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Arsenic species and their accumulation features in green turtles (*Chelonia mydas*)

Tetsuro Agusa, Kozue Takagi, Hisato Iwata, Shinsuke Tanabe *

Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan

Abstract

Total arsenic (As) and its compounds were determined in liver, kidney, muscle, and stomach contents of green turtles (*Chelonia mydas*). Total As concentrations in the muscle were higher than those in the kidney and liver. Arsenobetaine (AB) was the predominant compound in all the three tissues and its levels were positively correlated with total As concentrations. This indicates that AB greatly contributes to As accumulation in green turtles. Higher concentrations of remaining As in the sample after extraction were detected in the liver, implying that lipid-soluble or protein bound As compounds accumulate in the liver of green turtles. Total As levels in tissues showed significant negative correlations with standard carapace length. The size-dependence of As accumulation in green turtles may be related to their feeding habit, shifting from carnivore to herbivore at different growth stages. Concentrations of AB and dimethylarsinic acid (DMA) were low in the stomach contents but high in the tissues, implying bioaccumulation of these arsenicals in green turtles.

Keywords: Arsenic; Arsenic compounds; Green turtle; Size-dependent accumulation; Bioaccumulation

1. Introduction

It is well known that arsenic (As) exists in various chemical forms in the marine environment, and its bioavailability and toxicity depend on speciation. Thus, chemical analysis of As compounds is needed to understand the behavior or risk of As in the marine environment. Although concentrations of As in marine animals and sea algae are comparable, speciation of As is greatly different between both phyla: in marine animals, arsenobetaine (AB) is the predominant As compound, while the major As form in sea algae is arsenoribosides (arsenosugars) (Francesconi and Edmonds, 1993, 1997). Furthermore, arsenocholine (AC), tetramethylarsonium ion (TETRA), trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), As[V], and As[III] are also detected in marine animals as minor As species (Francesconi and Edmonds, 1993, 1997). On the other hand, studies regarding As speciation in high-order marine animals such as marine mammals and sea birds, especially sea turtles, are still lacking.

Sea turtles are widely distributed from tropical to temperate waters in the world. Green turtles (*Chelonia mydas*) inhabit Japanese coastal waters (Kamezaki and Matsui, 1997). Some studies have addressed accumulation of contaminants such as trace elements and organic pollutants in green turtles because of the concern on their decreased population by chemical contamination (Aguirre et al., 1994; Gordon et al., 1998; McKenzie et al., 1999; Miao et al., 2001; Lam et al., 2004). Our previous studies have reported accumulation features of trace elements including As in green turtles (Saeki et al., 2000; Sakai et al., 2000a,b; Anan et al., 2001; Kubota et al., 2002a, 2003a,b; Fujihara et al., 2003). However, limited data are available on As speciation in green turtles and the sample size in the previous studies was small ($n = 5$) (Kubota et al., 2002a, 2003a,b; Fujihara et al., 2003). In the present study, total As and its compounds were determined in liver, kidney, and

* Corresponding author. Tel./fax: +81 89 927 8171.
E-mail address: shinsuke@agr.ehime-u.ac.jp (S. Tanabe).

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muscle of 20 green turtles collected from Ishigaki Island, Okinawa, Japan to understand the accumulation of As. Also, stomach contents were analyzed to make clear the bioaccumulation of As in green turtles.

2. Materials and methods

2.1. Sample details

Green turtles ($n = 20$) from Ishigaki Island, Okinawa, Japan in November 2000 and January 2005 were caught by fishermen under permission for commercial use and scientific. Standard carapace length (SCL) along a straight line, carapace width (CW), and body weight (BW) were measured and sex was confirmed before dissection (females, $n = 17$ and males, $n = 3$). The means of SCL, CW, and BW were 43.4 cm (range: 38.6–53.4 cm), 37.2 cm (33.0–43.9 cm), and 10.2 kg (3.3–19.8 kg), respectively. Kidney ($n = 10$), liver ($n = 20$), muscle ($n = 20$), and stomach contents ($n = 3$) were collected and were kept at −80 °C in the Environmental Specimen Bank (*es*-BANK) for global monitoring at Center for Marine Environmental Studies (CMES), Ehime University, Japan (Tanabe, 2006) until chemical analyses.

2.2. Analysis of total As

Analysis of total As concentration was conducted following the method of Kubota et al. (2001) with a slight modification. Kidney, liver, muscle, and stomach contents were freeze-dried and uniformly homogenized. About 0.05–0.15 g of freeze-dried tissue was digested with acid mixture (HNO_3:H_2SO_4:$HClO_4$ = 5 ml:5 ml:10 ml) by heating at over 300 °C. Total As concentration was measured by a hydride generation (HVG-1 Hydride System, Shimadzu, Kyoto, Japan) – atomic absorption spectrometry (AA680, Shimadzu, Kyoto, Japan) (HG-AAS). Accuracy of these analyses was checked by using a standard reference material, dogfish muscle (DORM2) provided by National Research Council Canada (NRCC). The precision of this procedure was more than 95% for total As concentration.

