

# バイオアクチノイド化学研究グループの主な成果

## Highlights of Research Group for Bioactinides Chemistry

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### 概要

微生物細胞表面におけるナノ粒子生成機構と鉱物生成への微生物の関与を解明する研究に取り組んだ。酵母を用いた実験から細胞表面にナノサイズのYbリン酸塩鉱物が成長することを発見した。ウランの耐性にはリン酸輸送に関連する遺伝子が関与することから、細胞表面に吸着したウランや希土類元素が細胞内のリン酸輸送系により運搬されたリン酸と反応して、ウランあるいは希土類元素-リン酸塩鉱物が成長する機構を明らかにした。真菌から排出される生体物質がMn酸化物に吸着したCe(IV)を溶解させることから、希土類パターンにおけるCeの負の異常が生じる機構を発見した。福島第一原子力発電所事故以降では、放射性Csの移行挙動の解明と修復に関する研究に取り組み、オートラジグラフ解析による可視化に基づく樹木中での放射性Csの挙動の解明や微生物及び土壌への放射性Csの濃集機構を解明する研究に取り組み、樹木に降下した放射性Csの初期分布と年オーダーの移行挙動及び土壌に降下した放射性Csが雲母様鉱物への強い吸着とともに雲母以外の鉱物への特異的な吸着が起こることを示した。

### 1. 研究の背景・経緯

アクチノイド (AN) は原子力発電所、再処理施設や核実験などで生成している。ANの放射線毒性は環境において長期に渡って影響する。そのため、環境中における長期的な移行を予測する必要がある[1,2]。環境中では、ANは移行中に土壌や岩石中の無機物(鉱物)や有機物(微生物)の固相界面での反応によりその化学状態を変える。反応の一つが固相表面での沈殿であり、沈殿によりANは可溶体から不溶体に化学状態を変える。我々は、ウランが休眠中の酵母表面において鉱物化することを見いだした。しかし、鉱物化の機構についてはまだ不明であった。

福島第一原子力発電所事故以降では、ANの鉱物化に関する研究に加えて、森林や土壌中における放射性Csの移行挙動の解明や、汚染した土壌の生物の特異性を活用した修復手法の開発を研究対象として加えた。

### 2. 研究の内容

我々はまず酵母 (*Saccharomyces cerevisiae*) を用いてYbのリン酸塩鉱物に関するポスト吸着現象の解明に取り組んだ[3-5]。リン酸含有培地で培養した酵母を1.44 ×

### Abstract

The mechanism and microbial response of nano-particle formation on the cell surface of microorganisms have been studied. We found that nano-particles of Yb-phosphate mineral was developed on the cell surface of *Saccharomyces cerevisiae*. The phosphate transporter genes concerned with phosphate transport contribute to uranium tolerance. Biomolecule exudates from fungi dissolved the Ce(IV) on the biogenic manganese dioxides in the solution, resulting in the negative anomaly of Ce in REEs pattern. After the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, we have conducted to elucidate migration of radioactive Cs in environment. We found very tight association of radioactive Cs to the soil collected in Fukushima. This tight association was resulted from the fixation not only by mica like minerals, but by non-mica like minerals because of low concentration of radioactive Cs.

### 1. Background

Actinides (ANs) have been generated in nuclear power plants, reprocessing facility, nuclear weapons tests. The radiotoxicity of the ANs affects long-duration in the environment. Therefore, prediction of actinide behavior in natural environments is of serious concern [1,2]. In the environment ANs are reacted with solid phase in soil and rock during migration in surface water. The solid phases contain minerals (inorganic material) and microorganisms (organic one). The ANs are changed their chemical species at the interface between these solids and water. One of the reactions is precipitation on the solid, and the precipitation changes chemical species of ANs from dissolved one to insoluble ones. We found that uranium was precipitated on the cell surface of *Saccharomyces cerevisiae* in the resting condition. However, the mechanisms of the formation of the precipitation has not been understood.

After the FDNPP accident, we started the research

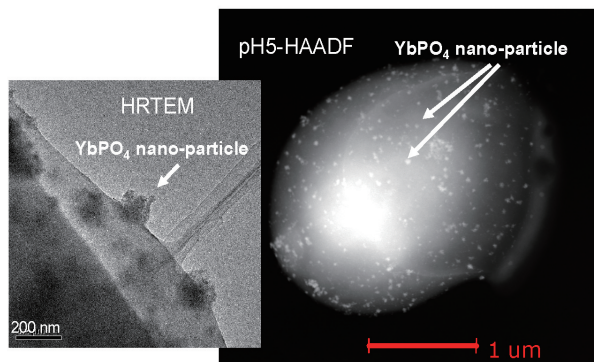


図1 Yb水溶液に接触させた酵母のTEM像  
ナノサイズの非晶質Ybリン酸塩が酵母細胞表面に直接生成した。

Fig.1 TEM photographs of yeast exposed to Yb solution

Nano-sized Yb phosphate with an amorphous phase was directly developed from the surface.

