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Subcellular Distribution of Naturally Elevated Cadmium in the Antarctic Clam *Laternula elliptica*

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Antarctic marine environment, though one of the most pristine environments on earth, shows high levels of Cd in surface water accounted for by the upwelling of nutrient rich deep water (Honda et al. 1987). Cadmium which has a high affinity to phosphate seems to be taken up by phytoplankton, and accumulated in various organisms of higher trophic levels through the food web. Elevation of Cd, as a result, is likely a unique feature of Antarctic marine organisms (Honda et al. 1987; De Moreno et al. 1997).

Antarctic clam, *Laternula elliptica*, has been recognized as a sentinel organism for metal pollution monitoring in Antarctic shallow water by virtue of its high metal accumulating capacity, wide distribution, and high population density (Ahn et al. 1996; SCAR 1996). This slow growing and long-lived (>20 years, Brey and McKenson 1997) cold-water bivalve species is known to strongly accumulate Cd in their tissues throughout their lifetime (Ahn et al. 2001). Especially in the kidney, the Cd level is extremely high (~350 $\mu g \cdot g^{-1}$ dry weight), comparable to those reported from the kidneys or digestive glands of temperate bivalves deliberately exposed to unnaturally high levels of Cd in experimental media. The high accumulation of Cd in the kidney reaching a peak at a shell length of approximately 80 mm suggests that *L. elliptica* may have developed specific Cd detoxifying and controlling mechanisms (Ahn et al. 2001). In molluscs, cellular metal detoxification commonly involves sequestration to soluble metal binding ligands or compartmentalization into insoluble particles (Viarengo and Nott 1993; Mason and Jenkins 1995; Langston et al. 1998). In the present study, we examined subcellular distribution of Cd in different tissues with a focus on Cd partitioning in soluble or insoluble fractions of cells.

MATERIALS AND METHODS

Laternula elliptica were collected from 20-30m depths at a station in Marian Cove near King Sejong Station (62°13′S, 58°47′W), King George Island between mid January to early February, 1999. Clams were depurated for 2 days and dissected into siphon-mantle, gill, digestive gland, gonad, and kidney. Each tissue from an individual clam was homogenized in 2 to 3 volumes of ice-cold 150 mM

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NaCl, 20 mM Tris-HCl (pH 8.6) containing 0.5 mM phenylmethylsulphonyl fluoride and 1.4 mM β-mercaptoethanol. The homogenate was centrifuged at 100,000 g at 4°C for 2 hours to separate cytosolic fraction (supernatant) from insoluble fraction (pellet). Both the cytosolic and insoluble fractions were kept at -70°C until further analysis.

Aliquots of supernatant (2 ml) and pellet (about 0.1 ~ 0.4 g wet wt) were digested in 30 ml Teflon tubes containing 6 ml of concentrated HNO_3 (Suprapur*, Merk) at room temperature for 1 hour followed by boiling at 100°C for about 6 hours until the solution became clear. Drying at 100°C evaporated excess HNO_3 and the residue was dissolved at 100°C in 14 ml of 1% HNO_3 for one hour. Concentrations of Cd were determined by inductively coupled plasma (ICP) – mass spectrometry (MS) (Perkin Elmer, Elan 6100) and/or ICP-atomic emission spectrometry (AES) (JOBIN YVON, JY 138 Ultrace) at Korea Basic Science Institute (KBSI, Seoul Branch). The accuracy of the metal analysis was checked using the standard reference materials for oyster (SRM 1566a, NIST, USA) and mussel tissues (CRM 278, IRMM, Belgium). The recovery rates of SRM 1566a and CRM 278 were 103.8 ± 3.3% and 103.3 ± 2.0%, respectively. Kidney and digestive gland were selected for examining distribution of soluble Cd in different sizes of molecules in the cytosol. These two organs were noted as the major Cd accumulation sites for Cd in the previous studies (Ahn et al. 1996). Low pressure gel filtration chromatography was performed using Sephacryl S-100 (LKB Pharmacia) based on the method described in Choi et al (2001). All the data were analyzed using a statistical program MINITAB13 (MINITAB Inc).

