The influence of zinc, aluminum and cadmium on the uptake kinetics of iron by algae

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Abstract

The uptake of iron by the marine diatom Thalassiosira weissflogii and the green algae Dunaliella tertiolecta in seawater has been investigated. Computations were made of the rate constants for the binding of iron to specific cell surface-associated transport ligands and the subsequent slow active uptake into the cytoplasm of the cells. Although both algae transport Fe, the sorption constants and the kinetic parameters are markedly different and temperature dependent. The sorption constants, which are one order of magnitude higher for T. weissflogii versus D. tertiolecta (2.5 \times 10^{-9} \text{ cell}^{-1} \text{ vs.} 2.2 \times 10^{-10} \text{ cell}^{-1}) at 25°C, give us a measure of the theoretical number of transport sites needed by each cell. The effects on the iron speciation and on the rate of iron uptake due to the addition of different concentrations of Zn(II), Al(III) and Cd(II) have also been determined. The results confirm that while iron is taken up via specific sites on the cell surfaces, synergistic and antagonistic effects on the iron rate uptake for T. weissflogii and D. tertiolecta, respectively, have been found and evaluated. © 1997 Elsevier Science B.V.

Keywords: iron; uptake; kinetic; algae; trace metals; synergism; antagonism

1. Introduction

Metals are required micronutrients in many of the enzyme and electron transport systems present in all living organisms. Our understanding of trace metal concentrations and distributions in the oceans has led us to the hypothesis that trace metals may limit phytoplankton growth and biomass (Barber and Chavez, 1991; Coale, 1991; Martin et al., 1991; Martin, 1992). Iron is arguably the most important of all the bioactive trace metals in the oceans (Bruland et al., 1991). Fe is potentially limiting to phytoplankton growth and is not known to be toxic to organisms at concentrations found in seawater (Martin and Fitzwater, 1988; Martin and Gordon, 1988; Martin et al., 1991). The major source of iron in the remote areas of the world’s oceans is deposition of atmospheric dust particles (Donaghey et al., 1991; Duce and Tindale, 1991). However, Finden et al. (1984), Rich and Morel (1990), and Wells et al. (1991) have demonstrated that iron oxyhydroxide particles and iron colloids are not directly available to phytoplankton. Iron particles and colloids must become soluble
thermally or photochemically to be taken up and, thus, to support phytoplankton growth (Rich and Morel, 1990). Little is known of the mechanisms of Fe transport in marine phytoplankton. In prokaryotes, extensive studies have shown that transport of Fe involves the formation of coordination complexes with specific chelators either exuded into the organism’s milieu or localized in its plasma membrane (Neilands, 1984; Ratledge et al., 1983). Production of marine siderophores is known to increase iron solubility and bioavailability in some organisms (Trick, 1989; Reid et al., 1993). However, the presence of siderophores in natural seawater has not been reported and few marine organisms with the capability to produce them have been identified (Sunda, 1989; Wells, 1989; Bruland et al., 1991).

Anderson and Morel (1982) have addressed the question of iron availability to eucaryotic phytoplankton through direct measurement of iron uptake by the coastal diatom Thalassiosira weissflogii in the presence and absence of chelators. They suggested that the iron uptake rate is determined by the extent of Fe binding to membrane-bound iron binding complexes (phytotransferrin), which is controlled by pseudo-equilibrium with free iron in the medium. This mechanism is thought to occur even when the medium is not at equilibrium due to the photo-reduction of Fe(III) or dissolution. Hudson and Morel (1990) demonstrated that cell surface-bound Fe is directly transported into the cell which implies that the transport occurs at specific, surface-associated sites.

In this paper we investigate the kinetics of Fe transport in the presence and absence of three trace metals, Zn(II), Al(III) and Cd(II) for two marine phytoplanktons: (the diatom Thalassiosira weissflogii and the green algae Dunaliella tertiolecta). Although the effect of metals on phytoplankton have been studied, the interactive effects have received insufficient attention. As it was pointed out by Morel et al. (1991), many trace elements in solution will indeed be found to colimit in some way the growth of oceanic plants. Minimum metal cellular quotas for a given metal can be affected by the concentration of other trace metals. This is clear from the coordinate nature of the uptake system. No ligand is ever perfectly specific so that some degree of competition for ligand binding must occur among all trace metals (Sunda, 1989; Bruland et al., 1991). Three metals were selected for these studies. Zn which is an essential element, but at high concentrations is toxic due to the interference in the metabolism of other essential metals (Sunda, 1989). Cadmium which is normally thought of as a nonessential metal, can nutritionally replace zinc-limited cultures (Price and Morel, 1990) and aluminum which is a non-essential element to plant metabolism. Folsom et al. (1986) found that the complexing of Al and Fe to algae cell surfaces is important in preventing toxic cytoplasmic levels of aluminum. The uptake of iron (iron bound to phytotransferrin plus internalized iron) was followed by taking into consideration its binding to surface ligands and the subsequent transfer across the cell membrane. The effects of the addition of other metals together with Fe in different ratios were determined by following the changes in iron speciation and rate of iron uptake.

2. Experimental

2.1. Culturing and preparation of inoculum

Axenic cultures of T. weissflogii and D. tertiolecta were supplied and incubated from the collection of Dr. Brand. The cultures were grown in polycarbonate flasks at 20°C under a light intensity of 59 fJinstein m$^{-2}$ s$^{-1}$ (cool-white fluorescent bulbs) with a 12:12 photoperiod. Seawater for the cultures was collected from the northern Tongue of the Ocean, Bahamas. The seawater and individual nutrient stock solutions were all tyndallized separately in Teflon bottles. Gulf Stream seawater ($S = 35.53$) filtered through a 0.2 μm Nucleapore filter was used for the kinetics experiments.