$10^{-4}$  mol/LのYb溶液に $25 \pm 1$  °C、pH3, 4及び5で2時間から2ヶ月間接触させた。Yb濃度は時間の経時に伴い減少した。接触後初期の吸着平衡から、pH2.5から吸着濃度がpH4まで徐々に増加し、その後pH5にかけて急激に増加した。吸着した化学状態をEXAFS（広域X線吸収微細構造解析）により分析した結果、pH3-5ではリン酸基へ吸着した化学種はほぼ一定であることから、pH5までの吸着上昇は解離していないリン酸基に配位した化学種の増加によるものであることが明らかとなった。

2日後から鉍物細胞表面にナノサイズの非晶質のYb-リン酸塩鉍物が生成することが（図1）、電子顕微鏡解析やEXAFS解析により明らかとなった。生成したナノ粒子は、2ヶ月後も $25 \pm 1$  °Cで中性のpH領域で安定であった。EXAFSスペクトルの経時変化から、Ybは接触直後のリン酸基への吸着からリン酸塩鉍物へと変化した。この結果は、Yb-リン酸塩鉍物生成がYbの酵母表面におけるポスト吸着現象の結果であることを示している。さらに、Ybを吸着した酵母をYb及びPを含まない溶液に移した場合でも、7日後にはYb-リン酸塩鉍物が生成した。これらの結果は、Pが細胞内から供給されて、細胞表面で吸着したYbと反応して鉍物化が生じたことを示している。一方、Ybを吸着した細胞表面官能基の模擬物質にリン酸溶塩溶液に添加してもリン酸塩沈殿物は生成しなかった。これらの結果は、細胞表面が官能基を提供するだけでなくリン酸塩鉍物生成の特異な基質であることを示している。したがって、ポスト吸着現象であるナノ粒子生成機構から、細胞表面が重元素の環境中における長期挙動において重要なプロセスを司る場であることが明らかとなった。

希土類元素やウランなどの重元素の鉍物化は微生物の活動によるものかとの疑問が生じる。そこで、6価の $UO_2$ イオンを用いて、4908種の一遺伝子欠損酵母によ

which subjects to elucidate the environmental behavior of radionuclides in land and forest, and to remediate the contaminated land areas by specific biological properties.

## 2. Contents of the study

We first conducted the study on the post-adsorption process of ytterbium (Yb) phosphate nano-particle formation by *Saccharomyces cerevisiae* (yeast) [3, 4, 5]. The yeast grown in P-rich medium were exposed to  $1.44 \times 10^{-4}$  mol/L Yb(III) solution for 2-120 h, and two months at  $25 \pm 1$  °C at an initial pH of 3, 4, or 5, respectively. Yb concentrations in solutions decreased as a function of exposure time. At the sorption of Yb on the cell surface, the sorption isotherm of Yb on the yeast cells between pH 2.5 and 5.5 showed that the fraction of adsorbed Yb increased gradually up to pH 4, and then increased steeply as the pH increases to 5. The Yb adsorption species on the yeast cell wall of the *S. cerevisiae* was determined by extended X-ray absorption fine structure (EXAFS) spectroscopy combined with a linear combination analysis at various pHs. The results indicated that Yb was associated with phosphoryl functional groups on the cells. Since solution pH was lower than pKa of phosphoryl deprotonation, and since the contribution of Yb-phosphoryl species was constant between pH 3 and 5, our results strongly suggest that most of the Yb was associated with undeprotonated phosphoryl functional groups.

After two days, nano-sized Yb phosphate with an amorphous phase formed on the yeast cells surfaces in the solutions with Yb (Fig. 1) by the analyses using field-emission scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (FESEM), transmission electron microscopy (TEM), and EXAFS. These nano-sized precipitates that formed on the cell surfaces remained stable even after two months of exposure at  $25 \pm 1$  °C around neutral pHs. The EXAFS data revealed that the chemical state of the accumulated Yb on the cell surfaces changed from the adsorption on both phosphate and carboxyl sites at 30 min to Yb phosphate precipitates at 5 days, indicating the Yb-phosphate precipitation as a major post-adsorption process. In addition, the precipitation of Yb phosphate occurred on cell surfaces during 7 days of exposure in Yb-free solution after 2 h of exposure (short-term Yb adsorption) in Yb solution. These results suggest that the released P from the inside of yeast cells reacted with adsorbed Yb on cell surfaces, resulting in the formation of Yb precipitates, even though no P was added to the exposure solution. In an abiotic system, the EXAFS data showed that speciation of the sorbed Yb on the reference

り  $UO_2$  への感受性の高い遺伝子を探査した [6]。その結果、0-2mM の U を添加しても遺伝子欠損酵母は成長した。0.5mM の U を含む培地中での生育実験から、13 の遺伝子欠損酵母が成長阻害を示す感受性の高い酵母であった。遺伝子欠損酵母は、リン酸輸送に関する遺伝子、PHO86, PHO84, PHO2 及び PHO87 が欠損したものであった。この結果は、リン酸輸送系がウラン耐性に係わっていることを示唆している。

