Biometals (2009) 22:659–669
DOI 10.1007/s10534-009-9235-2

The role of siderophores in iron acquisition by photosynthetic marine microorganisms

Brian M. Hopkinson · François M. M. Morel

Received: 18 November 2008 / Accepted: 23 March 2009 / Published online: 3 April 2009

Abstract The photosynthetic picocyanobacteria and eukaryotic microorganisms that inhabit the open ocean must be able to supply iron for their photosynthetic and respiratory needs from the subnanomolar concentrations available in seawater. Neither group appears to produce siderophores, although some coastal cyanobacteria do. This is interpreted as an adaptation to the dilute oceanic environment rather than a phylogenetic constraint, since there are cases in which related taxa from different environments have the capacity to produce siderophores. Most photosynthetic marine microorganisms are presumably, however, capable of accessing iron from strong chelates since the majority of dissolved iron in seawater is complexed by organic ligands, including siderophores. Rather than direct internalization of siderophores and other iron chelates, marine organisms primarily appear to use uptake pathways that involve a reduction step to free bound iron, closely coupled with transport into the cell.

Keywords Siderophores · Phytoplankton · Ocean · Iron uptake · Cyanobacteria

B. M. Hopkinson (✉) · F. M. M. Morel
Department of Geosciences, Princeton University, Princeton, NJ 08544, USA
e-mail: bhopkins@princeton.edu

Introduction

The low solubility of iron in oxic seawater in conjunction with minimal external supplies results in subnanomolar iron concentrations throughout most of the ocean (Johnson et al. 1997; Measures et al. 2008). In approximately one-third of the surface ocean iron supply is so low that it limits the growth of resident phytoplankton, the photosynthetic prokaryotes and eukaryotes which are responsible for carbon fixation in pelagic waters and form the base of open-ocean food chains. Phytoplankton have high iron requirements due to the pervasive use of heme co-factors and iron-sulfur clusters for electron transport in photosynthetic proteins (Raven 1990). Acquisition of the scarce iron that is present in seawater is complicated by the fact that it is nearly completely complexed to organic ligands of unknown structures (Gledhill and van den Berg 1994; Rue and Bruland 1995; Wu and Luther 1995). It has been hypothesized that siderophores may be used by microorganisms to access iron from this ligand-bound pool, or that the natural ligands may themselves be siderophores, leading to an interest in the role of siderophores in the ocean.

Siderophores are important in iron acquisition for diverse organisms in environments as different as the human body and soils. However, the dilute nature of the pelagic marine environment promotes large diffusive losses and renders the efficiency of siderophore-based iron uptake strategies problematic. Nonetheless many heterotrophic marine bacteria are

known to synthesize siderophores, and transport both their own siderophores as well as those produced by other organisms (Reid et al. 1993; Granger and Price 1999; Martinez et al. 2000). The use of siderophores in the marine environment is not well understood, and it may be that these marine bacteria use siderophores primarily when attached to particles where losses by diffusion could be reduced and particulate sources of iron may be available.

The use of siderophores by marine phytoplankton has also been the subject of much research, although the findings have been somewhat complicated. The phytoplankton, which dominate primary production in the open-ocean, are unicellular and free-living, relying on diffusive fluxes of nutrients for growth. For such organisms the problem of diffusive loss of secreted siderophores is most significant. A theoretical study of this problem suggests that the use of siderophores may not be prohibitively wasteful for individual cells greater than several microns in size or for populations greater than roughly 1,000 cells/ml (Volker and Wolf-Gladrow 1999). These results indicate that siderophore production is not out of the question for the many photosynthetic microorganisms that satisfy one of these conditions in the ocean. Nonetheless, it seems that only a small number of marine photosynthetic organisms produce their own siderophores. In contrast many photosynthetic microorganisms are capable of acquiring iron from organic chelates, including siderophores, through mechanisms that are beginning to be understood.

Siderophore production

Both prokaryotes and eukaryotes are known to produce siderophores, notably heterotrophic bacteria and fungi which are rich sources of novel iron binding compounds. The enzymatic pathways by which bacteria and fungi biosynthesize selected siderophores have been determined. Like many secondary metabolites, modular nonribosomal peptide synthetase (NRPS) systems and in some cases mixed NRPS-polyketide synthetase (PKS) systems are used to produce numerous hydroxamate and catecholate siderophores (Crosa and Walsh 2002; Haas et al. 2008). Several common bacterial siderophores, including citrate-based siderophores and ferrioxamines, are synthesized via an unrelated pathway termed the NRPS-independent siderophore (NIS) biosynthetic pathway (Challis 2005). Graminaceous plants have independently developed the use of siderophores, producing tricarboxylic acid chelators such as mugineic acid which form weaker iron complexes than hydroxamate or catecholate siderophores but play a similar physiological role (Curie and Briat 2003). The knowledge that siderophore production has been acquired or evolved in multiple prokaryotic and eukaryotic lineages has enhanced interest in the potential for siderophore production and uptake by marine microorganisms who must survive in an environment with limited iron availability.

Heterotrophic marine bacteria often produce siderophores and a substantial number of these compounds have been isolated and structurally characterized (Reid et al. 1993; Martinez et al. 2000; Butler 2005). A distinctive characteristic of marine siderophores is the frequent presence of a hydrophobic fatty acid tail imparting an amphiphilic character to the compound (Martinez et al. 2003). Marine siderophores produced by a single organism are commonly found in suites, their structures differing only by the length of the fatty acid tail, a feature that regulates the hydrophobicity of the compounds, modulating diffusion from the organism itself or the particle it inhabits (Martinez et al. 2000).

