ナンヨウブダイの消化管内容物の毒

誌名	日本水産學會誌
ISSN	00215392
著者	安元, 健 ほか3名,
巻/号	43巻1号
掲載ページ	p. 69-74
発行年月	1977年1月

Bulletin of the Japanese Society of Scientific Fisheries 43 (1) 69–74 (1977)

Toxins in the Gut Contents of Parrotfish

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(Received June 28, 1976)

The gut contents and the liver of a parrotfish *Scarus gibbus* contained ciguatoxin but not scaritoxin in spite of the fact that the latter toxin is dominant in the flesh. Absence of scari toxin in the diet suggested the possibility that the toxin was produced by the fish from a precursor in the diet. The conversion of the precursor, however, will not take place in the liver.

The gut contents contained maitotoxin and acetone soluble toxin(s) besides ciguatoxin. This newly found acetone soluble toxin was assumed to be a basic compound of small molecular size having strong paralytic action.

Algae content in the gut contents of the parrotfish was estimated to be very low on the basis of chlorophyll concentration. It was concluded that algae are not the organisms which produce the toxin(s).

Ciguatera is a disease caused by ingestion of a variety of fishes associated with coral reefs. A fat soluble toxin named ciguatoxin[1] has long been considered to be responsible for the most of the posioning. It has been also proposed that the toxin is produced by a benthic alga, presumably a blue green alga, and is taken up by herbivorous fishes. These, in turn, are eaten by carnivores and the toxin is transmitted to many other fishes of higher trophic level through the web of food chain.[2]

Recently, analysing clinical and symptomatological data of human cases poisoned by ingestion of a parrotfish *Scarus gibbus,* BAGNIS has reached to a conclusion that a toxin different from diguatoxin should be present in the flesh of this species. The toxin was named scaritoxin[3] and the prediction was soon evidenced by isolation of the toxin.[4] The discovery of scaritoxin promptly raised a question as to its origin and led us to examine whether scaritoxin is produced, like ciguatoxin, by a benthic organism and taken up by the fish or whether it is a product converted from a precursor such as ciguatoxin in some organ of the fish. Attempts were also made to see if the gut contents contain other toxins such as maitotoxin and acetone soluble toxins found in a herbivorous surgeonfish[5] and turban shell[6]. Since the parrotfish is known as a coral feeder and therefore is not likely to take up sufficient amount of algae, the alleged source of toxin, determination of chlorophyll content was made to estimate the amount of the algae ingested.

The present paper reports the absence of scaritoxin in the gut contents and liver

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of a parrotfish *Scarus gibbus* and occurrence of ciguatoxin, maitotoxin, and acetone soluble toxin(s) in the gut contents. Some preliminary tests on the properties of the acetone soluble toxin(s) were also included.

Materials and Methods

Materials Specimens of *S. gibbus* were collected at Gambier Islands, French Polynesia, in 1974. The fish were dissected soon after catch and the livers and the gut contents were kept frozen until used.

Fractionation of the toxins The samples were first extracted with boiling methanol and the extracts were fractionated into the following four fractions by our routine method: diethyl ether soluble, acetone soluble, acetone precipitates, and water soluble fractions.[6] Toxicity of each fraction was tested by intraperitoneal injection into mice and expressed by mouse unit (MU) as defined in our previous paper.[5]

Column chromatography In order to test the presence of scaritoxin, the diethyl ether soluble fraction was chromatographed first on a silicic acid column and then on a DEAE-cellulose acetate column after the manner described by CHUNGUE *et al.*[4] Elution of the toxin was carried out with chloroform containing varying concentration of methanol. Chromatography of the acetone precipitates was performed on a silicic acid column with solvent consisting of chloroform and methanol of various ratios in a manner as employed for purification of maitotoxin. Ion exchange chromatography of the acetone soluble toxin was carried out on a column of Amberlite CG–50 (Na^+ form) after the manner described by CHANTZ *et al.* for purification of saxitoxin.[7]

Determination of chlorophyll content The gut contents (47 g) were extracted with 80% aqueous acetone and the extracts were made up to 500 ml. Aliquots of the solution were subjected to photometrical determination at 663 and 645 nm. The chlorophyll contents were calculated by the equations proposed by MACKINNEY[8] as follows:

$$\log (I_0/I)_{663} = 82.04\ Ca + 9.27\ Cb$$

$$\log (I_0/I)_{645} = 16.75\ Ca + 45.6\ Cb$$

where Ca and Cb respectively represent the concentration of chlorophyll *a* and *b*. The $\log (I_0/I)_{663}$ and $\log (I_0/I)_{645}$ are the absorbances of the sample solution at 663 and 645 nm.

