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Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups

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Abstract

Siderophores, high-affinity Fe(III) ligands produced by microorganisms to facilitate iron acquisition, might contribute significantly to dissolved Fe(III) complexation in ocean surface waters. In previous work, we demonstrated the photoreactivity of the ferric ion complexes of several α-hydroxy carboxylic acid–containing siderophores produced by heterotrophic marine bacteria. Here, we expand on our earlier studies and detail the photoreactivity of additional siderophores produced by both heterotrophic marine bacteria and marine cyanobacteria, making comparisons to synthetic and terrestrial siderophores that lack the α-hydroxy carboxylate group. Our results suggest that, in addition to secondary photochemical reaction pathways involving reactive oxygen species, direct photolysis of Fe(III)-siderophore complexes might be a significant source of Fe(II) and reactive Fe(III) in ocean surface waters. Our findings further indicate that the photoreactivity of siderophores is primarily determined by the chemical structure of the Fe(III) binding groups that they possess—hydroxamate, catecholate, or α-hydroxy carboxylate moieties. Hydroxamate groups are photochemically resistant regardless of Fe(III) complexation. Catecholates, in contrast, are susceptible to photooxidation in the uncomplexed form but stabilized against photooxidation when ferrated. α-Hydroxy carboxylate groups are stable as the uncomplexed acid, but when coordinated to Fe(III), these moieties undergo light-induced ligand oxidation and reduction of Fe(III) to Fe(II). These photochemical properties appear to determine the reactivity and fate of Fe(III)-binding siderophores in ocean surface waters, which in turn might significantly influence the biogeochemical cycling of iron.

In the oceans, the speciation of dissolved iron, an important limiting micronutrient for marine phytoplankton, has been shown to be dominated by complexation with strong organic ligands (Gledhill and Van den Berg 1994; Rue and Bruland 1995; Powell and Donat 2001). Factors that control the production, loss, and transformation of these organic ligands are therefore important parameters in the oceanic biogeochemical cycling of iron and the regulation of marine primary productivity. One process that is likely to affect ligand cycling in the upper ocean is photochemistry. Direct photochemical reactions of free ligands or Fe(III)-ligand complexes, or secondary reactions of free and ferrated ligands with photochemically produced radical species, can lead to ligand decomposition and accumulation of Fe(II) and Fe(III)′—the sum of all inorganic Fe(III) species—thereby serving to increase the net bioavailability and reactivity of iron.

Studies of the photochemically mediated redox cycling of iron in ocean surface waters (e.g., O'Sullivan et al. 1991; Johnson et al. 1994; Waite et al. 1995) have provided evidence for the photoreactivity of naturally occurring Fe(III) species in seawater but little mechanistic information as to the relative importance of direct versus secondary photochemical reactions of Fe(III)-ligand complexes, or the significance of photolysis as a sink for Fe(III)-binding organic

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ligands. Despite recent advances in the chemical characterization of dissolved organic matter (DOM) in marine systems (e.g., Aluwihare et al. 2002), lack of knowledge about the chemical nature of naturally occurring Fe(III) ligands in seawater has been a barrier to progress in studies of iron cycling.

Although uncertainties remain about the chemical identity of organically bound Fe(III) and the excess Fe(III) ligands present in seawater, several lines of evidence indicate that siderophores or their breakdown products are likely to make up some component of the organic ligands that dominate dissolved Fe(III) speciation. Siderophores are low–molecular weight, high-affinity Fe(III)-binding ligands secreted by bacteria under conditions of iron stress to scavenge and transport iron. Marine cyanobacteria and heterotrophic bacteria have been shown to produce siderophores under iron-limiting culture conditions (e.g., Trick 1989; Haygood et al. 1993; Wilhelm and Trick 1994), and the conditional stability constants for Fe(III) reported for naturally occurring Fe(III) ligands—$\sim 10^{12}$ L mol^{-1} with respect to Fe(III)′—are similar to those determined for siderophores produced by cultured marine bacteria (Lewis et al. 1995; Witter et al. 2000; Barbeau et al. 2001). Most recently, low–molecular weight Fe(III)-binding ligands isolated from coastal California upwelling waters have been shown to possess hydroxamate and catecholate groups, Fe(III)-coordinating moieties characteristic of siderophores (Macrellis et al. 2001).

Given the evidence that siderophores contribute to the Fe(III) ligands present in seawater, we have used siderophores isolated from cultures of heterotrophic marine bacteria as model ligands to investigate the influence of photolysis on the natural Fe(III) ligand pool. Figure 1 depicts the structurally characterized siderophores employed as model ligands in our studies. In previous work with the aquachelins, bis-hydroxamate α-hydroxy carboxylate siderophores produced by the marine bacterium *Halomonas aquamarina,* we have shown that Fe(III)-aquachelin complexes undergo a light-induced ligand-to-metal charge transfer reaction that results in partial ligand oxidation and reduction of Fe(III) (Barbeau et al. 2001). Similarly, petrobactin, a bis-catecholate α-hydroxy carboxylate siderophore produced by the oil-degrading marine bacterium *Marinobacter hydrocarbonoclasticus,* also undergoes a photo-induced oxidation when ferrated, resulting in decarboxylation of the ligand (Barbeau et al. 2002).

Herein, we present a synthesis of our studies of the photochemistry of marine siderophores on the basis of their characteristic Fe(III) binding functionalities: hydroxamate, catecholate, and α-hydroxy carboxylate groups. The photoreactivity of the aquachelins and petrobactin is described in the absence of Fe(III) complexation and in the context of the photoreactivity of additional siderophores produced by marine bacteria, including several isolated from cultures of the marine cyanobacterium *Synechococcus.* The photochemical reactivity of α-hydroxy acid–containing marine siderophores and model ligands without the α-hydroxy acid group is compared in the presence and absence of Fe(III) complexation. Results from these studies indicate that siderophore photoreactivity is largely determined by the nature of the Fe(III) binding groups present. These findings underscore the utility of structurally characterized ligands as a tool in mechanistic studies and the need for further structural characterization, not only of siderophores produced by marine bacteria, but also of the naturally occurring Fe(III) ligands in seawater.

