Comparative Biochemistry and Physiology, Part C 149 (2009) 281–288

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Multi-biomarker responses in the freshwater mussel *Dreissena polymorpha* exposed to polychlorobiphenyls and metals

Melissa Faria [a], Luis Carrasco [b], Sergi Diez [b,c], Maria Carmen Riva [a], Josep Maria Bayona [b], Carlos Barata [b,*]

[a] Laboratory of Environmental Toxicology (UPC), CN 150 Km 14.5, Terrassa 08220, Spain
[b] Department of Environmental Chemistry, IDAEA, (CSIC), Jordi Girona 18, Barcelona 08034, Spain
[c] Environmental Geology Department, ICTJA-CSIC, Lluis Solé i Sabarís, s/n, Barcelona 08028, Spain

ARTICLE INFO

Article history:
Received 20 June 2008
Received in revised form 28 July 2008
Accepted 29 July 2008
Available online 3 August 2008

Keywords:
Oxidative stress
Dreissena polymorpha
Methyl mercury
Mercury
PCB
Cadmium
EROD

ABSTRACT

Contaminant related changes in behavioral, phase I and II metabolizing enzymes and pro-oxidant/antioxidant processes in the freshwater mussels *Dreissena polymorpha* exposed to metals and PCBs were assessed. Behavioral and biochemical responses including filtering rates, key phase I, II and antioxidant enzymes and levels of metallothioneins, glutathione, lipid peroxidation and DNA strand breaks were determined in digestive glands of mussels after being exposed to sublethal levels of mercury chloride, methyl mercury, cadmium and Aroclor 1260 during 5 days. In 7 out of 12 responses analyzed, mussels showed significant differences across treatments. Unusual properties of measured ethoxyresorufin-*O*-deethylase (EROD) activities indicated that mussels lack an inducible CYP1A enzymatic activity. Despite of using similar exposure levels, inorganic and organic mercury showed different biomarker patterns of response with methyl mercury being more bio-available and unable to induce metallothionein proteins. Mussels exposed to Cd presented higher levels of metallothioneins and an enhanced metabolism of glutathione, whereas those exposed to Aroclor showed their antioxidant glutathione peroxidase related enzyme activities inhibited. Although there was evidence for increased lipid peroxidation under exposure to inorganic and organic mercury, only mussels exposed to Aroclor had significant greater levels than those in controls.

1. Introduction

Aquatic organisms are currently being exposed to multiple chemical contaminants with different mechanisms of toxicity, each contributing to a final overall adverse effect. Consequently, in ecological quality monitoring programs, the integration of chemical data with biological responses (biomarkers) is strongly recommended to characterize effects of contaminants to organisms (den Besten, 1998; Clements, 2000). Biomarkers can offer a more complete and biologically more relevant information on the potential impact of toxic pollutants on an organism's health (van der Oost et al., 2003). Furthermore, the use of a large set of biochemical responses may allow us to identify potential hazardous contaminants in the field. This approach has been successfully used mostly in fish and bivalve marine mollusks (see reviews of Di Giulio et al., 1995; Livingstone, 2001; van der Oost et al., 2003) and include biochemical response that are related with the metabolism and toxicity modes of action of contaminants.

Due to its abundance in many European and American fluvial habitats, its relative long life span and great ability to bio-concentrate toxic chemicals, the freshwater bivalve zebra mussel (*Dreissena polymorpha*) has been used extensively as sentinel species to monitor persistent organic contaminants and metals (Kraak et al., 1991; De Lafontaine et al., 2000; Binelli et al., 2001; Camusso et al., 2001; Berny et al., 2002). More recently, the use of biochemical responses of *D. polymorpha* has also allowed to detect specific biological response to particular contaminants or environmental pressures (De Lafontaine et al., 2000; Lecoeur et al., 2004; Minier et al., 2006; Binelli et al., 2005, 2006a,b; Giamberini and Cajaraville, 2005; Ricciardi et al., 2006; Voets et al., 2006; Marie et al., 2006; Osman et al., 2007; Guerlet et al., 2007; Binelli et al., 2007, 2008). Nevertheless, the above mentioned studies have been focused in most occasions on just a few biochemical responses and restricted to few contaminant substances, thus their use as diagnosis toxicity tools of field populations exposed to multiple pollutants is limited and can be misleading. Indeed contradictory results exist within zebra mussel biomarker studies and between them and those conducted with other related bivalve species. For example in laboratory exposures, Lecoeur et al. (2004) reported that in zebra mussel metallothionein proteins were induced by Cd but not by Cu, whereas in an early field work De Lafontaine et al. (2000) found a positive relationship between metallothionein levels and Cu. Moreover, De Lafontaine et al. (2000) and Binelli et al. (2005, 2006a,b; 2007) reported measurable and inducible CYP1A-ethoxyresorufin-*O*-deethylase (EROD) like activity in zebra mussels collected from polluted sites or exposed in the laboratory to polychlorobiphenyls (PCBs), polybrominated diphenyl

