Metal Oxyhydroxide Dissolution as Promoted by Structurally Diverse Siderophores and Oxalate

by

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Submitted in revised form to
Geochimica et Cosmochimica Acta
June 17, 2014

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Abstract. Siderophores, a class of biogenic ligands with high affinities for Fe(III), promote the dissolution of metal ions from sparingly soluble mineral phases. However, most geochemical studies have focused on quantifying the reactivity of DFOB, a model trishydroxamate siderophore. This study utilized three different siderophores, desferrioxamine B, rhizoferrin, and protochelin, with structures that contain the most commonly observed binding moieties of microbial siderophores to examine the siderophore-promoted dissolution rates of FeOOH, CoOOH, and MnOOH in the absence and presence of the ubiquitous low molecular mass organic acid oxalate by utilizing batch dissolution experiments at pH = 5–9. Metal-siderophore complex and total dissolved metal concentrations were monitored for durations of one hour to fourteen days, depending on the metal oxyhydroxide identity and solution pH. The results demonstrate that MnOOH and CoOOH generally dissolve more quickly in the presence of siderophores than FeOOH. Whereas FeOOH dissolved exclusively by a ligand-promoted dissolution mechanism, MnOOH and CoOOH dissolved predominantly by a reductive dissolution mechanism under most experimental conditions. For FeOOH, siderophore-promoted dissolution rates trended with the stability constant of the corresponding aqueous Fe(III) complex. In the presence of oxalate, measured siderophore-promoted dissolution rates were found to increase, decrease, or remain unchanged as compared to the observed rates in single-ligand systems, depending on the pH of the system, the siderophore present, and the identity of the metal oxyhydroxide. Increases in observed dissolution rates in the presence of oxalate were generally greater for FeOOH than for MnOOH or CoOOH. These results elucidate potential dissolution mechanisms of both ferric and non-ferric oxyhydroxide minerals by siderophores in the environment, and may provide further insights into the biological strategies of metal acquisition facilitated by coordinated exudation of low molecular weight organic acids and siderophores.

1. Introduction

All organisms require transition metals, such as Co, Mn, and Fe, for use in the metalloproteins and enzymes that are necessary for proper metabolic function (Frausto da Silva and Williams, 1991). Under oxic conditions at circumneutral pH, these metals tend to be entrained in insoluble oxide and hydroxide minerals that are not readily available for biological uptake (Adriano, 2001). To overcome the potential limits on organism nutrition caused by the low bioavailability of iron and possibly other metals, graminaceous plants, bacteria, and fungi exude organic molecules, such as low molecular mass organic acids and siderophores, that promote the dissolution, solubilization, and uptake of nutrient metals (Kraemer, 2004; Marschner et al., 1986; Winkelmann, 2007).

Siderophores (derived from Greek meaning “iron carrier”) are specialized chelating agents exuded by microorganisms during periods of iron stress to enhance iron bioavailability. Currently, about 500 different types of siderophores have been discovered (Hider and Kong, 2010). Despite this structural diversity, microbial siderophores are typically characterized as tetra- or hexa-dentate ligands with oxygen-bearing moieties (commonly hydroxamate, catecholate, salicylate, α -hydroxycarboxylate, or carboxylate) that directly bind a central metal ion [viz. Fe(III)] (Crumbliss and Harrington, 2009). These configurations result in thermodynamically stable, soluble complexes that can be taken up via specific cellular receptors (Harrington and Crumbliss, 2009; Stintzi et al., 2000). Although most geochemical studies to date have utilized the trishydroxamate siderophore desferrioxamine B (DFOB), it is reasonable to assume that siderophores may display a range of reactivity that will be largely dictated by the nature of the ligand moieties that interact directly with the metal center.

An important proposed role of siderophores in soils is promoting the dissolution of iron from low solubility minerals. Desferrioxamine B promotes iron dissolution from a number of different phases, including oxides, hydroxides, phosphates, sulfides, and clay minerals (Cervini-Silva et al., 2012; Cervini-Silva and Sposito, 2002; Cornejo-Garrido et al., 2008; Haack et al., 2008; Liermann et al., 2005; Liermann et al., 2000; Rosenberg and Maurice, 2003; Watteau and Berthelin, 1994; Wolff-Boenisch and Traina, 2007a). The DFOB-promoted dissolution of FeOOH has been shown to proceed by a surface-controlled mechanism, resulting in the formation of a Fe(III)HDFOB⁺ complex (Cocozza et al., 2002). Interestingly, in the presence of DFOB and low molecular mass organic acids (such as oxalate), the dissolution rate of goethite is enhanced beyond a simple combination of the single-component dissolution rates (Cervini-Silva and Sposito, 2002; Cheah et al., 2003; Wolff-Boenisch and Traina, 2007b). This “synergistic” effect results from oxalate acting as an iron shuttle that delivers Fe(III) to dissolved siderophores, with rate of detachment of a labile pool of Fe(III)-oxalate surface complexes enhanced by the presence of the siderophore (Loring et al., 2008; Reichard et al., 2007a; Reichard et al., 2007b).

Recent studies have suggested that siderophores may play diverse roles in the environment beyond promoting Fe(III) solubilization and uptake (Duckworth et al., 2009a; Duckworth et al., 2009b; Kraepiel et al., 2009; Schalk et al., 2011). Despite the assumed high specificity of siderophores for Fe(III), strong interactions between DFOB and other metals have been recognized for over 50 years (Anderegg et al., 1963). In fact, metal ions such as Mn(III) and Co(III) may have stability constants with siderophores that rival or exceed those of Fe (Duckworth et al., 2009b; Duckworth and Sposito, 2005a; Harrington et al., 2012a; Harrington et al., 2012b; Parker et al., 2004). Interestingly, in the small number of studies that have probed the siderophore-promoted dissolution rates of Mn- and Co-bearing minerals, both reductive and

ligand-promoted dissolution mechanisms have been observed (Bi et al., 2010; Duckworth and Sposito, 2005b; Duckworth and Sposito, 2007; Peña et al., 2007). Whether a reductive or ligand-promoted dissolution mechanism predominates may be controlled by the electrode potential of the mineral (Bi et al., 2010).

