J Radioanal Nucl Chem (2011) 290:447–451
DOI 10.1007/s10967-011-1228-2

Biochemical fractionation and cellular distribution of americium and plutonium in the biomass of freshwater macrophytes

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Received: 19 May 2011 / Published online: 9 June 2011

Abstract Accumulation of americium (^{241}Am) and plutonium (^{238,242}Pu) and their distribution in cell compartments and biochemical components of the biomass of freshwater aquatic plants *Elodea canadensis, Ceratophyllum demersum* and *Myrioplyllum spicatum* and aquatic moss *Fontinalis antipyretica* have been investigated in laboratory experiments. Americium and plutonium taken up from water by *Elodea canadensis* apical shoots were mainly absorbed by structural components of plant cells (90% for ^{241}Am; 89% for ^{238}Pu and 82–87% for ^{242}Pu). About 10–18% of isotope activity was recorded in the cytosol fraction. The major concentration (76–92%) of americium was bound to cell wall cellulose-like polysaccharides of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Fontinalis antipyretica*, 8–24% of americium activity was registered in the fraction of proteins and carbohydrates, and just a minor concentration (<1%) in the lipid fraction. The distribution of plutonium in the biomass fractions of *Elodea* was similar to that of americium. Hence, americium and plutonium had the highest affinity to cellulose-like polysaccharides of cell walls of freshwater submerged macrophytes.

Keywords ^{241}Am · $^{238, 242}$Pu · Fractionation · Submerged macrophyte · Carbohydrates · Cellulose · Protein

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Introduction

Isotopes of plutonium and americium are typical components of radioactive nuclear wastes discharged into aquatic ecosystems. These nuclides dominate the long term radiotoxicity. The Yenisei River is contaminated with transuranium elements due to the operation of the Mining-and-Chemical Combine (ROSATOM), which has been producing weapon-grade plutonium since late 1950th. Actinides ^{239}Np, ^{239,240}Pu, ^{241}Am and ^{243,244}Cm have been detected in sediments, aquatic weeds of the Yenisei River, flood plain soils and berry shrubs in the flood plain [1–3]. Aquatic plants are an important component in migration of artificial radionuclides in the aquatic environment. Freshwater plants can accumulate transuranium elements in their biomass under natural [4, 5] and experimental conditions [6–8]. ^{241}Am and ^{242}Pu taken up from water by aquatic plant *Elodea canadensis* can tightly bind to the organic fraction of biomass [6, 8]. It has been shown that artificial radionuclides (^{137}Cs, ^{60}Co, ^{51}Cr) bind with different biochemical molecules of plant biomass [9]. The role of biochemical components of biomass in biosorption of ^{241}Am was estimated for yeasts [10] and fungi [11]. There are experimental data indicating that assimilation of artificial radionuclides by grazers depends on radionuclide distribution in microalgae cell compartments [12]. Hence, the data on the distribution of americium and plutonium in fractions of plant biomass can be used to understand the role of certain cell structures in uptake of actinides from water and to estimate further migration of these actinides in the aquatic environment. The purpose of this study was to estimate and compare the distributions of americium and plutonium in the intra- and extracellular compartments and biochemical components constituting the biomass of common freshwater macrophytes.

Experimental

Materials

The apical shoots (3–4 cm long) of common submerged macrophytes: vascular aquatic plants *Elodea canadensis* Michx., *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. and aquatic moss *Fontinalis antipyretica* Hedw. (5 cm long) were used for laboratory experiments. The plants were sampled in the Yenisei River upstream of the contaminated zone and acclimated to laboratory conditions during a week. In experiments we used filtered (0.2 μm RC-membranes, Schleicher & Schuell) Yenisei River water. ^{241}Am ($T_{1/2} = 434$ y) was added to water as a 2 M HNO_3 solution, ^{242}Pu ($T_{1/2} = 373000$ y) and ^{238}Pu ($T_{1/2} = 88$ y)—as 1 M HNO_3 solutions. Isotopes were obtained in Rimex Ltd. (St. Petersburg, Russia).

