Ubiquitous Presence of Fe(II) in Aquatic Colloids and Its Association with Organic Carbon

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*Supporting Information

ABSTRACT: Despite being thermodynamically less stable, small ferrous colloids (60 nm to 3 μm in diameter) remain an important component of the biogeochemical cycle at the Earth’s surface, yet their composition and structure and the reasons for their persistence are still poorly understood. Here we use X-ray-based Fe L-edge and carbon K-edge spectromicroscopy to address the speciation and organic−mineral associations of ferrous, ferric, and Fe-poor particles collected from sampling sites in both marine and freshwater environments. We show that Fe(II)-rich phases are prevalent throughout different aquatic regimes yet exhibit a high degree of chemical heterogeneity. Furthermore, we show that Fe-rich particles show strong associations with organic carbon. The observed association of Fe(II) particles with carboxamide functional groups suggests a possible microbial role in the preservation of Fe(II). These findings have significant implications for the behavior of Fe(II) colloids in oxygenated waters, and their role in different aquatic biogeochemical processes.

INTRODUCTION

Iron minerals are common in soils and sediments, where they represent important sources of Fe for biological processes and, because of their large and highly reactive surface area, they act as strong sorbents for various contaminants and nutrients.3,4 In aquatic environments, Fe species can persist as colloids in the micrometer to submicrometer size range, where their stability is governed by water composition and mineral surface chemistry. Photochemical transformations, mineral dissolution and precipitation, and changes to the Fe redox state can further modify the fate of these phases and their associated contaminants and nutrients.3−5 While the importance of colloidal Fe in various biogeochemical processes has gained considerable recognition,6 little attention has been paid to the speciation, stability, and mineralogical associations of Fe phases in the colloidal fraction of oxygenated natural waters.

With the advent of synchrotron-based X-ray spectromicroscopy methods, the structures of Fe colloids, their chemical and mineralogical characteristics, and the factors affecting Fe colloid stability can now be probed directly.7,8 Several researchers have examined the chemistry of natural Fe-containing particles in the particulate fraction using X-ray imaging and speciation techniques at both the K- and L-edges of Fe.9−16 The X-ray absorption spectromicroscopy at the Fe K-edge, which is highly sensitive to Fe concentration and has a lower spatial resolution (>100−150 nm to a few micrometers), has been utilized to determine the average Fe speciation in heterogeneous samples3,17 and to determine particle-specific speciation in larger micrometer-sized particulates.15 In comparison, spectromicroscopy at the Fe L-edge has a much higher spatial resolution (down to a few nanometers) and has successfully been applied to study Fe biomineralization11,12 and the speciation of Fe colloids from hydrothermal vents14 and the open ocean.16

In this study, we applied Fe L-edge and C K-edge scanning transmission X-ray microscopy (STXM) to identify the common forms of Fe in the colloidal fractions of natural

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waters and the association of different Fe pools with natural organic moieties. This zone-plate-based X-ray spectromicroscopy is ideal for the study of aquatic colloids as samples can be examined in their native hydrated state, down to a spatial resolution of 10–15 nm without any special sample preparation. Furthermore, the Fe L-edge is characterized by a large absorption cross section (which results in high-contrast images) and X-ray absorption near-edge structure (XANES) spectra that are rich in chemical and structural information.16,18,19 von der Heyden and co-workers16 showed the identification of some of the Fe mineral phases using the energy difference between the two L3 peaks (ΔE value) and the quotient of their peak intensities (intensity ratio value) (Figure 1). Although many Fe mineral phases have unique Fe coordination environments, and thus XANES spectral shapes, and can be characterized by their unique combination of ΔE value and intensity ratio values (Figure 2), the technique is limited when Fe phases are structurally and chemically similar (e.g., akaganeite and goethite). Here we exploit the information contained in these L3-edge spectral parameters to evaluate the speciation of Fe in colloids collected from both marine and fresh waters and show the ubiquitous presence of thermodynamically unstable Fe(II) in association with organic carbon moieties.