Nutrients added to the seawater were: $10^{-4}$ M NaNO$_3$, $10^{-5}$ M NaH$_2$PO$_4$, $10^{-7}$ M MnSO$_4$, $10^{-4}$ M Na$_2$SiO$_3$, $10^{-5}$ M NH$_4$Cl, $10^{-8}$ M ZnSO$_4$, $10^{-9}$ M CoCl$_2$, $10^{-9}$ M CuSO$_4$, $10^{-7}$ M Fe-EDTA, $10^{-7}$ M thiamine, $10^{-8}$ M biotin, and $10^{-8}$ M vitamin B$_{12}$. The cells were harvested just before the stationary phase by filtration ($< 20$ mm of Hg) through a 3 μm Nucleopore filter and then resuspending them in trace-metal clean, 0.2 μm filtered seawater, followed by another filtration. The washing procedure was repeated three times. After resus-
pension in a small volume of seawater, the concentration was about 50,000–100,000 cells cm\(^{-3}\) for the experiments used to evaluate the HCl washing procedure efficiency (see below), the cell concentrate was killed by treating with 0.5% Formalin (Aldrich) for one hour. For the other samples, one hour later, the culture was diluted (ca. 100 \(\times\)) into 0.2 \(\mu\)m filtered seawater containing the desired trace metals. The final volume was 1.5 1 with a concentration of 1–10 \(\times\) \(10^7\) cells l\(^{-1}\). Cells were counted optically with a hemacytometer (Fuchs Rosenthal). There was very little growth during the short term (4–5 h) iron uptake experiments, but it resumed in response to the added iron 8–14 h after the experiments began, (showing that the cultures were viable).

2.2. Iron uptake

The iron uptake experiments were made with Fe(III), Zn(II), Al(III) and Cd(II) atomic absorption standard solutions (Sigma). The Fe(III) standards were prepared by the dilution of a 30 \(\mu\)M Fe(III) solution kept at pH 8 to oxidize any Fe(II) present in the stock solution (about 10% of the Fe(III) atomic absorption standard solution was found to be Fe(II)). The solutions were then acidified to pH 1 with HCl 0.1 M before use. Under these conditions the Fe(II) blank was negligible.

All the uptake experiments were made at 25°C or 8°C in a water bath under laboratory light conditions with aseptic techniques. The temperature was controlled to ±0.02°C with a Forma temperature bath and monitored with a Guildline Pt thermometer. Polycarbonate flasks were conditioned to the iron concentrations used in our studies in order to minimize adsorption and used prior to any washing procedure. Uptake experiments were begun by diluting the appropriate volume of the trace metal standards into the 0.2 \(\mu\)m filtered seawater. One hour was allowed between the addition of the trace metal and the addition of the cell stock solution to attain an equilibrium distribution of the hydrolysis, polymerized and organically complexed of Fe(III) (Van der Giessen, 1968). After the cells were added, the decrease in Fe(III) concentration in the solution was followed by iron chemiluminescence determination. The zero time corresponds to when the cell solution was added. Prior to this addition, the total Fe(III) concentration (iron added plus iron initially in the sample) was analyzed. Experiments reported elsewhere (Johnson et al., 1994; Van den Berg, 1995) have demonstrated that there is no a loss of iron to the preconditioned carboy walls and total iron can be determined by acidifying subsamples of the solution to pH = 3. Undetectable changes in total Fe(III) concentration were observed when this method was used (see Fig. 1, 3 \(\mu\)m, no cells).

Table 1 describes the fractionation scheme followed in this work, including whether the variable was directly measured or obtained by the difference from measured variables. Each time, 15 cm\(^3\) water samples were spiked and split into 10 cm\(^3\) and 5 cm\(^3\) subsamples. Prior to filtration, the 5 cm\(^3\) sample was treated with HCl 1 M (final pH = 2) for 30 min to dissolve any colloidal hydrous ferric oxide (see discussion below) and filtered through a 3 \(\mu\)m filter to remove the cells. The HCl labile fraction was then determined, which represents dissolved iron and colloidal iron including that precipitated on the cell walls. The 10 cm\(^3\) seawater sample was first filtered using a 3 \(\mu\)m filter, and the colloidal and dissolved Fe(III) in solution determined. A 5 cm\(^3\) sample of this 10 cm\(^3\) subsample, was filtered with 0.1 \(\mu\)m filter in order to determine dissolved Fe(III). According to our scheme, four Fe(III) determinations were carried out for each sample: Fe(III) prior the addition of cells, after HCl (pH = 2, 30 min) treatment and 3 \(\mu\)m filtration, Fe(III) after 3 \(\mu\)m filtration, and Fe(III) after 0.1 \(\mu\)m filtration. When the cells were removed, there was no difference between the dissolved iron determined after the 3 \(\mu\)m and 0.45 \(\mu\)m filtration. In this study it was considered that colloidal iron can be determined as the difference between 3 \(\mu\)m and 0.1 \(\mu\)m filtration. Using these four factors it was possible to determine the total iron, HCl labile fraction including the fraction precipitated on the cell walls (< 3 \(\mu\)m, HCl treatment), colloidal and dissolved Fe(III) in solution (< 3 \(\mu\)m), and dissolved Fe(III) (< 0.1 \(\mu\)m). It was, also, possible to calculate precipitated Fe(III) as the difference between HCl labile fraction and colloidal and dissolved Fe(III) in solution, colloidal iron as the difference between colloidal and dissolved Fe(III) in solution and dissolved iron, and iron uptake as the difference between total iron and HCl labile fraction. Mass balance was checked for D. tertiolecta cul-
Fig. 1. Time-course of iron concentration after 0.1 and 3 μm Nucleopore filtration in presence and absence of viable and Formalin-treated cells. At time 0, 1.0 × 10^7 cells l−1 T. weissflogii were added to 100 nM Fe(III). Insert: Time-course of iron loss when samples of a culture, previously incubated in 100 nM Fe(III) are acidified to pH 2 with HCl.

tures. In this case, after HCl treatment and 3 μm filtration, the cells were desegregated in concentrated HNO₃:HCIO₄ (1:1) mixture, and iron sorbed on the cells was analyzed. Iron sorbed on the cells directly determined was similar to that theoretically determined as the difference in the analytical error.