2.3. Analysis of As compounds

Arsenic compounds were extracted by methanol/Milli-Q water (9:1 v/v) from powdered freeze-dried samples (0.05–0.15 g) (Kubota et al., 2002a). Supernatant was concentrated until methanol was evaporated. After evaporation, samples were diluted by Milli-Q water and then filtered by a 0.20 μm filter. Arsenic that was remaining in the sample after extraction (residue As) was measured by HG-AAS after acid digestion mentioned above. A Hamilton PRP-X100 anion-exchange column (Hamilton, Reno, NV, USA; $NH_4H_2PO_4$ buffer, pH 6.0) and a Supelcosil LC-SCX cation-exchange column (Supelco, Bellefonte, PA, USA; pyridine buffer, pH 2.6) were used to separate the As compounds. As internal standards, Rb and Rh were added to both mobile phases to monitor analytical interference. Eight As compounds (AB, AC, TETRA, TMAO, DMA, MMA, As[III], and As[V]) were measured by a high-performance liquid chromatography (HPLC; Shimadzu, LC10A Series, Kyoto, Japan)/inductively coupled plasma mass spectrometry (ICP-MS; HP4500, Hewlett–Packard, Avondale, PA, USA) (HPLC/ICP-MS). Results of accuracy for As compounds in this study were in general agreement with the NRCC and other studies (Goessler et al., 1998; Mattusch and Wennrich, 1998; Kuehnelt et al., 1997; Kubota et al., 2002a). In this study, the concentrations of As compounds were represented in units of μg As/g dry wt. The sum of eight As species and residual As was shown as ΣAs.

2.4. Analysis of statistics

One half of the value of the respective limit of detection was substituted for those values below the limit of detection and was used in statistical analyses. Variables with less than 50% of detection rates were not used in this study. All available data was tested by Kolmogorov–Smirnov's one sample test and by drawing a histogram, and was confirmed to be normal distribution. Pearson's correlation coefficients and regression analyses were used to assess the strength of the association between As levels and SCL and between concentrations of As compounds. Tukey–Kramer method, along with one-factor ANOVA, was conducted to detect differences in concentrations of As in kidney, muscle, and liver. A p value of less than 0.05 was considered to indicate statistical significance in this study. StatView (version 5.0 for windows, SAS® Institute, Cary, NC, USA) and SPSS (version 12.0 for windows, SPSS Japan, Tokyo, Japan) were used for all statistical analyses in this study.

3. Results and discussion

3.1. Total As in tissues

Total As concentrations in the muscle, kidney, and liver of green turtles are shown in Fig. 1 and Table 1. Arsenic was detected in all the tissues of green turtles and its levels were 0.94–165 μg/g dry wt. Although concentrations of As in kidney and liver were comparable ($p > 0.05$), remarkably high accumulation of As was observed in muscle compared to kidney ($p < 0.05$) and liver ($p < 0.05$). Previous studies on As in sea turtles also reported relatively high accumulation of As in the muscle (Saeki et al., 2000; Storelli and Marcotrigiano, 2000; Agusa et al., in press). On the other hand, concentrations of As in muscles were lower than those in liver or kidney of marine mammals (Muir et al., 1988; Ebisuda et al., 2002; Kubota et al., 2005) and sea birds (Kubota et al., 2002b). Therefore, high concentrations in the muscle of green turtles might indicate species-specific accumulation of As (for more discussion, see Agusa et al., in press). Concentrations of As in muscle and kidney showed a positive

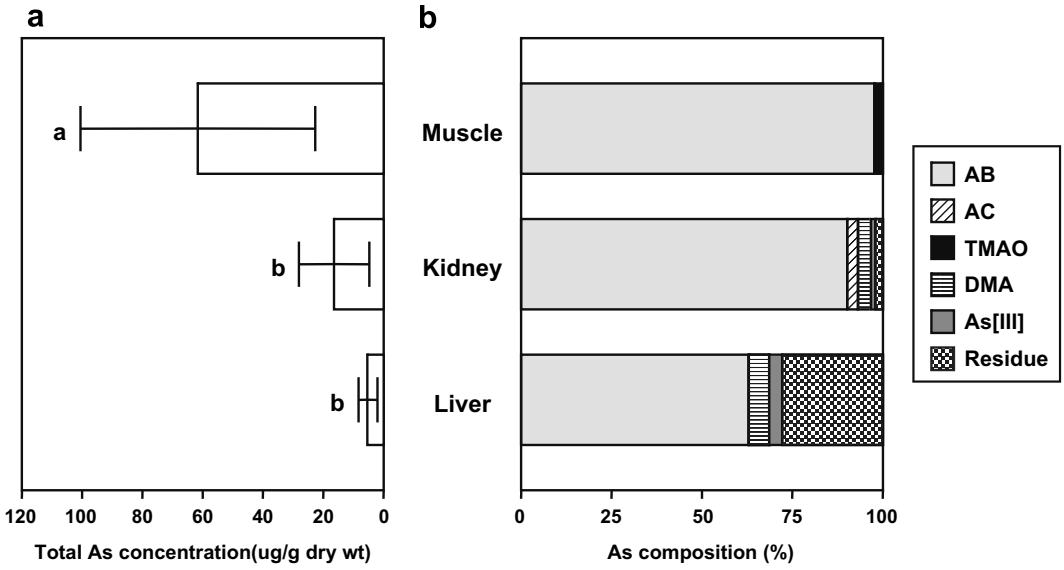

Fig. 1. (a) Concentrations (mean and SD) of total As and (b) compositions (mean) of As compounds (arsenobetaine, AB; arsenocholine, AC; trimethylarsine oxide, TMAO; dimethylarsinic acid, DMA; arsenite, As[III]; As in residue of the tissues after extraction; residue) in muscle, kidney, and liver of green turtles. Concentrations with the same letter are not significantly different at $p < 0.05$.

