Vol. 11: 233–237, 1983 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published March 24

Accumulation and cellular distribution of ^{241}Am, ^{210}Po, and ^{210}Pb in two marine algae

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ABSTRACT: Accumulation and cellular distribution of ^{241}Am, ^{210}Po, and ^{210}Pb were studied in the marine diatom *Thalassiosira pseudonana* and the marine green alga *Dunaliella tertiolecta*. Both species concentrated Am, Po, and Pb from artificially spiked cultures, resulting in wet weight concentration factors of approximately 1 to 4 × 10^5 for ^{241}Am, 3 to 7 × 10^4 for ^{210}Po, and 5 to 17 × 10^3 for ^{210}Pb. These concentration factors are comparable to those based on analyses of natural plankton assemblages. In contrast to ^{210}Po, ^{241}Am and ^{210}Pb appeared to associate almost exclusively with structural components (cell walls and plasmalemmae) and showed no evidence of protein association. The data, together with field evidence, suggest that ^{241}Am and ^{210}Pb are not bound to cell material easily assimilated by herbivorous zooplankton, while ^{210}Po associates with cellular organic compounds and is assimilated in animals. This may lead to high ^{210}Po turnover rates in surface waters.

INTRODUCTION

Deposition and toxicity of heavy metals in phytoplankton cells have been reviewed by Davies (1978), Huntsman and Sunda (1980), and others. Despite a voluminous literature, there are comparatively few studies on the cellular localization of metals in algae, and certainly none that include any of the actinide metals. It is known that phytoplankton concentrate some transuranic elements from surrounding waters (Fisher et al., 1980; Yen, 1981; Fisher et al., in press), and the cellular localization of transuranics and other metals may have some bearing on the role organisms play in mediating the distribution of these metals in the sea. For example, metals associated with inorganic matrices and with poorly digested algal cell walls may be expected to pass rapidly through herbivores grazing on phytoplankton, and be transported to deep water and sediments via sinking fecal pellets. Metals associated with utilizable proteins present in algal cytoplasm, organelles or membranes, may pass more effectively through a herbivore's gut lining and be retained by the animal.

In this introductory study we have examined the site of deposition in algal cells of americium-241, a transuranic element which is introduced into the marine environment through anthropogenic activities, and which rapidly accumulates in algae by passive adsorption (Fisher et al., in press). Two algal species were compared: the diatom *Thalassiosira pseudonana* and the naked green alga *Dunaliella tertiolecta*. We have also compared the behaviour of ^{241}Am in these 2 algae with that of 2 natural uranium decay series nuclides: polonium-210 and lead-210. Previous analyses of environmental samples have shown that ^{210}Po is relatively reactive in marine ecosystems and is the most significant source of natural radiation doses experienced by marine organisms (Heyraud, 1982). High concentrations of ^{210}Po have been observed in tissues of several classes of marine animals, with especially high concentrations in the hepatopancreas of planktonic invertebrates (Heyraud and Cherry, 1979; Heyraud, 1982; Cherry and Heyraud, 1981). There have been no studies reported on the biokinetics of ^{210}Po in phytoplankton, although it appears from the few field measurements of suspended particles that this element is accumulated by these microorganisms (see below). The behaviour of ^{210}Pb, the radioprecursor of ^{210}Po, was also examined since measurements of this nuclide in marine invertebrates suggest that ^{210}Pb is less reactive for organic compounds than ^{210}Po and that it associates

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0171-8630/83/0011/0233/$ 02.00

differently with biological materials (Heyraud and Cherry, 1979; Heyraud 1982; Heyraud and Cherry, in press).

MATERIALS AND METHODS

Cells of the diatom *Thalassiosira pseudonana* (Clone 3H) and the chlorophyte *Dunaliella tertiolecta* (Clone Dun) were maintained axenically in sterile-filtered (0.2-µm Nuclepore filter) Mediterranean surface seawater enriched with f/2 nutrients (Guillard and Ryther, 1962) but without added Cu, Zn or EDTA. Experimental inocula, taken from stock cultures in late log phase, were transferred to sterile 1-l Erlenmeyer flasks containing 800 ml of unenriched sterile-filtered Mediterranean seawater. The flasks were stoppered with polyurethane plugs and wrapped with aluminium foil to prevent illumination of cells and possible cell division, thereby ensuring a constant biomass in each flask. The initial cell density was set at 8×10^4 cells ml^{-1} for each species for the Po and Pb experiments (corresponding to about 10 µg and 8.2 µg wet weight ml^{-1} for *D. tertiolecta* and *T. pseudonana*, respectively) and 3×10^4 cells ml^{-1} for the Am experiments. Subsequent cell counts using a modified Fuchs-Rosenthal hemacytometer confirmed that no cell growth had occurred during the experiments. Immediately after inoculation, 110 µl of a 0.2-µm filtered solution containing ^{210}Pb and ^{210}Po (dissolved in dilute HNO_3) in approximate equilibrium, or 50 µl of filtered ^{241}Am solution (^{241}Am dissolved in dilute HNO_3) were introduced to flasks with an Eppendorf pipet. The radioactivity was measured in the water as 0.037, 0.040 and 46 Bq ml^{-1} for ^{210}Po, ^{210}Pb and ^{241}Am, respectively. Control flasks contained the same seawater and isotopes but no cells. All flasks were maintained without shaking for 4 d at 17 °C ± 1 C°.

