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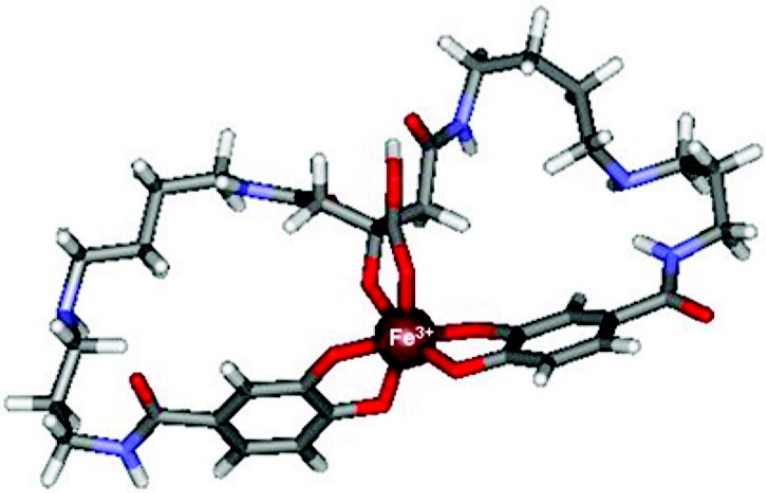
J. Am. Chem. Soc., **2008**, 130 (7), 2124-2125 • DOI: 10.1021/ja077202g



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Published on Web 01/26/2008

Petrobactin-Mediated Iron Transport in Pathogenic Bacteria: Coordination Chemistry of an Unusual 3,4-Catecholate/Citrate Siderophore[1]

Rebecca J. Abergel, Anna M. Zawadzka, and Kenneth N. Raymond*

Department of Chemistry, University of California, Berkeley, California 94720-1460

Received September 17, 2007; E-mail: raymond@socrates.berkeley.edu

Because bacterial growth and infection rely on adequate iron supply, the ability to scavenge iron in the iron-limited environment of the host is a virulence trait for pathogenic microorganisms.[2] To mediate their mandatory iron acquisition, the zoonotic causative agent of anthrax, *Bacillus anthracis*, and the human opportunistic pathogen *Bacillus cereus* both synthesize, by independent pathways, two low-molecular-weight ferric ion chelators, the hexadentate siderophores bacillibactin (BB) and petrobactin (PB) (Figure 1).[3] While BB is built on a central trilactone scaffold, linked to the common 2,3-dihydroxybenzoyl iron-chelating subunits, and exhibits a phenomenally high and selective affinity for iron ($K_f = 10^{48}$),[4] it is specifically bound by the mammalian protein siderocalin, a component of the antibacterial iron-depletion defense of the innate immune system.[5] Conversely, PB, first isolated from the marine bacterium *Marinobacter hydrocarbonoclasticus*,[6] is an unusual citrate- and 3,4-catecholate-based ligand,[7] the only natural hexadentate 3,4-catecholate siderophore observed in a pathogenic bacterium. Moreover, the full pathogenicity of *B. anthracis* in mice is observed only when PB is produced.[8] The protein siderocalin cannot accommodate 3,4-catecholate moieties within its binding pocket, precluding sequestration of PB. Hence, incorporation of such iron-binding moieties into a siderophore has been proposed as a bacterial strategy to evade the immune system.[5] However, little is known about the iron coordination chemistry of 3,4-catecholamide fragments.[9] Are the thermodynamic and kinetic properties of the ferric PB complex advantageous as compared to other more common hydroxamate and citrate siderophores?

Another notable feature of PB is the photoreactivity of its ferric complex.[6] Photolysis of the complex into the citrate ligand-to-metal charge-transfer band is reported to result in decarboxylation and oxidation of the ligand, forming a 3-ketoglutarate residue at the former site of the citryl moiety (Figure S1).[6] This process also involves reduction of the metal center;[10] in fact when 0.1 mM solutions of $[Fe^{III}(PB)]^{3-}$ and $[Ga^{III}(PB)]^{3-}$ contained in quartz cuvettes are exposed to sunlight, no spectral change is observed for $[Ga^{III}(PB)]^{3-}$ whereas the photolysis of $[Fe^{III}(PB)]^{3-}$ is characterized by significant changes (Figure S2).[11]

