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Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002

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Abstract

The coastal marine cyanobacterium *Synechococcus* sp. PCC 7002 produces three amphiphilic siderophores, synechobactins A-C, under iron-limiting growth conditions. The synechobactins are comprised of a citric acid backbone linked to two 1, 3-diaminopropane units. The terminal amine of one diaminopropane is acetylated and hydroxylated forming one hydroxamate group. The terminal amine of the other diaminopropane is appended to one of a series of fatty acids and *N*-hydroxylated on the fatty acid amide forming the second hydroxamate linkage. Synechobactins A-C differ among themselves in the identity of the fatty acid residue as dodecanoic acid, decanoic acid, or octanoic acid, respectively. They are the first structurally elucidated siderophores from marine cyanobacteria and are related to schizokinen, a previously identified siderophore that lacks amphiphilic character, isolated from other bacteria including freshwater cyanobacteria.

Iron is an important limiting nutrient for many marine microorganisms. Although recent reports demonstrate that growth of marine autotrophic and heterotrophic microorganisms are limited by the low iron concentration (0.02–1 nmol L^{-1}) in much of the world's surface ocean waters, the dominant form of this iron is not yet fully defined. Most significantly, the dissolved Fe(III) in the ocean is tightly complexed by an organic ligand or a class of organic ligands of unknown structure with conditional stability constants for Fe(III) (Rue and Bruland 1995; Van den Berg 1995; Wu and Luther III 1995) in a range similar to that of microbial siderophores. Siderophores are low–molecular mass, high-affinity Fe(III)-coordinating ligands secreted by microorganisms under iron-deficient conditions to facilitate iron acquisition. Typical Fe(III) coordinating groups in siderophores include the hydroxamate and catecholate moieties, although the discovery of α-hydroxy carboxylic acid–containing siderophores is particularly prevalent in marine bacteria (*see below*). The Fe(III)–siderophore complex, once formed, is recognized by its cognate receptor expressed on the bacterial outer membrane, which catalyzes the internalization of the Fe(III)–siderophore complex (Neilands 1995). Although microbial siderophores have not yet been isolated directly from natural seawater, low–molecular mass compounds isolated from coastal California upwelling waters have been shown to contain hydroxamate and catecholate groups by chemical assays specific for each group (Macrellis et al. 2001).

Several hundred structures of terrestrial siderophores are known, although relatively few structures of marine siderophore have been identified. The structures of marine siderophores from heterotrophic marine bacteria that are known have certain interesting chemical properties in common. Marinobactins from *Marinobacter* spp. strain DS40M6, aquachelins from *Halomonas aquamarina* (Martinez et al. 2000), and amphibactins from *Vibrio* sp. R-10 (Martinez et al. 2003) are amphiphilic peptidic siderophores that each contain one of a series of fatty acid appendages attached to their hydrophilic peptide head groups that coordinate Fe(III) (Fig. 1). Membrane partitioning experiments with artificial lipid bilayers indicate that cell association of the amphiphilic marine siderophores depends on the length and hydrophobic nature of the lipid tail, possibly forming a gradient of siderophores emanating out from the cell (Xu et al. 2002; Martinez et al. 2003). In aquatic natural environments, such a gradient could be an efficient strategy to limit diffusion of secreted siderophores. In contrast to the relative preponderance of amphiphilic siderophores produced by marine bacteria, fatty acid–containing amphiphilic siderophores are relatively rare among terrestrial siderophores.

The other common feature among many of the siderophores from oceanic bacteria is the presence of an α-hydroxy carboxylate moiety, such as citrate or β-hydroxy aspartate, which is one of the Fe(III)-binding groups. In natural sunlight or ultraviolet (UV) light, the Fe(III) complex of these siderophores undergoes a light-induced ligand-to-metal charge transfer reaction that results in reduction of Fe(III) to Fe(II) and decarboxylation of the ligand (Barbeau et al. 2001*a*,*b*, 2003). Although the α-hydroxy carboxylate moiety is found in many terrestrial siderophores, the bacteria producing these siderophores are enteric bacteria that would not normally experience sunlight or other UV light conditions. The presence of the photoreactive α-hydroxy carboxylic acid moiety in siderophores when coordinated to Fe(III) could then have a significant influence on the availability of iron in natural aquatic environments. Thus, further elucidation of structures of α-hydroxy carboxylic acid–containing siderophores, as well as their role in cycling of iron, is important.