Materials and methods

Model ligands—Aquachelins A–D, marinobactins A–E, petrobactin, and alterobactins A and B were isolated and purified from cultures of marine heterotrophic bacteria using previously described techniques (Reid and Butler 1991 [alterobactins]; Martinez et al. 2000 [aquachelins and marinobactins]; Barbeau et al. 2002 [petrobactin]). Siderophores were isolated in the Fe-free (apo) form and used without further de-ferration. The hydroxamate siderophore des-ferrioxamine B (DFOB) was obtained from CIBA-GEIGY. The synthetic catecholate siderophores 3,4-LICAM and 3,4-LICAMS were a gift from C. J. Carrano, Southwest Texas State University. DFOB, 3,4-LICAM and 3,4-LICAMS were also obtained in apo form and used without further de-ferration.

Cyanobacterial siderophores were isolated and semipurified from Fe-limited laboratory cultures of the halotolerant *Synechococcus* strain PCC 7002 using previously described methods (e.g., Wilhelm and Trick 1994; Wilhelm et al. 1996). These siderophores were not fully structurally characterized, but siderophore-containing extracts were assayed for the presence of catechol and hydroxamate functional groups using the procedures of Arnow (1937, catechols) and Csaky (Gillam et al. 1981, hydroxamate). The siderophore in extract O12 tested positive only for the presence of the hydroxamate group, and the siderophore in extract C413 tested positive only for the presence of the catecholate group.

Photochemical experiments—For the structurally characterized siderophores used in this study (Fig. 1), irradiations were carried out on solutions of ferrated or apo ligands (concentration 20–40 μmol L^{-1}) in 0.2-μm-filtered, ultraviolet (UV)-irradiated Sargasso seawater (UVSSW) at pH 8. Solutions were placed in acid-washed quartz flasks ($\sim$50 ml volume) for sunlight exposure in a temperature-controlled water bath (18–20°C) for a duration of 6–8 h on cloudless sunny days. Identical solutions shielded from sunlight served as dark controls in all experiments.

For photochemical experiments with the cyanobacterial siderophores, solutions of the apo siderophores were made up to a concentration of $\sim$5 nmol L^{-1}, assuming a molecular mass of 500 for the organic chelators. Solutions were made up in 500 ml of UVSW (seawater from Monterey Bay from which trace metals and trace metal–binding organic compounds have been removed as described by Macrellis et al. 2001). Solutions were placed in an acid-cleaned quartz still and exposed to natural sunlight on sunny days for 5.5 h between 1000 and 1530 h. An identical quartz still containing only 500 ml UVSW was exposed at the same time as a control for metal contamination.

Isolation and structural characterization of photoproducts—Isolation and partial structural characterization of photoproducts was carried out only for those irradiations in-

