Biotransformation of inorganic arsenic in a marine herbivorous fish *Siganus fuscescens* after dietborne exposure

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**ABSTRACT**

Arsenic (As) is well known to be biodiminished along marine food chains. The marine herbivorous fish at a lower trophic level are expected to accumulate more As. However, little is known about how marine herbivorous fish biotransform the potential high As bioaccumulation. Therefore, the present study quantified the biotransformation of two inorganic As species (As(III) and As(V)) in a marine herbivorous fish *Siganus fuscescens* following dietborne exposure. The fish were fed on As contaminated artificial diets at nominal concentrations of 400 and 1500 µg As(III) or As(V) g−1 (dry weight) for 21 d and 42 d. After exposure, As concentrations in intestine, liver, and muscle tissues of rabbitfish increased significantly and were proportional to the inorganic As exposure concentrations. The present study demonstrated that both inorganic As(III) and As(V) in the dietborne phases were able to be biotransformed to the less toxic arsenobetaine (AsB) (63.3–91.3% in liver; 79.0–95.2% in muscle). The processes of As biotransformation in rabbitfish could include oxidation of As(III) to As(V), reduction of As(V) to As(III), methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), and subsequent conversion to AsB. These results also demonstrated that AsB synthesis processes were diverse facing different inorganic As species in different tissues. In summary, the present study elucidated that marine herbivorous fish had high ability to biotransform inorganic As to the organic forms (mainly AsB), resulting in high As bioaccumulation. Therefore, marine herbivorous fish could detoxify inorganic As in the natural environment.

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1. Introduction

Marine organisms have been shown to accumulate high concentrations of arsenic (As) (1–1000 µg g−1) in many studies (Maher and Batley, 1990; Francesconi and Edmonds, 1997, 1998). They accumulate and transform As species inside the body when exposed to As in the diet and other routes/sources such as water, soil, particles, etc. (Edmonds et al., 1997; Hasegawa et al., 2001; Suhendrayatna and Maeda, 2001). Therefore, arsenic species in marine organisms are not only as inorganic forms or methylated forms (monomethylarsonic acid (MMA), dimethylarsinic acid (DMA)), but also as a variety of complicated organic As forms, e.g. several As-containing ribosides in marine algae (Morita and Shibata, 1990; Francesconi and Edmonds, 1994, 1997, 1998), arsenobetaine (AsB) in marine fish *Fundulus heteroclitus*, *Terapon jarbua*, *Salmo salar*, and *Gadus morhua* (Amlund et al., 2006; Bears...
et al., 2006; Zhang et al., 2012).

However, the biosynthesis of AsB is not completely understood although it has been reported more than 30 years ago (Edmonds and Francesconi, 1977). It was postulated that arsenosugars (AsS) were precursors of AsB, and the finding of dimethylarsinousol, a possible intermediate, supported this hypothesis (Francesconi and Edmonds, 1994; Edmonds and Francesconi, 2003). In addition, since AsB was also found in the marine organisms living in the environments where algae were not the dominant food source (Kokkatiwong et al., 2009), it was hypothesized that other biosynthetic pathways (not from AsS) existed for AsB biosynthesis. In our recent study, we quantified the biotransformation of two inorganic As species (As(III) and As(V)) in a marine carnivorous juvenile grunt *T. jarbua* following waterborne and dietborne exposures for 10 d. We demonstrated that both inorganic As(III) and As(V) in the dietborne and waterborne phases were rapidly biotransformed to the less toxic AsB (89–97%) (Zhang et al., 2012). Therefore, in the present study, we speculated that As may also gradually biotransformed from inorganic forms into AsB in different tissues of marine herbivorous fish. Additionally, a common practice when investigating As compounds in marine organisms is to examine the muscle tissue only, which is usually high in AsB (Francesconi and Edmonds, 1993; Maher et al., 1999). It may result in an underestimation of the importance of other As compounds in other tissues. Therefore, a comprehensive analysis on As biotransformation in different tissues of rabbitfish was performed in the present study.

