Environmental Pollution 206 (2015) 202–208

Contents lists available at ScienceDirect

Environmental Pollution

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

Isotopic fractionation during the uptake and elimination of inorganic mercury by a marine fish

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ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
1 July 2015
Accepted 3 July 2015
Available online xxx

Keywords:
Stable mercury isotopes
Inorganic mercury
Mass dependent fraction
Internal distribution
Liver

ABSTRACT

This study investigated the mass dependent (MDF) and independent fractionation (MIF) of stable mercury isotopes in fish during the uptake and elimination of inorganic species. Mercury accumulation during the exposure led to re-equilibration of organ isotopic compositions with the external sources, and elimination terminated the equilibrating with isotope ratios moving back to the original values. Generally, the isotopic behaviors corresponded to the changes of Hg accumulation in the muscle and liver, causing by the internal transportation, organ redistribution, and mixing of different sources. A small degree of MDF caused by biotransformation of Hg in the liver was documented during the elimination, whereas MIF was not observed. The absence of MIF during geochemical and metabolic processes suggested that mercury isotopes can be used as source tracers. Additionally, fish liver is a more responsive organ than muscle to track Hg source when it is mainly composed of inorganic species.
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1. Introduction

Elevated mercury (Hg) concentrations have been observed in diverse fish species over the past 50 years (US FDA, 2014), and have become an important public health issue. Recently the stable Hg isotopes in fish tissues are increasingly used as a tracer for sources and exposure pathways of Hg in the environment (Bergquist and Blum, 2007; Estrade et al., 2010; Gehrke et al., 2011; Kritee et al., 2009; Kwon et al., 2012, 2013; Yin et al., 2013). Both field and controlled studies were performed to unravel its complex biogeochemical process. Given the growing interest of applying this technique, it is vital to first understand Hg isotopic fractionation during various biological and ecological processes.

Variations of Hg isotopic compositions in field samples are the combined results of mixed reservoirs with different isotopic signatures and systematical fractionations during reactions. Two types of isotope fractionations have been observed, namely the mass-dependent fractionation (MDF) via the nuclear mass selectivity, and the mass-independent fractionation (MIF) via the magnetic isotope effect and the nuclear volume effect (Buchachenko et al., 2008; Schauble, 2007). The preserved MDF and MIF in samples make it possible to apply the Hg isotope ratios to trace the pollution sources, quantify the exposure pathways, and study the Hg behavior in the environment. Earlier, Kwon et al. (2012) studied the Hg isotope fractionation during trophic transfer and internal distribution in the fish. They found no evidence of MDF in juvenile yellow perch (*Perca flavescens*) after 2 months of feeding on commercial food pellets containing methylmercury (MHg), and in lake trout (*Salvelinus namaycush*) after 6 months of feeding on bloater (*Coregonus hoyi*). In another study using marine fish (Kwon et al., 2013), they observed a shift of δ^{202}Hg values in amberjack (*Seriola dumerili*) due to the small internal fractionation during excretion of shrimp diet that contained low level of inorganic Hg. Meanwhile, some field results suggested correlations between the MDF in fish muscle and their Hg concentrations (Bergquist and Blum, 2007), but no direct result proved the production of *in vivo* MIF (Kritee et al., 2009; Kwon et al., 2012, 2013). Das et al. (2009) reported a progressive enrichment in the odd-mass Hg isotopes with increasing trophic level, which was explained by the discrepancy of Hg species and the preference of assimilation and accumulation of organic Hg (Tsui et al., 2012).

In the present study, we explored the stable Hg isotope fractionations during the uptake and elimination of Hg(II) in a marine fish. Although MHg is usually accumulated through trophic webs and considered as the most toxic form in ecological systems (Oken et al., 2005, 2008), Hg(II) is the most commonly released chemical form by anthropogenic activities (Manohar et al., 2002; US EPA, 1997) and contributes more than 95% of the total Hg in aquatic

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http://dx.doi.org/10.1016/j.envpol.2015.07.008
0269-7491/© 2015 Published by Elsevier Ltd.

systems (Watras et al., 1998). Additionally, Hg(II) and MHg undergo different exposure pathways and vary in their assimilation efficiencies, dissolved uptake rates and elimination, as well as detoxification (Wang and Wong, 2003; Pentreath, 1976; Sorensen, 2010). All of the mentioned mechanisms would influence the *in vivo* isotope fractionation relative to different chemical forms of Hg exposure. Therefore, Hg(II) exposure is also important in exploring the biological processes of Hg isotopes, especially in highly contaminated systems where fish %MHg was low (Horvat et al., 2003).

A marine teleost, thorn fish (*Terapon jarbua*), was chosen in this study because of its strong adaptability under the laboratory conditions. We exposed the fish to either dietary or aqueous source of Hg(II). During the 4 weeks of uptake and subsequent 8 weeks of depuration, the Hg biodynamics (accumulation, transportation and distribution), speciation, and isotopic behaviors in fish were measured. The Hg isotopic behavior during trophic transfer was determined by comparing the isotope ratios of exposure sources to the ratios in fish. The isotopic behavior during uptake was determined by comparing the *in vivo* isotope ratios at week-0 to the values at week-4. Finally the isotopic behavior during elimination was determined by comparing the isotope ratios between week-4 and at week-12. Variations of Hg isotopic compositions were explained based on the changes of Hg concentrations and other biological processes.

