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Alteration of the kidney membrane proteome of *Mizuhopecten yessoensis* induced by low-level methyl parathion exposure

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ABSTRACT

Methyl parathion (MP) is a widely used organophosphorus pesticide that causes severe health and environmental effects. We investigated the alteration of the proteomic profile in the membrane enriched fraction of the kidneys of the scallop *Mizuhopecten yessoensis* exposed to low-level MP. Gas chromatography analysis showed that MP residues were significantly accumulated in the kidneys and the digestive glands of the scallops. According to two-dimensional electrophoresis, 17 proteins were differentially modulated under MP exposure. The mRNA expressions of 12 differential proteins were analyzed using quantitative PCR, and 10 showed consistent alteration of mRNA level with that of protein expression level. Altered expressions of two proteins (mitochondrial processing peptidase and α-tubulin) were also examined using Western blotting, showing that the mitochondrial processing peptidase was down-regulated but α-tubulin remained unchanged in response to MP exposure. Subcellular locations of all the identified proteins that were predicted using bioinformatics tools indicate that few of them are permanently located in the membrane. The differentially expressed proteins are involved in several critical biological processes, and their relevance to human health has been illuminated. These data taken together have provided some novel insights into the chronic toxicity mechanism of MP and have suggested mitochondrial processing peptidase as a potential biomarker for human health and environmental monitoring.

1. Introduction

Organophosphorus (OP) compounds have been used widely as pesticides and herbicides since World War II, and have made great contributions to maintaining world food production. Nevertheless, their excessive use in agricultural programs has caused severe environmental pollution and health hazards (Abdollahi et al., 1995; Konstantinou et al., 2006). Although since the 1980s OPs have largely replaced the more persistent organochlorine pesticides due to their relatively lower stability and because of their environmental lability, some of them (including methyl-parathion; MP) are still considered extremely dangerous pesticides (Eto, 1974). MP is one of several OP insecticides that are widely applied in agriculture and it is a highly toxic insecticide classified by the United States EPA as a class I toxicant (EPA, 1999). Due to it potentially causing damage to both human health and the environment, its use has been restricted in many countries including China and the USA. In spite of its high toxicity, MP was once considered to pose less risk to the ecosystem than organochlorine pesticides due to its short half-life (approximately 1.2 d) in sediment, low bioaccumulation factor and high biotransformation rate (Schimmel et al., 1983). However, this compound is found in food and potable water near farms spreading MP (Fanta et al., 2003; Fenske et al., 2002). MP residues in seawater or sediments can produce serious public health risks due to the consumption of contaminated organisms (Serrano et al., 1995). Moreover, in view of the lack of study on vulnerable biotopes, it is probable that the potential impacts of MP on the marine ecosystems have been substantially underestimated (Bennett and Bennett, 1990; Da Silva et al., 1993). Therefore it is necessary to reevaluate the hazards of MP and to monitor its behavior in the marine environment following a more comprehensive analytical protocol.

Bivalves are widely used as sentinels for monitoring pollution of the marine environment (Goldberg et al., 1978; Walker and

Abbreviations: MP, methyl parathion; 2DE, two-dimensional gel electrophoresis; PMF, peptide mass fingerprinting; OP, organophosphorus; EST, expressed sequence tag; DG, digestive gland; MEF, membrane enriched fraction; WC, whole cell; MPP, mitochondrial processing peptidase; MAPRE1, microtubule-associated protein, RP/EB family, member 1; PEPCK, phosphoenolpyruvate carboxykinase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; TA, transcriptional activator; ITI, inter-alpha-trypsin inhibitor; IOX, inositol oxygenase; GRAVY, grand average of hydrophobicity; TMR, transmembrane region.

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Livingstone, 1992). As sessile, filter-feeding and low metabolism organisms they may take up and concentrate contaminants to levels well above those present in the surrounding waters or sediment, thus being able to provide information on local pollution sources (De La Vega Salazar et al., 1997; Solé et al., 2000). *Mizuhopecten* (*M.*) *yessoensis* which was used in this study is a commercially valuable cold water scallop species in China and Japan. Its advantages include a mature cultivation technique, high abundance and high stress tolerance. Moreover, the rapid development of the expressed sequence tag (EST) database on *M. yessoensis* during recent years has also supported us in toxicological and genetic studies (Wang et al., 2008).

As with other OP insecticides, the most prominent clinical effects of poisoning with MP result from the inhibition of acetylcholinesterase (AChE) (Abou-Donia, 2003; Eddleston et al., 2004). Moreover, the biotransformation to the reactive metabolite methyl paraoxon can enhance its AChE inhibiting activity (Bharate et al., 2010; Paudyal, 2008). Compared with the acute toxicity of MP, which is relatively well understood, the chronic effects of low-level exposure to MP are equivocal. MP exposure has been implicated as a causal factor in a variety of different forms of human ill-health (Garcia et al., 2003). More specific relatively low-level effects have been described in animals, such as neurodevelopmental disruption (Qiao et al., 2002), reproductive toxicity (Uzunhisarcikli et al., 2007) and oxidative stress (Delgado et al., 2006). However, classical epidemiological studies relating to low-level exposures have still been unable to identify specific OP pesticides associated with ill health, mainly because the studies concerning mechanisms are far from complete (Carter et al., 2007). Therefore there is a clear need to use a more global method such as transcriptomics and proteomics to profile cellular gene expression changes in response to chronic OP exposure rather than using symptom-dependent diagnoses.