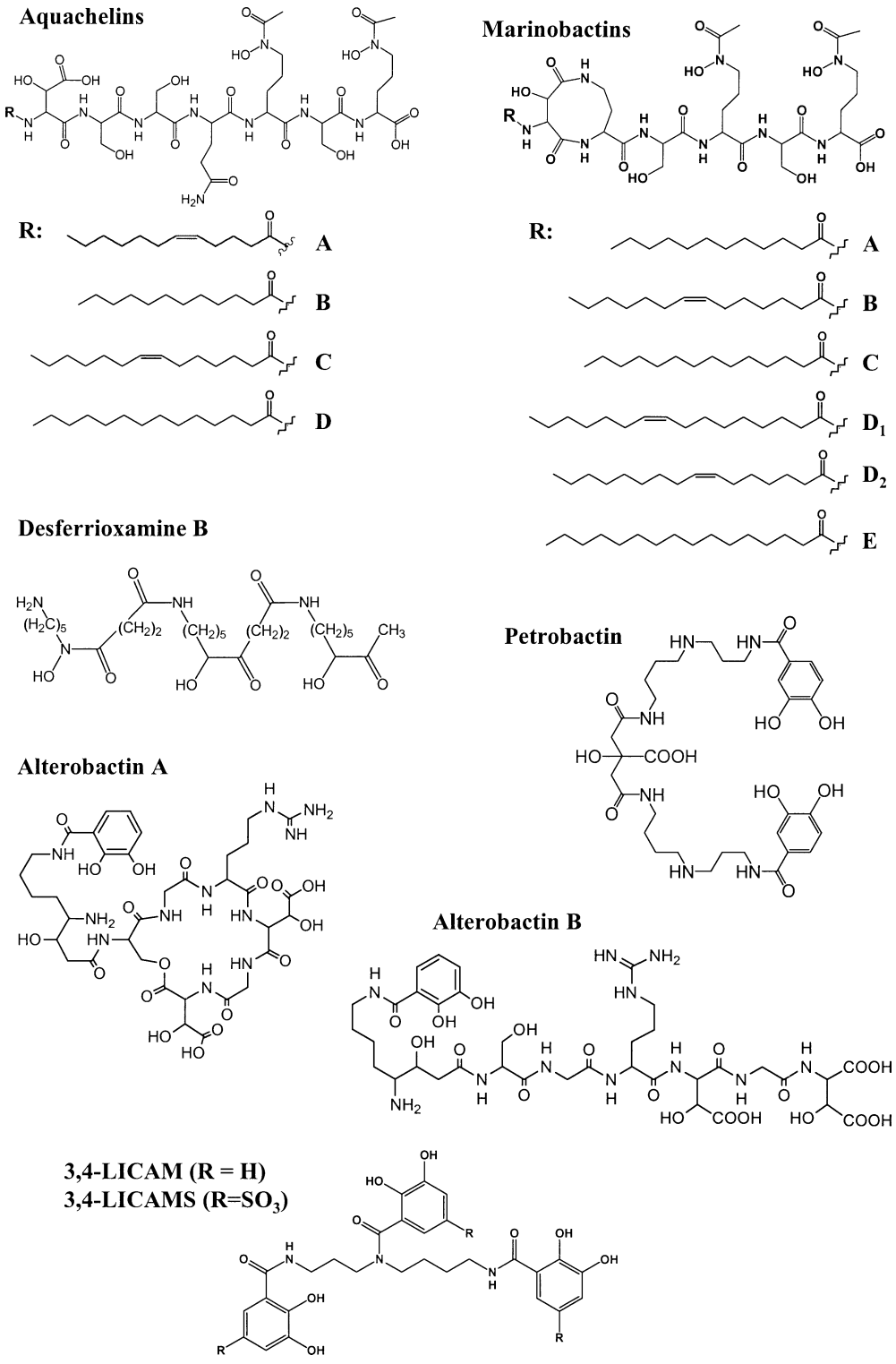

Fig. 1. Structurally characterized siderophores used in this study: aquachelins from *Halomonas aquamarina* and marinobactins from a *Marinobacter* species (Martinez et al. 2000); petrobactin from *Marinobacter hydrocarbonoclasticus* (Barbeau et al. 2002; Bergeron et al. 2003); alterobactins A and B from *Alteromonas luteoviolacea* (Reid et al. 1993); desferrioxamine B, a terrestrial bacterial siderophore; 3,4-LICAM and 3,4-LICAMS are synthetic siderophore analogs.

volving structurally characterized siderophores (Fig. 1). Immediately following photolysis, photolyzed solutions and dark controls were filtered (0.2-μm–pore size filter) and frozen at −70°C until analysis within 1–2 d. UV-visible spectrophotometry of filtered control and photolyzed siderophore solutions in UVSSW were obtained on a Cary 3e spectrophotometer. Photoproducts were isolated and purified directly from photolyzed solutions using reversed-phase high-performance liquid chromatography (RP-HPLC) on Vydac C-4 and C-18 columns using gradients from 0.1% trifluoroacetic acid (TFA) to 80% acetonitrile/0.1% TFA. Eluent absorbance was monitored at 215, 310, or 420 nm depending on the compounds being isolated. Isolated photoproducts were concentrated in vacuo and used for electrochemical analysis or mass spectrometry. Electrospray ionization mass spectrometry (ESI-MS) of isolated photoproducts was performed with a standard ESI source, with single quadrupole mass analyzer in positive ion mode and a VG-Fisons (Micromass) Platform II quadrupole mass spectrometer coupled to a Michrom BioResources HPLC unit for direct injection.

Electrochemical analyses—Electrochemical analysis of the cyanobacterial siderophores before and after sunlight exposure was carried out by direct titration of the photolyzed and nonphotolyzed 5–nmol L^{-1} UVSW solutions. Increasing iron additions were performed on aliquots of the samples, and these aliquots were left to equilibrate for 1 h. The strength of Fe(III) complexation by the siderophores was determined by competitive ligand equilibration (CLE) followed by adsorptive cathodic stripping voltammetry (ACSV) with salicylaldoxime (SA) as the competing ligand, as described in detail in Rue and Bruland (1995). This CLE-ACSV technique is a highly sensitive indirect method that detects the concentration of the electrochemically active, metal-added ligand complex $Fe(SA)_2$. The measurement for iron is made after a competing equilibrium has been established between Fe(III)′ (the sum of all inorganic Fe(III) species), the added ligand salicylaldoxime, and the siderophore or siderophore degradation product. The $Fe(SA)_2$ complex formed during competitive ligand equilibration is adsorbed onto a hanging mercury drop electrode, and the analytical signal is derived from the reduction current as the Fe(III) in the adsorbed complex is reduced during the cathodic stripping step. Differential pulse was used during the stripping step to enhance the analytical signal. Total dissolved Fe was analyzed by UV oxidation followed by ACSV (Rue and Bruland 1995). Subsamples (50 ml) were placed in acid-cleaned Teflon containers with a quartz window and irradiated in a Jelight UV oxidation system for 90 min at a wavelength of 254 nm with an intensity of 25,000 W cm^{-2}. Total dissolved Fe was then measured using ACSV with SA as the competing ligand.

Electrochemical analysis of petrobactin and its photodegradation products was carried out using similar methods. Photoproducts were generated by sunlight exposure of solutions of petrobactin and Fe(III)-petrobactin in UVSSW as described above. CLE-ACSV analysis was carried out directly on UVSSW solutions, both the dark control (petrobactin) and the irradiated solutions (petrobactin and Fe[III]-petrobactin). In addition, several photoproducts of petrobactin and Fe(III)-petrobactin were isolated from sunlight-exposed solutions via RP-HPLC, freeze-dried, and used in electrochemical analysis. These freeze-dried isolates were redissolved in 500 μl of methanol (Fisher Optima) prior to making up standard solutions in UVSW for CLE-ACSV titration as described above. Total dissolved iron was measured by acidifying 50-ml aliquots of samples (pH 1.7), briefly microwaving (~20 s), and, once cooled to room temperature, bringing the pH back up to 8.0 and applying the ACSV technique with SA (Rue and Bruland 1995). An average standard deviation for conditional stability constants of ±0.3 log units is reported for all siderophores and siderophore photoproducts. This number represents a conservative estimate of deviation from a series of titration experiments on the same ligand where the analytical competition strengths were varied in an attempt to verify a particular binding strength.

