Chemosphere 168 (2017) 860–869

Contents lists available at ScienceDirect

Chemosphere

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

Metabolomic responses of *Haliotis diversicolor* to organotin compounds

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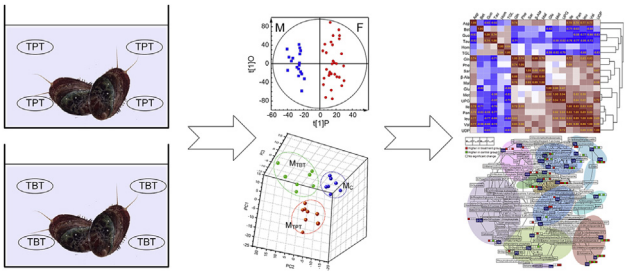
HIGHLIGHTS

- Metabolic responses of abalone induced by tributyltin/triphenyltin were studied.
- Obvious gender-, tissue- and compound-specific responses were found.
- Tributyltin/triphenyltin disturbs energy metabolism and osmotic regulation.
- Immune and oxidative stress was induced by tributyltin/triphenyltin exposure.
- Metabolomics is useful to elucidate organotin compound-induced toxic effects.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form
24 September 2016
Accepted 29 October 2016
Available online 10 November 2016

Handling Editor: Caroline Gaus

Keywords:
Metabolomics
Haliotis diversicolor
Hepatopancreas
Gill
Organotin compounds

ABSTRACT

Organotin compounds, especially tributyltin (TBT) and triphenyltin (TPT), are a group of hazardous pollutants in marine environments. *Haliotis diversicolor* is an important marine model organism for environmental science. In this study, ^{1}H NMR spectroscopy together with pattern recognition methods was used to investigate the responses of hepatopancreas and gill of *Haliotis diversicolor* to TBT and TPT exposure. It was found that obvious gender-, tissue- and compound-specific metabolomic alterations were induced after a 28-day exposure. TBT and TPT exposure not only caused the disturbance in energy metabolism and osmotic balance in hepatopancreas and gill tissues with different mechanisms, but also induced oxidative stresses. These metabolic alterations were highlighted in the accumulation of aspartate, uridine diphosphate-N-acetylglucosamine, uridine diphosphate glucose, guanosine and the depletion of leucine, isoleucine, valine, malonate, homarine, trigonelline in all exposure gills, as well as in the depletion of ATP, AMP, betaine in male exposure gills and pantothenate in male exposure hepatopancreases. The significant decreased aromatic amino acids (AAAs), lysine and glutamate in gills and increased betaine in hepatopancreases for TPT exposure together with increased glutamate and decreased betaine in gills and increased glutamate and glycine in hepatopancreases for TBT exposure demonstrated their specific metabolic characteristics. Among these characteristic metabolites, AAAs, lysine and glutamate in the gill as well as pantothenate in the hepatopancreas might be identified as

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http://dx.doi.org/10.1016/j.chemosphere.2016.10.124

potential biomarkers for TPT or TBT exposure in *Haliotis diversicolor*. The results provide a useful insight into the toxicological mechanisms of organotin compounds on *Haliotis diversicolor*.

1. Introduction

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been extensively used as biofungicides, wood preservatives and antifouling agents in paints applied to ships since the 1960s. Because of their highly toxic effects on many target and non-target aquatic life, the International Maritime Organization (IMO) imposed a worldwide ban on the application of organotin compounds as antifouling agents for ships from 2008. However, many developing countries or regions of the IMO member states, such as India, South East Asia, and China, have not issued any national laws on organotin pollution. The levels of TBT in China's coastal environments range from below the detection limit to 976.9 ng (Sn)/L (Cao et al., 2009; Jiang et al., 2001). Although organotin compounds readily degrade with UV radiation, they are persistence in anaerobic sediment, lasting decades (Antizar-Ladislao, 2008). Given the persistence of organotin chemicals in the sediment it is likely their presence affects the health of benthic organisms (Yu et al., 2013).