The interactions between DFOB and Fe(III)-bearing minerals have been well studied, but only a limited number of studies exist that have explored mineral dissolution rates and mechanisms as promoted by other siderophores (Cervini-Silva, 2008; Dehner et al., 2010; Haack et al., 2008; Hersman et al., 1995; Reichard et al., 2005; Wolff-Boenisch and Traina, 2007b), a fact that limits our understanding of the function of siderophores in soils and natural waters (Kraemer, 2004). In addition, to our knowledge there have been no published studies that examine the interactions of siderophores other than DFOB with Mn(III) and Co(III)-bearing minerals. The goals of this study were to quantify and compare the dissolution rates of FeOOH, MnOOH, and CoOOH as promoted by several structurally diverse microbial siderophores, including DFOB, rhizoferrin, and protochelin (Fig. 1), in the absence and presence of oxalic acid. The results were then discussed in terms of the possible fate of siderophores in the environment and their role in the biological uptake of metals.

2. Materials and Methods

2.1. Materials

All solutions were prepared in ASTM Type I deionized water (DI; resistivity > 18 M Ω cm). Unless otherwise noted, all chemicals were obtained from Fisher Scientific or VWR international and were of reagent or ACS certified grade.

The three siderophores examined in this study, which were chosen for their structural diversity (Fig. 1), are all produced by soil-based bacteria (Bickel et al., 1960; Cornish and Page, 1995; Münzinger et al., 1999). The mesylate salt of the trishydroxamate siderophore DFOB was purchased from Sigma Aldrich and used without any further purification. Both rhizoferrin (Rhiz; α -hydroxycarboxylate siderophore) and protochelin (Proto; a triscatecholamide siderophore) were synthesized by the Duke University Small Molecules Custom Synthesis Facility. The rhizoferrin sample used in this study is a racemic mixture of both naturally occurring enantiomers (Drechsel et al., 1992; Münzinger et al., 1999).

The Fe and Mn oxyhydroxides that were used in this study were chosen to represent common oxide and hydroxide minerals. Heterogenite (β -CoOOH), although not environmentally common, was chosen as a model for Co found in solid solution in oxyhydroxide minerals and to facilitate comparison with previous studies (Bi et al., 2010). Goethite (α -FeOOH) was synthesized by reacting 1 M $\text{Fe}(\text{NO}_3)_3$ solution with 5 M KOH solution in a 1 L Erlenmeyer flask (Cornell and Schwertmann, 1996; Schwertmann and Cornell, 2000). After ageing the resulting precipitate for 6 hours, the mixture was centrifuged and washed with DI water five times. The solution was filtered and the solid residue lyophilized until dry (ca. 48 hrs.). The dry solid was disaggregated with a mortar and pestle and stored in a freezer until use.

Manganite (γ -MnOOH) was synthesized according to literature methods (Duckworth and Sposito, 2005b; Giovanoli and Luenberger, 1969; McArdell et al., 1998). Solutions of 0.2 M NH_4OH , 0.06 M MnSO_4 , and 30% H_2O_2 were each purged for 1 h with N_2 . A 20.4 mL aliquot of N_2 -purged 30% H_2O_2 was added to 1 L of continuously stirred MnSO_4 solution. After stirring for 15 minutes, 300 mL of N_2 -purged NH_4OH solution was slowly added to the 2 L flask with vigorous stirring, resulting in the formation of a dark-brown precipitate. The suspension was

rapidly heated to the boiling point, and then maintained at 98 °C under reflux with constant stirring for 6 h. The resulting mixture was then filtered while hot and washed with 500 mL of hot DI water during filtration. The residue was then cold-washed by suspension and sonication in deionized water for 15 min, followed by centrifugation for 15 min at $\sim 25000 \times g$ RCF. The washing process was repeated five times. The washed solid was then freeze dried for 48 hrs, after which it was disaggregated with a mortar and pestle, and stored in a freezer until use.

The CoOOH utilized in this study is the same sample used by Bi et al. (2010), and its synthesis and properties have previously been described.

2.2. Characterization of Oxyhydroxide Minerals

Dried Fe(III) and Mn(III) oxyhydroxide minerals were characterized to determine morphology, phase, and specific surface area. Samples for transmission electron microscopy (TEM) analysis were prepared by dispersing solid particles in methanol. A drop of suspension was placed onto the surface of a carbon film coated Cu grid (Ted Pella Scientific). After drying, TEM micrographs of FeOOH and MnOOH were collected on a Hitachi HF-2000 transmission electron microscope (TEM). Both minerals exhibited particles with a uniform needle-like shape with average lengths of approximately 700 nm and 400 nm, respectively (Fig. S1). Powder X-ray diffraction (XRD) patterns of solid samples were collected from 15-95° 2 θ with a Rigaku SmartLab X-ray diffractometer equipped with a Co-K α source, which confirmed the mineral phase of synthesized solids as goethite and manganite (Fig. S1). Brunauer–Emmett–Teller (BET) surface area analyses of the three minerals determined by nitrogen adsorption using an Autosorb-1-MP surface pore analyzer (Quantachrome Corp.) measured 69 and 49 m² g for FeOOH and

MnOOH, respectively. Bi et al. (2010) reported a BET surface area of 59.8 m² g for the CoOOH used in this study.