Cyanobacteria are among the most important primary producers in aquatic environments. Cyanobacteria are also known to produce and utilize siderophores to acquire iron

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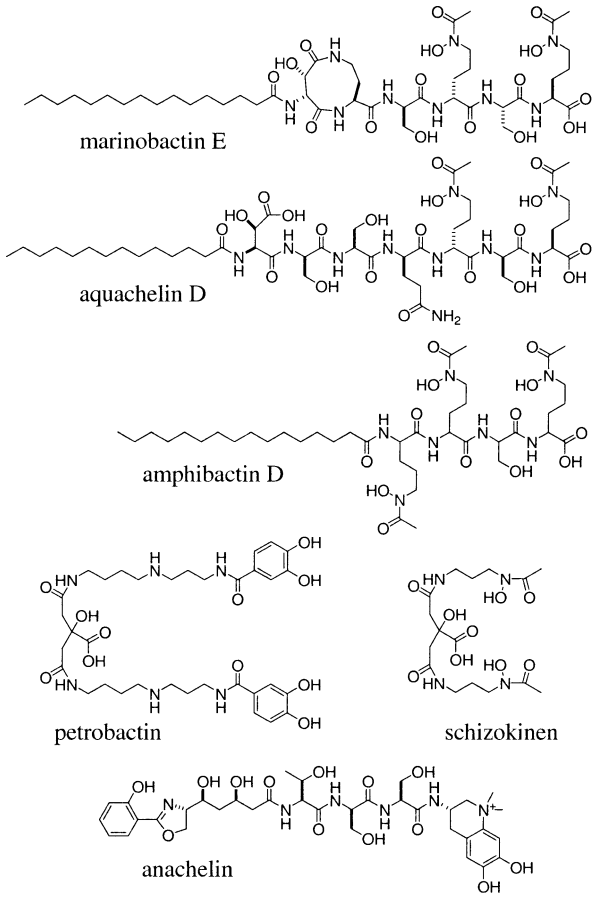

Fig. 1. Structures of siderophores from marine bacteria and freshwater cyanobacteria.

under iron-deficient conditions (Murphy et al. 1976). The first structural report of a cyanobacterial siderophore was schizokinen (Fig. 1) from the freshwater *Anabaena* sp. PCC 7120 (Simpson and Neilands 1976), although schizokinen was originally isolated from the terrestrial bacterium *Bacillus megaterium* (Mullis et al. 1971). Various other cyanobacterial species are also reported to produce siderophores; however, the structures have not been reported (Wilhelm and Trick 1994). Recently, the anachelins (Fig. 1) have been isolated from the freshwater strain *Anabaena cylindrica* (Ito et al. 2001, 2004). Anachelins are peptidic structures that contain certain unique moieties not found previously in other siderophores. Surprisingly, the structures of siderophores from marine cyanobacteria have not been previously reported, although their competitive advantage among prokaryotic and eukaryotic phytoplankton species has been suggested (Hutchins et al. 1999). The coastal marine cyanobacterium *Synechococcus* sp. PCC 7002 (formerly named *Agmenellum quadruplicatum* PR-6) has been previously reported to produce an amphiphilic hydroxamate-type siderophore (Armstrong and van Baalen 1979). It therefore prompted us to elucidate the structure of the siderophore produced by this strain. Herein we report the isolation and structure of synechobactins A–C, a family of photoreactive amphiphilic siderophores produced by *Synechococcus* sp. PCC 7002.

