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Sorption of Eu(III) on *Pseudomonas fluorescens* in the Presence of Citric Acid

Yoshinori Suzuki,*[,a,b] Takuya Nankawa,[b] Takahiro Yoshida,[b] Takuo Ozaki,[b] Toshihiko Ohnuki,[b] Arokiasamy J. Francis,[c] Satoru Tsushima,[a] Youichi Enokida,[d] and Ichiro Yamamoto[a]

[a]*Department of Materials, Physics, and Energy Engineering, Graduate School of Engineering, Nagoya University, Furocho, Chikusa-ku, Nagoya 464-8603, Japan*
[b]*Advanced Science Research Center, Japan Atomic Energy Research Institute, Tokai, Ibaraki 319-1195, Japan*
[c]*Environmental Sciences Department, Brookhaven National Laboratory, Upton, New York 11973, USA*
[d]*Ecotopia Science Institute, Nagoya University, Furocho, Chikusa-ku, Nagoya 464-8603, Japan*

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We studied the sorption of Eu(III) on *Pseudomonas fluorescens* in the absence and presence of citric acid by a batch method. The cells were placed in a solution containing 2 μM of Eu(III) and 0, 100, or 1000 μM of citric acid at pH 3–9 for 5 hours. In the absence of citric acid, almost 100% of Eu(III) was sorbed on *P. fluorescens* at pHs below 7; above 7, sorption decreased with an increase in pH. The time course of Eu(III) sorption on *P. fluorescens* showed that a fraction of it was desorbed into the solution at alkaline pHs, suggesting that the bacterium may release some exudates. With citric acid present, we found that at higher concentrations there was lower sorption of Eu(III), reflecting the formation of Eu(III)-citrate complexes competes with the Eu(III)-cell-surface complexes. This decrease in Eu(III) sorption was significant in alkaline pHs. These findings suggest that citric acid which is ubiquitously found in the environment enhances migration of trivalent actinides in the alkaline environment.

1. Introduction

Naturally occurring organic substances and microorganisms in the environment interact with heavy metals, including actinides.[1–3] Elucidation of their interactions is important in estimating the mobility of actinides in the environment, and in using microorganisms to remediate contaminated soils containing actinides.[4, 5] Many investigations have assessed the effect of the naturally-occurring organic acids, fulvic acid and humic acid on the sorption of metal ions on inorganic substances, such as metal oxides and clay minerals.[6–8] They either increase or decrease the metal-ion sorption depending on the pH of the soil solution and properties of the inorganic substances. For example, fulvic acid increases Yb(III) sorption on alumina in acidic solutions and decreases it in alkaline solutions.[9] The hydrated Yb(III) ion did not significantly sorb on alumina at acidic pH while it showed a high affinity with it at alkaline pH. The uptake of Yb(III) in acidic solutions was enhanced due to the sorption of the fulvic acid complexed with Yb(III) on alumina; in alkaline solutions it decreased due to the formation of Yb(III)-fulvate complexes that have a low affinity for alumina. Citric acid, which is a low molecular organic substance ubiquitously found in the environment,[10] also affects the sorption of metal ions on inorganic substances. Redden et al.[11] reported that citric acid enhances the sorption of U(VI) on goethite, but reduces its sorption on kaolinite at acidic pH. These findings demonstrate that the presence of organic substances affects the mobility of metal ions.

Sorption behavior of metal ions on microorganisms has been investigated widely. Several experiments have suggested that the predominant functional groups on cell surfaces involved in the sorption of metal cations are carboxyl and phosphate functional groups.[12, 13] However, the effects of organic acids on metal-ion sorption on microorganisms still are not clearly understood. Yoshida et al.[14] reported the negative effect of a siderophore, desferrioxamine B (DFO), on the sorption of Fe(III), Eu(III), and Hf(IV) on *Pseudomonas fluorescens*, the degree of which correlated well with the stability of the DFO complexes.

