

# Arsenic in Marine Mammals, Seabirds, and Sea Turtles

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## 1 Introduction

Arsenic, a chalcophilic element, is widespread in the environment. Although arsenic may possibly be an essential element for life (Cox 1995) and some micro-organisms are known to use arsenic for energy generation (Oremland and Stolz 2003), no firm data are available on its essentiality for biological systems (Francesconi 2005). In contrast to its possible essentiality in life, many studies have focused on its high toxicity, which has been well known from various cases of poisoning throughout the ages (Nriagu 2002). The toxicity is especially high for inorganic arsenic; trivalent inorganic arsenic [arsenite; As(III)] is known to bind readily to sulfhydryl groups of enzymes leading to enzyme inhibition, whereas pentavalent inorganic arsenic [arsenate; As(V)], which is structurally similar to phosphate, may disrupt metabolic reactions that require phosphorylation (Cox 1995). Symptoms of acute intoxication in humans by inorganic arsenic include severe gastrointestinal disorders, hepatic and renal failure, and cardiovascular disturbances, whereas chronic exposure causes skin pigmentation, hyperkeratosis, and cancers in the lung, bladder, liver, and kidney as well as skin (Gorby 1994; WHO 2001). At present, arsenic contamination of groundwater is a worldwide problem (Mandal and Suzuki 2002), particularly in the Bengal Delta where chronic ingestion of arsenic in groundwater poses a significant health risk to about 36 million people (Nordstrom 2002). Thus, the development and use of techniques to remove arsenic from polluted groundwater is an urgent necessity (Chowdhury 2004). In contrast to the hazards of arsenic, it is useful in medicine. For example, arsenic trioxide ( $\text{As}_2\text{O}_3$ ) has recently attracted considerable attention as a therapeutic agent for treatment of acute promyelocytic leukemia and other cancers, although the precise mechanisms by which it produces results are not fully understood (Zhu et al. 2002).

Arsenic is used in agriculture, livestock, medicine, electronics, industry, and metallurgy (Azcue and Nriagu 1994). Worldwide anthropogenic emission of arsenic was estimated to be ~5,000 t/yr in the mid-1990s, of which more than half was accounted for by nonferrous metal production (Pacyna and Pacyna 2001). Emission from natural sources, estimated to be ~12,000 t/yr (Pacyna and Pacyna 2001), is more than twice that from anthropogenic sources. The major natural source is volcanoes (Nriagu 1989). Therefore, both anthropogenic and natural

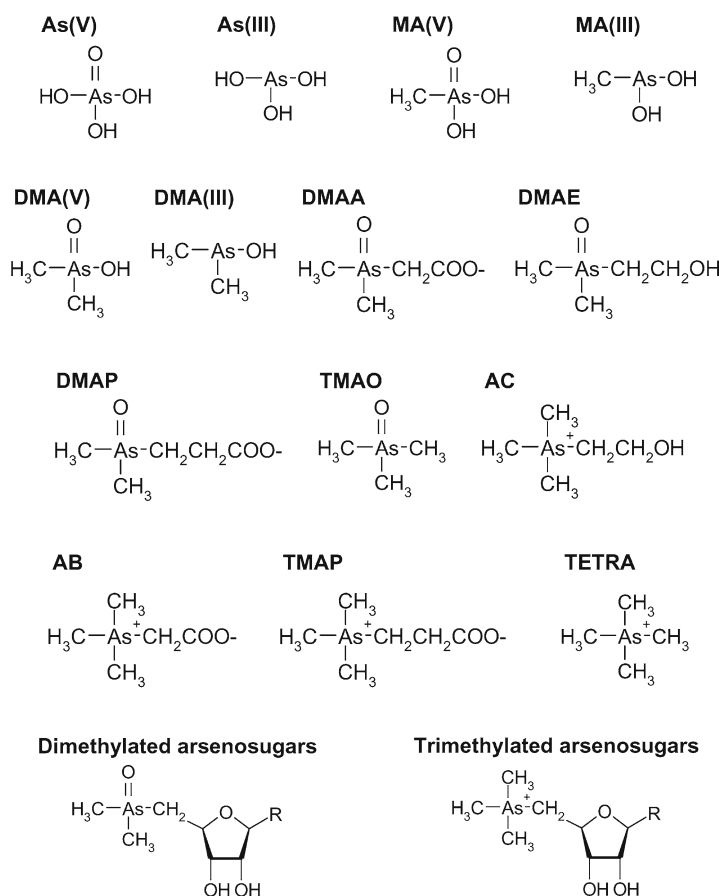
sources should be factored into evaluations when assessing the environmental risk of arsenic.