Dose-death time relationship The dose-death time relationship for the acetone soluble toxin was determined on mice following the description of KONOSU *et al.*[9] The resultant curve was compared with that obtained with the acetone soluble toxin of a turban shell.

Stability test of the acetone soluble toxin Aliquots of acetone soluble toxin were dissolved in 1 N hydrochloric acid, 1 N acetic acid, and 1 N ammonium hydroxide solutions. Each solution was heated in a boiling water bath for 2 hr, evaporated to dryness, and the residue was tested for toxicity on mice.

Results

The results of the toxicity test of each fraction are shown in Fig. 1. Only the water soluble fraction was nontoxic and other three fractions were toxic, indicating the multiplicity of the toxins. In the case of the liver, only the diethyl ether soluble fraction was toxic, toxicity being 42 MU/g tissue.

When the diethyl ether soluble fractions from both the gut contents and liver were subjected to silicic acid column chromatography, toxicity was recognized in the chloroform-methanol (9: 1) fractions. In the next chromatography on DEAE-cellulose column, the toxin appeared only in the chloroform-methanol (1: 1) eluates as shown in Figs. 2 and 3. The chromatographic behavior of the toxin was compatible with that of ciguatoxin. The signs in mice such as nausea, paralysis in limbs, and dificulty in respiration also agreed with those of ciguatoxin. Failure in detecting toxicity in the chloroform eluate from the DEAE-cellulose column indicated absence of scaritoxin in the samples.

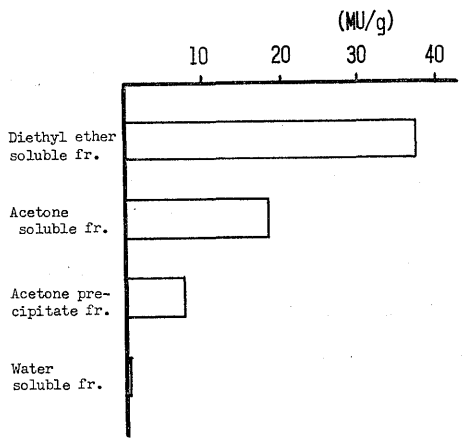

Fig. 1. Toxicity of the fractions obtained from the gut contents.

As shown in Fig. 1, the gut contents contained acetone soluble toxin(s) in a remarkable amount. The toxin(s) was found to be dialyzable through a cellophane membrane and was stable in acidic media but was unstable in the alkaline medium. The mice died of strong paralysis in a short period. The toxin was retained by the column of Amberlite CG–50 resin and was eluted from the column with 0.5 N acetic acid. These properties

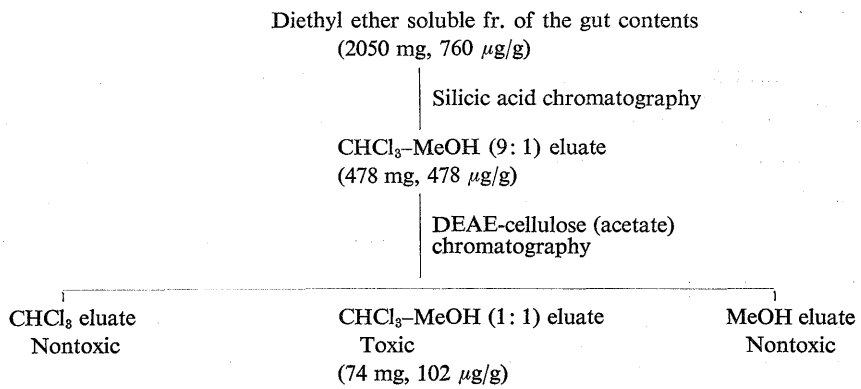

Fig. 2. Fractionation of the diethyl ether soluble fraction of the gut contents by column chromatography.