■ MATERIALS AND METHODS

Lacustrine samples were collected from freshwater systems in New Jersey and Puerto Rico, and the marine samples focused...
on the Southern Ocean and the western South Pacific Ocean. To attain a sufficient number of particles for STXM analysis, a range of site-specific sampling techniques was employed and detailed descriptions are documented in the Supporting Information (section 1). Ocean water samples, typically characterized by a low suspended particle load, required preconcentration procedures using either McLane pumps (nominal pore size of 1 µm, Whatman QMA) or vacuum filtration (nominal pore size of 0.2 µm, Millipore PTFE), whereas freshwater samples, with high particle loads, could be collected without the need for prior filtration. In all instances, trace metal clean techniques were followed, and because of sensitivity to contamination, marine water samples were collected following the rigorous guidelines set out in the GEOTRACES protocol.20

All Fe L-edge and C K-edge spectromicroscopic analyses were conducted at the Molecular Environmental Sciences endstation at the Advanced Light Source.9 Samples were prepared on silicon nitride (SiN3) membrane windows according to the procedures outlined in the Supporting Information (section 2). During STXM experimentation, Fe-rich particles were located on the SiN3 sample window using coarse 10 µm × 10 µm or 30 µm × 30 µm image maps generated by subtracting an edge region (709–710 eV) X-ray image from an image of the same area collected at an energy below the absorption Fe L-edge (700 eV). Once located, Fe-rich regions were analyzed for their XANES spectra using either line scans or image stacks (10–50 nm spatial resolution) over the Fe L-edge region. Energy increments of 0.5 eV were used above and below the edge (695–703 and 715–730 eV), and a step size of 0.2 eV was used close to the Fe L-edge (703–715 eV).

Once Fe-enriched particles had been identified and analyzed, the corresponding regions on the SiN3 window were evaluated at the carbon K-edge for the prevalence of organic phases (Figure 1) by applying the procedures described above. XANES spectra were collected for both Fe-enriched and Fe-poor organic particulates by generating either line scans or image stacks, using energy increments of 0.2 eV at the C K-edge (283–300 eV) and 0.5 eV above and below the edge (280–283 and 300–315 eV). All collected C K-edge spectra were calibrated using the adventitious carbon absorption feature at 284.8 eV. Although beam damage effects have been studied in detail for some homogeneous aqueous solutions of simple organic molecules (e.g., siderophores and amino acids),21 the stability of organics in the X-ray beam varies with the type of functional groups, the size of molecules, the presence of water, and the energy of excitation. For example, humic and fulvic acids are more stable than catechols, and hydroxamate siderophores are more stable at the C K-edge than at the N K-edge.21 While heterogeneous natural samples containing organics are typically more stable than aqueous homogeneous solutions containing the same organics, beam damage effects are more difficult to quantify for heterogeneous samples because no two particles are identical. However, Fe-rich particles were tested with repeat C K-edge XANES before and after the collection of Fe L-edge XANES to test the effects of sample alteration. These tests showed minimal alteration in the C K-edge XANES features (Figure S3 of the Supporting Information).

Although this study focused on the Fe L-edge and C K-edge, we also strengthened our data set with complementary nitrogen K-edge and Fe K-edge XANES data. For identified Fe-enriched particles, XANES analyses were conducted at the N K-edge region (385–420 eV) following the analysis protocols described for the Fe L-edge and the C K-edge. The collected N K-edge XANES spectra for Fe-enriched particles, however, typically exhibited weak signals because of low N abundances. Because of larger sample volumes, bulk Fe K-edge analyses could be conducted on the sediment trap samples collected from Lake La Plata, Puerto Rico. Analyses were conducted at the X-18B bend magnet beamline of the National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY), and multiple repeat XANES scans showed that no radiation-induced sample alteration was incurred. Further analysis protocol and beamline specifications are described in the Supporting Information (section 3).

RESULTS AND DISCUSSION

The collection of high-quality microscopic and spectroscopic data for small colloids on a particle-by-particle basis is a time-consuming process. The presented data set (>60 particles) was collected using >20 days of synchrotron facility beam time, as each SiN3 sample window requires 1–3 days for complete spectroscopic and microscopic characterization at the Fe and C X-ray absorption edges. Fe(III) particles commonly occurred as the smaller-sized colloids (down to 20 nm in diameter), whereas Fe(II)-rich particles were typically larger (0.06–2.6 µm) and were observed in all of the sampling sites considered in this study. In the open ocean domain, Fe can be a limiting nutrient, and consequently and despite our particle-concentrating sampling protocol, Fe-enriched particles were sparse and difficult to locate (e.g., only 3–10 particles or aggregates per SiN3 window for some marine samples). Open ocean Fe-rich particles and particle aggregates were typically quasi-spherical in shape and tended toward the smallest size domains (from 20 to ~800 nm), with some larger particles collected at depth. In stark contrast, SiN3 sample plates prepared using freshwater samples were highly enriched in Fe, with much of the Fe associated with organic flocs and mineral aggregates up to several micrometers in diameter.

