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Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anadonta cygnea*

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Abstract

Swan mussels (Anodonta cygnea) were exposed to a toxic strain of the cyanobacterium *Oscillatoria agardhii*. Mussels accumulated large amounts of the peptide *Oscillatoria* toxin which was present in low concentrations within the cyanobacterial cells in the test aquaria (40–60 μg *Oscillatoria* toxin/l). The toxin concentration in the mussels increased during the experiment and after 15 days of exposure the concentration was 70 ± 2 μg/g freeze dried tissue (mean ± range of values). The highest concentration of the toxin (130 μg/g of freeze dried tissue) was found in the hepatopancreatic tissue. The toxin did not seem to be metabolized in the mussels and they were not killed by the high toxin concentrations within them. After two months in clean water still detectable amounts of toxin were present in the mussels.

Introduction

The cyanobacterium (blue-green alga) *Oscillatoria agardhii* is common in eutrophic lakes and reservoirs and like many other cyanobacteria it has been frequently associated with various kinds of water management problems (Silvey *et al.*, 1972; Berger, 1975; Skulberg, 1978; Feuillade & Feuillade, 1981; Zevenboom *et al.*, 1981; Post *et al.*, 1985; Berg *et al.*, 1986). There are several reports on toxic strains of this species (Østensvik *et al.*, 1981; Leeuwangh *et al.*, 1983; Berg *et al.*, 1986; Eriksson *et al.*, 1986; Eriksson *et al.*, 1988a). The toxic principle of this cyanobacterium has been characterized as extremely poisonous to the liver with properties closely resembling those of peptide toxins isolated from the cyanobacterium *Microcystis aeruginosa* (Berg & Søli, 1985; Eriksson *et al.*, 1988a). Preliminary investigations have shown that the toxin of *Oscillatoria* is also a peptide which is structurally related to the toxins of *M. aeruginosa* (Eriksson *et al.*, 1987; Eriksson *et al.*, 1988a). A general structure for the toxins of *Microcystis aeruginosa* has been described as cyclo (-Ala-X-β-methyl-Asp-Y-Adda-Glu-N-methyl-dehydro-Ala). In this structure X and Y refer to variable amino acids and Adda to a β-amino acid with an unsaturated side chain (Botes *et al.*, 1984; Botes *et al.*, 1985; Botes, 1986). Although the final structure of *Oscillatoria* toxin has not been presented it contains at least the β-amino acid Adda and N-methyldehydro-Ala (Krishnamurthy *et al.*, 1986b; Meriluoto *et al.*, 1988). Both *Microcystis* and *Oscillatoria* toxin exhibit lipophilic properties (Botes, 1985; Meriluoto & Eriksson, 1988) which are partly due to the unsaturated side chain in Adda.

Kills of stock and wildlife animals have been reported in connection with blooms of toxic cyanobacteria (Schwimmer & Schwimmer, 1968; Skulberg *et al.*, 1984; Carmichael *et al.*, 1985). A field study of a bloom of toxic *O. agardhii* showed heavy mortalities of fish with simultaneous occurrence of dead birds and muskrats (Eriksson *et al.*, 1986). These findings indicated possible accumulation of the toxin from *O. agardhii* in the food chains. Alkaloid toxins from various marine algae are known to accumulate at higher trophic levels. The aim of this study was to establish whether accumulation of peptide cyanobacterial toxins is also possible.

Material and methods

Cyanobacterial material

The toxic *O. agardhii* (strain CYA-38) was received from the culture collection of Norwegian Institute for Water Research (NIVA) and was originally isolated from Lake Gjersjön in Norway (Skulberg & Skulberg, 1985). It was cultured in 20 l polyacrylic vessels with the water from the lake from which the mussels were taken as medium (see below). Possible other cyanobacteria and algae in the water were killed by repeated deep-freezing of the water. Nutrients were added to the medium so that the final concentration corresponded to approximately 20% Z8 (Staub, 1962). The experiments were carried out at 20 °C under continuous illumination (20–40 μE $m^{-2}s^{-1}$).