In this study, we investigated Eu(III) sorption on *P. fluorescens* and the effect of citric acid on the sorption by a batch method. *Pseudomonas fluorescens* is a ubiquitous soil bacterium. Europium(III) is a good analogue for trivalent actinides, such as Am(III) and Cm(III), which are problematic elements in high-level radioactive waste. We previously reported that *P. fluorescens* did not degrade the 2:2 Eu(III)-citrate complex at pH 7 when the cell concentration was quite low.[15] In the present study, we examined the pH dependence (3–9) of Eu(III) sorption on *P. fluorescens* at high cell concentrations in the absence and presence of citric acid to assess the effect of biosorption on the migration behavior of Eu(III) and Eu(III)-citrate complexes.

2. Experimental

2.1. Culture. *Pseudomonas fluorescens* (ATCC 55241) isolated from the low-level radioactive waste disposal site at West Valley, NY, USA[16] was used. It was aerobically grown in 250 mL of nutrient medium containing the following ingredients per liter: beef extract, 3 g; polypeptone, 5 g; and, NaCl, 5 g. The culture was incubated at 30 ± 1 ˚C on a rotary shaker at 100 rpm in the dark. Cells were harvested during the early stationary phase by centrifugation at 10,000 *g* for 5 min, washed three times with a 0.1 M NaCl solution, and resuspended in the same solution.

2.2. Eu(III) sorption on *P. fluorescens*. One milliliter of the cell suspension and 50 mL of a solution containing 2 μM $EuCl_3$, 0.1 M NaCl, 5 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), and 0–1 mM citric acid were mixed in a 100-mL Erlenmeyer flask. The cell concentration was 167 ± 9 $mg_{dry\ weight}\ dm^{-3}$. The initial pH of the solution was adjusted at 3–9 with HCl or NaOH. During the five-hour exposure of the cells to Eu(III), the solution was

*Corresponding author. E-mail: h042306d@mbox.nagoya-u.ac.jp. FAX: +81-52-789-3785.

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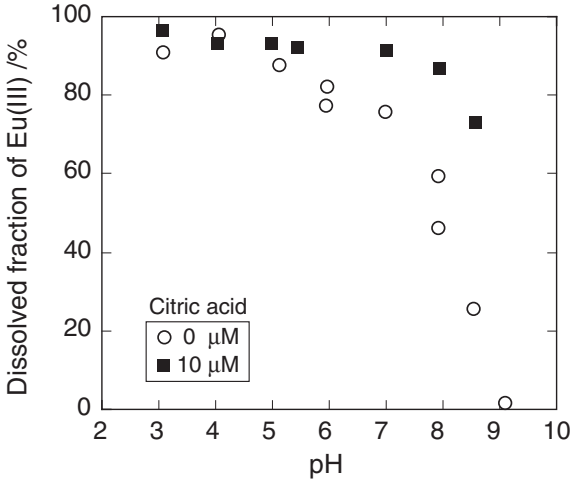

Figure 1. The pH dependence of the dissolved fraction of Eu(III) in the absence of cells. The solutions contained 2 μM Eu(III), 0.1 M NaCl, and 5 mM HEPES with or without 10 μM citric acid.

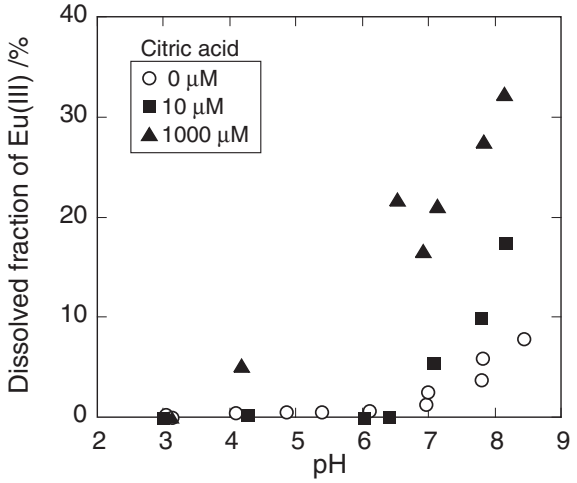

Figure 2. The pH dependence of the dissolved fraction of Eu(III) in the presence of 167 ± 9 $mg_{dry\ weight}$ L^{-1} cells. The solutions contained 2 μM Eu(III), 0.1 M NaCl, 5 mM HEPES, and 0, 100 or 1000 μM citric acid. The aqueous phase was separated with a 0.20-μm membrane filter.