Rabbitfish (family Siganidae or Teuthididae) are a group of marine herbivorous fishes widely distributed around the world. They are considered to be excellent food fish and have been used for mariculture in the Mediterranean and Indo-Pacific Regions (Lam, 1974). Rabbitfish *Siganus fuscescens* is evaluated to be a good candidate for aquaculture because of its high tolerance to environmental stresses (Harahap et al., 2002). Thus, when facing high inorganic As in diets, whether AsB would be the main compound in the rabbitfish, and the pathway of As biotransformation in this marine herbivorous fish still needs to be established.

Therefore, the aims of this research were to investigate the biotransformation processes of As(III) and As(V) in different tissues of marine herbivorous fish *S. fuscescens* following dietborne exposure. We consequently analyzed As speciation to comprehend the mechanisms of As biotransformation and uncover if AsB would be also the main compound in marine herbivorous fish. Overall, we tried to link inorganic As exposure and its biotransformation in order to verify our hypothesis, the potential pathway for AsB formation.

2. Materials and methods

2.1. Fish and experimental design

Rabbitfish (6–7 cm in length) were obtained from a fish farm at Shenzhen, China. They were maintained in natural sand-filtered seawater (25 °C, 33%) and fed artificial diets (purchased from a feed company in Shenzhen, China) twice a day at 2% of their body weight. They were acclimated to the test conditions for 2 weeks prior to the exposure experiment.

As(III) and As(V) were added to the artificial diets as an aqueous solution of arsenite and arsonate (NaAsO₂ and Na₂HAsO₄·2H₂O, Sigma, USA), to achieve a nominal concentration of 400 and 1500 μg g⁻¹ diet, respectively. When the diet pellets were completely soaked within the As solution, they were dried at 60 °C for 1–2 h to constant weight. The diets were then stored at −20 °C in sealed polyethylene bags until they were used. The measuring concentrations of exposed artificial diets were 377.8, 1661.4, 369.4, 1474.9 μg g⁻¹ (dry weight), respectively. The As species concentrations and distribution in exposed artificial diets are shown in Table 1.

100 rabbitfish were exposed to 5 dietborne As treatments (control, 400 μg g⁻¹ As(III), 1500 μg g⁻¹ As(III), 400 μg g⁻¹ As(V), and 1500 μg g⁻¹ As(V)) individually. Fish were fed twice per day (1 h for each feeding) for 21 d or 42 d and any uneaten food and feces were removed to prevent negligible waterborne As exposure. The tanks were under a light:dark cycle of 12:12 h. At the end of the exposure, fish were starved for approximately 24 h to allow the depuration of gut contents. Fish from each treatment (*n* = 7–10) were then collected and placed in sealed polyethylene bags after washing off the seawater on the body surface. The standard length and wet weight of each individual fish were immediately measured, and then the fish were frozen at −80 °C for further analysis.

2.2. Total arsenic concentrations

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.

About 0.05–0.2 g of samples were weighted in 15 mL volumetric flasks and digested with 2 mL of concentrated HNO₃ (65%, analytical reagent grade, Fisher Scientific) in heating block at 80 °C for 24 h until clarification. After cooling, the samples were diluted to 5 mL with Milli-Q water. A blank digest was processed using the same procedure. The samples were analyzed for total As by using inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700 series). The standard solution was prepared by serial dilution from a stock solution (National China Standard, National Institute of Metrology, China). The accuracy of our digestion method was tested by analyzing the standard reference materials (SRM) BCR-627 Tune Fish Tissue (Institute for Reference Materials and Measurements, Geel, Belgium). The artificial diets were also digested and the total As concentrations were simultaneously measured. The total As recovery rate of the SRM was 100.3%. The As concentrations were expressed as μg g⁻¹ dry weight.

2.3. Arsenic speciation analysis

As speciation analysis was described in our previous article (Zhang et al., 2015). The extraction efficiencies and analysis methods were evaluated by the analysis of SRM tuna fish tissue. BCR-627 tuna fish tissue (0.05 g) was used for AsB and DMA analyses. The BCR-627 reference material contained an average AsB concentration of 3.68 ± 0.35 μg g⁻¹ (94% recovery of certified value, *n* = 3). The measured DMA values were 1.21 ± 0.26 μg g⁻¹ (81% recovery of certified value, *n* = 3). Spikes were used to confirm the recovery of other As species detected during speciation analysis. In our study, the recovery rates of As(III), As(V) and MMA were 85–92%, 81–95%, and 79–91%, respectively.

