Accumulation of Heavy Metals in the Antarctic Clam, Laternula elliptica, and in the Korean coastal Clam, Ruditapes philppinarum

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Abstract

Immunohistochemical and ultrastructural experiments were conducted to find out heavy metal accumulation in some selected organs such as the kidney, the digestive gland, and the gill of the Antarctic clam Laternula elliptica and R. philippinarum.

According to the immunohistochemical study the subject organs of the clam showed reactions indicating the presence of MT (metallothionein), a metal-binding protein involved in metal detoxifying process. Examination under the transmission electron microscope also revealed that other ligands may play a role in metal accumulating and detoxifying process in L. elliptica and R. philippinarum.

In the artificial exposure of the clam to Cd, the clams showed immediate subcellular response s. The level of the anti-MT reactions became higher in the proportion to the degree of pollutio n of their habitat and to the period of Cd exposure. These suggest that the two species can be used as efficient biomarkers for Cd exposure in the natural environment.

Introduction

The bivalved mollusks such as clams, mussels, oysters, and scallops are filter feeders inhabiting usually on the bottom of the sea. Some species of the benthic communities have been studied on the metal accumulation in their tissues.

The Antarctic soft-shelled clam Laternulla elliptica is an endemic species in the Antarctica, and widely distributed around the Antarctic continent. This deep-burrowing and large sized clam species (>10cm in shell length) occurs frequently as dense patches (>100 individuals

m-2) in shallow and sheltered areas, dominating benthic communities. Despite its wide distribution and high biomass, this species has rarely been studied until recently, due apparently to the difficulties in sampling and experimental manipulation in its physically unstable habitats.

And Ruditapes philppinarum is an endemic and edible clam inhabiting in the mud flats of the West Sea of Korea.

This study was conducted to find out the patterns of heavy metal accumulation and metallothionein distribution in some selected organs of the clams Laternulla elliptica collected from the Antartic ocean, and Ruditapes philppinarum.

The three visceral organs (kidney, digestive gland, and gill) which have strong metal accumulation tendencies were chosen for the analysis. Throughout the above experiments some meaningful results were obtained.

Material and Methods

(1) Material

The first species used for the present study was an antarctic clam Laternula elliptica collected from Marian Cove at sites close to the King Sejong Station (62°13'S, 58°45'W) on King George Island. The another species examined was an edible clam, Ruditapes philppinarum which was collected from three collecting sites, the Cheonsu-bay (clear area), the Sihwa-lake (intermediate area), and the Tando-island (polluted area) of the West Sea, Korea.

The specimens, transported from the King Sejong Station to the laboratory, and collected from the Korean mud flats, were examined as followings:

① Control Specimens

To keep ultrastructural normal condition, the specimens collected from the King Sejong Station were fixed with Karnovsky's fixative immediately after collection and kept in a box at 4℃ for transportation from there to our laboratory. The specimens collected from the coastal mud flats of Korea also treated under the same conditions.

② Specimens for Bioassay

The specimens for Cd exposure were kept in an aquarium containing 50 ppb of CdC12 for 4 hours to 6 days and were fixed with Karnovsky's fixative buffered with 0.1M cacodylate buffe r at 4℃.

(2) Methods

① Immunohistochemical Study

Thick paraffin sections of the subject organs were obtained through the routine processes for the light microscopic observations. And for the immunohistochemical study the paraffin sections stained with the DAKO's LSAB (Labelled Streptavidin Biotin) kit.

② Transmission Electron Microscopy

The specimens prefixed with the Karnovsky's fixative during transportation were post-fixed with OsO4 buffered with 0.1M phosphate buffer. The fully fixed specimens were washed and dehydrated in a series of alcohol, and embedded in Epon 812 mixture. Thin sections (70-80 ㎚ thick) were obtained with LKB ultramicrotome, double stained with uranyl acetate and lead citrate, and observed with a transmission electronmicroscope (JEM-1010)

③ Analysis of the Chemical Elements with SEM-EDS

The processes taken were same to those of the scanning electron microscopy. The subject che mical elements for analysis were 11 such as Al, Si, Cl, Ca, Cr, Fe, Cu, Zn, Cd, Hg, and Pb.

Results and Discussion

① Immunohistochemistry

In L. elliptica, the apical cytoplasm of the renal epithelial cells, the interdiverticular connective tissues of the digestive glans and the outer epithelium of gill lamellae of the two species strongly reacted to anti-MT, indicating the presence of MT (metallothionein), a metal-binding protein involved in metal detoxifying process.

In R. philippinarum, the level of the anti-MT reactions became higher in the proportion to the degree of pollution of their habitat and to the period of Cd exposure.

② Electron Microscopy (SEM and TEM)

Examination under the TEM revealed that other ligands (e.g. metal-rich granules in the kidney and digestive gland) may play a role in metal accumulating and detoxifying process in L. elliptica and R. philippinarum.

In a short-term experiment where the clams were exposed to a high Cd concentration 50 ppb (50μg L-1), ultrastructural changes were examined under SEM. The epithelial cells of the

three organs showed some changes such as swollen rER, swollen nucleus membrane and inclusion bodies in the nuclei after 8-hour Cd exposure in L. elliptica, and after 4-hour exposure in R. philippinarum.

In R. philippinarum, the epithelial cells of the digestive gland of the clams collected from polluted area (Tando-island) also showed certain changes such as swollen rER, swollen nucleus envelope and inclusion bodies in the nulcei.

③ Analysis of the Chemical Elements with SEM-EDS

In L. elliptica, the kidney showed relatively high Cd concentrations in the apical cytoplasm and connective tissues beneath the epithelium, suggesting that Cd is involved in induction of MT in this organ. In the digestive gland, on the other hand, Cu predominated, implicating that Cu may be responsible for formation of MT. The relative proportion of Cu was the highest also in the gill, but other metals such as Cr and Fe were relatively higher than the other organs. Thus, the results of the SEM-EDS analysis showed that metal distribution is highly variable among the body parts. This seems to indicate that metal accumulating and detoxifying process should be different depending on the function of the body organs.

And in R. philippinarum, tissue of the digestive gland showed relatively higher concentration of S, Zn, and Cd. These elements are supposed to be concerning with the MT-reaction in the gland.

The immediate subcellular responses of the clams to Cd exposure suggest that the two species, L. elliptica and R. philippinarum can be used as rapid and efficient biomarkers for Cd exposure in natural environment.

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