Materials and methods

Strains and media—The cyanobacterium *Synechococcus* sp. PCC 7002 was obtained from Dr. E. A. Webb (Woods Hole Oceanographic Institution), and cells were maintained in ASP-2 medium as modified by Armstrong and van Baalen (1979). ASP-2 basal medium contained 18 g NaCl, 5 g $MgSO_4 \cdot 7H_2O$, 0.05 g KH_2PO_4, 0.37 g $CaCl_2 \cdot 2H_2O$, 0.60 g KCl, 1 g $NaNO_3$, 1 g Tris, 0.1 g NH_4Cl, 30 mg Na_2EDTA, 2.7 mg $FeCl_3 \cdot 7H_2O$ (10 μmol L^{-1}), 1 mL P-1 trace metal stock solution containing 3.426 g L^{-1} H_3BO_3, 432 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 31.5 mg L^{-1} $ZnCl_2$, 3.0 mg L^{-1} MoO_3, 3.0 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 0.3 mg L^{-1} $CoCl_2 \cdot 6H_2O$, and 4 μg L^{-1} vitamin B_{12} per liter of doubly deionized water (Nanopure®). The medium was adjusted to pH 8.08 before autoclave sterilization. The culture (750 mL) was grown in a flat-bottom boiling flask with a long neck (Pyrex, 1.0 L) on 24-h light with cool white fluorescent lamps at 30°C; filtered air was continuously bubbled through the cultures. The growth of cells was monitored by measuring the optical density at 750 nm on a Varian-CARY 3E UV-visible spectrometer with a 1-cm path length cell.

Iron-limited culture—To prepare iron-deficient ASP-2 medium (ASP-2[-Fe]), the medium with the exception of $FeCl_3 \cdot 7H_2O$, Na_2EDTA, P-1 trace metals, and vitamin B_{12} was treated with Chelex-100 to remove residual iron; then, vitamin B_{12} and one tenth of the normal concentration of P-1 trace metals were added. Cells grown in complete ASP-2 medium for 2 weeks were harvested and washed four times with ASP-2(-Fe) medium with centrifugation then inoculated into ASP-2(-Fe) medium to a final optical density of 0.12 at 750 nm. As a result of the observation by Armstrong and van Baalen that increased siderophore production occurred on addition of NH_4Cl (2 mmol L^{-1}) as the nitrogen source at 30°C, but not at 26°C or 39°C, we also cultured *Synechococcus* sp. PCC 7002 in accordance with their procedure.

Siderophore assay—Siderophores were detected by the chromazurol S (CAS) shuttle assay (Schwyn and Neilands 1987). Typically, each 500-μL sample solution was incubated with 500 μL of CAS shuttle solution for 1 h, at which point the absorbance at 630 nm was recorded. To monitor cell growth and the production of siderophores in iron-sufficient or iron-deficient cultures, small aliquots of the culture were collected daily for 7 d. To increase the sensitivity of the CAS assay, the cell-free medium was concentrated 10-fold by lyophilization and diluted with 50% methanol (MeOH) to dissolve amphiphilic compounds. Undissolved solid material was removed by centrifugation, and the resultant supernatant was subjected to the assay. (NB: A false positive CAS response could be obtained with 100% MeOH; however, 50% MeOH, as used here, does not affect the CAS response.) The Csaky test was used to detect the presence of hydroxamate compounds, and the Arnow test was used to detect the presence of dihydroxyphenolic compounds (*see* Macrellis et al. 2001).

Isolation of siderophores—The siderophores were isolated from the supernatant following centrifugation of the iron-

deficient culture (30 min, 5,000 × g) and adsorbed onto Amberlite XAD-2 resin. The resin was vigorously stirred with the supernatant for 12 h and sequentially washed with two column volumes of Nanopure water, one of 50% MeOH and two of 100% MeOH. Siderophore-containing fractions corresponding to 100% MeOH were pooled, dried in vacuo, and applied to a C18 reversed-phase high-performance liquid chromatography (HPLC) column (Vydac 10 mm ID × 250 mm length). The siderophores were purified for 15 min in an acetonitrile gradient (from 20% to 50%) containing 0.05% trifluoroacetic acid (TFA). The absorbance of the eluent was monitored at 215 nm. The fractions containing siderophores were lyophilized, and the white solid of siderophores were stored at −20°C until analyzed. Schizokinen was prepared from *Anabaena variabilis* NIES-23 as described in a previous study (Ito 2003).

