Toxicon 78 (2014) 68–77

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Cumulative effects of exposure to cyanobacteria bloom extracts and benzo[a]pyrene on antioxidant defence biomarkers in *Gammarus oceanicus* (Crustacea: Amphipoda)

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A R T I C L E I N F O

Article history:
Received 21 July 2013
Received in revised form 19 November 2013
Accepted 26 November 2013
Available online 4 December 2013

Keywords:
Antioxidant enzymes
Nodularin
Benzo[a]pyrene
Gammarus oceanicus
Mixture toxicity
Baltic Sea

A B S T R A C T

The Baltic Sea suffers from extensive blooms of the toxic cyanobacteria *Nodularia spumigena* that produces nodularin toxin (NOD). Additionally, intensification of oil transportation and related activities in the area increase the risk of oil spills. The current experiment was designed to mimic a situation where an oil spill occurs during a cyanobacterial bloom by exposing the amphipod *Gammarus oceanicus* to a NOD-rich cyanobacterial extract and the polycyclic aromatic hydrocarbon compound benzo[a]pyrene (B[*a*]P), a common constituent of oil. The activity of the antioxidant enzymes glutathione *S*-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were examined after 48 and 96 h of exposure. Exposure to low and high levels of the NOD-rich extract produced a time-dependent activation of GST, GPx and SOD. CAT levels were elevated only by high NOD treatment. Also the toxicity of B[*a*]P was indicated by significantly elevated antioxidant response. In the combined exposures treatment-dependent additive increases in the activity of GPx and SOD were observed as well as inhibitory (antagonistic) effects on GST, CAT and GPx. Rapid concentration-dependent accumulation of NOD by *G. oceanicus* was observed. The addition of B[*a*]P reduced the accumulation of NOD and resulted in different biomarker response patterns compared to single exposures demonstrating the effects of mixture toxicity.

1. Introduction

In the marine environment, both natural and anthropogenic stressors cause multiple detrimental effects on organisms. The combined effects of different stress factors can be anything from antagonism to high synergy. The poor understanding of these effects occurring under natural conditions significantly complicates the making of relevant risk assessments for environmental chemicals. Thus, the issue of mixture toxicity is high up in the current ecotoxicological research agenda worldwide (Devier et al., 2011).

In the Baltic Sea organisms are subjected to regularly occurring toxic cyanobacterial blooms. These blooms (consisting mainly of *Nodularia spumigena*, *Aphanizomenon* sp. and *Anabaena* sp.) contain toxic nodularin (NOD,

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0041-0101/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.toxicon.2013.11.015

Kankaanpää et al., 2009) and microcystin-LR (MC-LR, Halinen, 2007, Karlsson, 2005) as major phycotoxins. In particular, the filamentous NOD producing brackish water cyanobacterium *N. spumigena* exhibits extensive blooms in the northern Baltic Sea during late summer (Sivonen et al., 1989b), covering areas of up to 100,000 km^2 (Hansson, 2006). NOD is a cyclic pentapeptide hepatotoxin structurally and functionally related to microcystins (Zurawell et al., 2005). It accumulates in fish, bivalves and other invertebrates (Karjalainen et al., 2008; Sipiä et al., 2001) and causes harmful effects by transferring through the food web (Karjalainen et al., 2007). Both NOD and MC-LR cause liver damage by inhibiting essential enzymatic functions in hepatic tissue (Yoshizawa et al., 1990).

In addition to toxic NOD, the most common form of NOD, cyanobacterial blooms occurring in the Baltic Sea may contain, as minor components, an array of less toxic nodularin variants, such as, the geometrical isomer of NOD, demethylated nodularins and linear nodularins (e.g. Karlsson et al., 2003; Mazur-Marzec et al., 2006, 2013). Neurotoxins such as saxitoxins and related gonyautoxins are known to occur only in a confined area within the Åland archipelago (Hakanen et al., 2012) and occurrence of anatoxin-a has not been reported in the Baltic Sea. Diarrhoeic shellfish toxins (DSTs) may also occur (Kuuppo et al., 2006) during dinoflagellate and late-summer cyanobacterial successions. However, the contribution (Kuuppo et al., 2006; Kankaanpää et al., 2009) and the toxicity (Aune et al., 2007; Miles et al., 2004) of DSTs to the overall phycotoxin budget is known to be small compared to that of NOD. Hence, the toxic NOD is the most abundant toxin in the Baltic Sea, with production and sedimentation far greater in quantity than the introduction of any anthropogenic substance (Kankaanpää et al., 2009), and therefore possesses high ecological relevance for and impact on aquatic organisms.

