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Journal of Alloys and Compounds 408–412 (2006) 1334–1338

Journal of ALLOYS AND COMPOUNDS

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Interactions of rare earth elements with bacteria and organic ligands

Takuo Ozaki [a,*], Yoshinori Suzuki [b], Takuya Nankawa [a], Takahiro Yoshida [a], Toshihiko Ohnuki [a], Takaumi Kimura [c], Arokiasamy J. Francis [d]

[a] *Advanced Science Research Center, Japan Atomic Energy Research Institute, Tokai, Ibaraki 319-1195, Japan*

[b] *Department of Materials, Physics and Energy Engineering, Nagoya University, Furocho, Chikusa, Nagoya 464-8603, Japan*

[c] *Department of Materials Sciences, Japan Atomic Energy Research Institute, Tokai, Ibaraki 319-1195, Japan*

[d] *Environmental Sciences Department, Brookhaven National Laboratory, Upton, NY 11973, USA*

Available online 13 June 2005

Abstract

We investigated the interactions of rare earth elements (REEs) Eu(III) and/or Ce(III, IV) with the common soil bacterium *Pseudomonas fluorescens* and organic ligands, such as malic acid, citric acid, a siderophore (DFO), cellulose, chitin, and chitosan. Malic acid formed complexes with Eu(III), but degradation of malic acid was observed when the ratio of malic acid to Eu(III) was higher than 100. Citric acid formed a stoichiometric complex with Eu(III) that was not degraded by *P. fluorescens*. Adsorption of Eu(III) from the DFO complex occurred as a free ion dissociated from DFO and not as the Eu(III)–DFO complex. Cerium(III) was oxidized to Ce(IV) during complexation with DFO to form the Ce(IV)–DFO complex. Time-resolved laser-induced fluorescence spectroscopy (TRLFS) analysis showed that cellulose, chitin, and chitosan, respectively, formed a weak complex, an inner-spherical complex, and an outer-spherical complex with Eu(III). This method also demonstrated that the coordination environment of Eu(III) adsorbed on *P. fluorescens* possessed similar characteristics to that of chitin, and revealed that adsorption of Eu(III) on *P. fluorescens* was through a multidentate and predominantly inner-spherical coordination.

Keywords: Rare earth elements; Bacteria; Organic ligands; Coordination environment; Biodegradation

1. Introduction

Rare earth elements (REEs) are widely used in industry [1], agriculture [2], and medicine [3]. Both radioactive and stable REEs can enter the food chain, resulting in their intake by humans. Thus, it has become increasingly important to know the behavior of these elements in the environment. There is particular concern about Cm(III) and Am(III), which are highly toxic because of their emission of high-energy α particles. Consequently, studies of the environmental behavior of Eu(III) that has similar chemical properties are very useful in predicting the behavior of Cm(III) and Am(III).

The environmental behavior of REEs is affected by biotic and abiotic factors. The former include interactions with microorganisms, plants, and their originated substances, such as citric acid and humic substances. Plant roots adsorb REEs on their surfaces [4]. The affinities of REEs in soil increases with a rise in pH, with substantial adsorption taking place at alkaline pHs [5] as well as complexation with humic acids [6]. We stress that little is known of the interactions of REEs with microorganisms and organic ligands. However, having such knowledge would help us to clarify the behavior of radionuclides with similar chemical properties.

Studies of the coordination environment of metals adsorbed on microorganisms facilitate our understanding of their associations. Time-resolved laser-induced fluorescence spectroscopy (TRLFS) is a non-destructive technique that has been used to determine the coordination environment of Eu(III) adsorbed on the surface of a solid or microorganisms in the presence of water [7,8]. For example, using TRLFS to evaluate the coordination environment of Eu(III) adsorbed

* Corresponding author.
E-mail address: tozaki@popsvr.tokai.jaeri.go.jp (T. Ozaki).

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doi:10.1016/j.jallcom.2005.04.142

on *Chlorella vulgaris* showed that functional groups in cellulose play an important role in the adsorption of Eu(III) on cell walls [9].

In this paper, we summarize our findings on the interactions of REEs with the common soil bacterium *Pseudomonas fluorescens* and organic ligands. The coordination environment of Eu(III) on *P. fluorescens* was determined by TRLFS, and compared with that on cellulose, chitin, and chitosan.

