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iTRAQ-based proteomic profiling of the marine medaka (*Oryzias melastigma*) gonad exposed to BDE-47

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ABSTRACT

A recent study demonstrated that 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) may have an adverse effect on the reproduction in marine medaka (*Oryzias melastigma*), but the molecular mechanisms remain largely unknown. In this study, we investigated the protein expression profiles of male and female gonads of *O. melastigma* exposed to dietary BDE-47 at two dosages (0.65 and 1.30 μg/g/day, respectively) for 21 days. Extracted proteins were labeled with iTRAQ and analyzed on a MALDI TOF/TOF analyzer, as results, 133 and 144 unique proteins were identified in testis and ovary, respective, and they exerted dose- and sex-dependent expression patterns. In testis, among the 42 differentially expressed proteins; down-regulation of histone variants and parvalbumins implicated BDE-47 may disrupt the spermatogenesis and induce sterility in fishes. In ovary, 38 proteins were differentially expressed; the elevation of vitellogenins and apolipoprotein A–I expression indicated BDE-47 acts as an estrogen-mimicking compound and led to reproductive impairment in *O. melastigma*.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a class of brominated flame retardants, are widely used in products to reduce the incident of fire (Watanabe and Sakai, 2003). PBDEs occur in the natural environment as a mixture of congeners, of which PBDE 47 is the most abundant (Kuiper et al., 2004). Because of the endocrine and thyroid hormone disrupting characteristics of PBDE 47 (Jugan et al., 2010), exposure to this compound in the environment has raised concerns in recent years. Reports also indicate that chronic exposure to PBDEs can cause neural degenerative problems and affect neural development in animals (Costa and Giordano, 2007). Investigations into the accumulation and physiological effects of PBDEs is therefore an important area of environmental research. The marine medaka (*Oryzias melastigma*) has recently been developed as a universal model for investigating toxicology in marine fish (Kong et al., 2008). It has a short generation time, distinct sexual characteristics and small genome size. It is also highly sensitive to estrogenic pollutants, making it a good model for studying the toxicological effects of endocrine disrupting compounds (Chen et al., 2008).

Previous studies have showed that adult female fish have an additional pathway for elimination or sequestration of organic contaminants through maternal transfer to their eggs, which may result in sex-specific accumulation and tissue distribution of PBDEs, leading to differential effects of these chemicals in male and female fish (Nyholm et al., 2008; van de Merwe et al., 2011). Specifically, van de Merwe et al. (2011) found that female *O. melastigma* exposed to BDE 47 in their diet at 1.3 ± 0.2 μg/day for 18 days accumulated significantly lower growth-corrected BDE-47 concentrations compared to males. Subsequent studies have also showed anti-estrogenic potency of hydroxylated BDE-47 on the modulation of the sexual hormone receptor (Liu et al., 2011), and sex-specific toxicological effects of BDE-47 in *O. melastigma* (Bo et al., 2011, 2012; Ye et al., 2012). These studies suggest that maternal transfer is an important offloading mechanism for female

Abbrevations: apoa1, apolipoprotein A–I; cct, T-complex protein 1 subunit; fhl1, four and half LIM domains protein 2 isoform b; hnrpdl, heterogeneous nuclear ribonucleic protein; jph1a, junctophilin 1a; pabpc1b, polyadenylate-binding protein; pvalb, parvalbumin; vtg, vitellogenin.

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fish, and that PBDEs may have adverse effects on reproduction. However, the underlying molecular mechanisms remain largely unknown.

The aim of this study was to investigate the proteomic responses in *O. melastigma* gonads following dietary exposure to BDE-47. Molecular approaches in ecotoxicology have greatly enhanced mechanistic understanding of the impact of aquatic pollutants in organisms. The use of isobaric tags for relative and absolute quantification (iTRAQ) and two-dimensional differential in-gel electrophoresis (DIGE) are commonly used for detecting protein expression change and identifying potential biomarkers in ecotoxicology studies (e.g. Martyniuk et al., 2012). Studies utilizing iTRAQ, the non-gel based quantitative proteomics can greatly improved the accuracy of proteomic studies, allowing quantification of the expression of different proteins as well as their potential interactions (Schneider and Hall, 2005). The most significant advantage of iTRAQ over more traditional proteomics approaches is that up to eight different samples can be simultaneously labeled and analyzed, and a recent study of the proteome response of the marine diatom *Thalassiosira pseudonana* after benzo(a)pyrene exposures with an iTRAQ-8 plex quantification analysis also demonstrated the strength of the iTRAQ approach that many utilized protein bioindicators can be quantified at once, providing a wealth of information that can be used to assess adverse effects in aquatic organisms (Carvalho and Lettieri, 2011). In this study, the iTRAQ-based proteomic approach was used to investigate the protein expression profiles of male and female *O. melastigma* gonads exposed to BDE-47. This information is critical to understanding the molecular responses underlying the reproductive impairments in *O. melastigma* upon PBDEs exposure.