Generally, no significant difference is observed between arsenic concentrations in seawater and freshwater: arsenic concentration is about  $1.5 \mu\text{g L}^{-1}$  in seawater,  $0.1\text{--}2.0 \mu\text{g L}^{-1}$  in river water with absence of significant nearby emission sources (e.g., mining activity and geothermal sources) and  $< 1 \mu\text{g L}^{-1}$  in lake water (Plant et al. 2005). However, it is widely known that marine organisms contain arsenic at much higher concentrations than do terrestrial organisms (Lunde 1977), and some marine species show arsenic levels exceeding  $2,000 \mu\text{g g}^{-1}$  dry wt on a whole-body basis (Gibbs et al. 1983). Hence, many studies have been conducted on arsenic levels and its speciation in marine organisms at low trophic levels (e.g., algae and shellfish). In contrast, few studies are available for marine mammals and seabirds occupying higher trophic levels, or sea turtles. It is known that marine mammals and seabirds accumulate organochlorine compounds (e.g., polychlorinated biphenyls) at high levels by biomagnification through the marine food chain (O'Shea 1999; Braune et al. 2005; Tanabe and Subramanian 2006). In particular, marine mammals have a unique tissue, blubber, which serves as the main repository for organochlorine compounds. Furthermore, it has been reported that marine mammals (Thompson 1990; Law 1996; O'Shea 1999), seabirds (Thompson 1990), and sea turtles (Anan et al. 2001) accumulate certain metals, such as cadmium, mercury, and copper, at high concentrations in their tissues. For example, some marine mammals show hepatic mercury levels of  $13,000 \mu\text{g g}^{-1}$  dry wt and a renal cadmium level of  $800 \mu\text{g g}^{-1}$  dry wt (O'Shea 1999). Such high accumulation of metals seems to depend not only on biomagnification through the food chain but also on various biological factors, such as species, feeding habits, and lifespan (Thompson 1990). Indeed, it has been suggested that organic mercury is virtually the only metal that can be biomagnified through the food chain (Langston and Spence 1995). Although many studies have been conducted on the accumulation of metals such as cadmium, copper, mercury, and zinc in tissues of marine mammals, seabirds, and sea turtles, there have been few efforts to study the presence of arsenic species in these animals. To illustrate, although more than 18,000 papers have been published on the accumulation of organochlorine compounds and metals in marine mammals since the 1960s (O'Shea and Tanabe 2003), no report was published on arsenic species in marine mammals (Eisler 1994; Law 1996) until the study of Goessler et al. (1998); this is probably because sensitive speciation techniques for arsenic were unavailable for many years. Because marine mammals, seabirds, and sea turtles display unique features in metal accumulation, it may be useful to characterize arsenic accumulation in these animals. Furthermore, studying arsenic species and their presence in high-trophic-level marine animals is crucial for understanding arsenic cycling in the marine ecosystem. In this review, we focus attention on the pattern of accumulation of arsenic species in marine mammals, seabirds, and sea turtles and also summarize the state of current knowledge related to this topic (e.g., newly identified arsenicals in other marine organisms).

