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Antioxidant responses in the nereidid *Laeonereis acuta* (Annelida, Polychaeta) after cadmium exposure

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Abstract

The objective of this study was to evaluate the effects of the exposure to cadmium on the antioxidant responses in the polychaeta *Laeonereis acuta*. The worms were submitted to 0, 5, and 100 μg of Cd/L during a period of test of 7 days. Cadmium was significantly ($p<0.05$) accumulated in *L. acuta* in both concentrations assayed, but the concentration of reactive oxygen and nitrogen species (RONS) increased ($p<0.05$) only in the group submitted to the highest concentration of cadmium (100 μg/L). At this concentration, a decrease in the activity of the superoxide dismutase and an increase of glutathione-*S*-transferase activity ($p<0.05$) was observed. The levels of both lipid peroxides and the activities of catalase and glutathione peroxidase were not affected ($p>0.05$) by the exposition to cadmium. Thus, cadmium can augment RONS levels and can interfere with the antioxidant defense system of the polychaete *L. acuta*, although cadmium does not directly induce oxidative stress unlike copper and iron.

Keywords: Cadmium; Polychaeta; Reactive oxygen and nitrogen species; Antioxidant defense

1. Introduction

Annelids are commonly used in toxicological studies (Klerks and Bartholomew, 1991; Marcano et al., 1996; Lucan-Bouché et al., 1999). In the group of the polychaeta, many species seem to exhibit an extraordinary tolerance to various environmental contaminants, being also the most common invertebrates found in polluted areas (Eriksen et al., 1988). The nereidid polychaete *Laeonereis acuta* is a common infaunal species in the Patos Lagoon estuary (Southern Brazil), being, according to Pagliosa and Barbosa (2006), one of the dominant species in urbanized rivers of Southern Brazil. It is characterized as a selective deposit feeder and, therefore, lives in close contact with the sediment (Bemvenuti, 1998). In addition, this species has been used as a model in toxicological assays, which have showed alterations in the antioxidant defense system of *L. acuta* after it has been exposed to pollutants such as cadmium, copper, and hydrogen peroxide (Geracitano et al., 2004; Rosa et al., 2005; Sandrini et al., 2006).

Cadmium is a non-essential metal, which can be found in soils, sediments, air, and water as a result of industrial activities (Waisberg et al., 2003). In humans, this metal is accumulated primarily in the liver and in the kidney and it is, clearly, a potent multi-tissue animal carcinogen (Waalkes, 2000). The mechanisms responsible for the toxicity of cadmium are not well understood. Some studies have been published linking the toxicity of cadmium to the generation of oxidative stress (Stohs and Bagchi, 1995; Sandrini et al., 2006). However, cadmium is not a redox active metal, unlike copper and iron, and it cannot generate reactive oxygen species (ROS) through Harber–Weiss and

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Fenton reactions. Nevertheless, many authors have reported an increase in the oxidative damage to macromolecules after their exposition to cadmium (Sarkar et al., 1998; Liu and Jan, 2000). According to Wang et al. (2004), cadmium can inhibit the electron transfer chain in the mitochondria, which may increase its ROS generation. Alternatively, many studies have attributed the generation of ROS, after cadmium exposure, to the interference of cadmium in the cellular antioxidant defense system. Cadmium can decrease the activity of antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which would increase the ROS concentration in the cells (Stohs and Bagchi, 1995; Waisberg et al., 2003). In addition, some authors have attributed the toxic effects of cadmium to a decrease in the cellular reduced glutathione (GSH) and a concomitant increase in the oxidized glutathione (GSSG), altering the redox state of the cell (Pourahmad and O'Brien, 2000).

Within this context, the objective of the present study was to analyze the effects of the exposure of the polychaeta *L. acuta* to cadmium evaluating the ROS generation, the content of lipid peroxide (LPO) and the activities of the antioxidant enzymes (CAT, GPx, glutathione-*S*-transferase (GST), and SOD).

2. Materials and methods

2.1. Animals and exposure conditions

The worms (~250 mg) were collected from a reference site, which was previously classified ('Saco do Justino'; 32°05′S–52°12′W) inside the Patos Lagoon (Southern Brazil), as it was done before by Geracitano et al. (2004). In the laboratory, they were individually maintained (6 days) in glass dishes (6.0 cm diameter) with sand and water at 10‰ (pH 8.00; 20 °C), which were also collected from the reference site. After this acclimation period, worms were transferred into dishes without sand, where they were maintained for another 4 days before the beginning of the assays. Over the whole period, on alternate days, the worms were fed with frozen *Artemia salina* and the water was 100% replaced. Photoperiod was fixed at 12L:12D.

