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Biological nano-mineralization of Ce phosphate by *Saccharomyces cerevisiae*

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ABSTRACT

In order to understand the nanoscale mineralization process of REE phosphate on microorganism surfaces, we have investigated the mechanism underlying Ce sequestration by yeast *Saccharomyces cerevisiae* after exposure to Ce(III) solution for 4–120 h at pH 3, 4, or 5. A variety of analytical techniques have been employed, including field-emission scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (FESEM-EDS), transmission electron microscopy (TEM), inductively coupled plasma atomic emission spectrometry (ICP-AES), and synchrotron-based X-ray absorption fine structure (XAFS).
Cerium concentration in solutions decreases as a function of exposure time and more rapidly at pH 5 than at pH 3 or 4. Analyses of the yeast cells by FESEM, TEM, and XAFS show that needle-shaped Ce(III) phosphate nanocrystallites with a monazite structure formed on the yeast cells by exposure to Ce(III) for 42 h, even though the initial solutions did not contain any P species. The Ce(III) phosphate nanocrystals grew from about 50 nm to hundreds of nanometers when pH increased from 3 to 5. Lower pH resulted in higher P concentration in the solution after the yeast cells were inoculated, indicating the release of P from the yeast cells. These results suggest that the sorbed Ce on the cell surfaces reacted with P released from inside the yeast cell, resulting in the formation of Ce(III) phosphate nanocrystallites. This post-sorption nanocrystallization on the microbial cell surface should play a key role in constraining the long-term migration of REEs and trivalent actinides in geological repositories.

1. Introduction

The application of rare earth elements (REEs) to medicine and industry has dramatically increased in the past two decades (Haxel et al., 2002). Furthermore, fission-genic REEs and trivalent actinides, which behave similarly in chemistry (Choppin, 1983, 1995), are contained in high-level radioactive waste. These short- and long-lived radionuclides immobilized as a waste form are eventually stored in geological repositories. These anthropogenic REEs can be potentially released into ambient environments in case of package failure and will make contact with groundwater within a geological period.

The mobility of REEs in groundwater is greatly influenced by their interaction with inorganic and organic components and by complex formations with inorganic and organic ligands (e.g., Takahashi et al., 1997; Ingri et al., 2000; Yoshida et al., 2004). In addition, several recent studies have reported that the migration of toxic metals is affected by microorganisms present in nature due to their high capacity to adsorb metal cations including REEs and actinides (e.g., Beveridge and Doyle, 1989; Fein et al., 1997; Daughney and Fein, 1998; Fein et al., 2001; Châtellier and Fortin, 2004; Ohnuki et al., 2005a; Ozaki et al., 2005, 2006; Tsuruta, 2006; Ngwenya et al., 2009). They have indicated that REEs are adsorbed on the functional groups of cell surfaces and that the adsorption equilibrium can be achieved within 60 min. However, the question remains whether or not the adsorbed REEs change their chemical states on a geological time scale.

The mineralization of Fe, Mn, and U by different kinds of microorganisms has been reported (Macaskie and Dean, 1985; Mann et al., 1987; Lovley et al., 1991, 1993; Fortin et al., 1997; Tebo et al., 1997; Gillan and Ridder, 2001; Tebo et al., 2005; Ohnuki et al., 2005b, 2008; Sturm et al., 2008). These facts suggest that the biomineralization of REEs is a possible chemical state after adsorption. However, to our knowledge there is no report on the biomineralization of REEs. To elucidate the biomineralization of REEs, we should understand in detail the mechanism, kinetics, and subsequent phase formation in REE immobilization.

In the present study, the mechanism of Ce(III) biomineralization by *Saccharomyces cerevisiae* has been investigated in detail utilizing field-emission scanning electron microscopy (FESEM) with energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), and X-ray absorption near-edge structure (XANES) analysis. Cerium was selected as a representative element of light REEs and as a multivalent fission product (Ce(III)/Ce(IV)) (Johnson and Kyker, 1966; Ohnuki et al., 2008). In general, Ce(III) can be oxidized to form insoluble CeO_2 (Braun et al., 1990), causing anomalies in REE patterns in the

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environment (Ohta and Kawabe, 2001; Davranche et al., 2008). Migration of LREE including Ce in soil and surface water systems is closely related to the occurrence of phosphate minerals such as monazite (Aubert et al., 2001). *Saccharomyces cerevisiae* was selected as a test organism because the physiological transport of other cations (U(VI), Cs(I), Cd(II), Pb(II), Sr(II), Zn(II)) by this organism has been well studied (Ohnuki et al., 2003, 2005a; Naeem et al., 2006).