Despite their occurrence in marine heterotrophic bacteria, siderophores do not appear to be commonly produced by marine cyanobacteria and no clear cases of classical siderophore production have been documented in eukaryotic marine phytoplankton. Marine cyanobacteria fall into several phylogenetic lineages (Honda et al. 1999; Shi and Falkowski 2008). Ecologically the most important are a monophyletic group of small ($\sim$1 μm) unicellular cyanobacteria composed of species of *Prochlorococcus* and *Synechococcus*, which dominate regions of low-productivity in the ocean. This group will be referred to as the "picocyanobacteria" to avoid confusion with other lineages that also contain species called *Synechococcus* despite varying degrees of relatedness among these groups. The nitrogen fixers also form a distinct lineage amongst cyanobacteria; among those a few species such as *Trichodesmium* sp. and *Crocosphaera watsonii* are important in the open ocean.

The only marine cyanobacterium from which a siderophore has been isolated and structurally characterized is *Synechococcus* sp. PCC 7002, a coastal

organism distinct from the picocyanobacteria, which produces a suite of amphiphilic siderophores termed synechobactins differing only in the lengths of the fatty acid tails (Ito and Butler 2005). The iron-binding portion of the synechobactins is identical to schizokinen, a citrate-based siderophore which provides hexadentate chelation for Fe(III) through a single α-hydroxy acid group and two hydroxamate functional groups (Fig. 1a). Schizokinen has previously been isolated from a nitrogen-fixing freshwater cyanobacterium *Anabaena* sp. PCC 7120, also known as *Nostoc* sp. PCC 7120 (Simpson and Neilands 1976). While synechobactins are currently the only siderophores that have been structurally characterized from marine cyanobacteria, iron-binding compounds with hydroxamate and catecholate moieties typical of siderophores have been reported to be produced by additional marine species (Wilhelm and Trick 1994). Cyanobacteria as a group have not traditionally been known to produce many siderophores. In fact only two other siderophores have been structurally characterized from cyanobacteria, the anachelins, which are peptide siderophores with unusual functional groups obtained from the freshwater cyanobacterium *Anabaena cylindrica* (Ito et al. 2001, 2004).

The recent proliferation of genome sequences for marine cyanobacteria has provided a new perspective on siderophore use and production in these organisms. Although the biosynthetic pathways of siderophore synthesis have not been explored in cyanobacteria, the genes involved have been thoroughly studied in heterotrophic bacteria as summarized above. Citrate-based siderophores, which include schizokinen and the synechobactins, are synthesized via the NIS biosynthetic pathway whose key genes are well-conserved (Challis 2005). Homologs of NIS genes were found in the genomes of cyanobacteria from which citrate-based siderophores have been isolated providing a plausible route for their biosynthesis (Table 1). The schizokinen producing *Anabaena* sp. PCC 7120 and synechobactin producing *Synechococcus* sp. PCC 7002 each contain in their genomes two members of the IucA/IucC protein family that catalyze amide bond formations between the citrate backbone and hydroxamate containing amine units, as well as a single copy of an IucD homolog involved in formation of the hydroxamate iron-binding groups. The only other cyanobacterial genome in which these NIS genes were found was *Anabaena variabilis* ATCC 29413, a relative of *Anabaena* sp. PCC 7120, indicating the synthesis of these siderophores is restricted to selected cyanobacterial lineages (Table 1; Palenik et al. 2003).

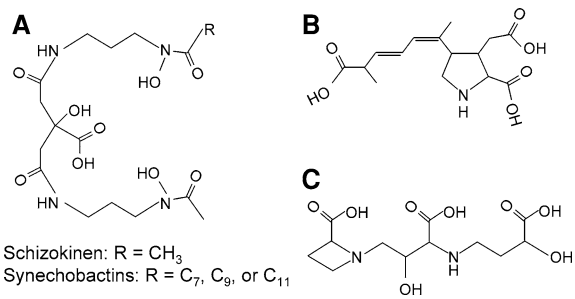

Fig. 1 **a** The structure of schizokinen/synechobactin siderophores which have been isolated from freshwater and marine cyanobacteria (Simpson and Neilands 1976; Ito and Butler 2005). **b** The structure of domoic acid, a neurotoxin and hypothesized metal chelator, produced by diatoms of the genus *Pseudonitzschia*. **c** The structure of a phytosiderophore, mugineic acid, is shown to illustrate similarities previously noted between this compound and domoic acid (Rue and Bruland 2001)

Other siderophores are commonly synthesized by NRPS or mixed NRPS/PKS pathways. Genes for components of these pathways are more commonly found in cyanobacterial genomes, including the marine nitrogen fixers *T. erythraeum* IMS101 and *Crocosphaera watsonii* WH8501, although only one marginal hit was identified in the marine picocyanobacteria (Table 1). In the case of *Anabaena* sp. PCC 7120, knockouts of certain NRPS clusters resulted in impairments in growth under iron stress and decreased production of siderophores implicating these clusters in siderophore biosynthesis (Jeanjean et al. 2008). However, NRPS and PKS pathways are used to synthesize a great diversity of compounds and it is currently difficult to predict the exact product synthesized based solely on genetic information. The presence of NRPS and PKS genes in cyanobacterial genomes leaves open the possibility that they produce siderophores via these pathways, but by no means requires it. One notable feature of the distribution of NRPS/PKS and NIS genes is their absence (except for one weak hit to an NRPS domain) from the picocyanobacterial lineages which dominate primary production in oligotrophic regions.