2.3. Iron determination

The system used for the detection of Fe(III) is the one used in a previous study (Millero et al., 1995a). The flow injection system with chemiluminescence detection for the analysis of Fe(II) has been modified by the addition of a reductant column to allow for the analysis of acidified samples for total Fe (II + III). The Fe(II) present in the samples prior to the addition of the reductant was undetectable or was rapidly oxidized to Fe(III) during filtration (half-life of few minutes, Millero and Sotolongo, 1989). The sample was analyzed for Fe(II) after the reduction of the Fe(III) with sulfite (Millero et al., 1995a). The reagent (luminol solution) and carrier solution (NaCl solution) that entered the instrument was separately driven by a Rabbit peristaltic pump through a six-channel

<table>
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<th>Operation definition</th>
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<tr>
<td>Initial</td>
<td>total Fe</td>
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<td>&lt; 3 μm, HCl treatment</td>
<td>HCl labile fraction</td>
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<td>&lt; 3 μm</td>
<td>colloidal and dissolved Fe(III) in solution</td>
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<td>&lt; 0.1 μm</td>
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<td>&gt; 3 μm, HCl insoluble</td>
<td>iron uptake (surface bound + internalized)</td>
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<td>&gt; 3 μm, HCl soluble</td>
<td>precipitated iron</td>
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<td>0.1 μm &lt; size &lt; 3 μm, HCl</td>
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electrical Valco two-position valve. The reactants are continuously mixed in a coiled reaction chamber positioned directly in front of a photomultiplier tube (Hamamatsu PMT HC124-02 biased at 1000 V) to measure the chemiluminescent emission. The rate of Fe(III) reduction by sulfite, S(IV), in seawater has been investigated by Millero et al. (1995a), and found to be first order with respect to Fe(III) and S(IV). The rate of reduction is pH dependent with the highest rate around pH 4.5 for S(IV) = 300 µM and giving a reaction time of 30 min. Under these conditions, we achieved a reduction of Fe(III) over 98%. The Fe(III) reducing reagent was 0.1 M NaHSO$_3$ in a 2.0 M ammonium acetate buffer (Buffer pH 4, final sample pH 4.5). The Luminol reagent (0.5 mM) (Sigma) was prepared in 0.2 M borate buffer and adjusted to a pH of 12.6. The carrier solution was a 0.7 M NaCl (Sigma ultra pure) solution.

Prior to the analysis, the Fe(III) reducing reagent was added to the seawater samples and allowed to react for at least 30 min. Iron standards in seawater were treated in a similar manner to the samples. The system response determined from standard additions using Fe(B) or Fe(II) was not statistically different, which is consistent with the complete reduction of Fe(II) by sulfite. This same sample treatment was recently used by Powell et al. (1995) with good levels of precision (±0.5 mM) and low detection limits (±0.1 mM) in at-sea iron analysis.

3. Model development

The phenomenon of metal uptake by microorganisms is quite complex. The actual attachment of metal ions on the cellular surface may include physical adsorption, ion exchange, or chemisorption. In this work, the term sorption is used to describe any of these possible modes of interaction between the metal ion and the surface of the cells without distinction.

In the mathematical model, it is assumed that metal uptake by the phytoplankton cells consists in two stages; an initial rapid uptake (passive uptake) followed by a much slower one (active uptake) (Gonzalez-Davila, 1995). During the passive uptake, the metal ions sorb onto the surface of the cells within a few seconds to minutes. In the second uptake stage, the metal ions are transported across the cell membrane and into the cytoplasm. Ting et al. (1989, 1991) developed and tested a mathematical description of these processes, and the model has now been refined and further tested to take into account the speciation behavior of Fe(III) in seawater. The following assumptions are made in the modeling of the uptake process:

(i) The metal in solution at the cell surface, $m$ (nM), is in equilibrium with the metal sorbed onto the cell, $C_s$ (nmol/cell), excluding the ferric hydroxide particles, possible adsorbed onto the cells. A linear relationship between these two forms is assumed

$$C_s = K_m$$

where $K_m$ is the sorption constant (1 cell$^{-1}$).

(ii) The metal ion sorbed on the cell surface transverses the cell membrane through the agency of carrier molecules present on both sides of the membrane. The carrier-mediated mechanism is analogous to an enzyme–substrate coupling scheme

$$C_s + E \overset{k_1}{\underset{k_{-1}}{\leftrightarrow}} CE \overset{k_2}{\underset{k_{-2}}{\leftrightarrow}} C_i + E$$

where $C_s$ = extracellular metal concentration (nmol/cell); $C_i$ = intracellular metal concentration (nmol/cell); $E$ = carrier concentration (nM); CE = metal–carrier complex concentration (nM); $k_1, k_{-1}$ = chemical reaction rate constants (cells/nmol h); $k_2$ = chemical reaction rate constants (h$^{-1}$).

(iii) The diffusion of the metal–carrier complex CE through the cell membrane is very rapid and the concentrations of $E$ and CE are small compared with $[C_s]$ and $[C_i]$.

(iv) Pseudo-steady state is assumed and there is no net accumulation of the metal–carrier complex and $d(CE)/dt = 0$.

