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Expression profiles of different glutathione S-transferase isoforms in scallop *Chlamys farreri* exposed to benzo[a]pyrene and chrysene in combination and alone

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ABSTRACT

Aquatic organisms are increasingly exposed to polycyclic aromatic hydrocarbons (PAHs) due to anthropogenic pressure. This study aimed at evaluating the response of Glutathione S-transferases (GSTs) in scallop *Chlamys farreri* against benzo[a]pyrene (BaP) and chrysene (CHR) exposure under laboratory conditions. Nine published GST genes were classified into six subfamilies and a new member of rho family was identified for the first time. Twelve GSTs (including nine published GST genes and three in transcriptome established by our laboratory) *m*RNA transcript levels in the gills, digestive glands, adductor muscle, mantle, testis, ovaries, blood cells of scallops were measured by real-time PCR. The results showed that the mRNA transcript levels of twelve GSTs, except GST-zeta, GST-mu and GST-microsomal, were highest in digestive gland. Accordingly, the mRNA expression levels of GSTs were measured in digestive glands of scallops exposed to BaP (0.1 μg/L and 1 μg/L), CHR (0.1 μg/L and 1 μg/L) and their mixtures (0.1 μg/L BaP +0.1 μg/L CHR and 1 μg/L BaP +1 μg/L CHR). The results indicated that different GST had specific response to different pollution exposure. In BaP exposure experiment, the mRNA expression level of GST-theta was a potential suitable biomarker. GST-sigma-2 and GST-3, which belonged to sigma class, were sensitive to CHR exposure while GST-microsomal was considered a potential ideal bioindicator to joint exposure of BaP and CHR. In summary, this study investigated the classification of GSTs and provided information about the expression profiles of different class GSTs after PAHs exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are regarded as one of the most important classes of persistent toxic substances. In recent years, PAHs are of global environmental concern because of their biotoxicity. Many PAHs are hypertoxic, mutagenic, carcinogenic to various organisms (Boffetta et al., 1997; Chen and Liao, 2006; Perez-Cadahia et al., 2004). In the past decades, PAHs have been found in a great quantity of aquatic ecosystems (Luchmann et al., 2014), such as Boston Harbor, Black Sea and Baltic Sea (Balkis et al., 2012; Lang et al., 2015; Wang et al., 2001). In China, PAHs pollution is becoming more serious with the increase of industrial and domestic sewage discharge and the development of offshore petroleum exploration and ocean shipping (Jin et al., 2014). Benzo[a]pyrene (BaP), which has been observed to accumulate in marine organisms (Meador et al., 1995), is widely used as a reference compound in studies on the toxicity of PAHs in natural communities (Aoyama et al., 2003). The metabolic process of B[a]P in mammals was studied more clearly than that in other organisms (Barhoumi et al., 2011; Cavalieri and Rogan, 1995; Harris et al., 2009; Jacques et al., 2010; Ramesh et al., 2001). Chrysene (CHR), another PAH, is composed of four condensed benzene rings and has two highly reactive Bay-regions where the main carcinogenic species can be formed. Unfortunately, the existence of CHR has been detected in many marine samples (Anyakora et al., 2005; Hellou et al., 1991; Ma et al., 2001; Pino et al., 2000). Guillen et al. (1997) reported that CHR occurred at the highest content among 16 priority PAHs in bivalve mollusks and other aquatic animals from polluted seawaters. What's more, the European Food Safety Authority (EFSA) concluded that the sum of the four specific PAH compounds, namely, benzo[a]anthracene (BaA), BaP, benzo[b]fluoranthene (BbF) and CHR, was the most suitable indicator for assessing PAHs in food in order to minimise the health risk from dietary PAHs exposure (2008). Scallop *Chlamys farreri*, one of the main commercial aquatic animals cultured in China, always inevitably cope with various organic contaminants because they filter large amounts of water and sediments for their nutritional and respiratory needs. Accordingly, *C. farreri* is frequently used as "sentinel

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organism" to monitor aquatic pollution (Pan et al., 2005). Therefore, studies focused on detoxification and metabolism of BaP and CHR exposed *C. farreri* are of noticeable importance.