The incorporation of 3,4-catecholate and citrate units in PB therefore presents a challenge for a full description of the thermodynamic properties of the ligand. Indeed, while the 3,4-dihydroxybenzamide units are susceptible to oxidation and decomposition at high pH, compromising accurate pK_a determination by potentiometric methods,[12] the photoreactivity of the ferric−citrate moiety requires rigorous dark conditions for spectrophotometric competition titrations. However, the affinities of PB and its photoproduct (PB^{ν}) for Fe(III) at physiological pH were measured via competition titration against EDTA. Titrations were performed on PB samples stored in the dark over 24 h, in which the pH was fixed at 7.4 and the total concentration of ligand and Fe(III) were kept equal and constant, whereas the concentration of EDTA was varied. For PB^{ν}, a stock solution of $[Fe^{III}(PB)]^{3-}$ was first prepared and exposed to sunlight in a quartz flask for 3 h. Aliquots of the photolyzed solution were then treated with EDTA and stored on the bench over 24 h. The concentrations of free and complexed ligands were then determined by UV−vis spectroscopy (Figure 2 and S3). The proportion of transmetalation resulting from the titration of PB and PB^{ν} against EDTA is shown in Figures 2 and S3, respectively. Since the stability constants of EDTA are well-known ($pM(Fe^{III}) = 23.42$),[13] the concentration of competing ligand necessary to generate an equal partition of Fe(III) between EDTA and the ligand (log([EDTA]/[L]) when log([Fe-EDTA]/[Fe-L]) = 0) directly gives the pM of the titrated ligand at physiological pH (eq S1). The refined relative stabilities ($\Delta pM = pFe_L = pFe_{EDTA}$) are −0.45(1) and 0.94(2) for $[Fe^{III}(PB)]^{3-}$ and $[Fe^{III}(PB^{\nu})]^{2-}$, respectively, which suggests that the photoproduct of PB ($pM(Fe^{III}) = 24.4(1)$) has a higher affinity for ferric ion than the parent ligand ($pM(Fe^{III}) = 23.0(1)$) at physiological pH. These numbers are in agreement with the trend previously observed on other citrate siderophores.[14] While the pM values of PB and its photoproduct are surprisingly low (same order of magnitude as aerobactin) for catecholate compounds, they are superior to that of the iron transport protein transferrin ($pM(Fe^{III}) = 21.6$).[15]

Literature precedent has shown that *B. anthracis* and *B. cereus* use transferrin-bound iron as a source of the metal during infection, uniquely in the case of the anthrax pathogen, which demonstrates the efficacy of PB at mobilizing iron from the host-binding protein.[18,19] The kinetics of iron removal from human diferric

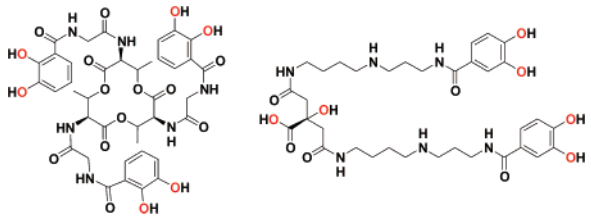

Figure 1. Bacillibactin (BB, left) and petrobactin (PB, right); the iron-coordinating atoms are indicated in red.

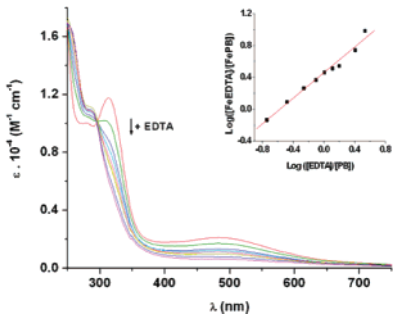

Figure 2. Spectrophotometric competition titration of PB against EDTA ($[Fe^{3+}] = [PB] = 0.1$ mM, [EDTA] from 0 to 0.5 mM, 0.1 M KCl, 0.01 M Hepes, pH 7.4, 25 °C, 1 cm cell). The inset shows the difference in $pM(Fe^{III})$ (*x* intercept).