2.4. Statistical analyses

Statistical analyses were performed using SPSS version 16.0. The differences of the corresponding values among different treatments 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. Arsenic bioaccumulation

Table 2 shows the As bioaccumulation in the intestine, liver, and muscle tissues of marine rabbitfish after 21 d and 42 d dietborne exposure. There were significant increases in As bioaccumulation between the control and the dietborne exposure treatments. Tissue As concentrations increased proportionally to the dietborne As concentrations in the As(III) and As(V) exposure treatments after 21 d and 42 d exposure. In our recent study, although there were significant differences in As bioaccumulation between the control and the dietborne As exposed T. jarbua, total As concentrations accumulated by T. jarbua were not proportional to the As exposure dosage after 10 d exposure (Zhang et al., 2012). Our another study employed a radiotracer technique to quantify the As(V) assimilation in T. jarbua. We found that the calculated As assimilation efficiencies (AEs) were low and constant (2.5–4.3%) at different dietborne As concentrations (0.05–100 μg g⁻¹), suggesting that the dietborne AEs were independent of the As(V) concentrations in the artificial diets (Zhang et al., 2011), thus the As accumulation increased with its content in the diets. The results in the present study were consistent with our previous study. Arsenic is well known to be bioavailable along marine food chains, thus marine herbivorous fish at a lower trophic level could accumulate more As than carnivorous fish. Therefore, further study is needed to explore the differences of As bioaccumulation in marine herbivorous and carnivorous fish.

In the present study, there were no significant differences between As(III) exposed treatments and As(V) exposed treatments. Similarity in As concentrations of rabbitfish after different inorganic As exposure suggested that the ability of rabbitfish to assimilate and retain As was not related to inorganic As speciation. This result was inconsistent with our recent study about bioaccumulation of inorganic As in Bombay oyster Saccostrea cucullata. As bioaccumulation was higher in 1 mg L⁻¹ As(III) exposure than 1 mg L⁻¹ As(V) exposure, suggesting that As(III) may be more bioavailable than As(V) in oysters when facing high As exposure concentrations (Zhang et al., 2015). Additionally, in As(V) 400 μg g⁻¹, As(III) and As(V) 1500 μg g⁻¹ exposure treatments, As bioaccumulations in liver after 42 d exposure were significantly higher than that after 21 d exposure, which were not found in other exposure treatments, indicating that changes in time series only occurred in liver.

3.2. Relationships between total arsenic and arsenic speciation distribution

Fig. 1 shows the correlation between total As concentration and As speciation distribution (%) in rabbitfish after 21 d and 42 d exposure. Negative correlation was observed between total As concentration and organic As distribution, while positive correlation was observed between total As concentration and inorganic As distribution in intestine, indicating that inorganic As contributed to the accumulation of total As. Correspondingly, when environmental inorganic As increased, the percentages of inorganic As of the total As were significantly higher in intestine (23.2–43.4%) than those in liver (4.52–21.6%) and in muscle (5.09–14.0%) (Figs. 2 and 3). These results were similar with previous studies, concentrations of inorganic As (As(III) and As(V)) and the unextracted arsenicals present in the mussel Mytilus edulis tissue both increased as the total concentration in the tissue increases (Whaley-Martin et al., 2012). The higher accumulation of inorganic As in digestive tissues of benthic feeding fish has been reported by Maher et al. (1999). However, Hong et al. (2014) reported that concentrations of organic As in fish, bivalves, crab, shrimp, except for gastropods, were directly proportional to the total concentrations of As. In the present study, there were no significant differences between total As concentrations and organic/inorganic As distribution in liver and muscle tissues of rabbitfish. However, Bears et al. (2006) reported that a 3-fold increase in total As in fish liver after killifish (F. heteroclitus) exposure to As was accompanied by a dramatic decrease