RESULTS AND DISCUSSION

Table 1 shows the concentrations of Cd and its percentage body burden in the various tissues of *L. elliptica*. Kidney and digestive gland were the target organs for Cd accumulation in *L. elliptica* as found in the previous studies (Ahn et al. 1996, 2001). Kidney showed the highest concentration, reaching up to 85 $\mu g \cdot g^{-1}$ dry weight, followed by the digestive gland ($p<0.001$, Tukey method). The concentration of Cd in the digestive gland was about one fifth of those found in the kidney. However, it is noteworthy that the highest body burden was retained in the digestive gland, which contained much larger tissue portion (31% of total tissue dry weight) than the kidney (2%). This indicates that a substantial amount of Cd is taken up as particulate form via feeding. On the other hand, Cd accumulation in the gills was not very significant in terms of both tissue concentration and percentage burden, suggesting that dissolved form is not the major source of Cd. The muscle part (siphon and mantle) and gonad, which contributed large portions of body mass (siphon-mantle-43.5%, gonad-30.3%), also showed relatively high percentage body burdens in spite of low Cd concentrations in their tissues.

Results from gel filtration chromatography performed on the cytosol of the kidney and digestive gland showed that most of the cytosolic Cd was associated with the

Table 1. Cadmium distribution in the soluble cytosolic fraction (supernatant), insoluble particulate fraction (pellet), and total homogenate in the various organs of *L. elliptica* collected from Marian Cove, King George Island.

	Concentration (μg/g dry weight) (Mean ± S.D., n=9)			% of total body
Organ	superntant	pellet	total	burden
Kidney	13.2 ± 8.09	42.5 ± 13.6***	53.3 ± 13.4	22.5
DG	3.83 ± 0.73	5.50 ± 2.13 *	9.33 ± 2.60	32.9
Gill	1.76 ± 0.46	1.93 ± 1.01	3.69 ± 0.97	3.3
Gonad	1.19 ± 0.30	1.68 ± 0.54	2.87 ± 0.74	18.0
SM	0.49 ± 0.18	1.88 ± 0.54***	2.39 ± 1.34	21.7

*The shell length of clams varied from 64.9 to 68.0 mm (66.0 ±1, n=9). Non-parametric Mann-Whitney *U*-test was used to test the significance of the difference between the values from the supernatant and pellet (* at $0.01 < p < 0.05$, *** at $p < 0.001$). DG: digestive gland, SM: siphon-mantle

protein eluted at 8-9 kDa (Figure 1). The size of this protein coincides with the size of metallothioneins (MTs) found in various marine molluscs. MTs are major metal-binding ligands found in many molluscs and are known to play an important role in metal detoxification by rapidly responding to metal exposures (Roesijadi 1992; Mason and Jenkins 1995; Langston et al. 1998). Previous studies reported immuno-histochemical responses to an antibody of MT in the kidney and digestive gland (Choi et al. 2001; Lee et al. 2001) of *L. elliptica*, supporting the presence of MT in these organs. Thus, the results of this study clearly show that MT or MT-like proteins play an important role in Cd sequestration in the cytosol of *L. elliptica* kidney and digestive gland as in many temperate molluscs. Further studies, however, are needed to assure the inducibility of this protein to Cd exposures.