Several metal binding compounds have been detected in cultures of eukaryotic marine phytoplankton, but none of these compounds have been shown to be siderophores. There are early reports of iron-binding compounds with hydroxamate or catecholate functional

Table 1 Putative siderophore biosynthesis and transport proteins in cyanobacterial genomes

Organism	IucA/IucC	IucD	NRPS (condensation domain)	TonB dependent receptors
Freshwater cyanobacteria				
Anabaena sp. PCC 7120	2	1	14	23
Anabaena variabilis ATCC 29413	2	1	21	10
Cyanothece sp. ATCC 51142	0	0	13	1
Cyanothece sp. CCY0110	0	0	14	1
Gloeobacter violaceus PCC 7421	0	0	0	32
Microcystis aeruginosa NIES-843	0	0	19	0
Nostoc punctiforme PCC 73102	0	0	51	2
Synechococcus elongatus PCC 6301	0	0	0	0
Synechococcus elongatus PCC 7942	0	0	0	0
Synechococcus sp. JA-2-3B'a(2-13)	0	0	0	2
Synechococcus sp. JA-3-3Ab	0	0	0	2
Synechocystis sp. PCC 6803	0	0	0	4
Thermosynechococcus elongatus BP-1	0	0	0	0
Marine cyanobacteria				
Acaryochloris marina MBIC11017	0	0	10	12
Crocosphaera watsonii WH 8501	0	0	19	0
Lyngbya aestuarii CCY9616	0	0	0	0
Nodularia spumigena CCY9414	0	0	30	3
Synechococcus sp. PCC 7002	2	1	0	7
Trichodesmium erythraeum IMS101	0	0	2	0
Marine picocyanobacteria				
Synechococcus spp.	0/10	0/10	0/10	0/10
Prochlorococcus spp.	0/12	0/12	1/12	0/12

PFAM Hidden Markov Models were used to search cyanobacterial genomes for components of NIS siderophore biosynthesis (IucA/IucC-PF04183; IucD-PB1759), NRPS condensation domains (PF0688), and TonB dependent outer membrane receptors (PF00593). In all the marine picocyanobacteria searched there was only a single weak hit to an NRPS condensation domain in one organism (*Prochlorococcus* sp. MIT9303) and so the results were grouped together and reported as number of hits for total number of genomes searched. These genomes were: *Synechococcus* BL107, *S.* CC9311, *S.* CC9605, *S.* CC9902, *S.* RCC307, *S.* RS9917, *S.* WH5701, *S.* WH7803, *S.* WH7805, *S.* WH8102, *Prochlorococcus* AS9601, *P.* CCMP1375, *P.* CCMP1986, *P.* MIT9211, *P.* MIT9215, *P.* MIT9301, *P.* MIT9303, *P.* MIT9312, *P.* MIT9313, *P.* MIT9515, *P.* NATL1A, *P.* NATL2A. Cutoff thresholds to determine significant hits for each HMM were: IucA/IucC: 1E-100; IucD: 1E-100; NRPS condensation domains: 1E-14; TonB dependent receptors: 1E-18

groups typical of siderophores being extracted from cultures of various eukaryotic phytoplankton including dinoflagellates and diatoms (Trick et al. 1983a, b). However, no compounds were structurally characterized in these studies and subsequent work has failed to reproduce the results (Soria-Dengg and Horstmann 1995). It is suspected that contaminating bacteria may have been responsible for production of the compounds with characteristics of siderophores (Sunda 2001). Using electrochemical ligand competition methods, production of iron binding compounds of unknown chemical identity has been detected in response to iron addition in cultures of the coccolithophore *Emiliania huxleyi* (Boye and van den Berg 2000). This response is opposite to that expected of siderophore production, which is upregulated under iron stress and turned off when iron is sufficient. Thus it seems unlikely that the secreted compounds are classic siderophores, but the response may be adaptive in the surface ocean where newly available iron is rapidly lost by precipitation and settling to deep seawaters if not complexed to dissolved chelators.

One compound secreted by a phytoplanktonic marine eukaryote, the diatom toxin domoic acid, has been implicated in metal uptake. This neurotoxin, produced by species of *Pseudonitzschia*, is a small organic compound containing three carboxylic acid groups similar to siderophores of graminaceous plants

such as mugineic acid (Rue and Bruland 2001; Fig. 1b, c). The compound complexes iron but with an affinity too weak to effectively out-compete natural seawater ligands except at domoic acid concentrations approaching the highest observed in nature. Domoic acid also chelates copper and there is evidence that it may serve both to detoxify copper at high copper concentrations and to aid in copper uptake at low copper concentrations (Rue and Bruland 2001; Maldonado et al. 2002; Wells et al. 2005). The production of domoic acid is enhanced when copper is limiting and its release increases copper bioavailability (Wells et al. 2005). Domoic acid could consequently play an indirect role in alleviating iron limitation as copper may be involved in diatom iron uptake systems, likely as a component of a multi-copper oxidase (Maldonado et al. 2006).

To summarize, siderophore production is uncommon in marine phytoplankton. The abundant marine picocyanobacteria and eukaryotic phytoplankton which are responsible for much of the primary production in the open ocean have not been found to produce siderophores nor have genes involved in siderophore biosynthesis been identified in their genomes. Although many of these organisms can be challenging to work with and extensive investigations of their secondary metabolites have not been carried out, their susceptibility to iron limitation by means of the relatively weak aminocarboxylate chelators, such as EDTA, in addition to the strong trihydroxamate siderophore desferrioxamine B (DFB), indicates that only small quantities of siderophores or weakly binding ones could be produced (Hudson and Morel 1990; Wells et al. 1994; Sunda and Huntsman 1995; Henley and Yin 1998; Hutchins et al. 1999a, b). Despite their inability to produce siderophores, phytoplankton are capable of accessing siderophore-bound iron in many cases though usually not by direct internalization of the siderophore compounds but rather through generalized mechanisms to acquire multiple iron forms as discussed below.

Presence of siderophores in the marine environment

It is known that >99% of dissolved iron is bound to organic ligands in surface waters of the open ocean and it has long been speculated that some or all of these ligands may be siderophores (Rue and Bruland 1995; Wu and Luther 1995). The conditional binding strengths of natural iron ligands are similar to those of many isolated marine siderophores, but the electrochemical competitive ligand techniques which are used to obtain these data provide little information on the identity of the organic ligands involved, other than their concentrations and binding strengths. Iron binding compounds with hydroxamate and catecholate functionalities typical of siderophores have been detected in natural organic matter extracts from seawater (Rue and Bruland 1995; Macrellis et al. 2001). The distribution of natural iron ligands, however, is not what might naively be expected of siderophores, which are generally produced in response to iron stress. Dissolved iron and ligand concentrations are positively correlated with each other throughout the ocean and ligand concentrations in iron-limited regions are lower than or similar to those in iron replete areas (Buck and Bruland 2007). Consistent with this distribution, rapid ligand production has consistently been found in response to experimental addition of iron to iron-limited regions of both the equatorial Pacific and Southern Ocean (Rue and Bruland 1997; Boye et al. 2005). Buck and Bruland (2007) also observed elevated ligand concentrations in a region of natural iron fertilization in the Bering Sea, where oceanic iron-limited waters were mixed with iron-rich shelf waters. While these results clearly indicate a biological source for the ligands produced, the observed distribution is not obviously consistent with what would be expected for siderophores.

