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Comparative contribution of trophic transfer and biotransformation on arsenobetaine bioaccumulation in two marine fish

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ABSTRACT

Marine fish can accumulate high arsenic (As) concentrations, with arsenobetaine (AsB) as the major species in the body. However, whether the high AsB accumulation in fish occurs mainly through trophic transfer from diet or biotransformation in the fish body remains unclear. This study investigated the trophic transfer and biotransformation of As in two marine fish (seabream *Acanthopagrus schlegeli* and grunt *Terapon jarbua*) fed artificial and clam diets for 28 d. The different diets contained different proportions of inorganic [As(III) and As(V)] and organic [methylarsenate (MMA), dimethylarsenate (DMA), and AsB] As compounds. Positive correlations were observed between the accumulated As concentrations and AsB concentrations in both fish, suggesting that AsB contributed to the accumulation of total As in marine fish. Based on the calculated total input of AsB and detected AsB concentrations in the muscle of the seabream and grunt, the ingested amounts of AsB accounted for 0.1–0.3%, 8.1–14.4% of detected AsB concentrations, respectively, in the muscle of seabream and grunt fish species, suggesting that AsB was mainly biotransformed versus trophically transferred in these marine fish. In summary, this study demonstrates that marine fish prefer to biotransform inorganic As forms into AsB, resulting in high bioaccumulation of total As.

1. Introduction

Arsenic (As) is the most common toxic substance in the environment, ranking first on the superfund list of hazardous substances (http://www.atsdr.cdc.gov/cercla/07list.html), that has created worldwide human health problems. Arsenic contamination in the environment has been reported worldwide (Li et al., 2011; Sohel et al., 2009). Although As is widely distributed in all organisms (Edmonds and Francesconi, 2003), total As concentrations are higher in marine fish (1–10 μg/g) than in freshwater fish (<1 μg/g) (Amlund and Berntssen, 2004; Ciardullo et al., 2010; Schaeffer et al., 2006). In a recent study on trace element contamination in wild marine fish collected from the coastal waters of China (from north to south), As was the trace element of greatest concern. The highest As concentration (134 μg/g dw) in marine fish was detected in *Trypauchen vagina* from southern China, and the concentrations detected in this study were generally 30 times higher than the safe limit established in China (1.0 μg/g ww) (Zhang and Wang, 2012). Onsanit et al. (2010) investigated the As levels in a cultured marine fish, seabass (*Lateolabrax japonicus*), from eight fish cages along the coast of Fujian Province in China. Among all the trace elements analyzed, only the As levels (1.5–4.5 μg/g ww in seabass muscle) at all sites were generally higher than the safe limit in China. Thus, marine fish have a strong ability to accumulate As. Based on previous studies, arsenobetaine (AsB) is the major As species in marine fish, typically accounting for more than 90% of the total As (Amlund et al., 2006a; Francesconi and Edmonds, 1997; Zhang et al., 2012; Kirby and Maher, 2002). Therefore, we aimed to determine whether AsB contributes to the higher As accumulation in marine fish.

Since the identification of AsB in marine fish, much research has been conducted to determine how AsB is formed in these organisms with differing results. Some earlier studies investigated the accumulation and distribution of As compound in marine fish species in relation to their trophic position, they speculated that the absorption of large amounts of AsB from the diet (Bedford et al., 1998; Francesconi and Edmonds, 1994; Kirby and Maher, 2002). A recent review stated that AsB has not been detected in many unicellular marine algae, even though arsenoribosides are the major As form present in these species, which supports the hypothesis that AsB is formed in marine animals via the ingestion and further

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metabolism of arsenoribosides (Duncan et al., 2015). Additionally, another possibility that marine fish ingest inorganic As and subsequently biotransform these components themselves to produce AsB (Bolan et al., 2006; Francesconi and Edmonds, 1993; Zhang et al., 2012). Thus, biotransformation could be the primary source of AsB in marine fish. One identified important factor modifying the As related health effects is its biotransformation (Vahter, 2002). To prove our hypothesis, we selected two carnivorous marine fish to attain a potential diversity of As bioaccumulation and biotransformation.

Recognition of the need to determine environmentally realistic exposures is currently growing. Therefore, the purpose of this study was to examine whether AsB contributed to the high As accumulation in marine fish, as well as to the source of AsB, and whether the AsB in present fish was contributed to by marine organisms at lower trophic levels through trophic transfer or by the fish themselves through biotransformation. To resolve these two questions, we investigated the trophic transfer and biotransformation of As in two carnivorous marine fish, seabream (*Acanthopagrus schlegeli*) and grunt (*Terapon jarbua*), following a series of exposures to artificial and clam diets. To understand the behavior of As in these marine fish, we selected artificial and clam diets to elucidate the effect of prey types containing different As species on the bioaccumulation, trophic transfer and biotransformation of As. Understanding the main source of less-toxic AsB in marine fish could assess the potential hazard to human beings, and may assess the natural risk to this potential hazard.