Exposure to PAHs results in adaptive biochemical responses, growing attention has been concentrated on PAH metabolic mechanism in organisms, especially on their metabolites and toxicity (Barhoumi et al., 2011; Cavalieri and Rogan, 1995; Ramesh et al., 2001). The detoxification responses of PAHs are a complex process that involves AhR pathway, phase I, phase II xenobiotic metabolizing enzymes and phase III transporters (Cai et al., 2016; Paetzold et al., 2009; Pereira et al., 2011). Glutathione-S-transferases (GSTs), important Phase II enzymes, are very suitable biomarkers used in exposure to environmental contaminants (Pan et al., 2005; Wang et al., 2011). Meanwhile, GSTs widely exist in almost all living species, including microorganisms, plants and animals (Pearson, 2005). Changes in GST activity and transcriptional induction in response to PAHs exposure have been investigated in several aquatic mollusks (Goldberg and Bertine, 2000; Le Pennec and Le Pennec, 2003; Liu et al., 2012). Functionally, GSTs are members of a protein superfamily involved in the cellular detoxification of reactive electrophilic compounds, including various intracellular metabolites, pollutants, drugs and pesticides, and in protecting tissues against oxidative damage (Ketterer et al., 1983; Konishi et al., 2005).

GSTs have been particularly considered in mammals (Wilce and Parker, 1994) and three major families of GSTs have been identified, including the cytosolic GSTs (namely alpha, mu, pi, theta, sigma, omega and zeta), the mitochondrial GST (kappa class) and microsomal GSTs (Hayes et al., 2005). In fishes, most of the members in GST family have been identified (Doi et al., 2004; Fan et al., 2007; Trute et al., 2007). For example, nine GST genes (alpha, rho, mu, theta, pi, kappa, microsomal-1, microsomal-2 and microsomal-3,) were cloned from common carp *Cyprinus carpio* (Fu and Xie, 2006) and three (omega1, omega-2 and zeta-1) were cloned from zebrafish *Danio rerio* (Timme-Laragy et al., 2013). As compared to vertebrates, studies of their presence and classification in mollusks are still increasing (Hoarau et al., 2001; Khessiba et al., 2001; Vidal and Narbonne, 2000). For instance, GST-alpha has been reported in mussel *Mytilus galloprovincialis* (Wang et al., 2013) but not in manila clam *Venerupis philippinarum* and *C. farreri*; two classes of GSTs (named GST-A and GST-B on NCBI), cloned from *V. philippinarum,* belonged to a new member of sigma and pi classes GSTs protein family by sequence alignments and phylogenetic analysis finally (Li et al., 2013). In *C. farreri*, nine kinds of GSTs have been cloned and recorded in GenBank, but four GSTs (named GST-1, GST-2, GST-3 and GST-4) have not been classified and further research on their respective functions in specific PAH exposure is limited.

In this study, we worked on the classification of all known GSTs in *C. farreri* for the first time. The expression patterns of GSTs (including the transcriptome of *C. farreri* mapped by our laboratory (SRA Accession No.: SRP018007, unpublished)) *m*RNA in different tissues of normal *C. farreri* were analyzed by real-time PCR. According to the results of the above, we detected the expression levels of GSTs in digestive glands of BaP and CHR single and combined exposed scallops. In summary, the aim of this study was to investigate the response of scallops to PAHs exposure and provide some information about the expression profiles of different class GSTs.

2. Materials and methods

2.1. Scallops and treatments

Healthy scallops *C. farreri*, aged 2 years and with average shell length of 6.06 ± 0.47 cm, were collected from rope-growing cultures at *Shazikou* (Yellow Sea, Qingdao, China) and acclimated to laboratory conditions in tanks with aerated seawater (salinity 30.7 ± 1.1%, pH 8.1 ± 0.8) at 19.7 ± 1 °C for 10 days before test. During the acclimation period, scallops were fed with dried powder of the blue green algae *Spirulina platensis* (30 mg d^{-1} for each individual) , and the water was totally exchanged daily. After the acclimatization, scallops were divided randomly into seven groups, each of which consisted of three replicate aquariums (n = 100 scallops/aquarium) in 100 L seawater. The concentrations in the culture seawater of BaP (0.153 ng/L) and CHR (0.139 ng/L) were measured by HPLC before the experiment and they had no impact on scallops by pre-experiment.

