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Quantitative Relationship between PSP Components of *Protogonyaulax tamarensis* and of Scallops, from Funka Bay, Hokkaido

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Abstract

Attempts were made to investigate the quantities of PSP (paralytic shellfish poison) in a dinoflagellate, *Protogonyaulax tamarensis*, and in toxic specimens of a scallop, *Patinopecten yessoensis*, from Funka Bay, Hokkaido.

PSP was partially purified by the following methods. In the case of *P. tamarensis*, the method employed consisted of extraction with 0.1N HCl, defatting with chloroform, and column chromatography on Bio-Gel P-2. In the case of the scallop, the method employed consisted of extraction with 80% ethanol (pH 2.0), defatting with chloroform, activated charcoal treatment, and column chromatography on Bio-Gel P-2. The toxins thus purified were analyzed by cellulose acetate membrane electrophoresis and assayed by using male mice of ddY strain.

The PSP in both organisms was similar in that gonyautoxins were the major components, but inconsistent in that the components differed in quantity. Namely, *P. tamarensis* contained gonyautoxin I (27%), gonyautoxin II (7%), gonyautoxin III (44%) and neosaxitoxin plus saxitoxin (22%), whereas the scallop contained gonyautoxin I (14%), gonyautoxin II (14%) gonyautoxin III (32%), gonyautoxin IV (13%), gonyautoxin V (2%) and neosaxitoxin plus saxitoxin (25%).

From these results, it was concluded that conversions of PSP components had taken place in the scallop, resulting in the quantitative changes. In addition, since gonyautoxin V, which is known to be specific to the western part of Japan, was detected, it was also concluded that low toxic components other than gonyautoxin V might be present.

Introduction

The plankton responsible for the toxification of bivalves such as the scallop, *Patinopecten yessoensis*, in Funka Bay, Hokkaido, was known to be a dinoflagellate, *Protogonyaulax tamarensis*, which produces toxins named paralytic shellfish poisons (PSP). PSP are accumulated in shellfish that feed on this plankton, which flourishes mainly from early summer to mid-summer. Toxification of bivalves in that bay has been reported yearly since 1978 and presents serious problems both to public health and to shellfish industries. In 1979, the outbreak of mussel poisoning in Asahikawa, Hokkaido, was reported to have resulted in 3 victims, including one death[1]. It was disclosed that the poisoning was caused by ingestion of PSP accumulated in the mussel, *Mytilus* sp., gathered at Funka Bay.

Furthermore, recent studies have revealed that a number of PSP, namely, gonyautoxin I-VIII (GTX_{1-8}), neosaxitoxin (neoSTX) and saxitoxin (STX), have been found in the causative dinoflagellate or toxic shellfish collected at various

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parts of the world. In Japan, multiplicity of PSP components has been demonstrated in shellfish, particularly in bivalves derived from Senzaki Bay (Yamaguchi Prefecture)[2], Seto Inland Sea[3], Owase Bay (Mie Prefecture)[4] and Funka Bay[5]. But as for PSP components in Funka Bay shellfish, many points are yet obscure. In our previous paper[6], we reported that the PSP components (GTX_{1-3}, neoSTX, STX) of *P. tamarensis* were different from those (GTX_{1-5}, neoSTX, STX) of the scallop, and that GTX_5, a low toxic component specific to the samples from the western part of Japan, was detected in those from Hokkaido for the first time. From these results, the presence of a conversion process of PSP components in shellfish was considered possible.

Therefore it seemed to be an interesting task to clarify the quantitative relation between PSP components of *P. tamarensis* and the scallop, and the aim of the present paper is thus to present a quantitative analysis of PSP components of both.

Materials and Methods

Materials

Cells of *Protogonyaulax tamarensis* from Funka Bay were cultured under the conditions given in Table 1.

Table 1. Culture conditions of *Protogonyaulax tamarensis* cells.

Temperature (°C)	12
Illumination (lx)*	3000
Culture period (days)	18
Culture medium	BSW-4

* Illuminated 16 h/day under cool white fluorescent light and left 8 h/day with the light off.

Frozen digestive glands of the scallop, *Patinopecten yessoensis*, collected in August, 1981 at Ohfune in Minamikayabe-cho on the coast of Funka Bay, were used.

Preparation of Toxins from P. tamarensis Cells and from the Scallop

The cultured *P. tamarensis* cells were harvested by filtration through a 0.45 μm membrane filter (Toyo). Extraction of *P. tamarensis* was done following the method of Noguchi *et al.* (1982).[7] The harvested cells were extracted with 0.1 N HCl and then centrifuged. The extract concentrated *in vacuo* was applied to a column chromatography on Bio-Gel P-2 (Bio-Rad Laboratories), and then eluted with 0.15 N acetic acid after washing in water.

The digestive glands of toxic scallops were extracted with 80% ethanol (pH 2.0) according to the method described in the previous paper[6]. The extract was treated with activated charcoal after defatting with chloroform and purified using Bio-Gel P-2.

