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The detoxification process, bioaccumulation and damage effect in juvenile white shrimp *Litopenaeus vannamei* exposed to chrysene

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ABSTRACT

This study aimed to evaluate the effect of chrysene (CHR) on detoxification enzymes, bioaccumulation and effect of CHR on biomolecule damage in different organs of the juvenile white shrimp *Litopenaeus vannamei*. In this study, juvenile white shrimp *L. vannamei* were exposed to CHR for 21 days at four different concentrations as 0, 0.3, 2.1 and 14.7 μg/L. Results showed that CHR bioaccumulation increased rapidly at first then reached a plateau. The activities of aryl hydrocarbon hydroxylase (AHH), 7-ethoxyresorufin O-deethylase (EROD), epoxide hydrolase (EH), glutathione-S-transferase (GST), sulfotransferase (SULT) and uridinediphosphate glucuronyltransferase (UGT) were induced and then became stable gradually. Moreover, 2.1 and 14.7 μg/L CHR treatments increased activity of superoxide dismutase (SOD) in gills and hepatopancreas, while total antioxidant capacity (T-AOC) and GSH/GSSG were suppressed after CHR exposure. Additionally, lipid peroxidation (LPO) levels, protein carbonyl (PC) contents and DNA damage were induced throughout the exposure period, and different trends were detected with time of exposure. Overall, these novel findings of CHR bioaccumulation and resulted toxicity demonstrate that CHR could affect the physical status of *L. vannamei*. This study will form a solid basis for a realistic extrapolation scientific data for aquaculture water monitoring and food security.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are persistent hydrophobic organic pollutants ubiquitously found in the environment and they originate mainly from incomplete combustion of organic materials and fossil fuels. As a kind of global persistent organic pollutants (POPs), PAHs have also been included in the group of persistent toxic substances (PTS), and 16 unsubstituted PAHs have been listed as "Priority Pollutants" by the United States Environment Protection Agency (US EPA), due to their well-known carcinogenic and mutagenic properties (Chung et al., 2007).

In 2008, the European Food Safety Authority (EFSA) introduced a system of four specific PAHs, namely, benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF) and chrysene (CHR), assessing that the sum of the four PAH compounds was the most suitable indicator for PAHs in food (EFSA, 2008). In recent years, PAH pollution has been much aggravated by the development of offshore oil and marine traffic, making the concentrations of CHR in the marine environment increasingly higher (Xiu et al., 2014) According to a survey, the CHR contents in surface sediment from Zhanjiang Bay, China, was up to 39.65 ng/g dry weight (Huang et al., 2012).

To the best of our knowledge, the mechanisms of PAHs toxicity to crustaceans have been rarely studied. while the mechanisms of PAHs toxicity concentrated in fishes and bivalves in aquatic animals (Pan et al., 2006; Yin et al., 2007; Liu et al., 2014). The biotransformation of PAHs is a complex process that involves xenobiotic-metabolizing phase I and phase II enzymes. In phase I, PAHs is introduced a functional group (e.g. –OH, –COOH, $–NO_2$) by the multienzymatic system cytochrome P450 (CYP450) (Dam et al., 2008), of which the induction of phase I P450 enzyme, measured as an increase in ethoxyresorufin-O-deethylase (EROD) activities have been widely used as biomarkers (Rewitz et al., 2006). In phase II, the PAHs metabolites conjugated with polar endogenous constituents such as glutathione-S-transferase (GST), to produce water-soluble conjugates that are easily excreted (Rey–Salgueiro et al., 2011). PAHs exerted their toxic effects by either the parent compound or subsequently, during metabolism resulting in production of reactive oxygen species (ROS) (Parrilla–Taylor et al., 2013). Although ROS plays an important role in host defense, overproduction and residuals can cause cellular damage. Most cells have protective mechanisms to balance ROS production and avoid oxidative stress, namely antioxidants. Antioxidant enzyme systems are a well-developed regulatory mechanism scavenging ROS, including non-enzymatic small antioxidant molecules (such as

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reduced glutathione (GSH)) and a cascade of enzymes (such as superoxide dismutase (SOD)) (Blokhina et al., 2003; Nordberg and Arnér, 2001; Valko et al., 2007). SOD is crucial in preventing the formation of lipid peroxidation by catalyzing the disproportionation of the lipid peroxidation initiator and the transformation of superoxide radical (O^{2-}) into H_2O_2 and O_2 (Tao et al., 2013). The enzyme plays an important role in protecting organisms from oxidative stress. GSH is reported to act as an effective antioxidant in marine animals and as a reactant in conjugation with electrophilic substances. Thus, changes in GSH levels are useful indicators of the detoxification ability of marine animals (Hannam et al., 2009). Overwhelming of the antioxidant capacity can result in mass oxidation of GSH leading to excretion of the oxidized molecule (GSSG) from the cell resulting in a reduced intracellular concentration of total glutathione (Hannam et al., 2010).

Exposure to some organic contaminants may result in cellular oxidative stress due to redox cycling and disruption of mitochondrial membranes. Organisms can adapt to increasing ROS production by up-regulating antioxidant defenses, such as the activities of antioxidant enzymes (Livingstone, 2001). But failure in antioxidant defense to detoxify excess ROS production can lead to significant oxidative damage including protein degradation, DNA damage and lipid peroxidation (LPO). Moreover, biotransformation processes not only affect residual contaminant body burden but can also alter the toxicity of certain chemicals (e.g. certain PAH compounds such as BaP), for which metabolites can be more toxic than the parent compound (Buhler and Williams, 1988; Lech and Bend, 1980), and these reactions might bring about the synthesis of more reactive molecules that can interact with the genetic material and cause DNA damage and protein carbonyls.