The natural iron binding ligands likely have multiple sources. Dissolved organic matter is a complex mixture of fresh and chemically reworked compounds some of which may by happenstance chelate iron (Laglera and van den Berg 2009). The fact that many trace metals studied are complexed to organic matter throughout the ocean supports this hypothesis (e.g. Bruland 1992; Saito and Moffett 2001). Organic exudates which happen to have iron-binding functionalities released by phytoplankton and other organisms may also contribute to the ligand pool. Such ligands could account for the observed increases in ligand concentrations when iron is added to iron-limited regions as photosynthetic rates intensify after relief of iron limitation. Additional likely sources for iron binding ligands include sedimentary

and riverine organic matter, in which humic substances contribute to iron binding (Laglera and van den Berg 2009). But given that marine heterotrophic bacteria synthesize siderophores it is likely that the more hydrophilic ones contribute to the natural iron ligand pool. A recent study by Mawji et al. (2008) is the first to convincingly report the detection and concentration of specific siderophores in the ocean. Both ferrioxamines and amphibactins, classes of siderophores known to be produced by marine bacteria, were detected throughout the North and South Atlantic. Only ferrioxamines were quantified and the trihydroxamate siderophores ferrioxamine E and G were found at concentrations up to 10 pM each binding as much as 5% of the total dissolved iron in surface waters. The concentration of these siderophores was correlated with the abundance of heterotrophic bacteria, implying local production by these organisms. Iron binding ligands are likely a complex mix of siderophores, organic exudates, and humic materials which combine to stabilize dissolved iron in seawater.

Siderophore uptake

Since the bulk of iron in seawater is bound to strong chelating agents, there is a clear incentive for organisms to evolve mechanisms to access organically bound iron. There is currently little evidence for direct internalization of siderophores by either eukaryotic or prokaryotic phytoplankton. However, both groups appear to be able to acquire iron from organic chelates using reductive mechanisms with varying degrees of efficiency depending on the iron complex and species.

Bacteria typically transport iron bound siderophores through their outer membranes using TonB-dependent outer membrane receptors, a class of transporters named for their reliance on the TonB protein which spans the periplasmic space powering uptake across the outer membrane (Postle and Kadner 2003). Once inside the periplasmic space ABC transporters are generally used to internalize siderophores, though in some cases iron is directly taken up without the siderophore (Schalk 2008). However, the specificity of ABC transporters is difficult to determine using bioinformatics so recent genome-based work has focused on identification of TonB-dependent receptors. TonB-dependent receptors have not been studied extensively in cyanobacteria but several with homology to siderophore receptors have been tentatively characterized in the freshwater cyanobacterium *Synechocystis* sp. PCC 6803 (Katoh et al. 2001). This organism does not produce its own siderophores and likely uses its TonB-dependent receptors to steal siderophores produced by other species, a common strategy practiced by many bacteria (Poole et al. 1990; Guan et al. 2000). The receptor for schizokinen in *Anabaena* sp. PCC 7120 has recently been identified based on its homology to known hydroxamate siderophore receptors and collocation in the genome with the schizokenin biosynthesis genes, and its function was characterized through knock-out mutation (Nicolaisen et al. 2008). The use of TonB-dependent receptors to transport siderophores, as occurs in *Anabaena* sp. PCC 7120 and *Synechocystis* sp. PCC 6803, may be common among freshwater and coastal marine cyanobacteria as such receptors are frequently present in their genomes (Table 1). However, TonB-dependent receptors have so far not been found in the genomes of marine picocyanobacteria and open-ocean nitrogen fixers (e.g. *C. watsonii*, *T. erythraeum* IMS101), indicating the only known pathway for siderophore transport by bacteria is absent from the open-ocean cyanobacteria (Webb et al. 2001; Palenik et al. 2003, 2006; Table 1).

Although the mechanisms are not always understood, the bioavailability of siderophores to many marine cyanobacteria has been investigated. Both the near-shore *Synechococcus* sp. PCC 7002 and the open-ocean *Synechococcus* sp. WH7803, despite its lack of a traditional siderophore uptake pathway, appear to be capable of accessing iron from a variety of hydroxamate and catecholate siderophores (Hutchins et al. 1999a, b). Colonies of *Trichodesimum* sp. collected in the subtropical Atlantic Ocean were able to access iron from a range of siderophores including desferrioxamine, rhodotorulic acid, and ferrichrome, though it should be noted that other bacterial species associated with the colonies could have contributed to uptake (Achilles et al. 2003). The widespread ability to acquire iron from chelates regardless of siderophore structure by organisms which appear to lack TonB-dependent outer membrane receptors suggests that an alternate uptake pathway is used. The most likely mechanism involves a reduction of Fe(III) to Fe(II) which weakens the metal binding and facilitates uptake (Fig. 2).

