SUBCELLULAR PARTITIONING OF HEAVY METALS IN GILLS AND VISCERAL MASS OF BIVALVES FROM THE NEW CALEDONIAN LAGOON

M. Metian [1], L. Hédouin [1-3], C. Barbot [4], O. Cotret [1], J.-L. Teyssié [1], S.W. Fowler [1] *, F. Goudard [4], J.-P. Durand [4], P. Bustamante [2] and M. Warnau [1]

[1] International Atomic Energy Agency – Marine Environment Laboratory, 4 Quai Antoine Ier, MC-98000 Principality of Monaco
[2] IRD-Noumea Center, New-Caledonia
[3] LBEM, UPRES-EA 3168, Université de La Rochelle, France
[4] GERMETRAD, SMAB UPRES-EA 2160, Université de Nantes, France

Abstract

The present work examined subcellular distribution of 5 metals and 2 radionuclides in two bivalve species in order to assess the potential toxicity of these elements in the organisms. The results indicate that Ag and ^{241}Am are preferentially associated with the cell membranes and organelles whereas Cr, Zn, Cd, Co, and ^{134}Cs are predominantly found in the cytosolic fraction of the cells.

Keywords: Metals, Radionuclides, Subcellular Fractioning, Bivalves.

Introduction

New Caledonia is the third producer of nickel in the world and this small South Pacific island is estimated to contain no less than 20% of the total stock of Ni on the planet. Metal contamination resulting from the nickel mining industry and related activities constitutes a long lasting threat for the marine ecosystems sheltered by the second largest reef system in the world [1]. However, as almost a rule when it concerns tropical ecotoxicology, available information on metal contamination in New Caledonia waters is extremely scarce and very little is known about the extent of local contamination and possible environmental impacts [1]. Moreover, a new extraction process of Ni (lixiviation, *viz.* acidic extraction) has recently been tested at the industrial level and should be implemented in the near future (2006-2007). This process will result inevitably in increased discharges of co-occurring metals in Ni ores (e.g. Co and Cr). Thus, information is needed in order to assess the possible impact of these additional metal inputs on local ecosystems.

The objective of the present study was to determine the potential toxicity of metals in two species commonly found in the lagoon: the edible clam *Gafrarium tumidum* and the oyster *Isognomon isognomon*. Therefore, subcellular distribution of five metals (Cd, Co, Cr, Zn, Ag) and two anthropogenic radionuclides (^{134}Cs, ^{241}Am) was examined in the gills and visceral mass of both species following seawater exposure using highly sensitive radiotracer techniques.

Materials and Methods

Both bivalve species were acclimated to laboratory conditions (open circuit aquaria; water renewal 10% hr^{-1}; S, 36 p.s.u.; T, 26 ± 0.5°C) for 6 weeks prior to experimentation. The organisms were then experimentally exposed for 28 days to radiotracers of five heavy metals (^{109}Cd, ^{57}Co, ^{51}Cr, ^{65}Zn, ^{110m}Ag) and two radionuclides (^{134}Cs, ^{241}Am) directly via seawater. At the end of the experiment, 6 individuals of each species were collected and dissected. The gills and visceral mass were separated, pooled, and processed for subcellular fractioning according to a previously described method [2]. Four different fractions were isolated using differential centrifugation (see Table 1). Distribution of the radiotracers among the different subcellular fractions was determined using high efficiency gamma spectrometry [2].

