We believe that extremely high Fe(II) concentrations (10 μmol L^{-1}) used in Maldonado et al. (2006), and the high growth rate of their culture (μ/μ_{max} = 0.62, 1.89 dd^{-1}), may account for the conflicting result. It is plausible that under these mildly Fe-limited growth conditions few Fe(II) transporters are present. Moreover, at a concentration of 10 μmol L^{-1} Fe(II), Fe(II) might have been able to access and saturate the MCOs of the HAFeT. Given that in our study, *T. oceanica* was severely Fe limited and that the Fe(II) concentration in the uptake media was much lower than that in Maldonado et al. (2006), in our study Fe(II) was most likely internalized by a putative nonspecific divalent metal transporter, independent of the HAFeT.

Interaction between Fe(III) and Cd—Our results suggest that there is no interaction between Cd and inorganic Fe(III) transport, given that neither Fe(III) nor Cd uptake was affected by the presence of the other metal (Tables 1, 2). It has been suggested that both organic and inorganic Fe(III) are transported via the HAFeT and must be reduced before uptake (Shaked et al. 2005). Since Cd had no effect on Fe(III) uptake in our experiments ($p = 0.275$, paired *t*-test, Table 1), it suggests that Cd does not interfere with the HAFeT. These results are consistent with those of Kustka et al. (2007), showing that μmol L^{-1} concentrations of Cd and other divalent metals (Mn and Zn) did not inhibit inorganic Fe(III) uptake by *T. pseudonana*. As in yeast (Stoj and Kosman 2003), the only metal inhibiting the HAFeT was Cu(II), a biologically reducible metal. Thus Cd, a nonreducible metal, must enter the cell through an alternative transporter.

Interaction between Fe(II) and Cd—In contrast to Fe(III) uptake, inorganic Fe(II) uptake rates at pH 6.6 were reduced by ~50% in the presence of Cd ($p = 0.036$, paired *t*-test, Table 1), suggesting that diatoms have separate transporters for inorganic Fe(III) and Fe(II) and that Cd and Fe(II) enter the cell through the same transporter. Further confirmation of the antagonistic effect of Cd on Fe(II) was obtained for two Fe-limited cultures, where Fe(II) uptake at pH 7.5 was reduced by 34.4% in the presence of Cd (data not shown).

Given the inhibitory effect of Cd on Fe(II) uptake, we expected Cd uptake rates to be similarly inhibited by Fe(II). Indeed, the presence of Fe(II) inhibited Cd uptake by ~35% ($p = 0.005$, paired *t*-test, Table 2). However, under our experimental conditions, absolute Cd uptake rates,

were approximately two times faster ($27.3 \pm 2.93 \times 10^{-21}$ mol Cd μm^{-2} h^{-1}) than the rates of Fe(II) uptake ($12.4 \pm 2.77 \times 10^{-21}$ mol Cd μm^{-2} h^{-1}, Table 2). These findings suggest that the putative, nonspecific divalent metal transporter has a higher specificity for Cd(II) than Fe(II) or that there are multiple Cd transporters. Yeast and plants are known to have two different families of nonspecific divalent metal transport proteins: NRAMPs and ZRT, IRT-like proteins (ZIPs), with family members differing in their substrate range and specificity. For example, in plants several members of the ZIP family have been identified and transport Fe(II), Mn(II), Cd(II), and/or Zn(II) (for reviews *see* Guerinot 2000; Hall and Williams 2003). In contrast, members of the NRAMP family may also mediate transport of Fe(II), Mn(II), and/or Cd(II), but no Zn(II) (for reviews *see* Hall and Williams 2003; Pittman 2005). Interestingly, various members of the ZIP and NRAMP families are upregulated under Fe deficiency, and some have been linked to enhanced Cd uptake and sensitivity (Guerinot 2000; Hall and Williams 2003; Pittman 2005). Our study suggests that, like yeast and plants, phytoplankton may have more than one nonspecific divalent metal transporter. These transporters seem to be upregulated under Fe limitation, therefore leading to the hyperaccumulation of Cd. Sunda and Huntsman (1996, 1998, 2000) also suggested that cellular uptake of Cd is controlled by two inducible transport systems: the Mn and the Zn uptake system. Given our knowledge of divalent trace metal transporters in yeast and plants (*see* above), it is likely that the high-affinity Mn(II) transporter proposed by Sunda and Huntsman (1996, 1998, 2000) is a NRAMP-like transporter and the system induced at low Zn is perhaps a ZIP-like transporter. Phytoplankton genomic work is needed to elucidate the divalent metal transporters and their degree of metal specificity.

Data in a recent manuscript suggest that Cd : P ratios are species specific and may change (increase or decrease) as function of the irradiance-dependent relative growth rate. Even though no compelling physiological mechanism was proposed to explain the findings, they indicated that their data were not supportive of the divalent metal transporter mechanism and that further studies were needed, given that Cd : Fe concentrations in their cultures were low relative to field conditions. Indeed, according to our findings, in their medium (pFe 19.9, as reported in Ho et al. 2003), phytoplankton would have had few nonspecific divalent metal transporters relative to Fe(III) transporters; therefore Fe acquisition should not have affected their Cd quotas.

This study provides a physiological mechanism for enhanced Cd accumulation by Fe-limited marine phytoplankton and suggests that Fe(II), if available, could prove to be a significant source of Fe for Fe-limited phytoplankton. Upregulation of Fe(II) transporters would therefore be advantageous under various ambient physical and chemical conditions that favor the stability of Fe(II), especially during an episodic Fe(II) supply from dust or rain. The upregulation of nonspecific divalent metal transporters may lower the concentration of Cd as well as other divalent metals in the surface ocean. These findings have major implications for the use of the Cd/Ca paleonutrient proxy for surface nutrient inventories, which relies on the assumption that dissolved Cd vs. PO_4^{3-} concentrations are constant in oceanic surface waters. Further investigation into how Fe-limited marine phytoplankton control divalent metal chemistry in seawater is needed.

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