2.3. Dissolution Experiments

Dissolution rates and products of metal oxyhydroxides were investigated at pH = 5–9 in the absence and presence of siderophores. Batch experiments were utilized to conserve siderophore samples. To maintain a constant pH for each experiment, 10 mM buffer solutions of acetate (Ac), 2-(*N*-morpholino)ethanesulfonic acid (MES) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Arcos Organic), were utilized for pH 5, 6, and 7–9, respectively. These buffers have previously been shown not to affect DFOB-promoted dissolution of metal oxyhydroxides (Bi et al., 2010; Cheah et al., 2003; Duckworth and Sposito, 2005b); however, no specific effort was made in this study to determine their effect on siderophore-promoted dissolution rates. A solution of 0.1 M NaCl electrolyte solution with 10 mM buffer was prepared and an aliquot (50 mL) was pipetted into an Erlenmeyer flask to which a known mass of siderophore was added (final concentration = 100 μM). For protochelin, this solution was briefly sonicated to help to dissolve the siderophore. Next, 0.03 g of metal oxyhydroxide was added to 100 mL of buffer and electrolyte solution, and sonicated for approximately 15 min to disperse particles. The flask was then wrapped in aluminum foil to exclude stray light, placed into a temperature-controlled water bath maintained at 25°C with constant stirring. After allowing the reaction temperature to equilibrate, the solution containing buffer, electrolyte, and siderophore was added to the mineral suspension. For FeOOH, a 2 mL aliquot was collected using a syringe and immediately filtered using a 0.2 μm nitrocellulose filter (Millipore). Subsequent aliquots were taken at 12 hour intervals over a 14 day period. For

MnOOH, the aliquots were collected periodically over 1 hr, whereas samples for CoOOH were collected at 15 min intervals up to three hours. At each solution pH, three replicates were conducted for experiments containing DFOB, and two replicates were performed for experiments containing protochelin, rhizoferrin, or no siderophore (control experiments). A biocide (e.g., azide) was not added to discourage microbial growth, but the formation of biofilms was not observed in any of the reaction vessels. Drift in the measured solution pH was found to be less than 0.4 units in all cases.

The dissolution rates of each metal oxyhydroxide as promoted by siderophores in the presence of oxalate were also determined by using a batch reactor approach. Experiments were conducted by the addition of oxalate to a mineral suspension followed by spiking of the system with a siderophore solution (Reichard et al., 2005). Because the effect of oxalate on FeOOH dissolution rate in the presence of siderophores has been shown to be dependent not only on the concentration of oxalate, but also on the initial duration of exposure to oxalate (Reichard et al., 2007b), the durations of the experiments with different minerals were determined by their relative dissolution rates in the absence of oxalate. Stock solutions of DFOB, rhizoferrin, protochelin, and oxalate were prepared immediately prior to the initiation of the experiments. A solution containing 0.1 M NaCl electrolyte and 10 mM buffer was prepared. Metal oxyhydroxide (0.03 g) was added to a volume of the buffered electrolyte solution, and the solution was sonicated for 15 minutes. The flask was then wrapped with aluminum foil and placed into a temperature-controlled water bath maintained at 25°C with constant stirring. After allowing the reaction temperature to equilibrate, the buffer and electrolyte solution was spiked with oxalate stock solution, resulting in a final oxalate concentration of 1 mM. For FeOOH dissolution experiments containing oxalate, aliquots were collected at $t = 0$ and every 24 hours for 96 hours.

The remaining mineral suspension was spiked with siderophore stock solution to produce a total concentration of 100 μM . Following siderophore addition, sample aliquots were collected at 8 hour intervals for up to 12 days. For MnOOH dissolution experiments containing oxalate, aliquots were collected every five minutes starting a half hour prior to spiking with each siderophore stock solution to provide a final concentration of approximately 100 μM siderophore. After spiking, aliquots were collected at 5 min intervals for 1 h. For CoOOH dissolution experiments containing oxalate, aliquots were collected every 15 min for an hour prior to spiking with the siderophore stock solution to provide a final concentration of 100 μM ; after which, aliquots were collected at 15 minute intervals for 3 h. All aliquots were immediately filtered through a 0.2 μm nitrocellulose filters. Two replicates were conducted for each experimental condition and experimental control reactions were replicated similarly with oxalate in the absence of siderophores.

2.4. Determination of Dissolution Products and Rates

The pH of each aliquot was measured with a Fisher Scientific XL 25 pH meter. The siderophore-metal complex concentration was measured immediately after sampling by UV-visible spectroscopy. Spectra were measured using an Ocean Optics Jaz UV-visible spectrophotometer to determine the complex concentration. The Beer-Lambert extinction coefficients for each M(III) -siderophore complex that was detected in our experiments is listed in Table S1. The subsample was returned to the original aliquot and stored in the dark at 4°C for total dissolved metal analysis. Because of the possible redox instability of Mn(III) - and Co(III) -siderophore complexes (Harrington et al., 2012a), spectral measurements were performed within 60 min of the completion of experiments for all dissolution experiments. Quantification of

Fe(III)-siderophore complexes, which are generally more redox stable than Mn(III)- and Co(III)-siderophore complexes, was conducted on a daily basis after sampling. In experiments containing FeOOH and protochelin at pH = 8–9, the Fe(III)-protochelin complex was not quantified because a brown colloid associated with the degradation of dissolved protochelin at alkaline pH (Harrington et al., 2012a) interfered with the spectrophotometric measurement.

The samples were acidified with trace metal grade HCl and diluted to bring sample concentrations within linear range of a Thermo Scientific iCE 3300 flame atomic absorption spectrometer (FAAS). The total dissolved metal concentration was quantified by FAAS to determine trends in dissolved metal concentration as a function of time.