Speciation of Iron. Southern Ocean Samples. Iron-rich particles in the South Atlantic and Southern Oceans have previously been classified into five distinctly different chemical categories according to their ΔE and intensity ratio spectral parameters.16 Despite the oxic nature of surface seawater, a remarkable 12% of particles analyzed in this study were classified as either purely ferrous or Fe(II)-rich mixed-valence phases. The majority of these particles were mixed-valence, and their spectral characteristics did not match those of any known Fe mixed-valence mineral phases (Figure 2B). Similarly, the ΔE versus intensity ratio values of purely Fe(II) phases did not match with any of the plotted points for standard mineral phases reported in the literature (e.g., pyrite). These mismatches serve to highlight the complexity of natural colloids, which typically have spectral features that are impacted by the presence of mixed phases and aggregates, chemical impurities (e.g., cation and ligand substitution; surface interactions, such as adsorbed ions and precipitates on nanoparticles), structural amorphism, and variable degrees of oxidation of pure phases.

Pacific Ocean Samples. More than 60 particles were analyzed from various depths (30–1000 m) at the three sampling sites in the South Pacific Ocean (section 1 of the Supporting Information). The majority of these particles (~80%) had iron in its ferrous form with mostly pure Fe(II) and a few mixed-valence phases identified (Figure 2B). Given
the proximity of the sampling locations to sites of active tectonism22 (Figure S2 of the Supporting Information), a proportion of the reduced Fe flux could be from associated hydrothermal sources.14,23,24

Figure 2B shows that the spectral parameters of Fe(II)-rich particles sampled from the Pacific Ocean have the largest range in distribution (ΔE value range of 1.5–2.8; intensity ratio range of 1.0–5.4) and that data points did not cluster around any of the standard mineral phases reported in the literature. Particles analyzed in this study ranged in size from 60 nm to 2.3 μm, with the average particle size tending toward larger values (mean = 0.70 μm; N = 50). The smallest particles were characterized by the smallest intensity ratio and ΔE values, and as the particle size increased, there was a slight positive and increasing trend with both of the spectral parameters (section 4 of the Supporting Information). Although the increase in intensity ratio with particle size (or thickness) can partially be explained by STXM saturation effects, the observed variation in the ΔE value is a definitive indication of chemical or structural differences between the smallest colloids and larger particles.

**Lacustrine Samples.** Between the temperate Pine Barrens and the tropical Lake La Plata, only samples from the Pine Barrens showed appreciable Fe(II) abundance upon being examined using Fe L-edge XANES spectroscopy. Only three discrete Fe-rich particles, ranging in size from 500 nm to 1 μm, were investigated in detail at this site, and all had spectra indicative of Fe(II) (Figure 2B). Although the spectral parameters could not be definitively matched to any of the examined standard ferrous mineral phases, one of the particles had spectral features similar to those of Fe(II)PO4, whereas the other two had Fe coordination environments most similar to that of Fe in biotite (Figure 2B).

In Lake La Plata, the installed sediment trap system ensured that relatively large sample volumes could be collected, allowing for additional X-ray diffraction (XRD) analysis of particles collected at this site. Although these samples showed 2–5% Fe, the XRD of these samples indicated only the presence of K-mica, kaolinite, and quartz, suggesting that the Fe phases are either X-ray diffraction amorphous or nanocrystalline. L-edge spectromicroscopy of particulate samples collected from the top and bottom parts of the water column showed only Fe(III). Comparison of the spectral features of these natural ferric phases to those of standard Fe oxide and oxyhydroxides showed that ~21% of these particles have ΔE value and intensity ratio values characteristic of the crystalline standards. The majority (two-thirds) of these had spectral features typical of goethite (α-FeOOH), a phase that has previously been shown to be a dominant Fe mineral phase in lacustrine waters.25

In contrast to the Fe L-edge, the bulk Fe XANES absorption spectra of particulates at the Fe K-edge indicated the presence of a significant fraction of Fe(II). Linear combination fitting of bulk Fe K-edge XANES spectra indicated that there was appreciable (16–38%) Fe(II) associated with the lacustrine Fe particulates, irrespective of sampling season and sampling depth (section 3 of the Supporting Information). This disagreement in the L-edge and K-edge data comes from the sizes of particulates probed; K-edge XANES is conducted at 7000 eV where the X-ray beams can penetrate deeper into the sample. Because the particulate concentration is very high for Lake La Plata samples, the particulates form large aggregates (several micrometers in diameter) that can be probed for their bulk chemistry by the K-edge XANES, whereas the L-edge XANES could be used to focus on the smaller nanometer-sized fraction away from the large aggregates.

