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Magnetic susceptibility of Mn(III) complexes of hydroxamate siderophores

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ABSTRACT

The hydroxamate siderophores putrebactin, desferrioxamine B, and desferrioxamine E bind Mn(II) and promote the air oxidation of Mn(II) to Mn(III) at pH > 7.1. The magnetic susceptibility of the manganese complexes were determined by the Evans method and the stoichiometry was probed with electrospray ionization mass spectrometry (ESIMS). The room temperature magnetic moments (μ_{eff}) for the manganese complexes of desferrioxamines B and E were 4.85 BM and 4.84 BM, respectively, consistent with a high spin, d^4, Mn(III) electronic configuration. The manganese complex of putrebactin had a magnetic moment of 4.98 BM, consistent with incomplete oxidation of Mn(II), as confirmed by X band EPR spectroscopy. Mass spectra of the Mn(III) desferrioxamine B and E complexes showed complexes at m/z 613.26 and 653.26, respectively, consistent with 1:1 complexation. Mass spectral peaks for manganese putrebactin at m/z 797.31 and 1221.41 corresponds to 1:2 and 2:3 Mn:putrebactin complexation. This study directly confirms the Mn(III) oxidation state in hydroxamate siderophore complexes.

1. Introduction

Siderophores are low molecular weight chelating agents produced by microbes in low iron environments to facilitate iron uptake [1]. In addition to Fe(III), siderophores have been shown to chelate other hard metal ions, such as Al(III) [2], Ga(III) [2], Mo(VI) [3], Ti(IV) [4], and V(V) [3,5,6], among others. Manganese is of particular interest because it is often found in the environment in oxide minerals. In a similar fashion to siderophore-mediated extraction of Fe(III) from iron oxide minerals, siderophores have been shown to dissolve manganite (γ-MnOOH) and hausmannite (Mn_3O_4) through pH-dependent processes [7–9]. Thus siderophores may be involved in biogenic cycling of manganese [10–12]. Mn(III) is not stable in aqueous solutions and readily disproportionates to Mn(II) and a variety of Mn(IV) oxides and hydroxides [13]. However Mn(III) can form stable complexes in aqueous solutions with oxygen donating ligands, including hydroxamates [14,15].

Mn(III) complexes of the linear and cyclic tris-hydroxamate siderophores, desferrioxamine B (DFOB) and desferrioxamine E (DFOE), respectively, were first investigated as mimics of superoxide dismutase [16–18]. Upon complexation of Mn(II) by DFOB or DFOE, air oxidation of Mn(II) under basic conditions (pH 8.5) produced a clear green solution [19], similar to the complex formed on addition of $Mn(OH)_3$ to DFOB and DFOE, as well as on addition of MnO_2 to DFOB and DFOE in the presence of a reductant [16]. Duckworth and Sposito found that the rate of oxidation of Mn(II)-DFOB by O_2 is fastest in the fully deprotonated complex [7,19]. They also found that the proton-independent stability constant of Mn(III)-DFOB is remarkably high, at log*K* 29.9 (±0.5) [19], and approaches that of Fe(III)-DFOB at log*K* 30.5 [20].

To further characterize the Mn(III) complexes of hydroxamate siderophores, we have measured the magnetic susceptibility of the air-oxidized Mn(II)-siderophore complexes to directly probe the Mn(III) oxidation state in aqueous solution, using the Evans NMR technique [21–23]. EPR spectroscopy was also employed to detect if any Mn(II) remained. We selected three structural hydroxamate archetypes for this study: the linear and cyclic *tris*-hydroxamate siderophores, DFOB, DFOE, and the cyclic *bis*-hydroxamate siderophore, putrebactin (Fig. 1). Comparison of the *tris*-hydroxamate to *bis*-hydroxamate siderophores is of particular interest due to differences expected in the stoichiometry of coordination, analogous to the Fe(III)-siderophore complexes [24–27]. Possible coordination complexes of Mn(III) and putrebactin could include the neutral bridged 2:3 Mn-putrebactin or 1:2 Mn-putrebactin complexes shown in Fig. 2, as well as multiple 1:1 Mn-putrebactin aqua or hydroxo complexes, or possibly 2:2 Mn-putrebactin μ-oxo or μ-hydroxo dimers (Fig. 2).

