Short Paper

Identification of Paralytic Shellfish Toxins
in Shellfish from Inland Sea*¹

Occurrence of toxic shellfish related to the toxic dinoflagellates *Gonyaulax* spp. has been reported in many parts of the world. The complexity and heterogeneity of the shellfish toxins and of toxins in *Gonyaulax* organisms themselves have been recently recognized^{1,2}. In Japan, six toxins including gonyautoxins-I, II, III, IV, V and saxitoxin have been isolated from shellfish and plankton samples collected during a bloom of *Gonyaulax* sp. in Owase Bay³.

In June, 1976, an elevated level (55 cells/ml) of *Gonyaulax* sp. was observed in the seawater from Oda Bay, Kagawa Prefecture, and several kinds of bivalves from the neighboring area were found to be toxic. Especially the toxicity of hepatopancreas of mussels, *Mytilus* spp., reached as high as 1950 MU/g⁴. Here we wish to report the results of analysis on the toxins in the mussels.

The collection of the specimen was described in the preceding report⁴. Extraction and isolation of toxins were carried out according to the methods used for the Atlantic paralytic shellfish toxins^{1,2}. Toxins were identified by comparing with the authentic samples isolated from *G. tamarensis* on tlc and electrophoresis².

The 80% ethanol extract (9.5 g, 7000 MU) from hepatopancreas was washed with chloroform and chromatographed on a Bio-Gel P-2 column (2.5 × 60 cm). About 95% of the toxin was bound in water and eluted with 0.03 N acetic acid. This fraction (68 mg) was purified by Bio-Rex 70 column chromatography (Fig. 1). Gonyautoxins-II, III, and V (GTX_{2,3,5}) were eluted with 0.03 N and saxitoxin with 1.0 N acetic acid. Relative quantity of toxins is as follows; GTX₂ 50, GTX₃ 25, GTX₅ 15 and saxitoxin 10%. Gonyautoxins-I and IV (originally coded as GTX_{1,4}) and neosaxitoxin were not detected. Only gonyautoxin-V (coded JGX1 in the previous report³) was enough amount to measure the specific toxicity. The value, 260 MU/mg, was far lower than other paralytic shellfish toxins.

Like the case in Owase Bay, although the causative organism in Inland Sea was identified as *G. catenella* on the basis of morphology⁴, the four toxins were identical to those from *G. tamarensis*. Discrepancy between the composition of toxins from Owase Bay and Inland Sea is the absence of gonyautoxins-I and IV in the latter. This may reflect the difference of

Fig. 1. Elution pattern of Bio-Rex 70 column chromatography. Partially purified toxin from the mussels was chromatographed on a 0.6 × 100 cm column, at a flow rate of 0.75 ml/min. Fractions of 3 ml were collected.

organisms at the level of strain or different physiological conditions of organisms or the mussels which accumulated the toxins.

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