Biosorption experiments

Initial activity concentration of ^{241}Am in water was 1.1–1.2 kBq/l, ^{238}Pu—630 ± 11 Bq/l; ^{242}Pu—4 ± 1 (exp. No. 1) and 128 ± 13 (exp. No. 2) Bq/l. Isotopes were added to water, pH was adjusted to 7 with 0.1 M NaOH, and plants were placed in experimental vessels. The vessels were illuminated from the top for 14 h per day, 2 klx on the surface. For dark incubation, vessels were wrapped in aluminium foil. Water temperature was 19 °C.

The shoots were taken out of water after being maintained in the presence of isotopes for 2–6 days and rinsed with distilled water at pH 7 (adjusted with 0.1 M NaOH) for about 30 s.

Fractionation of plant biomass

To determine the dissolved intracellular portion of ^{241}Am, the washed fresh shoots were homogenized in a glass cylinder homogenizer with a glass pestle in the presence of a tiny amount of distilled water, pH 7. The homogenized biomass was separated by filtering through membranes (0.2 μm, RC, Schleicher & Schuell).

For subsequent extraction of lipids, proteins and carbohydrates, rinsed shoots were dried at 60 °C. The dry shoots were finely ground and lipids were extracted from the samples with a mixture of isopropyl alcohol and trichloromethane (1:1 v/v) for 24 h at room temperature. Extract of lipids was separated from particulate biomass by filtration through glass fibre filters (25 mm dia., Millipore, Ireland) [13]. The biomass remaining on the filters was treated with 1 M NaOH for 1 h in boiling water bath to extract protein. The alkali extracts were separated from the biomass residue by filtration as described above; the glass fibre filters of dia. 47 mm were used. The filters with the biomass residue were washed with distilled water at the end of filtration. The biomass residue was not separated from the filters prior to measurements of γ-activity.

Sample preparation and measurement of isotope concentration

For mass spectrometry and γ-counting the samples of biomass were digested in the mixture of H_2O_2 (30%) and HNO_3 (conc.) under heating. Liquid extracts were evaporated and the dry residues were also mineralised. The mineralised samples were transferred into scintillation vials and the final volume of samples was adjusted to 10 ml with distilled water. To avoid loss of activity, all glassware was rinsed with 1 M HNO_3 after contact with extracted fractions of the biomass, and the rinse fluids were added to the corresponding biomass fractions. The loss of isotopes during fractionation did not exceed a few percent except for ^{242}Pu, which will be discussed further in this paper.

^{241}Am and ^{238}Pu activities were measured in samples using a "Wallac 1480 Wizard 3" Gamma-counter (Perken Elmer, Finland). The activity of ^{238}Pu was recorded using the characteristic X-ray line. The statistic standard deviation of γ-counting did not exceed 10%. Concentrations of ^{242}Pu in samples (exp. No. 1) were measured on an α-spectrometer (7184, Eurisys Mesures, France) coupled to a PLUS-300 Si-low-background semiconductor detector. Sample preparation for α-spectrometry and the measurement technique were described by Bolsunovsky et al. [6]. Concentrations of ^{242}Pu in samples (exp. No. 2) were measured on an Agilent-7500a ICP mass-spectrometer (Agilent Technologies, USA). Detection limit for ^{242}Pu was 3 ng/l (or 0.4 Bq/l).

