

## The role of unchelated Fe in the iron nutrition of phytoplankton

**Abstract**—The important question of iron bioavailability in the sea has become complicated by the discovery that marine phytoplankton can take up Fe bound in very stable chelates via reductive processes, and some particular Fe species through specialized transport mechanisms. As a result there is some question of whether the small fraction of Fe that is “free” or unchelated in seawater is important in the nutrition of natural phytoplankton assemblages. A careful examination of published laboratory studies on Fe uptake by model organisms all support the idea that unchelated Fe(III) is highly available for uptake and that it is an important source of the Fe taken up by phytoplankton under a variety of experimental conditions. Comparing these results with field data on Fe speciation shows that unchelated Fe can be an important source of Fe to the phytoplankton in the sea: it is likely sufficient to contribute the bulk of the Fe supporting primary production in regions that are not limited by Fe and a significant fraction everywhere, including high-nutrient low-chlorophyll areas.

The revolution in our understanding of ocean productivity started by Martin 20 yr ago (Martin and Fitzwater 1988) is continuing. In addition to high-nutrient low-chlorophyll (HNLC) oceanic areas, some coastal ecosystems (Hutchins et al. 1998) and deep chlorophyll *a* maxima of oligotrophic regions (Hopkinson and Barbeau pers. comm.) have now been shown to be limited or colimited by iron. To go beyond observations or phenomenological experiments and truly understand the link between Fe inputs and productivity, we need to elucidate the relation between the concentration of various chemical forms of Fe in seawater and its uptake by phytoplankton; that is, we need to define chemically the “availability” of Fe species to marine phytoplankton.

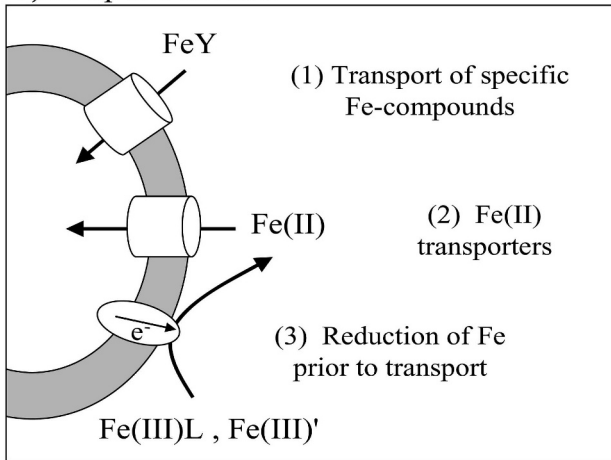
For the purpose of our discussion, despite their variety and complexity, we can classify the Fe uptake systems of microorganisms into three categories (Fig. 1A): (1) transport systems that are specific for particular Fe compounds or families of compounds, such as Fe citrate, Fe siderophores, or hemes; (2) Fe(II) transporters of various specificities, including divalent metal ion transporters and oxidase–permease complexes that oxidize Fe(II) while transporting it across the external membrane; (3) transport systems that include reductases able to reduce various Fe(III) species at the cell surface and deliver Fe(II) to (2). Notably absent from our list are transporters of unchelated Fe(III), Fe(III)', which have been reported to exist at the inner but not the outer membranes of gram negative bacteria. The existence of such transporters in the outer membrane of some phytoplankton would, of course, not affect our argument regarding the importance of unchelated Fe(III).

We have at present no firm information regarding either the existence in phytoplankton of transport systems that