TBT is one of the most toxic chemicals released into marine environments. It was well documented that TBT could lead to a series of adverse effects in marine invertebrates, including imposex (Horiguchi et al., 1997, 2002; Swennen et al., 2009), endocrine disruption (Inoue et al., 2006; Siah et al., 2003), neurotoxicity (Oberdörster and McClellan-Green, 2002), apoptosis induction and cytogenetic damage (Cima et al., 1999; Hagger et al., 2002; Jha et al., 2000). However, the toxicological mechanisms of TBT remained elusive. Although TPT and TBT often co-exist in the environment, fewer studies focus on the toxicity of TPT, as the input of TBT as an antifouling chemical is higher than that of TPT. TPT, which has been used as fungal biocide since the 1960s, can enter rivers after rain as agricultural runoff (Champ, 2000). In addition, since TPT based paints are cheaper, fishermen and aquaculturists have been starting to use TPT based paints as opposed to TBT based ones in recent years. An increasing number of studies have reported unexpectedly high levels of TPT in fishes (from 132.4 to 485.7 ng (Sn)/g wet weight) around the world (Yi et al., 2012). Consequently, research on the biological effects of TBT and TPT remains a necessity.

Haliotis diversicolor is one of the most commercially important cultured abalones in southern coastal areas of China. As a typical marine benthic gastropod, abalones are highly sensitive to environmental stresses, so they are ideal bio-indicators of the health of marine benthic environment (Jia et al., 2009). In gastropod mollusks, the hepatopancreas, as a metabolic organ, is the main defense organ against the external environmental stresses (Wu et al., 2010). The gill, mainly involved in respiration, osmoregulation, detoxification, immune function and neuronal signaling, is the first tissue to be affected by organotin compounds in the external environment (Cappello et al., 2015). To our knowledge, no studies attempted to compare the gender-specific responses induced by organotin compounds in *H. diversicolor* at metabolite levels. Numerous studies have confirmed the applicability of NMR-based metabolomics to characterize environmental stresses induced biological effects in organisms (Fasulo et al., 2012; Hines et al., 2007; Jones et al., 2008; Lankadurai et al., 2011; Viant et al., 2003). This study was therefore designed to investigate the toxicological effects of TBT and TPT on hepatopancreas and gill tissues of male and female abalones by NMR-based metabolomics and gain an insight into the metabolic mechanism involved.

2. Materials and methods

2.1. Animals handling and sampling

Adult abalones *H. diversicolor* (body length 4.1 ± 0.2 cm, wet weight 7.08 ± 1.27 g) were collected in December 2014 from Peiyang abalone farm (Xiang'an, Xiamen, Fujian Province, China). The abalones were acclimatized in polyethylene tanks (80 cm × 75 cm × 60 cm) with 300 L aerated sand-filtered seawater for 7 days.

After acclimatization, the animals were randomly divided into three groups: solvent control, TPT and TBT groups (each containing 50 females and 50 males). Each group was then exposed either to seawater with 5 μL/L ethanol, 100 ng (Sn)/L TPT or 100 ng (Sn)/L TBT, respectively. Tributyltin chloride (TBT) and triphenyltin chloride (TPT) (purity ≥ 98.0%) were purchased from Wako (Osaka, Japan). The concentrations of TPT and TBT were chosen according to our pre-experiment and other report (Yu et al., 2004), which are within the range of natural concentrations of TPT and TBT in environment (Cao et al., 2009). Since our previous study confirmed that there was no significant metabolomic difference in abalone *H. diversicolor* samples between seawater control group (abalones cultured in the filtered seawater) and solvent (ethanol) control group (unpublished data), no seawater control group was set in this study. Throughout the experiments, abalones were fed with appropriate amount of fresh kelp (*Laminaria japonica*) once a day in the afternoon. About an hour later, the seawater with specific ethanol, TPT or TBT concentration was completely renewed to remove the influence of food debris or fish excreta and ensure a stable exposure concentration. Temperature (24.0–25.0 °C), salinity (35.7–36.3), pH (8.2–8.3) and oxygen saturation (96.9–97.8%) were also measured. After 28 days of exposure, the hepatopancreas and gill tissues were taken from at least nine female and eight male abalones of each group (the limited number is mainly due to the high mortalities of the abalones during the 28-day exposure of TPT and TBT and the uncertain sex confirmation), and frozen in liquid nitrogen and then stored at −80 °C before metabolite extraction. Abalone sex was assessed by observing the color of the reproductive tissue, which appears beige for male and green for female.

2.2. Preprocessing of the biosamples and ^{1}H NMR measurements

Polar metabolites in hepatopancreas and gill tissues were extracted using the methanol/water/chloroform method described previously (Wu et al., 2008) with a slight modification. One-dimensional ^{1}H NMR spectra of all the samples were acquired using the NOESYGPPR1D pulse sequence on a Bruker Avance III 600 MHz spectrometer at 296 K. More details were described in the Supplemental materials.

2.3. Spectral processing and statistical analyses

All ^{1}H NMR spectra of tissue extracts were processed and then converted to a data matrix using MestReNova 8.1.2 software