Results and discussion

Concentration of actinides in plant biomass

Activity concentration of americium taken up by apical shoots of macrophytes from water ranged from 528 to 1990 Bq/g of dry mass and was the highest in aquatic moss *F. antipyretica* (Table 1). The differences in americium biosorption by aquatic macrophytes could be due to differences in the surfaces areas of the plant species, resulting in dissimilarities in the number of binding sites on cell wall surface. The biosorption capacity also depends upon the plant's epiphytic activity and metabolic stress response. The ability of aquatic bryophytes (*F. antipyretica* in particular) to absorb high concentrations of heavy metals, radionuclides and organic pollutants is well known [14]. Well developed detoxication mechanisms allow survival of bryophytes at high concentrations of pollutants in water

Table 1 Activity concentrations of ^{241}Am, ^{242}Pu and ^{238}Pu in dry biomass of macrophyte species (Bq/g), taken up from water for 2–6 days

	Days	^{241}Am	^{242}Pu (exp. No. 1)	^{242}Pu (exp. No. 2)	^{238}Pu
Elodea canadensis	2	882 ± 74	13	424 ± 32	339 ± 73
Elodea canadensis	4	882 ± 194	19	466 ± 69	406 ± 94
Elodea canadensis	6	923 ± 153	18	388 ± 101	322 ± 26
Elodea canadensis	6	947 ± 172	17 ± 3	428 ± 28	329 ± 24
Elodea canadensis—dark incubation	2–6	915 ± 123	–	–	–
Ceratophyllum demersum	5	528 ± 74	–	–	–
Myriophyllum spicatum	5	960 ± 29	–	–	–
Fontinalis antipyretica	5	1990 ± 170	–	–	–

Mean for 3–6 replicates

[14, 15]. The accumulation of organic matter (epiphytic microorganisms and their exometabolites) and mineral (mainly carbonate) incrustations, which occur on vegetative organs of submerged macrophytes naturally, can increase the absorption of metals by plant surface [16]. This was proved experimentally for absorption of ^{241}Am by leaves of *E. canadensis* [13].

Plutonium accumulation was estimated for *E. canadensis* only. Activity concentration of ^{238}Pu in the biomass of *E. canadensis* was 322–406 Bq/g, ^{242}Pu—13–19 and 388–466 Bq/g in exp. No. 1 and No. 2, respectively. Activity concentrations of americium and plutonium accumulated in the biomass of macrophytes from water for 2–6 days did not differ considerably, indicating the equilibrium state in the system. Activity concentration of americium in the biomass of *Elodea* in the light was similar to its activity concentration in the dark, indicating the energy-independent (biosorption) mechanism of americium uptake from water. It has been shown experimentally that the biosorption mechanism of ^{241}Am by *Saccharomyces* from water involves ion exchange, complexation and nonspecific adsorption in cell wall owing to static electricity [10]. We can expect similar mechanisms for submerged macrophytes.

Actinides in intra- and extracellular compartments of *E. canadensis*

Microscopy showed that homogenization of *Elodea* shoots destroyed the cells and most of the chloroplasts. The major concentrations of americium and plutonium were registered in the fraction of particles of homogenized biomass larger than 0.2 μm (Table 2), i.e. cell walls, membranes and organelles. Up to 10% of americium and up to 18% of plutonium activities were registered in the filtrate (dissolved fraction), which contained particles smaller than 0.2 μm, i.e. cytosol. Similar results were reported for americium in marine microalgae [17, 18]: up to 7% of ^{241}Am was registered in cytoplasm, and the major portion of the radionuclide was bound to structural components (cell walls and plasmalemmae). Major role of cell walls in biosorption of ^{241}Am was reported also for yeasts [10] and fungi [11]. Reinfelder and Fisher [19] referred to higher affinity of ^{241}Am, as well as plutonium, to oxygen than to nitrogen or sulfur [20] when explained the low penetration of ^{241}Am into cytoplasm of microalgae.

It was shown experimentally [19] that the larger concentration of the metal is dissolved in cytoplasm of microalgae, the more effectively it is assimilated by crustaceans. Due to low penetration of ^{241}Am in cytoplasm of microalgae, the assimilation of ^{241}Am by copepods was low (0.9%). Marine bivalves, however, assimilated up to 40% of ^{241}Am from labeled microalgae [12]. The retention of ^{241}Am ingested by calanoid copepod with labeled algae (4.5%) was more efficient than the retention of ^{237}Pu (0.8%) [18].