Structure determination—Initial mass measurements for the siderophores from *Synechococcus* sp. PCC 7002 was determined by electrospray ionization mass spectrometry (ESI-MS) performed with a quadrupole time-of-flight (Q-TOF) mass spectrometer (P.E. Biosystems) and a Pesciex ion spray source. The Q-TOF mass spectrometer was also used for accurate mass determination. ^{1}H, ^{13}C, and various two-dimension nuclear magnetic resonance (NMR) spectra were recorded on a Varian 500-MHz instrument with the solvent dimethylsulfoxide-d_6 (d = 2.49 ppm). For identification of amine functionalities, the siderophore was hydrolyzed in 50% hydrogen iodide (HI) solution at 110°C for 48 h. After removal of excess HI by repeated cycles of evaporation to dryness followed by addition of water, the sample was dissolved in pentafluoropropionic anhydride to derive amine moieties. The mixtures were incubated for 30 min, then dried and subjected to gas chromatography–mass spectrometry (GC-MS) analysis (Hewlett-Packard model HP-5890). For identification of citric acid, the dried HI hydrolysate was heated in 3 mol L^{-1} HCl-MeOH solution at 110°C for 1 h. The esterified acid was extracted with ethyl acetate and subjected to GC-MS analysis. For identification of acyl units, purified siderophores were oxidized with 0.1% periodic acid (Emery and Neilands 1960), and the carboxylic acid released was extracted with diethyl ether. After evaporation of organic solvent, the residue was esterified with trimethylsilyldiazomethane in MeOH and subjected to GC-MS analysis.

Results

Synechococcus sp. PCC 7002 is a halotolerant unicellular cyanobacterium that has previously been reported to produce siderophores (Armstrong and van Baalen 1979; Wilhelm and Trick 1994). Siderophore production as monitored by the CAS assay tracks with cell growth under iron-deficient growth conditions (Fig. 2). When *Synechococcus* sp. PCC 7002 was cultured under iron-replete conditions, the added iron masked the iron-binding activity of the CAS assay, thereby obviating the CAS response. However, HPLC analysis of the supernatant from this iron-satiated culture did not show any trace of synechobactin production (data not shown). Accumulated siderophore activity reached a maximum about 5 d after inoculation of cells into iron-deficient medium; therefore, the supernatant of 5 d of iron-deficient culture (2.5 L) was pooled and treated with XAD-2 resin to adsorb the organic compounds. The siderophores eluted with 100% MeOH, suggesting they were relatively hydrophobic in nature. HPLC purification of the 100% MeOH fraction yielded three siderophores: (1) synechobactin A (7.5 mg), (2) synechobactin B (1.5 mg), and (3) synechobactin C (0.3 mg), in order of decreasing hydrophobicity (Fig. 3). In addition, some synechobactins were isolated by extraction of the cyanobacterial cell pellet with MeOH.