(v) On a unit volume basis, the carrier content of each cell is essentially constant, and so the total carrier concentration in the system, $E_t$, is given by

$$E_t = [E] + [CE]$$

(vi) One mole of carrier reacts with 1 mol metal ion to produce 1 mol metal carrier complex.
From assumption (iv), the reaction velocity $v$ for the formation and decomposition of the metal–carrier complex is given by

$$v_f = k_1 [C_i][E] - k_{-1} [CE]$$
$$v_b = k_2 [CE] - k_{-2} [C_i][E]$$

(4)

Using Eqs. (3) and (4), we obtain

$$\nu = E_1 \frac{Z_1 [C_i] - Z_2 [C_i]}{1 + Z_3 [C_i] + Z_4 [C_i]}$$

(5)

and

$$[CE] = E_1 \frac{Z_1 [C_i] - Z_2 [C_i]}{1 + Z_3 [C_i] + Z_4 [C_i]}$$

(6)

where

$$Z_1 = \frac{k_1 k_2}{k_{-1} + k_2}, \quad Z_2 = \frac{k_{-1} k_{-2}}{k_{-1} + k_2},$$

$$Z_3 = \frac{k_2}{k_{-1} + k_2}, \quad Z_4 = \frac{k_{-2}}{k_{-1} + k_2}$$

(7)

From assumption (iii), $[CE]$ is small, so from Eq. (6), $Z_3 [C_i] + Z_4 [C_i] \approx 0$. Hence Eq. (5) approximates to

$$\nu = E_1 (Z_1 [C_i] - Z_2 [C_i])$$

(8)

Replacing $E_1$ by the product $np$, where $n$ is the cell number (cell$^{-1}$) and $p$ is the carrier content of the cells (nmol cell$^{-1}$),

$$v = np (Z_1 [C_i] - Z_2 [C_i])$$

(9)

By defining $R_1$, the carrier rate constant, as $p(Z_1)$ and $R_2$, the ratio of the rate constants, as $Z_2 / Z_1$ and writing $v$ as $d(n[C_i]) / dt$, one obtains

$$\frac{d(n[C_i])}{dt} = nR_1 (Z_1 [C_i] - R_2 [C_i])$$

(10)

The metal mass balance, taking into account the adsorption of iron hydroxide colloids gives

$$A = m + H + n([C_i] + [C_i])$$

(11)

where $A$ is the total metal concentration in a unit volume of the system, $m$ is the concentration of the metal ion in solution and $H$ is the ferric hydroxide colloid concentration.

Substituting Eqs. (1) and (11) into Eq. (10), one obtains

$$\frac{d(n[C_i])}{dt} = \frac{nR_1}{K^{-1} + n} (A - H - [C_i] (n + R_2 (K^{-1} + n)))$$

(12)

This final expression provides a description of the model from which an analytical solution can be obtained. At the initial stage of the metal uptake, only surface sorption is significant so that $[C_i]$ may be assumed to be zero in Eq. (11). At time $t = 0$, $n = n_0$. Hence, with Eq. (1), one obtains

$$K = \frac{A - m - H}{m} n_0$$

(13)

An estimate of the sorption constant $K$ can therefore be made using results obtained at the beginning of the experiments.

From Eqs. (1) and (11), the intracellular metal concentration $n[C_i]$ may be writing as

$$n[C_i] = \frac{K^{-1} (A - H) - m (K^{-1} + n)}{K^{-1}}$$

(14)

Substituting Eqs. (1) and (14) into Eq. (10) yields

$$\frac{d(K^{-1} (A - H) - m (K^{-1} + n)) / K^{-1}}{dt} = \frac{nmR_1}{K^{-1}} - \frac{K^{-1} (A - H) - m (K^{-1} + n)}{K^{-1}} R_1 R_2$$

(15)

By defining two variables $\alpha$ and $\beta$ as

$$\alpha = \frac{K^{-1} (A - H) - m (K^{-1} + n)}{K^{-1}}, \quad \beta = \frac{nm}{K^{-1}}$$

(16)

Eq. (15) becomes

$$\frac{d\alpha}{dt} = \beta R_1 - \alpha R_1 R_2$$

From the experimental data, $\alpha$ can be expressed as a power series of $t$, and thus the gradient can be expressed as a function of $t$. Likewise, $\beta$ can be calculated using the values of $n$ and $m$ at different
times. By substituting $d\alpha/dt$, $\alpha$ and $\beta$ evaluated at different times into Eq. (16), the carrier rate constant $R_1$ and the ratio of the rate constants $R_2$ were estimated using a linear least-squares analysis routine. Having obtained an estimate of the three constants from the experimental data, Eq. (12) was solved in the case of viable cells without growth ($dn/dt = 0$), by defining

$$U(t) = \frac{R_1(A - H)}{K^{-1} + n}$$

$$m = \frac{K^{-1}(A - H)}{K^{-1} + n_0} \times \left(1 - \frac{U(t) n_0}{V(A - H)} \left[1 - \exp(-Vt)\right]\right)$$

Rewriting Eq. (12) gives

$$\frac{dn[C_1]}{dt} = n(U(t) - V[C_2]) (19)$$

If $[C_1] = 0$ and $n = n_0$ at $t = 0$, the solution becomes

$$[C_1] = \frac{U(t)}{V} (1 - \exp(Vt))$$

$$[C_3] = \frac{A - H}{K^{-1} + n_0} \times \left(1 - \frac{U(t) n_0}{V(A - H)} \left[1 - \exp(-Vt)\right]\right)$$

and

$$m = \frac{K^{-1}(A - H)}{K^{-1} + n_0} \times \left(1 - \frac{U(t) n_0}{V(A - H)} \left[1 - \exp(-Vt)\right]\right)$$