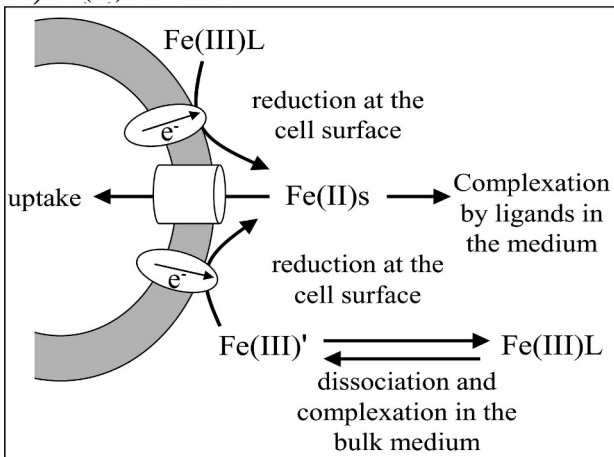
belong to category (1) or the concentration of the corresponding compounds in seawater. As a result, the question of Fe bioavailability in seawater is focused on uptake via systems (2) and (3), which are supported by genomic and transcriptomic data in two marine diatoms (Kustka et al. 2007). Early studies demonstrated a correspondence between the concentration of unchelated Fe in the medium and Fe uptake by phytoplankton (the Fe' model; Hudson and Morel 1990). Following the demonstration that Fe bound in some strong complexes can be taken up by some species of phytoplankton and that Fe(III) must be reduced for uptake (Soria-Dengg and Horstmann 1995; Maldonado and Price 2001, Shaked et al. 2005), newer studies have focused on the role of Fe(II) in uptake. Recently two models have been proposed to describe the kinetics of Fe uptake by phytoplankton and effectively quantify Fe availability in seawater: (1) the Fe(II)s model (Fig. 1B; Shaked et al. 2005) uses the surface concentration of reduced iron, Fe(II)s, as the parameter controlling uptake; it is based on experimental data with diatoms and explicitly incorporates the previous Fe' model by making unchelated Fe(III), an important source of reduced Fe at the cell surface. (2) The FeL model (where L represents an Fe-chelating ligand; Fig. 1C; Salmon et al. 2006) makes uptake dependent on the concentration of Fe(II) in the bulk medium and considers chelated Fe(III) to be the only source of reduced Fe; it is based on data with natural samples of the cyanobacterium *Lyngbya majuscula* (but is presented as applicable to other phytoplankton including diatoms) and emphasizes the reoxidation of Fe(II), particularly that of the chelated form, Fe(II)L, as a key process competing with uptake. An examination of the similarities and differences between these two models and a comparison of their predictions with available experimental data provide a good basis for exploring the question of Fe availability to phytoplankton.

The most obvious difference between the Fe(II)s and FeL models is that one considers the surface concentration of Fe(II) and the other its bulk concentration as the controlling parameter. This difference stems from the difference in the principal reduction mechanisms assumed to generate Fe(II) as a substrate for uptake: cell surface reductases in one case, bulk reduction by  $O_2^-$  in the other. This difference is immaterial in the mathematical formulation of the models since they both ignore the diffusion of Fe(II) species between the cell surface and the bulk solution. But the question of what processes are responsible for Fe reduction is in fact important. Because of the necessary diffusion to the cell surface and of the fast reoxidation of the unchelated Fe(II), Fe(II)', it matters greatly if reduction occurs at the surface of cells or in the bulk solution and by what mechanism. For example, the photoreduction of an Fe(III)chelate (such as Fe(III)ethylenediaminetetraacetate [EDTA] in culture medium) results

## A) Fe uptake models



## B) Fe(II)s model



## C) FeL model

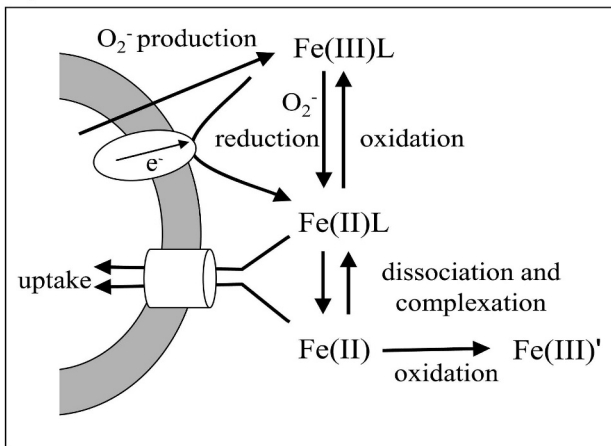


Fig. 1. Schematic illustrations of Fe uptake systems in phytoplankton. (A) Three major pathways of Fe uptake from the external milieu. The important differences in the uptake systems of eukaryotes and prokaryotes are intentionally left out, as are the detailed steps involved in each of the pathways. (B) Fe(II)s model modified from Shaked et al. (2005). Both the chelated and unchelated Fe(III), FeL, and Fe(III)' serve as sources of Fe(II) at the surface for uptake. (C) FeL model modified from Salmon et al. (2006). Only the chelated Fe(III), Fe(III)L, serves as a source of Fe(II), either free or chelated, for uptake.

