Short Paper

Ichthyotoxicity of a Flagellate *Uroglena volvox**¹

In May 1977, a massive waterbloom of a flagellate *Uroglena volvox* occurred along the north and west coasts of Lake Biwa, Shiga Prefecture, and continued for about two weeks. Any mass mortality of fish was not observed during the waterbloom. However, we found experimentally that *U. volvox* shows a fairly strong ichthyotoxicity to the ayu-fish *Plecoglossus altivelis*, whereas it is weakly active against the killifish *Oryzias latipes*. In this paper, we describe the characterization of the toxic principle in *U. volvox*.

A frozen sample (70 g) of the flagellate was repeatedly extracted with 5 volumes of hot methanol for 30 min. The ether-soluble portion of the extract was divided into acidic, neutral, and alkaline fractions. The acidic fraction having most of the ichthyotoxic activity was subjected to Florisil column chromatography. The toxin was mostly eluted with diethyl ether-methanol (98:2) and diethyl ether-acetic acid (96:4). Those eluants were combined, and applied to silicic acid column chromatography. The toxin (480 mg) was eluted with *n*-hexane-diethyl ether (95:5). The final preparation suspended at a level of 50 ppm was found to kill all of the five ayu-fish tested (7–8 cm in total length) between 31 and 84 min. The activity of fish was hindered immediately after being placed in the suspension. About 20 min later, the fish lay on their side or swam up and down, while gasping. These behaviors lasted for some time, and then suddenly they started agonizing and finally died. Similar results were obtained when the ayu-fish were put in a 0.5% flagellate suspension. The hemolytic activity of the toxin was 1.2 Saponin Unit per mg, when estimated by the method of OSHIMA *et al.*¹⁾

The IR spectrum of the toxin resembled that of a fatty acid. The toxin was then methylated and analyzed by gas liquid chromatography on 10% diethylene glycol succinate. As shown in Table 1, the main fatty acids were recognized to be myristic (C_{14:0}), palmitic (C_{16:0}), and docosatetraenoic acid (C_{22:4}).

From these results and the related phenomenon with green alga *Chaetomorpha minima*²⁾, the ichthyotoxicity of *U. volvox* appears to be attributable to free fatty

Table 1. Fatty acid composition of *U. volvox*

C _{m:n}	%	C _{m:n}	%
12:0	1.8	18:2	5.0
14:0	37.6	18:3	2.5
14:1	1.9	20:0	trace
15:0	trace	20:1	trace
16:0	10.1	20:3	1.1
16:1	1.3	22:0	3.8
16:2	3.2	22:2	7.7
18:0	2.6	22:4	9.6
18:1	7.7		

acids present in a high quantity. Incidentally, it was noticed that the flagellate-free lake water was also toxic to the ayu-fish. This suggests that *U. volvox* secretes fatty acids to the outer environment. It is necessary to pay more attention to *U. volvox* waterbloom in Lake Biwa because the ayu-fish, one of the most important culture fishes in this lake, is highly sensitive to fatty acids.

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References

- 1) Y. OSHIMA, K. SHIOMI, and Y. HASHIMOTO: *Bull. Japan. Soc. Sci. Fish.*, **40**, 223–230 (1974).
- 2) N. FUSEYANI, C. OZAWA, and Y. HASHIMOTO: *Bull. Japan. Soc. Sci. Fish.*, **42**, 941 (1976).

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*¹ 黄緑鞭毛虫 *Uroglena volvox* の魚毒性

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