4. Results and discussion

The oceanic chemistry of Fe is highly complicated and still not fully understood. Dissolved Fe can exist in two different oxidation states in seawater, Fe(III) and Fe(II). Fe(III) is the thermodynamically stable form in oxygenated waters. However, in surface waters, there are several processes that reduce Fe(III) leading to measurable steady concentrations of Fe(II). This inorganic Fe(II) is oxidized back to Fe(III) with a half-life of a few minutes (Millero and Sotolongo, 1989). Inorganic Fe(III) speciation is dominated by the hydrolysis products, with Fe(OH)$_2^+$ and possibly Fe(OH)$_3^-$, as the dominant inorganic species in seawater (Millero et al., 1995b). The free hydrated Fe$^{3+}$ ion is not only an extremely rare species, but it is also the slowest of the inorganic species to react with ligands or surface sites due to its slow exchange of water (Hudson et al., 1992). Dissolved inorganic Fe(III) is currently thought of as the chemically relevant form of Fe for phytoplankton uptake (Hudson and Morel, 1990). Rich and Morel (1990) using well-defined iron colloids showed that for the marine diatom *T. Weissflogii*, colloids can only be dissolved under photoreduction and thermal conditions to satisfy their need for iron. Until recent years, there was a lack of knowledge concerning the degree to which Fe(III) is complexed with natural organic ligands in the oceans. Recent studies by Gledhill and Van den Berg (1994), Van den Berg (1995), Wu and Luther (1995) and Rue and Bruland (1995) have shown that it is likely that iron occurs organically complexed throughout the oceanic environment with an important excess of Fe(III) complexing organic ligands. It has been found that there are at least two natural Fe-chelators in existence in surface seawater which can complex iron at concentrations over 1–2 nM. Wu and Luther (1995) using a kinetic approach have found that organic ligands from different oceanic environments have different rates of complexing Fe(III) added to the sample. Fe additions of around 10 nM are needed to titrate the Fe-chelator concentration and presumably be partially precipitated as colloidal hydrous ferric oxide. This excess of strong iron-binding ligands in the oceans can alter the fundamental basis of our understanding of oceanic Fe speciation and biological availability. They have suggested that some portion of the organically-bound iron must become biologically accessible for phytoplankton growth.

In our study, we have applied an acid-washing technique in order to examine the effect of the iron speciation on the sorption. In the absence of algae, the 100 nM iron added will be present as free iron, inorganic iron including the most important ferric
hydroxide colloidal particles and natural organic Fe-complexed. In our studies, an hour of Fe(III) incubation time was used prior to the addition of the cell concentrate. After this time, Fig. 1 shows that Ferric hydroxide precipitation was evidenced by the reduction in the total iron concentration after filtering with 3.0 μm and 0.1 μm Nucleopore filters, and that these concentrations do not change appreciably with time. This shows that no adsorption took place. As can be seen in Fig. 1 (0.1 μm, no cells), less than 10 nM Fe(III) is present as dissolved iron. According to Van den Berg (1995) and Wu and Luther (1995), 90 to 95% of this Fe(III) can be organically-complexed providing sufficient organic ligands are available. From Fig. 1, we find that around 90 nM Fe(III) is colloidal hydrous ferric oxide. In the presence of algae, part of the ferric hydroxide particles are going to be adsorbed on the cells. This was shown by using Formalin-treated (0.5%) and 3 μm filtration (3.0 μm, dead cells in Fig. 1). These results indicate that an important fraction of the total colloidal iron is being retained on the cell surface via nonphysiological processes. This fraction needs to be determined in order to compute the true sorption constants in assimilation studies. Anderson and Morel (1982) used a technique that exploits the Fe(III) reducing property of ascorbate in pH = 2.5 to dissolve filterable colloidal iron, which produces the same but faster results than the HCl washing procedure. This technique could not be applied in our case due to the interference of ascorbic acid in the chemiluminescence determination of iron. Instead, we have applied the technique which dissolved the colloidal adsorbed iron by using an acidification with HCl (final pH = 2) for 30 min. After this time, a steady state concentration of Fe(III) was approached (Fig. 1 insert). In experiments carried out using viable and dead cells filtered by 3.0 μm, a decrease can be seen (Fig. 1) in the Fe(III) concentration due to both incorporation on the cell and precipitation on the cell surface. When this technique was applied to the viable (HCl, viable cells) and Formalin-treated cultures (HCl, dead cells), only the viable culture showed significant decrease in the iron concentration. This study also shows that even when the precipitated iron increases with time (see 3.0 μm, dead cells), the HCl-washing technique dissolves this precipitate giving the same result as when there are no cells added to the solution. This indicates that acid-washed technique effectively removes all the ferric hydroxide. It should be pointed out, however, that a pH = 2 may not be tolerated by some species and may put a severe osmotic stress on natural phytoplankton populations.

Fig. 2. Time-course of iron (A = 97.22 nM) with and without HCl treatment before filtering according to the fractionation scheme in Table 1 on 1.22 × 10^7 cells l^{-1} T. weissflogii in the absence and in the presence of 382 nM Zn(II) added at 25°C. The sorption plus membrane model profiles are presented as full lines.
In all the figures where metal uptake kinetics data are shown, the model profiles (using Eq. (12)) are presented as full lines while points denote values obtained from experimental results. The metal ion was added at time $t = 0$ (min). Fig. 2 shows the uptake of Fe(III) initially at 97.2 nM by *T. weissflogii* $1.22 \times 10^7$ cells $l^{-1}$ at 25°C under standard conditions. Upon mixing the culture with iron ions, the colloidal and dissolved Fe in the solution determined after filtration using 3 μm filters dropped by about 30% within the first 1–5 min. This decrease is due to both passive uptake and deposition of colloidal iron on the cell surface. When the cell was first acid-washed, a higher HCl labile fraction is observed which allows us to determine the real Fe sorbed concentration which was used to determine sorption constants (Eq. (13)). Subsequently, a slow metal uptake was observed. The increase in the colloidal accumulation on the surface cell with time (difference between HCl labile fraction, and colloidal and dissolved Fe in solution) should also be noted. The decrease in the dissolved iron after filtering with a 0.1 μm is due to the uptake of iron by the cells. The model proposed in this work is consistent with the observed instantaneous decrease in the residual metal concentration in the solution brought about by metal ion sorption onto the algal cell walls and the simultaneous membrane transport of the metal ions into the cytoplasm of the cells. As can be seen from all the figures, there is a good agreement between the experimental results and the model calculations. According to other authors (Anderson and Morel, 1982; Sunda, 1989; Hudson and Morel, 1990), it would appear that the uptake of iron is mediated by a transport protein in the membrane, ($E$ in Eq. (2)), named ‘phytotransferrin’. The uptake is controlled by the kinetics of metal exchange between the free or the complexed iron in the medium and phytotransferrin on the cell surface which may depend on the kinetics of the chelate dissociation in line with the results of Rich and Morel (1990) and Wu and Luther (1995). The process of iron exchange may proceed through one of the two mechanisms, as discussed by Jackson and Morgan (1978). Either the iron chelate may first dissociate and the hydrated or hydrolyzed Fe(III) species may then react with phytotransferrin or a ternary complex, Chelator–Fe–phytotransferrin, may form leading to direct metal exchange. Both processes are considered in Eq. (2). However, as has been pointed out by Hudson and Morel (1990), ligand exchange reactions through the formation of intervening ternary complexes involving an exuded chelator do not appear to be the mechanism for iron exchange.