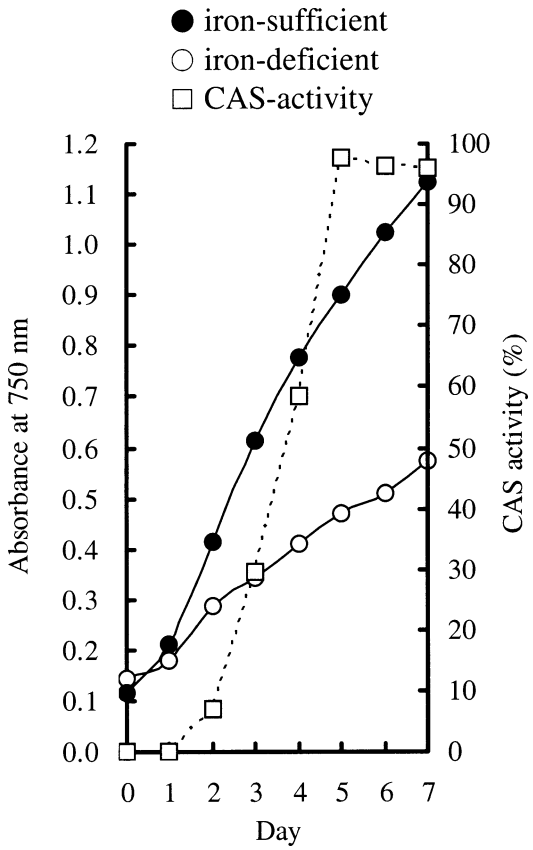

Fig. 2. Time course of *Synechococcus* sp. PCC 7002 growth and siderophore production, including growth under iron-sufficient conditions, growth under iron-deficient conditions, and CAS activity under iron-deficient conditions. CAS activities are represented as an inverse ratio of the control (fresh medium) because the absorbance at 630 nm of CAS solution decreases in proportion to increased siderophore in sample.

Synechobactin A, the major compound, was positive in the Csaky assay for the hydroxamate moiety but not in the Arnow assay for the catecholate group, indicating that it is a hydroxamate-type siderophore. ESI-MS analysis of synechobactin A gave an intense parent ion peak at m/z 561 and 583 for $(M + H)^+$ and $(M + Na)^+$, respectively. Although synechobactin A had a negative ninhydrin reaction, its HI hydrolysate showed a ninhydrin-active spot by thin-layer chromatography. GC-MS analysis of the pentafluoropropionate derivative of the HI hydrolysate of synechobactin A revealed that the amine unit was 1,3-diaminopropane. Furthermore, GC-MS analysis of the methylester derivative of the HI hydrolysate of synechobactin A revealed the presence

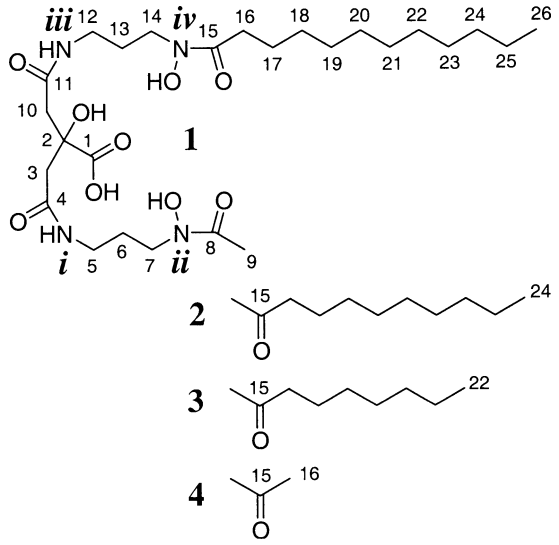

Fig. 3. Structure of synechobactins A (1), B (2), and C (3) and schizokinen (4). *See Table 1* for ^{1}H and ^{13}C NMR chemical shifts for corresponding atoms. Numbers for nitrogen atoms are noted in Roman numerals.

Table 1. ^{13}C and ^{1}H NMR chemical shifts (**δ**, ppm) and coupling constants (*J*, Hz) of synechobactin A in dimethylsulfoxide-d_6. br, broad; brt, broad-triplet; d, doublet; m, multiple; t, triplet).

Position	δ_C	δ_H (m, Hz)
1	175.0	
2	73.4	
3, 10	43.3	2.48 (d, J = 14.5)
4, 11	169.5	2.55 (d, J = 14.5)
5, 12	36.2	3.02 (brt)
6, 13	26.6	1.62 (m)
7, 14	45.1 or 45.3	3.48 (t, J = 6.9)
8	170.3	
9	20.3	1.97 (m)
15	172.8	
16	31.7	2.32 (brt)
17	24.2	1.46 (m)
18–23	28.7, 28.8, 28.9 28.9, 29.0, 29.1	1.24 (m)
24	31.3	1.24 (m)
25	22.1	1.24 (m)
26	14.0	0.85 (t, J = 6.9)
N*i*,*iii*		7.94 (br)
N*ii*,*iv*-OH		9.65 (br) or 9.78 (br)

of 1,5-dimethyl citrate, implicating it as a citrate-based hydroxamate siderophore.

