Available online at www.sciencedirect.com
ScienceDirect
Marine Environmental Research 65 (2008) 437–444
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Metallothioneins and cytosolic metals in *Neomysis integer* exposed to cadmium at different salinities

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Received 23 October 2007; received in revised form 8 February 2008; accepted 8 February 2008

Abstract

In the present study the induction of metallothioneins (MTs) and its relation to cytosolic metal concentrations (Zn, Cu and Cd) in the euryhaline crustacean *Neomysis integer* exposed to Cd at different salinities was studied. *N. integer* was exposed to the same free cadmium ion activity of 5.74×10^{-9} mol l^{-1} (i.e. 1/5 of the 96 h LC_{50} value expressed as cadmium activity) in hypo-osmotic (5 psu), isosmotic (16 psu) and hyper-osmotic media (25 psu) for 7 days. In this way, the effect of salinity on cadmium speciation was eliminated and therefore the physiological effect of salinity on Cd accumulation and MT induction could be studied.

The accumulation of cytosolic Cd in *N. integer* changed with salinity from 1.11 ± 0.05 μmol l^{-1} at 5 psu up to 1.43 ± 0.17 μmol l^{-1} at 25 psu. This could indicate that the physiological response of euryhaline estuarine invertebrates like *N. integer* to salinity changes can influence the rate of trace metal uptake from solution. While the salinity changes did not cause significant differences in cytosolic Zn concentrations (mean value of all tested salinities: 34.4 ± 2.8 μmol l^{-1}), an inverse relationship between salinity and cytosolic Cu concentration was observed. The highest concentration of 15.7 ± 2.3 μmol Cu l^{-1} was determined at 5 psu and the lowest 10.9 ± 1.4 μmol Cu l^{-1} at 25 psu. This could point to a possible relationship between the copper concentration and the hemocyanin metabolism in *N. integer*.

This is the first time that differential pulse voltammetry method was applied to MT assays with *N. integer*. Although the exposure to Cd resulted in a higher Cd cytosolic concentration, no subsequent MT increase was detected. The significant positive correlation between MT levels and cytosolic Cu concentrations (Spearman correlation coefficient $r_s = 0.356$, $p = 0.009$) implies a strong relationship between MT and Cu in *N. integer*.

Keywords: Mysid; *Neomysis integer*; Cadmium; Salinity; Metallothionein; Cytosolic metals

1. Introduction

Beside natural sources, human activities significantly contribute to the presence of cadmium in the environment (Hutton, 1983). Because of its toxicity, persistence and accumulation in the environment (Cole and Volpe, 1983; Herber, 2004) cadmium is identified as priority hazardous substance within the water framework directive (WFD, 2000). Anthropogenic metal contamination influences both freshwater and coastal water bodies (Charlesworth and Service, 2000; Ibhadon et al., 2004). In this respect, molluscs, crustaceans and other marine invertebrates living in the littoral zone are known to accumulate high levels of metals in their tissues and yet survive in these polluted environments (Bryan et al., 1985; Rainbow, 2002; Fränzle, 2003). This tolerance depends on the ability of these animals to regulate metal in many of their tissues and to accumulate excess metal in non-toxic forms in other particular

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doi:10.1016/j.marenvres.2008.02.002

tissues (Viarengo and Nott, 1993). One of the proposed metal homeostasis and detoxification mechanisms in marine invertebrates is binding to specific soluble ligands, the most important of which are metallothioneins (MTs) (Viarengo and Nott, 1993; Amiard et al., 2006). Metals bound to metallothioneins, may be more available to predators than metals associated with insoluble cellular constituents (Wallace and Luoma, 2003; Zhang and Wang, 2006). Studies of the trophic transfer of metal contaminants have shown that the trophic transfer of certain metals in aquatic systems may be controlled by the internal distribution of metal within prey and that this distribution may be influenced by detoxification mechanisms (Wallace and Lopez, 1996; Fisher and Reinfelder, 1995). The ecological significance of these findings is that detoxification mechanisms in prey organisms may mediate the bioreduction or bioaccumulation of toxic metals along food chains by altering metal bioavailability (Wallace and Lopez, 1997).

