J|A|C|S
COMMUNICATIONS

Published on Web 12/27/2001

Petrobactin, a Photoreactive Siderophore Produced by the Oil-Degrading Marine Bacterium *Marinobacter hydrocarbonoclasticus*

Katherine Barbeau,[‡] Guangping Zhang,[‡] David H. Live,[†] and Alison Butler*,[‡]

Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, and Structural Biology NMR Resource, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455

Received August 7, 2001

Crude oil is one of the most important organic pollutants in the marine environment, and consequently much research has been devoted to marine oil spill remediation. Bioremediation is gaining increased acceptance as a strategy for the treatment of marine systems impacted by petroleum hydrocarbon release.[1] Siderophores, highly specific Fe(III) transport ligands produced by bacteria, may play a role in the biodegradation of petroleum hydrocarbons in marine systems by facilitating microbial acquisition of iron, a limiting nutrient. Here we report the first structural characterization of a siderophore produced by an oil-degrading marine bacterium. Petrobactin, produced by *Marinobacter hydrocarbonoclasticus*,[2] is a bis-catechol α-hydroxy acid siderophore that readily undergoes a light-mediated decarboxylation reaction when bound to Fe(III).

M. hydrocarbonoclasticus SP.17 (ATCC 49840) was aerobically cultivated in a synthetic seawater medium.[3] Siderophores were isolated and purified from the culture supernatant as previously described.[4] Electrospray ionization mass spectrometry (ESI-MS) of apo petrobactin produced a fragmentation pattern consistent with dihydroxybenzoyl, spermidinyl, and citryl residues, with a molecular ion peak $(M + H)^+$ at *m*/*z* 719 (Figure 1).

The presence of spermidinyl and citryl moieties was confirmed by acid hydrolysis followed by derivitization with methanolic HCl and pentafluoropropionic anhydride; derivitized fragments were compared to derivitized standards via GC-MS. The molecular formula of petrobactin ($C_{34}H_{50}N_6O_{11}$) was established via high-resolution fast-atom bombardment mass spectrometry: $(M + H)^+$ peak at *m*/*z* 719.3614. Structure elucidation was completed by NMR (Table 1). The connectivity of the spermidinyl, citryl, and dihydroxybenzoyl moieties was established via heteronuclear multiple bond correlation spectroscopy (HMBC): the carbonyl carbon (C7) in the 2,3-hydroxy-benzoate is coupled to H5, H6 from the benzene ring and H8, N1H (8.31 ppm) from the spermidine; the carbonyl carbon (C15) in the citrate is coupled to both the H16 from the citrate and the N3H proton (8.41 ppm) from the spermidine. The 1:1 stoichiometry of Fe(III):petrobactin was established by spectrophotometric titration at 484 nm.[5]

The presence of the citryl functionality in petrobactin suggested that the ferrated form of this siderophore might be photolabile, given that light-mediated decarboxylation reactions of α-hydroxy acids complexed to transition metal ions are well-known.[6] Fe(III)-petrobactin was in fact found to be readily photolyzed in natural sunlight, under conditions typical of near-surface ocean waters.[7] Although the primary photoproduct of Fe(III)-petrobactin photolysis had a retention time on reverse-phase HPLC similar to that of petrobactin, ESI-MS of the iron-free photoproduct indicated a molecular ion peak $(M + H)^+$ at *m*/*z* 673, a mass difference of 46 from the $(M + H)^+$ molecular ion of petrobactin. The fragmentation pattern of the photoproduct observed via ESI-MS (Figure 2) indicates that the alteration of the petrobactin molecule is localized to the citrate moiety: the presence of *m*/*z* fragments 137, 194, and 282 suggests that the dihydroxybenzoyl and spermidinyl residues remain unchanged, while the appearance of *m*/*z* fragment 392 and new molecular ion 673 (and absence of *m*/*z* fragment 438, see Figure 1) indicate that the citryl moiety is the site of decarboxylation and oxidation, accounting for the loss of 46 mass units.