The ^{13}C and ^{1}H NMR spectral values of synechobactin A in dimethylsulfoxide-d_6 are summarized in Table 1. The ^{1}H NMR spectrum showed the presence of an amide proton resonance at δ_H 7.94, a singlet methyl peak at δ_H 1.97, a triplet methyl peak at δ_H 0.85, and a huge peak at δ_H 1.24 that presumably resulted from an alkyl group. The ^{13}C NMR spectrum and heteronuclear multiple quantum coherence (HMQC) analysis of synechobactin A revealed the presence of a quaternary carbon signal at δ_C 73.4 and four carbonyl carbon signals at δ_C 175.0, δ_C 172.8, δ_C 170.3, and δ_C 169.5; this last ^{13}C resonance is likely an overlap of two nearly identical carbonyl signals (*see below*). A ^{1}H–^{1}H correlation spectroscopy (COSY) experiment allowed us to assign a spin system from an amide signal at δ_H 7.94 to a methylene signal at δ_H 3.48 corresponding to a 1,3-diaminopropane moiety detected by GC-MS analysis. The absence of another amide proton signal of the diamine indicated that a hydroxamate group existed at this position as a 1-amino-3-*N*-hydroxypropane moiety. Interestingly, the HMQC spectrum showed that a methylene signal at δ_H 3.48 coupled to two adjacent carbon signals at δ_c 45.1 and δ_c 45.3, implying two 1-amino-3-*N*-hydroxypropane moieties are present that exist in slightly different environments in synechobactin A. Two broad signals corresponding to hydroxamic acid protons were observed at δ_H 9.65 and 9.78, also supporting the presence of two units. The ^{1}H–^{1}H COSY experiment also gave a long alkyl group from a triplet methyl signal (H26) to a methylene signal (H16) via a huge methylene signal at δ_H 1.24. Heteronuclear multiple bond correlations (HMBCs) from H16 and H17 signals to a carbonyl carbon C15 signal indicated that this alkyl chain was a saturated fatty acid. The number of peaks seen in the ^{13}C NMR spectrum of synechobactin A indicates the fatty acid is dodecanoic acid. GC-MS analysis of the hydrolyzed fatty acid confirmed the presence of dodecanoic acid. The citrate moiety was confirmed by HMBCs from two doublet methylene signals (H3 or H10) to a quaternary carbon signal (C2) and to two carbonyl carbon signals (C1 and C4 or C11). A singlet methyl and a carbonyl carbon remaining to be assigned were also identified as an acetyl group by HMBC.

Connectivity of the identified subunits was accomplished by HMBC spectroscopy. The HMBC between amide protons of the 1-amino-3-*N*-hydroxypropane moieties and two terminal carbonyl carbons (C4 or C11) of the citrate moiety revealed the connection of both units through amide bonds. The HMBC spectrum shows the correlation from a proton signal at δ_H 3.48 (H7 and H14) of the two 1-amino-3-*N*-hydroxypropane moieties to two carbonyl carbons of an acetyl and a dodecanoyl moieties, indicating that each of the 1-amino-3-*N*-hydroxypropane moieties is attached to each acyl moiety. Consequently, the constructed structure of synechobactin A is similar to that of schizokinen, a symmetrical siderophore. Comparison of the ^{1}H NMR spectrum of synechobactin A to that to schizokinen from *A. variabilis* NIES-23 showed that they are similar, differing only by the presence of the fatty acid resonances and the lack of identical hydroxamate groups in synechobactin A (Fig. 4). The unsymmetrical structure of synechobactin A explains the presence of two distinct hydroxamate protons and the nonoverlapping ^{13}C resonances of C7 and C14. Finally, the molecular formula of synechobactin A was demonstrated by accurate mass analysis to be m/z 583.3337 for $(M + Na)^+$ consistent with $C_{26}H_{48}N_4O_9Na$ (Δ 4.02 ppm). Thus, the structure of synechobactin A is unambiguously defined.