次に、微生物起源 (バイオ) Mn 酸化物への希土類元素の濃集について調べる実験に着手した [7, 8]。真菌 KR21-2 とバイオ Mn 酸化物が共存する系では、希土類元素を含む溶液から除かれる濃度が溶液の pH に依存した。すなわち、低 pH 領域では Ce が特異的に除かれる、いわゆる Ce の正の異常が見られた。pH の上昇に伴い Ce の正の異常は減少し、中性付近では負の異常となった。XANES 解析からいずれの場合でもバイオ Mn 酸化物に濃集した Ce の酸化数は 4 価であった。この原因を調べるため、溶液中の生体物質をサイズ排除 (SEC) HPLC カラム-ICPMS による分析で調べた。その結果、Ce を伴う生体物質が中性付近で排出されることが分かった (図 2)。Ce の酸化数が 4 価であることから、生体物質が Mn 酸化物に濃集した Ce(IV) を溶解させることが分かった。

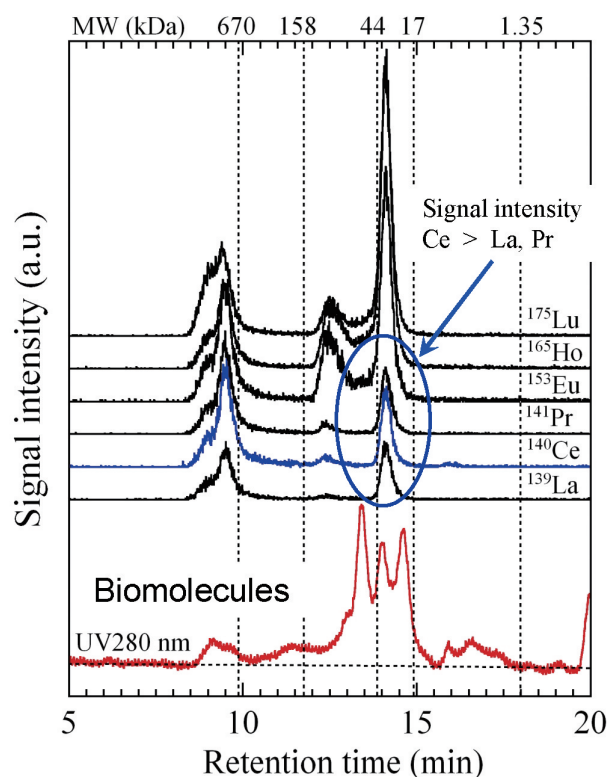


図 2 バイオ Mn 酸化物と真菌に希土類水溶液を接触させた後の液中希土類の SEC-HPLC-ICP-MS プロファイル

Fig.2 Profiles of REE signals with UV 280 nm absorbance of the solution containing biogenic  $MnO_2$  and fungus hyphae by SEC-HPLC-ICP-MS

materials, carboxymethyl cellulose and Ln resin, did not change even when the Yb was exposed to P solution, without forming Yb phosphate precipitates. This result strongly suggests that the cell surface of the yeast plays an important role in the Yb-phosphate precipitation process, not only as a carrier of the functional groups but also as a substrate inducing the nucleation of phosphate nanoparticles. This study also implies that this post-adsorption nano-particle formation process caused by microbial cells should be one of the important processes governing the long-term migration of heavy rare earth elements and presumably trivalent actinides in geological repository.

It is question that how the microorganisms response to the stimulation of lanthanides and actinides to form phosphate minerals. We screened 4908 non-essential gene deletion mutant yeast strains for uranium sensitivity and low accumulation by growth in agar medium containing uranium [6]. All mutant strains grew successfully on agar media containing 0 or 0.2 mM uranium for one week at 30°C. Thirteen strains with single gene deletions showed reduced growth in the agar medium containing 0.5 mM uranium and were identified as uranium-sensitive mutant strains. The phosphate transporter genes of PHO86, PHO84, PHO2, and PHO87 were among the deleted genes in the uranium-sensitive mutant strains, suggesting that genes concerned with phosphate transport contribute to uranium tolerance. Seventeen single-deletion strains showed lower uranium accumulation than the wild type after exposure to agar medium containing 0.5 mM uranium, and were identified as mutant strains with low uranium accumulation.

We conducted sorption experiments of REEs on biogenic Mn oxides produced by *Acremonium* sp. strain KR21-2 [7,8]. The distribution coefficients,  $K_d(REE)$ , between biogenic Mn oxide (plus hyphae) and 10 mmol/L NaCl solution showed larger  $K_d$  for Ce than neighboring Ln and Pr called as “a positive Ce anomaly” at pH 3.8 caused by oxidation of Ce(III) to Ce(IV) by the biogenic Mn oxide, which was confirmed by analysis of the Ce L<sup>III</sup>-edge XANES spectra. With increasing pH, the positive Ce anomaly became less pronounced. Furthermore, negative Ce anomalies were observed at a pH of more than 6.5, suggesting that Ce(IV) was stabilized in the solution (< 0.2 μm) phase, although Ce(III) oxidation to Ce(IV) on the biogenic Mn oxide was confirmed by XANES analysis. It was demonstrated that no Ce(III) oxidation occurred during sorption on the hyphae of strain KR21-2 by the  $K_d(REE)$  patterns and XANES analysis. The analysis of size exclusion HPLC-ICP-MS (Fig. 2) showed that some fractions of REEs in the filtrates (< 0.2 μm) after sorption experiments were bound to organic molecules (40 kDa and < 670 kDa fractions), which were possibly released from the hyphae. Our data indicates that the negative Ce