Table 1
Concentrations (mean and range, μg/g dry wt) of total As in tissues of green turtles from various locations

Location	Muscle	Kidney	Liver	References
Ishigaki Island, Japan ($n = 20$)	61.6 (11.2–165)	16.5 (4.6–44.3)	5.3 (0.9–9.7)	This study
Ishigaki Island, Japan ($n = 5$)			4.0 (2.7–7.7)	Fujihara et al. (2003)[a]
Yaeyama Islands, Japan ($n = 19$)	24.1 (2.58–74.9)	5.72 (0.15–9.99)	1.76 (0.44–5.34)	Saeki et al. (2000)
Yaeyama Islands, Japan ($n = 5$)			3.65	Kubota et al. (2003b)
Hawaian Islands ($n = 13$)		(<2.0–22.7)	(<2.0–21.3)	Aguirre et al. (1994)[a]
Moreton Bay, Australia ($n = 23$)		0.63 (0.00–2.30)	0.87 (0.13–2.47)	Gordon et al. (1998)[a]
South China Sea (Adults, $n = 3$)	14.61		19.57	Lam et al. (2004)
South China Sea (Juvenile, $n = 2$)	14.45	6.97	4.65	Lam et al. (2004)

[a] Wet weight concentration was converted to dry weight concentration assuming that moisture content was 70%.

correlation ($r = 0.831$, $p < 0.01$). However, there were no significant correlations ($p > 0.05$) between total As levels in liver and muscle, and liver and kidney.

3.2. As compounds in tissues

Compositions of As compounds in muscle, kidney, and liver of green turtles are shown in Fig. 1. Arsenobetaine was major As compound in the three tissues, especially in muscle (more than 97% to ΣAs). Concentrations of AB were the highest in the muscle ($p < 0.05$). A positive linear correlation between concentrations of AB and ΣAs was observed in all tissues ($R^2 = 0.998$, $p < 0.001$, $[\Sigma As] = 1.0 \times [AB] + 1.5$) (Fig. 2). Also, tissues of green turtles with higher concentrations of ΣAs contained higher percentages of AB (Fig. 2). These results suggest that AB largely contributes to the accumulation of As in green turtles and this was consistent with previous studies conducted in marine mammals and sea birds (Fujihara et al., 2003, 2004; Kubota et al., 2003b). Similar to results of total As, positive correlation between concentrations of AB in muscle and kidney was found ($r = 0.932$, $p < 0.001$), but not for liver–muscle and liver–kidney. These results were different from the observations in black-footed albatrosses (Fujihara et al., 2004), black-tailed gulls (Kubota et al., 2002b), and ringed seals (Ebisuda et al., 2002) which showed positive correlations among AB concentrations in muscle, kidney, and liver. Therefore, tissue distribution of AB in green turtles might be different from that in other higher trophic marine animals such as marine mammals and sea birds.

For other arsenicals, DMA was detected in muscle, kidney, and liver (Fig. 1) and the levels were the highest in kidney ($p < 0.05$). In muscle and liver, concentrations of DMA showed positive correlations with those of ΣAs (muscle: $r = 0.630$, $p < 0.05$; liver: $r = 0.615$, $p < 0.05$), indicating that DMA also influences As accumulation in green turtles although the contribution is less than AB. Arsenocholine levels were high in the kidney but not detected in liver and muscle of almost all specimens. Although the levels were low, As[III] was detected in all the three tissues. Concentrations of TETRA, TMAO, MMA, and As[V]

