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4.14 HYPERACCUMULATION OF RADIOACTIVE ISOTOPES BY MARINE ALGAE

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ABSTRACT

Hyperaccumulators are effective indicator organisms for monitoring marine pollution by heavy metals and artificial radionuclides. We found a green algae, *Bryopsis maxima* that hyperaccumulate a stable and radioactive isotopes such as Sr-90, Tc-99, Ba-138, Re-187, and Ra-226. *B. maxima* showed high concentration factors for heavy alkali earth metals like Ba and Ra, compared with other marine algae in Japan. Furthermore, this species had the highest concentrations for Tc-99 and Re-187. The accumulation and excretion patterns of Sr-85 and Tc-95m were examined by tracer experiments. The chemical states of Sr and Re in living *B. maxima* were analyzed by HPLC-ICP/MS, LC/MS, and X-ray absorption fine structure analysis using synchrotron radiation.

KEYWORDS: hyperaccumulation, algae, Tc-99, Re-187, Ra-226

INTRODUCTION

Hyperaccumulator plants are effective for mineral exploration, or biorecovery and detoxification of metal-contaminated soils [1 and 2]. We found a new hyperaccumulator useful for estimating marine pollution by heavy metals or radioisotopes and for studying the mechanisms of bioaccumulation of elements by organisms.

MATERIALS AND METHODS

Fifty species of marine algae were collected from the coast of Japan in 2000-2002. Ba and Sr were determined by ICP-AES (detection limits=0.1 ppb), and Re-187 ($T_{1/2}$=4.35x10^{10}y, abundance ratio=62.6%) was analyzed by ICP-MS (detection limit=0.1 ppt) after ca. 1 g of dry matter was wet-digested in high-purity of a nitric acid and hydrogen peroxide. Sr-90 ($T_{1/2}$=28.78y) and Tc-99 ($T_{1/2}$=2.111x10^5y) were measured by low background β-ray spectroscopy. Ra-226 ($T_{1/2}$=1600y) was analyzed by a liquid scintillation counter. Before measuring radioactivity, we digested 10-50 g of ash samples and then performed radiochemical procedures such as adding of a yield tracer, ion exchange, solvent extraction, etc. Gamma ray spectroscopy was applied to 50 g of ash samples of marine algae (counting time=200,000 sec). X-ray absorption fine structure analyses were carried out to clarify the chemical forms of Re and Sr in living bodies of algae at BL01B1 with a SPring-8 of JASRI and at BL12C with a Photon Factory of KEK. HPLC-ICP/MS and LC/MS were also applied to the supernatant of the homogenate of living bodies of *B. maxima*. Accumulation and excretion experiments of Sr-85 ($T_{1/2}$=64.84d) and Tc-95m ($T_{1/2}$=61.2d) were also performed using living six species of marine algae to compare data from stable and radioisotope analyses for field samples.

RESULTS AND CONCLUSIONS

Concentrations of stable and radioisotopes

Table 1 shows concentrations of elements in green algae *Bryopsis maxima* and *Ulva pertusa*. The concentrations of all elements in *B. maxima* are considerably higher than those in *U. pertusa*. In particular, the Re concentration (Re-185+187=5,490,000 pg/g dry, Re-187=5.6 mBq/g dry) was 22,000 times higher in *B. maxima* than in *U. pertusa*. The Re concentrations in 48 species of algae, excluding the genus *Bryopsis*, ranged from 51 to 29,100 pg/g on a dry weight basis (mean$\pm$sd; 5,200$\pm$4,800 pg/g). Although there is little information on the Re contents in land plants and marine algae, a brown alga *Pterygophora californica* was reported to have the highest Re concentration (11,700 pg/g dry) among 21 species from the California coast [3]. Therefore, *B. maxima* was defined as a true Re hyperaccumulator in the plant kingdom. The concentration factor (CF) was calculated to be 7.4×10^5 (7.1×10^4 on a wet basis) from the Re concentration (7.42 pg/mL) in Pacific Ocean seawater [4]. *B. maxima* also showed a high concentration (1.03 pg/g dry=660 μBq/g dry) of Tc-99 belonging to the same Ⅶ/7 group as Re in the periodic table. Although Tc-99 was detected in some species of marine algae, such as *Sargassum thunbergii* (18-270 μBq/g dry), *Sargassum hemiphyllum* (130 μBq/g dry), and *Hizikia fusiformis* (42 μBq/g dry), the concentration of Tc-99 in almost all of Japanese marine algae collected in 2001-2002 was less than the minimum detectable amount (15 μBq/g dry=3 mBq/200g dry).