2.5. Determination of Dissolution Rates

In the presence of siderophores and the absence of oxalate, dissolution of metal oxyhydroxides may proceed by proton-promoted (R_H), ligand-promoted (R_L), or reductive dissolution pathways (R_R), all of which may occur simultaneously. As such, the total dissolution rate (R_T) is the sum the rate of dissolution from each pathway:

$$R_T = R_H + R_R + R_L \quad (1)$$

All three trivalent metals are thought to be insoluble or unstable (with respect to other redox states) over the pH range of the experiments when not complexed by high-affinity ligands. Additionally, control experiments conducted in the absence of siderophores demonstrate that the proton promoted dissolution rates for MnOOH, CoOOH, and FeOOH were negligible at all pH values ($R_H \approx 0$; data not shown). Hence, dissolution in the presence of siderophores (Sid) is assumed to occur only by reduction of the metal ion center (reductive dissolution, R_R , eq. 2) or

by formation of a complex where the oxidation state of the metal center is retained (ligand-promoted dissolution, R_L , eq. 3):

where reductive dissolution results in concomitant oxidation of the siderophore (Sid_{ox}) (Duckworth and Sposito, 2005b). We note that it is also possible for surface redox reactions to result in metal reduction, followed by rapid reoxidation and complexation of the dissolved metal (Dhungana et al., 2007; Simanova et al., 2010). Because we only measure the formation of complexes spectrophotometrically in solution, we are unable to distinguish this potential dissolution pathway from ligand-promoted dissolution.

In the presence of oxalate (Ox), the surface metal atoms may dissolve by oxalate-promoted and reductive pathways (R_{Ox} , eqs. 4-5):

Whereas Fe(III) minerals may dissolve by both eq. 4 and 5 depending on the environmental conditions (Martin, 2005), eq. 5 is expected to be predominant for MnOOH (Stone, 1987; Xyla et al., 1992). In addition to dissolution via eq. 2-5, in the presence of siderophores and oxalate there may also be a “synergistic effect”, where the dissolution rate is promoted beyond the sum of the individual dissolution rates in the corresponding single component systems (Cervini-Silva and Sposito, 2002; Cheah et al., 2003; Reichard et al., 2007a; Wolff-Boenisch and Traina, 2007b).

For all experiments, plots of both metal-siderophore complex (when detected) and total metal concentration initially increased monotonically as the experiment progressed (Fig. S2-S4).

To calculate initial dissolution rates in single ligand systems, linear least-square regression analysis was performed on the first 5–10 points, with the duration chosen by picking the maximum number of linear points, as judged by changes to the regression coefficient ($R^2 > 0.85$); the overall dissolution rates were then calculated and tested at a 95% confidence interval, which was used to estimate error. For experiments containing oxalate (Fig. S5), $[\text{Fe}]_{\text{T}}$ (total iron concentration) reached a steady level in the presence of oxalate, followed by a rapid increase upon the addition of siderophore, consistent with previous studies that have used similar methodology (Reichard et al., 2005; Reichard et al., 2007a; Reichard et al., 2007b). The total dissolution rates ($R_{\text{Sid-Ox}}$) of the metal oxyhydroxides by siderophores in the presence of oxalic acid were also calculated and tested at a 95% confidence interval by performing linear regression analysis on the first 3–5 points after siderophore addition, with $R^2 > 0.80$ for these regressions.

3. Results and Discussion

3.1. Trends in Siderophore-promoted Dissolution Rates for Metal Oxyhydroxides.

The siderophore-promoted total dissolution rates of FeOOH, MnOOH, and CoOOH in the presence of DFOB, rhizoferrin, and protochelin are shown in Fig. 2A-C and Table 1. The total dissolution rates of CoOOH and MnOOH at all pH values were faster than those observed for FeOOH. For MnOOH and CoOOH, dissolution rates decreased with increasing pH in the presence of all three siderophores. In contrast, FeOOH dissolution rates were pH-independent for DFOB and rhizoferrin but increased with increasing pH for protochelin. Observed dissolution rates in the presence of protochelin for each mineral were greater than those observed in the presence of DFOB and rhizoferrin at all pH values, with the exception of FeOOH at pH = 5.

It has been previously noted that CoOOH and several Mn oxyhydroxide and oxide minerals dissolve more rapidly than Fe oxyhydroxide and oxide minerals in the presence of the trishydroxamate siderophore DFOB (Bi et al., 2010; Duckworth et al., 2009a). Our results with protochelin and rhizoferrin also follow this trend. Because the siderophores utilized in this study incorporate the range of common siderophore moieties, the results suggest that the observed siderophore-promoted dissolution rates for Mn(III) and Co(III) oxyhydroxides may generally exceed those of Fe(III) minerals.

3.2. Siderophore-promoted Dissolution of FeOOH.

Figure 2A shows the initial siderophore-promoted dissolution rates of FeOOH at 25 °C. In all cases, the observed total dissolved metal concentration did not exceed the total siderophore concentration, and Fe(III)-siderophore complexes were detected spectrophotometrically at all pH values (see Fig. S2A-C). For all situations where Fe(III)-siderophore complexes were quantified, the total dissolution rates and ligand-promoted dissolution rates are within experimental error, indicating a predominantly ligand-promoted dissolution mechanism, which is consistent with previous studies (Cervini-Silva, 2008; Coccozza et al., 2002; Kraemer et al., 1999; Lloyd, 1999; Wolff-Boenisch and Traina, 2007b).

Observed concentrations of $[\text{Fe}]_{\text{T}}$ and Fe(III)-siderophore complexes initially increase linearly and reach a steady maximum (Fig. S2A-C). For DFOB, this maximum value is $> 80 \mu\text{M}$ in all cases, suggesting a reaction stoichiometry between Fe and DFOB that approaches unity. In contrast, the maximum $[\text{Fe}]_{\text{T}}$ values for rhizoferrin are $< 70 \mu\text{M}$, suggesting a significantly lower reaction stoichiometry despite the fact that Fe(III)-rhizoferrin is known to form a 1:1 complex (Carrano et al., 1996; Harrington et al., 2012b). For protochelin, the maximum $[\text{Fe}]_{\text{T}}$ values

range from 15-25 μM , which may reflect the limited solubility of the neutral H_6Proto and $\text{Fe(III)H}_3\text{Proto}$ species that form at $\text{pH} < 7$ or the instability of deprotonated Proto species that form at $\text{pH} > 7$ (Harrington et al., 2012b).