Interestingly, insoluble Cd comprised significantly higher proportion than soluble Cd in the kidney, muscle, and digestive gland ($p<0.05$, Mann-Whitney U-test), while it showed a similar proportion with soluble fraction in the gill and gonad (Table 1). In particular, about 80% of the total Cd was associated with insoluble fraction of the cells in the kidney and muscle parts, indicating that Cd sequestration to insoluble forms was especially important in these organs. In a preliminary experiment on this species, numerous electron dense concretions were microscopically observed in its kidney epithelial cells (Lee et al. 2000), suggesting that these may play an important role in sequestering Cd, accumulated at such an extremely high level in the kidney. Previous studies have also reported that metal-rich granules and concretions are involved in metal sequestration in some marine molluscs (Viarengo and Nott 1993; Mason and Jenkins 1995, Langston et al. 1998; for reviews). Furthermore, the overall percentages of insoluble fractions were much higher in *L. elliptica* than those in

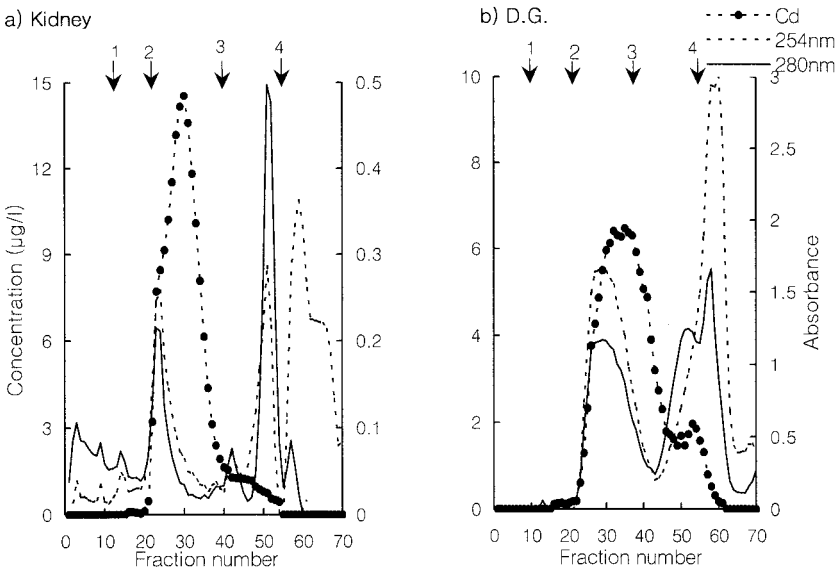

Figure 1. Elution profiles of Sephacryl S-100 chromatography of cytosolic fractions obtained from a) the kidney and b) the digestive gland (D.G.) of *L. elliptica*. Size markers are shown in numbers (1: BSA-66 kDa, 2: α-chymotrypsin-25 kDa, 3: rabbit liver metallothionein-6.6 kDa; 4. Vitamin B_{12}-1.35 kDa).

Table 2. Percentage proportions of insoluble Cd in different organs of marine bivalves collected from Antarctic and temperate waters.

Species	D.G.	Gill	Muscle	Kidney	Gonad	Reference
Antarctic speices						
Laternula elliptica	42	47	21	20	41	This study
Adamussium colbecki	30					1
Temperate species						
Mytilus edulis	78	83	79			2
M. galloprovincialis	56	63	62			3
Crassostrea gigas	72	64				4
C. virginica		82				5
Mizuhopecten yessoensis	93	82		84		6
Ruditapes decussatus	79	70				7

* Data for comparisons were obtained from natural populations or the animals chronically exposed to low levels of Cd. No data on gonads from other bivalves were available for comparison. 1. Viarengo et al. (1993), 2. Geret and Cosson (2002), 3. Isani et al. (1997), 4. Geret and Cosson (2000), 5. Roesijadi and Klerks (1989), 6. Lukyanova et al. (1993), 7. Roméo and Gnassia-Barelli (1995).