On the other hand, the brown algae *Fucus vesiculosus* from Sellafield in England had very high concentration of Tc-99 (17 Bq/g wet weight) [5]. Although the concentration of Tc-99 in *Ascophyllum nodosum* from Cape Wrath in Scotland was 410 mBq/g wet weight [5], *A. nodosum* from Norway and

Table 1 Concentrations (pg/g dry weight) of elements in green algae collected from the same location and at the same month (March in 2001)

Element (Isotope)	*Bryopsis maxima* (N=3) (mean±sd)	*Ulva pertusa* (N=3) (mean±sd)
Ba	$4.63 \times 10^9 \pm 1.9 \times 10^8$	$1.72 \times 10^6 \pm 1.1 \times 10^5$
Ra (as Ra-226)	$2.14 \times 10^1 \pm 4.5 \times 10^0$	$1.95 \times 10^{-2} \pm 1.6 \times 10^{-3}$
Re	$5.49 \times 10^6 \pm 3.7 \times 10^5$	$2.51 \times 10^2 \pm 2.3 \times 10^1$
Sr	$1.31 \times 10^{10} \pm 1.1 \times 10^9$	$3.21 \times 10^7 \pm 2.7 \times 10^6$
Tc (as Tc-99)	$1.03 \times 10^0 \pm 3.5 \times 10^{-1}$	$<2.36 \times 10^{-2} \pm$

Canada indicated low concentrations of Tc-99 (40 mBq-Norway, 0.4 mBq-Canada on a wet weight basis). It was estimated that a wide difference in Tc-99 concentration occurred in seawater of Atlantic Ocean.

As shown in Table 1, *B. maxima* had the highest accumulation of alkali earth elements. For example, the Ba concentrations (4,630 μg/g dry) in *B. maxima* were 2,700 times higher than in *U. pertusa. B. maxima* was defined as a Ba hyperaccumulator because the average concentration of Ba in 50 species of marine algae in Japan was 5.19 ± 4.89 μg/g dry wt. Since the Ba concentration in seawater is 0.015 μg/g, the concentration factor of Ba by *B. maxima* was calculated to be 310,000 (29,000 on a wet basis). The average concentration of Ra-226 in 12 species of Japanese marine algae was 0.225 ± 0.178 pg/g dry wt. In contrast, *B. maxima* showed a very high concentration of Ra-226 (21.4 pg/g dry=780 mBq/g dry). Since the mean concentration of Ra-226 in ocean seawater is 0.00013 pg/g, the concentration factor of Ra-226 by B. maxima was calculated to be 165,000 (15, 800 on a wet basis)

Gamma ray spectra of *B. maxima* and *U. pertusa* are shown in Fig. 1. 185.7155 kev of U-235 must be overlapped with 186.1010 kev of Ra-226. We think that no significant amount of U-235 was present in *B. maxima*, because 143.762 kev and 163.332 kev of U-235 were not observed. Furthermore, clear peaks of Bi-214 and Pb-214 derived from Ra-226 were found in the spectrum of *B. maxima*. As can be seen in Table 1 and Fig.1., *B. maxima* was recognized to be a Ra hyperaccumulator. The Sr in *B. maxima* had the highest value (13,100 μg/g dry) among algae. We think that *B. maxima* is a Sr hyperaccumulator since the "Hyperaccumulation level of Sr" is 5,000 μg/g dry [6]. Usually, the Ca concentrations in marine organisms are 10-100 times higher than the Sr concentrations. It was unique that the Sr concentrations in *B. maxima* were almost equal to the Ca concentrations (10,800-20,810 μg/g dry). As a result of radiochemical analyses of Sr-90, *B. maxima* showed the highest value (2.8 mBq/g dry= 0.27 mBq/g wet) among algae. Its CF value for a wet basis was calculated to be 160, since the radioactivity of Sr-90 in the seawater around Japan was approximately 1.61 mBq/L (0.79-2.2 mBq/L) [7].

<u>Chemical forms of Re and Sr in the living condition</u>

XAFS analysis indicated that the Re in *B. maxima* existed in the Re (VII) state, but its tetrahedral symmetry was broken. As results of XAFS analyses, HPLC-ICP/MS and LC/MS, it was estimated that Re and Sr in *B. maxima* were bonded to constituent materials such as polysaccharides and proteins, although they are present in ionic forms in seawater.

<u>Accumulation and excretion of radioisotopes</u>

Figure 2 illustrate accumulation and excretion curves or calculated concentration factors of Tc-95m for six species of algae. *B. maxima* had the highest CF (28,000) among them. In the case of Sr-85, *B. maxima* also showed the highest CF value (120) of Sr-85 among species.