The DFOB-promoted dissolution rates measured in this study are significantly higher than the reported surface area-normalized dissolution rates, which vary from 7.3×10^{-13} to $2.5 \times 10^{-12} \text{ mol m}^{-2} \text{ s}^{-1}$ for DFOB-promoted dissolution of FeOOH . Although it is not completely clear why the rates in this study are higher than those previously reported, it is possibly related to the experimental conditions of the reported rates. The rates reported in this work are initial dissolution rates derived from batch experiments, whereas most literature rates are steady-state rates derived from continuous flow experiments. Initial dissolution rates may reflect contributions from high-energy sites (MacInnis and Brantley, 1992) and commonly exceed steady-state dissolution rates (Duckworth and Sposito, 2005b; Kraemer, 2004). When compared to initial DFOB-promoted FeOOH dissolution rates derived from batch experiments (Table S2), our rates are approximately an order of magnitude greater than literature rates (Carrano et al., 1996; Lloyd, 1999; Neubauer et al., 2002; Wolff-Boenisch and Traina, 2007b) determined at similar DFOB concentrations (Table S2). We do not have an explanation for this discrepancy between our dissolution rates and those in the literature, although differences in experimental conditions (solids loading, ionic strength, concentration, etc.) may account for some variation in rates. Our results for protochelin at $\text{pH} = 6$ agree well with initial FeOOH dissolution rates promoted by enterobactin, which is also a triscatecholate siderophore (Wolff-Boenisch and Traina, 2007b).

It has previously been reported that dissolution rates trend with complex stability constants (β) for a group of structurally homologous DFOB derivatives (Cervini-Silva, 2008).

This trend is also evident in our data, as the Fe(III)Proto³⁻ complex stability constant ($I = 0$) of $\beta = 10^{48.6}$ (Duhme et al., 1997) exceed those for Fe(III)HDFOB⁺ ($\beta = 10^{32.0}$) and Fe(III)Rhiz³⁻ ($\beta = 10^{25.3}$) (Carrano et al., 1996; Schwarzenbach and Schwarzenbach, 1963). A plot (Fig. 3) of initial dissolution rates for FeOOH derived from batch reactions with siderophores from our study and from the literature (Table S2) against literature stability constants demonstrates a positive correlation ($R^2 = 0.70$; $p < 0.05$). The trend, which extends a previous analysis (Cervini-Silva, 2008) to a wider range of structures and stability constants, suggests that siderophore-promoted dissolution processes may be related to mechanisms of complex formation, and that the siderophore-promoted dissolution rate of FeOOH may be estimated from the ligand-metal stability constant.

3.3. Siderophore-promoted Dissolution of CoOOH and MnOOH

Dissolution rates of CoOOH and MnOOH in the presence of siderophores are shown as a function of pH in Fig. 2B-C and Table 1. Dissolution rates generally decreased with increasing pH for all siderophores. The rates for protochelin exceed those of DFOB and rhizoferrin, with the greatest differences observed at $\text{pH} \leq 7$. In all cases except DFOB-promoted dissolution at alkaline pH (for MnOOH, $\text{pH} \geq 7$; for CoOOH, $\text{pH} \geq 8$), no metal siderophore complexes were detected spectrophotometrically, suggesting that dissolution occurs exclusively by a reductive pathway (Fig. S3-S4). For DFOB, both pH-dependent trends in and values of dissolution rates agree well with those of previous workers, who reported dissolution rates of $0.20\text{--}1.7 \times 10^{-9} \text{ mol m}^{-2} \text{ s}^{-1}$ for MnOOH (Duckworth and Sposito, 2005b) and $0.55\text{--}2.7 \times 10^{-9} \text{ mol m}^{-2} \text{ s}^{-1}$ for CoOOH (Bi et al., 2010) at $\text{pH} = 5\text{--}9$.

For MnOOH and CoOOH, reaction stoichiometries were highly variable for different siderophores. For DFOB, maximum $[\text{Co}]_{\text{T}}$ and $[\text{Mn}]_{\text{T}}$ values at low pH were approximately 3-4-fold higher than the initial DFOB concentration but decreased to a value near unity with increasing pH, consistent with previous results studying the DFOB-promoted dissolution of CoOOH and MnOOH (Bi et al., 2010; Duckworth and Sposito, 2005b). For protochelin, a very redox active siderophore (Harrington et al., 2012a), ratios of maximum $[\text{Co}]_{\text{T}}$ and $[\text{Mn}]_{\text{T}}$ values to initial protochelin concentrations varied from ~1–13 and decreased monotonically with increasing pH. Interestingly, ratios of maximum $[\text{Co}]_{\text{T}}$ and $[\text{Mn}]_{\text{T}}$ values to initial rhizoferrin concentrations were less than unity and varied from ~0.25-0.75, similar to stoichiometry observed for rhizoferrin-promoted FeOOH dissolution. These results, coupled with the dissolution rates, indicate significant variation in reactivity of siderophores with redox-active mineral surfaces.

Only reductive dissolution is observed for protochelin- and rhizoferrin-promoted dissolution of CoOOH and MnOOH. Previous work has shown that Co(III)-protochelin complexes are unstable at all pH values, and Mn(III)-protochelin complexes are only transiently stable at alkaline pH (Harrington et al., 2012a). It is thus not surprising that protochelin dissolved both CoOOH and MnOOH solely by reductive pathways. It is more surprising that rhizoferrin only undergoes reductive dissolution with CoOOH and MnOOH. Although previous reports of attempts to produce a Co(III)-rhizoferrin complex were unsuccessful (Carrano et al., 1996), a Co- rhizoferrin complex that was synthesized by oxidation of Co(II) with H_2O_2 was found by extended X-ray absorption fine structure (EXAFS) spectroscopy to be structurally consistent with Co(III)Rhiz^{3-} (Fig. S6 and Table S3) and determined by potentiometric titration (Fig. S7) to have a stability constant of $\log\beta = 38.9$ at $I = 0$. For MnOOH, rhizoferrin has been shown to form

a stable complex with Mn(III) at alkaline pH (Harrington et al., 2012b). In addition, high concentrations of citrate, an α -hydroxycarboxylate ligand, have been shown to promote dissolution of MnOOH (feitknechtite with some hausmannite impurity) at $\text{pH} \geq 7$ by both reductive and non-reductive processes (Klewicki and Morgan, 1999). It is possible that a higher concentration of rhizoferrin may produce Mn(III)Rhiz³⁻ or Co(III)Rhiz³⁻ complexes at alkaline pH, although we did not test this hypothesis.