At 72 h, aliquots of cells were filtered onto 1-µm Nuclepore filters and washed with 10 ml of unlabelled glass fiber filtered seawater (Millipore filters were found to give less satisfactory blank values, particularly for ^{210}Po and ^{210}Pb). The radioisotope content of cells after 72 h exposure was used to calculate 72 h concentration factors, defined as atoms of isotope g^{-1} cell wet weight (or for vol/vol concentration factors as atoms μm^{-3}) divided by atoms of isotope dissolved per ml (or μm^{-3}) of ambient seawater. (Preliminary experiments showed that isotope uptake was essentially complete by 72 h). The wet weight and volume of each *Thalassiosira pseudonana* cell were determined to be 102 pg and 61 μm^3, respectively; and 125 pg and 91 μm^3, respectively for each *Dunaliella tertiolecta* cell (Fisher et al., in press). After filtration the 72 h cells were resuspended into unlabelled sterile-filtered seawater and allowed to stand for 24 h to remove loosely bound isotope (Fisher et al., in press). An aliquot of the cells was then assayed for radioactivity. The remaining cells were refiltered and resuspended into distilled water, which caused virtually all of them to burst. The cell soups were then centrifuged at 4 °C at 745 g (5 min), 2000 g (15 min) and 10 000 g (15 min). Each pellet and the final supernatant were collected separately. Radioactivity was measured in each fraction by standard techniques (see below). The protein content of each fraction was determined by the Coomassie Blue technique (Bradford, 1976) using modifications for dilute solutions (Setchell, 1981).

In a preliminary study of the binding strength of ^{241}Am to phytoplankton, the first pellet in the *Thalassiosira pseudonana* differential centrifugation scheme was suspended into 6 ml of 0.05 M Tris buffer pH 7.4. Half the suspension was placed into each of 2 dialysis bags made of membrane with a molecular weight cut-off of approximately 12 000. One bag was dialyzed against 45 ml of buffer, the other against buffer containing 0.1 M β-mercaptoethanol. After 2 h dialysis at ice temperature and gentle shaking, the contents of the bags and buffers were counted for radioactivity.

Radioactivity of ^{241}Am-containing samples was determined by detecting 60 KeV photons with a multichannel analyzer coupled to two 7.6-cm well-type NaI (Tl) crystals. ^{210}Po and ^{210}Pb were measured by a standard technique involving acid digestion of samples and plating of ^{210}Po on silver discs prior to alpha-counting (e.g. Heyraud and Cherry, 1979). Counting errors (1 σ) for samples analyzed to determine concentration factors were ≤ 6 % for all isotopes.

RESULTS AND DISCUSSION

Both algal species accumulated all 3 isotopes from seawater (Table 1). ^{210}Po uptake was essentially complete after 1 d; ^{210}Pb and ^{241}Am uptake continued through Day 3, but the rate of change in cellular nuclide association after 3 d had dropped to about 10 % d^{-1}. Typical time course results are described in detail elsewhere (Fisher et al., in press). Results using cell-free control cultures indicated that approximately 4 % of ^{210}Po associated with particles >1 µm, while approximately 38 % of the ^{210}Po associated with particles <1 µm but >0.2 µm. In contrast, only 0.4 % and 0.5 % of ^{210}Pb activity and 2 % and 4 % of ^{241}Am activity were retained by 1-µm and 0.2-µm filters, respectively.