Results and discussion

Mixed hydroxamate/α-hydroxy carboxylate siderophores—In previous work, we reported the photoreactivity of Fe(III)-aquachelin complexes, which readily undergo oxidative cleavage of the ligand and reduction of Fe(III) when photolysis occurs in the ligand-to-metal charge transfer band (Barbeau et al. 2001). The marinobactins are structurally related to the aquachelins, and when bound to Fe(III), they undergo a similar photochemical transformation, cleaving at the site of the β-hydroxyaspartate-diaminobutyrate ring to form two photoproducts: a hydrophilic peptide fragment and a hydrophobic fatty acid tail fragment. The major peptide photoproduct has a characteristic molecular ion peak $(M + H)^+$ at a mass to charge ratio of $m/z = 691.0$, when analyzed by ESI-MS, corresponding to the mass of the peptidic headgroup minus the fatty acid tail and some component of the β-hydroxyaspartate-diaminobutyrate ring (Fig. 2). As is the case with the aquachelins, the marinobactin peptide photoproduct retains the ability to coordinate Fe(III) via the two hydroxamate groups, as evinced by the detection of iron adducts via ESI-MS ($m/z = 743.8$ for the iron adduct, mass difference of 53 from molecular ion peak, *see Fig. 2*) and by the UV-visible absorption spectrum of the Fe(III)-complexed photoproduct. As expected on the basis of the molecular structure of the marinobactins, all of the marinobactins in series A–E primarily form this same hydrophilic peptide photoproduct on photolysis of their ferric ion complexes. However, some of the marinobactins with shorter fatty acid tails (e.g., marinobactins B and C) have a tendency to form an additional photoproduct, in which some portion of the molecule is lost (mass loss 58 by ESI-MS) but the fatty acid tail is retained. This is likely because of photodecarboxylation and incomplete cleavage of the nine-member β-hydroxyaspartate-diaminobutyrate ring in these molecules.

Photochemical experiments were also performed on the iron-free aquachelins and marinobactins, irradiating 30–μmol L^{-1} solutions at pH 8 in UVSSW for a full day in natural sunlight. UV-visible spectrophotometry, RP-HPLC analysis, and ESI-MS of dark controls versus photolyzed so-

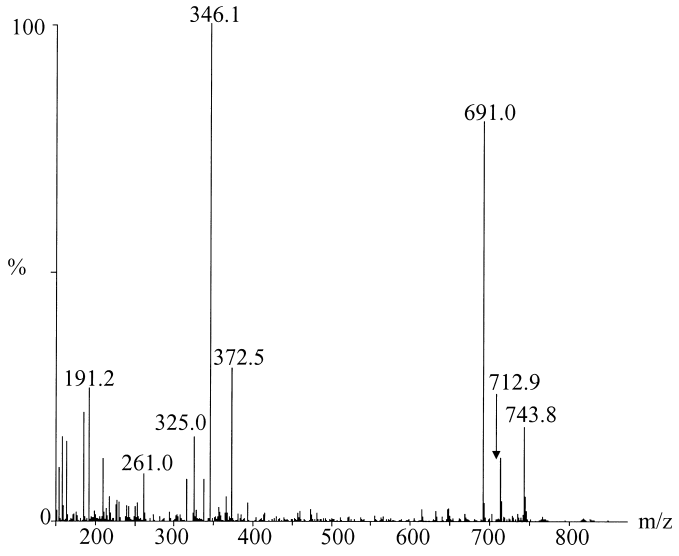

Fig. 2. Electrospray ionization mass spectrum of Fe(III)-marinobactin E peptide photoproduct (positive mode, cone voltage 35 V). Molecular ion peak $(M + H)^+$ at $m/z = 691.0$; iron adduct at $m/z = 743.8$ (mass difference of 53 from molecular ion peak).

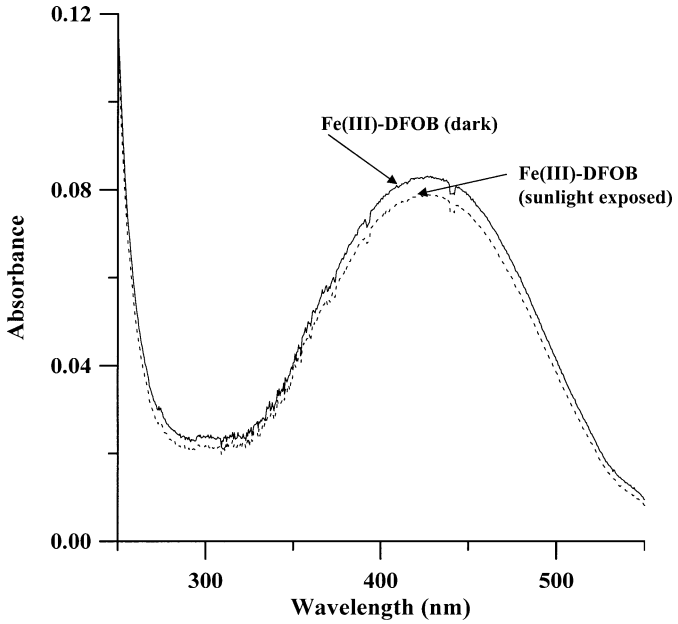

Fig. 3. Ultraviolet-visible absorption spectra of Fe(III)-DFOB; dark control and sunlight exposed in UVSSW (30 μmol L^{-1}, pH 8).

lutions revealed no apparent direct photodegradation of the iron-free aquachelins and marinobactins. This result is consistent with the UV-visible absorption spectra of these compounds, which do not extend above 240 nm (the solar radiation cutoff is wavelengths >295 nm). As previously reported for the ferrated aquachelins and marinobactins, the iron-free siderophores are also relatively stable to thermal degradation on timescales of weeks to months (determined by incubation in UVSSW [pH 8] in the dark at 50°C with periodic analysis via RP-HPLC and ESI-MS). Thus, photolysis of the aquachelins and marinobactins as Fe(III) complexes could be one of the primary loss processes for these and similar compounds in the upper ocean.

Hydroxamate siderophores—DFOB is a tris-hydroxamate terrestrial bacterial siderophore produced by gram-positive actinomycetes and by several gram-negative species (Keller-Schierlein et al. 1965; Mucha et al. 1999). Recently, desferrioxamine G, a siderophore very similar in structure to DFOB, was shown to be produced by a coastal marine *Vibrio* species (Martinez et al. 2001). Like the aquachelins and marinobactins, the iron-free form of DFOB does not absorb light within the solar spectrum; thus, it is not photochemically reactive in natural sunlight. Previous work in freshwater systems (Finden et al. 1984; Gao and Zepp 1998) has demonstrated that, unlike the ferrated aquachelins and marinobactins, ferrated DFOB is not photoreactive.