Table 1

<table>
<thead>
<tr>
<th>As species concentrations (μg g⁻¹) and Distribution (%)</th>
<th>As(III) 400 μg g⁻¹</th>
<th>As(V) 400 μg g⁻¹</th>
<th>MMA</th>
<th>DMA</th>
<th>AsB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>190.3 ± 6.49</td>
<td>67.6 ± 4.62</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Liver</td>
<td>1005.9 ± 94.5</td>
<td>303.7 ± 8.09</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Muscle</td>
<td>22.5 ± 0.76</td>
<td>340.3 ± 8.55</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Total As concentrations in intestine, liver, and muscle tissues of marine rabbitfish after 21 d and 42 d dietborne exposure. Values are mean ± SD (n = 7–10).</th>
</tr>
</thead>
<tbody>
<tr>
<td>As concentrations (μg g⁻¹)</td>
</tr>
<tr>
<td>Intense</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>As(III) 400 μg g⁻¹</td>
</tr>
<tr>
<td>As(V) 400 μg g⁻¹</td>
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<td>As(III) 1500 μg g⁻¹</td>
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<td>Muscle</td>
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<td>As(III) 400 μg g⁻¹</td>
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<td>As(III) 1500 μg g⁻¹</td>
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<td>As(V) 1500 μg g⁻¹</td>
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<tr>
<td>42 d</td>
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<tr>
<td>As(III) 400 μg g⁻¹</td>
</tr>
<tr>
<td>As(V) 400 μg g⁻¹</td>
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<tr>
<td>42 d</td>
</tr>
<tr>
<td>As(III) 400 μg g⁻¹</td>
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<tr>
<td>As(V) 400 μg g⁻¹</td>
</tr>
<tr>
<td>As(III) 1500 μg g⁻¹</td>
</tr>
<tr>
<td>As(V) 1500 μg g⁻¹</td>
</tr>
</tbody>
</table>

Fig. 1. The relationship between total As concentrations and organic As distribution (%) in intestine of marine rabbitfish after 21 d and 42 d exposure.
in the relative percentage and absolute concentration of AsB, and an increase in percentages and absolute concentrations of the toxic species methylarsenicals (MMAs and DMAAs), As(III) and As(V).

3.3. Biotransformation of arsenic

3.3.1. Biotransformation between As(V) and As(III)

In both As(V) exposure treatments after 21 d and 42 d exposure, the concentrations and proportions of tissue As(III) increased significantly in intestine and muscle, indicating that As(V) reduction to As(III) occurred in intestine and muscle tissues of rabbitfish. However, the ability of As(V) reduction to As(III) in liver was related to exposure time and As(V) concentrations (Figs. 2 and 3; Tables 3 and 4). As(V) reduction was considered as a detoxification process by enzymatically reacting with GSH or nonenzymatically (Shiomi et al., 1996; Mrak et al., 2008).

The concentration of tissue As(V) increased significantly in intestine and liver in 1500 μg g⁻¹ As(III) treatments, and it increased significantly in muscle in 400 and 1500 μg g⁻¹ As(III) treatments after 21 d and 42 d exposure compared to the ones in the control treatment (Tables 3 and 4). These results demonstrated that As(III) could also be oxidized to As(V) in rabbitfish. Such results were seldom reported in fish, yet there were some results in other organisms. For instance, in our recent study on Bombay oyster S. cucullata, the concentration of tissue As(V) had a 20% increase in the As(III) exposed oysters, although its proportion was comparable to the control (Zhang et al., 2015). A higher intracellular proportion of As(V) was also observed in the freshwater green alga C. reinhardtii under As(III) exposure (Wang et al., 2013). As(III) was completely oxidized to As(V) no matter whether C. reinhardtii were pre-exposed to ampicillin or not (Wang et al., 2014). In a yellowstone thermoacidophilic eukaryotic alga

![Figure 2](image-url)
Cyanidioschyzon sp., both the oxidation and reduction between As(III) and As(V) were found in parallel (Qin et al., 2009). The mechanism of As(III) oxidation in organisms has been poorly understood. In the present study, it should be noted that in control rabbit fish, the ratios of As(III) to As(V) were closely to 1:1 in muscle, 1.1–1.9 in liver, and 0.2–1.9 in intestine (10 times difference) after 21 d and 42 d exposure (Tables 3 and 4), indicating that there was a balance between As(III) and As(V) at constant redox potential in muscle and liver, yet not in intestine. As an external ingestion part, intestine might be influenced by surrounding environment and intestinal microorganisms.