Freshwater mussels

The freshwater mussels were collected by scuba diving from the mesotrophic Lake Littoistenjärvi, near Turku (SW Finland), in August 1986 (water temperature 18–20 °C). Mussels with a mean size of 9 ± 2 cm were used in the experiments (interval indicates the range of sizes). All the mussels were active and motile at the beginning of the experiment. Experiments were carried out in 40 l glass aquaria and clean sand was used as bottom substrate for the mussels. The *Oscillatoria* was administered to the aquaria with a peristaltic pump which also recirculated the water back to the culture vessel (Fig. 1). The flow was adjusted so that the dry weight concentration of *Oscillatoria* in the test aquaria was 10–20 mg/l. This concentration agrees well with concentrations which can be found in natural eutrophic environments. Microscopical examination of the cyanobacterial cells showed them to be viable and intact during the experiment, thus indicating that no significant lysis of the cells occurred. The toxic *O. agardhii* contained 2000–3000 μg toxin per gram freeze-dried material. With the dry weight concentrations of *Oscillatoria* ranging from 10–20 mg/l, the aquaria thus contained 40–60 μg toxin/l. After 15 days of exposure the remaining mussels were transferred to aquaria containing untreated lake water. Control mussels received untreated lake water only.

When mussel samples were taken from the aquaria, the mussels were opened and the tissues thoroughly rinsed with clean water. Each sample of mussels consisted of the pooled soft tissues of three mussels. The range of dry weights of the soft tissues of individual mussels used in the experiments was 1–4 g. The mussels used for the determination of the tissue distribution of the *Oscillatoria* toxin were sampled after thirty days of

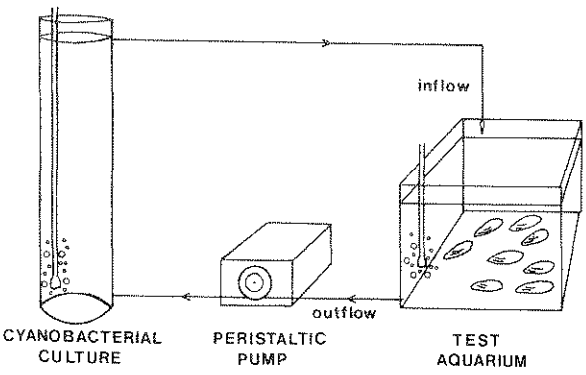

Fig. 1. Experimental conditions. Freshwater mussels, *Andonta cygnea*, were kept in glass aquaria to which toxic *Oscillaria agardhii* (CYA-38) was administered. The dry weight concentration of toxic *Oscillatoria* in the test aquaria was kept at a level of 10–20 mg/l during the whole experiment.

exposure in a separate trial with lower algal densities and were then kept in clean water for five days after which their soft tissues were removed. The sample for determination of the tissue distribution consisted of the pooled organs from five mussels. The mussel tissue homogenates were deep-frozen (−20 °C) after collection, then freeze-dried.

HPLC analysis

A method modified (Eriksson *et al.*, 1988a) from earlier described methods was used (Siegelman, 1984; Krishnamurthy *et al.*, 1986a). The cyanobacterial toxin was extracted from the mussel tissue homogenates by 30 min. bath sonication with 50 ml 5% butanol-20% methanol-75% water per gram freeze-dried homogenate. After centrifugation (1 hr., 48000 G) the pellets were reextracted. After partial purification on Bond-Elut C-18 columns, the toxin was analyzed on a reversed phase C-18 HPLC column (Nucleosil 7 C 18, 10 × 250 mm; Macherey-Nagel) with a μ-Bondapak guard column. The extracts were eluted with 27% acetonitrile in 0.01 M ammonium acetate (flow rate 3.0 ml/min.) on a Kratos pump system with the detector set at 240 nm. Highly purified toxin isolated from *Oscillatoria* CYA-38 was used as standard.

Bioassays

When the mussel extracts were initially analyzed on HPLC the toxicity of the peaks with the same retention time as the purified toxin from *Oscillatoria* was tested by mouse bioassays. The eluate was rotary evaporated, dissolved in water and injected intraperitoneally in male SVR mice (20–25 g). Characteristic signs for intoxication with this type of toxin, i.e. swollen, enlarged and dark liver (Berg & Søli, 1985; Eriksson *et al.*, 1988a), were looked for.