## 2 Arsenic Species and Cycling in the Marine Ecosystem

### 2.1 Arsenic Species

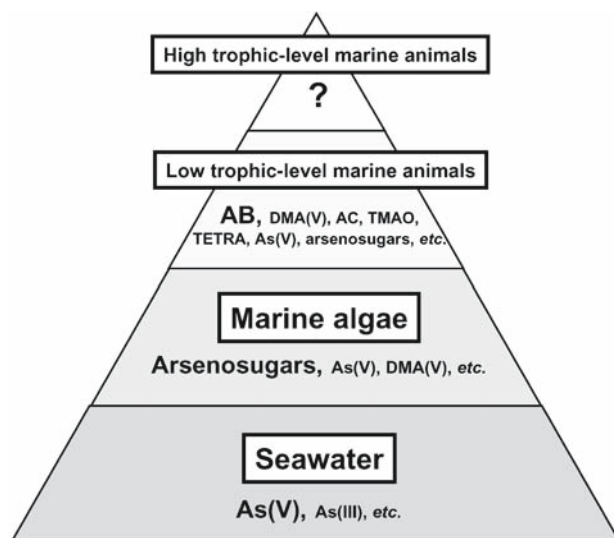
Arsenic is present in various chemical forms (Fig. 1), and its toxicity depends on the particular chemical form. Therefore, an understanding of arsenic speciation is essential to understanding its environmental behavior and ecotoxicological effects. Recently, the new scientific field, “metallomics,” which focuses on identification of metallomes (metalloproteins, metalloenzymes, and other metal-containing biomolecules)



**Fig. 1** Water-soluble arsenicals found in the marine ecosystem: As(V), arsenate; As(III), arsenite; MA(V), methylarsonic acid; MA(III), methylarsonous acid; DMA(V), dimethylarsinic acid; DMA(III), dimethylarsinous acid; DMAA, dimethylarsinoyl acetate; DMAE, dimethylarsinoyl ethanol; DMAP, dimethylarsinoyl propionate; TMAO, trimethylarsine oxide; AC, arsenocholine; AB, arsenobetaine; TMAP, trimethylarsoniopropionate; TETRA, tetramethylarsonium ion

and the elucidation of their functions in biological systems, has received increasing attention (Haraguchi 2004). Arsenic speciation is also of interest from the point of view of this new field (Suzuki 2005). High performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) is the technique most employed to determine arsenic speciation. Arsenic speciation is conducted using ICP-MS as a detector after separation of arsenic species by HPLC. The HPLC-ICP-MS technique is the most sensitive method for detecting arsenic species and often allows a detection limit lower than  $0.5 \mu\text{g L}^{-1}$  (e.g., Hirata et al. 2006). The choice of the HPLC column (e.g., anion-exchange, cation-exchange, or reversed-phase) affects the separation of arsenic species, because different arsenic species behave differently on each column. In part, this variability may occur because the dissociation constant, functional groups, and molecular size are largely different among arsenic species. Therefore, HPLC columns should be selected based on characteristics of the expected analytes and coexisting arsenicals. It should be noted that the HPLC-ICP-MS technique requires the appropriate authentic reference material for successful identification of arsenic species because no structural information is inherently provided by this method. Identification of arsenic species depends on a comparison of the retention times of unknowns versus such authentic standards using selected HPLC column(s).

Seawater is considered as the starting point for arsenic cycling in the marine ecosystem. In seawater, most arsenic exists in the inorganic form (Fig. 2), with pentavalent arsenic,  $\text{HAsO}_4^{2-}$ , predominating in oxygenated surface water (Cullen and Reimer 1989). Arsenic shows a nutrient-like vertical profile in the water column (i.e., depletion at the euphotic zone), suggesting biological uptake of arsenic

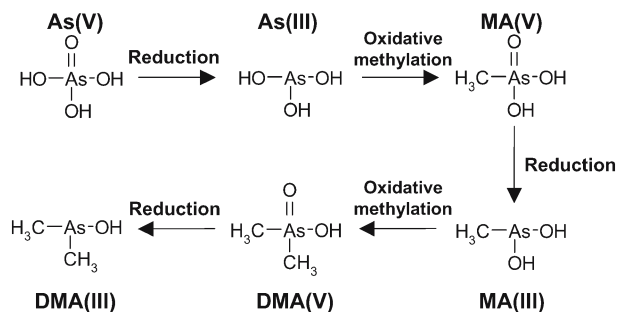
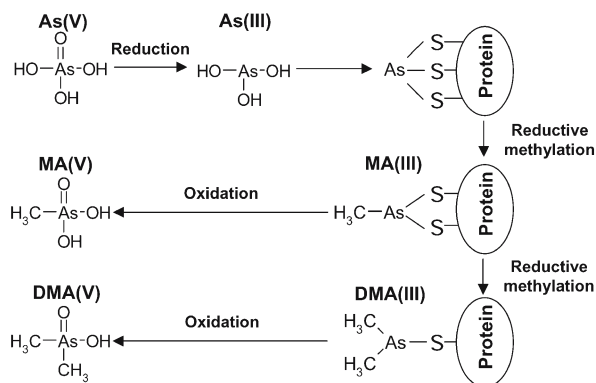