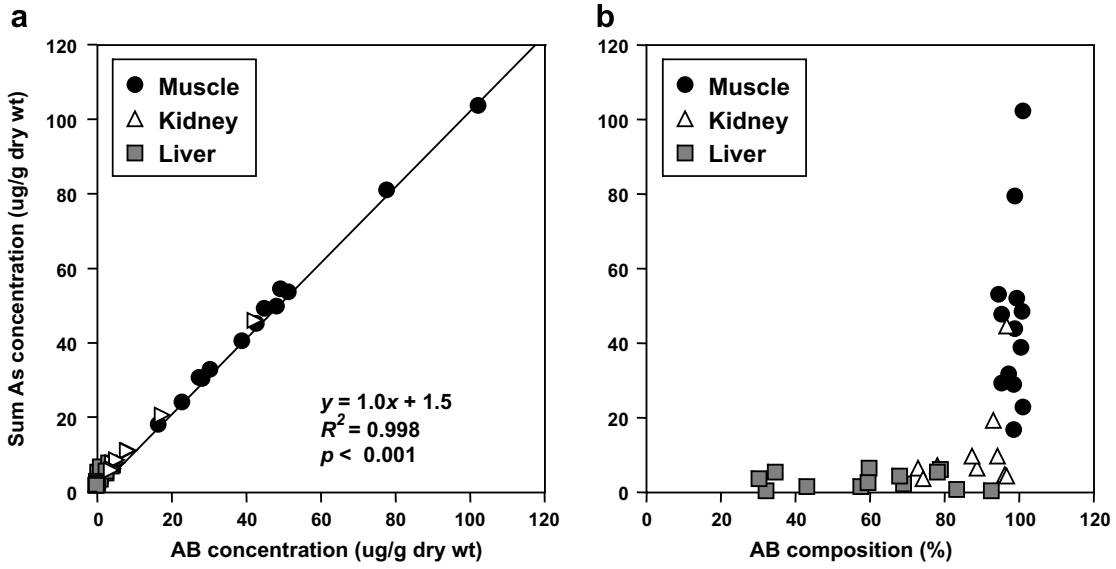

Fig. 2. Relationships (a) between concentrations of arsenobetaine (AB) and ΣAs, and (b) between percentage of AB to ΣAs concentration in muscle, kidney, and liver of green turtles.

were quite low or below detection limits in this study. Therefore, these compounds are not considered in further discussion.

In this study, significant concentrations of As were detected in residue of the tissues after methanol/water extraction (Fig. 1) and the levels were remarkably high in the liver ($p < 0.05$). Lipid-soluble As compounds and trivalent As compounds which bind to proteins may exist in this fraction (Francesconi et al., 1994; Styblo et al., 1996). It is known that trivalent arsenicals such as As[III], methylarsonous acid, and dimethylarsinous acid have high toxicity and show high affinity with –SH groups in proteins. Especially, cytotoxicity or genotoxicity of trivalent organic arsenicals are known to be higher than other As species (Petrick et al., 2000; Styblo et al., 2000; Mass et al., 2001). Further studies on identification of As compounds in the residue fraction and assessment of the toxicological impacts in green turtles are required.

3.3. Regional difference in As concentrations

To understand regional difference in As levels, concentrations of total As in the present study were compared to those from other locations (Table 1). Concentrations of total As in muscle, kidney, and liver of green turtles in this study were higher or comparable to those in other regions (Aguirre et al., 1994; Gordon et al., 1998; Saeki et al., 2000; Fujihara et al., 2003; Kubota et al., 2003b) except for the result on liver by Lam et al. (2004). Total As levels in kidney and liver of green turtles from Australia (Gordon et al., 1998) were much lower than those in this study and other studies. It has been reported that mean concentrations of As in seawater from Pacific Ocean and South Australia were 1.8 μg/l (1.6–2.1 μg/l) and 1.3 μg/l (1.1–1.6 μg/l) (WHO, 2001), respectively, suggesting non-significant regional difference in background As levels in seawater. Therefore, the different composition of prey may be associated with As accumulation in green turtles from each location. Indeed, based on analyses of the stomach contents, Bjorndal (1997) has shown that feeding habit of green turtles may be different in each habitat.

On the contrary in studies on total As accumulation, there were few available data on As speciation in green turtles. Fujihara et al. (2003) and Kubota et al. (2003b) have reported accumulation features of As compounds in liver of green turtles from Yaeyama and Ishigaki Islands, Japan and the results showed that AB was the predominant species of As. Relatively high concentrations of residual As in liver of green turtles were observed in their studies (Fujihara et al., 2003; Kubota et al., 2003b). Hepatic concentration and composition of As compounds in this study were similar to those in previous studies (Fujihara et al., 2003; Kubota et al., 2003b).

3.4. Size-dependent accumulation of As

Since there is no method to determine age of green turtles, biological information such as SCL, CW, and BW were used as indicators of growth stages. Positive correlations were observed among SCL, CW, and BW, ($p < 0.001$, respectively) and thus we used SCL as a representative indicator of age. A significant negative correlation was found between total As concentrations in liver and SCL of green turtles ($p < 0.01$, $R^2 = 0.321$, $[\text{Total As}] = 6.8 \times 10^8 \times [\text{SCL}]^{-5.0}$) (Fig. 3). Furthermore, relationship between muscular total As levels and SCL was negative but not statistically significant ($p > 0.05$). For kidney, no clear correlation was found, possibly due to the small sample size ($n = 10$). Negative correlation between total As concentrations and SCL was also found in green

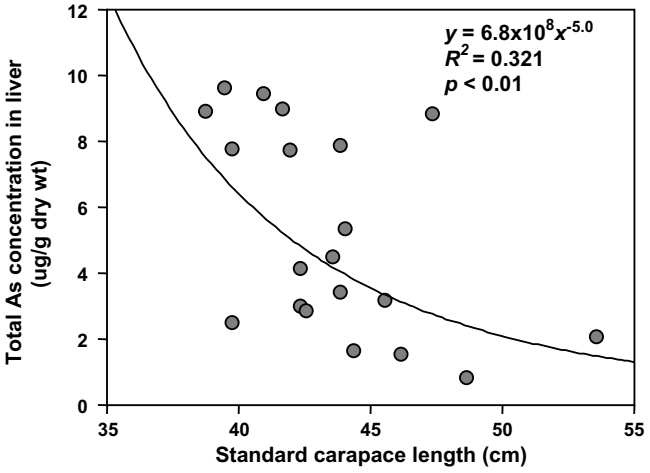

Fig. 3. Relationship between total As concentrations in liver and standard carapace length of green turtles.