環境中におけるナノ粒子について調べるため、幌延サイトの地下500mの地下水中の元素をSECHPLC-ICPMSにより調べた[9]。その結果、Uは数十ppt程度の濃度で低分子量のシリカ粒子とともに存在することが分かった。シリカ粒子はNa,K,Mg,Ca等の元素を含まなかった。無機的なシリカナノ粒子に取り込まれたUの地下水中での存在を示すデータは世界初であり、Uの環境中移行を解明する上で重要な発見である。

福島第一原子力発電所事故により降下した放射性Csの挙動を解明する研究として、森林及び土壌中の放射性Csの挙動解明に取り組んだ[10-13]。事故後2ヶ月及び22ヶ月経過後に福島県内で樹木を採取して、オートラジオグラフィ（AR）解析を行った。事故2ヶ月後に飯舘村で採取したカヤ（*Torreya nucifera*）及びスギ（*Cryptomeria japonica*）では、ほとんどの放射性Csが事故以前に成長した（古い）枝葉に沈着し、事故後に成長した新しい枝葉にはほとんど移行していなかった（図3）。この結果から、事故後数ヶ月は降下した放射性Csが降下位置に留まっていることが分かった。次に、22ヶ月経過後に大熊町においてスギ試料の採取したAR解析結果から（図4）、古い枝葉には放射性Csが留まっていることが分かった。一方、新しい枝葉でも放射性Csが検出され、特に成長が著しい先端部分では濃度が高かったことから、一部の放射性Csの移動が確認された。これらの結果は、樹木に降下した放射性Csは年オーダーの速

anomalies under circumneutral pH conditions arose from Ce(III) oxidation on the biogenic Mn oxide and subsequent complexation of Ce(IV) with organic ligands. These results demonstrate that the coexistence of the biogenic Mn oxide and hyphae of strain KR21-2 produces a specific redox chemistry which cannot be explained by inorganic species.

The chemical forms of actinides and lanthanides in the groundwater are still unclear after released as chemical species of cation and/or particles. The saline groundwater collected at a depth of about 500 m in Horonobe, Japan, where an underground research laboratory has been built, was rich in salinity (Na 4900ppm, Cl 7600ppm), iodine (42 ppm), and methane gas. We analyzed colloids and ions of this groundwater mainly by employing the SEC-UV-Vis-ICP-MS technique and focused on speciation of actinide (uranium) and iodine [9], both of which are of particular importance for radioactive waste disposal. For this, the groundwater sample was introduced to SEC columns after being passed through a 0.45 µm filter but without further pretreatment such as isolation of colloids. Chromatographic profiles obtained with two different SEC columns were compared. This study revealed that uranium contained in the groundwater at several tens of ppt was associated with low molecular weight silica species with neutral charge. The silica species were virtually free of metal elements such as Na, K, Mg, Ca, and Al. This study also found that almost all of the iodine in the groundwater was iodide (I<sup>-</sup>). The groundwater contained an unidentified organic colloid that was not a carrier for the radioactive waste-relevant elements Se, Sr, I, Cs, Th, and U.

The nuclear accident at the FDNPP occurred as a consequence of the massive earthquake and associated tsunami that struck the Tohoku and north Kanto regions of Japan on 11 March 2011. A series of hydrogen explosion was occurred from 13 March to 15 March at the units 1, 2, and 3. The release rate of <sup>137</sup>Cs on 15 March is estimated between 10<sup>12</sup> and 10<sup>15</sup> Bq/h. This fallout radioactive Cs were dispersed from FDNPP to ocean and land. Some of the released radioactive Cs was deposited on the ground of the area located north-west direction from FDNPP. The spatial concentration distribution and depth profiles of radioactive Cs were measured to estimate dose rate and to estimate the fate in the terrestrial environment.

How were radionuclides attached to and transported in forest plants and soil after fallout of radionuclides as a result of the accident at FDNPP. This question must be resolved to quantify present and future forest contamination. Distributions of radionuclides in plants and soil samples are usually measured by cutting small fractions from the samples. Unfortunately, this technique requires considerable time and effort. Thus, we have applied an autoradiography technique to analyze the distribution of radionuclides within the plants and soil [10, 11].