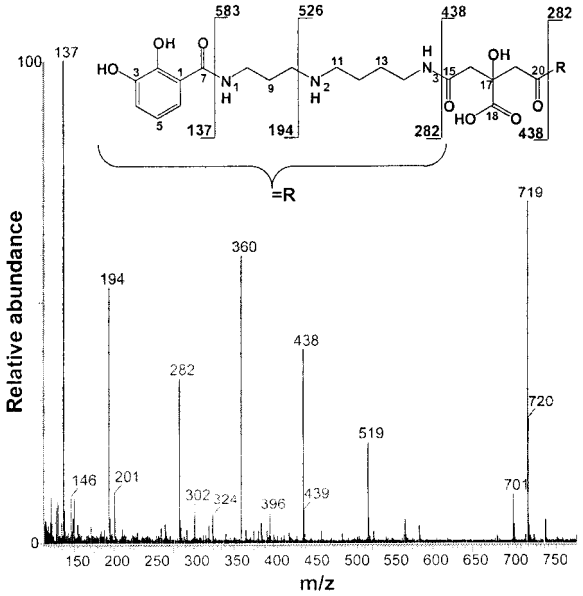


Figure 1. Electrospray ionization mass spectrum of petrobactin (positive mode, cone voltage 110 V), with molecular structure and mass fragment analysis diagram.

Photolysis of Fe(III)-petrobactin is consistent with formation of a 3-ketoglutarate residue at the former site of the citryl moiety, initiated by ligand-to-metal charge transfer of the Fe(III)-citrate complex. The photoproduct retains the ability to complex Fe(III) as evinced by its UV−visible spectrum in the presence of Fe(III), which is similar to that of Fe(III)-petrobactin (see Supporting Information). Thus, in addition to the coordination of the catecholate groups, we speculate that an enolate moiety of the 3-ketoglutarate fragment in the photoproduct could also coordinate Fe(III), given that the metal-coordinative properties of β-ketoenolates are well-known.

In summary, we have structurally characterized the first siderophore produced by a known hydrocarbon-degrading marine bacterium, *M. hydrocarbonoclasticus*. This report is one of the first to

* To whom correspondence should be addressed: butler@chem.ucsb.edu.
† University of Minnesota.
‡ University of California.

10.1021/ja0119088 CCC: $22.00

Table 1. ^{13}C and ^{1}H Resonances for Petrobactin

carbon no.	^{13}C	^{1}H [J (Hz)]	^{13}C–^{1}H long-range couplings
C1 (*C29*)	125.37		H6, H4 (*H32, H34*)
C2 (*C30*)	148.47		H4, H5, H6 (*H32, H33, H34*)
C3 (*C31*)	144.86		H4, H5, H6 (*H32, H33, H34*)
C4 (*C32*)	114.81	6.76 [2]	H5 (*H33*)
C5 (*C33*)	118.95	7.18 [2, 10]	H6 (*H34*)
C6 (*C34*)	115.07	7.28 [10]	H5 (*H33*)
C7 (*C28*)	166.65	N1H 8.31 (*N6H 8.31*)	H5, H6, H8, N1H 8.31 (*H27, H33, H34, N6H 8.31*)
C8 (*C27*)	36.15	3.27	H9, N1H 8.31 (*H26, N6H 8.31*)
C9 (*C26*)	26.23	1.80	H8 (*H27*)
C10 (*C25*)	44.77	2.90, N2H 8.41 (*2.90*)	H8, H9 (*H26, H27*)
C11 (*C24*)	46.45	2.90 (*2.90, N5H 8.41*)	H12, H13 (*H22, H23*)
C12 (*C23*)	26.03	1.43	H13 (*H22*)
C13 (*C22*)	22.98	1.55	H12 (*H23*)
C14 (*C21*)	37.69	3.04	H12, H13, N3H 8.01 (*H22, H23, N4H 8.01*)
C15 (*C20*)	169.54	N3H 8.01 (*N4H 8.01*)	H16, H19, N3H 8.01 (*H16, H19, N4H 8.01*)
C16 (*C19*)	43.32	2.50, 2.58	H19 (*H16*)
C17	73.47		H16, H19
C18	175.02		H16, H19

[a] Numbering corresponds to Figure 1. Symmetric atoms listed in italics are in parentheses. Spectra were acquired on ~10 mg petrobactin in 700 μL of d_6-DMSO, using Varian Inova 500, 600, and 800 MHz NMR spectrometers.