We calculated wet weight concentration factors (WCF) by subtracting the appropriate control value from the cellular activity, dividing by the wet biomass and then dividing this number by the amount of nuclide remaining in solution. These are shown in Table 1 along with the 72 h volume concentration factors

Table 1. Concentration factors, at 72 h, for ^{241}Am, ^{210}Po, and ^{210}Pb in marine phytoplankton, expressed on volume/volume (VCF) and wet weight (WCF) bases. Also shown are WCF's in natural phytoplankton samples, for comparison. All values are to be multiplied by 10^4

Specimen	Reference	^{241}Am		^{210}Po		^{210}Pb	
		VCF	WCF	VCF	WCF	VCF	WCF
T. pseudonana	This report	69	41	12.3	7.4	2.87	1.72
D. tertiolecta	This report	18	13	4.3	3.1	0.76	0.55
Natural particulates, Irish Sea	Pentreath et al. (1982)	–	*22±3	–	–	–	–
Natural diatoms, Cape of Good Hope	Shannon et al. (1970)	–	–	–	5±3	–	0.9±0.8
Natural phytoplankton, California coast	Martin and Knauer (1973)	–	–	–	–	–	*†2.8±0.5
Natural phytoplankton, Peru	Heyraud and Cherry (1979)	–	–	–	0.9	–	0.12
Natural phytoplankton, Washington coast	Bennett and Carpenter (1979)	–	–	–	*0.9±0.4	–	*▽0.16±0.08

* Data originally expressed on dry weight basis; we assume dry weight = 10 % wet weight for this comparison
† Organic fraction of cells only; based on stable Pb measurements; assumes [Pb] in seawater = 30 ng l^{-1} (Brewer, 1975)
▽ Assumes [^{210}Po] in seawater = 0.925 mBq l^{-1} and [^{210}Pb] = 1.85 mBq l^{-1} (Shannon et al., 1970)

(VCF) for each nuclide in both species. The concentration factors ranged from WCF's of 5.5×10^3 for ^{210}Pb in *Dunaliella tertiolecta* to 4.1×10^5 for ^{241}Am in *Thalassiosira pseudonana*; VCF's ranged from 7.6×10^3 for ^{210}Pb in *D. tertiolecta* to 6.9×10^5 for ^{241}Am in *T. pseudonana*. For both species, ^{210}Po concentration factors were intermediate between those for ^{241}Am and for ^{210}Pb. Where comparative data were available, the WCF's measured in these culture experiments were of the same order of magnitude as those measured in natural plankton samples, although the latter exhibit considerable variation in both ^{210}Po and ^{210}Pb WCF's (Table 1). The high concentration factors for ^{241}Am are comparable with data for other transuranic isotopes such as ^{237}Pu and ^{252}Cf (Fisher et al., in press), and higher than those reported for transition metals (Lowman et al., 1971). Differences in the reactivity of these elements may possibly reflect differences in their speciation in seawater, although all were added as nitrates.

Bioaccumulation of ^{210}Pb and of transuranic elements, including ^{241}Am, in marine phytoplankton proceeds by passive adsorption onto cell surfaces (Schultz-Baldes and Lewin, 1976; Fisher et al., 1980; Yen, 1981; Fisher et al., in press). Generally, 2 cellular pools are observed for these metals: an easily lost pool and a tightly-bound fraction, with the percentage in the latter increasing with exposure time (Schultz-Baldes and Lewin, 1976; Fisher et al., in press). There are no data in the literature on ^{210}Po uptake by phytoplankton, though our data for bioaccumulation of this element in phytoplankton maintained in the dark suggest that it also proceeds passively, or at least requires no illumination.

Nearly all the ^{241}Am and ^{210}Pb was associated with the heavy cell fractions contained in the first 2 pellets (Table 2). Thus 94 % of total cellular ^{241}Am in both species was in the first 2 pellets, and 100 % and 96.5 % of the ^{210}Pb in *Thalassiosira pseudonana* and *Dunaliella tertiolecta*, respectively, were in these pellets. In contrast, only 62 % and 69 % of the cellular ^{210}Po in *T. pseudonana* and *D. tertiolecta*, respectively,

Table 2. Fractionation of cellular protein, ^{241}Am, ^{210}Po, and ^{210}Pb in *Thalassiosira pseudonana* (3H) and *Dunaliella tertiolecta* (Dun). Counting errors ≤ 10 % unless noted

Cell fraction	% total cell protein (weighted mean ±1 SD)		% total activity in each cell fraction					
	(n = 3)	(n = 4)	^{241}Am		^{210}Po		^{210}Pb	
	3H	Dun	3H	Dun	3H	Dun	3H	Dun
1st pellet (754 g, 5 min)	31±2	16±3	83	89	43.5	30	82.5	59
2nd pellet (2000 g, 15 min)	9±4	7±4	11	5	18.5	39	17.5†††	37.5
3rd pellet (10 000 g, 15 min)	5±5	5±3	4	1	7.5	2.5•••	0	2††
Supernatant	55±11	72±9	2	5	30.5	28.5	0	1.5••
Total per cell	7.6±3.0•	12.8±1.7•	667†	133†	0.20†	0.13†	0.08†	0.03†