in the formation of Fe(II)'. During the charge transfer reaction that follows the absorption of a photon, the ligand is oxidized to some byproduct while the complexed Fe(III) is reduced to Fe(II), which is released to solution. Because the rate of oxidation of Fe(II)' by oxygen, which may be accelerated further by  $H_2O_2$  or  $O_2^-$ , is faster than the rate of complexation by ligands in solution or than the rate of diffusion to the surface of cells, the net result of such photoreduction is an increase in the Fe(III)' in solution. This does not necessarily occur if the reduction is caused by an external electron donor such as  $O_2^-$  in a manner that leaves the ligand intact, keeping Fe(II) bound and allowing reoxidation of Fe(II) coordinated to the ligand. Studies with the diatom *Thalassiosira weissflogii* showed that  $O_2^-$  produced by the organism was ineffective at increasing Fe uptake (Kustka et al. 2005). The data of Salmon et al. (2006) showing a relatively fast Fe uptake rate in the presence of diethylenetriaminepentaacetate (the Fe(III) complex of which is apparently not reduced by  $O_2^-$ ; Rose and Waite 2005) also seem to contradict the conclusion that Fe(III) reduction by  $O_2^-$  determines uptake in *L. majuscula*.

Another apparent difference between the two models is that one considers a unique parameter Fe(II)s as controlling uptake while the other explicitly differentiates between Fe(II)' and Fe(II)L, both serving as substrates for uptake. In the Fe(II)s model, the parameter Fe(II)s does not represent the concentration of unchelated Fe(II) at the surface, but all Fe(II), chelated or unchelated, that can react with uptake ligands (Shaked et al. 2005). Indeed, some Fe(II) transporters are likely able to capture Fe(II) bound to an external ligand, not just  $Fe^{2+}$  or its complexes with major anions. In this particular respect, the Fe(II)s and FeL model are not different. The use of a single parameter, Fe(II)s, in the Fe(II)s model is functionally equivalent to the use of a single uptake constant in the FeL model for both free and chelated Fe(II)—a simplification made necessary by the absence of pertinent data and that obviates the difficulty in quantifying ligand exchange kinetics. The net result is that both models make similar predictions for Fe uptake in media where Fe(III) is chelated to an extremely strong chelator such as desferrioxamine B (DFB). In this situation the concentration of unchelated Fe(III), Fe(III)', is negligible and only Fe(III)L (L = DFB) serves as a source of Fe(II) for uptake. Clearly both the rate at which the iron can be reduced in the chelate and its rate of reoxidation are then critical factors in determining uptake. A similar result may obtain in a situation where Fe(III) is bound in a weak and highly reducible complex such that the rate of reduction of Fe(III)L dominates the production of Fe(II). An example might be that of Fe(III)citrate in the case of *Lyngbya* (Salmon et al. 2006); but data obtained under well-defined conditions (where the ligand does not affect the kinetics of dissolution of Fe oxide) and with cells that are known not to have an Fe citrate transporter are necessary to prove the point.

At bottom, the fundamental difference between the Fe(II)s and the FeL models that leads to dissimilar predictions is that the Fe(II)s model explicitly includes the unchelated Fe(III) in the medium as an important

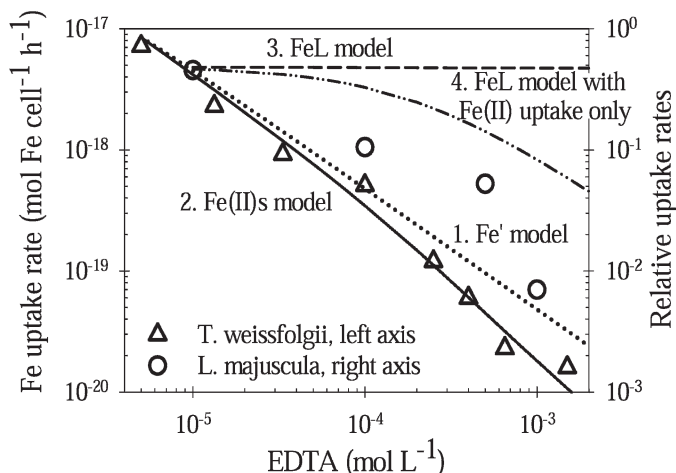


Fig. 2. Comparison of models with experimental data on iron uptake at varying EDTA concentrations in iron-limited diatoms (*Thalassiosira weissflogii*; one experiment reproduced from Shaked et al. 2005) and cyanobacteria (*Lyngbya majuscula*; reproduced from Salmon et al. 2006 and multiplied by a constant to match the scales). Line 1: Fe' model (Hudson and Morel 1990); line 2: Fe(II)s model (Shaked et al. 2005); line 3: FeL model (Salmon et al. 2006); line 4: modification of the FeL model in which only Fe(II)' is taken up. See Web Appendix 1, [www.aslo.org/lo/toc/vol\\_53/issue\\_1/0400a1.pdf](http://www.aslo.org/lo/toc/vol_53/issue_1/0400a1.pdf).

source of Fe(II) for phytoplankton uptake, while the FeL model explicitly excludes it as irrelevant. Let us consider the predictions of the two models for the common laboratory situation where the iron is complexed to EDTA in excess. In this situation, the two models ascribe the Fe uptake to two completely different sets of reactions: in the Fe(II)s model, the bulk of the Fe(II) that is taken up comes from the reduction of Fe(III)'; in the FeL model, it comes from the reduction of Fe(III)L (L = EDTA). This difference leads to divergent predictions regarding the effect of increasing the concentration of L on the rate of Fe uptake by phytoplankton.