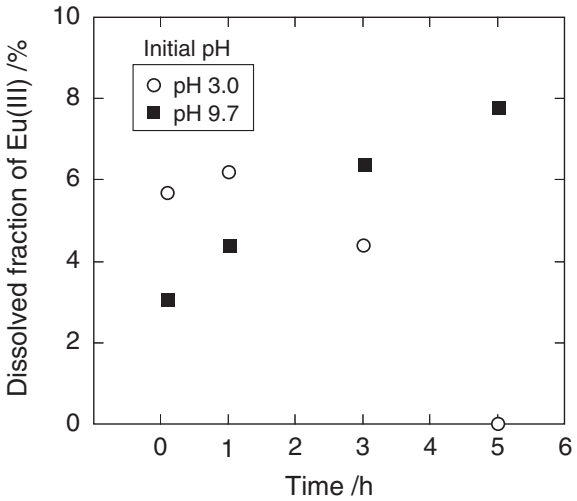

Figure 3. The time dependence of the dissolved fraction of Eu(III) in the presence of 158 $mg_{dry\ weight}$ L^{-1} cells at pH 3.0 and 9.7. The solutions contained 2 μM Eu(III), 0.1 M NaCl and 5 mM HEPES. The pH of solutions changed from 3.0 and 9.7, to 3.1 and 8.4, respectively, during 5 hours of agitation.

agitated by a stirrer. Then, an aliquot of the solution was withdrawn. The optical density at 600 nm (OD_{600}) of the aliquot was measured as an indicator of cell concentration. The withdrawn aliquot was filtered through a 0.20-μm membrane filter. The concentration of Eu(III) in the filtrate was analyzed by inductively-coupled plasma-atomic emission spectroscopy (Shimadzu ICPS-7000, Shimadzu Corporation, Kyoto, Japan). Blank tests, i.e. without cells, were conducted by the same procedures.

3. Results and Discussion

3.1. Effect of citric acid on solubility of Eu(III). Figure 1 shows the pH dependence of the dissolved fraction of Eu(III) in the absence and presence of citric acid without bacterial cells. The pH values were measured after 5 hours of agitation. In the absence of citric acid, approximately 90% of Eu(III) was dissolved in solution at pH 3. The percentage decreased slightly with an increase of pH in the acidic pH region, and had risen to approximately 75% at pH 7. The dissolved fraction decreased dramatically with a rise in pH from 7 to 9 when it was almost 0%. It was reported that the decline in the dissolved Eu(III) fraction with an increase of pH is due to the formation of Eu(III) precipitates, such as $EuOHCO_3$ or $Eu(OH)_3$.[17, 18]

In the presence of 10 μM citric acid, at acidic pHs, the dissolved Eu(III) fraction was almost comparable to that in the solution without citric acid, but above pH 6, the dissolved fraction rose. At alkaline pHs, the formation of stable Eu(III)-citrate complexes prevented the formation of insoluble Eu(III) species. These results signify that solubility of Eu(III) increases in the presence of citric acid at neutral and alkaline pHs.

3.2. Eu(III) sorption on *P. fluorescens* in the absence of citric acid. Open circles in Figure 2 represent the pH dependence of the dissolved fraction of Eu(III) in the absence and presence of citric acid at a cell concentration of 167 ± 9 $mg_{dry\ weight}$ dm^{-3}. Europium(III) was not detected in solution below pH 7, showing that almost 100% of dissolved Eu(III) was sorbed on *P. fluorescens* at these pHs. The sorption fell slightly with an increase of pH above pH 7. The sorption of Eu(III) on minerals has been widely investigated.[19, 20] Goethite and boehmite did not greatly sorb Eu(III) below pH 6, though significant sorption was observed above it.[21] On the other hand, *P. fluorescens* showed a substantially higher affinity with Eu(III) at acidic pH under the present experimental conditions. It was reported that transport of *P. fluorescens* was highly retarded in the porous medium, such as alluvium sand, quartz sand, or silica beads.[22–24] These facts indicate that *P. fluorescens* may play an important role in regulating the migration of Eu(III) in an acidic environment, wherein those minerals show less association with Eu(III).