Fig. 2 also shows the uptake of 97.2 nM iron by $1.33 \times 10^7$ cells $l^{-1}$ *T. weissflogii* when spiked with 382 nM Zn(II) concentration (Fe:Zn, 1:4) at 25°C (black inverse triangles). Upon the addition of the metal ions to the culture, the concentration of iron taken up by the alga increases with time at a greater rate than in the presence of iron alone. The sorption constants $K$ (Table 2), which are dependent on the environmental conditions such as pH, medium composition, and cell physiology, do not vary appreciably throughout the experiments. These results show that the sites for the iron sorption are not affected by

### Table 2

<table>
<thead>
<tr>
<th>Conditions</th>
<th><em>T. weissflogii</em></th>
<th><em>D. tertiolecta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td></td>
<td></td>
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<tr>
<td>Fe:Me (1:0)</td>
<td>2.464 ± 0.147</td>
<td>2.029 ± 0.465</td>
</tr>
<tr>
<td>Fe:Zn (1:1)</td>
<td>2.306 ± 0.245</td>
<td>2.261 ± 0.115</td>
</tr>
<tr>
<td>Fe:Zn (1:4)</td>
<td>2.476 ± 0.302</td>
<td>2.181 ± 0.098</td>
</tr>
<tr>
<td>Fe:Al (1:5)</td>
<td>2.654 ± 0.134</td>
<td>2.221 ± 0.127</td>
</tr>
<tr>
<td>Fe:Cd (1:8)</td>
<td>2.251 ± 0.183</td>
<td>2.230 ± 0.217</td>
</tr>
</tbody>
</table>

| 8°C         |                 |                  |
| Fe:Me (1:0) | 1.333 ± 0.164   | 1.675 ± 0.089    |
| Fe:Zn (1:4) | 1.318 ± 0.204   | 1.613 ± 0.189    |
| Fe:Al (1:5) | 1.398 ± 0.345   | 1.648 ± 0.098    |
the presence of different metals at different concentrations which, in turn, shows the specificity of the sorption sites of the cells for iron.

Fig. 3 shows the iron kinetics in the absence of other added metals and in the presence of Zn(II) (Fe:Zn 1:4) at 25°C by the green algae *D. tertiolecta* 9.4 × 10^7 cells l^{-1}. Again, the sorption plus membrane transport model agrees well with the results found experimentally, both in respect to the residual metal concentration in the solution and for the quantity of metal taken up by the algal cells. It is clear that significantly less iron is removed from the solution in the presence of increasing amount of Zn added to the solution (black inverse triangles). Although the metal sorption process (both in the absence and presence of other metal ions added) may not appear to be very dissimilar (Table 2), it must be realized that the overall specific uptake is the sum of the two components: the surface sorption and the intracellular fraction. While the sorption capacity of the cells by iron shows a relatively constant behavior, the membrane transport must be much smaller in the presence of other metals, due to interference in the metabolism of other essential metals. These issues have been discussed by Sunda et al. (1991), Bruland et al. (1991) and Morel et al. (1991).

With the high iron concentration used in our experiments, the sorption constant allows us to determine the theoretical number of transport sites needed by each cell. Direct uptake from the cell surface of 1.6 × 10^{-16} mol Fe cell^{-1} is found for *T. weissflogii* and 1.5 × 10^{-17} mol Fe cell^{-1} for *D. tertiolecta* at 25°C. Hudson and Morel (1990) found a value of 10^{-17} mol Fe cell^{-1} at 20°C which is similar to the values we have found.

Figs. 4–6 show, respectively, the uptake of Fe(II1) with different Zn(II), Al(III) and Cd(II) concentrations at 8°C and 25°C in seawater in the presence of 1.3 × 10^7 cells l^{-1} *T. weissflogii*. Fig. 7 shows the results for the same experiments of iron uptake in the presence of 1.0 × 10^8 cells l^{-1} *D. tertiolecta*. In the *T. weissflogii* experiments it is clearly shown that the presence of a second metal ion effectively favors the forward carrier reaction thereby resulting in the observed synergism. The effect is more important for the presence of the essential element zinc which plays an important role in many enzyme systems (Sunda, 1989) than is the case for Al. The additions of Cd have no appreciable effect on the iron uptake for the alga *T. weissflogii*. Harrison and Morel (1983) also found cadmium inhibition on the growth of *T. weissflogii* when the overall cellular iron is not
reduced. As expected, the decrease in temperature decreases the sorbed iron concentration with $K = 2.48 \times 10^{-9}$ cell$^{-1}$ to $K = 1.35 \times 10^{-9}$ cell$^{-1}$ (Table 1) ($\Delta H^0 = 25$ kJ mol$^{-1}$) and the effect of the synergistic behavior.