Reduction may occur directly at the membrane surface as in eukaryotes, or perhaps indirectly though an electron shuttle. Experiments with the benthic marine cyanobacterium, *Lyngbya majuscula*, have been interpreted as evidence that the radical anion superoxide serves as such a shuttle (Rose et al. 2005). Recent work with open-ocean *Synechococcus* spp. and *Trichodesmium erythraeum* IMS101 shows that both organisms

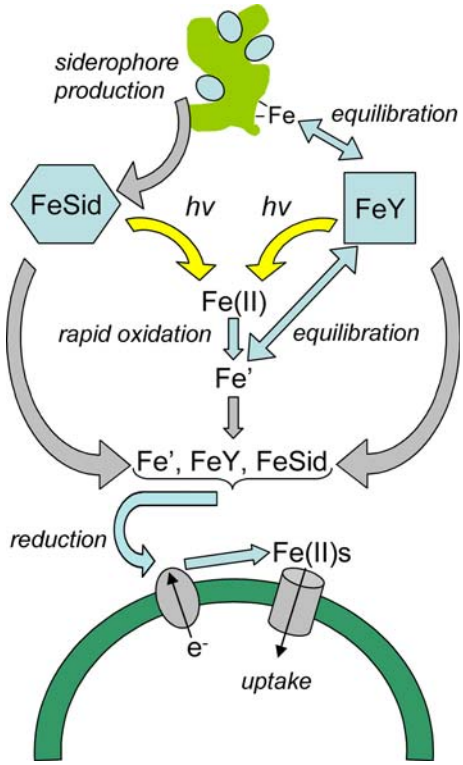

Fig. 2 A diagram of important iron forms in the ocean and their interaction with the reductive iron uptake pathway (Shaked et al. 2005). Siderophores (FeSid) are most likely produced by heterotrophic bacteria and bind iron either from the particulate or dissolved phase. Siderophore-bound iron becomes bioavailable to phytoplankton either by cell surface reduction and uptake or by photoreduction (hv) of iron complexed to photolabile siderophores. At seawater pH, reduced iron, Fe(II), is rapidly reoxidized to an inorganic form (Fe' denotes all inorganic iron forms). Similarly, iron complexed to other natural organic ligands (FeY) may be taken up directly via the reductive uptake pathway or released to the inorganic pool by photoreduction. Many natural iron ligands are weaker than siderophores such that Fe' in equilibrium with these ligands is a small but readily available source of iron to the cell. The reducible forms of iron accessible to the cell surface by diffusion include Fe', FeY, and FeSid. These iron forms are reduced by a cell surface reductase, weakening chelates and facilitating transport of Fe(II)s (reduced iron at the cell surface)

are capable of reducing iron and, in the case of *T. erythraeum* IMS101, the addition of iron(II) chelators reduces rates of iron uptake from siderophores indicating a reduction step is involved in uptake (Roe and Barbeau personal communication; Lis and Shaked personal communication). If these reductive mechanisms are common in cyanobacteria, the observed differences in siderophore bioavailability may be the result of differences in iron-chelate reduction potential, the susceptibility of the iron-siderophore complex to reduction by an electron shuttle, or the ability of the siderophores to diffuse through the outer-membrane. Being near the approximate size limit of compounds that can pass through porins, iron-siderophore complexes may diffuse slowly through the outer-membrane of marine cyanobacteria, but at rates sufficient to satisfy their relatively slow iron uptake. Alternatively, a ligand exchange process between siderophores in the environment and a transporter-bound siderophore could be occurring (Stinzti et al. 2000). This mechanism, termed a "siderophore shuttle", has been proposed to explain indiscriminate siderophore transport in heterotrophic bacteria, but the current model for this mechanism requires at least one TonB-dependent outer-membrane receptor to which the membrane associated siderophore binds. Although the mechanisms are just beginning to be understood, the ability to access strong iron chelates appears common in marine cyanobacteria.

The mechanism by which siderophore iron is acquired by eukaryotic phytoplankton has been most well studied in diatoms of the genus *Thalassiosira*. The current model for the diatom iron uptake pathway, and its molecular underpinnings, closely follows that of the yeast, *Saccharomyces cerevisiae* (Fig. 2). In this yeast, iron species, including organic chelates, are reduced by a ferric reductase at the cell surface releasing Fe(II) that is then oxidized by a multi-copper ferroxidase in the cytoplasmic membrane and internalized by a permease (Kosman 2003). All steps in the process are closely coupled. Similarly, diatoms reduce siderophores and other organic iron complexes, a step that weakens iron binding and facilitates uptake (Soria-Dengg and Horstmann 1995; Maldonado and Price 2001; Shaked et al. 2005). Although the production of superoxide by diatoms results in iron reduction in the bulk medium, the rapid reoxidation by oxygen results in a futile reduction–oxidation cycle that does not aid uptake (Kustka et al. 2005). Instead, the diatom uptake pathway likely includes cell-surface ferric reductases,

several of which have been identified in the genomes of *Thalassiosira pseudonana* and *Phaeodactylum triconutum* and are regulated by iron availability (Kustka et al. 2007). Homologs to a multi-copper ferroxidase and an iron permease were also found in *T. pseudonana* (Maldonado et al. 2006; Kustka et al. 2007). If the structure and characteristics of natural iron binding ligands are diverse or variable, as seems likely, the non-specific nature of a reductase mechanism may make it advantageous despite the potential waste of energy from the rapid reoxidation of Fe(II) in seawater.

Little work has been done on siderophore uptake and bioavailability outside of the diatoms, but the genomes of two picoeukaryotic green algae, *Ostreococcus tauri* and *Ostreococcus lucimarinus*, indicate that this marine phytoplankton lack a reductase-type iron uptake system (Palenik et al. 2007). However, they are reported to have genes with similarity to prokaryotic siderophore uptake pathways suggesting they have different strategies for iron uptake than diatoms possibly targeting specific iron chelates.

Overall it appears that the few phytoplanktons that have been studied in some detail have the ability to access iron from strong organic chelates, generally via a reductive uptake pathway. However, in many cases, particularly for strong iron chelators such as DFB, uptake from iron-ligand complexes is quite slow and in fact DFB is commonly used to induce iron limitation in field populations of both eukaryotes and prokaryotes (Wells et al. 1994, Hutchins et al. 1999a, b). In the environment, weak iron chelates or inorganic iron forms are likely the most important sources of iron for phytoplankton when they are available while strong iron chelates serve as supplemental iron sources (Morel et al. 2008).