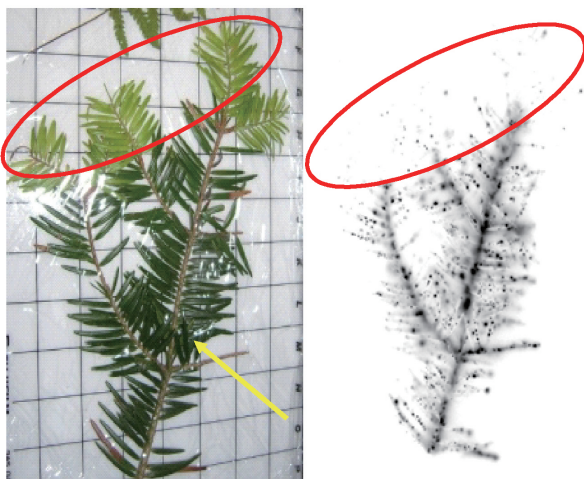


図3 事故の2ヶ月後に採取したカヤ(*Torreya nucifera*)の(左)写真と(右)オートラジオグラフ像

赤丸の部分は、事故後に成長した葉(薄緑色)。赤丸の部分には黒い点がほとんど無い。これは、古い葉(黄色の線で示す)に含まれていた放射性Csは若い葉にほとんど移行しなかったことを示す。

Fig.3 Autoradiograph image of *Torreya nucifera* sampled after 2 months (left) Photograph (photo) of *Torreya nucifera*, (right) autoradiograph image (AR)

Red circles indicate the leaves (light green colored leaves in photo) that grew after the accident. Black spots are practically zero within the red circles, indicating that the radioactive Cs in old leaves (yellow arrow) were rarely transported to the young leaves.

度で樹木内を移行することを示唆している。

土壌中の放射性Csの化学状態を調べるため、事故1ヶ月後に飯館村で採取した3種類の土壌を用いて1M NH<sub>4</sub>Cl溶液及び1M CH<sub>3</sub>COOH溶液を用いた脱離試験を行った。さらに、試薬溶液による脱離実験後の残渣土壌

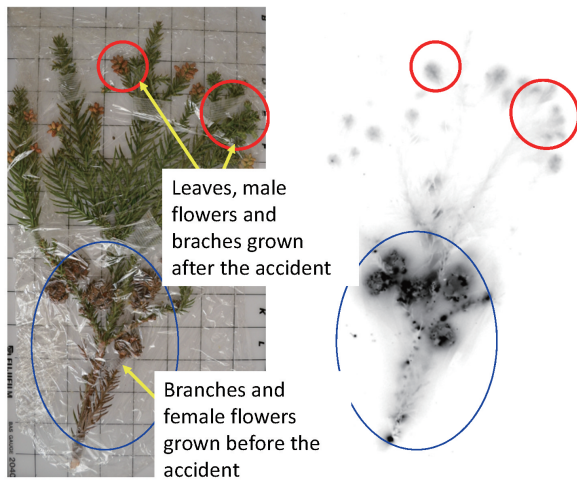


図4 1F事故約2年後に採取したスギの写真(左)と放射能分布像(右) スギ表面に沈着した放射性Csは、○で囲んだ1F事故以前に生育していた枝葉や雌花部分には斑点状に分布します。一方、1F事故後に生育した○で囲んだ枝葉や雄花部分に分布しています。

Fig.4 4 Autoradiograph image of *Cryptomeria japonica* sampled after about 2 years

(left) Photo of *Cryptomeria japonica*, (right) AR image. Blue circles indicate the branches and female flower and old leaves in photo (yellow arrows) that grew before the FDNPP accident. Red circles indicate that male flower and leaves emerged after the FDNPP accident contained some amounts of radioactive Cs inside them.

We collected *Torreya nucifera* and *Cryptomeria japonica* in Iitate-mura, Soma-gun, Fukushima in May, 2011 and Ookuma-machi, Futaba-gun, Fukushima in December, 2012, respectively. We placed the samples on an imaging plate (IP) coated with fluorescent paint that reacts to radiation and photographed the spatial distribution of radionuclides. The distribution of radionuclides in the branches and leaves of *T. nucifera* (Fig. 3 (left)) was analyzed by the autoradiograph image where many black spots were present (Fig. 3 (right)). These black spots appeared on the IP in response to radiation originating from radioactive Cs. Note that <sup>131</sup>I was decayed out in May. Figure 3 (left) illustrates dark and light green leaves that grew before (old) and after (young) the accident, respectively. Black spots were present on the dark green leaves but not on the light green leaves, indicating that radionuclides attached to the old leaves were rarely transported to the young leaves. The same result was obtained from analysis of *C. japonica*.

The autoradiograph image (Fig. 4 (right)) in *C. japonica* (Fig. 4 (left)) Sampled on December, 2012 shows many black spots on the branches and female flowers emerged before the accident. On the contrary, broadened black areas were observed in the leaves and the female flowers emerged after the accident, indicating that radioactive Cs is translocated from the tree surface to the new branches and leaves. Since the top of the leaves and male flowers are in active for growing, and since K ions are known to be accumulated in the active areas, radioactive Cs may be transported from the old branches to the newer area.

These results clearly shows that radioactive Cs deposited on the tree surface is slowly translocated to new growing areas at the rate of year order.