Results

HPLC analysis of the extracts of the mussels exposed to toxic *Oscillatoria* showed a large peak with the same retention time as *Oscillatoria* toxin (Fig. 2). When this fraction was isolated it had the

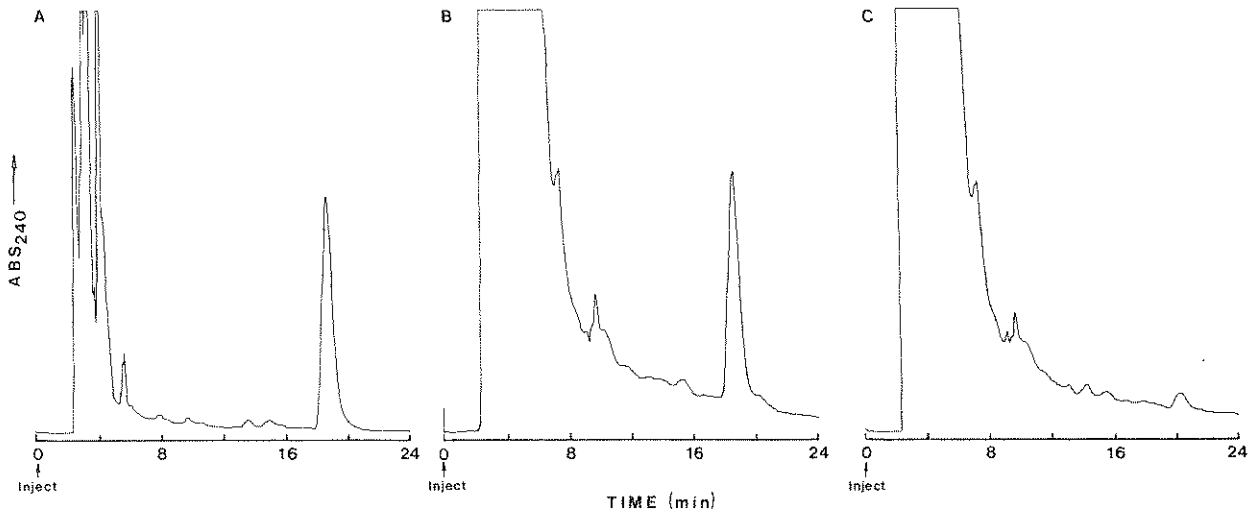

Fig. 2. HPLC chromatographs of extracts of (A) toxic *Oscillatoria agardhii*, (B) mussels exposed to toxic *Oscillatoria agardhii* and (C) control mussels. The fraction with the *Oscillatoria* toxin was eluted at about 18.6 min; (the big peak on chromatograms (A) and (B)). HPLC conditions: A Nucleosil 7 C 18 column was eluted with 27% acetonitrile in 0.01 M ammonium acetate with a flow rate of 3.0 ml/min. (detector set at 240 nm).

same toxic potential per absorbance unit and caused similar symptoms in mice as the toxin isolated from *Oscillatoria* CYA-38. Both mice injected with the purified toxin and the fraction isolated from the mussel extracts died within 1–3 hours. It was concluded that the toxin was probably not metabolized in the mussels.

The mussels accumulated *Oscillatoria* toxin quite rapidly during the experiment (Fig. 3). The mean toxin concentration after 15 days of exposure was 70 μg/g dry weight. With dry weights of the soft tissues ranging from 1–4 g, the calculated total amount of toxin in individual mussels thus correspond to 70–280 μg per mussel. After 15 days the mussels were transferred to clean water. After two months in clean water the mussels still contained detectable amounts of toxin. The high concentration of the toxin in the mussels had no considerable effects on them since they survived and were motile during the whole experiment.

The highest toxin concentration was detected in the hepatopancreatic tissue (Fig. 4). The dry weight of this organ is quite low, average 3.5% of the total dry weight of the soft tissue. However, the amount of toxin in the hepatopancreas was about 40% of the total toxin content in the mussels. The intestine and gonads and the muscles contained only minor quantities of the toxin. Most of the rest of the toxin was in the remaining tissue (kidneys, connective tissue) although the concentrations in these parts of the body were quite low.