<u>Future problems</u>

1. The presence of special genes: The genus *Bryopsis* may possess a special ability to hyperaccumulate heavy elements, since *Bryopsis corticulans* had the same tendency as *B. maxima*.

It remains to be clarified whether groups of *Bryopsis* have special genes controlling the hyperaccumulation mechanisms of elements or protection systems against the DNA damage from large quantities of radiation from natural or artificial radioisotopes.

2. The fate of Rn-222: How does Rn-222 derived from Ra-226 behave in a living body?
3. Constituent materials with high affinity: What kinds of substances in algae are related to the high accumulation of elements. Is it possible that effective collecting reagents for rare metals such as Sr, Ba, and Re, and artificial radioisotopes such as Sr-90 and Tc-99, will be developed if chemical forms of these elements in *B. maxima* are clarified?

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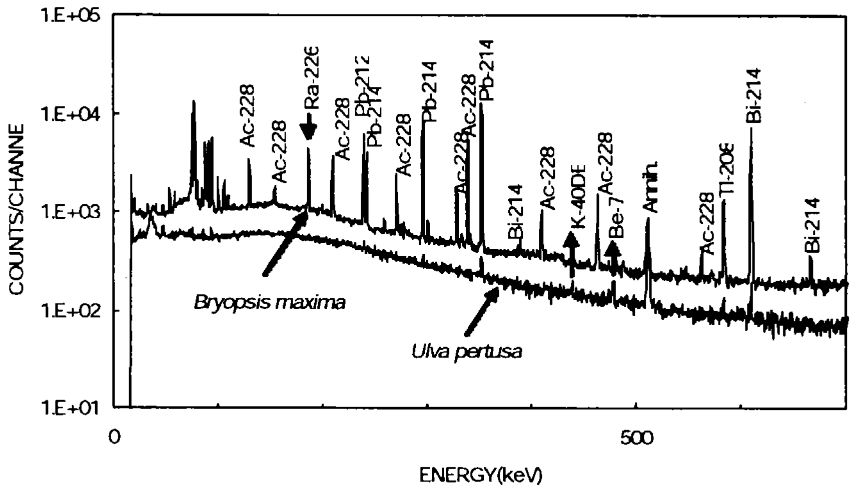

Figure 1. Gamma ray spectra of *Bryopsis maxima* and *Ulva pertusa*

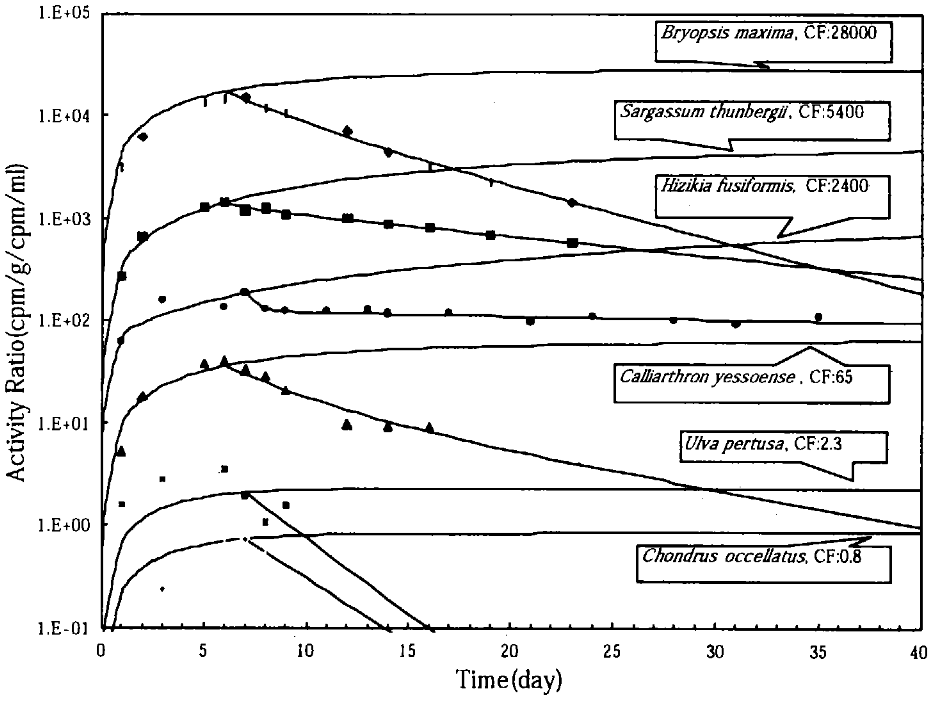

Figure 2. Accumulation and excretion of Tc-95m by algae