新研究グループの発足に際して

Heavy Elements Microbiology Research

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Microorganisms play a major role in the transformations of radionuclides and toxic metals in nature. There is a paucity of information on the mechanisms biotransformation of radionuclides by microorganisms. An understanding at the fundamental level the mechanisms of mobilization, immobilization, and bioavailability of actinides is important from the standpoint of mobility and stability in the environment, disposal of radioactive wastes in deep geological formations, and remediation of contaminated sites and materials.

The microbiology research group is conducting basic scientific research on interactions of microorganisms with actinides. The overall objective of this research is to elucidate at the fundamental level the mechanisms of interaction of heavy elements (lanthanides and actinides) with microorganisms. Pure and mixed cultures of known microorganisms and microorganisms isolated from contaminated sites are being investigated under environmentally relevant conditions (aerobic and anaerobic) and in the presence of appropriate electron donors and acceptors. The heavy elements that are examined include Eu, Cm, U, Pu, Np and Am.

We are investigating the mechanisms of (i) association of selected actinides and lanthanides with microorganisms and (ii) biotransformation (dissolution, precipitation and fate) of the various chemical forms of the actinides. State-of-the-art spectroscopic techniques include, X-ray absorption near edge structure (XANES) and X-ray Photoelectron Spectrometry (XPS) to determine the oxidation states, extended X-ray absorption fine structure (EXAFS) to determine coordination with the nearest neighbor, time-resolved laser-induced fluorescence spectroscopy (TRLFS) to determine the coordination number and nature of the complex (inner-sphere, outer-sphere complex), laser-induced breakdown spectroscopy (LIBS) to determine the colloids and biocolloids, and electrochemical techniques to determine the transport of actinides in artificial biomembrane systems.

This is a multi-disciplinary research team involving expertise in microbiology, biochemistry, geochemistry, molecular biology, radiochemistry, and electrochemistry. Selected areas of on going research are highlighted here.

Coordination of Eu(Ⅲ) and Cm(Ⅲ) with the Cell Surfaces of Microorganisms. The kinetics and distribution coefficients of Eu(Ⅲ) and Cm(Ⅲ) on the cell surfaces of *Chlorella vulgaris, Bacillus subtilis, Pseudomonas fluorescens, Halomonas* sp., *Halobacterium salinarum*, and *Halobacterium halobium* were determined by batch experiments. The coordination

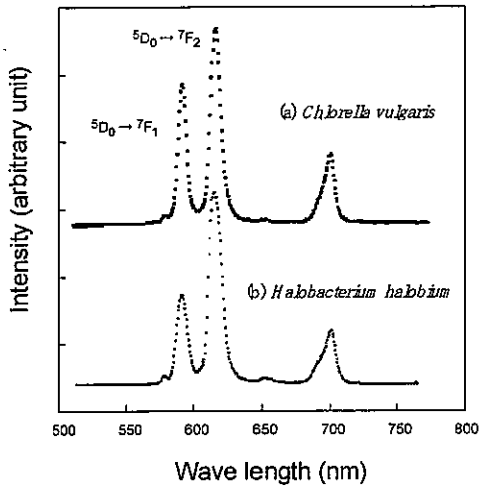

Fig.1 Typcal emission spectra of Eu(Ⅲ) excited at 394 nm obtained for (a) *Chlorella vulgans* and (b) *Halobacterium halobium*. Δ N_{H2O} was determined by the lifetime measured at 615nm. $R_{E/M}$ was estimated from the relative peak intensity of $^5Do \rightarrow {}^7F_2$ (615nm) to $^5Do \rightarrow {}^7F_1$ (592nm).

environments of the adsorbed Eu(Ⅲ) on the microorganisms were examined by TRLFS (1). In Figure 1 typical TRLF spectra for an alga and a bacterium is shown. *C. vulgaris, Halomonas* sp., and *Halobacterium halobium* exuded metabolites with affinity for Eu(Ⅲ) and Cm(Ⅲ). The pH dependence in the distribution coefficients showed that the adsorption of Eu(Ⅲ) and Cm(Ⅲ) was not fully attributable to the exchange with $3H^+$ on the functional groups in the cell surfaces. It is considered that the exchange with Na^+ on the functional groups was involved in the adsorption on the halophilic bacteria (*Halomonas* sp., *Halobacterium salinarum, Halobacterium halobium*). TRLFS indicated that the coordination environments on the halophilic bacteria were more complicated than that on the other three microbial strains. This suggests some common properties in the cell surfaces of the halophilic bacteria, which is related to their adaptability to high saline conditions.

EXAFS analysis showed the local structure around the Eu(Ⅲ) adsorbed on cellulose and with *C. vulgaris* was similar. These results indicate that the reactions both at cell surfaces through the adsorption as well as in solution through chelation with the exudates are important in estimating the environmental behavior of Eu(Ⅲ) and Cm(Ⅲ) in aqueous environments (2).

Microbial Immobilization of Uranium. Microorganisms are known to immobilize uranium by

Fig.2 Secondary electron image of U(Ⅳ) accumulated in yeast. EDS Analysis of needle like precipitates around cells by EDS confirmed the formation of uranyl phosphate.

(i) bioaccumulation and biosorption by microbial biomass and biopolymers; and (ii) reductive precipitation from higher to lower oxidation state by enzymatic action. These processes have received considerable attention because of their potential application in the remediation of uranium con-taminated soils, aqueous waste streams, and wastes. (3).