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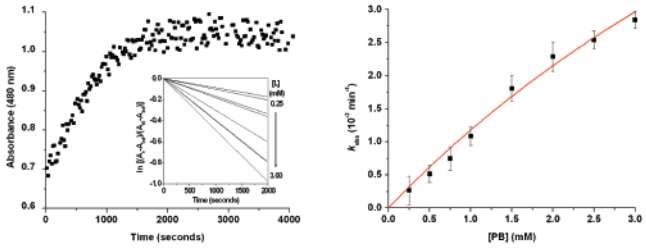

Figure 3. (Left) absorbance at 480 nm for the reaction of 0.1 mM Fe_2-Tf with 3 mM PB at 37 °C in Tris buffer; (insert) plots of $\ln[(A_t - A_\infty)/(A_{t0} - A_\infty)]$ versus time for various concentrations of PB; (right) observed rate constant of iron removal from Fe_2-Tf as a function of PB concentration.

Table 1. Siderophore Ability to Remove Iron from Fe_2-Tf

competing ligand	k_{max} (10^{-2} min^{-1})	K' (M^{-1} min^{-1})
petrobactin	12.3(7)	13(2)
enterobactin[16]	2.1	8.1
aerobactin[17]	0.3	3.4

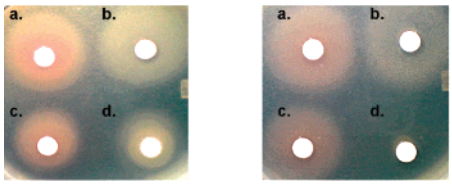

Figure 4. Growth of *B. subtilis* HB5600 (left) and HB5625 (right) under iron-limited conditions around discs infused with 20 nmoles of (a) $[Fe^{III}(PB)]^{3-}$, (b) apo-PB, (c) $[Fe^{III}(PB^{\nu})]^{2-}$, and (d) apo-BB.

transferrin (Fe_2-Tf) by PB were followed at 37 °C by monitoring the UV−vis spectral changes upon addition of the ligand into a solution of Fe_2-Tf in Tris buffer at physiological pH. The band maximum at 480 nm corresponding to $[Fe^{III}(PB)]^{3-}$ shifts as the complex is formed (Figure 3). Pseudo-first-order rate constants, k_{obs}, were calculated from nonlinear least-square fits of the absorbance versus time (eq S2). A hyperbolic relationship between k_{obs} and the ligand concentration is observed, indicating saturation behavior with respect to the concentration of PB. As the rate reaches a plateau as a function of ligand saturation, k_{obs} is equal to the maximum rate constant k_{max}; on the other hand when the ligand concentration is small, the rate is first order in ligand concentration and k_{obs} is equal to the second-order rate constant k' (Figure 3, eq S3). Fitting of the data provided k_{max} and k' values, as reported in Table 1. Comparison of the rate constants obtained for PB to those reported for the 2,3-catecholate siderophore archetype enterobactin[16] and the citrate/di-hydroxamate aerobactin[17] shows that 3,4-catecholate units can remove iron from Fe_2-Tf faster than both 2,3-catecholate and hydroxamate iron-binding moieties.

Siderophore disc diffusion assays were performed with *Bacillus subtilis* HB5600 (*dhbA*::*spc*), a strain that cannot produce siderophores,[20] showing that both PB and its photoproduct PB^{ν} can be used as xeno-siderophores by the soil organism *B. subtilis* and promote its growth under iron-depleted conditions (Figure 4). In addition, deletion of the BB receptor *feuA* in *B. subtilis* HB5625 (*feuA*::*spc dhbA*::*mls*) did not affect PB- and PB^{ν}-mediated iron uptake. Thus, *B. subtilis* possesses a separate uptake system for the ferric complex of PB and its photoproduct that has not been reported previously as such.[20] Correspondingly, we speculate that *B. anthracis* and *B. cereus* can acquire both $[Fe^{III}(PB)]^{3-}$ and $[Fe^{III}(PB^{\nu})]^{2-}$, which raises the question of whether the incorporation of a photoreactive citrate scaffold in PB plays a role in the life cycle of pathogenic *Bacilli* as it does for pelagic marine bacteria[10] and whether both ferric complexes are recognized and transported by the same proteins.