In the Fe(II)s model, increasing [L] decreases the uptake rate through two separate mechanisms: (1) by decreasing Fe(III)' and hence the rate of Fe(II)s formation; and (2) by competing with the cells for Fe(II) at the surface. Because the supply of Fe(II)s from Fe(III)EDTA is small, the first effect results in an Fe uptake rate that is inversely proportional to [L]—this is the same prediction as the Fe' model. The second effect slightly exaggerates the decrease in uptake rate at very high [L]. Those predictions are in accord with experimental data obtained with diatom cultures (Fig. 2).

The FeL model ascribes the decrease in uptake rate at high [L] entirely to the decrease in Fe(II)' resulting from the complexation of the free Fe(II) by L and the fast reoxidation of Fe(II)L. Since Fe(II)' is also lost directly by oxidation (and cellular uptake), the complexation by L must be at least as fast as the oxidation to result in a significant decrease in uptake rate. This occurs for EDTA when  $[L] \geq 200 \mu\text{mol L}^{-1}$  ( $= k_{\text{ox}}^{\text{Fe(II)}}/k_{\text{compl}}^{\text{Fe(II)L}} = 4 \times 10^{-3} \text{ s}^{-1}/20 \text{ L mol}^{-1} \text{ s}^{-1}$ , considering only the oxidation

by oxygen). But at this high EDTA concentration, Fe(II)' is only a small fraction of Fe(II) ( $\text{Fe(II)'} = k_{\text{dissoc}}^{\text{Fe(II)L}}[\text{Fe(II)L}]/(k_{\text{ox}}^{\text{Fe(II)}} + k_{\text{compl}}^{\text{Fe(II)L}}[L]) \approx 1\% [\text{Fe(II)L}]$ , where  $k_{\text{dissoc}}^{\text{Fe(II)L}} \approx 8 \times 10^{-5} \text{ s}^{-1}$ ), so there is no measurable effect of increasing [L] on the uptake rate (Fig. 2). Even if, contrary to the basic premises of the FeL model, one considered the rate of uptake of Fe(II)' to be much larger than that of Fe(II)L (e.g., by making the latter negligible), the decrease in uptake rate resulting from the increase in [L] would occur at ligand concentrations much higher than observed experimentally (Fig. 2). No matter what reasonable set of parameters one might try, the predictions of the FeL model, or of any model that ignores the role of unchelated Fe(III) as a source of Fe for uptake, are at odds with experimental data obtained with diatoms in the presence of EDTA.

One is forced to conclude that Fe(III)' is an effective direct source of Fe(II) for uptake by diatoms, but that Fe(III)EDTA is not. This is presumably caused by the rapid reoxidation of the reduced Fe bound to EDTA in the presence of oxygen and other oxidants (Seibig and van Eldik 1997) and is consistent with the fact that an Fe(II) sequestering agent such as ferrozine captures very little Fe when Fe(III)EDTA is reduced by  $\text{O}_2^-$  (Rose and Waite 2005). Thus the complex Fe(III)EDTA can serve as a significant source of Fe for uptake by diatoms only via prior dissociation to form Fe(III)'. This was verified by Shaked et al. (2005) by demonstrating that, at high cell concentrations, the total uptake rate of the culture becomes limited by the rate of Fe(III)EDTA dissociation. No such limit is predicted if direct reduction of Fe(III)EDTA provides Fe(II) for uptake as is assumed in the FeL model.