Actinides in biochemical fractions of plant biomass

The distribution of americium in biochemical fractions of biomass was estimated for four macrophyte species: vascular aquatic plants *E. canadensis, M. spicatum* and *C. demersum* and aquatic moss *F. antipyretica* (Fig. 1). The lipid fraction of the biomass contained 0.1–0.8% of

Table 2 Activity percentages in the dissolved intracellular fraction (cytosol) and the particulate biomass of *E. canadensis* (mean ± SD, $n = 3$)

Cell fraction	^{241}Am	^{238}Pu	^{242}Pu (exp. No. 1)	^{242}Pu (exp. No. 2)
Dissolved	10 ± 2	11 ± 3	13 ± 4	18 ± 12
Particulate	90 ± 2	89 ± 3	87 ± 4	82 ± 12

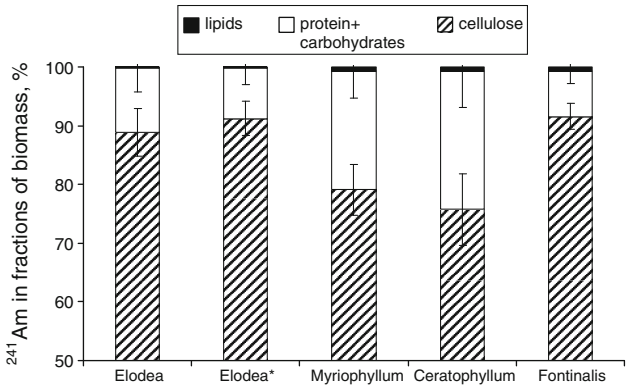

Fig. 1 Distribution of ^{241}Am (% of total activity) among fractions of the biomass of four macrophyte species: *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Fontinalis antipyretica*. Mean ± SD, $n = 3$. * *Elodea* was incubated in the dark

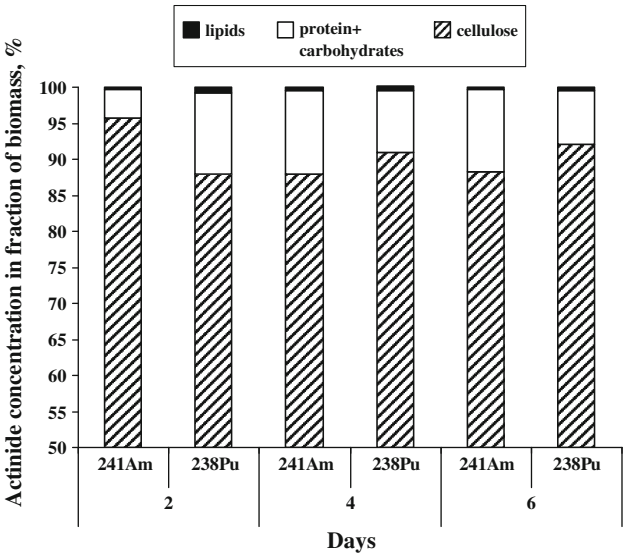

Fig. 2 Distributions of ^{241}Am and ^{238}Pu (% of total activity) among biochemical fractions of the biomass of *Elodea canadensis* after 2–6 days of incubation in water containing radionuclides

americium. As we estimated before, the total concentration of lipids in the biomass of *E.canadensis* was 3.6% [13]. The contribution of lipids to the adsorption of ^{241}Am by fungi and yeasts was not considerable [10, 11], that correspond with our data for submerged macrophytes (Fig. 1).

The concentration of americium in alkali extract of the biomass of macrophytes was 8–24% (Fig. 1). Alkali extract of *E. canadensis* biomass contained the major concentration of cellular protein (95 ± 5%) and the major concentration of carbohydrates (89 ± 13%) [13]. Total concentration of protein in the biomass of *E. canadensis* was 30%, carbohydrates—6% [13]. The effect of protein and carboxyl functional groups on the adsorption of americium was demonstrated for yeasts and fungi [10, 11]. The extraction of carboxyl functional groups from biomass of yeasts and fungi reduced the adsorption capacity of these microorganisms most effectively [10, 11], implying that the carboxyl groups may play more important role in adsorption of americium than protein. The contribution of protein to the biosorption of americium was most essential for yeasts [10] than for fungi [11].