3.4. Siderophore-promoted Dissolution Rates of Metal Oxyhydroxides in the Presence of Oxalate.

The dissolution rates of FeOOH in the presence of oxalate and siderophores are shown in Fig. 4A and Table 2. At $\text{pH} = 6$, DFOB in the presence of oxalate exhibits an ~4-fold enhancement of the sum of both the DFOB- and oxalate-promoted dissolution rates at corresponding conditions, which is roughly consistent with literature results (Cheah et al., 2003; Reichard et al., 2007a). At $\text{pH} = 8$, the rates corresponding to the reaction with DFOB in the presence of oxalate are equal to the sum of the oxalate- and siderophore-promoted rates within experimental error. In the presence of oxalate, the dissolution of FeOOH by protochelin is greatly enhanced compared to the sum of the dissolution rate of each ligand in the system, with dissolution increasing by a factor of approximately 7 at $\text{pH} = 6$; a 2.5-fold enhancement is observed at $\text{pH} = 8$. However, for rhizoferrin at $\text{pH} = 6$ and 8, dissolution rates are equal to the sum of the oxalate- and the rhizoferrin-promoted rates within experimental error, indicating a lack of a synergistic effect on dissolution.

The synergistic effects of oxalate on siderophore-promoted dissolution result from oxalate acting as an iron shuttle that delivers Fe(III) to dissolved siderophores, with detachment

of a labile pool of Fe(III)-oxalate surface complexes favored by the presence of the siderophore (Loring et al., 2008; Reichard et al., 2007a; Reichard et al., 2007b). This conceptual model may be applied to assist with the interpretation of our results. In our results, the extent to which the dissolution rate is promoted depends on the solution pH as well as the stability constant of the Fe(III)-siderophore complex. At pH = 6, dissolution rates trend with the stability constant of the siderophore. The stability constant of the trisoxalato-Fe(III) complex is $\log \beta = 30.75$ (Mollar et al., 1991). Although this stability constant is > 10 orders of magnitude lower than that of protochelin, it is similar in magnitude to that of DFOB and exceeds that of rhizoferrin by several orders of magnitude. This suggests that at high concentrations of oxalate, trisoxalato-Fe(III) complexes may persist transiently in solution containing DFOB (as noted by Cheah et al. (2003) in UV-visible measurements), slowing the synergistic process. In addition, it suggests that it is not thermodynamically favorable for rhizoferrin to remove Fe(III) from trisoxalato complexes, explaining the lack of a synergistic effect on total dissolution rates in the presence of rhizoferrin and oxalate. At pH = 8, the sorption of oxalate onto FeOOH has been shown to be very weak, with only low surface concentrations observed (Eick et al., 1999; Mesuere and Fish, 1992). If we assume that the presence of siderophores does not markedly enhance the adsorption of oxalate (Reichard et al., 2007a), the absence of labile adsorbed oxalate species at pH = 8 is consistent with the lack of an effect on the dissolution rates of FeOOH by DFOB and rhizoferrin, and marked reduction of its effect on the dissolution rate of protochelin.

As compared to FeOOH, oxalate exhibited a smaller impact on the siderophore-promoted CoOOH and MnOOH dissolution rates (Table 2). Protochelin increased dissolution rates by approximately a factor of two for MnOOH at pH = 6 and for CoOOH at pH = 8; however, no increase in rate was observed for MnOOH at pH = 8, and a decrease in the dissolution rate

occurred for CoOOH at pH = 6. Similarly, dissolution rates in the presence of oxalate and DFOB or rhizoferrin were not enhanced as compared to the sum of the single-ligand systems. Although the studies use different Mn minerals with different average Mn oxidation numbers, this result is somewhat surprising because Saal and Duckworth (2010) noted a 2-3 fold increase in reductive dissolution rates for Mn(IV)O₂ in the presence of oxalate. It should be noted that, in addition to the siderophore, oxalate may promote reductive dissolution of Mn oxides (eq. 5) (Stone, 1987; Xyla et al., 1992), and it is possible that the multi-ligand effects, such as the synergistic effect of oxalate and siderophore on ligand-promoted FeOOH dissolution, may function by differing reaction mechanisms for ligands which promote reductive dissolution. However, more studies of reductive dissolution in multi-ligand systems are needed to better understand these effects.

4. Summary and Conclusion

This paper described the dissolution rates and mechanisms of Fe(III), Mn(III), and Co(III) oxyhydroxides promoted by siderophores with a variety of metal-binding moieties in the presence and absence of oxalate. By incorporating experimental dissolution rates of FeOOH at pH = 6 with literature values of Fe(III) stability constants, a correlation between mineral dissolution kinetics and complex thermodynamics was demonstrated, extending a previous result derived for a small set of DFOB-derivatives (Cervini-Silva, 2008). In general, siderophore-promoted dissolution rates followed the order MnOOH > CoOOH > FeOOH (Fig. 5), which is consistent with previous results obtained for DFOB. These results extend prior results obtained with DFOB to siderophores comprised of differing functional groups, filling a significant knowledge gap in the literature and allowing us to better estimate the reactivity of siderophores in the environment. If we assume that the range of siderophore moieties used in this study spans

most of the reactivity of bacterial siderophores with specific metal oxyhydroxides, we can estimate that in the presence of siderophores (100 μM), total siderophore-promoted dissolution rates for MnOOH , CoOOH , and FeOOH are in the range of 4×10^{-8} – $6 \times 10^{-10} \text{ mol m}^{-2} \text{ s}^{-1}$, 3×10^{-8} – $4 \times 10^{-11} \text{ mol m}^{-2} \text{ s}^{-1}$, and 4×10^{-11} – $3 \times 10^{-12} \text{ mol m}^{-2} \text{ s}^{-1}$, respectively. This suggests that, depending on their relative environmental abundance, Co and Mn, whose dissolution rates exceed those for Fe by up to 4 orders of magnitude, may compete with Fe for a limited pool of siderophores during siderophore-promoted dissolution of minerals in soils, sediments, and other marine and terrestrial environments.