The results obtained with diatoms are apparently also applicable to other eukaryotic phytoplankton that have been studied (Hudson and Morel 1990; Sunda and Huntsman 1995). Are they valid for *L. majuscula* or other cyanobacteria? Most published experiments on Fe uptake by cyanobacteria that use EDTA to control Fe availability have only used a single concentration of the ligand, making it impossible to distinguish the relative roles of Fe(III)' and Fe(III)L as sources of Fe reduction and uptake. One exception is the experiment reported for *L. majuscula* by Salmon et al. (2006) at constant total Fe and increasing EDTA concentrations (Fig. 2). As the EDTA increased from 10 to 1,000  $\mu\text{mol L}^{-1}$ , the Fe uptake rate decreased by a factor of about 60, nearly inversely proportional to [L] as would be expected from the Fe(II)s model if Fe(III)' were the sole source of Fe(II) at the surface. Conversely, as discussed above, the observed decrease in uptake rate with increasing EDTA is much larger than can be accounted for by the FeL model (Fig. 2) and clearly inconsistent with the idea that Fe(III)L is the only substrate for reduction. Fe(III)' must thus have been an important source of the reduced Fe taken up by *L. majuscula* in this experiment.

Besides experiments done at varying concentrations of chelating agents such as EDTA, the only ones that bear on the question of the availability of unchelated Fe(III) are those performed at varying light intensity in the presence of EDTA. As explained above, the net effect of the

photochemical degradation of Fe(III)EDTA is an increase in Fe(III)' in the medium. For example, in the experiments of Sunda and Huntsman (1997) performed with two diatoms, two dinoflagellates, and one cyanobacterium, increasing the light intensity from 50 to 500  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  resulted in a doubling in Fe(III)' for a given Fe and EDTA concentration. The result was a doubling in the Fe uptake rate of the organisms, as expected if Fe(III)' is the dominant form of Fe taken up by the cells. So, incomplete as they are, all the available data support the idea that unchelated Fe(III) is highly available for uptake, regardless of the organisms involved or the mechanisms they use to take up the iron—as might be expected from basic thermodynamic and kinetic considerations.

The interesting practical question is to what extent Fe uptake by various phytoplankton species in the sea depends on Fe(III)' or on other pools of dissolved Fe. We can obtain an estimate of the possible contribution of the unchelated Fe by putting together field data on Fe speciation and laboratory data on Fe requirements and uptake in model species. Taken at face value, the available data on Fe complexation in the surface ocean indicate Fe(III)' values in the ranges 0.01–1.2  $\text{pmol L}^{-1}$  in HNLC regions (Rue and Bruland 1995, 1997; Boye et al. 2001) and 0.04–50  $\text{pmol L}^{-1}$  in North Atlantic waters (Powell and Donat 2001; Boye et al. 2006). The high values are known to be sufficient for near-maximum growth rates of model oceanic species of diatoms and coccolithophores (Sunda and Huntsman 1995). The low values could only support very slow growth rates even for picoplankton taking up Fe near the limit of diffusion ( $<0.05 \text{ d}^{-1}$ , considering a minimum cellular Fe concentration around 20  $\mu\text{mol L}^{-1}$ ; Sunda and Huntsman 1995). But, during the day, the low Fe' values may be increased significantly above those estimated from electrochemical titrations as a result of photoreduction of Fe(III) complexes (Maldonado et al. 2005; Barbeau 2006). For example, Rue and Bruland (1997) estimated a daytime Fe' of  $\sim 2 \text{ pmol L}^{-1}$  (up from 0.01  $\text{pmol L}^{-1}$ ) in the equatorial Pacific. Values of Fe' in the 2–3  $\text{pmol L}^{-1}$  range are sufficient to support growth rates of  $\sim 0.5\text{--}0.7 \text{ d}^{-1}$  in *Thalassiosira oceanica* and *Emiliana huxleyi* (Sunda and Huntsman 1995), comparable with growth rates of diatoms in the Fe-limited regions of the subarctic and equatorial Pacific (Blain et al. 1997). We can extrapolate to lower concentrations by considering that the uptake rate of the organisms is proportional to Fe' and scales with their surface area (Sunda and Huntsman 1995), and by assuming that the minimum cellular Fe concentration (mol/cell volume) remains the same. The net result is an Fe-limited growth rate proportional to Fe' and inversely proportional to the cell radius,  $R$ . So at a concentration of unchelated Fe in the range 0.2–0.3  $\text{pmol L}^{-1}$ , cyanobacteria or picoeukaryotes ( $R \approx 0.6 \mu\text{m}$ ) should be able to achieve a growth rate of 0.25–0.35  $\text{d}^{-1}$ . This growth rate is

about half that measured for *Prochlorococcus* in the equatorial Pacific (Mann and Chisholm 2000). Thus, even under these Fe-limited conditions, the unchelated Fe may sustain a sizeable fraction of the production by the picoplankton. As we design and carry out experiments to unravel the difficult question of Fe availability to phytoplankton in seawater, we should not ignore the potential importance of the small fraction of dissolved Fe that is unchelated.

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