The percent of ^{241}Am in the biomass residue of macrophytes was 76–92% (Fig. 1). The biomass residue was considered to be mainly cellulose-like polysaccharides of cell walls. The biomass residue of *Elodea* in our experiments also contained 5% of cellular protein and 11% of carbohydrates [13]. The biomass residue comprised 19% of *Elodea* biomass [13] and 20% of *F. antipyretica* biomass. Other authors reported the content of cellulose in the tissues of *Elodea* and *Fontinalis* about 15%, lower concentration of cellulose was detected in *Myriophyllum* (5–10%) [21]. This proves our assumption of the cellulose basis of the biomass residue in our experiments. The results for *Elodea* are consistent with our results obtained previously [13]. The biochemical fractionations of americium absorbed by *Elodea* under dark and light conditions were similar (Fig. 1), supporting the mechanism of biosorption.

The distribution of ^{238}Pu in the biochemical fractions of the biomass of *Elodea* (Fig. 2) was similar to the distribution of ^{241}Am: the major portion of plutonium (88–96%) was detected in the biomass residue, i.e. bound to cellulose-like polysaccharides of cell walls. From 7 to 11% of ^{238}Pu was recorded in the fraction of proteins and carbohydrates and 0.5–0.8% in the fraction of lipids. Hence, of the molecules constituting *Elodea* biomass, cellulose-like polysaccharides are principal concentrators of ^{241}Am and ^{238}Pu. The distributions of americium in the biomass of three vascular plant species and aquatic moss do not differ essentially (Fig. 1). The distribution of ^{241}Am and ^{238}Pu in the biochemical fractions of the biomass of *Elodea* did not change essentially during plant exposure in water containing actinides (Fig. 2). The results of biochemical fractionation of ^{242}Pu are not presented here, because of essential loss (up to 60%) of ^{242}Pu concentration during the preparation of the samples for mass spectrometry.

Other authors reported an important role of extractable polysaccharides and lipids in retention of such artificial isotopes as ^{137}Cs, ^{60}Co and ^{51}Cr in the biomass of *Elodea canadensis* [9]. Compared to macrophytes, the biosorption of radionuclides in the biomass of cyanobacteria and microalgae was much higher due to higher concentration of these isotopes in the fraction of sugars [9]. But the concentration of isotopes in cellulose of cell walls was not taken into consideration.

Conclusions

Transuranium elements americium and plutonium taken up from water by *E.canadensis* apical shoots were mainly

(90% for ^{241}Am; 89% for ^{238}Pu and 82–87% for ^{242}Pu) absorbed by structural components of plant cells (mostly cell walls). About 10–18% of the activity was recorded in the dissolved fraction including cytosol.

The major portion of ^{241}Am (76–92%) was bound to cellulose-like polysaccharides of cell walls of *E. canadensis*, *M. spicatum*, *C. demersum* and *F. antipyretica*, 8–24% of americium activity was registered in the fraction of proteins and carbohydrates, and just a small portion (<1%) in the lipid fraction. The distribution of ^{238}Pu in the biochemical biomass fractions of *Elodea* was similar to that of ^{241}Am. Hence, americium and plutonium had the highest affinity to cellulose-like polysaccharides of cell walls of freshwater submerged macrophytes.

Acknowledgments The authors are grateful to D. Dementyev (IBP SB RAS, Krasnoyarsk), A. Zhyzhaev (ICCT SB RAS, Krasnoyarsk) and M. Melgunov (IGM SB RAS, Novosibirsk) for measurements of actinides in samples. The research was supported by Grant of Russian Foundation for Basic Research and Krasnoyarsk Scientific Foundation No. 09-04-98010.

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