3.3.2. Biotransformation of inorganic As to methylated forms

In intestine, methylated As (MMA and DMA) accounted for a large percentages (17.9–56.0%), except the 1500 μg g⁻¹ As(V) treatment after 42 d exposure. However, the percentages of MMA and DMA were low in liver (3.5–27.8%) and muscle (2.0–10.2%) (Figs. 2 and 3). These observations might be due to that intestine had lower biotransformation ability of methylated forms to AsB than liver and muscle tissues. Moreover, the intestine is the first step of As biotransformation, facing the dietborne inorganic As directly, while the liver and muscle face the complex As forms (more proportion of organic As) absorbed through the intestine. Therefore, although the ability of methylation differences existed, methylation of As(III) to MMA and DMA might be a critical process of As biotransformation from the inorganic forms to the organic forms in intestine, liver, and muscle tissues of rabbit fish. According to previous studies, it has been well known that carnivorous fish and other organisms can convert the toxic inorganic As in their bodies into methylated forms. For example, the carnivorous killifish can accumulate As directly from water and partially biomethylate it (Kuroiwa et al., 1994). It has been revealed that bacteria (Klebsiella

![Fig. 3. The proportion of different As species (%) in the rabbitfish after dietborne As exposure for 42 d. Data are mean ± SD (n = 6). As(III), arsenite; As(V), arsenate; MMA, monomethylarsonate; DMA, dimethyarsinate; AsB, arsenobetaine.](image-url)
oxytoca and Xantthomonas sp.), eukaryotic algae (Cyanidioschyzon sp.), phytoplankton (C. reinhardtii), and protozoan (Trachelomycetes thermophile) have the ability to biotransform inorganic As species to methylated forms (MMA and DMA) (Maeda et al., 1992; Qin et al., 2009; Yin et al., 2011; Wang et al., 2013). Therefore, As(V) reduction (from As(V) to As(III)) and subsequent methylation (from inorganic As to MMA and DMA) are the two steps of biotransformation in some aquatic organisms (Vahter, 2002).

3.3.3. Biotransformation to AsB

Rabbitfish exhibited a predominance of AsB (57.3–89.77%, 51.6–92.3% in liver; 80.6–89.5%, 79.0–95.2% in muscle for 21 d and 42 d, respectively). AsB was predominated in the intestine of rabbitfish (<0.5%) after 400 and 1500 μg g⁻¹ As(V) exposure for 21 d and 42 d. However, it was not the predominant compounds after 1500 μg g⁻¹ As(III) treatment for 21 d (16.8%) or after 400 μg g⁻¹ As(III) treatment for 42 d (24.5%) (Figs. 2 and 3). Therefore, the present study demonstrated that rabbitfish was apt to subsequently biotransform MMA and DMA to AsB, one of the less toxic and reactive As species. It has been found that the less-toxic AsB often constituted more than 95% of all As compounds accumulated in marine fish (Francesconi and Edmonds, 1997). Simultaneously, the biosynthesis of AsB compounds in the marine and freshwater food chains has also been reported in a number of studies (Hanakka et al., 1995; Ochsenkühn-Petropulu et al., 1997; Goessler et al., 1998; Francesconi et al., 2000).