2. Materials and methods

2.1. Experimental design

The five-month-old thorn fish (*T. jarbua*) was supplied by a fish farm from Yung Shue O (Sai Kung, Hong Kong) and acclimatized in the Coastal Marine Laboratory of Hong Kong University of Science and Technology for 2 weeks in filtered seawater with continuous aeration at water temperature of 25–28 °C and salinity of 31–32 psu. They were fed once a day with commercial food pellets (New Life, Spectrum, 2 mm) at approximately 2% of their mean dry body weight. All fish were then transferred to 80 L tanks and randomly divided into two groups: the group exposed to aqueous Hg(II) (A-group), and the group exposed to dietary Hg(II) (D-group). We included two exposure routes because fish take up both aqueous and dietary Hg(II) through gills and digestive systems (Boudou and Ribeyre, 1985). We specifically tested whether different routes influenced the biological process of Hg isotope fractionation. Five replicated tanks were used in each group and 16 individuals were included in each tank (total number = 240). We used 4-week uptake and 8-week elimination to study the Hg accumulation, organ distribution, speciation, and fractionation of isotopes. Circulating seawater was terminated after acclimation and changed manually every day during the 12-week experimental periods. During the uptake, fish in the A-group was fed with unexposed pellets and maintained in spiked seawater, and fish in the D-group were fed with exposed food pellets and maintained in control seawater. To avoid the aqueous Hg(II) binding to the food pellets, we only fed the fish immediately after changing the water, and the aqueous Hg(II) was spiked after fish finished their food, which usually took 5–30 min. During the elimination, all fish were fed with unexposed pellets and maintained in control seawater.

2.2. Exposure treatments

The working solution of Hg(II) was prepared by dissolving $Hg(NO_3)_2 \cdot H_2O$ (Sigma, USA) in Milli-Q water, purged for 2 h with nitrogen to remove the dissolved gaseous Hg, wrapped with aluminum foil to avoid the Hg isotope fractionation caused by photochemical reactions (Bergquist and Blum, 2007), and stored until it was needed. The nominal concentration of the dietary Hg(II) was 50 μg/g, which was high enough to produce measureable results. Food pellets were soaked in 50 μg/mL Hg(II) solution for 6 h, dried at room temperature, and kept in −80 °C freezer prior to use. The actual total Hg concentrations were 45.9 ± 3.7 μg/g (2SD, n = 5). Since there were no reference relative to Hg fractionation in fish exposed to aqueous Hg, we used a nominal concentration of 0.5 μg/L Hg(II) which was usually employed in kinetic studies of fish (Wang and Wong, 2003), and the actual dissolved concentration was 0.5 ± 0.08 μg/L (2SD, n = 5). Such concentration was about hundred times lower than that used by Bergquist and Blum (2007) which proved photoreduction-induced MIF. Thus, the MDF and MIF of aqueous Hg(II) in the tank seawater were minimized, especially when all tanks were kept in dark throughout the experimental periods.

2.3. Sample collection

Two or 3 individuals from each tank were randomly selected and sacrificed at the start of experiment (0 week), and at the end of the 2nd week, 4th week, 6th week, 8th week, 10th week, and 12th week. The body weight and length of each individual were measured, and the liver and muscle were dissected, weighted, and freeze dried at −80 °C (Freeze-Dryer, ilShinBioBase Co. Ltd., South Korea), followed by dry weight measurements. Fish samples collected from the same tank were pooled in case of small sample amount and individual heterogeneity, and divided into two portions for Hg concentration and isotope analysis. The initial body weight was 4.6 ± 1.5 g (wet weight, n = 14), and the body length was 53.8 ± 7.0 mm (fork length, n = 14) and 66.8 ± 8.7 mm (total length, n = 14). The growth dilution of Hg concentration and its influence on isotopic behaviors in fish were ignored because the final body weight (4.47 ± 0.99 g, n = 14) and size (fork length: 54.5 ± 4.2 cm, total length: 68.2 ± 6.5, n = 14) were not statistically different from the initial data as a result of individual heterogeneity and the influence of dark conditions on fish growth. Mortality rates were lower than <5% throughout the experimental periods.

2.4. Mercury concentration analysis

The total mercury (THg) concentration was analyzed by cold vapor atomic fluorescence spectrometry (Quick Trace M-8000, CETAC Technologies, USA). Fish tissue was completely digested in ultrapure acid (H_2SO_4:HNO_3 = 1:4, v/v, Sigma, USA) on block heater (BT5D, Grant Instruments, UK) at 95 °C for 3–4 h (Yin et al., 2013). The digested samples were appropriately diluted, oxidized to Hg(II) with a hydrochloride/bromate/bromide mixture, reduced with hydroxylamine hydrochloride to destroy the free halogens, and converted to Hg(0) with stannous chloride (US EPA, 2007). The Hg(0) vapor was then separated from solution by purging with nitrogen, collected by gold traps, and thermally desorbed from the analytical trap into a fluorescence detector. Standard curves were established using a liquid Hg standard (PerkinElmer, USA) for each analytical session. Precision and accuracy for the analytical system were quantified with blanks, 10% standard reference material DORM-4 (fish protein, National Research Council of Canada), and 10% replicates. Method precision expressed as relative percent difference for duplicate samples averaged 1.05 ± 4.9% (2SD, n = 20), and the mean percent recovery of DORM-4 samples was 97.3 ± 7.9% (2SD, n = 24).

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