Figure 3 shows the UV-visible absorption spectrum in UVSSW of Fe(III)-DFOB, which does exhibit absorption within the solar spectrum: there is a broad absorption maximum at 430 nm, characteristic of the hydroxamate to Fe(III) charge transfer band (Albrecht-Gary and Crumbliss 1998). We have confirmed the photostability of Fe(III)-DFOB, which exhibits little change in UV-visible absorption properties (Fig. 3) or ESI-MS spectrum upon photoirradiation. The photostability of Fe(III)-DFOB is consistent with our observations of the photostability of the Fe(III)-hydroxamate moieties of the ferrated aquachelins and marinobactins. A key variable appears to be the ease with which individual Fe(III) ligand groups within the siderophore complex can be irreversibly oxidized on a timescale of the lifetime of the excited state of the Fe(III)-ligand complex. Carboxylate ligands, as observed for the β-hydroxyaspartate chelating moieties of the aquachelins and marinobactins, are readily oxidized irreversibly via decarboxylation, as previously shown for simpler Fe(III)-carboxylate complexes (Balzani and Carassiti 1970; Faust and Zepp 1993 and references therein).

The semipurified hydroxamate siderophore extract (O12) produced by *Synechococcus* PCC 7002 has not yet been fully structurally characterized, but its photochemical behavior as assessed by CLE-ACSV is consistent with that described above for the hydroxamate-containing aquachelins, marinobactins, and DFOB. CLE-ACSV analysis of O12 siderophore solutions indicated no significant changes in the siderophore's conditional binding strength with respect to Fe(III)′ as a result of a 5.5-h sunlight exposure: $K^{\text{cond}}_{\text{FeL,Fe}'}$ for the O12 siderophore was $10^{12.3\pm0.3}$ L mol^{-1} prephotolysis and $10^{12.0\pm0.3}$ L mol^{-1} postphotolysis (Table 1). These values are not significantly different, confirming the photostability of iron-free hydroxamate siderophores in seawater, even at low (nmol L^{-1}) concentrations under full sunlight irradiation.

Mixed catecholate/α-hydroxy carboxylate siderophores—The structure of petrobactin, a bis-catecholate α-hydroxy carboxylate siderophore, offers several possibilities for photochemical reaction, both as an Fe(III)-ligand complex and as a free ligand. In previous work, we have described the photoreactivity of Fe(III)-petrobactin, in which photolysis into the α-hydroxy carboxylate ligand-to-metal charge transfer band results in decarboxylation and oxidation of the li-

Table 1. Results of CLE-ACSV analyses on siderophores and siderophore photoproducts generated in photochemical experiments. Errors on the $K^{cond}_{FeL,Fe'}$ from these titrations averaged ±0.3 log units on the basis of multiple titrations employing several different analytical competition strengths or ''windows.''

Sample	$K^{cond}_{FeL,Fe'}$ (L mol^{-1})
Cyanobacterial siderophore photolysis experiments	
O12 (hydroxamate)	$10^{12.3}$
O12 sunlight exposed	$10^{12.0}$
C413 (catecholate)	$10^{12.0}$
C413 sunlight exposed	$10^{10.9}$
Petrobactin photolysis experiments	
Petrobactin (catecholate/α-OH-carboxylate)	$10^{11.4}$
Petrobactin sunlight exposed (whole solution)	$10^{10.7}$
Fe(III)-petrobactin sunlight exposed (whole solution)	$10^{11.1}$
Petrobactin sunlight exposed, RP-HPLC isolate m/z = 717	$10^{10.8}$
Petrobactin sunlight exposed, RP-HPLC isolate m/z = 719	$10^{10.5}$
Fe(III)-petrobactin sunlight exposed, RP-HPLC isolate m/z = 673	$10^{11.1}$

CLE-ACSV, competitive ligand equilibration–adsorptive cathodic stripping voltametry; RP-HPLC, reversed-phase high-performance liquid chromatography.

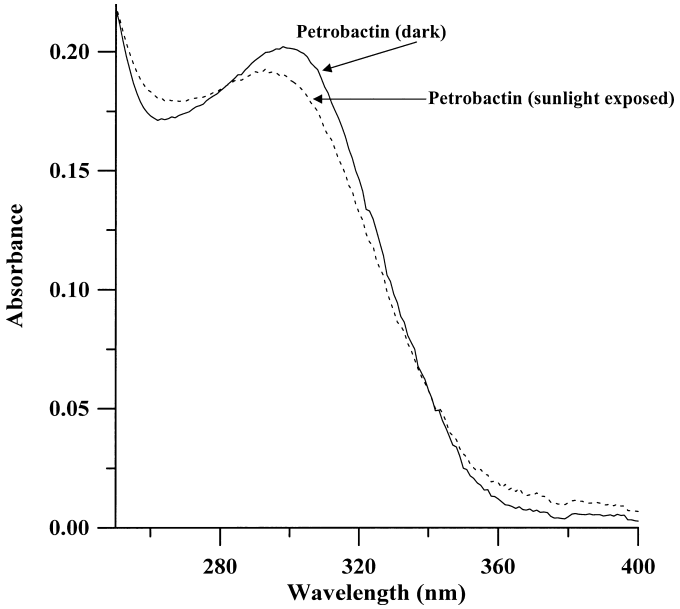

Fig. 4. Ultraviolet-visible absorption spectra of petrobactin (i.e., the apo ligand without Fe[III]); dark control and sunlight exposed in UVSSW (22 μmol L^{-1}, pH 8).

gand at the site of the α-hydroxy acid group, forming a 3-ketoglutarate moiety. Structural analysis indicated that photolyzed Fe(III)-petrobactin retained Fe(III)-complexing capacity via its two catecholate groups, and we speculated that the enolate form of the 3-ketoglutarate moiety could contribute to Fe(III) complexation by the photoproduct (Barbeau et al. 2002). Here, we report CLE-ACSV data that supports our structural analysis (Table 1). Petrobactin was shown by CLE-ACSV to have a conditional stability constant with respect to Fe(III)′ of $K^{cond}_{FeL,Fe'} = 10^{11.4\pm0.3}$ L mol^{-1}. When Fe(III)-petrobactin was photolyzed, the binding strength of the photoproduct formed was similar to that of the original ligand: $K^{cond}_{FeL,Fe'} = 10^{11.1\pm0.3}$ L mol^{-1}. The imperceptible loss in binding strength indicates that the catechol groups remain intact and lends support to the hypothesis that the photochemically transformed backbone of the molecule, the 3-ketoglutarate residue, contributes to Fe(III) binding as an enolate (the metal-coordinative properties of β-ketoenolates are well known).