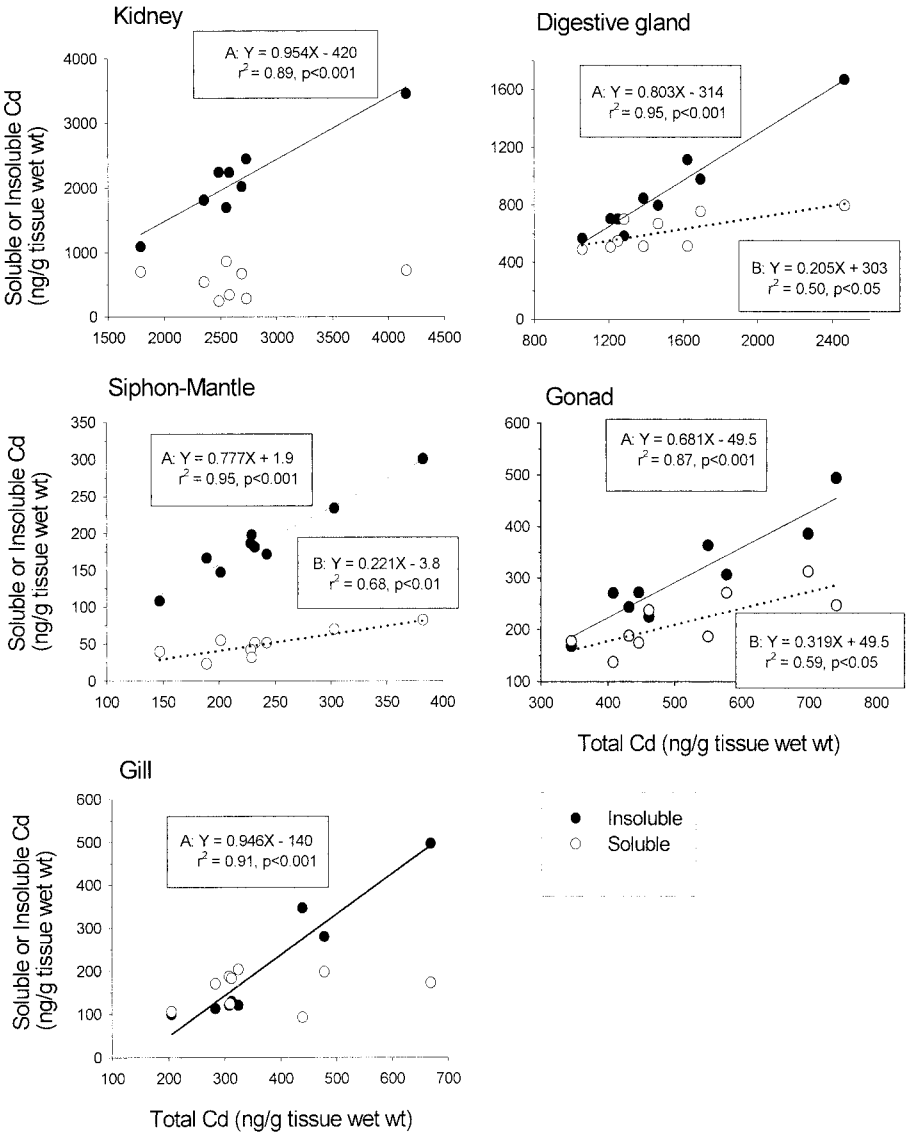

Figure 2. Relations of both soluble and insoluble Cd concentrations to total Cd concentrations in different organs of *L. elliptica*. Regression lines are shown when they are statistically significant. A: regression equations for insoluble Cd, B: regression equations for soluble Cd.

temperate bivalves (Table 2). These differences between *L. elliptica* and other temperate bivalves may be attributed to different exposure concentrations and period that the clams have experienced (Johansson et al. 1986; Bordin et al. 1994) Alternatively, this may be a unique feature of cold-water species with slow

growth rate and long life span. The Antarctic scallop *Adamussium colbecki* also showed a high proportion of insoluble Cd in its digestive gland (Table 2), suggesting that Cd sequestration to insoluble ligands is a major Cd detoxifying process in Antarctic bivalves that have adapted in the naturally Cd-enriched environment. In strong support of this speculation, the absolute amount of Cd present in insoluble fraction increased considerably in all organs as the total concentration increased, while those in soluble fraction showed little change or slight increase (Figure 2). In future studies, the insoluble Cd-binding ligands are to be characterized.

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