ESI-MS analyses of synechobactins B and C revealed peaks at m/z 533 and 505 for $(M + H)^+$, respectively versus m/z 561 $(M + H)^+$ for synechobactin A. The successive decrease of each by 28 mass units corresponding to two methylene units suggested that structural difference in the

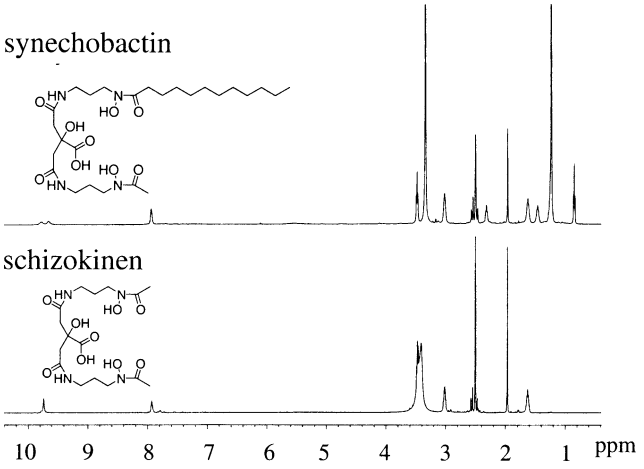

Fig. 4. ^{1}H NMR spectra of synechobactin A (1) and schizokinen (4) in dimethylsulfoxide-d_6. The intense peaks at 2.49 and 3.35 ppm correspond to dimethylsulfoxide and H_2O, respectively.

synechobactins could reside in the chain length of a fatty acid tail. GC-MS analysis revealed that synechobactins B and C contained a decanoic and an octanoic acid, respectively. The molecular formulas of synechobactins B and C were confirmed by accurate mass analyses as *m*/*z* 555.3005 and 527.2667 for $(M + Na)^+$ consistent with $C_{24}H_{44}N_4O_9Na$ (Δ 0.80 ppm) and $C_{22}H_{40}N_4O_9Na$ (Δ −3.88 ppm), respectively.

The ESI-MS of the ferric complex of synechobactin A gives an intense molecular ion peak at *m*/*z* 614, which is 53 mass units higher than the free ligand. The exact mass, *m*/*z* 614.2586 for $(M - {}^3H + Fe + H)^+$ is consistent with $C_{26}H_{46}N_4O_9Fe$ (Δ −4.60 ppm). Two hydroxamate groups and the α-hydroxy carboxylate of the citrate moiety likely coordinate Fe(III), analogous to the well-characterized Fe(III)–aerobactin complex. Ferric complexes of citrate-type siderophores are photoreactive, resulting in decarboxylation of the siderophore and reduction of Fe(III) (Barbeau et al. 2001*b*, 2003). When an approximately 1.6 μmol L^{-1} solution of Fe(III)–synechobactin A complex dissolved in H_2O is placed under fluorescent lights at 30°C used for continuous culture of *Synechococcus* sp. PCC 7002, ESI-MS reveals that after 3 h, all the ferric complex of synechobactin A at *m*/*z* 614 had disappeared and that a new peak at *m*/*z* 568 appeared. The loss of 46 mass units, which is also seen in the ferric-petrobactin photoproduct (Barbeau et al. 2001*b*), is consistent with loss of CO_2 and two protons, yielding a 3-ketoglutarate backbone in place of citrate. The photoproduct of synechobactin A still coordinates Fe(III), as observed by *m*/*z* of 568 which is 53 mass units higher than that of the apo-photoproduct.

