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Natural organic matter (NOM) has the potential to modify the multixenobiotic resistance (MXR) activity in freshwater amphipods *Eulimnogammarus cyaneus* and *E. verrucosus*

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Abstract

Based on the chemical features of natural organic matter (NOM) with its variety of functional groups, we hypothesized that NOM will modify the multixenobiotic-resistance (MXR) of an organism as xenobiotic chemicals do. The MXR system is a general first rather non-specific line of defense against environmental contaminants. The aim of this study was to compare the impacts on MXR activity in amphipod species (*Eulimnogammarus cyaneus* and *E. verrucosus*, from Lake Baikal) stressed by cadmium chloride or dissolved NOM for 24 h. NOM exposure concentrations were environmentally realistic. MXR activity was assessed based on rhodamine B efflux; its specificity was proven by a verapamil inhibition assay. It was shown that both NOM and $CdCl_2$ lead to substantial reduction of the rhodamine B efflux. This suggests that NOM may be regarded as a chemosensor which is able to reduce the efficiency of the MXR system. Possible mechanisms of direct NOM impact on MXR processes are discussed, such as peroxidation of the membranes (including P-glycoproteins) or internal blockage of the MXR pump by bioconcentrated NOM. In general, our results show that well-developed depuration pathways of freshwater organisms in contaminated environments may be impaired by strong chemical stressors and, more important, by natural biogeochemical matrices such as humic substances — humic substances are present in all freshwater systems.

Keywords: MXR; P-glycoprotein; Natural organic matter (NOM); Amphipods; Baikal; Chemosenzitation; *Eulimnogammarus cyaneus*; *Eulimnogammarus verrucosus*

1. Introduction

Natural Organic Matter (NOM) has a wide distribution in aquatic environments. Humic substances (HS) are the major component of NOM representing more than 60–80% of the total dissolved organic carbon (Thurman, 1985; Steinberg and Münster, 1985). Recently, several reports on the function of NOM as environmental signals to freshwater organisms have been published (Steinberg et al., 2006). Münster (1985) was the first to postulate that NOM compounds may have the potential to penetrate biomembranes. Later Petersen and Persson (1987) reported on such direct adverse effects of dissolved NOM on aquatic organisms and discussed the pH-dependent lipophilicity of at least one fraction of NOM as the potential mechanism. It was also shown that NOM could modulate the activity of biotransformation enzymes of organisms and also directly inhibits photosynthetic oxygen production of plants (Pflugmacher et al., 1999, 2001, 2006). Wiegand et al. (2004) found elevated levels of the chaperon HSP70 in fish after exposure to isolated HS; the impact of some NOM to several freshwater amphipods resulted in changes of peroxidase activity and HSP70 expression (Timofeyev et al., 2004). Steinberg et al. (2003) categorized the effects on aquatic plants and animals as (i) non-specific, such as expression of heat shock proteins (HSP) and

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modulation of biotransformation enzymes, or (ii) specific, such as inhibition of photosynthetic oxygen release in plants, or changes in the sex ratio of fishes and amphibians. Basic ecotoxicological requirements are fulfilled: several mechanisms apply to a variety of aquatic organisms, dose–response relationships and quantitative structure effect relationships may be established where applicable. It was concluded that HS are natural environmental chemicals that exert a chemical stress and, thus, are able to structure aquatic guilds by various modes of action (Steinberg et al., 2003). Furthermore, evidence is accumulating that HS even have the potential of hormone-like compounds (Meinelt et al., 2004; Steinberg et al., 2004; Lutz et al., 2005). Very recently, it was shown that both an NOM source and a synthetic humic-like substance act as an attractant for the nematode *Caenorhabditis elegans* and induce genes encoding for antioxidant and biotransformation enzymes (Menzel et al., 2005).