Conclusions

Although an iron uptake system involving extracellular siderophore release and uptake of the iron siderophore complex is used by many organisms in many different environments, the best current data indicate that marine phytoplankton do not commonly employ such a system to acquire iron. This is presumably a result of the dilute nature of the open-ocean pelagic environment where diffusive loss of siderophores is significant. It is also possible that pelagic phytoplankton are in many instances more limited by nutrient availability than they are by light energy and are thus able to release electrons more readily than organic compounds.

The majority of phytoplankton probably do not use siderophores simply because reduction based mechanisms are more efficient and are capable of accessing the majority of iron species in the ocean. To access dissolved iron a reductive strategy is likely more efficient since the electron transfer occurs directly at the cell surface minimizing diffusional loss and eliminating the cost of synthesizing a complex siderophore. Iron reduction and uptake are closely coupled at the cell membrane to reduce reoxidation of iron increasing the mechanism's efficiency (Shaked et al. 2005). A substantial portion of iron in the ocean is in a colloidal form, which could in principle be solubilized through the production and release of siderophores (Wu et al. 2001). But unlike the situation in soils, apparently this reservoir of iron is not large enough to justify the cost of siderophore production. It is also possible that the colloidal pool of iron is in a dynamic equilibrium with the dissolved phase on timescales which make it bioavailable to marine organisms without the help of siderophores (Hurst and Bruland 2007). Siderophore production in the marine pelagic environment seems to be restricted to heterotrophic bacteria, which likely employ siderophores when attached to particles such as marine snow, phytoplankton, or zooplankton (Fig. 2). In these conditions loss to diffusion is reduced in part due to the amphiphilic character of most marine siderophores (Martinez et al. 2000). In this context siderophores would serve to scavenge iron from within the particle matrix allowing bacteria to compete in the nutrient rich microenvironment.

Inevitably some of the siderophores produced by heterotrophic bacteria are released into the bulk dissolved phase forming a portion of the natural iron-binding ligands in the ocean (Mawji et al. 2008). The presence of siderophores and other strong iron chelators in the marine environment has driven the development of phytoplankton iron acquisition mechanisms capable of accessing these organically complexed iron forms (Fig. 2). Characterization of iron uptake systems in phytoplankton is still ongoing, but reduction of Fe(III) in organic chelates to Fe(II) seems to be an important component of all uptake pathways studied (Maldonado and Price 2001; Rose

et al. 2005; Shaked et al. 2005). Photoreduction and subsequent release of iron from siderophores and other organic chelates is also likely to be an important process for increasing inorganic and bioavailable iron concentrations in surface waters, supplementing the active reduction of iron by phytoplankton (Price and Morel 1998; Barbeau et al. 2001).

With the marine organisms responsible for siderophore production and the structures of these siderophores now better understood, the task remains to determine the sources and role of these compounds in seawater. The restriction of siderophore production to heterotrophic marine bacteria and the amphiphilic character of many marine siderophores suggest particles are hotspots for siderophore production. Future work to understand the distribution of siderophores in seawater and factors influencing their production (iron availability, quorum sensing and bacterial density) will help define the role of siderophores in the dilute marine environment.

Acknowledgments We would like to thank Kathy Barbeau and Yeala Shaked for providing data prior to publication and for helpful comments on the manuscript. This work has been supported by the National Science Foundation.

References

Achilles KM, Church TM, Wilhelm SW, Luther GW, Hutchins DA (2003) Bioavailability of iron to *Trichodesmium* colonies in the western subtropical Atlantic Ocean. Limnol Oceanogr 48:2250–2255

Barbeau K, Rue EL, Bruland KW, Butler A (2001) Photochemical cycling of iron in the surface ocean mediated by microbial iron(III)-binding ligands. Nature 413:409–413. doi:10.1038/35096545

Boye M, van den Berg CMG (2000) Iron availability and the release of iron-complexing ligands by *Emiliania huxleyi*. Mar Chem 70:277–287. doi:10.1016/S0304-4203(00)00032-3

Boye M, Nishioka J, Croot PL, Laan P, Timmermans KR, de Baar HJW (2005) Major deviations of iron complexation during 22 days of a mesoscale iron enrichment in the open Southern Ocean. Mar Chem 96:257–271. doi:10.1016/j.marchem.2005.02.002

Bruland KW (1992) Complexation of cadmium by natural organic-ligands in the central North Pacific. Limnol Oceanogr 37:1008–1017

Buck KN, Bruland KW (2007) The physicochemical speciation of dissolved iron in the Bering Sea, Alaska. Limnol Oceanogr 52:1800–1808

Butler A (2005) Marine siderophores and microbial iron mobilization. Biometals 18:369–374. doi:10.1007/s10534-005-3711-0

Challis GL (2005) A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. ChemBioChem 6:601–611. doi:10.1002/cbic.200400283

Crosa JH, Walsh CT (2002) Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol Rev 66:223–249. doi:10.1128/MMBR.66.2.223-249.2002

Curie C, Briat JF (2003) Iron transport and signaling in plants. Annu Rev Plant Biol 54:183–206. doi:10.1146/annurev.arplant.54.031902.135018

Gledhill M, van den Berg CMG (1994) Determination of complexation of iron(III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry. Mar Chem 47:41–54. doi:10.1016/0304-4203(94)90012-4

Granger J, Price NM (1999) The importance of siderophores in iron nutrition of heterotrophic marine bacteria. Limnol Oceanogr 44:541–555

Guan LL, Onuki H, Kamino K (2000) Bacterial growth stimulation with exogenous siderophore and synthetic N-acyl homoserine lactone autoinducers under iron-limited and low-nutrient conditions. Appl Environ Microbiol 66:2797–2803. doi:10.1128/AEM.66.7.2797-2803.2000