Discussion

In this study, we report the structures of three new photoreactive and amphiphilic siderophores from the *Synechococcus* sp. PCC 7002 strain: synechobactins A, B, and C. This is the first structure elucidation of cyanobacterial siderophores from marine species and from unicellular species; all previously known cyanobacterial siderophores were isolated from only freshwater and filamentous cyanobacteria. *Synechococcus* sp. PCC 7002 is a coastal isolate, in contrast to the small oceanic *Synechococcus* strain WH8102 that has been predicted to lack a siderophore-mediated iron uptake system based on whole genome analysis (Palenik et al. 2003). Catecholate-containing siderophores have also been reported in cultures of *Synechococcus* sp. PCC 7002 (Wilhelm and Trick 1994), although under the growth and isolation conditions employed in our investigations, catecholate siderophores were not detected.

Synechobactins A–C are related to schizokinen, a previously identified siderophore from *B. megaterium* (Mullis et al. 1971), as well as the freshwater cyanobacteria *Anabaena* sp. PCC 7120 and *A. variabilis* NIES-23 (Simpson and Neilands 1976; Ito 2003), although the synechobactins are amphiphilic whereas schizokinen is not. In addition, two other schizokinen-type amphiphilic siderophores have been reported from terrestrial bacteria. One is acinetoferrin from *Acinetobacter haemolytis*, in which each acetyl moiety is replaced by (*E*)-2-octenoic acid (Okujo et al. 1993) and the other is rhizobactin 1021 from *Rhizobium meliloti* 1021, in which one acetyl moiety is replaced by (*E*)-2-decanoic acid (Persmark et al. 1993).

From the proposed biosynthetic scheme of rhizobactin 1021, it is predicted that the (*E*)-2-decanoic acid is incorporated after the schizokinen unit has been completely constructed by the products of four biosynthetic genes (Lynch et al. 2001). In this respect, the addition of a fatty acid appendage could be used for a strategic advantage. The role of the fatty acid tail in synechobactins A–C is presumed to enhance the affinity of siderophores for the bacterial membrane surface like other amphiphilic siderophores from marine bacteria (Fig. 1), although further investigations on the biological significance of the amphiphilic nature of these another siderophores is still in progress. In fact, under the bacterial growth conditions employed herein, some of the synechobactins were extracted from the bacterial cell pellet with MeOH.

The photoreactivity of the Fe(III)–synechobactin complex is another interesting feature of the synechobactins. As presented above, exposure of Fe(III)-synechobactin A to fluorescence light yielded an iron-bound product with a loss of 46 mass units. The Fe(III) complex of the siderophore petrobactin (Fig. 1), which also has a citrate backbone like the synechobactins, is photoreactive, resulting in the reduction of Fe(III) to Fe(II) and loss of 46 mass units, corresponding to loss of CO_2 and two protons (Barbeau et al. 2001*b*, 2003). The synechobactin photoproduct likely coordinates Fe(III) via the two remaining hydroxamate groups and the β-ketoenolate derived from a 3-ketoglutarate moiety formed in the decarboxylation reaction, although full structural analysis is in progress.

The production of a suite of photoreactive amphiphilic siderophores by marine cyanobacteria is intriguing and raises questions about the form of iron that the cells take up. The presence of the fatty acid appendage that can anchor the siderophores in the bacterial membrane could be important by reducing a three-dimensional search in space of a siderophore for its receptor to a two-dimensional search (Martinez

et al. 2003). The amphiphilicity could also be important if a two-step process of iron acquisition occurs, initiated by photolysis followed by uptake of Fe(II). Furthermore, the photoreactive α-hydroxy carboxylic acid moiety, when coordinated to Fe(III), could have a significant influence on the availability of iron in natural aquatic environments and affect both the cycling of iron and the growth of cyanobacteria, as well as other microorganisms. Thus, future investigations are planned to investigate the biological significance of the photoreactivity and amphiphilicity of the synechobactins in particular, as well as the other photoreactive amphiphilic marine siderophores (Fig. 1).

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