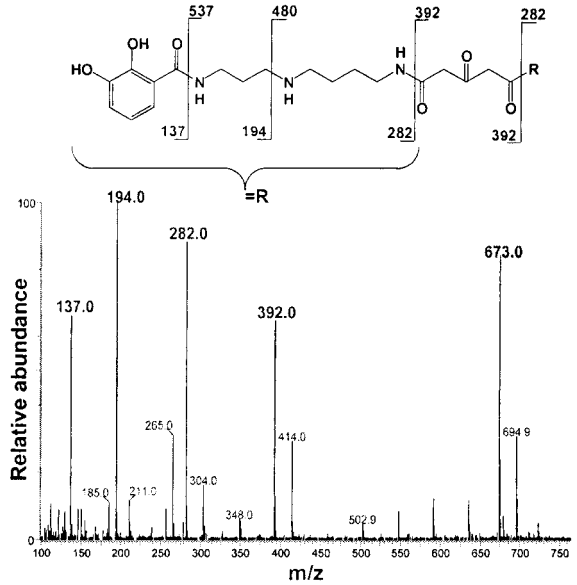
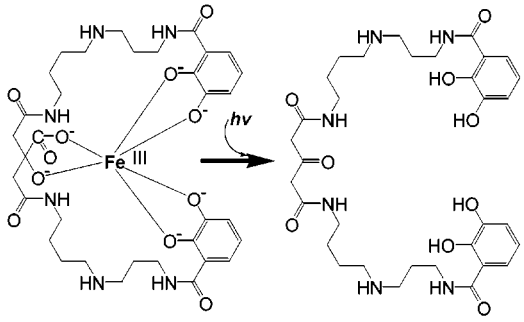


Figure 2. Electrospray ionization mass spectrum of Fe(III)-petrobactin photoproduct (positive mode, cone voltage 110 V), with molecular structure and mass fragment analysis diagram.

demonstrate photodecarboxylation of Fe(III)-siderophore complexes containing an α-hydroxy acid group.[8] When the citrate moiety of petrobactin is complexed to Fe(III), a facile photolytic ligand-to-metal charge-transfer reaction occurs that results in decarboxylation and oxidation of the petrobactin ligand (Scheme 1). The wavelength-dependent quantum yield of this photoreaction and its potential role in the siderophore-mediated uptake of Fe(III) by *M. hydrocarbonoclasticus* are currently under investigation. We are also investigating the general implications of these results for the numerous other α-hydroxy-acid-containing siderophores that have been characterized previously.[9]

Scheme 1. Schematic of Photochemical Transformation of Fe(III)-Petrobactin



Acknowledgment. Supported by NSF/DOE CHE-9810248 under the Environmental Molecular Science Institute, CEBIC (Center for Environmental BioInorganic Chemistry) (A.B.), NIH GM38130 (A.B.), and the UC President's Postdoctoral Fellowship Program (K.B.). NSF BIR-961477 (D.H.L.) is acknowledged for the purchase of the 800 MHz NMR at UMn. Thanks to James Pavlovich, Ph.D. (UCSB) for assistance with mass spectrometry.

Supporting Information Available: UV−visible spectrum of Fe(III)-petrobactin vs the Fe(III)-complexed Fe(III)-petrobactin photoproduct and extinction coefficients (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Head, I. M.; Swannell, R. P. J. *Curr. Opin. Biotechnol.* **1999**, *10*, 234. (b) Prince, R. C.; Varadaraj, R.; Fiocco, R. J.; Lessard, R. R. *Environ. Technol.* **1999**, *20*, 891.

(2) (a) Gauthier, M. J.; Lafay, B.; Christen, R.; Fernandez, L.; Acquaviva, M.; Bonin, P.; Bertrand, J.-C. *Int. J. Syst. Bacteriol.* **1992**, *42*, 568. (b) Al-Mallah, M.; Goutx, M.; Mille, G.; Bertrand, J.-C. *Oil Chem. Pollut.* **1990**, *6*, 289.

(3) Medium contained 29.3 g/L NaCl, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L NH_4Cl, 3 g/L K_2HPO_4, 0.15 g/L $CaCl_2 \cdot 2H_2O$, and 5 g/L sodium succinate; pH 7.

(4) Reid, R. T.; Butler, A. *Limnol. Oceanogr.* **1991**, *36*, 1873.

(5) Petrobactin (82 μM) in Tris buffer at pH 8 was titrated with a stock solution of Fe^{3+}.

(6) (a) Balzani, V.; Carassiti, V. *Photochemistry of Coordination Compounds*; Academic Press: London, 1970; Chapter 10. (b) Faust, B. C.; Zepp, R. G. *Environ. Sci. Technol.* **1993**, *27*, 2517.

(7) Solutions of Fe(III)-petrobactin (22 μM) in 0.2 μm filtered, UV-irradiated oligotrophic seawater were irradiated in quartz flasks in natural sunlight at pH 8, 25 °C, for a 1-day photoperiod. See also Supporting Information.

(8) Barbeau, K.; Rue, E. L.; Bruland, K. W.; Butler, A. *Nature* **2001**, *413*, 409.

(9) Albrecht-Gary, A.-M.; Crumbliss, A. L. In *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1998; Vol. 35, pp 239−327.

JA0119088