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Nodularin induces oxidative stress in the Baltic Sea brown alga *Fucus vesiculosus* (Phaeophyceae)

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Abstract

In the Baltic Sea regular, intensive cyanobacterial blooms rich in the cyanobacterium *Nodularia spumigena* occur during the summer season. *N. spumigena* is known to produce the cyclic pentapeptide nodularin (NOD) in high concentrations. Marine macroalgae, together with sea-grass meadows, are an extremely important habitat for life in the sea. In addition to this, the decaying macroalgae substantially contribute to the substrate for the microbial loop in coastal food webs. Uptake of nodularin into the brown macroalga *Fucus vesiculosus* was assessed using an ELISA technique resulting in an uptake of up to 45.1 μg kg^{-1} fresh weight (fw). Nodularin was also detected in the reproductive part of the algae (receptacle) at 14.1 μg kg^{-1} fw. The induction of oxidative stress in *F. vesiculosus*, after exposure to NOD, was also shown by monitoring cellular damage as changes in lipid peroxidation and the activation of antioxidative defence systems (antioxidative capacity, superoxide dismutase and soluble glutathione *S*-transferase).

Keywords: Cyanotoxin; Macroalgae; Oxidative stress; Nodularin; Fucus

1. Introduction

During the past one hundred years the phosphorous load into the Baltic Sea has increased by about eightfold and the nitrogen load about fourfold (Larsson et al.,

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1985). As a consequence of this, the pelagic primary production has increased by an estimated 50% (Elmgren, 1989) and sedimentation of organic carbon 5- to 10-fold (Jonsson and Carman, 1994). In the northern Baltic Sea regular, intensive cyanobacterial blooms rich in the cyanobacterium *Nodularia spumigena* occur during the summer season (Sivonen et al., 1989). *N. spumigena* is known to produce the cyclic pentapeptide hepatotoxin nodularin (NOD) in high concentrations (Sivonen et al., 1989). The most common NOD is NOD-R, or cyclo(-D-*erythro*-β-methylAsp(*iso*)-L-Arg-Adda-D-Glu(*iso*)-2-(methylamino)-2-(*Z*)-dehydrobutyric acid), where Adda stands for 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid (Carmichael et al., 1988). The concentration of NOD in the Baltic Sea has been shown to range from 0.5 $\mu g\ L^{-1}$ to 25.8 $\mu g\ L^{-1}$ with a maximum cell-bound concentration of 18.1 $mg\ kg^{-1}$ (Kononen et al., 1993; Kankaanpää et al., 2001; Mazur and Pliński, 2003).

Several cases of animal poisonings in the southern Baltic Sea have been reported (Edler et al., 1985; Nehring, 1993). Changes in liver histopathology have also been observed in trout exposed in vivo with *Nodularia* cells containing NOD (Kankaanpää et al., 2002). Other recent studies have indicated that NOD can accumulate into blue mussels (*Mytilus edulis*; Sipiä et al., 2001a) and some Baltic fish species, e.g. flounder (*Platichthys flesus*; Kankaanpää et al., 2002), Atlantic cod (*Gadus morhua*; Sipiä et al., 2001b) and the three-spined stickleback (*Gasterosteus aculeatus*; Kankaanpää et al., 2001). One feature of rocky seashores in the Baltic Sea is the growth of macroalgae belonging to three different phyla: Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae). These algae, together with sea-grass meadows, are extremely important for life in the sea. Macroalgae are not to a great extent utilised by primary consumers as a food source, but for numerous smaller animals, such as isopods (especially *Idotea baltica*), benthic isopods (e.g. *Saduria entomon*), blue mussels, crustaceans, scallops, starfish and lobsters they do provide shelter. Several of the economically most important fish species, herring or pollock, are entirely dependent on the macroalgae zones in their early life stages. Macroalgae also increase the available substrate for benthic microalgae, which in turn are the main food for small herbivores. In addition to this, the decaying macroalgae are substantially contributing to the substrate for the microbial loop in coastal food webs (Mann, 1982; Lüning, 1990). It has been shown that cyanobacterial toxins can promote oxidative stress in freshwater macrophytes (Pflugmacher, 2004). To prevent cellular damage caused by reactive oxygen species (ROS), cells have an elaborate system involving antioxidative enzymes which include superoxide dismutase (SOD), glutathione peroxidase (GPx), ascorbate peroxidase (APx), catalase (CAT) and glutathione reductase (GR). SOD catalyzes the dismutation (reduction) of superoxide anions into oxygen and hydrogen peroxide. Hydrogen peroxide is reduced by CAT, GPx or APx to water, the latter using ascorbate as an electron donor. Glutathione reductase is part of an oxido-reduction cycle involving glutathione and ascorbate (Foyer and Halliwell, 1976; Smith et al., 1990). Glutathione reductase catalyses the reduction of glutathione disulfide (GSSG) in an NADPH-dependent reaction and therefore plays a pivotal role in protection against oxidative damage and the adjustment processes of metabolic pathways.

Marine macroalgae, many of which play an important role in the marine coastal ecosystem, have so far been overlooked in relation to cyanobacterial toxins. The aim of this study was to examine any effects of a NOD-containing crude cell extract of *N. spumigena* on the most abundant Baltic Sea brown algae, the bladder wrack *Fucus vesiculosus* and in