2. Materials and methods

2.1. BDE-47 bioencapsulation and sample collection

BDE-47 was bioencapsulated in brine shrimp (*Artemia* sp.) and fed to marine medaka at different doses following methods previously described by van de Merwe et al. (2011). Three doses of BDE-47 were prepared: (1) control (100% clean *Artemia*), (2) low dose, 0.65 μg/g daily intake of BDE-47 (50% BDE-47 *Artemia* + 50% control *Artemia*), and (3) high dose, 1.30 μg/g daily intake of BDE-47 (100% BDE-47 *Artemia*).

2.2. Dietary exposure of marine medaka to BDE-47

The marine medaka *O. melastigma* used in this experiment were from stock originally purchased from Interocean Industries (Taiwan) and reared in the State Key Laboratory in Marine Pollution, City University of Hong Kong for more than 30 generations. Glass

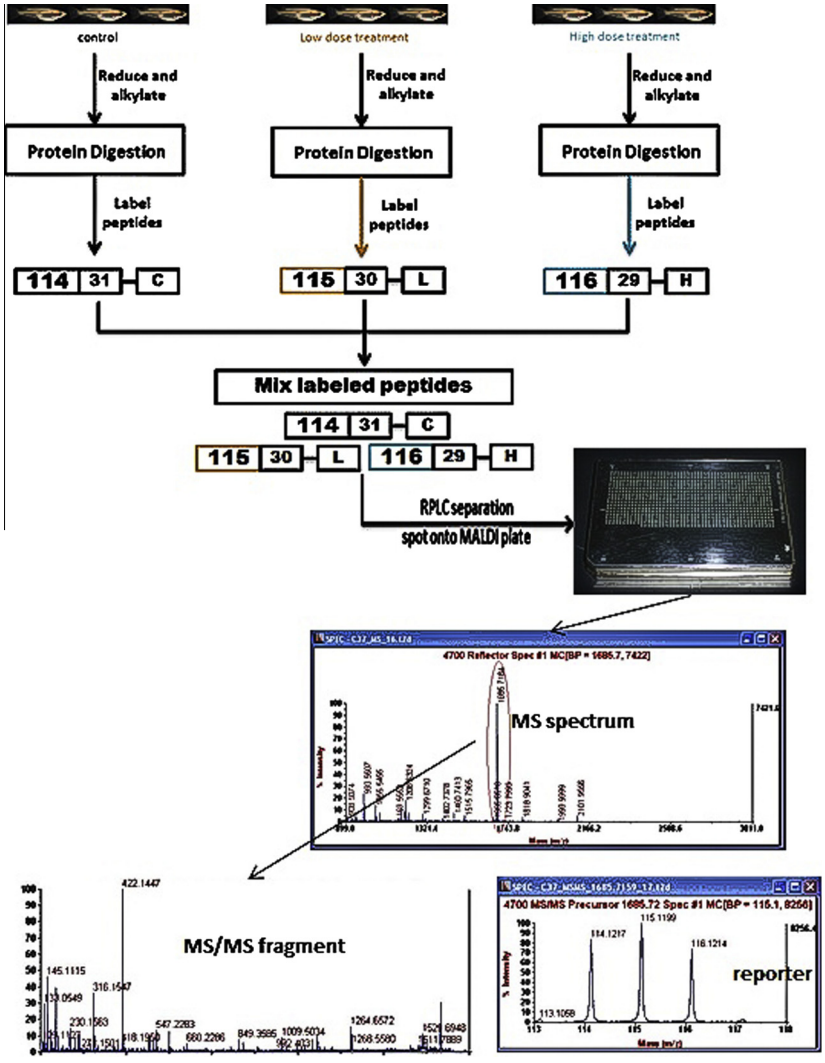

Fig. 1. Illustration of iTRAQ-based method for proteomic profiling of male and female marine medaka (*Oryzias melastigma*) gonads exposed to BDE-47.

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