**Fig. 2** Arsenicals found in seawater and organisms at each trophic level

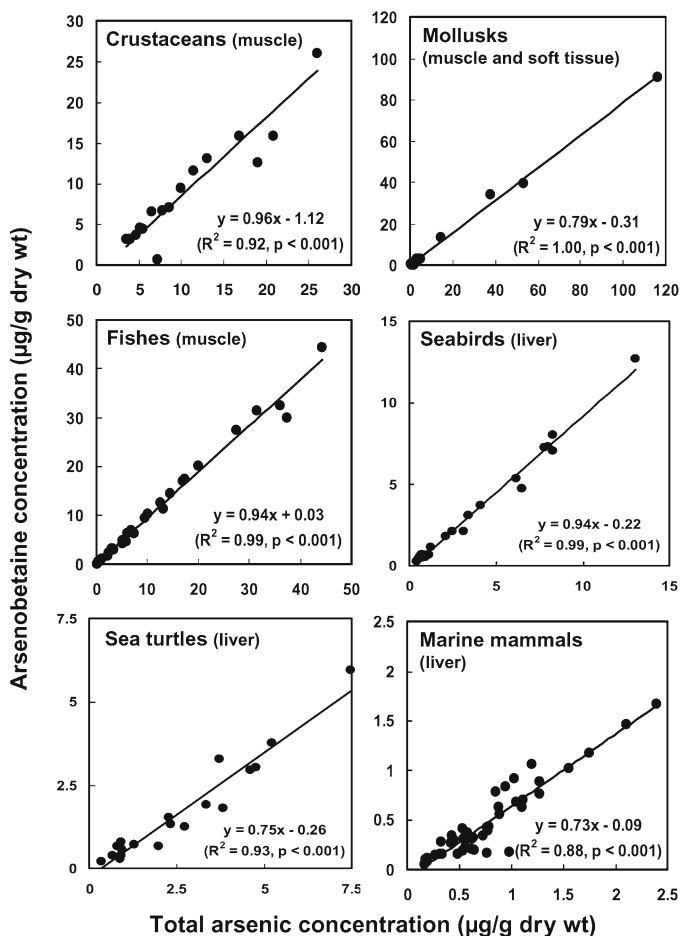
by marine phytoplankton, in spite of its high toxicity (Cullen and Reimer 1989; Shibata and Morita 2000). In addition to inorganic arsenic, pentavalent monomethylated and dimethylated arsenicals, methylarsonic acid [MA(V)] and dimethylarsinic acid [DMA(V)], respectively, are also present. Santosa et al. (1996) reported that the ratio of MA(V) + DMA(V) to total arsenic increased with water temperature and also was influenced by nutrient levels in Pacific Ocean surface waters, which suggests that the abundance of organoarsenicals reflect the biological activity [i.e., uptake of As(V) and subsequent methylation to MA(V) and DMA(V)] of phytoplankton in the surface water. Furthermore, trivalent methylarsonous acid [MA(III)] and dimethylarsinous acid [DMA(III)] were also detected in seawater (Hasegawa 1996). It should be noted that the methylation pathway of inorganic arsenic is not yet firmly established. It has been generally accepted that inorganic arsenic is methylated oxidatively (Fig. 3a), but recently a reductive methylation pathway has also been proposed (Hayakawa et al. 2005; Naranmandura et al. 2006). In the latter pathway, MA(V) and DMA(V) are shown as the end products of transformation (Fig. 3b), which is consistent with the abundant presence of these pentavalent arsenicals in animals (Aposhian and Aposhian 2006).