Haas H, Eisendle M, Turgeon BG (2008) Siderophores in fungal physiology and virulence. Annu Rev Phytopathol 46:149–187. doi:10.1146/annurev.phyto.45.062806.094338

Henely WJ, Yin Y (1998) Growth and photosynthesis of marine *Synechococcus* (Cyanophyceae) under iron stress. J Phycol 34:94–103. doi:10.1046/j.1529-8817.1998.340094.x

Honda D, Yokota A, Sugiyama J (1999) Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences from five marine *Synechococcus* strains. J Mol Evol 48:723–739

Hudson RJM, Morel FMM (1990) Iron transport in marine phytoplankton: kinetics of cellular medium coordination reactions. Limnol Oceanogr 35:1002–1020

Hurst MP, Bruland KW (2007) An investigation into the exchange of iron and zinc between soluble, colloidal, and particulate size-fractions in shelf waters using low-abundance isotopes as tracers in shipboard incubation experiments. Mar Chem 103:211–226. doi:10.1016/j.marchem.2006.07.001

Hutchins DA, Franck VM, Brzenzinski MA, Bruland KW (1999a) Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. Limnol Oceanogr 44:1009–1018

Hutchins DA, Witter AE, Butler A, Luther GW (1999b) Competition among marine phytoplankton for different chelated iron species. Nature 400:858–861. doi:10.1038/23680

Ito Y, Butler A (2005) Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002. Limnol Oceanogr 50:1918–1923

Ito Y, Okada S, Murakami M (2001) Two structural isomeric siderophores from the freshwater cyanobacterium *Anabaena cylindrica* (NIES-19). Tetrahedron 57:9093–9099. doi:10.1016/S0040-4020(01)00934-6

Ito Y, Ishida K, Okada S, Murakami M (2004) The absolute stereochemistry of anachelins, siderophores from the cyanobacterium *Anabaena cylindrica*. Tetrahedron 60:9075–9080. doi:10.1016/j.tet.2004.07.105

Jeanjean R, Talla E, Latifi A, Havaux M, Janicki A, Zhang CC (2008) A large gene cluster encoding peptide synthetases and polyketide synthases is involved in production of siderophores and oxidative stress response in the cyanobacterium *Anabaena* sp. strain PCC 7120. Environ Microbiol 10:2574–2585. doi:10.1111/j.1462-2920.2008.01680.x

Johnson KS, Gordon RM, Coale KH (1997) What controls dissolved iron concentrations in the world ocean? Mar Chem 57:137–161. doi:10.1016/S0304-4203(97)00043-1

Katoh H, Hagino N, Grossman AR, Ogawa T (2001) Genes essential to iron transport in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 183:2779–2784. doi:10.1128/JB.183.9.2779-2784.2001

Kosman DJ (2003) Molecular mechanisms of iron uptake in fungi. Mol Microbiol 47:1185–1197. doi:10.1046/j.1365-2958.2003.03368.x

Kustka AB, Shaked Y, Milligan AJ, King DW, Morel FMM (2005) Extracellular production of superoxide by marine diatoms: contrasting effects on iron redox chemistry and bioavailability. Limnol Oceanogr 50:1172–1180

Kustka AB, Allen AE, Morel FMM (2007) Sequence analysis and transcriptional regulation of iron acquisition genes in two marine diatoms. J Phycol 43:715–729. doi:10.1111/j.1529-8817.2007.00359.x

Laglera LM, van den Berg CMG (2009) Evidence for geochemical control of iron by humic substances in seawater. Limnol Oceanogr 54:610–619

Macrellis HM, Trick CG, Rue EL, Smith G, Bruland KW (2001) Collection and detection of natural iron-binding ligands from seawater. Mar Chem 76:175–187. doi:10.1016/S0304-4203(01)00061-5

Maldonado MT, Price NM (2001) Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae). J Phycol 37:298–309. doi:10.1046/j.1529-8817.2001.037002298.x

Maldonado MT, Hughes MP, Rue EL, Wells ML (2002) The effect of Fe and Cu on growth and domoic acid production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis*. Limnol Oceanogr 47:515–526

Maldonado MT, Allen AE, Chong JS, Lin K, Leus D, Karpenko N, Harris SL (2006) Copper-dependent iron transport in coastal and oceanic diatoms. Limnol Oceanogr 51:1729–1743

Martinez JS, Zhang GP, Holt PD, Jung HT, Carrano CJ, Haygood MG, Butler A (2000) Self-assembling amphiphilic siderophores from marine bacteria. Science 287:1245–1247. doi:10.1126/science.287.5456.1245

Martinez JS, Carter-Franklin JN, Mann EL, Martin JD, Haygood MG, Bulter A (2003) Structure and membrane affinity of a suite of amphiphilic siderophores produced by a marine bacterium. Proc Natl Acad Sci USA 100:3754–3759. doi:10.1073/pnas.0637444100

Mawji E, Gledhill M, Milton JA, Tarran GA, Ussher S, Thompson A, Wolff GA, Worsfold PJ, Achterberg EP (2008) Hydroxamate siderophores: occurrence and importance in the Atlantic Ocean. Environ Sci Technol 42:8675–8680. doi:10.1021/es801884r

Measures CI, Landing WM, Brown MT, Buck CS (2008) High-resolution Al and Fe data from the Atlantic Ocean CLIVAR-CO2 repeat hydrography A16 N transect: extensive linkages between atmospheric dust and upper ocean geochemistry. Global Biogeochem Cycles 22:GB1005. doi:10.1029/2007GB003042

Morel FMM, Kustka AB, Shaked Y (2008) The role of unchelated Fe in the nutrition of phytoplankton. Limnol Oceanogr 53:400–404

Nicolaisen K, Moslavac S, Samborski A, Valdebenito M, Hantke K, Maldener I, Muro-Pastor AM, Flores E, Schleiff E (2008) Alr0397 is an outer membrane transporter for the siderophore schizokinen in *Anabaena* sp. strain PCC 7120. J Bacteriol 190:7500–7507. doi:10.1128/JB.01062-08