Methods of Toxin Analysis

PSP obtained as mentioned above was adjusted to 1.0 MU/μl. Thirty microliters (=30MU) of the PSP solution were applied on a 12×12 cm cellulose acetate membrane (Chemetron), which had been soaked in 0.08 M Tris-HCl buffer (pH 8.7) beforehand, together with PSP standards which contained GTX_{1-4}, neoSTX and STX, and then separated by electrophoresis for 30 min. at 0.8 mA/cm width. After electrophoresis, only PSP standards were sprayed with 1% hydrogen peroxide, and then converted to fluorescent bodies by heating at 110°C for 10 min. Each part corresponding respectively to GTX_5, neoSTX and to STX, was scraped off from a cellulose acetate membrane with the guidance of fluorescent bodies, and extracted with 0.01N acetic acid. The extracts were subjected to mouse assay, which was carried out according to the method described in A.O.A.C.[8]. Male mice of ddY strain weighing from 18 to 20 g were used.

Results

Quantitative relations of PSP components between *P. tamarensis* and the scallop are shown in Table 2. The PSP composition of *P. tamarensis* was apparently different from that of the scallop, namely, the quantities of PSP components in *P. tamarensis* were as follows; GTX_1 (27%), GTX_2 (7%) GTX_3 (44%), neoSTX plus STX (22%). In the scallop they were as follows; GTX_1 (14%), GTX_2 (14%), GTX_3(32%), GTX_4(13%), GTX_5(2%), neoSTX plus STX(25%). GTX_3 comprised 44% of the toxicity in *P. tamarensis* but 32% of the toxicity in the scallop. On the other hand, GTX_2 in the scallop, which is an epimer of GTX_3, came to comprise 14% of the toxicity, with an increase by 7% in comparison with the quantity of GTX_2 in *P. tamarensis*. A similar phenomenon was observed for the two organisms in the case of GTX_1 and GTX_4.

Table 2. Quantitative relation between PSP components of *Protogonyaulax tamarensis* and of toxic scallops from Funka Bay.

Organism \ PSP	GTX_1	GTX_2	GTX_3	GTX_4	GTX_5	STX+neoSTX
Protogonyaulax tamarensis	27%	7%	44%	—	—	22%
Patinopecten yessoensis	14%	14%	32%	13%	2%	25%

* The figures show in toxicity percent of PSP components.

Discussion

In chemical structure PSP components are very similar. For example, GTX_1; 11α-(OSO_3) neosaxitoxin, GTX_4; 11β-(OSO_3) neosaxitoxin, GTX_2; 11α-(OSO_3) saxitoxin, GTX_3; 11β-(OSO_3) saxitoxin[9]. Furthermore, the equilibrium such that $GTX_1 \rightleftarrows GTX_4$ and $GTX_2 \rightleftarrows GTX_3$ was revealed by Shimizu *et al.*[10]. Therefore it is conceivable that PSP components of *Protogonyaulax* spp. eaten by plankton feeders

such as bivalves can undergo conversion. In the present experiment the point in dispute that the quantity of GTX_2 in the scallop increased in comparison with that of GTX_2 in *P. tamarensis*, is consistent with the report by Yasumoto *et al.*[11]. In addition, Yasumoto reported that the quantity of STX in the scallop increased in a similar manner as GTX_2[11]. From this experiment the change in quantity of STX cannot be explained. Judging from the quantitative difference between PSP components of the two organisms, the presence of a conversion process in shellfish is fully conceivable. But some people argue that the difference between PSP compositions is due to inadequate purification procedures, and therefore a close scrutiny of purification procedures of PSP is desirable.

Moreover, low toxic components such as GTX_5, GTX_6, GTX_8 and its epimer can convert into high toxic components such as STX, neoSTX, GTX_3 and GTX_2 respectively, by mild oxidation, one by one[12–14]. These low toxic components are thought to be precursors of their respective high toxic components[12–14]. Therefore the conversion from a low toxic into high toxic component, as well as from a high toxic into high toxic component, is within the bounds of possibility. We also reported in the previous paper that GTX_5, whose specific toxicity is thought to be 280 MU/mg, was detected in Funka Bay[6]. GTX_5 is specific to the western part of Japan and thought to be the precursor of STX[12]. Its presence in Funka Bay is noteworthy. Considering the presence of GTX_5 in toxic scallops from Ohfunato Bay, Iwate Prefecture, and also the presence of GTX_8 and its epimer in the toxic sea squirt *Halocynthia roretzi* from the same bay, it is conceivable that these low toxic but potentially high toxic components are present not only in the western part of Japan but also in eastern Japan, particularly Hokkaido. However, low toxic components corresponding to GTX_3 and GTX_2, namely GTX_8 and its epimer, have not yet detected in Funka Bay. There is another opinion that GTX_2 and GTX_3 may not exist as precursors.

It is necessary in the future to further the investigations of the forms of PSP existing in shellfish, and of their conversion.

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