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Complexation of Eu(III), Pb(II), and U(VI) with a *Paramecium* glycoprotein: Microbial transformation of heavy elements in the aquatic environment

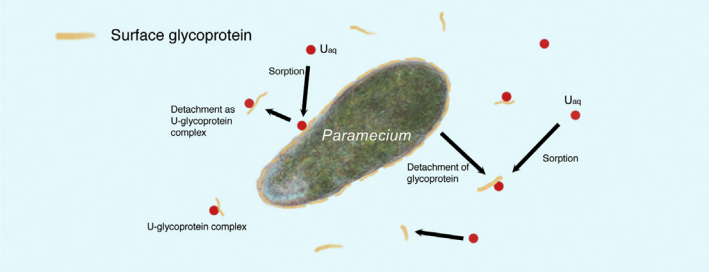
Naofumi Kozai [a, *], Fuminori Sakamoto [a], Kazuya Tanaka [a], Toshihiko Ohnuki [a, b], Takahiro Satoh [c], Tomihiro Kamiya [c], Bernd Grambow [a, d]

[a] Japan Atomic Energy Agency, Advanced Science Research Center, Tokai, Ibaraki, 319-1195, Japan
[b] Tokyo Institute for Technology, Laboratory for Advanced Nuclear Energy, Tokyo, 152-855, Japan
[c] National Institute for Quantum and Radiological Science and Technology, Takasaki Advanced Radiation Research Institute, Takasaki, Gunma, 370-1292, Japan
[d] SUBATECH, Mines Nantes, University of Nantes, CNRS-IN2P3, Nantes, France

HIGHLIGHTS

- Less Eu(III) and U(VI) were sorbed on living *Paramecium* cells than pre-killed cells.
- Eu and U formed an aqueous complex with a soluble *Paramecium* glycoprotein.
- This soluble glycoprotein is the one covering the entire cell surface of *Paramecium*.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the interaction of inorganic aqueous Eu(III), Pb(II), and U(VI) with *Paramecium* sp., a representative single-celled protozoan that lives in freshwater. Living and prekilled *Paramecium* cells were tested. The prekilled cells were killed with a fixative. After 24 h exposure of the cells to inorganic aqueous solutions containing Eu(III) or U(VI), analyses by microparticle-induced X-ray emission with a focused beam (<1 μm) did not detect Eu and U in the living cells, whereas Eu and U were detected in the prekilled cells. Size exclusion chromatography coupled with on-line ultraviolet–visible detection and elemental detection by inductively coupled plasma mass spectrometry of the aqueous phases collected after the living cell experiments revealed that a fraction of the Eu, Pb, and U in the aqueous phase bound to a large (*ca.* 250 kDa) *Paramecium* biomolecule and formed a metal-organic complex. The characteristics of the biomolecule were consistent with those of the soluble glycoproteins covering the surfaces of *Paramecium* cells. These results show that *Paramecium* cells transform inorganic aqueous Eu, Pb, and U to organic complexes. This paper discusses the relation between this novel complexation and the sorption of these heavy elements on *Paramecium* cells.

* Corresponding author.
E-mail address: kozai.naofumi@jaea.go.jp (N. Kozai).

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1. Introduction

For 70 years, severe contamination of the aquatic environment by radionuclides has occurred in the Northern Hemisphere from nuclear facilities and nuclear accidents. Typical examples of operational discharge to the environment include the Mayak nuclear facility, Russia, from the 1950s to the 1960s; the Hanford site, USA, since 1943; the Savannah River Site, USA, from the 1950s to the 1980s; and the British Nuclear Fuels reprocessing plant at Sellafield, UK, since the 1950s. Examples of accidental discharge include the Chernobyl Nuclear Power Plant, Russia, in 1986, and the Fukushima Daiichi Nuclear Power Plant (FDNPP), Japan, in 2011. Large amounts of radionuclides, including Pu, have been released into surface water, groundwater, and seawater (Lukashev, 1993; Cook et al., 1997; Myasoedova and Drozhko, 1998; Dai et al., 2002; Bailly de Bois et al., 2012; Kanda, 2013; Oikawa et al., 2013; Keum et al., 2015). The FDNPP accident also severely contaminated sewage water (Shibata et al., 2012; Kamei-Ishikawa et al., 2013; Kozai et al., 2015).

To evaluate the potential transfer pathways of radionuclides from the aquatic environment to the human food chain, the role of microbes has received intense attention because microbial transformation of radionuclides is very different from abiotic transformation. Typical microbial transformation processes involving radionuclides include 1) binding to functional groups on the cell surface (Gorman-Lewis et al., 2005; Kelly et al., 2002; Haas et al., 2001); 2) binding to aqueous biomolecules, such as siderophores, released by the microbes (Renshaw et al., 2003; Hémadi et al., 2011; Swedlund et al., 2015); 3) deposition inside or outside cells as oxides and phosphates (biomineralization), (Ohnuki et al., 2005; Jiang et al., 2012); 4) sorption onto biogenic minerals (Tanaka et al., 2010; Eickhoff et al., 2014; Sasaki et al., 2014); and 5) reduction or oxidation to different oxidation states (Francis and Dodge, 2015; Cherkouk et al., 2016; Hu et al., 2016). Microbes may either accelerate or retard the transfer of radionuclides to humans. Binding to aqueous biomolecules (process 2) may facilitate the transfer due to the high mobility and solubility of the biomolecules in water. Most known microbial transformation reactions of radionuclides have been identified by using bacteria and unicellular fungi such as yeast.