For the *D. tertiolecta* experiments (Fig. 7), an antigenic effect is clearly observed for the addition of increasing amounts of zinc and for additions of aluminum and cadmium both at 25°C and 8°C. That is, Fe(III) increases with the reduction of the concent-

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Fig. 4. Uptake kinetics of 100 nM Fe(III) in the absence and presence of Zn(II) at 8°C and 25°C in the presence of $1.3 \times 10^7$ cells l$^{-1}$ *T. weissflogii*. Sorbed Fe(III) was determined after HCl treatment, final pH = 2 during 30 min.

Fig. 5. Uptake kinetics of 100 nM Fe(III) in the absence and presence of Al(III) at 8°C and 25°C in the presence of $1.3 \times 10^7$ cells l$^{-1}$ *T. weissflogii*. Sorbed Fe(III) was determined after HCl treatment, final pH = 2 during 30 min.
Fig. 6. Uptake kinetics of 100 nM Fe(III) in the absence and presence of Cd(II) at 8°C and 25°C in the presence of 1.3 × 10⁷ cells l⁻¹ T. weissflogii. Sorbed Fe(III) was determined after HCl treatment, final pH = 2 during 30 min.

The uptake of Fe(III) in the presence of Cd(II) shows endothermic behavior with specific sorption energies. The sorption constants for 25°C and 8°C (Table 2) indicate a reduction in the sorption constants with temperature from 2.2 × 10⁻¹⁰ 1 cells⁻¹ to 1.6 × 10⁻¹⁰ 1 cells⁻¹, suggesting an endothermic process with ΔH⁰ = 13 kJ mol⁻¹. This positive specific sorption energy may be due to the heat of the hydration of iron being lower than the heat of sorption.

Fig. 7. Uptake kinetics of 100 nM Fe(III) in the absence and presence of Zn(II), Al (III) and Cd(II) at 8°C and 25°C in the presence of 1.0 × 10⁸ cells l⁻¹ D. tertiolecta. Sorbed Fe(III) was determined after HCl treatment, final pH = 2 during 30 min.
In order to understand the different behavior of the two algae, the *T. weissflogii*, with its siliceous cell walls and the biflagellate *D. tertiolecta* with a lack of a true cell wall (Van den Hoek et al., 1995), we have studied both the effect of the addition of different metals on the speciation of iron and the effect of these additions on the parameters in the sorption plus membrane transport model. Figs. 8 and 9 show the total colloidal Fe(III) concentrations determined as the difference between the HCl labile fraction (Fe(III) concentration after HCl (pH = 2, 30 min) treatment and 3 μm filtering) and dissolved...
Fe(III) concentration (after filtering by 0.1 μm) at 25°C for *T. weissflogii* experiments and *D. tertiolecta* experiments, respectively. The changes in the total colloidal concentrations can be attributed to (1) a decrease in the total iron in the solution as more iron is taken up due to polymerization into large polymers and crystalline ferric colloids, or (2) an increase in the organically complexed iron as more metal-binding proteins are exuded or excreted by the algae to the solution.

For the *T. weissflogii* experiments, the increase in the uptake of iron after the addition of different concentrations of zinc and aluminum (Figs. 2, 4 and 5) is followed by an important decrease in the total colloidal concentration (Fig. 8). According to Morel et al. (1991) and to Rue and Bruland (1995), the organically complexed iron, may be transported across the membrane. This may be the case if sufficiently lipophilic complexes form or if the specific transport ligands such as siderophores are released into the medium for subsequent transmembrane transfer of the metal-ligand complexes via specialized porter proteins. Moreover, according to Wells and co-workers (Wells et al., 1983; Wells, 1989) and Rich and Morel (1990), the photoreduction and thermal conditions on Fe oxides in seawater produce amorphous Fe(III)-oxide coatings which are more soluble and more labile than the original oxides. Such coatings increase the dissolved Fe(III) species concentrations and hence increase Fe bioavailability. In all our *T. weissflogii* experiments, the increase in the iron uptake is lower than the decrease in the colloidal iron which shows that both the reduction of Fe(II) to the highly soluble Fe(II) mediated by extracellular algal products and an increase in the organically complexed iron could take place when higher concentrations of zinc and aluminum are added to the seawater solution (Morel et al., 1991). The higher concentration of organic ligands could appear as a response of the cells to the high concentrations of zinc and aluminum added in our studies. At high Zn concentrations, this metal can act as a toxicant due to the interference in the metabolism of iron (Morel et al., 1991). In order to reduce this toxic effect, more ligands may be exuded by the alga. The decrease in colloidal iron increases the dissolved iron necessary for the Fe uptake. What is not clear at the present moment is the mechanism and the rate of Fe transformation reactions from stable Fe-organic complexes, inorganic crystalline Fe hydrolysis products and small Fe colloids to labile iron.

What this study shows is that the increase in iron uptake for the *T. weissflogii* must be due to the use of labile iron generated by photoreduction and thermal conditions or the use of organically complexed iron, as concluded by Rue and Bruland (1995). The cadmium additions to *T. weissflogii* (Fig. 6) show that it does not affect the total colloidal concentration, and the same amount of iron is available in the absence or presence of cadmium. At low temperatures (data not shown), the same behavior is observed. Higher colloidal concentrations (about 5–10 nM for iron alone and between 10–15 nM in the presence of a second metal) are found than at 25°C due to both the effect of temperature on the solubility of precipitates and the reduction in biological activity at lower temperatures.

For the studies of iron uptake with *D. tertiolecta* (Figs. 3 and 7), it is observed that the decrease in the uptake concentration of iron in the presence of a second metal ion is followed by an increase in the total colloidal iron concentration (Fig. 9). The addition of appreciable amounts of zinc, aluminum and cadmium to the algal solution competitively inhibits Fe uptake. The decrease in iron uptake produces an equivalent increase of iron in the solution which is precipitated, as seen in Figs. 7 and 9. It has been shown that the green flagellate *D. tertiolecta* excretes sulfide, which can strongly complex and precipitate most heavy metals (Van den Hoek et al., 1995). Such precipitation in the membrane as insoluble sulfide could further restrict the inward passage of ions. The increase in colloidal iron reduces the iron available to be assimilated by the alga. As pointed out in other studies (Sunda et al., 1991), the addition of these metals can also nutritionally replace iron. At 8°C, the colloidal iron concentration reaches 70 nM when only iron is added and increases to around 80 nM when zinc is added in a ratio 1:4. This increase in colloidal iron reduces the iron availability and, thus, the uptake of iron.