* Corresponding author. Tel.: +34 93 4006100; fax: +34 93 2045904.
E-mail address: cbmqam@cid.csic.es (C. Barata).

1532-0456/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2008.07.012

ethers (PBDEs), dichlorodiphenylthrichlor-oethanes (DDTs) and hexachlorobenzene, whereas in other bivalve species this activity was seldom detected (Livingstone et al., 1990a; Porte et al., 1995, 2001; Solé and Livingstone, 2005).

The aim of this study is to use a large set of biomarkers to identify specific and distinctive patterns of responses of *D. polymorpha* individuals to known contaminants. The biomarkers included the xenobiotic metabolizing phase I and II enzyme responses EROD and glutathione *S*-transferase, respectively (van der Oost et al., 2003); glutathione (GSH) levels and glutathione reductase, which aids maintenance of GSH levels recycling oxidized glutathione (Canesi et al., 1999); metallothionein proteins as a marker for metal exposure (Amiard et al., 2006); antioxidant enzymes involved in detoxifying reactive oxygen species such as superoxide dismutase (SOD EC 1.15.1.1 converts $O_2^{\cdot-}$ to H_2O_2), catalase (CAT EC 1.11.1.6—reduces H_2O_2 to water), glutathione peroxidase (GPX EC 1.11.1.9—detoxifies H_2O_2 or organic hydroperoxides) and markers of oxidative tissue damage (lipid peroxidation and DNA strand breaks) (Halliwell and Gutteridge, 1999). The selected contaminants included the metals cadmium, mercury in its inorganic and organic forms, which are known to alter GSH and metallothionein levels in mussels (Canesi et al., 1999; De Lafontaine et al., 2000; Lecoeur et al., 2004) and a PCB mixtures, Aroclor 1260, which has been reported to induce EROD activity in zebra mussel (Binelli et al., 2006a). The chosen contaminants may co-occur together in large amounts in heavy industrialized rivers and lakes and are susceptible to be bioaccumulated by mussels (Mikac et al., 1985; Rutzke et el., 2000; Hanari et al., 2004). Furthermore, by including different forms of mercury it will be possible to test if metal speciation matters. So far in common (*Mytilus galloprovincialis*) there is evidence that mercury speciation affected differently glutathione metabolism (Canesi et al., 1999), but there is little or no information in other mussel species nor on other biomarkers (Diez et al., 2008).

2. Materials and methods

2.1. Chemicals

The following chemicals were used for laboratory exposures: Aroclor 1260 (technical PCB mixture, Supelco, Bellefonte, USA), mercury chloride ($HgCl_2$; 99% purity, Sigma-Aldrich), methyl mercury chloride salt (CH_3ClHg, Pestanal-Riedel-de Haën, Sigma-Aldrich, Seelze, Germany) and cadmium ($CdCl_2$, 99% purity, Sigma-Aldrich).

Homogenization along with enzymatic activities, lipid peroxidation, DNA damage and protein assays was preformed with dithiothreitol (DTT); phenylmethanesulfonly fluoride (PMSF); ethylenediamine-tetra-acetic acid, disodium, salt, dihydrate (EDTA); hydrogen peroxide (H_2O_2); xanthine oxidase (EC 1.2.4.22); superoxide dismutase (EC 1.15.1.1); xanthine; β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt (NADPH); oxidized glutathione (GSSG); reduced glutathione (GSH); cumene hydroperoxide (CHP); sodium azide, 1-chloro-2,4-dinitrobenzene (CDNB); glutathione *S*-transferase (EC 2.5.1.18); monochlorobimane (mCB); resorufin ethyl ether (7-ethoxyresorufin); resorufin sodium salt; sodium hydroxide, 2,6-di-*tert*-butyl-4-methylphenol (BHT); 1-methyl-2-phenylindole; 1,1,3,3-tetramethoxypropane (TMP); sodium dodecyl sulfate (SDS); bisBenzimide H 33258 (Hoescht dye); deoxyribonucleic acid sodium salt from calf thymus, type 1, fibres and γ-globulins from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade and were obtained from Merck (Darmstadt, Germany).