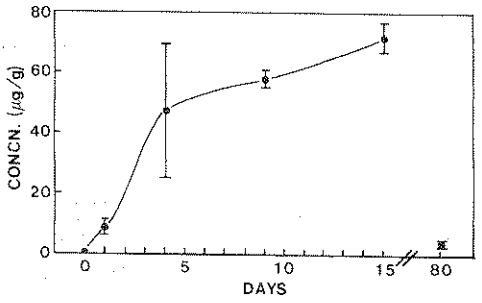

Fig. 3. Accumulation of *Oscillatoria* toxin in freshwater mussels, *Anodonta cygnea*, exposed to toxic *Oscillatoria agardhii*. The toxin concentrations in the mussels were analyzed by HPLC Purified *Oscillatoria* toxin was used as standard. The last measurement at 80 days was made after the mussels had been kept in pure lake water for 65 days after exposure. Vertical bars show the range of concentrations in 2 or 3 samples.

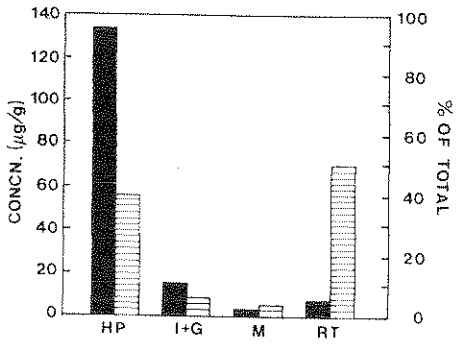

Fig. 4. Organ distribution of *Oscillatoria* toxin in freshwater mussels, *Anodonta cygnea*, after 30 days of exposure to low densities of toxic *Oscillatoria agardhii*. The black bars show the toxin concentrations (μg/g freeze dried tissue) in the different organs and the striped bars show the relative amounts (%) of toxin in the different tissues as related to the total toxin content in the mussels. The toxin concentration found in the whole mussels in this trial averaged 11.9 μg/g freeze dried tissue. Abbreviations: HP = hepatopancreas, I + G = intestine + gonads, M = muscles, RT = remaining tissues.

Discussion

Accumulation of algal toxins in marine ecosystems is well documented. Especially the accumulation of various dinoflagellate toxins in bivalves has been extensively studied (for reviews see: Baden, 1983; Carmichael *et al.*, 1985). So far accumulation of toxins from different freshwater cyanobacteria has not been reported although circumstantial evidence has pointed to this possibility (Schwimmer & Schwimmer, 1968; Keymer *et al.*, 1972; Skulberg *et al.*, 1984; Eriksson *et al.*, 1986). In the present study the freshwater mussels showed a remarkable ability to accumulate the peptide *Oscillatoria* toxin. The calculated total concentrations of toxin in the mussels were 70–280 μg per mussel, which implies that they can concentrate low toxin concentrations, corresponding to concentrations in natural environments, over a relatively short period of time. The unchanged characteristics of the toxin isolated from the mussel extracts indicate that the toxin

was not metabolized in the mussels. The fact that the highest toxin concentration was found in the hepatopancreas agrees well with earlier reports on the organ distribution of lipid-soluble dinoflagellate toxins in marine mussels (Baden, 1983). The decreased toxin concentration after two months in clean water could be due either to a degradation of the toxin or passive excretion of the toxin.

Since the freshwater mussel *Anodonta cygnea* accumulated the peptide toxin from *Oscillatoria* readily, it is reasonable to assume that also other filter feeders could accumulate this or similar peptide toxins. So far cyanobacterial species from four different genera, i.e. *Microcystis, Anabaena, Oscillatoria* and *Nodularia*, have been shown to contain this type of hepatotoxic peptides (Botes, 1986; Krishnamurthy *et al.*, 1986a; Berg *et al.*, 1987. Eriksson *et al.*, 1988a; Eriksson *et al.*, 1988b). As the question of accumulation of cyanobacterial toxins is of considerable importance in eutrophic environments, further investigations are required to clarify if accumulation of cyanobacterial toxins is common in aquatic food chains.

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