Short Paper

Specific Accumulation of Iodine by the Operculum
of the Strawberry Conch *Strombus luhuanus*

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Accumulators having a special ability to concentrate a specific element at a high level have been discovered in the animal kingdom.^{1–5)} They are regarded as being very effective indicator organisms for monitoring marine pollution by heavy metals and radionuclides. Since little data on the concentrations of iodine in gastropods was reported, a screening study for finding iodine accumulators was focused on gastropods.

Thirty species of gastropods belonging to Strombidae, Trochidae, Potamididae, Muricidae, Conidae and Turbinidae were collected from August in 1994 to September in 1995 from the shallow water off the coast of Japan and were dissected into several organs such as the operculum, foot, gills and intestine. Determinations of iodine in specimens were carried out by neutron activation analysis. Specimens and a standard were irradiated with thermal neutrons having $5.5 \times 10^{13} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ of JRR-3M at the Japan Atomic Energy Research Institute. The Leco model 932 CHNS analyzer was used to measure the concentrations of C, H, N, O, and S in the operculum of *Strombus luhuanus* (Fig. 1). Point (beam size = $10 \mu\text{m}$) analyses by the JEOL JXA-8900R electron probe X-ray microanalyzer (EPMA) were carried out for ten targets in the operculum of *S. luhuanus* to obtain the average concentrations of Br, Ca, and Cl. The BIO-RAD Digilab FTS-60A/896 Fourier transform infrared (FT-IR) spectrometer was used to know the major component in the operculum of four species of conch.

The concentrations of iodine in various organs of thirty species except the operculum of the conch ranged from 0.20 to $35.3 \mu\text{g/g}$ on a dry weight basis. In contrast, the operculum of the conch have very high concentrations of iodine, as shown in Table 1. In particular, *S. luhuanus* showed the highest concentration (mean $\pm$ SD = $22,800 \pm 5,200 \mu\text{g/g}$ dry wt, $n=10$) of iodine among four species of Strombidae. The specific accumulation of iodine was thought to be common and limited to Strombidae since gastropods belonging to other families show the low concentrations ($22\text{--}980 \mu\text{g/g}$ dry wt) of iodine in their operculum.

Quantitative analyses by neutron activation analysis or with the CHNS analyzer and EPMA revealed that the major elements in the operculum of *S. luhuanus* were: Br (1.7%), C (47.7%), Ca (0.30%), Cl (1.5%), H (6.2%), I (2.3%), N (15.6%), O (16.0%), and S (0.30%). The large percentages (85.5%) of C, H, N, and O indicated that the

Fig. 1. Photograph of *S. luhuanus*.

This species lives in a shallow water from subtropical to tropical zones. Its operculum (arrow) has a brown blade with saw-like teeth. Scale bar = 1 cm.

operculum was made of organic materials.

The operculum of the conch was mainly composed of protein since three characteristic absorption bands (amide I band at 1638 cm^{-1} , amide II band at 1517 cm^{-1} , and amide III band at 1236 cm^{-1}) indicating the presence of protein were observed from the result of FT-IR. In fact, most of the operculum of *S. luhuanus* could be digested (experimental conditions; 37°C , 72 hr) by a continuous treatment of an acid protease from *Aspergillus niger*, an alkaline protease from *Bacillus subtilis* and carboxypeptidase from a bovine pancreas. Secondly, VG PlasmaQuad PQ-2 inductively coupled plasma mass spectrometer was

Table 1. Concentrations of iodine in the operculum of four species of the family Strombidae

Species	Concentration ($\mu\text{g/g}$ dry wt)	<i>n</i>
<i>Strombus luhuanus</i>	22,800 $\pm$ 5,200	10
<i>Lambis truncata</i>	5,760 $\pm$ 1,580	5
<i>Lambis lambis</i>	3,680 $\pm$ 1,200	5
<i>Harpago chiragra</i>	3,630 $\pm$ 1,000	5

used to select only iodine-containing fraction from many peaks which were obtained by the application of HPLC equipped with an silica-ODS column to the protease-digested solution. Eleven kinds of amino acids were present in the iodine-containing fraction. In contrast, aminosugar, saccharides and lipid were not detected in it. LC-MS revealed that iodine was not bonded to valine, although only valine existed as a free amino acid. It was also found from X-ray absorption fine structure analysis by synchrotron radiation that iodine atoms were covalently bonded to carbon atoms and iodine did not exist as I_2 , I^- and IO_3^- (unpubl. data). From these results, iodine atoms were thought to be covalently bonded to carbon atoms of certain amino acids in the operculum of *S. luhuanus*. I have a plan to clarify an iodo-amino-acid and the amino acid sequence by means of LC/MS/MS after a pure peptide containing iodine will be obtained.

In this work, why the conch needs iodine when forming their operculum could not be clarified. Furthermore, the relationship between the physical properties of the operculum and the existence of iodine could not be disclosed. However, a possible physiological role of iodine judging

from the unique locomotion of the conch is suggested. When gastropods draw their soft part inside the shell, the aperture is closed by the operculum to protect the soft body against predators. The operculum of the conch is considered to have a different role. The conch crawl over sand in a very different fashion from other gastropods. The foot of the conch is somewhat reduced in size, and consequently its mobility is poor. To compensate for the atrophied foot, the saw-toothed operculum digs into the sand and the conch then "pole" forward by rapid contraction of the columella muscle.⁶⁾ The operculum needs some degree of the flexibility coupled with sufficient hardness to allow the conch to crawl on the sand. It is speculated that the iodine-containing protein may play an important role in acquiring this desired flexibility and hardness since the operculum of other species of gastropods except Strombidae has not both of the flexibility and hardness. In the future, comparing physical properties of an iodine-containing protein with those of an iodine-excluding protein is being planned.

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