2. Materials and methods

2.1. Exposure diets

The artificial diets were bought from a feed company in Shenzhen, China. The diets were labeled with As(III) and As(V) as an aqueous solution of arsenite and arsenate ($NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$, Sigma, USA), respectively, to achieve a nominal concentration of 267 nmol As/g diet. The clams *Asaphis violascens* were collected from Shenzhen, China, in March 2014. They were directly labeled with waterborne As(III) and As(V) (13.3 μmol/L) for 4–7 d. After the labeling, the clams were dissected to obtain their soft tissues. The handling methods of artificial diets and the tissues were described in our previous study (Zhang et al., 2016).

2.2. Experimental design

Seabream *Acanthopagrus schlegeli* (8.4–15.5 cm in length) and grunt *T. jarbua* (7.5–12.9 cm in length) were obtained from a fish farm at Shenzhen, China. The seabream and grunt are important commercial and cage-cultured marine fish in Shenzhen. These two fish are considered carnivorous, feeding on clams. They were maintained in natural sand-filtered seawater (24–26 °C, 33‰) and fed artificial and clam diets, respectively, at approximately 3% of their body weights daily in the laboratory, to allow acclimation to the food. The tanks were under a light:dark cycle of 12:12 h. Feces and uneaten food were removed twice a day. They were acclimated to the conditions for 2 weeks prior to the exposure experiment.

The seabream and grunt were randomly separated and placed in natural sand-filtered seawater. There were eight treatment tanks [two dietborne exposures for both As(III) and As(V) for each fish species] with a sample number of 10–12 fish per tank for both fish. In the control treatment, the fish were fed the unspiked artificial diet. In the exposure treatments, the fish were fed the spiked artificial diets and clams, respectively. Fish were fed twice per day and any uneaten food was removed to prevent negligible waterborne As exposure. The seabream and grunt were exposed to dietborne As for 28 d. The seabream fed As(V)-exposed artificial diets all died. At the end of the exposure, they were starved for approximately 24 h to allow the depuration of gut contents. The fish (n = 8–10 per treatment) from each tank were then collected and placed in a plastic bag, and seawater on the surface of whole fish body was blotted dried. They were immediately measured for length and wet weight, and then frozen at −80 °C for further analysis.

2.3. Chemicals, reagents, total As and As species analysis

The frozen fish were thawed on ice and their intestine, liver, and dorsal muscle tissues were carefully dissected. Then they were freeze dried (freeze drier), homogenized (grinding) and stored in small polyethylene bags for total As and As speciation analysis.

The analysis of total As was described in our previous study (Zhang et al., 2015). The accuracy of our digestion method was evaluated by analyzing a tuna fish standard reference material (SRM) (BCR-627, Institute for Reference Materials and Measurements, Geel, Belgium). The total As recovery rate of the SRM was 99.6%. The As concentrations in the marine fish were expressed as nmol/g dry weight. The As speciation analysis was also described in our previous article (Zhang et al., 2015). The extraction efficiency and analysis method were evaluated through analysis of the tuna fish SRM. The BCR-627 tuna fish tissue (0.05 g) contained an average AsB concentration of 48.7 ± 2.54 nmol/g (94% recovery, n = 6). The measured dimethylarsinic acid (DMA) values were 1.47 ± 0.67 nmol/g (73% recovery, n = 6). Spikes were used to confirm the recovery of other As species detected during the speciation analysis. In our study, the recovery rates of As(III), As(V) and monomethylarsonic acid (MMA) were 81–91%, 91–95%, and 82–90%, respectively.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 16.0. The differences in the corresponding values in the different treatment groups were tested by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test. A probability level (p-value) of less than 0.05 was regarded as statistically significant.

3. Results and discussion

3.1. As speciation distributions in exposed food

Table 1 presents the concentrations of various As species and their percentages in the different fish diets. They contained different proportions of inorganic [As(III) and As(V)] and organic (MMA, DMA, and AsB) As compounds. The As(III)- and As(V)-exposed artificial diets exhibited a predominance of As(III) (53.1%) and As(V) (94.1%), respectively, whereas AsB was only about 0.49–0.54%. The clam diets exhibited a predominance of AsB (70.2–79.7%), followed by DMA (15.4–24.6%), whereas the abundances of inorganic [As(III) and As(V)] (3.39–3.82%) were low. Therefore, the artificial and clam diets contained different As species distributions.

3.2. As bioaccumulation and trophic transfer in fish

The total As concentration ranges were 33.8–118 nmol/g, 62.6–159 nmol/g, and 81.0–156 nmol/g in the intestine, liver, and muscle tissues of seabream and 37.5–115 nmol/g, 65.9–255 nmol/g, and 18.4–69.0 nmol/g in the intestine, liver, and muscle tissues of grunt, respectively (Table 2). With a few exceptions, the As concentrations in the intestine, liver, and muscle tissues of seabream and grunt feeding on exposed clam diets were significantly higher than in those feeding on exposed artificial diets. These results indicate that As bioaccumulation is related to the food type. We further calculated the newly accumulated As concentrations as the total