2. Effect of Eu(III) on the degradation of malic acid and citric acid by *P. fluorescens*

The effects of metals on the biodegradability of organic substances have been widely examined. Most investigations focused on organic substances with high chelating ability, such as NTA and EDTA [10,11]; a few focused on ones with low chelating ability. However, organic substances, such as lactic acid and ascorbic acid, with relatively low chelating abilities with REEs are reported to affect their uptake by plants [12]. We found that the degradation rates of malic acid by the bacterium decreased with an increasing ratio of Eu(III) to malic acid concentrations in culture medium containing low concentrations of essential elements [13] and malic acid as a sole carbon source (Fig. 1). In the absence of malic acid, the decrease of Eu(III) from solution was not observed (data not shown). In the medium with the lowest ratio of malic acid to Eu(III) concentrations, malic acid was not degraded, even though an excess of the acid was present. This finding suggests that malic acid and Eu(III) did not form a stable complex, and that Eu(III) exerted toxic effects on the metabolic activity of *P. fluorescens* [13]. A decrease in the toxicity of metals by complexation with organic substances has been widely reported [14,15]. Malic acid can mask the toxicity of Eu(III) through complexation and degradation of malic acid was observed when the ratio of malic acid to Eu(III) was higher than 100.

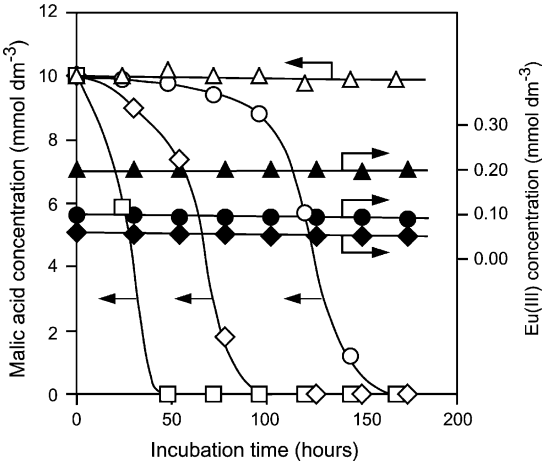

Fig. 1. Concentration of malic acid and Eu(III) after the inoculation of *P. fluorescens*. Malic acid concentration in the medium whose original Eu(III) concentrations were 0, 0.05, 0.10, and 0.20 mmol dm^{-3} is represented as (□), (◊), (○), and (△), respectively. Europium(III) concentration in the media whose original Eu(III) concentrations were 0.05, 0.10, and 0.20 mmol dm^{-3} is represented as (♦), (●), and (▲), respectively [9].

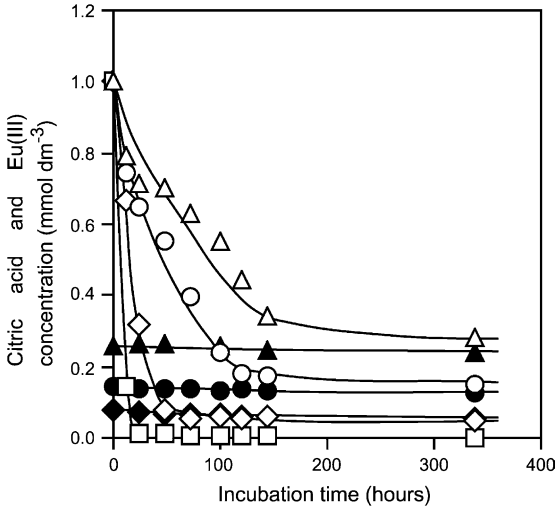

Fig. 2. Concentration of citric acid and Eu(III) after the inoculation of *P. fluorescens*. Citric acid concentration in the medium whose original Eu(III) concentrations were 0, 0.075, 0.15, and 0.25 mmol dm^{-3} is represented as (□), (◊), (○), and (△), respectively. Europium(III) concentration in the media whose original Eu(III) concentrations were 0.075, 0.15, and 0.25 mmol dm^{-3} is represented as (♦), (●), and (▲), respectively [13]. The data show that the final concentration of Eu(III):citric acid reached 1:1 for all treatments.

In contrast, the biodegradation of a complex of Eu(III)–citrate by *P. fluorescens* differed. In all culture media with citric acid as the sole carbon source, the concentration of citric acid decreased with time until it reached that of Eu(III) (Fig. 2). The rates of decrease were slower in the media with higher Eu(III) concentrations, while almost no change was observed in the Eu(III) concentration [16]. If the medium contained 10 mmol dm^{-3} citric acid for each Eu(III) concentration, as in the malic acid experiment, the rate of its decrease in the medium would be higher than the one in the comparable medium in the present study because the amount of free citric acid, which *P. fluorescens* can metabolize for growth, is greater, and thus, degradation would be accelerated. Attempts have been made to elucidate the factors that determine the degradability of a particular metal–organic acid complex. Klüner et al. [11] showed that the biodegradability of metal–EDTA complexes by certain bacteria is not completely in accordance with their stability. Firestone and Tiedje [10] suggested that the structure of complexes containing NTA determines their biodegradability. Joshi-Tope and Francis [17] also claimed that the biodegradability of metal–citric acid complexes by *P. fluorescens* depends on their structure, showing that mononuclear bidentate complexes are readily degraded, whereas mononuclear tridentate, binuclear, and polynuclear complexes are recalcitrant. The results of this present study show that a 1:1 Eu(III)–citric acid complex is recalcitrant to degradation by *P. fluorescens* [16].