• pg $cell^{-1}$ •• counting error = 33 % ••• counting error = 20 %
† µBq $cell^{-1}$ †† counting error = 14 % ††† counting error = 25 %

Table 3. $^{210}Po/^{210}Pb$ activity ratios in pelagic plankton and zooplankton fecal material. Values are means ± 1 SD and are shown with number of observations in parentheses

	Zooplankton feces•	Phytoplankton••	Mixed zooplankton+	Hepatopancreas of pelagic crustaceans△
$^{210}Po/^{210}Pb$ Activity ratio	2.3 ± 0.7 (n = 7)	4.6 ± 2.1 (n = 4)	26.7 ± 17.0 (n = 46)	131 ± 76 (n = 13)

• Beasley et al. (1978); Heyraud (1982)
•• Shannon et al. (1970); Heyraud and Cherry (1979)
+ Shannon et al. (1970); Turekian et al. (1974); Kharkar et al. (1976); Heyraud and Cherry (1979)
△ Heyraud and Cherry (1979); Cherry and Heyraud (1981)

were in the first 2 pellets with approximately 30 % of the isotope contained in the final supernatant. The first pellet, which contained most of the cell wall and plasmalemmae debris, held 2 to 3 times as much ^{241}Am or ^{210}Pb, on a percentage basis, as ^{210}Po for both species. The fractionation of total cellular protein is also given in Table 2. From these data it appears that ^{241}Am and ^{210}Pb associate with cell walls or membranes, and do not generally bind with cellular protein in these species. This is consistent with previous circumstantial evidence that suggests binding of transuranic elements to diatom cell walls (Fisher et al., 1980; Fisher et al., in press). The dialysis experiment with ^{241}Am showed that none of the nuclide in the material in the first pellet from *T. pseudonana* was removed when exposed to β-mercaptoethanol, supporting the hypothesis that this element is probably not bound by sulfhydryl groups in proteins. Transuranic metals tend to concentrate to higher levels and have longer biological half-lives in diatoms than in green algae, possibly reflecting differences in the metal affinities of different cell surfaces (Fisher et al., in press). In contrast to the siliceous frustule of the diatom, *D. tertiolecta* is a naked flagellate and has only an external protein-coated membrane (Jokela, 1969). The ^{210}Po data suggest that there was a more uniform distribution of this element in the various cell fractions, and that its cellular distribution more closely followed the protein distributions. Though ^{210}Po may initially attach to cell surfaces, once associated with certain proteins along a cell membrane it may be carried into the interior of the cell (see Williams, 1981, for general discussion of this topic).

^{210}Po has been found to concentrate in the hepatopancreas of several marine invertebrates (Heyraud and Cherry, 1979). Preliminary experiments with lobster and squid hepatopancreas have indicated that ^{210}Po is associated with protein in this organ (Cherry et al., 1979; Burns, Cherry and Heyraud, unpubl.). If, as in the invertebrate hepatopancreas, ^{210}Po associates with proteins in phytoplankton, and if ^{210}Pb and similarly reacting elements such as ^{241}Am are bound only to structural components of cells, then it may be expected that ^{210}Po would be assimilated to a greater extent by herbivores than ^{210}Pb or ^{241}Am. Consistent with this hypothesis are data indicating that $^{210}Po/^{210}Pb$ ratios in fecal pellets of zooplankton are half those of their phytoplankton food supply (Table 3) in field samples; more field data are needed to determine whether this difference is significant. It appears that these herbivores retain proportionately more of the ^{210}Po in algae than ^{210}Pb, ultimately assimilating the ^{210}Po into the hepatopancreas, while a higher proportion of the ^{210}Pb is excreted in fecal material. Similarly, Fisher et al. (in prep.) and Gorsky et al. (in press) have shown that euphausiids and appendicularians assimilate negligible amounts of ^{241}Am from a contaminated algal diet. The assimilation of ^{210}Po-containing molecules by herbivores could lead to a higher turnover rate for ^{210}Po than for ^{210}Pb or ^{241}Am in surface waters. Regeneration rates of ^{210}Po appear to be greater than those of ^{210}Pb in coastal waters off New York (Li et al., 1981) and in the neuston layer of the coastal Mediterranean (Heyraud and Cherry, in press).

Acknowledgements. The International Laboratory of Marine Radioactivity operates under a tripartite agreement between the International Atomic Energy Agency, the Government of the Principality of Monaco and the Oceanographic Institute at Monaco.

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This paper was presented by Dr. D. S. Woodhead; it was accepted for printing on January 29, 1983