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Interactions of the Rare Earth Elements–Desferrioxamine B Complexes with *Pseudomonas fluorescens* and γ-Al_2O_3

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We have studied the interactions of REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, and Er)–desferrioxamine B (DFO) complexes with *Pseudomonas fluorescens* cells and with γ-Al_2O_3, at pH 4–9. Higher adsorption of REEs was obtained at lower pHs on *P. fluorescens* cells and at higher pHs on γ-Al_2O_3. The degree of negative anomaly of Ce compared to its neighboring REEs, La(III) and Pr(III) increased with increasing pH. XAFS analysis showed that Ce exists as the Ce(IV)–DFO complex in pH higher than 6. Thus, the pH dependence of Ce anomaly is predominantly dependent on the stability of Ce(IV)–DFO complex.

The sequestration of metal cations by organic and inorganic particles can affect their mobility in the environment.[1] Biological materials possess functional groups able to complex with metal cations. Siderophores are microbial chelating agents produced to solubilize Fe(III).[2] Desferrioxamine B (DFO) is a trihydroxamate siderophore ubiquitously found in the environment,[3] and its interaction with various metal cations has been studied.[4] The stability constants of the REEs(III)–DFO (REEs: rare earth elements) complexes rise with increasing atomic number.[4] We previously showed that the percent adsorption of REEs(III) on an aerobic bacterium *Pseudomonas fluorescens* and on γ-Al_2O_3 at pH 7 in the presence of DFO decreased with an increase in atomic number.[5] In addition, we found that negative adsorption anomaly of Ce on *P. fluorescens* cells and γ-Al_2O_3 compared to the neighboring REEs(III), La(III) and Pr(III); this was because of the oxidization of Ce(III) to Ce(IV) during complexiation with DFO and the higher stability of the Ce(IV)–DFO complex than that of the Ce(III)–DFO complex and the La(III)– and Pr(III)–DFO complexes.

Carboxyl and phosphate groups mainly are responsible for metal binding on bacterial surfaces,[6] while surface hydroxy groups mostly contribute to binding of metal cations on minerals.[1] Charge of the functional groups is changed with pH in the solution[7] suggesting that adsorption behavior of REEs(III)–DFO complexes is changed with solution pH.

In this study, we have examined pH dependence of the adsorption of REEs on bacteria and aluminum oxide in the presence of DFO. *P. flurescens* is a Gram-negative bacterium widespread in the terrestrial environment. γ-Al_2O_3 was used as a model inorganic particle, which has a large surface area in its aluminum hydroxide phases. pH dependence was examined between pH 4–9, where most of the soil bacteria can survive.

Desferrioxamine B was purchased from Sigma Co., Ltd. and used without further purification. The growth conditions and the recovery of *P. fluorescens* (ATCC 55241) are shown elsewhere.[6] γ-Al_2O_3 (Degussa Aluminum Oxide C) was kindly supplied by Aerosil Co. (Japan). The reported surface area of this product is $100\,m^2\,g^{-1}$.[8]

We measured adsorption of eleven REEs, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, and Er, in the presence of DFO on *P. fluorescens* cells and on γ-Al_2O_3 by a batch method. *P. fluorescens* of $2.0\,g_{dry\ weight}\,L^{-1}$ or γ-Al_2O_3 of $4.0\,g\,L^{-1}$ were incubated in 10 mL of a $0.1\,mol\,L^{-1}$ NaCl solution containing $1.0\,mg\,L^{-1}$ of each REE (La, 7.20; Ce, 7.14; Pr, 7.10; Nd, 6.93; Sm, 6.65; Eu, 6.58; Gd, 6.36; Tb, 6.29; Dy, 6.15; Ho, 6.06; and Er, $5.98\,\mu mol\,L^{-1}$), $5.0 \times 10^{-4}\,mol\,L^{-1}$ DFO, and $1 \times 10^{-3}\,mol\,L^{-1}$ tris(hydroxylmethyl)aminomethane (Tris) at room temperature open to air. The suspensions were stirred while maintaining the pH at 4.0 ± 0.1, 5.0 ± 0.1, 6.0 ± 0.1, 7.0 ± 0.1, 8.0 ± 0.1, and 9.0 ± 0.1 during experiments with HCl or NaOH solution. After 30 min of incubation, the suspension was filtered through a 0.2 μm membrane filter made from mixed cellulose ester (ADVANTEC MFS, Inc., DISMIC-25). The concentration of REEs in the filtrate was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Shimadzu, ICPS-7000). The distribution coefficient (K_d) of REEs between the solid phase and water was defined as

$$K_d(\mathrm{REE}) = c([\mathrm{REE}]_{init} - [\mathrm{REE}]_{filt})/[\mathrm{REE}]_{filt}$$

where $[REE]_{filt}$ is the concentration of an REE in the filtrate, and $[REE]_{init}$ is the initial concentration of dissolved REE. The term c ($g\,L^{-1}$) describes the ratio of solid phase (dry weight) to water.