turtles from Yaeyama, Japan (Saeki et al., 2000). The variations of trace element accumulation with growth are influenced by various factors such as metabolic and feeding rates, reproductive stage, and growth dilution of the elements (Langston and Spence, 1995). For green turtles, the growth-dependent variation in As concentrations might be partially influenced by changes in feeding habit with growth stage. Juveniles of green turtles are carnivorous, while adults are herbivorous (Bjorndal, 1997). Predominant compounds of As were AB in zooplankton and arsenosugars in algae, while total As concentrations in both the organisms were similar (Francesconi and Edmonds, 1993). Although AB is easily absorbed through the digestive tract (Vahter et al., 1983; Yamauchi et al., 1986), absorption efficiencies of arsenosugars are low (Shiomi et al., 1990). Sakai et al. (2000b) also reported that concentrations of Cd in green turtles decreased with SCL and explained this as a result of different feeding habits between juveniles and adults; zooplankton, which is a primary diet for juveniles, contains much higher Cd concentrations than sea plants, the diet of adult green turtles. Therefore, growth-dependent decrease of total As concentrations in green turtles may be influenced by the change in prey items with growth.

3.5. Bioaccumulation of As compounds

Accumulation of As in marine animals is generally related to consumption of their diets (Maher and Butler, 1988). In this study, AB was the predominant compound in green turtles as referred to above (Figs. 1 and 2). It is interesting where AB in green turtles comes from, because green turtles feed on sea algae, which contains mainly arsenoribosides as total As (Francesconi and Edmonds, 1993, 1997). Recently, Nischwitz and Pergantis (2005) detected trace levels of AB in sea algae using a HPLC electrospray tandem mass spectrometry for the first time. In addition, green turtles may consume some small marine animals which contain AB together with marine algae and sea grasses (Bjorndal, 1997). Indeed, AB was detected in stomach contents of green turtles in this study (Fig. 4). Therefore, it is considered that green turtles ingest AB through ingestion of their diet. However, concentrations of AB detected in stomach contents were low (mean, 0.22 μg/g dry wt), whereas those in muscle (mean, 45.6 μg/g dry wt), kidney (mean, 11.3 μg/g dry wt), and liver (mean, 2.42 μg/g dry wt) of green turtles were high compared to marine mammals (Ebisuda et al., 2002; Kubota et al., 2003a,b, 2005). To understand trophic transfer efficiency of AB in green turtles, bioaccumulation factors of AB were calculated as ratios of AB concentrations in tissues (muscle, kidney, and liver) to those in

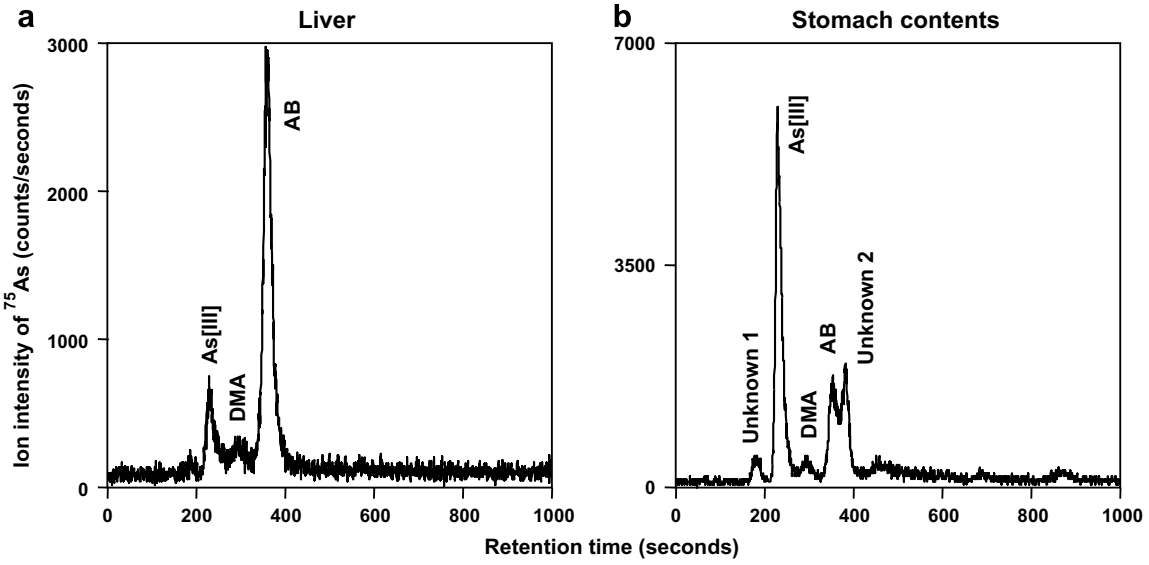

Fig. 4. HPLC/ICP-MS chromatograms of As compounds (arsenite, As[III]; dimethylarsinic acid, DMA; arsenobetaine, AB) in extracts from (a) liver and (b) stomach contents of green turtles obtained with Supelcosil LC-SCX cation-exchange column (mobile phase, 6.7 mM aqueous pyridine at pH 2.6; injection volume, 100 ml; flow rate, 1.5 ml/min).