In aquatic ecosystems, protozoa are key members of microbial communities and control bacterial populations (Finlay and Esteban, 1998). However, little is known about the microbial transformation of radionuclides by protozoa. Several papers have reported the sorption and accumulation of aqueous metal species by protozoa (Casiot et al., 2004; Mortuza et al., 2005; Rehman et al., 2010; Yin et al., 2011; Brockmann et al., 2014), although to our knowledge, other microbial transformations of metallic elements (processes 2–5) by protozoa have not been investigated.

In this paper, we investigated the microbial transformation of aqueous inorganic Eu(III), Pb(II), and U(VI) by *Paramecium*, a unicellular ciliated protozoan that is ubiquitous in freshwater, by simple sorption experiments and subsequent elemental analysis of cells and chromatographic analysis of aqueous Eu, Pb, and U species. Eu(III) was used as homolog for trivalent actinides. The geochemical behavior of trivalent lanthanides is very similar to that of trivalent actinides, such as Am(III), Cm(III), and Pu(III) (Chapman and Smellie, 1986; Krauskopf, 1986). Eu, Am, and Cm are stable in the +3 oxidation state over a naturally occurring pH and Eh range. Uranium is a naturally occurring actinide and its decay chain ends in stable isotope of Pb. Eu(III), Pb(II), and U(VI) were chosen by considering their different oxidation states. It is known that physicochemical behaviors of aqueous heavy metals vary with their oxidation states. We found that aqueous inorganic Eu, Pb, and U form complexes with a large soluble *Paramecium* biomolecule, and we discuss this finding in relation to the identity and function of the biomolecule.

2. Experimental

2.1. Paramecium sp.

Paramecium bursaria Strain Os1 with intracellular symbiotic *Chlorella* was used. This strain was kindly provided by Prof. I. Miwa, Ibaraki University, Japan. *P. bursaria* is ubiquitous in aerobic fresh water.

2.2. Reagents

All reagent solutions used in this study were prepared with ultrapure water (18.2 MΩ, total organic carbon < 4 ppb) and reagent-grade chemicals.

An aqueous solution (Solution A) containing essential elements for *Paramecium*, 10 mM NaCl, 0.8 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.1 mM $MgSO_4 \cdot 7H_2O$, and 0.02 mM β-glycerophosphoric acid disodium salt ($C_3H_7Na_2O_6P \cdot 5H_2O$) was used. This organic phosphorous salt was used instead of inorganic phosphorous salt (*e.g.*, NaH_2PO_4) to avoid metallic elements being precipitated with phosphate ions. To our knowledge, the complexation of this organic phosphate with Eu and U has not been reported. However, we found that β-glycerophosphoric acid forms complexes with Eu and U.

To prepare stock solutions containing 10 mM Eu(III) or U(VI), a portion of Solution A was acidified with a dilute aqueous HCl to a pH between 4 and 5, and then $Eu(OCOCH_3)_3 \cdot nH_2O$ or $UO_2(OCOCH_3)_2 \cdot nH_2O$ was dissolved in Solution A.

2.3. Paramecium cell culture

P. bursaria cells were cultured in Solution A with yeast cells (*Saccharomyces cerevisiae*) as the food source and collected between the late log growth phase and the late stationary phase. Organic membranes precipitated during the culture and the organic membranes were mainly composed of *Paramecium* excrement, which was observed by scanning electron microscopy. Many of the organic membranes were visible to the naked eyes. Most of the organic membranes were separated from the *Paramecium* cells and yeast cells by filtration with a 180-μm-mesh nylon net filter. The organic membranes were collected on the filter. The filtrate containing *Paramecium* cells was purified further by filtering with an 11-μm-mesh nylon net filter; *Paramecium* cells were collected on the filter and yeast cells (several micrometers in size) passed through. The *Paramecium* cells on the filter were washed gently with Solution A, and then they were transferred to a vessel with Solution A. Before the cells were used for the experiments, all cells in the high cell density suspensions were alive and swimming vigorously.

For some experiments, *Paramecium* cells killed with a fixative were used. Studies on the sorption and uptake of solutes by microbes often use cells prekilled by autoclaving. Because living *Paramecium* cells are easily ruptured by various stimuli including autoclaving, we prepared prekilled *Paramecium* cells with a chemical fixative. Live *P. bursaria* cells were submerged in 4% glutaraldehyde ($OHC(CH_2)_3CHO$) and 60 mM sodium cacodylate ($(CH_3)_2AsO_2Na \cdot 3H_2O$), and then the cells were repeatedly washed with purified water.

2.4. Exposure of Paramecium cells to aqueous Eu and U solutions

First, the tolerance (survival ratio) of living *Paramecium* cells in Eu and U aqueous solutions was investigated as a function of the