Palenik B, Brahamsha B, Larmier FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EA, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb EA, Waterbury J (2003) The genome of a motile marine *Synechococcus*. Nature 424:1037–1042. doi:10.1038/nature01943

Palenik B et al (2006) Genome sequence of *Synechococcus* CC9311: insights into adaptation to a coastal environment. Proc Natl Acad Sci USA 103:1355–13559. doi:10.1073/pnas.0602963103

Palenik B et al (2007) The tiny eukaryote Ostreococcus provides genomic insights into the paradox of plankton speciation. Proc Natl Acad Sci USA 104:7705–7710. doi:10.1073/pnas.0611046104

Poole K, Young L, Neshat S (1990) Enterobactin-mediated iron transport in Pseudomonas aeruginosa. J Bacteriol 172:6991–6996

Postle K, Kadner RJ (2003) Touch and go: tying TonB to transport. Mol Microbiol 49:869–882. doi:10.1046/j.1365-2958.2003.03629.x

Price NM, Morel FMM (1998) Biological cycling of iron in the ocean. In: Sigel A, Sigel H (eds) Iron transport and storage in microorganisms, plants, and animals. Metal ions in biological systems, vol 35. M. Dekker Inc, New York, pp 1–36

Raven JA (1990) Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway. New Phytol 116:1–18. doi:10.1111/j.1469-8137.1990.tb00505.x

Reid RT, Live DH, Faulkner DJ, Butler A (1993) A siderophore from a marine bacterium with an exceptional ferric ion affinity constant. Nature 366:455–458. doi:10.1038/366455a0

Rose AL, Salmon TP, Lukondeh T, Neilan BA, Waite TD (2005) Use of superoxide as an electron shuttle for iron acquisition by the marine cyanobacterium *Lyngbya majuscula*. Environ Sci Technol 39:3708–3715. doi:10.1021/es048766c

Rue EL, Bruland KW (1995) Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar Chem 50:117–138. doi:10.1016/0304-4203(95)00031-L

Rue EL, Bruland KW (1997) The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnol Oceanogr 42:901–910

Rue EL, Bruland KW (2001) Domoic acid binds iron and copper: a possible role for the toxin produced by the marine diatom *Pseudo-nitzschia*. Mar Chem 76:127–134. doi:10.1016/S0304-4203(01)00053-6

Saito MA, Moffett JW (2001) Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. Mar Chem 75:49–68. doi:10.1016/S0304-4203(01)00025-1

Schalk IJ (2008) Metal trafficking via siderophores in Gram-negative bacteria: specificities and characteristics of the pyoverdine pathway. J Inorg Biochem 102:1159–1169. doi:10.1016/j.jinorgbio.2007.11.017

Shaked Y, Kustka AB, Morel FMM (2005) A general kinetic model for iron acquisition by eukaryotic phytoplankton. Limnol Oceanogr 50:872–882

Shi T, Falkowski PG (2008) Genome evolution in cyanobacteria: the stable core and the variable shell. Proc Natl Acad Sci USA 105:2510–2515

Simpson FB, Neilands JB (1976) Siderochromes in cyanophyceae: isolation and characterization of schizokinen from *Anabaena* sp. J Phycol 12:44–48

Soria-Dengg S, Horstmann U (1995) Ferrioxamines B and E as iron sources for the marine diatom *Phaeodactylum tricornutum*. Mar Ecol Prog Ser 127:269–277. doi:10.3354/meps127269

Stintzi A, Barnes C, Xu J, Raymond KN (2000) Microbial iron transport via a siderophore shuttle: a membrane ion transport paradigm. Proc Natl Acad Sci USA 97:10691–10696. doi:10.1073/pnas.200318797

Sunda W (2001) Bioavailability and bioaccumulation of iron in the sea. In: Turner DR, Hunter KA (eds) The biogeochemistry of iron in seawater. Wiley and Sons, West Sussex, pp 41–84

Sunda WG, Huntsman SA (1995) Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar Chem 50:189–206. doi:10.1016/0304-4203(95)00035-P

Trick CG, Andersen RJ, Gillam A, Harrison PJ (1983a) Prorocentrin: an extracellular siderophore produced by the marine dinoflagellate *Prorocentrum minimum*. Science 219:306–308. doi:10.1126/science.219.4582.306

Trick CG, Andersen RJ, Price NM, Gillam A, Harrison PJ (1983b) Examination of hydroxamate-siderophore production by neritic eukaryotic marine phytoplankton. Mar Biol (Berl) 75:9–17. doi:10.1007/BF00392625

Volker C, Wolf-Gladrow DA (1999) Physical limits on iron uptake mediated by siderophores or surface reductases. Mar Chem 65:227–244. doi:10.1016/S0304-4203(99)00004-3

Webb EA, Moffett JW, Waterbury JB (2001) Iron stress in open-ocean cyanobacteria (*Synechococcus, Trichodesmium*, and *Crocosphaera* spp.): identification of the IdiA protein. Appl Environ Microbiol 67:5444–5452. doi:10.1128/AEM.67.12.5444-5452.2001

Wells ML, Price NM, Bruland KW (1994) Iron limitation and the cyanobacterium *Synechococcus* in equatorial Pacific waters. Limnol Oceanogr 39:1481–1486

Wells ML, Trick CG, Cochlan WP, Hughes MP, Trainer VL (2005) Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. Limnol Oceanogr 50:1908–1917

Wilhelm SW, Trick CG (1994) Iron-limited growth of cyanobacteria: multiple siderophore production is a common response. Limnol Oceanogr 39:1979–1984

Wu J, Luther GW (1995) Complexation of Fe(III) by natural organic ligands in the northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach. Mar Chem 50:159–177. doi:10.1016/0304-4203(95)00033-N

Wu J, Boyle E, Sunda WG, Wen LS (2001) Soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific. Science 293:847–849. doi:10.1126/science.1059251