In marine organisms, arsenic is known to exist mainly as organic forms, although elucidation of the actual structures involved only took place over many years. In 1977, arsenobetaine (AB; see Fig. 1) was first identified in the western rock lobster (*Panulirus cygnus*) (Edmonds et al. 1977). AB was first synthesized in the 1930s for pharmacological studies, but its presence in biota and the environment was not reported until 1977 (Edmonds et al. 1993). In 1981, arsenosugars (Fig. 1) were also identified in brown kelp, *Ecklonia radiata* (Edmonds and Francesconi 1981). Subsequent studies on arsenic species in various low-trophic-level marine organisms revealed that marine algae, which rest at the base of the marine food chain, accumulate arsenic (mainly as arsenosugars) at levels of 1,000–50,000 times that of seawater. Low-trophic-level marine animals contain arsenic mainly as AB (see Fig. 2) at levels comparable to those in marine algae (Francesconi and Edmonds 1993). Arsenosugars found in marine algae and in some marine animals comprise the largest group (more than 20) of naturally occurring arsenicals (Francesconi 2005). Interestingly, the composition of arsenosugars in marine algae is related to their phylogeny: red and green algae contain arsenosugars of rather simple structure, whereas in brown algae the structure of arsenosugars is more complicated (Morita and Shibata 1990). Although AB was not detected in marine algae until recently, a study by Nischwitz and Pergantis (2005a) revealed the presence of AB in these organisms.

Major arsenicals found in marine ecosystems are shown in Figs. 1 and 2. Arsenobetaine, arsenocholine (AC), trimethylarsine oxide (TMAO), and tetramethylarsonium ion (TETRA) are the arseno-analogues of the nitrogen-containing compounds, glycine betaine, choline, trimethylamine oxide, and tetramethylammonium ion, respectively (Shibata et al. 1992). Thus, in uptake and retention, marine animals do not discriminate these arsenicals from their natural nitrogen analogues (Shibata et al. 1992).

**(a) Oxidative methylation pathway****(b) Reductive methylation pathway****Fig. 3** Hypothesized oxidative (a) and reductive (b) methylation pathways of inorganic arsenic

Marine animals generally contain arsenic mainly as AB (Figs. 2, 4), with two exceptions: the first, a marine teleost fish, the silver drummer (*Kyphosus sydneyanus*), which digests its macroalgal diet by fermentation (Edmonds et al. 1997), and second, the dugong (*Dugong dugon*), which feeds on seagrass (Kubota et al. 2002a, 2003b). The proportion of AB in marine animals varies depending on their feeding habit and trophic position, with animals of higher trophic levels containing higher proportions of AB (Francesconi and Kuehnelt 2002). For example, AB comprises the major arsenic species in pelagic carnivorous marine fish, whereas various arsenicals are contained in detritivorous and herbivorous marine fish, with the corresponding proportion of AB being relatively low (Kirby and Maher 2002). In general, the arsenic composition in marine animals reflects the distribution found in their prey, because marine animals take up arsenicals mainly through their diets (Phillips 1990). However, the trophic transfer coefficient differs among species of arsenicals. Although inorganic arsenic predominates in seawater, dimethylated arsenosugars and



**Fig. 4** Relationship between total arsenic and arsenobetaine concentrations in marine animals. Total arsenic in marine mammals, seabirds, and sea turtles includes nonextractable arsenic in liver. Data on crustaceans, mollusks, and fishes are from references cited in Francesconi and Edmonds (1993); hepatic arsenic concentrations of marine mammals are from Kubota et al. (2003a), Fujihara et al. (2003), and Goessler et al. (1998); those of seabirds are from Kubota et al. (2003a) and Fujihara et al. (2003); and those of sea turtles are from Kubota et al. (2003a) and Fujihara et al. (2003)

trimethylated arsenicals (i.e., AB) are contained as major arsenicals in marine algae and marine animals, respectively (Fig. 2), with proportions of more methylated forms increasing with trophic level. Hence, the proportion of AB and degree of methylation of predominant arsenicals are related to the trophic position of the organism. However, the concentrations of total arsenic and AB vary greatly among species (Francesconi and Edmonds 1993) and are not related to the trophic position of the organism (Table 1).



