Accumulation of Heavy Metals in the Antarctic Clam *Laternula elliptica*

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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 hepatopancreas, and the gills of the Antarctic Clam *Laternula elliptica*

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(e.g. metal-rich granules in the kidney) may play a role in metal accumulating and detoxifying process in *L elliptica*. In the artificial exposure of the clam to Cd, it showed immediate subcellular responses, suggesting that this species can be used as rapid and efficient bioindicators for Cd exposure in natural environment.

Introduction

The Antarctic soft-shelled clam *Laternulla elliptica* is a filter feeding bivalves species endemic to the Antarctic, 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.

This study was conducted to find out patterns of heavy metal accumulation and metallothionein distribution in some selected organs of the clam *Laternulla elliptica* .

The three visceral organs(kidney, hepatopancreas, 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 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 parts of the clam's body used were the kidney, the hepatopancreas, and the gills. Speciemen transportation from the King Sejong Station to the laboratory was performed as followings:

① Control Specimens

To keep ultrastructural normal condition, the clams were fixed with Karnovsky's fixative immediately after collection and kept in a box at 4℃ for transportation from the collecting site of King Sejong Station to our laboratory.

② Specimens for Bioassay

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

(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 OsO_4 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 chemical elements for analysis were 11 such as Al, Si, Cl, Ca, Cr, Fe, Cu, Zn, Cd, Hg, and Pb.

Results and Discussion

① Immunohistochemistry

The immunohistochemical study showed that the apical cytoplasm of the renal epithelial cells, the interdiverticular connective tissues of the hepatopancreas and the outer epithelium of gill lamellae strongly reacted to anti-MT, indicating the presence of MT(metallothionein), a metal-binding protein involved in metal detoxifying process.

② Electron Microscopy(SEM and TEM)

Examination under the transmission electron microscope also revealed that other ligands(e.g. metal-rich granules in the kidney) may play a role in metal accumulating and detoxifying process in *L. elliptica*. Roles of these ligands however, are yet to be clarified by further studies on metal composition of these particles in relation to their distribution within the cells.

In a short-term experiment where the clams were exposed to a high Cd concentration (500μg 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 hours of the Cd exposure.

The results suggest that these immediate subcellular responses to Cd exposure can be used as rapid and efficient bioindicators for Cd exposure in natural environment.

③ Analysis of the Chemical Elements with SEM-EDS

The SEM-EDS analysis further identified the relatively high Cd concentrations in the apical cytoplasm and connective tissues beneath the epithelium in the kidney, 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 highest also in the gill, but other metals such as Cr and Fe were relatively high compared to 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.

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