Table 1. Subcellular partitioning (mean %) of radioisotopes in gills and visceral mass of two bivalves

	Gafrarium tumidum							*Isognomon isognomon*						
Gills	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am
Nuclei	18	28	28	30	73	20	25	17	16	22	14	23	17	27
Lysosomes + mitochondria	6	7	6	2	6	7	6	10	19	30	15	34	12	36
Membranes	10	17	16	1	6	13	25	19	8	15	10	23	16	10
Microsomes	10	22	19	1	5	13	27	10	5	0	6	7	11	4
Cytosol	57	25	31	67	10	48	17	44	52	33	54	13	45	22
Visceral mass														
Nuclei	28	9	24	10	49	27	42	25	20	28	24	43	26	35
Lysosomes + mitochondria	13	6	10	2	12	11	22	22	10	20	15	27	19	47
Membranes	7	3	15	1	3	6	19	6	2	5	3	19	6	6
Microsomes	6	3	12	1	2	5	13	7	3	6	3	4	7	3
Cytosol	45	79	40	87	35	51	4	39	65	41	54	7	42	9

Results and Discussion

Measurements of specific enzymatic markers (acid phosphatase for lysosomes; glucose-6-phosphatase for microsomes; 5' nucléotidase for plasmic membrane) indicated that the purity of the different subcellular fractions was good. Results of the subcellular distribution of the different metal radiotracers and radionuclides in gills and visceral mass are given in Table 1.

Globally, the distributions in both tissues were similar for each given bivalve species. The only main departure from this was observed for ^{57}Co in the clam: the cytosolic fraction was much lower in the gills (25%) than in the visceral mass (79%). Cr, Co, Zn, Cd and ^{134}Cs were mainly found in the cytosolic fraction (30 - 87%) whereas ^{110m}Ag and ^{241}Am were mainly associated with membranes and organelles (65 - 96%). These results are in agreement with those reported for other bivalves from temperate waters (e.g. the scallop *Chlamys varia* [3] and the oyster *Crassostrea gigas* [4]).

The predominant distribution of Ag in the insoluble fraction could be due to specific Ag storage/detoxification in the two bivalve species. Indeed, some bivalves are well known to be able to trap Ag as non toxic Ag_2S precipitates within their tissues [5].

Preferential distribution of most radioelements in the cytosol suggests that, once incorporated into the cells, a large part of these metals could be toxic, since they are susceptible to bind key soluble components of the cells (e.g. proteins, enzymes, DNA). However, in the case of Cd and Zn, a substantial part of the cytosolic metal is most probably detoxified as "metal-metalloprotein" complexes (approx. 40% in the case of Cd according to Boisson *et al.* [2]). Furthermore, the metals preferentially associated with the cytosolic fraction are likely to be readily bioavailable to higher trophic levels preying on these organisms [6]. This is of particular concern since the clam *G. tumidum* is consumed by local populations and could therefore be a non-negligible source of human exposure to metals through seafood consumption.

References

1 - Labrosse P., Fichez R., Farman R., and Adams T., 2000. New Caledonia. Pp. 723-736. *In:* Sheppard C.R.C. (ed.), Seas at the Millennium: An Environmental Evaluation, vol. 2. Pergamon Press, Amsterdam.

2 - Boisson F., Goudard F., Durand J.-P., Barbot C., Piery J., Amiard J.-C., and Fowler S.W., 2003. Comparative radiotracer study of cadmium uptake, storage, detoxification and depuration in the oyster *Crassostrea gigas* : potential adaptive mechanisms. *Mar. Ecol. Prog. Ser.*, 254: 177-186.

3 - Bustamante P., Guyot T., and Miramand P., submitted. Subcellular and body distributions of 17 trace elements in the variegated scallop *Chlamys varia* from the Charente-Maritime coast (France).

4 - Milcent M.C. *et al.*, 1996. Identification of Cs-137 and Am-241 binding sites in the oyster *Crassostrea gigas*. *Biochem. Molec. Biol. Intern.*, 39: 137-148.

5 - Berthet B., Amiard J.C., Amiard-Triquet C., Martoja R., and Jeantet A.Y., 1992. Bioaccumulation, toxicity and physico-chemical speciation of silver in bivalve mollusks : ecotoxicological and health consequences. *Sci. Total Environ.*, 125: 97-122.

6 - Reinfelder J.R., and Fischer N.S., 1991. The accumulation of element ingested by marine copepods. *Science*, 251: 794-796.