For the estimation of migration of radioactive Cs, sorption behavior of the deposited radioactive Cs should be clarified. Cesium at low concentration is strongly sorbed on mica like minerals. These suggest that some amounts of radioactive Cs are irreversibly sorbed with soils. However, the characteristics of the irreversible sorption of fallout Cs in the contaminated soils are scarcely known. We have carried out the desorption experiments of radioactive Cs using appropriate reagents solution to clarify the association of radioactive Cs with soils [12]. We also carried out the elutriation and sieve treatment for analyzing the size dependence of radioactive Cs sorption and the mineral composition. The AR image of thin-section of the contaminated soils showed that radiocesium was heterogeneously distributed in the soil. The desorption experiments with a 1 M NH<sub>4</sub>Cl solution and a 1 M CH<sub>3</sub>COOH solution showed that about 30% radiocesium was desorbed, indicating that about 70% radiocesium are present as an immobile one. Even though no mica-like minerals was detected in the soil, nearly the

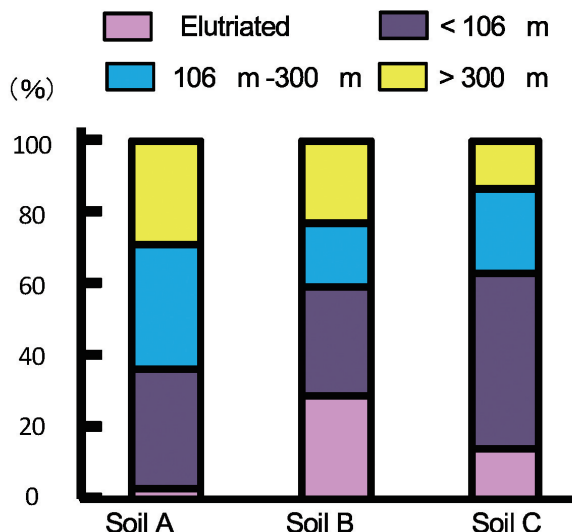


図5 脱離試験後の残渣土壌を粒子の大ききで分級した各粒子成分に含まれる放射性Cs

水ひして得た最も細かい粒子成分(粘土を含む)よりも大きな粒子成分に放射性Csが多く含まれていた。

Fig.5 Size fractions of radioactive cesium in the soils sampled in Fukushima-prefecture

Radioactive cesium was distributed in the size fractions larger than the clay fraction which is elutriated.

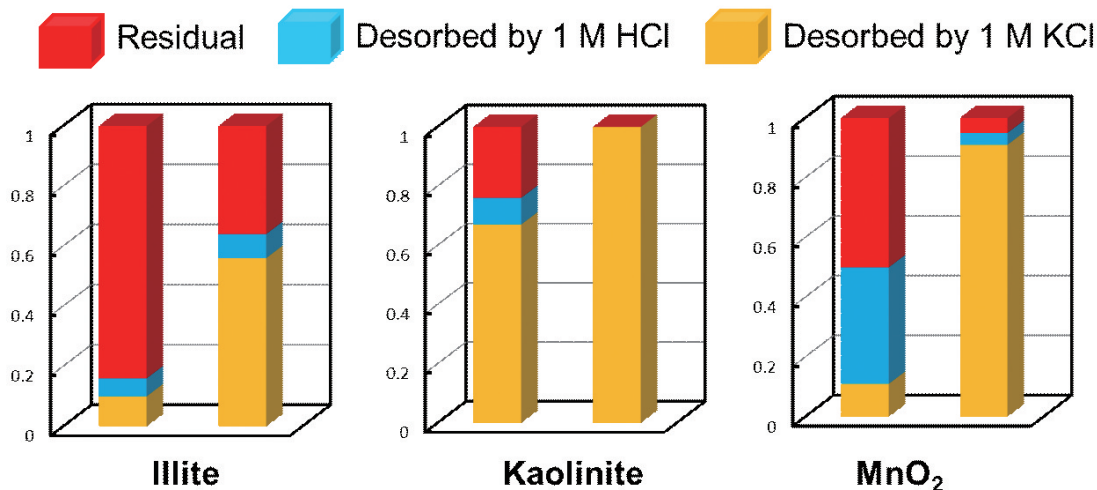


図6 鉱物に吸着した放射性Csを試薬溶液で処理したときに脱離したCsの割合  
をCs濃度が低くなると(10<sup>-9</sup>M)、脱離試薬で処理した後に固相に残ったCsの割合が多くなった。

Fig.6 Desorbed fraction of radiocesium from minerals at different cesium concentrations  
When Cs concentration is very low of 10<sup>-9</sup> M, higher amounts of Cs resided after the treatment with desorption reagents solution.

について篩い分けによる粒度別放射性Cs濃度の測定とX線回折による鉱物組成について分析した。その結果、2種類の試薬溶液で脱離した放射性Csは約30%であった。残りの70%については土壤に強く吸着されていると考えられる。残渣土壤の篩い分けによる粒度別の放射性Cs濃度から、放射性Csは粒子の小さい粘土画分だけでなく、比較的大きな粒度中にも存在した(図5)。X線回折による鉱物組成から、粘土画分には雲母などの層状鉱物が存在した。一方、比較的大きな粒子中には雲母様の鉱物が含まれないものもあった。この結果は、放射性Csは雲母様鉱物以外の鉱物にも強く吸着していることを示している。