Anthropogenic chemical contamination is another serious issue in the Baltic Sea where the highly populated catchment area and continually increasing tanker traffic, due especially to expansion in oil refining activities in the area, pose serious threats to the marine ecosystem (HELCOM, 2010). Oil-related compounds and particularly polycyclic aromatic hydrocarbons (PAH) affect marine organisms in various ways, including genotoxic, biochemical and physiological alterations (Frouin et al., 2007; Kirby and Law, 2010). PAH concentrations in surface waters of the Baltic Sea range between 0.7 and 16.6 ng l^{-1}, strongly depending on the time of the year (Witt and Matthäus, 2001). A few days after the occurrence of a major oil spill water concentrations of total PAH in various sea areas have been recoded to range between 0.53 and 100 μg l^{-1} (González et al., 2006). Due to the hydrophobic nature and high octanol–water partitioning of PAH their concentrations in sea water are usually low, but the compounds are readily associated with particulate matter and become finally deposited in the sediment. These properties also facilitate their accumulation in aquatic organisms (Livingstone et al., 1985; Lowe et al., 2006). Concentrations of PAH in biota depend on, among other factors, differences in bioavailability of different PAH, feeding behaviour, and metabolic capabilities of organisms (Baumard et al., 1998). Among the PAH observed in the marine environment, benzo[a]pyrene (B[*a*]P) is a common constituent of pyrolytic and petrogenic mixtures. In the Baltic Sea, B[*a*]P concentrations have been found to vary from 5 to 115 pg l^{-1} in surface water, 0.20–385 ng g^{-1} in sediments, and 1.8–12 ng g^{-1} in bivalves (Baumard et al., 1998; Pikkarainen, 2004a, 2004b; Witt, 1995).

Induction of the antioxidant defence machinery in cells is a universal response to elevated levels of reactive oxygen species (ROS). This can be caused by increased detoxification reactions as a result of exposure to organic contaminants and toxins. Exposure to NOD and to B[*a*]P has been shown to induce the formation of ROS in various organisms (Ding and Ong, 2003; Livingstone, 2001). During oxidative challenge cells usually increase their levels of antioxidant enzymes such as catalase (CAT), glutathione *S*-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD). The activity of these enzymes has therefore been successfully used in the assessment of effects of pollutants in aquatic ecosystems (Livingstone, 2001; Valavanidis et al., 2006).

Compared to fish and bivalve molluscs, relatively few studies on ROS mediated biological effects in crustacean amphipods have been conducted (Correia et al., 2003; Hatlen et al., 2009; McLoughlin et al., 2000). Species of the genus *Gammarus* are widely distributed, abundant and ecologically important in the food webs of marine coastal ecosystems. They are therefore highly relevant and potentially useful species also in monitoring and assessment of the state of the aquatic environment. Several species of gammarids are found all around the coastal areas of the Baltic Sea with *Gammarus oceanicus* standing out as one of the most common ones.

The main hypothesis of the present study was that exposure to a NOD-rich cyanobacterial extract increases the formation of ROS and results in elevated levels of antioxidant enzyme activities in the amphipod *G. oceanicus*. Furthermore, a combined exposure to the NOD-rich extract and B[*a*]P, was expected to cause further alterations in the measured parameters due to the increased toxicity and possible additive, synergistic or antagonistic effects of these compounds. To test these assumptions, two full-factorial experiments were carried out. The first one focused on the effects of exposure of the test species to low and high levels of a NOD-rich extract over a 96 h period. The second experimental scheme was designed to investigate the cumulative effects of NOD and B[*a*]P, mimicking a situation where an oil spill occurs during a period of a cyanobacterial bloom, i.e. late summer conditions in the northern Baltic Sea. A scenario where PAH from the oil spill adheres to the decaying cyanobacterial bloom and drifts to the shore demonstrates the risk of simultaneous exposure of organisms to high concentrations of both NOD and PAH (González et al., 2006; Henriksen, 2005).

2. Materials and methods

2.1. Collection of experimental organisms

Adult *G. oceanicus* sized 15–20 mm were collected by hand nets from a natural reserve area of Tvärminne outer