The concentration of Al(III) dissolved from γ-Al_2O_3 as the DFO complex after exposure to $1.0 \times 10^{-4}\,mol\,L^{-1}$ 1:1 Eu– or Ce–DFO for 180 min was analyzed by HPLC-ESI-MS. γ-Al_2O_3 was removed by filtration, and the concentration of the Al(III)–DFO complex in the filtrate was assessed by using the SIR mode at $m/z = +585.3$.

The oxidation state of Ce in the DFO complex was determined by X-ray absorption near-edge structure (XANES) spectroscopy in the fluorescence mode at the BL-27B line at KEK (Tsukuba, Japan). After adjusting the pH of the 0.5 mM 1:1 Ce–DFO complex solution to 4.0 ± 0.1, 7.0 ± 0.1, and 9.0 ± 0.1 A in a 0.1 M NaCl solution, XANES spectra for the Ce L_{III} edge were measured for Ce in the presence of DFO. XANES spectra for the samples of Ce adsorbed on *P. fluorescens* and γ-Al_2O_3, and standards of $Ce^{III}(NO_3)_3$ and $Ce^{IV}O_2$ were collected.

The K_d(REE) patterns for *P. fluorescens* cells (Figure 1a) in the presence of DFO at pHs 4 to 9 showed lower K_d(REE) in higher pH solution, i.e., K_d(Sm) was approximately $100\,mL\,g^{-1}$ at pH 4, 5, and 6, decreased to approximately $0.2\,mL\,g^{-1}$ with increasing pH to 9 (Figure 2). At pH 6, the K_d's of La, Pr, Nd, Sm, and Eu were approximately $100\,mL\,g^{-1}$, and for Ce it fell below these values. The K_d(REE) from La to Er on *P. fluorescens* cells decreased with increasing atomic number at pH 7

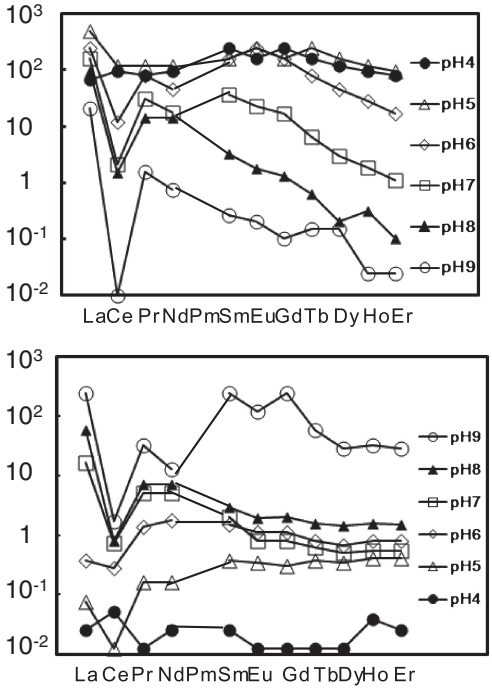

Figure 1. The K_d of 11-REEs on (a) *P. fluorescens* cells and (b) γ-Al_2O_3 in the presence of DFO.

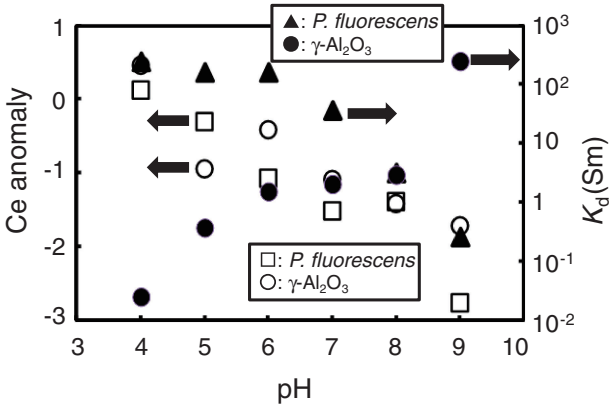

Figure 2. Degrees of Ce anomaly and K_d(Sm) on *P. fluorescens* cells and γ-Al_2O_3 as a function of pH.

to 9, except for Ce where it was significantly lower than that of the neighboring REEs, La(III) and Pr(III).

The K_d(REEs) of γ-Al_2O_3 was below 0.1 mL g^{-1} at pH 4 (Figure 1b). With increasing pH, their K_d(REEs) values rose (Figure 2 for Sm). The K_d(REEs) of γ-Al_2O_3 was around 100 mL g^{-1} at pH 9 except for Ce whose K_d was approximately 1 mL g^{-1}. At pHs above 5, the K_d of Ce on γ-Al_2O_3 apparently was smaller than that of La(III) and Pr(III). HPLC-ESI-MS analysis showed that the Al(III)–DFO complex dissolved in the solution of pH 7 were 4.5 and 2.7 μM in the Eu– and Ce–DFO samples, respectively.

The degree of Ce anomaly for the K_d(REE) patterns was expressed by: $DA = \log K_d(Ce) - [\log K_d(La) + \log K_d(Pr)]/2$.