(i) Biosorption and Bioprecipitation of Uranium. Microbes, whether living or dead, possess an abundance of functional groups such as carboxylate, hydroxyl, and phosphate on their cell surface that bind uranium. Polymers secreted by many actively metabolizing microbes also immobilize metals. We are investigating the mechanisms of bioprecipitation of U(Ⅵ) by the yeast *Saccharomyces cerevisiae*. Chemical form of the precipitated U(Ⅵ) was analyzed by SEM-EDS.

Yeast cells exposed to uranium released cellular phosphate, which resulted in the precipitation of uranium. SEM-EDS analyses of the U associated with yeast indicated the formation of uranyl phosphate mineral on the surface of cells (Figure 2). These results indicate that two different processes are relevant to U mineralization by S. cerevisiae. In one process U reacts in the solution with P released from *S. cerevisiae*, where the chemical composition in the solution is super-saturated with respect to H-autunite. In the other process U reacts with P on the surface of *S. cerevisiae*, where H-autunite is was formed by local saturation (3).

(ii) Immobilization of Uranium due to Reductive Precipitation. Reduction of an element from a higher to a lower oxidation state or to elemental form affects

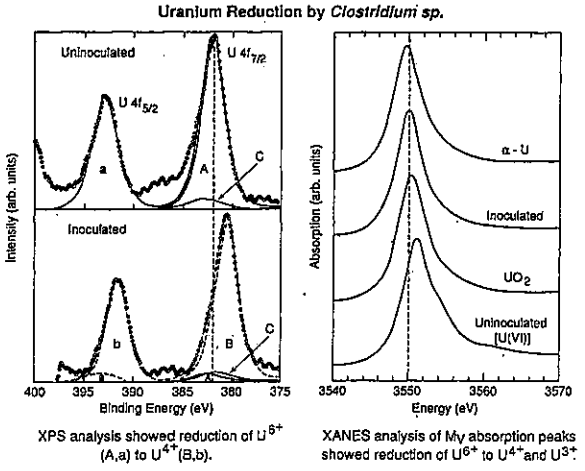

Fig.3 Reduction of uranium by anaerobic bascteria (5).

its solubility resulting in the precipitation of several metals.

$$U(VI)_{aq} \xrightarrow[\text{fermenters, sulfate reducers}]{\text{dissimilatory metal reducers}} > U(IV)_{aq}$$

For example, a variety of microorganisms convert hexavalent uranium to the tetravalent state by enzymatic action. The direct implication of microorganisms in the reduction of uranium is of considerable interest because of its potential application in bioremediation of contaminated sites by immobilizing the uranium *in situ*.

The reduction of uranium was reported in axenic cultures of iron-reducing bacteria, fermentative bacteria, sulfate-reducing bacteria, and cell-free extracts of *Micrococcus lactilyticus*, and in uranium wastes by *Clostridium sp*. (4, 5).

Reactive barrier technology is based on the activities of these anaerobic bacteria. However, the long-term stability of the immobilized uranium is not known.

Interaction of *Pseudomonas fluorescens* with Eu (III)- and Ce(IV)-Desferrioxamine Complexes. Naturally occurring chelating agents such as siderophores, are able to form complexes with actinides and enhance their solubility and mobility in the environment. Adsorption and/or biodegradation of chelated actinides by microorganisms are important processes, which regulate their mobility in the natural environment. In this study, association of Eu(III), Ce(IV), and Fe(III)-desferrioxamine B (DFO) complexes with aerobic bacterium, *Pseudomonas fluorescens* (ATCC 55241), was investigated Eu(III) and Ce(IV) were used as analogues to trivalent and tetravalent actinides, respectively. When equimolar Eu(III)- and Ce(IV)-DFO complexes were incubated with P. fluorescens in 0.1 M Tris-HCl buffer (pH=7.3), the metals were removed from solution, with no change in DFO concentration in solution. With decreasing metal/DFO molar ratio from 1 to 0.01, the accumulation of Eu(III) and Ce(IV) by *P. fluorescens* decreased. Kinetics study showed that accumulation of Eu(III) reached the maximum within 30 minutes, and then it decreased slightly with time. On the other hand, Ce(IV) accumulation proceeded in a parabolic process where the kinetics was slower than that of Eu(III) accumulation. In comparison to Eu(III) and Ce(IV), the removal of Fe(III) added as a DFO complex by *P. fluorescens* was not observed. The formation constants (log K) of Eu(III)-DFO and Fe(III)-DFO are 15 and 30.6, respectively. These results suggest that Eu(III)-DFO complex dissociated in the presence of bacterial cells and was readily biosorbed (6)

References

1. Ozaki, T.; Arisaka, M.; Kimura, T.; Francis, A.J.; Yoshida, Z.; Anal. Bioanal. Chem. in press.
2. Ozaki, T., J.B. Gillow, A.J. Francis, T. Kimura, T. Ohnuki, and Z. Yoshida. 2002. J. Nuc. Sci. Technol. (*in press*).
3. Ohnuki,T., T. Ozaki, T. Yoshida, N. Kozai, A.J. Francis and H. Iefuji. Mechanism of Uranium mineralization by *Saccharomyces cerevisiae.* Paper presented at the AGU Meeting, San Francisco, December 5-11, 2002.
4. Francis. A.J.; Dodge, C.J.; Lu, F.; Halada, G.P.; Clayton, C.R. Environ. Sci. Technol. 1994, 28, 636-639.
5. Francis, A.J. J. Alloys Compd. 1998, 271-273, 78-84.
6. Yoshida, T., T. Ozaki, T Ohnuki, and A.J. Francis. Interaction of *Pseudomonas fluorescens* with Heavy Elements-Desferrioxamine Complexes. Paper presented at the AGU Meeting, San Francisco, December 5-11, 2002.