Thus, as in the case with the Fe(III)-aquachelins and Fe(III)-marinobactins, photolysis of Fe(III)-petrobactin results in fairly localized oxidative damage to the ligand, allowing much of the ligand structure and significant Fe(III) binding strength to be maintained. Therefore, although these Fe(III)-siderophore complexes do readily undergo photoinduced ligand oxidation reactions along with Fe(III) reduction, the oxidized ligands appear to be fairly persistent and still coordinate Fe(III) with appreciable binding strength, thereby continuing to contribute to the pool of Fe(III) binding ligands in seawater. This observation is significant because it has been suggested that if photolysis of naturally occurring Fe(III)-ligand complexes were fast enough to maintain measurable (nmol L^{-1}) daytime Fe(II) concentrations in seawater, concentrations of these ligands would be unlikely to persist in the surface ocean because of photochemical degradation (Voelker and Sedlak 1995; Moffett 2001). The observation of such concentrations of strong Fe(III)-binding ligands in the oceanic euphotic zone (e.g., Rue and Bruland 1995) would therefore seem to argue against significant Fe(II) production via direct photolysis reactions of strong Fe(III)-organic complexes. However, our finding that Fe(III)-siderophore complexes are in several cases only partially oxidized by photolysis offers a possible explanation for the observed persistence of siderophore-like Fe(III) complexes in surface waters, despite the apparent photoreactivity of marine siderophores. Our results also suggest the potential importance of direct, photochemically mediated ligand-to-metal charge transfer reactions of strong Fe(III)-ligand complexes in seawater as a mechanism for generating diel increases in Fe(II) and reactive Fe(III)′, as previously observed in field studies (e.g., O'Sullivan et al. 1991; Johnson et al. 1994; Waite et al. 1995). This direct mechanism of photochemical iron redox cycling is likely to be most significant (relative to secondary photochemical reactions) in oligotrophic pelagic ocean waters, where concentrations of colored dissolved organic material (CDOM) as a source of photochemical radicals is greatly reduced, and most of the dissolved iron present occurs in the form of strong Fe(III)-ligand complexes, possibly with siderophores.

Another possible photochemical reaction pathway for petrobactin is via photooxidation of the iron-free ligand at the catechol groups. Catechols absorb light around 300 nm and are known to be readily photooxidized to quinones or semiquinones in oxygenated solution. Photolysis of Fe(III)-free petrobactin resulted in slight shifts in the UV-visible spectrum (Fig. 4), with an increase in absorption at wavelengths >360 nm. ESI-MS revealed the formation of photoproducts with molecular ion peaks $(M + H)^+$ either indistinguishable from (m/z = 719) or slightly less than