The DA for *P. fluorescens* and Al_2O_3 plotted as a function of pH (Figure 2) showed that DA was almost 0 at pH 4, indicating

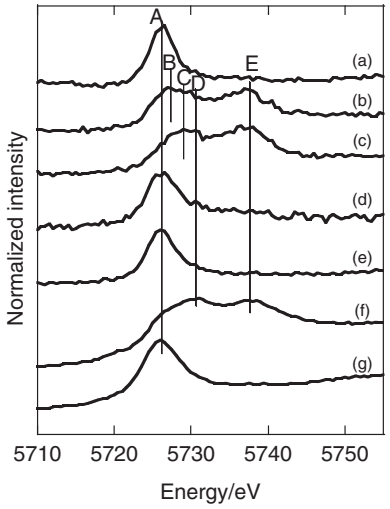

Figure 3. XANES spectra of Ce obtained for (a) 0.5 mM Ce–DFO complex at pH 4, (b) 0.5 mM Ce–DFO complex at pH 7, (c) 0.5 mM Ce–DFO complex at pH 9, (d) Ce adsorbed on γ-Al_2O_3 at pH 7, (e) Ce adsorbed on *P. fluorescens* cells at pH 7, (f) $Ce^{IV}O_2$, and (g) $Ce^{III}(NO_3)_3$.

that no Ce anomaly was observed, and the DA decreased with increasing pH. The pH dependence of the DA for *P. fluorescens* was nearly the same as Al_2O_3, even though pH dependence of K_d(REE) was different.

The XANES spectra of the Ce–DFO complex in the solutions of different pH (Figures 3a–3c) showed that there was one peak at 5726 eV (bar A) for Ce–DFO complex solution at pH 4. While Ce complexes at pH 7 (Figure 3b) and 9 (Figure 3c) had two peaks at ca. 5728 (bar B at pH 7) or 5729 (bar C at pH 7) and 5738 eV (bar E), indicating that both Ce(III) and Ce(IV) were present in the Ce–DFO complex. The amount of Ce(IV) was higher at pH 9 than pH 7, because of higher energy shift at the peak between 5726 and 5730 eV. The XANES spectra of Ce adsorbed on γ-Al_2O_3 at pH 7 (Figure 3d) and on *P. fluorescens* cells at pH 7 (Figure 3e) showed one strong peak at 5726 eV (bar A), indicating the presence of Ce(III). These results indicate that most of Ce was present as Ce(III) in the solutions of pH 4 even though DFO was present, and some fraction of Ce was oxidized to Ce(IV) in the solutions of neutral pH with DFO. In addition, the oxidation state of Ce adsorbed on *P. fluorescens* cells and γ-Al_2O_3 was trivalent.

The surfaces of *P. fluorescens* and *Bacillus subtilis* cells effectively adsorb Eu(III) at pH 3–5, possibly via carboxy groups.[6] The formation of stronger DFO complexes with REEs(III) at alkaline pHs masks their adsorption on the surface of *P. fluorescens* to a great extent. A higher affinity of the surface of the cells with REEs(III) at lower pHs reflects the fact that the stability of the REEs(III)–DFO complex falls with a decrease in pH more significantly than does the decrease in the affinity of REEs(III) with the cell surfaces.

The surface of γ-Al_2O_3 contains the aluminol functional group whose deprotonation constant (pK_{a1}) is approximately 7.[8] At lower pHs, these surface aluminol groups exhibit lower affinities with REEs(III). At pH 7–8, Eu(III) has a high affinity with aluminum oxide boehmite (γ-AlOOH), but low at pHs under 6.[9] These facts reflect the lower K_d(REE) by γ-Al_2O_3 in

lower pH. The stability constant ($\log K$) of the Al(III)–DFO complex is 24.5[10] that is larger than those of the REEs(III)–DFO complexes. Because of this, when the REEs(III)–DFO complexes were in contact with γ-Al_2O_3, the DFO favorably formed complexes with Al(III). Consequently, each REE(III) showed higher K_d at higher pHs wherein Al(III) competed more effectively with REEs(III) for DFO.

The degree of Ce anomaly for *P. fluorescens* cells and γ-Al_2O_3 increased with increasing pH, even though pH dependence of K_d(REE) patterns was opposite between *P. fluorescens* cells and γ-Al_2O_3. XANES analysis showed that higher fraction of Ce(IV)–DFO was present in solution at pH 9 than at pH 7, indicating that the stability of Ce–DFO complex increases with increasing pH. These facts strongly suggest that Ce anomaly depends on the stability of Ce–DFO complex but not on the functional groups of solid surface.

These findings indicate that the presence of DFO can contribute to extraction of information on environmental geochemical processes from the characteristic distribution of REEs, resulting from the competitive sequestration of REEs between DFO and solid phases (organic and inorganic particles). In addition, this study demonstrated that microorganisms and minerals can effectively adsorb and so retard mobilization of REEs(III) complexed with siderophores in acidic solution by microorganisms and in alkaline solution by minerals.

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