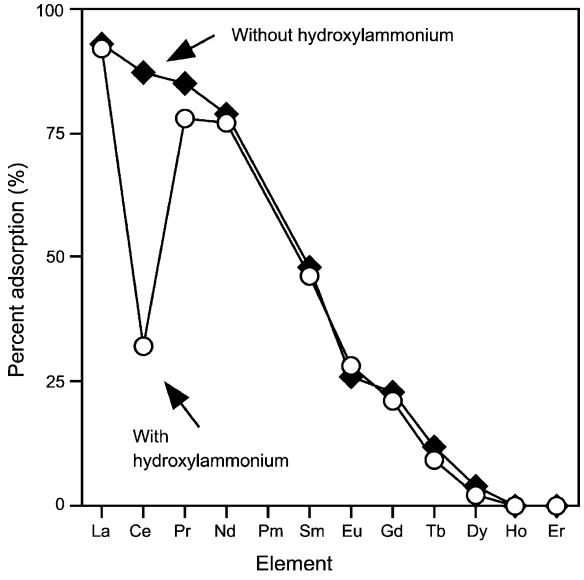

Fig. 3. Percent adsorption of rare earth elements on *P. fluorescens* cells after 30 min contact. (◯) and (♦) represent the percent adsorption of REEs in the absence and presence of hydroxylammonium, respectively [19].

3. Effect of DFO on the adsorption of rare earth elements on *P. fluorescens*

Various microorganisms excrete a siderophore to solubilize and then utilize Fe(III). Desferrioxamine B (DFO) is a trihydroxamate siderophore ubiquitous in the environment [18]. DFO is not available either as free DFO or the DFO–REEs complexes to *P. fluorescens* [19]. The stability constant ($\log K$) of the Eu(III)–DFO complex is 15 [20], whereas that of Eu(III)–citric and Eu(III)–malic acid complexes is 7.77 and 4.85, respectively [21]. We examined the effect of DFO on the adsorption of REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, and Er). The percent adsorption of REEs determined after 30 min contact with 1.0 mg dm^{-3} of each REE (7.2 (La(III))—6.0 mol dm^{-3} (Er(III)) and 0.5 mmol dm^{-3} DFO decreased roughly in relation to its atomic number, except Ce (Fig. 3)). The percent adsorption of Ce was significantly lower than those of its neighboring REEs, La(III) and Pr(III). We expected the stability constant of the Ce(III)–DFO complex to be intermediate or comparable to that of the La(III)–DFO and Pr(III)–DFO complexes. A XANES study showed that the oxidation state of Ce in the DFO complex was tetravalent [22]. Therefore, the anomalously lower Ce adsorption was due to the higher stability of the Ce(IV)–DFO complex than that of the Ce(III)–DFO complex. After adding hydroxylammonium (a reducing agent), the Ce anomaly disappeared. The spontaneous oxidation of Ce in the DFO complex is due to the extremely large ratio of the stability of Ce(IV)–DFO in comparison to that of Ce(III)–DFO [22]. The distribution of trivalent REEs between water and particles shows a gradual variation with their atomic number because of the lanthanide contraction. Cerium is the only REE with redox transformation at ambient aquatic conditions [23]. The REE patterns for environmental waters often show a lower abundance of Ce compared with the neighboring REEs, which is called the negative Ce anomaly, resulting from the higher tendency of Ce(IV) to adsorb on particles than the trivalent REEs [24]. We note that naturally occurring organic ligands, such as DFO, can contribute to the positive Ce anomaly in environmental waters.

4. Coordination environment of Eu(III) on *P. fluorescens*, cellulose, chitin, and chitosan

Using TRLFS, the coordination environment of Eu(III) adsorbed on the solid–water interface can be estimated. The fluorescence lifetime of excited Eu(III) is related to the number of water molecules in the inner-sphere (N_{H_2O}) [25]. The relative intensity ($R_{E/M}$) of the $^5D_0 \rightarrow {}^7F_2$ (electric dipole) and $^5D_0 \rightarrow {}^7F_1$ (magnetic dipole) emissions is related to the strength of ligand field of Eu(III) in both the inner-sphere and outer-sphere [26]. The unknown coordination environments of Eu(III) can be characterized by the location of $\Delta N_{H_2O}(=9 - N_{H_2O})$ and $R_{E/M}$ plotted on the coordination–environment diagram [27]. From published data, we assumed that the number of water molecules in the inner-sphere of hydrated Eu(III) ion was nine [28]. Therefore, ΔN_{H_2O} represents the number of coordination sites occupied in the inner-sphere of Eu(III) by ligands other than water molecules. In this study, the adsorption of hydrolyzed Eu(III) species was not observed: hydrolyzed Eu(III) exhibits extremely short luminescence lifetimes τ_{obs}^{-1} [29]. Fig. 4 shows the distribution on the coordination–environment

