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Chemosphere 64 (2006) 121–128

CHEMOSPHERE

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Metallothioneins induction and antioxidative response in aquatic worms *Tubifex tubifex* (Oligochaeta, Tubificidae) exposed to copper

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Received 11 May 2005; received in revised form 20 October 2005; accepted 24 October 2005
Available online 5 December 2005

Abstract

Metallothioneins (MTs), are low molecular weight proteins, mainly implicated in metal ion detoxification. Increase in MT contents is considered as a specific biomarker of metal exposure. Recently it has been demonstrated that MTs participate in several cellular functions such as regulation of growth, and antioxidative defences. *Tubifex tubifex* were exposed to different copper concentrations (50, 100, and 200 $\mu g\,l^{-1}$) for 7 and 15 days. MT levels in exposed worms increased significantly ($p < 0.05$) after 7 and 15 days of exposure to different concentrations of copper (maximum +208% for 100 $\mu g\,l^{-1}$ after 7 days of exposure). Also important perturbation in metal–metallothionein content occurred, along with an increase in total soluble protein content in all treated worms after 7 and 15 days (max. +88.49%). Catalase activities (CAT) in Cu treated-worms were significantly increased, and demonstrated a development of antioxidative defenses. Additionally a reduction of gulathione-S-transferase (GST) was observed in all treated worms after 7 days of exposure to Cu (max. −44.42%). The high induction of MTs observed during *T. tubifex* exposure to Cu make them potentially useful biomarkers to monitor metal pollution.

Keywords: Biomarkers; Aquatic worms; Oxidative stress; Total soluble protein; Gulathione-S-transferase (GST); Catalase activities (CAT); Metallothioneins

1. Introduction

Copper pollution appears in the aquatic environment from natural and anthropogenic sources such as mine washing or agricultural leaching. Although Cu is a trace element essential to life it is also one of the most toxic heavy metals (Tóth et al., 1996). In Champagne (France) copper had been used on vines as a fungicide for over 100 years, consequently it represents the major metallic contaminant in underground and surface waters. Copper concentration in retention basin collecting run off water from Champagne's vineyards can reach 400 $mg\,l^{-1}$ in the sediment and 120 $\mu g\,l^{-1}$ in the water (Reuil sur Marne, Marne, France) (G.E.R.B.E., 1998). The quality of aquatic ecosystems must often be monitored to assess ecotoxicological impact of pollution, and this requires methods simple and reliable (Lagadic et al., 1997).

Tubifex tubifex (Oligochaete, Tubificidae) is an aquatic oligochaete known to be very resistant to pollution and often the last to disappear from a contaminated site (Lucan-Bouché et al., 1999). Several characteristics of Tubificidae make them potentially useful test organisms for sediment bioassays. They are widely distributed, frequently dominating the macrobiotic community in freshwater habitats. Furthermore, they dwell in the sediment, borrowing and ingesting large volumes of sediment for feeding.

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0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.10.045

Worms are exposed to contamination both through interstitial water and through contact with sediment particles. Tubificidae also play a major role in bioturbation and in decomposition of organic matter. Although *T. tubifex* has been proposed as a test organism for ecotoxicology studies site (Lucan-Bouché et al., 1999).

Metallothioneins (MTs) are cysteine-rich, heat-stable, metal-binding, low molecular mass proteins which play a major role in the homeostasis of essential metals (such as Zn and Cu) and the detoxification of heavy metals including copper, and cadmium (Viarengo, 1989; Viarengo and Nott, 1993; Palumaa et al., 2005). High metal concentration in cell induce an increase in metallothionein concentration. The potentiality to use MTs as a biomarkers of metal contamination has been evaluated by several authors for different animal species (Cherian and Goyer, 1978; Hamer, 1986; Viarengo, 1989; Bebianno et al., 1993). Since MTs were first described (Margoshes and Vallée, 1957), their structure, physico-chemical properties and chemical characteristics have been investigated in most animal families (Kägi and Kojima, 1987). However, from a physiological point a view, clarification of their different biological roles in organisms remains a challenge (Davies and Cousins, 2000). It had been demonstrated that MTs participated directly to antioxidative defences and can be induced by some reactive species of oxygen (Davies and Cousins, 2000). Antioxidant systems have been studied for some years in fish, bivalves and Oligochaeta like earthworms and aquatic worms (*T. tubifex*) exposed experimentally to chemicals or collected from polluted areas (Di Giulio et al., 1989; Winston and Di Giulio, 1991; Lemaire and Livingstone, 1993; Mosleh et al., 2005a,b). The parameters studied include antioxidant enzymes such as catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR), could be useful as biomarkers reflecting not only exposure to contaminants, but also their toxicity. It is now well known that numerous pesticides can induce an increase in reactive oxygen species concentrations in cells and consequently a development of antioxidative defenses such as CAT, GST, and GR (Davies et al., 1994; Cossu et al., 1997; Mosleh et al., 2005a,b). GST is a well-known Phase II detoxification enzyme catalyzing the initial step of mercapturic acid synthesis and the conjugation of glutathione with xenobiotics and their metabolites such as the alkyl transferase and peroxide transferase, detoxifying PAH peroxide produced by P450. However it has been demonstrated that GST participate also in antioxidative defenses (Hajime et al., 2005). CAT is an intracellular antioxidants enzyme involved in defense systems against the radicals generated by the ambient oxidative pollutants. CAT is a peroxisomal hydroperoxidase that degrades H_2O_2 to H_2O and O_2 (Baumard et al., 1999).

