Occurrence of PSP-infested Scallops in Ofunato Bay during 1976–1979 and Investigation of Responsible Plankton*1

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(Received August 24, 1981)

Toxic specimens of the scallop Patinopecten yessoensis were collected from Ofunato Bay, Iwate Prefecture, in early or mid-summer during 1976–1979. The scallop specimens were dissected into various parts of organ and examined on distribution of the toxicity. Toxins were prepared from the digestive glands and analyzed by TLC and electrophoresis.

The highest toxicity was detected in digestive gland (19–110 MU/g), followed by rectum (12–83), foot (21–56), mantle (11–39), gill (3–13) and gonad (2–13). The lowest toxicity was found in adductor muscle (2–3 MU/g).

Irrespective of the year of collection, the toxins contained gonyautoxins 1–4 as the major, and saxitoxin and neosaxitoxin as the minor. The culture of Protogonyaulax tamarensis OF-1 cells presented essentially the same toxin composition as toxic scallops, indicating the infestation of this plankton to scallops and other bivalves in Ofunato Bay in recent years.

A great number of studies on paralytic shellfish poison (PSP) have been carried out in America, Canada and some European countries, but few in Japan until 1975, when a Protogonyaulax catenella red tide occurred in Owase Bay, Mie Prefecture, with the toxification of some bivalves such as mussel and short-necked clam. Chemical properties of the toxins from P. catenella and mussels were hardly distinguishable from each other, thereby indicating that mussel toxin might have come from this plankton. Before the Owase incidence, there were recorded three poisoning cases supposedly due to PSP. One of them occurred in 1961 at Ofunato Bay, Iwate Prefecture, with 20 victims including one death. The toxification of bivalves in the same bay was also observed in the following two years. In 1976, we found that some specimens of the scallop Patinopecten yessoensis collected there possessed the toxicity scores as high as 800 MU/g digestive gland. Fukuyo isolated a plankton suspect from the bay and designated it P. tamarensis OF-1 (formerly Gonyaulax excavata OF-1).

To elucidate further if this plankton can be closely associated with the infestation to scallops, a comparison was made on the toxins from both organisms. Anatomical distribution of the toxicity and annual variation in PSP compositions of the scallop were also investigated.

Materials and Methods

Materials

Specimens of the scallop Patinopecten yessoensis were collected in Ofunato Bay, in May 1976, June 1977 and 1978, and April 1979. The live scallop specimens were immediately frozen and kept at −20°C until used. For anatomical distribution survey, ten each of male or female scallops, after being thawed and shucked, were dissected into adductor muscle, digestive gland, mantle, rectum, foot, gill and gonad. The organs thus dissected were assayed for PSP by the method described below.

P. tamarensis OF-1 cells which were isolated from Ofunato Bay in 1977 by Dr. Y. FUKUYO of Kitasato University, were used as seed cells. They were cultured in SW II medium at 14–15°C under 3,000 lx of white fluorescent light for 12 h/day. The culture was harvested by filtration through a 0.45 μm membrane filter (Toyo), when they reached a cell density of about 20,000 cells/ml. It usually took a period of 4 weeks for harvesting of the cells.

*1 Presented at the Annual Meeting of the Japanese Society of Scientific Fisheries on April 4, 1980, Tokyo.
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Preparation of Toxins from Scallops and P. tamarensis OF-1 Cells

Scallop toxins were obtained from digestive glands by the method reported previously. P. tamarensis toxins were prepared as follows.

The cells harvested as above were extracted overnight with 0.1 N HCl and then filtered or centrifuged. The extract was concentrated and applied to a series of column chromatography on Sephadex G-15, Bio-Gel P-2 and Bio-Rex 70. The procedure was the same as that of scallop toxins except for using Sephadex G-15 in place of activated charcoal.

Methods of Toxin Analysis

For analysis of scallop and P. tamarensis toxins, electrophoresis on cellulose acetate strips was performed at 0.5 mA/cm for 1.5 h with a buffer system of 0.08 M Tris-HCl, pH 8.7. TLC analysis was carried out on silica gel 60 plates with a solvent system of pyridine: ethyl acetate: water: acetic acid (75: 25: 30: 15). Toxins were viewed under UV light (365 nm) after spraying with 1% hydrogen peroxide and heating at 110°C for 10 min. Reference standards of gonyautoxins and saxitoxins were prepared from scallop digestive glands as described before. Toxicity was assayed by the official method for PSP using 18–20 g male mice (ddY).

Results and Discussion

Distribution of the toxins in various scallop organs is shown in Table 1. The highest toxicity was observed in digestive gland, but lesser toxicity also in mantle, rectum, foot, gill and gonad. The adductor muscle showed the lowest toxicity. Some toxicity variation was found between both sexes. Fairly large differences in toxicity were recognized among four samples, particularly in digestive gland. This indicates that the toxicity of scallop depends largely upon the locality and/or depth of collection.