そこで、非雲母鉱物である、カオリナイト、ハロイサイト、クロライト、モンモリロナイト、モルデナイト、Mn酸化物、チタン酸化物、鉄酸化物、及び雲母鉱物であるイライトについて、吸着・脱離実験を行った。吸着実験からCs濃度が低い場合には、吸着係数の序列がmordenite > illite > montmorillonite, sericite, MnO<sub>2</sub>, kaolinite, and halloysite > chlorite, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and FeOOHとなった。脱離実験の結果から、montmorillonite, mordenite, MnO<sub>2</sub>及びkaoliniteについては、0.1 mole L<sup>-1</sup> LiCl溶液、1 mole L<sup>-1</sup> KCl溶液及び1 mole L<sup>-1</sup> HCl溶液による脱離後も、それぞれ40%、40%、50%及び25%が脱離されずに残った(図6)。この結果から、Cs濃度が低い場合には雲母様鉱物以外の鉱物にもCsは強く吸着されることが明らかとなった。福島土壤への放射性Csの吸着においても、雲母様鉱物や雲母以外のカオリナイトやMn酸化物などへの強い吸着が放射性Csの難移動性の原因と考えられる。

福島や関東圏での排水汚泥処理により生じた焼却灰には高い濃度の放射性Csが検出される。焼却灰中の放射性Csの化学状態と脱離挙動を明らかにする実験を行っ

same result was obtained, suggesting that the minerals other than mica-like one immobilize radiocesium (Fig. 5).

We studied the adsorption behavior of radioactive cesium (Cs) by the non-mica minerals kaolinite, halloysite, chlorite, montmorillonite, mordenite, MnO<sub>2</sub>, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and FeOOH [13]. The adsorption and desorption experiments of Cs on the minerals were carried out at the Cs concentrations 1x10<sup>-4</sup>, 1x10<sup>-5</sup> and 2x10<sup>-9</sup> mole L<sup>-1</sup> at pH 5.5. The desorption of Cs from the minerals was examined using 0.1 mole L<sup>-1</sup> LiCl, NaCl, KCl, RbCl, and CsCl solutions. The sequential desorption was examined using a 0.1 mole L<sup>-1</sup> LiCl solution, a 1 mole L<sup>-1</sup> KCl solution, and a 1 mole L<sup>-1</sup> HCl solution. The distribution coefficient (K<sub>d</sub>) for the minerals at the Cs concentration 10<sup>-9</sup> mole L<sup>-1</sup> was in the order of mordenite > illite > montmorillonite, sericite, MnO<sub>2</sub>, kaolinite, and halloysite > chlorite, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and FeOOH, differing from the order observed at higher Cs concentrations. After the sequential desorption by the three reagent solutions, the residual fraction of Cs was lower at the Cs concentration 10<sup>-9</sup> mole L<sup>-1</sup> than at higher concentrations. Approximately 40%, 40%, 50%, and 25% of the adsorbed Cs were residual in montmorillonite, mordenite, MnO<sub>2</sub> and kaolinite, respectively after the sequential desorption (Fig. 6). These results strongly suggest that (1) radioactive Cs at 10<sup>-9</sup> mole L<sup>-1</sup> is more strongly associated with the non-mica minerals than at higher concentrations of 1x10<sup>-4</sup> and 1x10<sup>-5</sup> mole L<sup>-1</sup>, and (2) the non-mica minerals montmorillonite, mordenite, kaolinite, and MnO<sub>2</sub> contributed to the fixation of the radioactive cesium fall-out on Fukushima soil.

We have analyzed the radioactive fallout cesium (<sup>137</sup>Cs) in the sewage sludge ashes (SSAs) produced in Japan after

た[14]。実験では、無機酸や有機酸による焼却灰の溶解を行い、溶解液中の放射性Cs濃度及び鉄などの元素濃度の測定を行った。その結果、濃塩酸により放射性Csの80-90%が溶離した。溶解液中にはリンと鉄が含まれていた。溶解残渣の電子顕微鏡分析及びX線回折の結果、残渣物中にはシリカ、長石、粘土鉱物などアルミノシリケートが存在していた。この結果から、焼却灰中の放射性Csは鉄鉱物やリン酸塩鉱物に取り込まれている。放射性Csの溶解回収量の粒径依存性調べるため、焼却灰をミルにより粉碎し小粒径化した。その結果、粒径が小さくなるほど放射性Csの回収量は増加した。さらに、鉄と放射性Csを含む酸溶液を中性化して鉄沈殿物を生成させる処理を行ったところ、放射性Csは溶液中に残った。これらの結果は、焼却灰の小粒径化、酸溶解、溶解液の中性化処理により95%以上の放射性Csが回収でき、鉄及びシリカなどの焼却灰残渣には5%以下の濃度に減容できることが分かった。

### 3. 成果の意義と波及効果

これまで報告されているバイオミネラリゼーションでは、溶液中に溶解した元素が細胞表面に集まることにより、沈殿物を生成する機構であった。微生物は反応の場を与えるか電子供与体として活用されてきた。我々の発見は、細胞表面を反応場として溶液中に溶解した元素の細胞表面への吸着と細胞の中から細胞外にイオン（ここではリン酸）が供給されることによりナノ粒子が形成される新しい反応の証拠を示した。これらの結果は、細胞の表面を新しい反応場として用いるナノ粒子生成方法の樹立に繋がると考えられる。我々の論文は、SCIENCE 2010のEditor's Choiceとして紹介された[15]。