Litopenaeus vannamei is a tropical species that has been widely cultured in extensive, intensive and semi-intensive systems, and it is the most popular shrimp for aquaculture in America, Thailand and China (Hou et al., 2014; Yang et al., 2010). For the past few years, environmental pollution has seriously affected the culture of *L. vannamei* (Bachère, 2000). Although a great number of studies concerning the effects of environmental pollutants on *L. vannamei* have been carried out, many aspects of environmental pollutant effects on crustaceans, particularly gills and hepatopancreas remain unclear. In the present study, we used the 4-ringed PAH chrysene (CHR) as representatives of PAHs. The purpose of this study was to determine potential toxic effects of CHR in gills and hepatopancreas on *L. vannamei* including (i) the accumulation profile of CHR; (ii) phase I detoxification enzyme activity of AHH, EROD, EH and phase II detoxification enzyme activity of GST, SULT, UGT; (iii) antioxidant defense system (T-AOC, SOD) and levels of non-enzymatic glutathione (both reduced (GSH) and oxidized (GSSG) forms); (iv) levels of biomolecule damage parameters (DNA damage, MDA contents and protein carbonyls).

2. Materials and methods

2.1. Chemicals

CHR (98% purity) form Supelco (Bellefonte, PA, USA). Acetone (Sigma, USA) was used in this study as a vehicle for CHR. All chemicals for sample preparation and HPLC detection were obtained from E. Merck (Darmstadt, Germany), and ultrapure reduced glutathione (GSH) was purchased from Amresco (American).1-Chloro-2,4-dinitrobenzene(CDNB),3′-phosphoadenosine-5′-phosphosulfate (PAPS), disodium salt of reduced form -nicotinamideadenine dinucleotide phosphate (NADPH), pyrogallicacid, 7-ethoxy-resorufin (ERF) were purchased from Fluka (USA). All other chemicals were analytical grade.

2.2. Animals and treatments

Healthy *L. vannamei* (physical integrity without injury, good viability), averaging 9.5 ± 0.5 cm in body length, were obtained from a commercial farm in Nanshan, Qingdao, China. The shrimp were acclimated in tanks (72 cm × 56 cm × 40 cm) with a water volume of 125 L each, containing aerated seawater (salinity 31‰, pH 8.2) at 25 ± 0.5 °C for one week prior to the experiment. During the acclimation period, one third to half of the water in each tank was replaced twice daily and the shrimp were fed with a formulated shrimp diet daily. Only apparently healthy animals at the inter-molt stage were used for the study. The molt stage was decided by the examination of uropoda in which partial retraction of the epidermis could be distinguished (Bonilla-Gómez et al., 2012).

In treatment aquarium, the shrimp were exposed to different CHR concentrations (0.3, 2.1 and 14.7 μg/L). There were three replicates for each level and control group, and 60 shrimp in each aquarium (equivalent to 480 shrimp m^{-3}). One third of the water was renewed twice daily, and seawater containing the same concentrations of CHR was added to maintain the corresponding concentrations of CHR during the experiment. CHR was first dissolved in acetone. The final acetone concentration was 0.001% in all tanks including the control ones (the acetone test has been done in a preliminary experiment with the result that there was no influence on shrimp). The exposure concentrations of CHR were based on the concentration of CHR in the coastal seawater, surface sediments in China, as well as CHR solubility (22–25 °C).

Shrimp were sampled 0, 1, 3, 6, 10, 15 and 21 days after the end of the acclimatization period, eight shrimp were sampled for each sampling time and concentration, including controls. Gills, muscle and hepatopancreas tissues were collected; 0.1 g hepatopancreas tissue for every sample was grinded in liquid nitrogen, and then was placed in 1.5 mL sterile centrifugal tube. The remaining tissue was dispensed into 5 mL collection tubes, and preserved at −80 °C for CHR concentration and enzyme activities in less than a week prior to use.

2.3. Chemical analysis

The analysis of CHR was conducted according to standard method procedures (USEPA, 1996). Freeze-dried shrimp tissue samples were Soxhlet extracted in 150 mL mixture of hexane and dichloromethane (1:1) at 70 °C for 18 h, and extracts for the shrimp tissues were prepared. All extracts were dried using anhydrous sodium sulfate and concentrated by a rotary evaporator. The concentrated extracts were concentrated to 5 mL. A chromatography column of length 50 cm, internal diameter of 1 cm was sequentially packed with glass wool, 12 cm of activated silica gel, 6 cm of alumina, which had been baked at 450 °C for 5 h, and 1 cm of anhydrous sodium sulfate (Wei et al., 2006). The extraction aliquot was added into the column, after that the column was preliminarily eluted with 15 mL dichloromethane and then 30 mL hexane, which were both discarded. 15 mL mixture of dichloromethane and hexane (2:1) were eluted thrice to obtain the combined eluate. The eluate was dried with anhydrous sodium sulfate, evaporated, resuspended in 5 mL acetonitrile and filtered (4 μm pore size).

HPLC analysis was performed with a Shimadzu LC20A system. CHR eluate was resolved on an Agilent ZORBAX Eclipse PAH column (4.6 mm × 250 mm × 5 μm) using a protocol gradient with solvent A: water and B: methanol. The gradient was: 95% B/5% A for 60 min. The column temperature was maintained at 30 °C and the flow rate was set at 1.0 mL/min, with fluorescence (excitation wavelength 295 nm, emission wavelength 430 nm) detection. 5 μL of concentrated extract were injected for each run.