2. Experimental

2.1. General experimental procedures

All UV–visible (UV–Vis) spectrophotometry was carried out on a Varian Cary-Bio 300 UV–Vis spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity Inova 400 MHz spectrometer (5 mm broad band probe) at room temperature. Mass spectra were obtained using electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) on a Micro Mass QTOF-2 mass spectrometer

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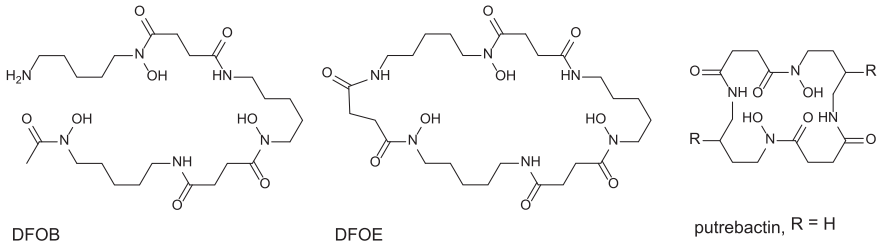

Fig. 1. Desferrioxamine B (DFOB), desferrioxamine E (DFOE) and putrebactin.

(Waters Corp.), using argon as the collision gas. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), measurements were taken on a Thermo iCAP 6300, and calibrated from dilutions of 1000 ppm Mn(II) or Fe(III) standard solutions (Fisher), using class A volumetric glassware. X-Band EPR spectra were obtained using a Bruker EMXplus Spectrometer with an aqueous flat cell.

2.2. Siderophores: DFOB, DFOE and putrebactin

DFOB mesylate was purchased from Sigma-Aldrich and used as obtained.

2.2.1. Isolation of putrebactin

Putrebactin was isolated from cultures of *Shewanella oneidensis* MR-1 [26]. *S. oneidensis* MR-1 was maintained on Difco 2216 marine broth plates, and cultured in an artificial sea water medium (ASG + Fe), containing 2 •L doubly deionized water, 20 g Bacto CAS amino acids, 2 g NH_4Cl, 2 g glycerol phosphate, 24.7 g $MgSO_47H_2O$, 2.9 g $CaCl_2•H_2O$, 33.1 g NaCl, 1.5 g KCl, 6 mL glycerol, and 2 μM (final concentration) of $FeCl_3$. Prior to inoculation, 20 mL of filter-sterilized 1.0 M HEPES buffer (pH 7.4), 4 mL of filter-sterilized 1.0 M $NaHCO_3$ buffer, and 20 mL filter-sterilized vitamin stock were added to the medium. Vitamin stock consisted of 4 mg niacin, 2 mg thiamin, 4 mg *p*-aminobenzoic acid, 2 mg calcium pantothenic acid, 20 mg pyridoxine HCl, 2 mg cyanocobalamin, 4 mg riboflavin, and 4 mg folic acid in 200 mL doubly deionized water.

2.2.2. Isolation of DFOE

DFOE was obtained from an uncharacterized bacterium isolated from seawater obtained from the Gulf of Mexico after the Deepwater Horizon oil spill in 2010; the bacterium was maintained on minimal nutrient seawater agar plates consisting of 0.5 g Bacto yeast extract, 5 g Bacto peptone, 15 g Bacto agar in 1 L filtered seawater. The bacterium was cultured in a minimal nutrient artificial seawater medium (ASW-Fe) composed of 30 g NaCl, 1.5 g KCl, 6 g $CaCl_2•2H_2O$, 24 g $MgSO_4•7H_2O$, 2 g NH_4Cl, 0.2 g glycerol phosphate, 4 g Bacto CAS amino acids, and 6 mL glycerol in 2 L doubly deionized water (Barnstead Nanopure II). Prior to inoculation, 20 mL of filter-sterilized 1.0 M HEPES buffer (pH 7.4), and 4 mL of filter-sterilized 1.0 M $NaHCO_3$ buffer were added to the medium.