Haas et al.[12] reported that U(VI) sorption on *Shewanella putrefaciens* decreases at alkaline pH due to formation of $UO_2(CO_3)_3^{4-}$ and $UO_2(OH)_3^-$. These species are repulsed by the cell surface that is negatively charged at alkaline pH. In this study, the decrease in Eu(III) sorption is not attributable to the formation of anionic complexes because concentrations of dissolved anionic hydrolytic and carbonate Eu(III) species at pH 7–9 were calculated to be less than 0.02 μM,[25] which corresponds to < 1% of the total Eu(III) concentration present in solution.

Figure 3 shows the time dependence of the dissolved fraction of Eu(III) in the presence of 158 $mg_{dry\ weight}$ dm^{-3} cells at pHs 3.0 and 9.7. In the solution whose initial pH was 3.0 (final pH, 3.1), 94% of Eu(III) was sorbed on *P. fluorescens* within 5 min, wherein the amounts of sorbed Eu(III) increased with time. After 5 hours contact, almost 100% was sorbed on the cells. In the solution whose initial pH was 9.7 (final pH, 8.4), 97% of Eu(III) was sorbed on *P. fluorescens* within 5 min; thereafter, the amount declined with time, and after 5 hours contact, the percentage of Eu(III) sorbed was 92%. The OD_{600} value of the solution before and after the 5 hours contact

were 0.455 and 0.450, respectively, showing the cell concentration in the solution was almost constant during the contact. Ozaki et al.[26] observed that the desorption of Eu(III) originally sorbed on *Chlorella vulgaris* occurred via exudates from the cells. In the present study, the decreasing trend in Eu(III) sorption with time at alkaline pH suggested that *P. fluorescens* excreted some organic substance which desorbed Eu(III) on the cell surface by complexation with Eu(III).

3.3. Eu(III) sorption on *P. fluorescens* in the presence of citric acid. Figure 2 shows the pH dependence of the dissolved fraction of Eu(III) in the presence of citric acid at a cell concentration of 167 ± 9 $mg_{dry\ weight}$ dm^{-3}. With 100 μM citric acid, Eu(III) was almost completely sorbed on the cells below pH 7; Eu(III) sorption decreased by 2–8% compared to that in the absence of citric acid above pH 7. In the presence of 1000 μM citric acid, Eu(III) was completely sorbed on the cells at pH 3, and the sorption of Eu(III) decreased with a rise in pH. This decrease in Eu(III) sorption on *P. fluorescens* with an increase of citric-acid concentration suggests that citric acid competes for Eu(III) with functional groups on the cell's surface. The fall in Eu(III) sorption on the cells was significant at alkaline pH, suggesting that stability of Eu(III)-citrate complexes increases with a rise in pH, and this increasing tendency is greater than that of Eu(III)-cell-surface complexes. Thus, citric acid apparently reduces the sorption of trivalent actinides on microorganisms, especially at alkaline pHs, by forming stable complexes with them.

4. Conclusions

We investigated Eu(III) sorption on *P. fluorescens* and the effect of citric acid on the sorption by a batch method. The cell walls of *P. fluorescens* displayed a high affinity for Eu(III) in acidic pHs, indicating *P. fluorescens* retained in geomedia may retard the migration of trivalent actinides in the acidic environment. In alkaline pHs, sorption of Eu(III) on *P. fluorescens* was inhibited by exudates released from the cells. In the presence of citric acid, citric acid competes for Eu(III) with functional groups on the cell's surface, consequently, sorption of Eu(III) decreased in alkaline pHs. These findings suggest that the both reactions, the biosorption and the complex formation with organic acids, are important for estimating the behavior of trivalent actinides in the environments.