For the exposure experiment, each group was treated with different toxicants at following concentrations: 0.1 μg/L BaP, 1 μg/L BaP, 0.1 μg/L CHR, 1 μg/L CHR, 0.1 μg/L BaP + 0.1 μg/L CHR, 1 μg/L BaP + 1 μg/L CHR. The exposure concentrations were based on the concentration of PAHs in the coastal seawater in China, and we set two relatively higher concentrations than the environmental concentration in order to elicit clearly distinguishable effects (Jin et al., 2014; Xiu et al., 2014). These concentrations also have been proved to impact scallops by pre-experiment. BaP (CAS#50-32-8) and CHR (CAS#218-01-9) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). BaP and CHR were dissolved in 0.001% DMSO (v/v) and the control groups were also containing 0.001% DMSO (v/v). And the DMSO test had been done in a preliminary experiment with the result that there was no obvious influence on scallops. During the experimental period, the scallops were also fed with dried powder of blue green algae daily in normal seawater for 2 h, and then transferred into renewed toxicant-laden seawater daily. The PAH concentration of exposure groups were determined every day before renewing the water during the experiment. The analyses of PAHs concentration were as below, 0.11 ± 0.009 μg/L BaP, 1.02 ± 0.03 μg/L BaP, 0.09 ± 0.012 μg/L CHR, 1.03 ± 0.06 μg/L CHR, (0.10 ± 0.008) μg/L BaP + (0.11 ± 0.007) μg/L CHR, (1.08 ± 0.09) μg/L BaP + (1.03 ± 0.013) μg/L CHR. The gills, digestive glands, adductor muscle, mantle, testis, ovaries, blood cells of nine untreated scallops were collected to determine the tissue distribution of 12 kinds of GSTs. After collection, the samples were flash-frozen in liquid nitrogen immediately and stored at −80 °C for subsequent total RNA extraction. According to the result of tissue distribution, digestive glands of 9 individuals from each treatment were separately sampled after exposure at 0, 1, 3, 6, 10 and 15 days respectively for subsequent examination.

2.2. Sequence analysis

Expect for GST-theta, GST-kappa and GST-mu, all GST protein sequences (GST-1, GST-2, GST-3, GST-4, GST-omega, GST-sigma-2, GST-zeta, GST-pi and GST-microsomal which are available on the NCBI) were compared to other species (GenBank accession numbers for the sequences are as follows: *Oreochromis niloticus* rho, ACT22666.1; *Oreochromis aureus* rho, ACT22668.1; *Sebastes schlegelii* rho, ANW83217.1; *Solea senegalensis* rho, BAG12568.1; *Siniperca chuatsi* rho, ACI32418.1; *Danio rerio* rho, NP_001038525.1; *Chlamys farreri* 2, ACF25903.1; *Kryptolebias marmoratus* zeta 1, NP_001316300.1; *Oplegnathus fasciatus* zeta, ADY80028.1; *Cyprinus carpio* zeta, BAS29981.1; *Xenopus laevis* zeta 1 L, NP_001088856.1; *Chlamys farreri* zeta, ADD82544.1; *Takifugu rubripes* omega, AAL08414.1; *Sebastes schlegelii* omega, ANW83218.1; *Oncorhynchus kisutch* omega, AGB56854.1; *Cyprinus carpio* omega, BAS29979.1; *Chlamys farreri* 1, ACF25902.1; *Chlamys farreri* omega, ADF32018.1; *Crassostrea ariakensis* omega, ACJ06747.1; *Chlamys farreri* 4, ACF25905.1; *Chlamys farreri* sigma 2, ADF32019.1; *Argopecten irradians* sigma, ANG56313.1; *Crassostrea ariakensis* sigma, ACJ06744.1; *Hyriopsis cumingii* sigma, AGU68336.1; *Chlamys farreri* 3, ACF25904.1; *Mytilus galloprovincialis* sigma 2, AFQ35984.1; *Mytilus edulis* pi, AAS60226.1; *Mytilus coruscus* pi, AGK07582.1; *Perna viridis* pi, AGN03945.1; *Corbicula fluminea* pi, AAX20374.1; *Ruditapes philippinarum* pi, ACM16805.2; *Chlamys Farreri* pi, ACL80138.2; *Esox lucius* pi, NP_001291057.1; *Mesitornis unicolor* microsomal 1, KFQ38301.1; *Tinamus guttatus* microsomal 1, KGL77014.1; *Xenopus laevis* microsomal 1 L, NP_001084720.1;