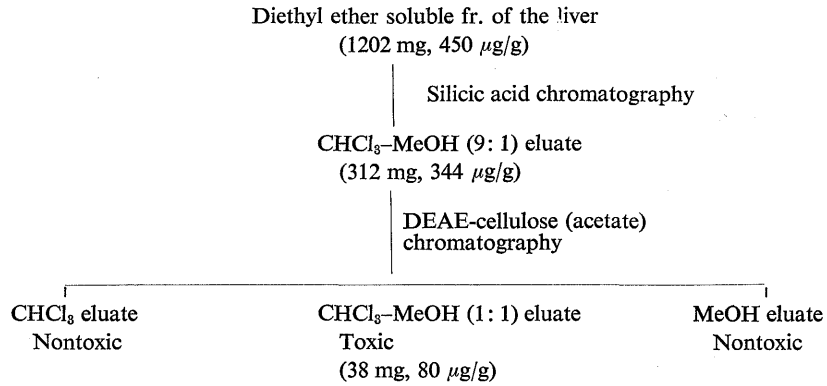

Fig. 3. Fractionation of the diethyl ether soluble fraction of the liver by column chromatography.

resemble those of acetone soluble toxins found in a turban shell[10-12]. However, the dose-death time relationship on mice proved that it was distinct from the turban shell toxin (Fig. 4).

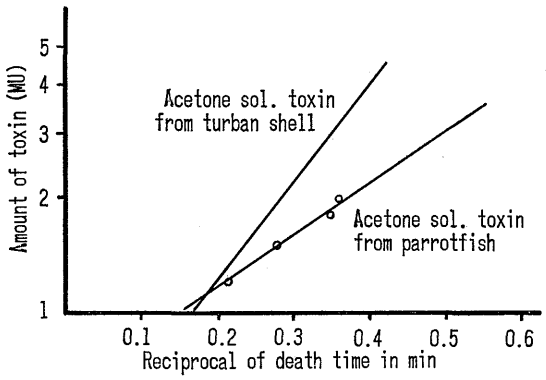

Fig. 4. Comparison of dose-death time relationships for acetone soluble toxins from parrotfish and turban shell.

The toxin in the acetone precipitates fraction was not dialyzable through a cellophane membrane. The result of the silicic acid column chromatography is given in Table 1. The toxin appeared in two fractions: chloroform-methanol (6: 4) and (4: 6). It killed mice only after several hr from injection. The signs were loss of activity, dificulty in respiration, and paralysis in limbs. These properties indicate that the toxin in the acetone precipitates is maitotoxin.

Spectrophotometric analysis of the acetone extract of the gut contents indicated that the concentration of chlorophyll *a* in the sample was about 0.79 μg/100 g. Since average chlorophyll contents of the ordinal algae are between 0.77 and 0.31%, the algae in the gut contents amount to only 103–260 μg/100 g of sample. These figures suggested that

Table 1. Silicic acid column chromatography of the acetone precipitates

Solvent	m*l*	Residue (mg)	Minimum lethal dose (μg/g)	Total MU
$CHCl_3$–MeOH (8: 2)	75	20	Nontoxic	0
$CHCl_3$–MeOH (7: 3)	75	26	Nontoxic	0
$CHCl_3$–MeOH (6: 4)	75	21	38	533
$CHCl_3$–MeOH (4: 6)	75	33	144	231
MeOH	75	39	Nontoxic	0

Sample: 0.2 g (310 μg/g, 652 MU)
Column: Silicic acid 15 g, 2.2×20 cm

the oridinal algae were negligible as the source of toxin.

Discussion

Despite the fact that scaritoxin is the dominant toxin in the flesh,[4] neither the gut contents nor the liver contained it in an appreciable amount. Failure in detecting scaritoxin in the gut contents suggests that the toxin is not acquired as such from the diet but is obtained by a conversion from a precursor. However, the fact that the parrotfish becomes toxic only in the endemic area indicates that such precursor may exist in the diets. The absence of scaritoxin in the liver denies that this organ is the place for the alleged transformation. Further study will be necessary to elucidate the origin of scaritoxin.

It has long been hypothesized that ciguatoxin is produced by a benthic alga of fine structure such as blue green alga. However, from the result obtained in the present study, it seems not likely the case. Presumably a benthic organism containing little or no chlorophyll may be responsible for producing the toxin.

Despite the distinct difference in feeding habit, the toxins found in the gut contents of the parrotfish were similar to those found in the viscera of a herbivorous mollusc *Turbo argyrostoma*[6,10–12]. This is the first time that the acetone soluble toxin(s) was detected in the gut contents of a ciguatoxic fish. Although the chromatographic behaviors and other properties of the acetone soluble toxin(s) of the parrotfish resembled those of the toxins from a turban shell, they were distinguishable from each other by the dose-death time curves. Attempts to identify the toxin(s) are being carried out.

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