Siderophores may promote dissolution via ligand promoted or reductive dissolution pathways. For FeOOH , dissolution by all three siderophores results exclusively in the production of Fe(III)-siderophore complexes. In contrast to results with FeOOH , reductive dissolution, resulting in divalent Co(II) and Mn(II) metal ions, was the dominant reaction for MnOOH and CoOOH at all solution pH values for rhizoferrin and protochelin and for DFOB at circumneutral to acidic pH. This observation suggests that the dissolution of metal oxyhydroxides may not be a significant source of Mn(III)- or Co(III)- complexes in the environment. In addition, because siderophores undergo concomitant oxidation during reduction of metal ions, the rapid reaction of siderophores with these minerals may be a significant sink for siderophores in soils, sediments, and aqueous environments.

Our results may help to shed light on microbial metal solubilization and uptake strategies. Siderophores are not produced in isolation but often in concert with low molecular mass organic acids. In principle, the presence of oxalate may enhance, inhibit, or have no effect on siderophore-promoted dissolution rates. In general, dissolution rates in the presence of oxalate and siderophores were enhanced (as compared to single-ligand systems) for FeOOH to a greater

extent than for CoOOH and MnOOH. For FeOOH, the presence of oxalate resulted in largest increase in rate for protochelin-promoted dissolution. Because protochelin is subject to degradation at $\text{pH} \leq 7$ (Harrington et al., 2012a), small organic acids increase dissolution rates relative to the lifetime of protochelin in alkaline soils. It has been previously suggested that timed exudation of siderophores with organic acids may be part of a biological strategy to maximize iron solubilization (Reichard et al., 2007a). Our observations lead us to suggest that the exudation of low molecular mass acids in concert with siderophores may be part of a cohesive biological strategy that provides additional benefits, including maximizing the effectiveness of short-lived siderophores in the environment, protecting siderophores from redox reactivity at surfaces (by delivering metals to dissolved species), and specifically increasing the solubilization of targeted metals (e.g., Fe(III)) that may be bound in complicated, multimetal-bearing mixtures of solid phases.

Acknowledgements. We are grateful for support received from the National Science Foundation Geobiology and Low-Temperature Geochemistry Program (award number EAR-0921313). We thank Jeff Maske, Erik Nelson, and Matthew Lattimer for help during data collection at the synchrotron. Portions of this research were carried out at the Stanford Synchrotron Radiation Lightsource, a Directorate of SLAC National Accelerator Laboratory and an Office of Science User Facility operated for the U.S. Department of Energy Office of Science by Stanford University. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institutes of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this

publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS, NCRR or NIH.

APPENDIX A. Supplementary data associated with this article can be found in the online version, at doi:XXXX

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Figures.

- Fig. 1.** Structures of the siderophores selected for use in this study, which were chosen to span the structural diversity of common binding moieties found in microbial siderophores.
- Fig. 2.** Average initial ligand-promoted (R_L) (open symbol) and total (R_T) (closed symbols) dissolution rates ($\text{mol m}^{-2} \text{s}^{-1}$) for (A) FeOOH, (B) MnOOH, and (C) CoOOH as promoted by DFOB (black triangles), rhizoferrin (gray squares), and protochelin (black diamonds). Conditions: 0.2 g L^{-1} metal oxyhydroxide; $100 \text{ }\mu\text{M}$ siderophore; 25°C ; 10 mM buffer; 0.1 M NaCl solution. Error bars represent 95% confidence intervals.
- Fig. 3.** Log-log plot of stability constant (Albrecht-Gary and Crumbliss, 1998; Carrano et al., 1996; Duhme et al., 1997; Hou et al., 1994; Loomis and Raymond, 1991; Murakami et al., 1989; Schwarzenbach and Schwarzenbach, 1963) and dissolution rates of α -FeOOH (squares) as promoted by siderophores used in this study and from literature at $\text{pH} = 6$. Open points indicate rates derived from this study. Other dissolution rates (closed points) are collected from experiments (Carrasco et al., 2007; Cervini-Silva, 2008; Cocozza et al., 2002; Neubauer et al., 2002; Wolff-Boenisch and Traina, 2007b) at similar conditions ($66\text{--}500 \text{ }\mu\text{M}$ siderophore, $\text{pH} = 6$; Table S2). Ent = enterobactin; DFOMTA, DFOD₁ = desferrioxamine derivatives; DMA = 2'-deoxymugineic acid.
- Fig. 4.** Average initial total dissolution rates ($\text{mol m}^{-2} \text{s}^{-1}$) for (A) FeOOH in the absence (R_T ; closed symbols) and presence of oxalate ($R_{\text{Sid-Ox}}$; open symbols) as promoted by (A) DFOB (triangles), (B) protochelin (squares), and (C) rhizoferrin (diamonds). Oxalate-promoted rates (R_{Ox}) in the absence of siderophores are shown as open circles. Conditions: 0.2 g L^{-1} metal oxyhydroxide; 0 or $100 \text{ }\mu\text{M}$ siderophore; 0 or $1000 \text{ }\mu\text{M}$ oxalate; 25°C ; 10 mM buffer; 0.1 M NaCl solution. Error bars represent 95% confidence intervals.
- Fig. 5.** Average initial total (R_T) dissolution rates ($\text{mol m}^{-2} \text{s}^{-1}$) for FeOOH (orange), MnOOH (pink), and CoOOH (green) as promoted by DFOB (triangles), rhizoferrin (diamonds), and protochelin (squares). Shaded areas show the bounds of dissolution rates as promoted by the trishydroxamate, triscatecholate, and α -hydroxycarboxylate siderophores in this study. Conditions: 0.2 g L^{-1} metal oxyhydroxide; $100 \text{ }\mu\text{M}$ siderophore; 25°C ; 10 mM buffer; 0.1 M NaCl solution.