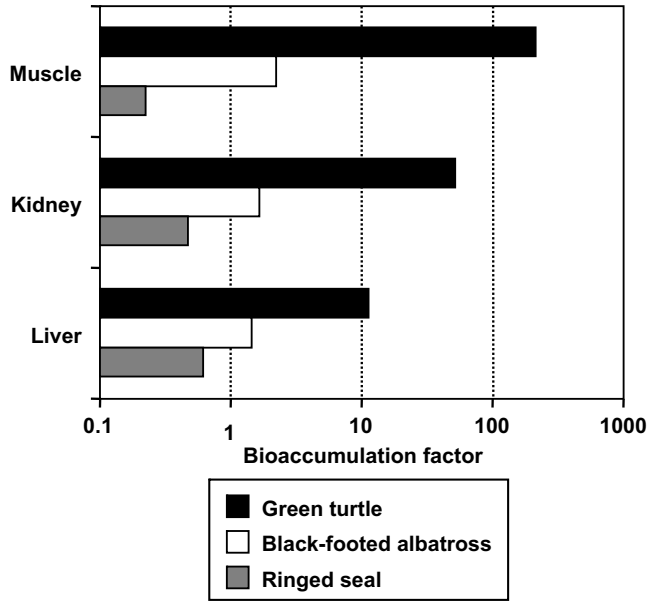

Fig. 5. Bioaccumulation factors (mean) of arsenobetaine (AB) in muscle, kidney, and liver of green turtles (this study), black-footed albatrosses (Fujihara et al., 2004), and ringed seals (Ebisuda et al., 2002). Bioaccumulation factors were defined as ratios of AB concentrations in muscle, kidney, and liver to those in stomach contents.

stomach contents (Fig. 5). Since data on AB concentrations in muscle, kidney, liver, and stomach contents are available for black-footed albatrosses (Fujihara et al., 2004) and ringed seals (Ebisuda et al., 2002), these bioaccumulation factors are also shown in Fig. 5 for comparison among higher-order marine animals. Interestingly, bioaccumulation factors of AB in tissues (210 in muscle, 52 in kidney, and 11 in liver) of green turtles were above one and were much higher than those of black-footed albatrosses (2.2 in muscle, 1.7 in kidney, and 1.4 in liver) and ringed seals (0.2 in muscle, 0.5 in kidney, and 0.6 in liver). It is well known that ingested AB is rapidly excreted through urine in rodents and humans (Shiomi, 1994; Vahter, 1994). These indicate that green turtles may have a specific mechanism for accumulation of AB; they may effectively absorb AB in diet and then accumulate AB in the body. Apart from direct intake of AB, accumulation of AB in green turtles may also result from transformation of arsenoribosides by green turtles themselves or by microorganisms in the intestine. Several pathways of biotransformation of arsenoribosides to AB have been suggested in several studies (Edmonds and Francesconi, 2003; Foster et al., 2006; Francesconi et al., 1998; Kirby et al., 2005; Shiomi et al., 1990). On the other hand, AB may be accumulated in higher-order marine animals including green turtles as an osmolyte along with glycine betaine, which is a predominant osmolyte in marine animals because the chemical structure and properties of AB are similar to those of glycine betaine (Fujihara et al., 2003).

Bioaccumulation factors of AB in muscle, kidney, and liver of black-footed albatrosses were above one (Fig. 5), suggesting the bioaccumulation of AB. On the other hand, lower bioaccumulation factors were observed in ringed seals (Fig. 5), thus ringed seals might not bioaccumulate AB through the ingestion of prey items and might rather excrete AB.

Other As compounds were also detected in stomach contents of green turtles (Fig. 4). Arsenite was detected in stomach contents at high concentration (mean, 1.71 μg/g dry wt) (Fig. 4), while concentrations of As[III] in muscle (mean, 0.04 μg/g dry wt), kidney (mean, 0.15 μg/g dry wt), and liver (mean, 0.14 μg/g dry wt) were much low (Figs. 1 and 4). Therefore, green turtles might be exposed to As[III] from the diet but available to metabolize As[III] and excrete rapidly from the body. Concentrations of DMA detected in stomach contents (mean, 0.02 μg/g dry wt) were lower than those in muscle (mean, 0.09 μg/g dry wt), kidney (mean, 0.45 μg/g dry wt), and liver (mean, 0.21 μg/g dry wt) (Figs. 1 and 4), and the bioaccumulation factors (2.2 in muscle, 1.7 in kidney and 1.4 in liver) were more than one, suggesting that green turtles might bioaccumulate DMA.