2. Experimental

2.1. Cerium uptake experiments

Saccharomyces cerevisiae X-2180 was grown in 150 mL of sterilized YPD medium made with 10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and 20 g L^{-1} dextrose. The yeast was incubated for 2 days on a rotary shaker at 110 rpm at 30 ± 1 °C. Then the cells were harvested by centrifugation for 10 min at 8.41×10^3 rpm; they were washed three times with 0.1 mol L^{-1} NaCl solution.

Harvested cells were transferred into polycarbonate tubes of a 30 mL aqueous solution containing 1.79×10^{-4} mol L^{-1} or 1.79×10^{-5} mol L^{-1} Ce(III), and 0.01 mol L^{-1} NaCl. The absorbance of the cells in this exposure solution reached 1.0 at 600 nm, as analyzed by a spectrophotometer, corresponding to a cell density of 0.56 dry g L^{-1} in all the exposure solutions. The pHs of the exposure solutions were adjusted to 3.0 ± 0.1, 4.0 ± 0.1, and 5.0 ± 0.1 by mixing 1.0 mol L^{-1} NaOH and 1.0 mol L^{-1} HCl solution in certain proportions. Duplicate exposure solutions were incubated stationary for 120 h at 25 ± 1 °C. To examine the reversibility of the accumulated Ce by the cells, exposure solutions containing 1.79×10^{-5} mol L^{-1} Ce were re-adjusted to pH of 3.0 ± 0.1 after exposure for 120 h, and were kept in stationary condition for 24 h at 25 ± 1 °C (Gorman-Lewis et al., 2005).

The supernatants were withdrawn at 2, 4, 20, 44, 68, 96, 120, and 144 h from the exposure solutions, from the control solutions containing 1.79×10^{-4} mol L^{-1} Ce without yeast cells, and from the yeast cell solutions containing only yeast cells without Ce. The supernatants were then centrifuged at 10^4 rpm for 10 min to remove most of the cells. The pH and the concentrations of Ce(III) and P in the supernatants were measured.

At 42, 68, and 120 h after exposure to a 1.79×10^{-4} mol L^{-1} Ce solution, the yeast cells were separated from the solution by centrifugation at 4000 rpm for 10 min and washed repeatedly with deionized water to remove any Ce-bearing solution retained through surface tension. The yeast cells were subsequently analyzed by FESEM for the samples obtained at 42 h and 120 h, by TEM for the samples obtained at 68 h, and by XANES for the samples obtained at 120 h.

2.2. Analytical methods

The concentrations of Ce(III) and P were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES; Shimadzu ICPS-7000) after the supernatants were filtered with a hydrophilic PTFE filter (0.20 μm; Advantec, Tokyo) and diluted appropriate times in 0.1 mol L^{-1} HNO_3. A TOA HM-30S pH meter with a combined electrode of TOA GS-5015C was used to measure the pHs of the solutions.

The yeast cell surfaces were analyzed by FESEM (JEOL-6330F) at 5 kV or 15 kV. The elemental compositions of the yeast cells were determined by FESEM equipped with an energy-dispersive X-ray analyzer (EDS; JED-2140) at an operating voltage of 15 kV.

Yeast cells exposed to a 1.79×10^{-4} mol L^{-1} Ce(III) solution at pH 5 for 68 h were analyzed by TEM (FEI TECNAI-F20) equipped with EDS at an acceleration voltage of 200 kV. Z-contrast imaging was performed in high-angle annular dark-field scanning TEM (HAADF-STEM) mode with FEI TIA software (Utsunomiya and Ewing, 2003). Three-dimensional textures of the exposed yeast cells were created by electron tomography in HAADF-STEM mode. Unstained samples were prepared by drying diluted (×200) aliquots of suspension on holey carbon films.