References

(1) Y. Takahashi and Y. Minai, J. Nucl. Radiochem. Sci. **5**, 37 (2004).
(2) C. Moulin, J. Wei, P. V. Iseghem, I. Laszak, G. Plancque, and V. Moulin, Anal. Chim. Acta **396**, 253 (1999).
(3) M. J. Keith-Roach and F. R. Livens, *Interaction of Microorganisms with Radionuclides* (Elsevier Science Ltd, Oxford, 2002).
(4) J. E. Banaszak, B. E. Rittmann, and D. T. Reed, J. Radioanal. Nucl. Chem. **241**, 385 (1999).
(5) A. J. Francis, J. Alloys Comp. **271-273**, 78 (1998).
(6) P. Reiller, V. Moulin, F. Casanova, and C. Dautel, Appl. Geochem. **17**, 1551 (2002).
(7) Y. Takahashi, T. Kimura, and Y. Minai, Geochim. Cosmochim. Acta **66**, 1 (2002).
(8) Y. Takahashi, Y. Minai, T. Kimura, and T. Tominaga, J. Radioanal. Nucl. Chem. **234**, 277 (1998).
(9) X. K. Wang, W. M. Dong, X. X. Dai, A. X. Wang, J. Z. Du, and Z. Y. Tao, Appl. Radiat. Isotopes **52**, 165 (2000).
(10) S. Buruckert, Ann. Agron. **21,** 421 (1970).
(11) G. D. Redden, J. Li, and J. O. Leckie, *Adsorption of Metals by Geomedia*, ed. E. A. Jenne, Academic Press, San Diego, CA. (1998), Ch. 13.
(12) J. R. Haas, T. J. Dichristina, and R. Wade Jr., Chem. Geol. **180**, 33 (2001).
(13) J. B. Fein, A. M. Martin, and P. G. Wightman, Geochim. Cosmochim. Acta **65**, 4267 (2001).
(14) Y. Yoshida, T. Ozaki, T. Ohnuki, and A. J. Francis, Radiochim. Acta **92**, 749 (2004).
(15) Y. Suzuki, T. Nankawa, Y. Yoshida, T. Ozaki, T. Ohnuki, A. J. Francis, S. Tsushima, Y. Enokida, and I. Yamamoto, J. Radioanal. Nucl. Chem. **266**, (2005) (in press).
(16) A. J. Francis, C. J. Dodge, and J. B. Gillow, Nature **356**, 140 (1992).
(17) W. Runde, G. Meinrath, and J. I. Kim, Radiochim. Acta **58/59**, 93 (1992).
(18) E. N. Rizkalla and G. R. Choppin, *Handbook on the Physics and Chemistry of Rare Earths*, Vol. 15, eds. K. A. Jr. Gshneidner and L. Eyring, Elsevier, New York (1991), Ch. 103.
(19) H. Catalette, J. Dumonceau, and P. Ollar, J. Contam. Hydrol. **35**, 151 (1998).
(20) T. Rabung, H. Geckeis, J. I. Kim, and H. P. Beck, J. Colloid Interface Sci. **208**, 153 (1998).
(21) S. M. Kraemer, J. Xu, K. N. Raymond, and G. Sposito, Environ. Sci. Technol. **36**, 1287 (2002).
(22) G. Chen, M. Rockhold, and K. A. Strevett, Res. Microbiol. **154**, 175 (2003).
(23) D. G. Jewett, B. E. Logan, R. G. Arnold, and R. C. Bales, J. Contam. Hydrol. **36**, 73 (1999).
(24) T. Kinoshita, R. C. Bales, M. T. Yahya, and C. P. Gerba, Wat. Res. **27**, 1295 (1993).
(25) Y. Takahashi, A. Tada, and H. Shimizu, Anal. Sci. **20**, 1301 (2004).
(26) T. Ozaki, T. Kimura, T. Ohnuki, Z. Yoshida, and A. J. Francis, Environ. Toxicol. Chem. **22**, 2800 (2003).