During the assay, worms were exposed to either 5 or 100 μg of Cd/L (as $CdCl_2$; Merck) for the period of 7 days. The concentration of 100 μg of Cd/L was chosen based on a previous work (Sandrini et al., 2006) and the concentration of 5 μg of Cd/L was the one considered safe for the preservation of aquatic fauna by the previous Brazilian legislation (Conselho Nacional do Meio Ambiente, CONAMA; resolution number 20, 07/30/1986; www.mma.gov.br/port/conama/res/res86/res2086.html). A control group was kept in saline water (10‰ at 20 °C and pH 8.0) without cadmium. During the exposure period, the worms were fed as described above. After 1 week, the worms were sacrificed and stored at −80 °C for biochemical and cadmium analysis. ROS concentration was measured immediately after the end of the experiment (see next section).

2.2. Cadmium analysis

The concentrations of cadmium were measured in the organisms as a whole, dried at 60 °C until constant weight was obtained and then digested, under pressure, with nitric acid and hydrogen peroxide (5:1 v/v) in a microwave digester system (CEM Mars 5, CEM Corporation, Matthews, NC, USA). Quality assurance and quality control were tested by processing blank samples and reference standard materials (Lyophilized Mussel Tissue Standard Reference Material SRM 2977; National Institute of Standards and Technology NIST, Gaithersburg, MD, USA). The cadmium was analyzed by atomic absorption spectrophotometry with electrothermal atomization (SpectrAA 300 Zeeman; Varian, Mulgrave, VIC, Australia). The concentrations of cadmium measured in the standard reference material were always within the 95% interval of confidence of certified values. The content of cadmium was referred to the dry weight of the worms ($n = 3$ for each treatment).

2.3. Assessment of reactive oxygen and nitrogen species (RONS) concentration

Fresh whole worms were homogenized (1:4, w/v) in phosphate buffer 50 mM, pH 7.5, plus NaCl (2.5%, w/v). Homogenates were centrifuged at 20,000*g* for 20 min (4 °C), and the supernatant obtained was used in the RONS assay. Before this, the total protein content of the supernatant was measured in triplicate, at 550 nm, using a commercial reagent kit (Doles Ltd., Brazil) based on the Biuret method. The assessment of RONS formation was done after the incubation of the supernatants (140 μg of protein) for 30 min, at 25 °C with 2′,7′-dichlorofluorescin diacetate (H_2DCF-DA, Molecular Probes) at a final concentration of H_2DCF-DA of 40 μM, according to the methodology employed by Viarengo et al. (1999). The acetates groups of H_2DCF-DA are cleaved by intracellular esterases and the non-fluorescent compound generated, H_2DCF, is oxidized by both ROS (particularly the hydroxyl radical, $HO^{\bullet}$) and reactive nitrogen species (particularly peroxynitrite, $ONOO^{-}$) present in the samples of the fluorescent compound, DCF (Myhre et al., 2003). The fluorescence intensity was determined using a fluorometer (Victor 2, Perkin Elmer), with an excitation and emission wavelength of 485 and 520 nm, respectively. Background fluorescence was determined before the addition of H_2DCF-DA. Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time of the measurement, after adjusting FU data to a second-order polynomial function (see Fig. 1a), the results were expressed as area of FU × min. Five–six organisms were measured per treatment.

2.4. Enzyme assays

For the antioxidant enzymes, whole worms (four–six animals per group) were homogenized (1:4, w/v) in phosphate buffer 50 mM, pH 7.5, plus NaCl (2.5%, w/v) and in a protease inhibitor cocktail (P2714; 1:200; Sigma). Homogenates were centrifuged at 500*g* for 15 min (4 °C) and the supernatant obtained was centrifuged at 12,000*g* for 30 min (4 °C). The supernatant of the second centrifugation was stored at −80 °C. Total protein content in the supernatant was measured as described above (Section 2.2). The activities of SOD, CAT, GPx, and GST were measured according to McCord and Fridovich (1969), Beutler (1975), Arun and Subramanian (1998), and Habig et al. (1974), respectively. All the methodologies applied spectrophotometric detection at 240 (CAT), 550 (SOD) or 340 nm (GPx and GST). The assays were performed, at least, in duplicate and the results were expressed in enzyme units. One CAT unit represents the amount of enzyme needed to degrade 1 μmol of H_2O_2 per min and per mg of proteins at 30 °C and pH 8.0. One SOD unit is defined as the amount of enzyme needed to inhibit 50% of cytochrome *c* (Sigma) reduction after superoxide anion generation (hypoxanthine/xanthine oxidase) per min and per mg of total proteins at 25 °C and pH 7.8. One GPx unit represents the amount of enzyme necessary to oxidize 1 μmol of NADPH (Sigma) per minute and per mg of protein, at 30 °C, pH 7.2, and 1 mM of sodium azide (to inhibit CAT activity) Finally, one GST unit represents the enzyme amount needed to conjugate 1 μmol of 1-chloro-2,4-dinitrobenzene (Sigma) per min and mg of total proteins at 25 °C and pH 7.0. Five worms were analyzed per treatment.

2.5. LPO determination

Oxidative stress damage was measured in terms of LPO, according to Hermes-Lima et al. (1995), and adapted to microplate reader for