福島における放射性Csの挙動解明に関する研究では、事故後2ヶ月における樹木などに降下した放射性Csの分布をAR法により可視化した。この結果は、放射性Cs等の分布調査とともに、放射性Csの環境中の挙動を理解する上で役だった。さらに、土壌中の放射性Csの化学状態分析及び鉱物組成との関係から、雲母様鉱物以外にも放射性Csが強く吸着することを明らかにした。この結果は、雲母が存在しない火山灰などに降下した放射性Csの難移動性を理解する上で重要な知見となった。土壌中の放射性Csの化学状態分析結果をまとめた論文は、JNSTの2012年度のMost Popular Article Awardを受賞した。

### 4. 今後の予定

我々は微生物細胞表面が特異の場となり、細胞内部元素の細胞外への排出による新しいナノ粒子生成機構を明らかにした。この結果は、細胞の内外を活用する新たな物質創成技術として活用する可能性がある。さらに、微生物と鉱物が共存する系では、鉱物あるいは微生物だけでは起こらないCe異常を発見した。また、鉄還元菌と鉄イオンが共存する系で、Se(IV)がSe(-II)に還元され

the FDNPP accident [14]. Five samples of SSAs produced in 2011 and 2012 were tested. Two of the samples contained  $^{137}\text{Cs}$  (23 and 9.6 kBq/kg, respectively) above the radioactivity criterion (8 kBq of radioactive Cs per kg of solid) for controlled landfill disposal in Japan. The mineral components of SSA are roughly divided into two groups. One is HCl-soluble phase mainly composed of phosphates and oxides, and the other is silicates including quartz, feldspar, and clay. Both the phases contained  $^{137}\text{Cs}$ . The major part (83-91%) of  $^{137}\text{Cs}$  was contained in the HCl-soluble phase. Among the HCl-soluble subphases, Fe-bearing phases, probably iron oxides, were most responsible for  $^{137}\text{Cs}$  retention, and no positive evidence was obtained to show that phosphate-bearing phases, which were included most in SSAs along with silicate phase, retained  $^{137}\text{Cs}$ . To facilitate dissolution of the HCl-soluble phase, pre-pulverizing SSAs and subsequent heating them at 95 °C in a 6M or a concentrated HCl aqueous solution was most effective. The radioactivity concentrations of  $^{137}\text{Cs}$  in all of the HCl-treatment residues were below the radioactivity criterion. This residue was mostly composed of silicates. After static leaching tests of the residue at 60 °C for 28d, no  $^{137}\text{Cs}$  was detected in simulated environment water leachates (pure water and synthetic seawater), demonstrating that the  $^{137}\text{Cs}$  in the residue are very stably immobilized in the silicates.

### 3. Importance of the result and its impact

Usual biomineralizations are derived by the sorption of the dissolved metal ions on the surface to form oxides, even for phosphate minerals where metals and phosphate ions dissolved in the solution were reacted on the cell surface to form the precipitation. Our finding showed that metals solved in the solution are reacted with counter ions present inside cells on the cell surface as inside out synthesis. Our finding of the nano-particles formation on the surface of microorganisms shows that the microbial surface functions as a new reaction fields to form nano-particles. These facts suggest the new method to synthesize nanomaterials from aqueous metal precursors by biomineralization. These results were introduced in SCIENCE, 2010 as Editor's choice [15].

For the study in Fukushima, AR analysis showed direct evidence of the initial distribution of fall-out radioactive Cs on the trees and soils, and slow translocation inside the trees. These results appeared in newspapers after the interview. The chemical states of the fall-out radioactive Cs in soil and minerals shows distinct behaviors of radioactive Cs in environments. These evidence supports for the remediation of the contaminated soil in Fukushima. The published paper in JNST was awarded as "Most Popular Article in 2012".

ることを明らかにした。Se(IV)は鉄還元菌あるいは2価鉄含有鉱物ではSe(0)までしか還元されない。このことは、微生物と鉱物の共存系では共同作用による新たな反応が存在することを示唆している。

福島における研究では、鉱物を中心として放射性Csの挙動を解明した。さらに、糸状菌などとの相互作用についても検討して、放射性Csの濃集について明らかにしている。これらの成果を活用して、生物を用いる修復やきのこへの移行を阻止する材料の開発を行う予定である。

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## 4. Perspectives

We showed specific functions of the microbial cell surface to form nano-materials by inside out synthesis. This inside out synthesis found to understand the environmental behavior of actinides. In addition, this inside out synthesis enables to produce nano-materials on the cell surface as nano-particle factory.

In the environments, organic materials involving microorganisms and inorganic materials of minerals are present. We have found the synergy effects of microorganisms and metal oxides to reduce Se(IV) to Se(-II), even though microorganisms or Fe oxides alone reduces Se(IV) to Se(0). This synergic reactions strongly suggest the importance of solid phase to dominate the transformation of actinides in the environments.

Fungi are known to accumulate Cs. This specific accumulation by fungi may cause high concentration of radioactive Cs in their fruiting body. Our findings of specific sorption of radioactive Cs by minerals, applying to reduce the transport of radioactive Cs in the contaminated medium and wood log to the fruiting body.