Production of the unusual 3,4-catecholate/citrate siderophore PB is a multipronged bacterial strategy to scavenge iron during infection. While insertion of 3,4-catecholamide subunits in the siderophore structure does not enhance its affinity for ferric ion as compared to corresponding hydroxamates, it promotes catalysis of iron removal from transferrin and evasion from siderocalin binding. The function of the photoreactivity of ferric PB as well as the corresponding uptake system remains unexplored in *B. anthracis* and *B. cereus*; however, our study shows that nonpathogenic *Bacillus* spp. can use exogenous PB, independent of light exposition. The siderophore pair BB/PB may therefore be to *Bacilli* organisms what the duo enterobactin/aerobactin is to enteric bacteria.

Acknowledgment. This work was supported by the National Institutes of Health (Grant AI11744). We thank Prof. Rowe Byers, Prof. Raymond Bergeron, and Prof. John Helmann for kindly providing us with samples of crude bacterial and synthetic petrobactin and bacterial strains, respectively.

Supporting Information Available: Experimental procedures for photolysis, spectrophotometric titrations, kinetics, and growth assays; additional spectral and titration data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Paper #82 in the series *Coordination Chemistry of Microbial Iron Transport* (for the previous paper, see ref 5).
(2) Byers, B. R.; Arceneaux, J. E. L. In *Iron Transport and Storage in Microorganisms, Plants, and Animals*; Sigel, A., Sigel, H., Eds.; Dekker, M.: New York, Vol. 1998; *35*, p 37−66.
(3) Wilson, M. K.; Abergel, R. J.; Raymond, K. N.; Arceneaux, J. E. L.; Byers, B. R. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 320−325.
(4) Dertz, E. A.; Xu, J.; Stintzi, A.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 22−23.
(5) Abergel, R. J.; Wilson, M. K.; Arceneaux, J. E. L.; Hoette, T. M.; Strong, R. K.; Byers, B. R.; Raymond, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 18499−18503.
(6) Barbeau, K.; Zhang, G. P.; Live, D. H.; Butler, A. *J. Am. Chem. Soc.* **2002**, *124*, 378−379.
(7) Bergeron, R. J.; Huang, G. F.; Smith, R. E.; Bharti, N.; McManis, J. S.; Butler, A. *Tetrahedron* **2003**, *59*, 2007−2014.
(8) Cendrowski, S.; MacArthur, W.; Hanna, P. C. *Mol. Microbiol.* **2004**, *51*, 407−417.
(9) El Hage Chahine, J. M.; Bauer, A. M.; Baraldo, K.; Lion, C.; Ramiandraosa, F.; Kunesch, G. *Eur. J. Inorg. Chem.* **2001**, *2001*, 2287−2296.
(10) Barbeau, K.; Rue, E. L.; Bruland, K. W.; Butler, A. *Nature* **2001**, *413*, 409−413.
(11) The charge of each metal complex was assigned based on the complexation reaction and its concomitant protonation steps only.
(12) Harris, W. R.; Amin, S. A.; Kupper, F. C.; Green, D. H.; Carrano, C. J. *J. Am. Chem. Soc.* **2007**, *129*, 12263−12271.
(13) Smith, R. M.; Martell, A. E. Critical Stability Constants; Plenum: New York 1977; Vol. 1−4.
(14) Kupper, F. C.; Carrano, C. J.; Kuhn, J.; Butler, A. *Inorg. Chem.* **2006**, *45*, 6028−6033.
(15) Raymond, K. N.; Bryan, B. L. In *Bioinorganic Chemistry An Inorganic Perspective of Life*; Kessissoglou, D. P., Ed.; Kluwer Academic Publishers: The Netherlands; 1995; Vol. 459, p 13−24.
(16) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097−6104.
(17) Konopka, K.; Bindereif, A.; Neilands, J. B. *Biochemistry* **1982**, *21*, 6503−6508.
(18) Garner, B. L.; Arceneaux, J. E. L.; Byers, B. R. *Curr. Microbiol.* **2004**, *49*, 89−94.
(19) Park, R. Y.; Choi, M. H.; Sun, H. Y.; Shin, S. H. *Biol. Pharm. Bull.* **2005**, *28*, 1132−1135.
(20) Ollinger, J.; Song, K.; Antelmann, H.; Hecker, M.; Helmann, J. D. *J. Bacteriol.* **2006**, *188*, 3664−3673.

JA077202G