The oxidation states of Ce precipitated on yeast cell surfaces were determined by XANES analysis. The Ce L_{III}-edge XANES spectra were obtained at beamline 27B at the Photon Factory of High Energy Research Organization (Tsukuba, Japan). XANES spectra of the powder samples of $Ce(III)Cl_3\cdot6H_2O$, $Ce(IV)(SO_4)_2$, and $Ce(IV)O_2$ were used as standards for Ce(III) and Ce(IV), respectively. The energy of the XANES spectrum of Ce was calibrated by assigning the first absorption edge at 5730 eV in the spectrum of Ce in CeO_2. All the measurements were carried out in fluorescence mode using a seven-element Ge solid-state detector (SSD) under ambient conditions.

2.3. Viability of yeast exposed to Ce solution

The viability of the yeast cells was estimated by the classical methylene blue straining technique (Paul, 1976). The yeast cells were exposed to 1.79×10^{-4} mol L^{-1} Ce solution at pH 3, 4, or 5 for 120 h. For a control, the yeast cells were exposed to Ce-free solution for 120 h. A yeast exposure solution (four replicates) was diluted with sterilized 0.9% physiological saline solution to the appropriate cell density when it was used to analyze cell viability. The measuring solution contained 1 ml diluted exposure solution, 2 ml phosphate-buffered saline (pH 4.6), and 2 ml methylene blue solution (0.02%). The viability of yeast cells was determined by microscopy with a hemocytometer (ERMA Inc., Tokyo, Japan). The dead cells were dyed blue. In the viability experiments, all solutions were sterilized in an autoclave for 20 min at 120 °C.

2.4. Thermodynamics of Ce(III) mineralization

Since the formation of Ce minerals depends on the chemical compositions of solution, the activity diagram of Ce was calculated by using the Geochemist workbench software package in Professional 6.0, Act2 (Bethke, 2001) based on the database from the current release of Visual Minteq (Felmy et al., 1984). The calculation was performed for the conditions of 0.01 mol L^{-1} NaCl at 25 °C at pH 3, 4, 5, or 6.

3. Results

3.1. Time-dependent accumulation of Ce(III) by S. cerevisiae

Fig. 1 shows time-dependent concentrations of Ce (a) and P (b) in the exposure solution containing yeast cells and Ce of 1.79×10^{-4} mol L^{-1} at pH 3, 4, or 5. Note that the errors bars of the Ce and P concentrations overlapped with the symbols. Hereafter, the error bars were not plotted unless the error bar was plotted beyond a symbol. The Ce concentration decreased with exposure time. At pH 3 or 4, more than 95% of Ce was dissolved in the solution within 2 h. The Ce concentration decreased slightly up to 96 h at pH 3 and then abruptly descended to ~60% at 120 h. At pH 4, the Ce concentration gradually decreased to approximately 27% of Ce after 120 h. At pH 5, the Ce concentration rapidly decreased and eventually fell below the detection limit after 96 h. No visible precipitates were observed in the exposure solution except for the yeast cells. The pH increased gradually to pH 3.7 ± 0.1, pH 6.0 ± 0.1, and pH 6.2 ± 0.1 after 120 h for the initial pH 3, pH 4, and pH 5 solutions, respectively.

On the other hand, the average concentrations of Ce in the control solutions containing Ce without yeast cells at pH 3, 4, and 5 (Fig. 1a) were constant for 120 h, indicating that the solutions were undersaturated with respect to solid Ce(III)-oxy/hydroxides. Further, Ce(III) sorption on the tube's walls was less than 0.1% of the initial concentration over 120 h.

The P concentration at pH 3 increased with increasing exposure time (Fig. 1b). At pH 3, approximately 1.9×10^{-6} mol L^{-1} of P was dissolved after 2 h of exposure, and the P concentration gradually increased to ~8.0×10^{-5} mol L^{-1} for 120 h. In the solutions at pH 4, the P concentration was kept around the detection limit of 1.1×10^{-6} mol L^{-1}, increasing slightly to about 2.0×10^{-6} mol L^{-1} at 120 h. At