2.2. Experimental animals and culture conditions

Following Binelli et al. (2006a), zebra mussels (*D. polymorpha*, Bivalvia: Dreissenidae) 2 cm long (shell length) tied on rocks, were collected from Riba-Roja reservoir in May 2006 by a scuba diver at 3–5 m depth and transported to the lab. Riba-Roja reservoir is localized in Ebro River, NE Spain and can be considered a reference site with low pollution levels (http://oph.chebro.es/DOCUMENTACION/Calidad/CalidadDeAguas.html). Mussels attached to rocks were rinsed and introduced into glass aquaria at a density of 0.5 L per individual (approx.) and maintained under constant oxygenation >90%, temperature (20 °C) and photoperiod (14 h; 10 h; light:dark). Animals were cultured in local field collected water, which was progressively replaced by artificial ASTM hard water, and fed with a suspension 1:1 of algae *Scenedesmus subspicatus* and *Chlorella vulgaris* (10^6 cells/mL, daily). The medium was renewed every other day for 10 days to allow the acclimatization of animals. After this period, 300 mussels with similar length (2 cm long) were selected for the experiments. They were gently cut off from rocks, placed on sheets of glass suspended in glass 30 L aquaria filled with of 20 L medium and maintained 5 days further in the same conditions described above, but exposed to the different treatments. Only specimens able to re-attach themselves by the byssus on the sheet glass were used in the experiments, the test medium was changed daily and mussels were fed adding food only 2 h before water renewal.

2.3. Experimental design

Five different treatments were performed: control (no exposure); Aroclor 1260 (150 ng/L); mercury chloride (40 μg/L total ion Hg), methyl mercury chloride (40 μg/L total ion Hg) and cadmium (34 μg/L total ion Cd). Exposure levels were selected since are known to alter biomarker responses in mussels (i.e. metallothioneins, glutathione metabolisms and EROD activities; Canesi et al., 1999; Lecoeur et al., 2004; Binelli et al., 2006a). Stock solutions of cadmium and inorganic mercury (expressed as total ion concentrations) were prepared by adding analytical reagent grade salts to 2 L deionized water (Milli-Q; 18 MΩ cm^{-1} resistivity) and sonicated during 1 h. Nominal test concentrations were subsequently prepared by adding aliquots of each metal stock solution to the aquaria filled with 20 L of ASTM. Concentrated (20×) aqueous solutions of methyl mercury and PCB mixtures were prepared by adding appropriate amounts in acetone (HPLC grade; <0.5 mL/L) to a borosilicate glass 2 L bottle container and allowing the acetone to completely evaporate with a stream of N_2, leaving behind a crystalline residue on the glass. Then the glass container was filled with the appropriate volume of ASTM hard water, sonicated during 1 h and mixed at 20 °C on an orbital shaker for 1 day. By using this procedure organic contaminants are dissolved directly in water without the aid of a carrier and hence the use of a solvent control is not needed (Barata and Baird, 2000). Nominal test concentrations were then subsequently prepared by adding the whole content of the solutions to the 20 L tanks pre-filled with 18 L of ASTM hard water to provide a total volume of 20 L.

2.4. Chemical analyses

The analysis of contaminants were restricted to their tissue levels in whole mussel samples collected at the end of exposures using groups of four to ten organisms. Levels of Cd were determined following the methods of Barata et al. (2005). Freeze-dried organisms were digested in concentrated nitric acid and hydrogen peroxide using Teflon bombs at 90 °C overnight. Within each digestion series, appropriate blanks with no animals and samples of similar weight of a certified reference material (lobster hepatopancreas, Tor 1, National Council of Canada, Ottawa) were also subject to the same procedure to account for background contamination levels and to validate the entire procedure. Cooled digested samples were diluted to a standard volume with deionized water. Trace Cd analyses were determined using a Perkin Elmer model Elan 6000 inductively coupled plasma mass spectrometer (ICP-MS). Calibration standards and a reagent blank were analyzed with every ten samples to monitor signal drift. In every instance, the signal typically changed by 3–5% throughout an analytical run. Additionally,