The key parameters in the sorption-membrane transport model are the sorption constant K, the carrier rate constant \( \bar{R}_1 \) (\( \bar{R}_1 = p(Z_1) \)), and the ratio of the rate constants \( \bar{R}_2 \) (\( \bar{R}_2 = Z_2/Z_1 \)) for each
experiment. The rate of membrane transport is proportional to $R_1$ and to $-R_2$ (Eq. (12)).

Table 2 provides a summary of these parameters for the iron uptake for the different experimental conditions. As mentioned above, the sorption constants $K$, appear to vary only slightly for the experiments at a given temperature. However, the values of $R_1$ and $R_2$ with only iron present are dependent on the metal added, the concentration of the second metal, the temperature and the type of cell. For *T. weissflogii* the increase in the uptake concentration of iron in the presence of increasing amounts of zinc or in the presence of aluminum is accompanied by an increase in $R_1$ and by a slight decrease in $R_2$ both at 25°C and 8°C. An increase in $R_1$ favors the forward carrier reaction. The $R_2$ is a function of both $Z_1$ and $Z_2$ thus a change in its value could either be due solely to the change in the forward reaction $R_1$ or a concurrent change in the reverse carrier reaction. Assuming that $p$, the carrier content of the cells remains approximately constant throughout a set of experiments, the observed increase in $R_1$ for all the experimental conditions for *T. weissflogii* suggests an enhancement of the forward carrier reaction, thereby resulting in a substantial increase in $Z_1$. The concomitant decrease in $R_2$ could be due to the increase of $Z_1$. As expected, at low temperatures, there is a decrease in the values of $R_1$ compared to that at 25°C and an increase in the $R_2$ values due to a decrease in $Z_1$. In contrast, the model parameters for the uptake of iron in the absence and presence of cadmium are similar at the two temperatures. The conclusions given above using the kinetics model parameters are in agreement with those obtained using the changes in the iron speciation in the presence of other ions. The increase in iron inorganically and organically complexed is accompanied by an increase in the carrier rate constants.

For *D. tertiolecta*, the values for the sorption constant are lower than those for the *T. weissflogii* showing that different phytoproteins are involved in the transfer process throughout the membrane. Also, the specific sorption energy of the sorption process is half that for *T. weissflogii*. Table 2 shows changes in both $R_1$ (the carrier rate constant) and $R_2$ (the ratio of rate constants) while $K$ remains relatively unaltered. In this case, $R_1$ decreases with increasing concentration of the competing zinc ions, with aluminum (ratio 1:5) and with cadmium (ratio 1:8). Assuming that the carrier content of the cells remains approximately constant, the decrease in $R_1$ suggests an inhibition of the forward carrier reaction due to both the competition from other ions and precipitation of insoluble sulfide thereby resulting in a decrease in $Z_1$. The sorption constants $K$ for these experiments also remained essentially constant. The competition between iron and other ions for surface sorption sites at different concentrations is insignificant. The important increase in $R_2$, however could either be due solely to the decrease in the forward reaction $R_1$ or a concurrent increase in the reverse carrier reaction. Nevertheless, the rate of membrane transport of iron is inhibited by the presence of the metal ions studied. *D. tertiolecta* shows an important antagonistic effect of cadmium on the iron uptake at both temperatures. The decrease in the rate of the forward reaction is followed by an important increase in the ratio of rate constant that could also be due to cadmium promoting the reverse carrier reaction for iron transport thereby leading to an inhibition in the uptake of iron. Price and Morel (1990) were the first to find the replacement of another essential trace metal, Zn, by Cd in Zn-starved phytoplankton. They pointed out that this substitution could be the cause of the Cd depletion observed in surface seawater.

Fe enrichment experiments (Martin et al., 1989) have been used to investigate whether phytoplankton are limited by low Fe concentrations in remote high nitrate, low-chlorophyll regimes. Wu and Luther (1995) and Rue and Bruland (1995) have shown that, in such studies, it is important not only to know the environmental conditions of the system which controls Fe speciation but also the concentration of Fe-binding organic ligand(s) and their conditional stability constant. Our studies show that the iron speciation is also affected by the type of phytoplankton cell present. The addition of iron will not only change the speciation of iron, but also will change the ratio of iron to other essential or non-essential trace metals. This change will affect the uptake of iron depending on the synergistic or antagonistic effect of different alga species present in the area. In our case, the increase in Zn and Al enhances Fe uptake by *T. weissflogii* and inhibits Fe uptake by *D. tertiolecta*, and the increase in Cd only inhibits Fe...
uptake by *D. tertiolecta*. We have shown that these additions were also followed by a decrease and by an increase in the total colloidal iron concentration, respectively, for *T. weissflogii* and *D. tertiolecta*. For *T. weissflogii*, it is possible that the photo-reduction of Fe(III) to Fe(II) at the cell surface could enhance not only the iron organically complexed but also enhance Fe uptake if they are part of a siderophore or other biomolecules such as porphyrin uptake system. Microorganisms isolated from coastal and oceanic waters have been shown to produce specific iron chelators (siderophores) in culture media (Trick, 1989; Reid et al., 1993). The antagonistic behavior showed by the alga *D. tertiolecta* may be the result of nutritional replacement of iron by other metal ions added and an increase of sulfide which precipitates most heavy metals, thereby increasing the total colloidal iron. Further studies are needed to completely elucidate these effects.

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