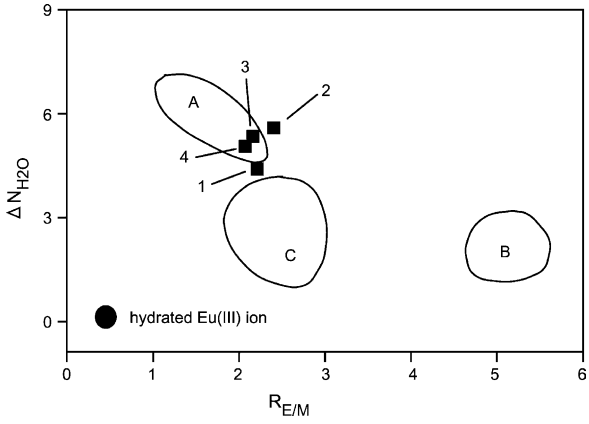

Fig. 4. Coordination–environment diagram obtained for Eu(III) adsorbed on *P. fluorescens* cells, (●) represents the results obtained for the hydrated Eu(III) ion with no interaction other than with water molecules. pHs are as follows: (1) 6.45, (2) 6.60, (3) 7.10, and (4) 7.16. Areas A–C show the distribution of $R_{E/M}$–ΔN_{H_2O} plots obtained for Eu(III) adsorbed on chitin, chitosan, and cellulose [27].

diagram of $R_{E/M}$–ΔN_{H_2O} plots of Eu(III) on the biopolymers cellulose, chitin, and chitosan. The number of water molecules removed from the inner-sphere of Eu(III) adsorbed on chitin is larger than those from cellulose and chitosan. The coordination of chitosan and chitin to metal cations occurs mainly through the amine nitrogen atom. The inner-spherical coordination of Eu(III) observed for chitin may reflect the existence of carbonyl oxygen, which has a hard character with a high affinity for the hard metal ion, Eu(III) [8]. The ligand field of Eu(III) adsorbed on chitosan is stronger than that on cellulose and chitin. The coordination environment of Eu(III) adsorbed on *P. fluorescens* showed the same characteristics as the one seen for chitin, indicating that the latter adsorption was both through multidentate and predominantly inner-spherical coordination [8]. *P. fluorescens* has carboxylic and phosphate functional groups on its surface. Texier et al. [30] examined the binding sites for Eu(III) on a Gram-negative bacterium, *Pseudomonas aeruginosa,* and demonstrated that carboxyl functional groups are mainly responsible for Eu(III) adsorption. Furthermore, Takahashi et al. showed that on weakly acidic ion-exchange resin, Eu(III) is adsorbed on these functional groups in an inner-spherical manner [31]. These findings suggest that the Eu(III) adsorbed on *P. fluorescens* cells is exchangeable with competing cations, as is the case for weakly acidic ion-exchange resins. The presence of an equimolar Na^+ inhibits the adsorption of La(III) on *P. aeruginosa* by about 23%, indicating an effective competition between these two elements [32]. Our findings show that TRLFS can reveal fine-scale complexity in the coordination environment of REEs and trivalent actinides adsorbed to bacteria.

5. Conclusions

Our study showed that Eu(III) preferentially adsorbs on bacterial cells in the presence of organic ligands with low chelating ability. The subsequent adsorption of Eu(III) on the surface of *P. fluorescens* cells is through an inner-spherical process, wherein its adsorption/desorption behavior may be greatly affected by competing cations. However, in some cases, organic substances with low chelating ability can effectively decrease the adsorption of Eu(III) on bacterial cells by their being bacterially transformed into ones with higher chelating ability. For organic ligands with high chelating ability, the biodegradation of free organic ligands proceeds until Eu(III) and the organic acid form a recalcitrant stoichiometric complex. An organic ligand that is not available to bacteria also affects the environmental behavior of REEs. A trihydroxamate siderophore, DFO, with high chelating ability forms a complex with Ce, generating a significantly stable structure, Ce(IV)–DFO, compared to Ce(III)–DFO. Organic ligands with high chelating ability can from a complex with Ce(IV), thereby leading to the anomalously lower percent adsorption reflecting the ionic-radius dependence among REEs.

Acknowledgments

The present study was partially supported by the REIMEI Research Resources of Japan Atomic Energy Research Institute and by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science to T.O.

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