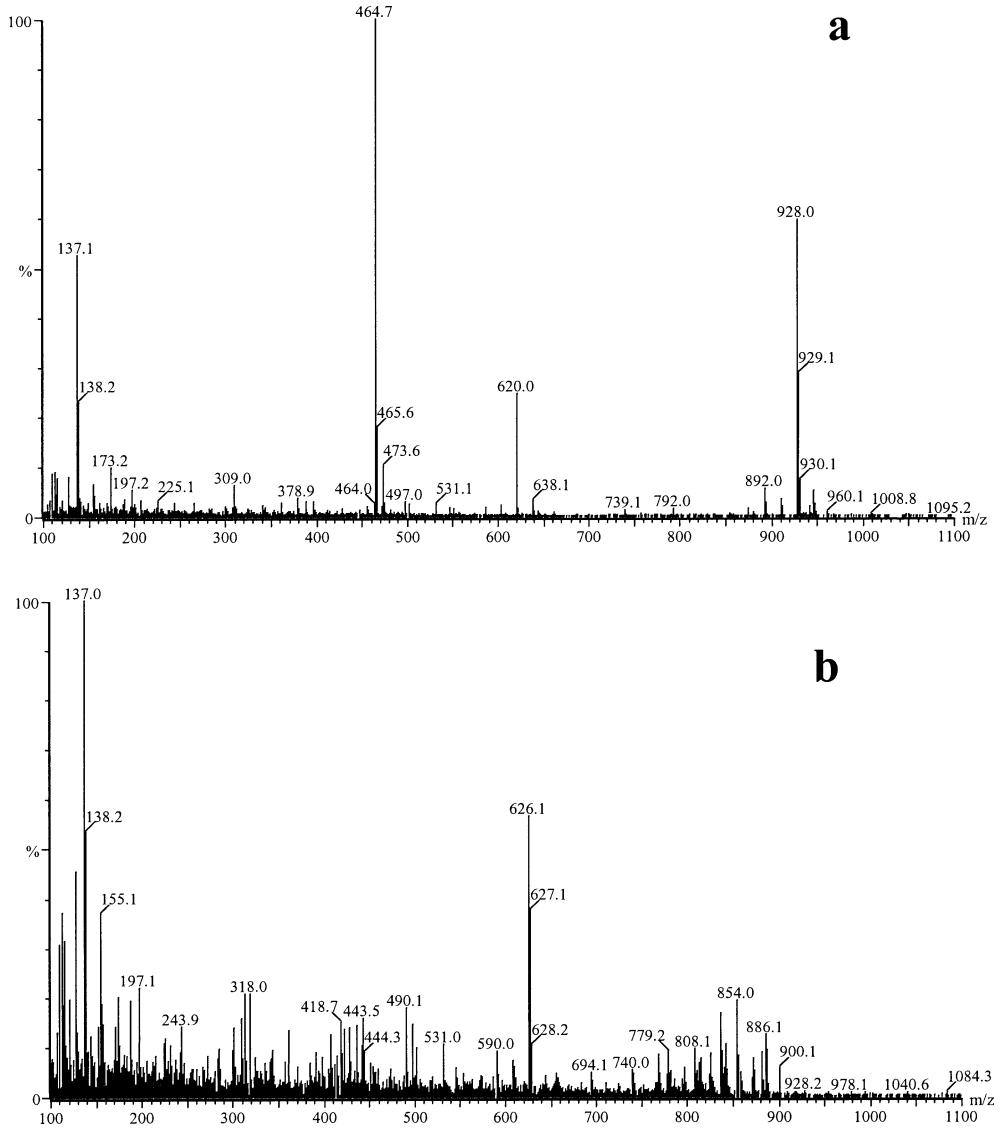

Fig. 5. Electrospray ionization mass spectrum of (a) alterobactin A and (b) RP-HPLC isolate (deferrated) of sunlight-exposed Fe(III)-alterobactin A solution (positive mode, cone voltage 110 V). Molecular ion peaks for alterobactin A at m/z = 928.0 $(M + H)^+$ and m/z = 464.7 $(M + 2H)^{2+}$.

petrobactin (m/z = 717). Such slight or undetectable mass differences are consistent with the photooxidation of the free catechols to quinones or semiquinones. In contrast to photolysis of Fe(III)-petrobactin, photolysis of petrobactin in the absence of Fe(III) also resulted in a significant decrease in ligand binding strength, from $K^{cond}_{FeL,Fe'} = 10^{11.4\pm0.3}$ L mol^{-1} for the original ligand to $K^{cond}_{FeL,Fe'} = 10^{10.7\pm0.3}$ L mol^{-1} for the whole photolyzed solution. This decrease in binding strength is likely a consequence of the oxidation of the catechol groups. These results indicate that for free catechol-containing Fe(III) ligands, photolysis can be a significant sink in ocean surface waters.

Although the aquachelins, marinobactins, and petrobactin all have discrete structurally altered photoproducts, for some marine siderophores, photodegradation can be more extensive and complex, as is the case for the Fe(III)-bound forms of alterobactins A and B. These ligands have one catecholate and two α-hydroxy carboxylate Fe(III)-binding moieties (Reid et al. 1993). Upon photolysis of Fe(III)-alterobactin A, large changes were observed in the UV-visible spectrum of the complex. ESI-MS analysis (Fig. 5) revealed that several photoproducts were apparently formed, most likely as a consequence of the presence of two photochemically susceptible α-hydroxy carboxylate groups and the complex cyclic structure of alterobactin A. Similar results were obtained for alterobactin B, which also has two α-hydroxy acid groups, but a linear structure. Thus far it has not been possible to characterize structurally the photodegradation products of the ferrated alterobactins because of the complexity of the reaction products formed.

Catecholate siderophores—The catechol siderophore extract (C413) produced by *Synechococcus* PCC 7002 was analyzed via CLE-ACSV for its photochemical reactivity in

the Fe-free form. Consistent with our results for petrobactin, the C413 siderophore was shown to be photoreactive as a free ligand, losing some of its Fe(III) complexing strength. For the nonphotolyzed ligand, $K^{cond}_{FeL,Fe'} = 10^{12.0\pm0.3}$ L mol^{-1}. After photolysis, the ligand's conditional stability constant with respect to Fe(III)′ was reduced by over an order of magnitude to $K^{cond}_{FeL,Fe'} = 10^{10.9\pm0.3}$ L mol^{-1} (Table 1). This reduction is likely from the photooxidation of the catechol group(s) to semiquinones or quinones. Such a decrease in the conditional stability constant will serve to increase the concentration of Fe(III)′ in equilibrium with the ligand, increasing the bioavailability of Fe.

Given the tendency of free catechol groups to readily photooxidize, the observed photostability of catechols when complexed to Fe(III) was somewhat surprising. To examine this phenomenon more closely in the absence of the photoreactive Fe(III)–α-hydroxy acid moiety, photochemical experiments were conducted with the synthetic tris-catecholate ligands 3,4-LICAM and 3,4-LICAMS. Fe(III)-free 3,4-LICAMS and Fe(III)-bound 3,4-LICAM (μmol L^{-1} concentrations) were exposed to natural sunlight for a full-day photoperiod (solubility considerations necessitated the use of the sulfonated compound in the Fe(III)-free experiment; 3,4-LICAM is not soluble in aqueous solution as the free ligand). The UV-visible absorption spectrum of Fe(III)-3,4-LICAMS (Fig. 6) showed changes characteristic of catechol oxidation, including a slight peak shift near the catecholate absorption maximum and increases in absorbance at wavelengths >360 nm, similar to the spectral changes observed for petrobactin on exposure to sunlight (Fig. 4). Fe(III)-bound 3,4-LICAM, in contrast, showed no apparent changes in absorption spectrum as a result of sunlight exposure, and this was confirmed by ESI-MS analysis, which indicated no change in the molecular ion peak of the compound. Although catechols are readily oxidized to semiquinones or quinones, this reaction is reversible. Thus, when complexed to Fe(III), the catechol ligand is not oxidized. Our observations are consistent with the work of Waite and Morel (1984), who noted that light had no effect on Fe(III)-catechol solutions in a fluorescence quenching study of iron interaction with various model ligands.