In the present study, AsB might be elevated in different strategies by As(III) and As(V) exposure in different tissues. In intestine, AsB levels and proportions were all significantly higher in 1500 μg g⁻¹ As(V) treatment than 1500 μg g⁻¹ As(III) treatment after 21 d, and its percentages were generally less than 50%. In liver, AsB levels and proportions were significantly higher in 1500 μg g⁻¹ As(V) treatment than 1500 μg g⁻¹ As(III) treatment after 21 d and 42 d, and its percentages ranged from 51.6% to 91.3%. In muscle, with a few exceptions, AsB levels and proportions were significantly lower in As(V) exposure than As(III) exposure, and AsB was predominant among all the treatments (Figs. 2 and 3; Tables 3 and 4). These results demonstrated that AsB synthesis processes were diverse after As(III) and As(V) exposure in different tissues. As(V) could induce more AsB than As(III) did in intestine and liver, but it was on the contrary in muscle. The proportion of AsB to the total As was increased with the digestive and transport process.
(diet-intestine-liver-muscle). It indicated that the biotransformation of AsB could be mediated by the microorganisms in the intestine, catalyzed by enzymes in liver and muscle, and finally stored in the mass storage tissue, muscle. The detail mechanisms of AsB formation in different tissue of rabbitfish needs to be explored in the future. Amlund et al. (2006) found that after three months of dietborne AsB exposure, AsB was the major As species in Atlantic salmon (Salmo salar L) and Atlantic cod (G. morhua L) muscle, representing more than 99% of total As presented. Kirby et al. (2005) reported that AsB was found to account for 90% of the As extracted from the muscle tissue of macrualgae-feeding marine animals, while 40% was present as AsB in the digestive gland. Besides, the presence of lower amounts of AsB in intestinal tissue, relative to muscle, was also demonstrated by Edmonds et al. (1997) in abalone Haliotis roreti. As compared to control, a significant decrease in AsB percentage was noticed in muscle tissue after As(III) and As(V) exposure, suggesting that the biotransformation capacity of muscle decreased when facing high inorganic As.

To date, the AsB synthesis pathways in marine fish has not been conclusively demonstrated. In the present study, we speculated that there was one potential way of AsB formation in rabbitfish. In the experimental conditions, rabbitfish was exposed to the inorganic As, thus the detected AsB in fish tissues should be biotransformed through inorganic As methylation and subsequently conversion to AsB. Previously, Edmonds and Francesconi (1987) suggested that a possible scheme for the conversion of inorganic As(V) to AsB. Thomas et al. (2004) demonstrated that As(V) is first converted into As(III) and then transformed into mono-, di-, and trimethylated products. Zhang et al. (2012) reported that inorganic As(III) and As(V) in the dietborne and waterborne phases were biotransformed to AsB in T. jarbua. Besides, several other pathways were proposed for the formation of AsB in previous study. Firstly, the formation of AsB involved the biotransformation of arsenobetaine to AsB (Edmonds, 2000; Edmonds and Francesconi, 2003; Ritchie et al., 2004). The main pathway is thought to be through the degradation of dimethylarsenobetaine to dimethylysinylethanol (DMAE), which is further converted into either dimethylarsinylacetic acid (DMAA) or arsenocholine (Asc). Further oxidation of Asc at the primary alcohol group results in the formation of AsB. Secondly, dimethylarsinylribosides and trimethylarsonioribosides have been assumed to be the precursors of AsB within the marine food chain (Francesconi and Edmonds, 1993; Edmonds and Francesconi, 2003). Thirdly, Edmonds (2000) has proposed an alternative pathway for the synthesis of AsB within marine organisms. He suggested that DMA might replace ammonium ions in the biosynthesis of amino acids. Thereby, ‘arsenylation’ of pyruvate, paralleling the biosynthesis of alanine, or glyoxylate could lead to the synthesis of AsB. However, the synthesis of AsB in marine fish has not been completely explained. Further research is thus needed to provide useful information on the formation of AsB in the marine herbivorous fish.

4. Conclusion

To our knowledge this is the first study to assess the biotransformation and AsB formation by different inorganic As (As(III) and As(V)) exposure in marine herbivorous fish. Arsenic biocaccumulation in rabbitfish increased significantly and proportionally to the inorganic As exposure concentrations. These results demonstrated that AsB was probably an end product of As biotransformation in marine herbivorous fish, and AsB was apt to be accumulated in the body rather than excreted in rabbitfish. Our findings highlighted one pathway of As biotransformation (the formation of AsB), including reduction of As(V) to As(III), methylation to MMA and DMA, and subsequent conversion to AsB in rabbitfish. These results also demonstrated that AsB synthesis processes were diverse facing different inorganic As species in different tissues. More studies are required to characterize the biotransformation, mainly the formation pathways of AsB, in marine herbivorous fish.

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