If HS are natural environmental chemicals and interact with the biotic defense systems (induction of stress proteins, biotransformation and antioxidant enzymes), we also should anticipate that they may interfere with the depuration (elimination) mechanisms. Many aquatic species are able to survive in environments which contain anthropogenic pollutants or natural toxins, and their resistance to pollution may be due, at least in part, to the function of their membrane glycoprotein extrusion pumps. The mechanism of this multixenobiotic resistance (MXR) (Kurelec and Pivcevic, 1989) is similar to the mechanism involved in multi-drug resistance (MDR) found in tumor cells (Juliano and Ling, 1976) and is associated with P-glycoproteins (P-gp). These proteins belong to a superfamily of membrane transporters termed ABC-proteins or traffic-ATPases (Ames et al., 1990; Higgins, 1992). P-gp acts as an energy-dependent pump to translocate a wide variety of structurally and functionally diverse compounds. P-gp-like proteins have been described as first line of defense against toxins (Epel, 1998) and detected in a variety of aquatic organisms including sponges, mussels, oysters, worms, and fishes (Waldmann et al., 1995; Smital and Kurelec, 1997; Minier et al., 1999; Kurelec and Pivcevic, 1991; Bard, 2000).

Taking into account the potential role of NOM as possible natural chemical with a variety of functional groups, we hypothesize that dissolved NOM may modify MXR activity as some xenobiotics would do. The aim of this study is to compare the modification of MXR activities in two freshwater amphipod species from the Siberian Lake Baikal, *Eulimnogammarus cyaneus* (Dyb) and *E. verrucosus* (Gerst.), stressed by presence of dissolved NOM components. CdCl$_2$ served as a representative of potentially toxic heavy metals and verapamil as a positive control.

2. Materials and methods

2.1. Animals

For this study, two freshwater amphipod species, *E. cyaneus* (Dyb) and *Eulimnogammarus verrucosus* (Gerst.) were used. These species are typical representatives of the fauna of the upper littoral zone in Lake Baikal. The specimens were collected at the shoreline in the area of the settlement Bol'shie Koty (southern Baikal). Before the experiments, the animals were maintained (pre-acclimated) in aerated aquariums at 6–8 °C at least for two, some for three days. Commercial food was used (Tetra-Min, Tetra GmbH, Germany) *ad libitum*. The water was continuously aerated, and the oxygen content was between 8–9 mg L^{-1}. The size of the collected individuals was 22–25 mm (*E. verrucosus*) and 15–18 mm (*E. cyaneus*). Age and sex of amphipods were not determined (in order to minimize handling of the delicate organisms). However, we assumed that most of the amphipods were adults because their size was close to maximal body size known for this species 30 mm for *E. verrucosus* and 20 mm for *E. cyaneus* (Bazikalova, 1951). For experiments only well-active animals were used.

2.2. Isolation of natural organic matter (NOM)

NOM was isolated by reverse osmosis from Lake Schwarzer See (a brown-water lake, Brandenburg State, north-eastern Germany) according to Serkiz and Perdue (1990); as a modification, we applied the sodium cation exchanger. The NOM comprised 24% organic carbon; the chemical characteristics of Lake Schwarzer See water and its NOM isolate are given in Table 1. The DOC concentrations were determined by high-temperature combustion (Shimadzu TC 5000) after acidification with phosphoric acid to remove inorganic carbonates (DIN EN 1484). The DOC was fractionated by a liquid chromatography fingerprint-technique with simultaneous UV- and DOC-detection according to Sachse et al. (2001, 2005) and classified as follows: HS 87.5%; low-molecular-weight acids 9.2%; polysaccharides 2.6%; low-molecular-weight substances 0%; specific UV absorption of 5.1 L/(mg m). Approximately 90% of the DOC was comprised of HS. For the sake of comparison, we applied this somewhat exotic NOM, because there have been several ecophysiological and ecochemical studies with this NOM (review: Steinberg et al., 2006; gammarids: Timofeyev

Table 1
Chemical characteristics of Lake Schwarzer See water and the NOM isolate from September 09, 2003

Lake Water, µmol L^{-1}	NOM features, ‰		
Total phosphorus	4.6	Ash content	76
Total nitrogen	140	C	204
Sulfate	250	H	25.2
Chloride	280	N	5.8
Dissolved silica	24.3	S	47.6
Dissolved inorganic carbon	790	Fe	0.44
Dissolved organic carbon	2,260	Mn	0.91
Sodium	200	Zn	0.05
Potassium	82	Cu	0.06
Magnesium	100	Al	0.12
Calcium	570	Na	157
Aluminum	n.d.	K	11.9
		Mg	8.3
		Ca	53.6

n.d. = not determined.