MT-like proteins have been reported in terrestrial earthworms *Eisenia foetida* (Suzuki et al., 1980), aquatic worms: *Lumbriculus variegatus* (Bauer-Hilty et al., 1989) and *T. tubifex* (Gillis et al., 2002, 2004; Mosleh et al., 2004, 2005a,b). Mosleh et al. (2005a,b) found that the exposure of *T. tubifex* to the some pesticides increased MTs levels in correlation to increase of antioxidative system in this worms. However very few studies concern the used of MTs or antioxidative systems as biomarkers of pollution in Tubificidae.

The aim of this study was to determine the effects of sublethal concentrations of the copper on MT content in the aquatic oligochaete *T. tubifex*. Total soluble protein contents and some antioxidative defenses (catalase, glutathione reductase, and glutathione-S-transferase) were studied to determine the toxic impact of the copper. Antioxidative defences were correlated with MT induction.

2. Materials and methods

2.1. Origin of the worms, culture maintenance and contamination with Cu

T. tubifex worms were collected from a site near Cormicy sur Marne (Marne, France). This site is a retention basin that receives and confines runoff water from a vineyard. Sediment samples containing *T. tubifex* were collected and brought back to the laboratory. The worms were carefully extracted and washed several times with tap water and then with a regional spring water (Source des Grands Bois, Fismes, France, Ca^{2+}: 124 mg l^{-1}; Mg^{2+}: 25 mg l^{-1}; Na^{+}: 6 mg l^{-1}; K^{+}: 2 mg l^{-1}; HCO_3^{-}: 399 mg l^{-1}, Cl^{-}: 22 mg l^{-1}; SO_4^{2-}: 80; NO_3: 0.5 mg l^{-1}; hardness: 300 ± 10 mg l^{-1} $CaCO_3$; pH: 7 ± 0.1). *T. tubifex* cultures made using an artificial sediment based on OECD Guideline 207 (OECD, 1984; Egeler et al., 1997). The water was continuously aerated and worms were held for 6 month before experiments. Worms were fed with TetraMin flakes (Tetra Werke, Melle, Germany) once a week. To assess the effects of the copper, the worms were exposed to various concentrations of copper as $CuSO_4$, $5H_2O$ (Sigma, Saint-Quentin Fallavier, France). The worms (approximately 1.5 g FW) were placed in crystallizing dishes ($\varnothing = 8$ cm) containing spring water and without sediment. $CuSO_4$ was added and the final concentration was 50, 100, and 200 μg Cu l^{-1}. The dishes were then incubated in the growth chambers as above for 7 and 15 days. Three dishes were prepared for each concentration and each duration. After incubation, animals from each dish were weighed before the three replicates were pooled together and frozen (−80 °C) for 7 days until analysis was performed.

2.2. Metallothionein analysis

For each treatment approximately 1 g of the frozen animals were homogenized at 4 °C in 2 ml of 2 mM Tris–HCl, pH 8, containing 20% glycerol, 2 mM mercaptoethanol, 6 μM leupeptin, and 0.5 mM phenyl methyl sulphonyl-fluoride (PMSF). The homogenate was then centrifuged at 18 000*g* for 90 min at 4 °C. The upper fatty layer was discarded and the supernatant was taken. Supernatant was heat-treated at 91 °C for 2 min immediately cooled in ice