In this study, two unknown peaks were detected in the stomach contents but not in the muscle, kidney, and liver (Fig. 4). Although these unknown arsenicals in stomach contents of green turtles might be arsenoribosides, further analysis is needed to confirm. Biotransformation of arsenoribosides to AB has been suggested in previous studies (Shiomi et al., 1990; Francesconi et al., 1998; Edmonds and Francesconi, 2003; Kirby et al., 2005; Foster et al., 2006). If these unknown arsenicals are identified as arsenoribosides, it may indicate that green turtles or their intestinal bacteria have capacity to convert arsenoribosides to AB. In future, measurement of arsenoribosides and verification of transformation of arsenoribosides are required to understand accumulation mechanism of As, especially AB in green turtles.

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References

Aguirre, A.A., Balazs, G.H., Zimmerman, B., Galey, F.D., 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia*

mydas) afflicted with fibropapillomas in the Hawaiian islands. Marine Pollution Bulletin 28, 109–114.

Agusa, T., Takagi, K., Anan, Y., Kubota, R., Iwata, H., Tanabe, S., in press. Specific accumulation of arsenic compounds in tissues of hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Ishigaki Island, Japan. Environmental Pollution. Available online 28 August 2007.

Anan, Y., Kunito, T., Watanabe, I., Sakai, H., Tanabe, S., 2001. Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. Environmental Toxicology and Chemistry 20, 2802–2814.

Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC, Boca Raton, FL, USA, pp. 199–231.

Ebisuda, K.-I., Kunito, T., Kubota, R., Tanabe, S., 2002. Arsenic concentrations and speciation in the tissues of ringed seals (*Phoca hispida*) from Pangnirtung, Canada. Applied Organometallic Chemistry 16, 451–457.

Edmonds, J.S., Francesconi, K.A., 2003. Organoarsenic compounds in the marine environment. In: Craig, P.J. (Ed.), Organometallic Compounds in the Environment. John Wiley and Sons, New York, USA, pp. 195–222.

Foster, S., Maher, W., Schmeisser, E., Taylor, A., Krikowa, F., Apte, S., 2006. Arsenic species in a rocky intertidal marine food chain in NSW, Australia, revisited. Environmental Chemistry 3, 304–315.

Francesconi, K.A., Edmonds, J.S., 1993. Arsenic in the sea. Oceanography Marine Biology Annual Review 31, 111–151.

Francesconi, K.A., Edmonds, J.S., Morita, M., 1994. Determination of arsenic and arsenic species in marine environmental samples. In: Nriagu, J.O. (Ed.), Arsenic in the Environment. Part I: Cycling and Characterization. John Wiley, New York, NY, USA, pp. 189–219.

Francesconi, K.A., Edmonds, J.S., 1997. Arsenic and marine organisms. Advanced Inorganic Chemistry 31, 111–151.

Francesconi, K.A., Goessler, W., Panutrakul, S., Irgolic, K.J., 1998. A novel arsenic containing riboside (arsenosugar) in three species of gastropod. Science of the Total Environment 221, 139–148.

Fujihara, J., Kunito, T., Kubota, R., Tanabe, S., 2003. Arsenic accumulation in livers of pinnipeds, seabirds and sea turtles: subcellular distribution and interaction between arsenobetaine and glycine betaine. Comparative Biochemistry and Physiology – C Toxicology and Pharmacology 136, 287–296.

Fujihara, J., Kunito, T., Kubota, R., Tanaka, H., Tanabe, S., 2004. Arsenic accumulation and distribution in tissues of black-footed albatrosses. Marine Pollution Bulletin 48, 1153–1160.

Goessler, W., Kuehnelt, D., Schlagenhaufen, C., Slejkovec, Z., Irgolic, K.J., 1998. Arsenobetaine and other arsenic compounds in the National Research Council of Canada Certified Reference Materials DORM 1 and DORM 2. Journal of Analytical Atomic Spectrometry 13, 183–187.

Gordon, A.N., Pople, A.R., Ng, J., 1998. Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. Marine and Freshwater Research 49, 409–414.

Kamezaki, N., Matsui, M., 1997. A review of biological studies on sea turtles in Japan. Japanese Journal of Herpetology 17, 16–32.

Kirby, J., Maher, W., Spooner, D., 2005. Arsenic occurrence and species in near-shore macroalgae-feeding marine animals. Environmental Science and Technology 39, 5999–6005.

Kubota, R., Kunito, T., Tanabe, S., 2001. Arsenic accumulation in the liver tissue of marine mammals. Environmental Pollution 115, 303–312.

Kubota, R., Kunito, T., Tanabe, S., 2002a. Chemical speciation of arsenic in the livers of higher trophic marine animals. Marine Pollution Bulletin 45, 218–223.

Kubota, R., Kunito, T., Tanabe, S., Ogi, H., Shibata, Y., 2002b. Maternal transfer of arsenic to eggs of black-tailed gull (*Larus crassirostris*) from Rishiri Island, Japan. Applied Organometallic Chemistry 16, 463–468.

Kubota, R., Kunito, T., Tanabe, S., 2003a. Is arsenobetaine the major arsenic compound in the liver of birds, marine mammals, and sea turtles? Journal De Physique IV 107, 707–710.

Kubota, R., Kunito, T., Tanabe, S., 2003b. Occurrence of several arsenic compounds in the liver of birds, cetaceans, pinnipeds, and sea turtles. Environmental Toxicology and Chemistry 22, 1200–1207.