**Speciation of Colloidal Organic Carbon and Its Association with Fe Phases.** Carbon K-edge XANES spectra were used to identify the predominant functional groups of organic molecules associated with Fe in marine and lacustrine particulates (Figures 1 and 3) and were further used in comparison against C XANES spectra from organics not associated with Fe (Figure 3B). All C spectral features were classified into the following broad classes because of their frequency: (1) unsaturated carbon, including both carbon double and triple bonds, associated with C 1s → π* transitions at approximately 285 eV; (2) aryl- and vinyl-keto functional groups at approximately 286.0–286.4 eV; (3) aliphatic carbon, heterocycles, and aldehydes at 286.5–287.4 eV; (4) amide, ketone, and carboxylate at 287.5–288.8 eV; and (5) alcohol, carboxamide, and carbonate at 289.5–290.5 eV (section 5 of the Supporting Information). Although ideal for mapping purposes, the STXM resolution of ~10 nm limits the specificity of the chemical probe for detecting a specific organic ligand or functional group binding Fe, and the C K-edge spectra derived from each pixel are averaged spectra of many organic molecules present in that region. The correlations presented here thus allude to associations of Fe-rich colloids with the prevalence of certain organic functional groups, and no comment is made with regard to the precise chemical bonding interactions between Fe and organic complexants.

Marine data represent 30 Fe(II) particles, five Fe mixed-valence particles, and two Fe(III) particles, whereas lacustrine data are averaged from four Fe(III) particles and three Fe(II) particles. In each instance, the corresponding number of analyses was conducted on Fe-poor regions of the SiN3 sample.
window to generate the data shown in Figure 3B. Irrespective of the particle chemical speciation or the aquatic sampling regime, all of the Fe particles evaluated showed significant association with organic carbon of heterogeneous functional group chemistry. Relative to Fe-poor regions, Fe-rich regions typically show greater variety and prevalence in organic functional group chemistry. Despite a host of chemical and biological differences between the two sampling regimes, marine and lacustrine Fe(II) particles show similar trends in their association with organic carbon functional groups. However, Fe(III) particulates collected from lacustrine settings have an absence of aryl- and vinyl-keto functional groups and are associated with fewer alcohol and carboxamide moieties than their marine Fe(III) counterparts. These alcohol and carboxamide moieties are also much more strongly associated with particulates containing Fe(II) (including mixed-valence phases), relative to Fe(III) particulates. Because carboxamide-related spectral features are common in microbial mats and biofilms, the presence of these spectral features in samples could imply regions of high microbial activity. This is supported by the N K-edge data for Fe(II) particulates that exhibit features between 400.8 and 401.8 eV (section 5 of the Supporting Information), suggesting the presence of amide and substituted N-heterocycles that are typically linked to biological activity. Alternative explanations for the preferential association of some organic functional groups (e.g., carboxamide) for Fe(II) are that these functional groups have a higher binding affinity for Fe(II) or that they play a role in stabilizing Fe in its reduced-valence state.14

Our X-ray spectroscopy and microscopy analyses have confirmed the ubiquity of Fe(II) colloids in a range of oxic aquatic environments, where the kinetics for oxidation to Fe(III) are known to be rapid.26,27 Our observations of Fe(II) colloidal associations with organic carbon are likely to have an impact on our understanding of Fe behavior in the natural systems, where Fe mineral phases are known to play important roles in biological growth,15 contaminant redox transformation processes,16,17 and nutrient and contaminant transport fluxes.18

**REFERENCES**


**ASSOCIATED CONTENT**

Supporting Information
Sample collection (section 1), soft X-ray spectromicroscopy analyses (section 2), Fe K-edge XANES analyses (section 3), spectral variations with particle size (section 4), and C K-edge and N K-edge spectral peak data (section 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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