Siderophore structure/photoreactivity relationships—On the basis of the studies presented here of siderophore photochemistry using a structurally diverse group of siderophores, we can now derive some general concepts for the photoreactivity of siderophores as Fe(III)-binding ligands in the marine environment, considering the characteristic Fe(III) binding groups that they possess: hydroxamate, catecholate, and α-hydroxy acid moieties. Our findings regarding the photoreactivities of these groups in the presence and absence of iron are summarized in Table 2. Hydroxamate groups are the most stable; therefore, tris-hydroxamate siderophores such as desferrioxamine G, which recently has been shown to be produced by a marine *Vibrio* (Martinez et al. 2001), should be relatively persistent in ocean surface waters, both as free ligands and as Fe(III)-siderophore complexes. As expected, catecholates are readily photooxidized in the free form, but the surprising photostability of these groups when complexed to Fe(III) suggests that catechols also have the potential to persist in ocean surface waters as part of Fe(III)-ligand complexes. The α-hydroxy carboxylate group is of key significance because of its photoreactivity when coordinated to Fe(III), thereby facilitating Fe redox cycling and leading to the production of reactive and bioavailable Fe(II) and Fe(III)′ in sunlit surface waters.

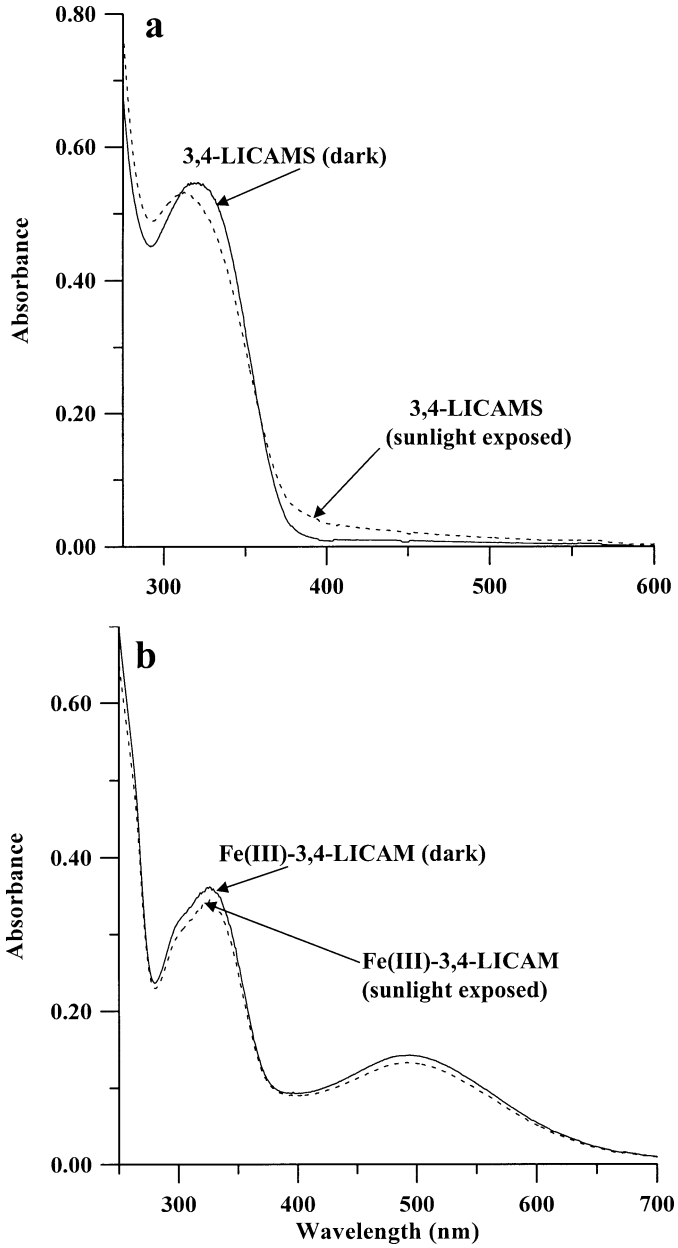

Fig. 6. (a) Ultraviolet (UV)-visible absorption spectra of 3,4-LICAMS; dark control and sunlight exposed in UVSSW (40 μmol L^{-1}, pH 8). (b) UV-visible absorption spectra of Fe(III)-3,4-LICAM; dark control and sunlight exposed in UVSSW (42 μmol L^{-1}, pH 8).

The existence of the α-hydroxy carboxylate group has been somewhat overlooked in the literature on marine siderophores, primarily because there is no qualitative chemical test for this group, like the Arnow and Csaky tests for catechols and hydroxamates, and because the α-hydroxy carboxylate group is comparatively rare in terrestrial sidero-

Table 2. Summary of the photochemical reactivity of the common siderophore Fe(III) binding groups as free ligands and as Fe(III)-ligand complexes.

Fe(III) binding group	Photochemcial reactivity as a free ligand	Photochemical reactivity as an Fe(III)-ligand complex
Hydroxamate	No reactivity	No reactivity
Catecholate	Oxidation of ligand with loss of Fe(III) binding ability	No reactivity
α-Hydroxy carboxylate	No reactivity	Oxidative decarboxylation of ligand; reduction of Fe(III) to Fe(II)

phores relative to the occurrence of catecholate and hydroxamate groups (Albrecht-Gary and Crumbliss 1998). Marine siderophores have in the past been characterized as simply hydroxamate or catecholate by qualitative chemical tests, but more extensive structural characterization is required to determine the presence or absence of the α-hydroxy carboxylate group. It is noteworthy that of the handful of siderophores produced by pelagic marine bacteria that have been fully structurally characterized (Haygood et al. 1993; Reid et al. 1993; Martinez et al. 2000; Barbeau et al. 2002), all are mixed-ligand siderophores, which contain not only hydroxamate or catecholate moieties, but also the α-hydroxy carboxylate group. Further structural studies of siderophores produced by marine microorganisms will likely provide more information about the general occurrence of the α-hydroxy carboxylate, hydroxamate, and catecholate moieties in these molecules, leading to a better understanding of how siderophores might mediate Fe photoredox cycling in the surface ocean.

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