Kubota, R., Kunito, T., Fujihara, J., Tanabe, S., Yang, J., Miyazaki, N., 2005. Placental transfer of arsenic to fetus of Dall's porpoises (*Phocoenoides dalli*). Marine Pollution Bulletin 51, 845–849.

Kuehnelt, D., Goessler, W., Irgolic, K.J., 1997. Arsenic Compounds in Terrestrial Organisms I: *Collybia maculata*, *Collybia butyracea* and *Amanita muscaria* from Arsenic Smelter Sites in Austria. Applied Organometallic Chemistry 11, 289–296.

Lam, J.C.W., Tanabe, S., Chan, S.K.F., Yuen, E.K.W., Lam, M.H.W., Lam, P.K.S., 2004. Trace element residues in tissues of green turtles (*Chelonia mydas*) from South China Waters. Marine Pollution Bulletin 48, 174–182.

Langston, W.J., Spence, S.K., 1995. Biological factors involved in metal concentrations observed in aquatic organisms. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley, Chichester, UK, pp. 407–478.

Maher, W., Butler, E., 1988. Arsenic in the marine environment. Applied Organometallic Chemistry 2, 191–214.

Mass, M.J., Tennant, A., Roop, B.C., Cullen, W.R., Styblo, M., Thomas, D.J., Kligerman, A.D., 2001. Methylated trivalent arsenic species are genotoxic. Chemical Research in Toxicology 14, 355–361.

Mattusch, J., Wennrich, R., 1998. Determination of anionic, neutral, and cationic species of arsenic by ion chromatography with ICPMS detection in environmental samples. Analytical Chemistry 70, 3649–3655.

McKenzie, C., Godley, B.J., Furness, R.W., Wells, D.E., 1999. Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters. Marine Environmental Research 47, 117–135.

Miao, X.-S., Balazs, G.H., Murakawa, S.K.K., Li, Q.X., 2001. Congener-specific profile and toxicity assessment of PCBs in green turtles (*Chelonia mydas*) from the Hawaiian Islands. Science of the Total Environment 281, 247–253.

Muir, C.G., Wagemann, R., Grift, N.P., Norstrom, R.J., Simon, M., Lien, J., 1988. Organochlorine chemical and heavy metal contaminants in white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala melaena*) from the coast of Newfoundland, Canada. Archives of Environmental Contamination and Toxicology 17, 613–629.

Nischwitz, V., Pergantis, S.A., 2005. First report on the detection and quantification of arsenobetaine in extracts of marine algae using HPLC–ES-MS/MS. Analyst 130, 1348–1350.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Vasken Aposhian, H., 2000. Monomethylarsonous acid (MMAIII) is more toxic than arsenite in Chang human hepatocytes. Toxicology and Applied Pharmacology 163, 203–207.

Saeki, K., Sakakibara, H., Sakai, H., Kunito, T., Tanabe, S., 2000. Arsenic accumulation in three species of sea turtles. Biometals 13, 241–250.

Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., Tatsukawa, R., 2000a. Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Marine Pollution Bulletin 40, 701–709.

Sakai, H., Saeki, K., Ichihashi, H., Kamezaki, N., Tanabe, S., Tatsukawa, R., 2000b. Growth-related changes in heavy metal accumulation in green turtle (*Chelonia mydas*) from Yaeyama Islands, Okinawa, Japan. Archives of Environmental Contamination and Toxicology 39, 378–385.

Shiomi, K., Chino, M., Kikuchi, T., 1990. Metabolism in mice of arsenic compounds contained in the red alga *Porphyra yezoensis*. Applied Organometallic Chemistry 4, 281–286.

Shiomi, K., 1994. Arsenic in marine organisms: chemical forms and toxicological aspects. In: Nriagu, J.O. (Ed.), Arsenic in the Environment. Part II: Human Health and Ecosystem Effects. John Wiley and Sons, New York, pp. 261–282.

Storelli, M.M., Marcotrigiano, G.O., 2000. Total organic and inorganic arsenic from marine turtles (*Caretta caretta*) beached along the Italian coast (South Adriatic sea). Bulletin of Environmental Contamination and Toxicology 65, 732–739.

Styblo, M., Hughes, M.F., Thomas, D.J., 1996. Liberation and analysis of protein-bound arsenicals. Journal of Chromatography B 677, 161–166.

Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Archives of Toxicology 74, 289–299.

Tanabe, S., 2006. Environmental Specimen Bank in Ehime University (es-BANK), Japan for global monitoring. Journal of Environmental Monitoring 8, 782–790.

Vahter, M., Marafante, E., Dencker, L., 1983. Metabolism of arsenobetaine in mice, rats and rabbits. Science of the Total Environment 30, 197–211.

Vahter, M., 1994. Species differences in the metabolism of arsenic compounds. Applied Organometallic Chemistry 8, 175–182.

WHO, 2001. Environmental Health Criteria 224: Arsenic and Arsenic Compounds, second ed. World Health Organization, Geneva.

Yamauchi, H., Kaise, T., Yamamura, Y., 1986. Metabolism and excretion of orally administered arsenobetaine in the hamster. Bulletin of Environmental Contamination and Toxicology 36, 350–355.