For siderophore isolation, each bacterial strain was grown for 24–48 h on an orbital shaker (180 rpm) and harvested via centrifugation. Purification of putrebactin and DFOE followed the same general procedure. Amberlite XAD-2 resin (Supelco) was combined with the cell free culture supernatant (100 g/L) and shaken for 2 h (120 rpm). Supernatant was then filtered away and the XAD-2 resin was rinsed with doubly deionized water (1 L/100 g), prior to packing the XAD-2 resin into a glass chromatography column (2 cm internal diameter). Siderophores were eluted in 100% methanol. The eluent was lyophilized, and stored at stored at −20 °C.

In the isolation procedure for putrebactin, the resulting yellow brown powder was rinsed approximately five times with ca. 10 mL doubly deionized water until the solid became white. The white solid was re-dissolved in 300 mL MeOH with gentle heating, at which point 25 mL of H_2O was added, followed by rotary evaporation to remove MeOH. Final purification via RP-HPLC was performed on a C_4 preparatory column (10 μm particle size, 250 mm column length, ×20 mm i.d., Higgins Analytical). A gradient of 100% H_2O (doubly deionized) with 0.05% trifluoric acetic acid (TFA) to 35% MeOH (0.05% TFA) over 30 min was used for putrebactin, and a 100% H_2O to 80% MeOH gradient (0.05% TFA) over 40 min was used for DFOE. The eluent was monitored continuously at 215 nm and putrebactin-containing fractions, as confirmed by mass spectrometry, were pooled, lyophilized and stored at −20 °C.

To analyze for adventitious iron contamination in the siderophore samples, the siderophore ligands (final concentration 0.1 mM) were dissolved in 1 mL of nitric acid (70%) and diluted into 100 mL in doubly de-ionized water. The ICP-AES spectrometer was calibrated in the linear range using a blank of 0.7% nitric acid and a 0.1 ppm Fe solution in 0.7% nitric acid prepared by dilution of a 1000 ppm Fe(III) standard (Fisher). Each sample was run in triplicate with an 80 s exposure time.

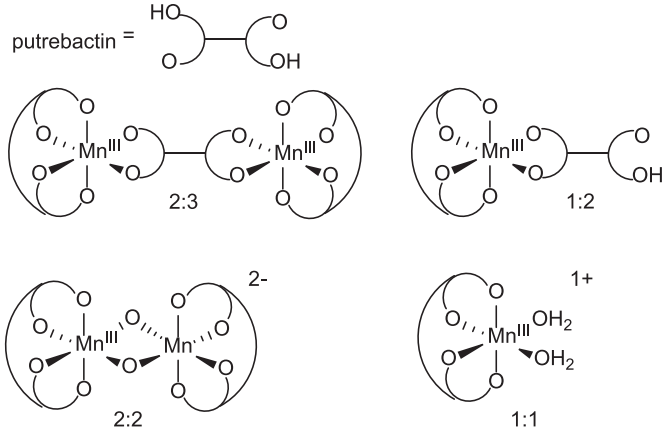

Fig. 2. Possible coordination modes of Mn(III) putrebactin complexes.

2.3. Formation of Mn(III)-siderophore complexes

A stock solution of 10 mM Mn(II) was prepared from $MnCl_2•4H_2O$ in D_2O (99.9%) and standardized by ICP-AES. Mn(II) (1 mM final concentration) was reacted in air with 2 mM DFOB or DFOE and 3 mM putrebactin, in water at pH 8–10 (or up to pH 9.5 for putrebactin) for 24 h. To adjust the pH, ~1 M NaOD or DCl was added, as needed. The NaOD and DCl solutions were prepared by dissolving solid NaOH and concentrated HCl (36%) into D_2O (99.9%). Solubilization of putrebactin in water required addition of NaOD to maintain the pH above 7.1. A 6 in. micro combination electrode (Accumet) with a calomel reference was used to monitor the pH of the solutions. The oxidation of Mn(II) to Mn(III) was followed at 310 nm which is in the range of the λ_{max} for the Mn(III) siderophore complexes. Solutions were filtered for EPR spectroscopic analysis and magnetic susceptibility measurements. The solutions for EPR spectroscopy and UV–Vis analysis were buffered by