Searching for protein biomarkers that undergo significant changes of volume or activities correlated with specific pollutant exposure in ecosystems has become a topic of increasing interest in the last few decades (Livingstone, 1993; Monserrat et al., 2007). Toxicoproteomics has emerged in order to use the proteomic approach to identify critical proteins and pathways that can specifically discern the mechanism of toxicity and search for biomarkers of exposure to the toxic substances (Wetmore and Merrick, 2004). However, the classic proteome analysis approach which aims to display the whole pool of expressed proteins within the studied system has some deficits. One of them is that certain classes of proteins, such as integral membrane proteins, are not represented proportionally to their abundance due to their highly hydrophobic characteristic, low abundance and heterogeneity (Tan et al., 2008). In order to overcome these limitations, a membrane proteomic approach which combines the first-line proteomic techniques with traditional approaches with respect to membrane fractionation protocols was introduced (Dreger, 2003). Analysis of the membrane proteome allowed a reduction in the complexity of the samples under investigation and yielded more information on the hydrophobic membrane protein components. Membrane proteins are coded for by approximately 1/3 of the coding portion of the human genome and present about 60% of the existing drug targets according to the results that reviewed by Hopkins and Groom (2002). This highlights the importance of studying the membrane proteome particularly in view of their fundamental roles in biological processes and finding potential action sites for interested toxicants.

Despite the importance of proteomics research into MP exposure effects on marine organisms, such reports were previously seldom mentioned. Chen and Huang (2011) report that eight proteins are down-regulated while 17 proteins are up-regulated after liver proteomics analysis of *Sparus latus* under acute MP exposure. Huang et al. (2011) investigated the alteration of protein profiles in *Danio rerio* brain in response to MP exposure, and found a reduced abundance of brain creatine kinase b and dihydropyrimidinase-like 3, but an increased abundance of heterogeneous nuclear ribonucleoprotein D-like. It is possible that some of these proteins would play a central role in future biomarker studies. However, none of the studies mentioned above focused on the kidney membrane proteome. Here, we attempted to study the alterations in the hydrophobic-enriched membrane proteome of the kidneys of *M. yessoensis* under low-level exposure to MP (50 μg/L, two to three orders of magnitude lower than the 48h-LC50 value of Solé et al., 2000) for 14 and 21 d, using two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF MS/MS) approaches. MP residues in the kidneys or the digestive glands (DGs) of *M. yessoensis* were measured using gas chromatography (GC) before the proteomics analysis. Our aim was to reveal novel biomarkers suitable for specifically monitoring MP pollution in seawater as well as to provide a specific explanation of the mechanism involved in the chronic effects of MP exposure on marine animals and humans.

2. Materials and methods

2.1. Animals and MP treatment

M. yessoensis was collected in inshore areas during winter (December each year to February next year) at temperature 12 °C) in Xiamen City. Three groups of scallops, 20 in each group, of size 18 ± 1.5 cm were kept in 60 L polyethylene aquaria filled with continuously aerated seawater (pH 8.2 ± 0.1, salinity 30.2 ± 0.2) and with a 12-h photoperiod. All experimental seawater was renewed in 24 h, and the scallops were fed with adequate chlorella as food before the renewed seawater during the cultivation period. After 1 week acclimation, two groups were exposed to 50 μg/L MP (Sigma, USA) for 14 d and 21 d, respectively. The remaining group was cultivated in clear seawater, as control. The MP stock was dissolved in acetone and diluted to 1000 mg/L with clear seawater before each delivery. MP was delivered into the aquaria of the treated groups each time after seawater renewal. Four scallops from each group were randomly killed and sampled at the end of the exposure periods in order to avoid individual difference. Both kidneys and DGs in the scallops were isolated and pooled together in the same group. Samples were immediately placed at −80 °C for further use.

2.2. Sample preparation and GC analysis of MP residues

The kidneys or the DGs of scallops were placed on ice, and homogenized in cold acetone (Sigma, USA) using a WERKE^{TM} T 10 basic disperser (IKA, Germany). The ratio of weight of the tissues to volume of acetone (w:v) was 1 mg: 10 μL. After centrifugation at 12,000 rpm for 10 min, the supernatants were collected in 5 mL tubes. The pellets were washed three times with cold acetone following a vortex and centrifuging procedure. All of the supernatants collected above were mixed and dried using a stream of nitrogen gas. The pellets were dissolved in 100 μL acetone and centrifuged at 3000 rpm for 5 min. The supernatants were used for GC analysis.

MP residues were analyzed in a Varian CP-3800 gas chromatograph (Varian, USA) equipped with a PFPD detector. A standard curve was drawn using an analytical standard of MP (100 μg/mL) purchased from the AEPI (Agricultural Environment Protection Institute, China). GC conditions were as follows: column, 30 m × 0.32 mm × 0.25 μm, CP-8752; oven temperature program, 150–220 °C at 20 °C/min, and 220 °C with 10 min hold; injection temperature, 250 °C; carrier gas, H_2; carrier flow rate, constant flow at 1 mL/min; injection volume, 1 μL; PFPD temperature, 300 °C.