Table 1. Average initial total (R_T) and ligand-promoted (R_L) dissolution rates ($\text{mol m}^{-2} \text{s}^{-1}$) for FeOOH, MnOOH, and CoOOH as promoted by DFOB, rhizoferrin, and protochelin. Conditions: 0.2 g L^{-1} metal hydroxide; $100 \text{ }\mu\text{M}$ siderophore; 25°C ; 10 mM buffer; 0.1 M NaCl solution. Error bars represent 95% confidence intervals. Conditions: 0.2 g L^{-1} FeOOH; $100 \text{ }\mu\text{M}$ siderophore; 25°C ; 10 mM buffer; 0.1 M NaCl. ND = not detected. NM = not measured.

total FeOOH dissolution rates $\times 10^{-11} (\text{mol m}^{-2} \text{s}^{-1})$						
	DFOB		Rhizoferrin		Protochelin	
pH	R_T	R_L	R_T	R_L	R_T	R_L
5	1.6 ± 0.4	1.6 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
6	1.3 ± 0.3	1.3 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	2 ± 1	1 ± 2
7	1.3 ± 0.1	1.3 ± 0.4	0.4 ± 0.1	0.5 ± 0.1	3 ± 1	2 ± 1
8	1.2 ± 0.4	1.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	4 ± 1	NM
9	1.2 ± 0.2	1.2 ± 0.5	0.3 ± 0.1	0.5 ± 0.1	3.0 ± 0.2	NM

total MnOOH dissolution rates $\times 10^{-9} (\text{mol m}^{-2} \text{s}^{-1})$				
	DFOB		Rhizo	Proto
pH	R_T	R_L	R_T	R_T
5	10.0 ± 0.7	ND	4 ± 1	34 ± 2
6	3.0 ± 0.2	ND	2.2 ± 0.3	17 ± 1
7	1.6 ± 0.2	1.9 ± 0.3	1.4 ± 0.2	13 ± 3
8	1.6 ± 0.2	1.5 ± 0.3	1.0 ± 0.1	11 ± 6
9	0.80 ± 0.04	0.8 ± 0.1	0.6 ± 0.1	9 ± 2

total CoOOH dissolution rates $\times 10^{-9} (\text{mol m}^{-2} \text{s}^{-1})$				
	DFOB		Rhizo	Proto
pH	R_T	R_L	R_T	R_T
5	2.6 ± 0.1	ND	0.30 ± 0.04	24 ± 10
6	1.6 ± 0.2	ND	0.20 ± 0.03	26 ± 6
7	1.3 ± 0.2	ND	0.10 ± 0.01	8 ± 1
8	1.1 ± 0.1	0.4 ± 0.2	0.10 ± 0.01	4 ± 2
9	1.0 ± 0.1	0.9 ± 0.3	0.04 ± 0.01	2.0 ± 0.4

Table 2. Total siderophore-promoted dissolution rates for FeOOH, CoOOH, and MnOOH in the absence (R_T) and presence of oxalate (R_{Sid-Ox}). Conditions: 0.2 g L⁻¹ metal oxyhydroxide; 0 or 100 μ M siderophore; 0 or 1000 μ M oxalate; 25°C; 10 mM buffer; 0.1 M NaCl.

total FeOOH dissolution rates $\times 10^{-11}$ (mol m ⁻² s ⁻¹)							
no oxalate (R_T)			oxalate (R_{Ox})		with oxalate (R_{Sid-Ox})		
pH	proto	rhizo	DFOB	oxalate	proto	rhizo	DFOB
6	2 $\pm$ 1	0.3 $\pm$ 0.1	0.6 $\pm$ 0.2	0.4 $\pm$ 0.3	14 $\pm$ 9	0.3 $\pm$ 0.1	4 $\pm$ 1
8	4 $\pm$ 1	0.3 $\pm$ 0.1	1.5 $\pm$ 0.2	0.1 $\pm$ 0.1	10 $\pm$ 4	0.3 $\pm$ 0.1	0.7 $\pm$ 0.1

total MnOOH dissolution rates $\times 10^{-9}$ (mol m ⁻² s ⁻¹)							
no oxalate (R_T)			oxalate (R_{Ox})		with oxalate (R_{Sid-Ox})		
pH	proto	rhizo	DFOB	oxalate	proto	Rhizo	DFOB
6	17 $\pm$ 1	2.0 $\pm$ 0.3	3.0 $\pm$ 0.2	0.8 $\pm$ 0.3	27 $\pm$ 6	2.1 $\pm$ 0.3	3.2 $\pm$ 0.2
8	11 $\pm$ 6	1.0 $\pm$ 0.1	1.6 $\pm$ 0.2	0.3 $\pm$ 0.2	9 $\pm$ 7	1.0 $\pm$ 0.3	1.0 $\pm$ 0.1

total CoOOH dissolution rates $\times 10^{-9}$ (mol m ⁻² s ⁻¹)							
no oxalate (R_T)			oxalate (R_{Ox})		with oxalate (R_{Sid-Ox})		
pH	proto	rhizo	DFOB	oxalate	proto	rhizo	DFOB
6	26 $\pm$ 6	0.2 $\pm$ 0.03	2.0 $\pm$ 0.2	0.2 $\pm$ 0.1	14 $\pm$ 6	0.3 $\pm$ 0.1	1.0 $\pm$ 0.1
8	4 $\pm$ 2	0.1 $\pm$ 0.01	1.0 $\pm$ 0.1	0.03 $\pm$ 0.02	6 $\pm$ 3	0.10 $\pm$ 0.01	1.0 $\pm$ 0.2

Desferrioxamine B

Protochelin

Rhizoferrin

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.