The adductor muscle holds a large portion (about 40%) of edible parts in scallop and is the most delicious part to be eaten. Although the toxicity in adductor muscle sometimes surpasses the quarantine limit of 4 MU/g, a possibility of poisoning from eating it might be slim. However, ingestion with other toxic organs such as mantle and gonad will cause intoxication. Commercial scallop products, if admixed in quantities with gill, foot or rectum, may also lead to a poisoning hazard. The digestive gland accounts for approximately 12% of the total weight of scallop organs. A man will be killed by ingestion of more than 28 g of digestive gland with a toxicity of 110 MU/g, assuming that oral minimum lethal dose in man is 3,000 MU.

Toxin extracts from scallop specimens collected yearly through 1976 to 1979 were purified by column chromatography. The resulting toxins were analyzed by TLC and electrophoresis.

A typical toxin pattern of scallop is illustrated in Fig. 1. Fractions I-IV were eluted with 0–0.03 M acetic acid. Fractions I and II contained two unknown toxins with Rf values of 0.18 and 0.75 (Fig. 2). Fraction III was a mixture of gonyautoxins 1–4 (GTX1–4). Fraction IV consisted of GTX2 and GTX3. Fraction V was eluted with an increased normality of acetic acid from 0.03 to 0.1 M. This fraction included saxitoxin (STX), neosaxitoxin (neoSTX) and an unidentified toxin with the Rf value of 0.72. All the scallop specimens tested contained GTX1, GTX2, GTX3 and GTX4 as the main components.

Table 1. Anatomical distribution of the toxicity (MU/g) in scallops.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Adductor muscle</th>
<th>Digestive gland</th>
<th>Mantle</th>
<th>Rectum</th>
<th>Foot</th>
<th>Gill</th>
<th>Gonad</th>
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<td>83</td>
<td>40</td>
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Scallops were collected in Ofunato Bay on August 9, 1979 and kept frozen at -20°C for 15 days. Ten each of respective organs of male or female scallops was assayed for toxicity.
PSP from Scallop and Protagonyaulax tamarensis

Fig. 1. Elution profiles of 1976 scallop (--●--) and P. tamarensis (--○--) toxins from a Bio-Rex 70 column.

Fig. 2. Thin-layer chromatographic and electrophoretic patterns of scallop and P. tamarensis toxins separated from a Bio-Rex 70 column. Relative mobility was calculated by assuming that of saxitoxin as 1.

Table 2. Toxin compositions*1 of scallops collected in Ofunato Bay during 1976–1979

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>GTX1+GTX4</th>
<th>GTX2+GTX3</th>
<th>STX+neoSTX</th>
<th>Other toxins</th>
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<td>May, 1976</td>
<td>20</td>
<td>76</td>
<td>3</td>
<td>1</td>
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<td>June, 1977</td>
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<td>3</td>
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<td>53</td>
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<tr>
<td>Apr., 1979</td>
<td>17</td>
<td>64</td>
<td>7</td>
<td>12</td>
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</tbody>
</table>

*1 The figures show percent toxicity of component toxins.

and STX and neoSTX as the minor (Table 2). GTX1 plus GTX4 accounted for more than half of the total toxicity in 1978, whereas GTX2 plus GTX3 were the case in other years.

The elution profile of P. tamarensis toxins on a Bio-Rex 70 column somewhat differed from that of scallop toxins, although both organisms possessed identical toxins (Figs. 1 and 2). As compared with the shellfish, the plankton gave a higher GTX1 content in fraction III but lower contents of GTX2 and GTX3 in Fraction IV.

We have reported in the preceding papers that the toxin compositions in oysters were altered during storage and/or purification of the toxins. The stability in aqueous media or frozen state was fairly different among the toxins. GTX1 was...
particularly unstable and underwent a gradual decomposition through purification steps. The difference in toxin compositions between both organisms seems to have been made by inconsistent purification procedures and/or varied storage condition.

Thus, it came to a conclusion that *P. tamarensis* OF-1 should be a causative organism for toxification of scallops and other bivalves in Ofunato Bay in recent years, supporting FUKUYO's supposition.10)

Acknowledgments

The authors are indebted to Dr. Y. FUKUYO, Kitasato University, School of Fisheries Sciences, for *P. tamarensis* OF-1 seed cells, and to Dr. H. TOKUDA, Faculty of Agriculture, the University of Tokyo, for useful advice on its cultivation. This work was supported in part by grants from the Ministry of Agriculture, Forestry and Fisheries, and the Ministry of Health and Welfare.

References