Furthermore, an important factor governing the accumulation and toxicity of metals in aquatic animals is the physico-chemical form in which the metal is present in the medium (Rainbow, 2002). Free cadmium species account for ca. 90% of the cadmium in the freshwater zone, whereas in marine systems chloro-complexes dominate the speciation distribution (Stumm and Morgan, 1996; Sadiq, 1992). It has been generally accepted that the toxicity of cadmium to aquatic animals changes as a function of ambient salinity with the metal generally being more toxic at low salinities. The effect of salinity on cadmium toxicity occurs primarily due to greater complexation of the free cadmium ion (Cd^{2+}) by the conservative ligand Cl^- (Sunda et al., 1978; De Lisle and Roberts, 1988). Study on the permeability of cadmium through lipid bilayer membranes suggested that cadmium transport and toxicity were protein mediated and correlated with Cd^{2+}, not $CdCl_2$, concentration (Gutknecht, 1983).

Next to physico-chemical factors, physiological factors modulate the response of organisms to metal challenge. For example, in a study with the euryhaline crustacean *Mysidopsis bahia*, the isosmotic point coincides with the salinity at which maximum tolerance to cadmium was observed (De Lisle and Roberts, 1988). For the euryhaline crustaceans *Orchestia gammarellus*, *Carcinus maenas* and *Necora puber* it is reported that decreased salinity is associated with reduced cadmium uptake (Rainbow et al., 1993; Rainbow and Black, 2005). This physiological response may include reductions in apparent water permeability with reduced salinities. Such physiological effects may be restricted to euryhaline organisms as opposed to aquatic invertebrates in general.

Neomysis integer used in this study is one of the most common mysids inhabiting estuaries along the European coasts and it has been shown to be sensitive to many toxicants at environmentally relevant concentrations (Roast et al., 2001; Verslycke et al., 2003; Wildgust and Jones, 1998). *N. integer* is a hyper- and hypo-osmoregulator with the isosmotic point at approximately 16 psu (De Lisle and Roberts, 1987). Thus, at low salinities it actively maintains its hemolymph hyper-osmotic to the external environment and at high salinities the hemolymph is maintained in a hypo-osmotic state. As a result of the water fluxes associated with hypo- and hyper-osmotic state of an organism, a number of physiological processes (e.g. uptake of major ions via ionic pumps, or excretion of unwanted salts) occur in order to keep the homeostasis of the organism (Rainbow, 1995; Roast et al., 2001). Consequently, the trace metal uptake can be facilitated due to increased activity of ionic pumps when an organism is hyper-osmoregulating. Uptake can also occur via the gut when the organism is hypo-osmoregulating.

This study is aimed at examining metallothionein induction in *N. integer* resulting from water–borne Cd exposure at different salinities. Concurrently, the Cd concentration in the cytosolic fraction was examined, as well as the constituent concentrations of the essential metals zinc and copper. Information on cytosolic metal concentrations enables the assessment of the relationship between cytosolic metals directly responsible for MT induction and the level of MT protein.

Expression of the exposure concentration in terms of Cd^{2+}, rather than total cadmium (Cd_T), reduces the apparent effect of salinity on cadmium toxicity (Engel and Fowler, 1979). In our study we used a similar approach. By using the same Cd^{2+} exposure concentration at different salinities, the effect of salinity on cadmium speciation was eliminated. Therefore, the true effect of salinity as an abiotic factor on Cd accumulation and the MT induction could be studied.

2. Materials and methods

2.1. Animal collection and maintenance

N. integer was collected by hand net (about 2500 animals were sampled) from the dock B3 in the harbour of Antwerp (Belgium). Dock B3, situated on the right bank of the river Scheldt, is connected to the river through the Berendrecht and Zandvliet sluices. Salinity at the sampling location was 5 psu. The animals were transported to the laboratory in 15 L buckets containing ambient water within 2 h after sampling.

In the laboratory the organisms were transferred to 200 L glass aquaria. Culture medium was artificial seawater (Instant Ocean®, Aquarium Systems, France) diluted with aerated deionized tap water to a final salinity of 5 psu. Water temperature was maintained at 15 ± 1 °C, and 12 h light:12 h dark photoperiod was used during culturing. Animals were fed *ad libitum* daily with 24–48 h old *Artemia* nauplii. Hatching of the *Artemia* cysts was performed in 1 L conical vessels under vigorous aeration and continuous illumination at 25 °C (Sorgeloos et al., 1986).

2.2. Exposure experiment

The experiments were performed at three different salinities: 5 psu (lower osmotic pressure than haemolymph