**Table 1** Arsenic concentrations in marine organisms ( $\mu\text{g g}^{-1}$  dry wt)

Organism	Range of means (Phillips 1990)	Geometric means (Neff 1997)	Means (Kubota et al. 2001)	Range of means (Saeki et al. 2000)	Range of means (Kubota et al. 2003b)
Marine algae	1.5–84	43.7			
Crustaceans	8.4–179	14.9			
Bivalve mollusks	5.0–1025	10.4			
Gastropod mollusks	9.0–407	52.0			
Cephalopod mollusks	6.4–99	16.1			
Fish	<0.2–216	5.6			
Marine mammals					
Pinnipeds (liver)			1.85		
Cetaceans (liver)			1.88		
Sea turtles (liver)				1.76–15.3	
Seabirds (liver)					2.25–12.2

The widely used HPLC-ICP-MS technique is effective in identification and quantification of arsenicals for which corresponding authentic standards are available, but it is not applicable in the absence of such standards. Recently, electrospray tandem mass spectrometry (ES-MS/MS) has been increasingly used for identification of arsenicals without using standard compounds, and has, therefore, contributed significantly to the understanding of arsenic metabolism in marine organisms and arsenic cycling in marine ecosystems (Edmonds and Francesconi 2003). For example, McSheehy et al. (2002) identified 15 organoarsenicals in the kidney of giant clams (*Tridacna derasa*), which have symbiotic unicellular algae in their tissues. These identifications were achieved using ES-MS/MS after successive chromatographic fractionation of the arsenicals by size-exclusion chromatography, anion-exchange chromatography, and cation-exchange chromatography (or anion-exchange chromatography with a high resolution column). More recently, Nischwitz and Pergantis (2006) established a HPLC-ES-MS/MS method that is capable of analyzing for 50 arsenic species, including various thio-arsenicals. Furthermore, in a recent study using ES-MS/MS, dimethylarsinoyl acetate (DMAA), dimethylarsinoyl propionate (DMP) and dimethylarsinoyl ethanol (DMAE), which are postulated intermediates in AB biosynthesis, were found in various marine animals and marine algae (Sloth et al. 2005a). Also, thio-arsenosugars containing As=S have been recently identified in mussels and marine algae using this method (Fricke et al. 2004; Schmeisser et al. 2004; Nischwitz et al. 2006) and HPLC-ICP-MS (Meier et al. 2005). It is noteworthy that these newly identified arsenicals have also been quantified for certified reference materials (CRMs) from marine animals (tuna fish, BCR-627; dogfish muscle, DORM-2; mussel tissue, CRM278R and oyster, 1566b) (Nischwitz and Pergantis 2005b).

## 2.2 Microbial Degradation of Arsenobetaine

Because AB is by far the dominant arsenical in most marine animals, degradation of AB to inorganic arsenic after its release into the environment from the decomposing dead animals is essential for completion of arsenic cycling in marine ecosystems. There are two possible pathways for AB degradation (Fig. 5): the conversion from AB to TMAO or from AB to DMAA. The TMAO or DMAA is further degraded to inorganic arsenic through DMA(V) in both pathways. The AB-degrading bacteria are ubiquitous in the marine environment. It was shown that microbial communities from marine sediments, marine algae, mollusk intestine, or suspended particles were able to convert AB to TMAO, DMA(V), and even to inorganic arsenic (Hanaoka et al. 1992). Microbial communities on suspended particles collected at a depth of 3500m were also able to degrade AB (Hanaoka et al. 1997). It is likely that aerobic microorganisms are primarily involved in degradation of AB, because AB is more rapidly degraded under aerobic than anaerobic conditions (Hanaoka et al. 1992). Khokiattiwong et al. (2001) suggested that AB is rapidly degraded when present at its environmentally relevant low level. In contrast, degradation took several weeks in an incubation experiment conducted by Hanaoka et al. (1992) in which a relatively high level of AB was employed. More than 95% of AB was converted to DMA(V) within 24 hr by microorganisms in seawater to which low levels of AB (100 and 750  $\mu\text{g As L}^{-1}$ ) were added and in which shore crabs (*Carcinus maenas*) were maintained (Khokiattiwong et al. 2001). A more detailed investigation revealed that AB was first converted to DMAA, reaching a maximum concentration after 3 hr incubation, and was then totally converted to DMA(V) after 48 hr. Thus, the authors expect that AB is not usually detected in seawater because of such rapid degradation. In these experiments, TMAO was not detected, suggesting that AB was degraded to DMA(V) primarily via DMAA.

In addition to studies on AB degradation by microbial communities, isolation and characterization of each AB-degrading bacterium have also been reported. Two

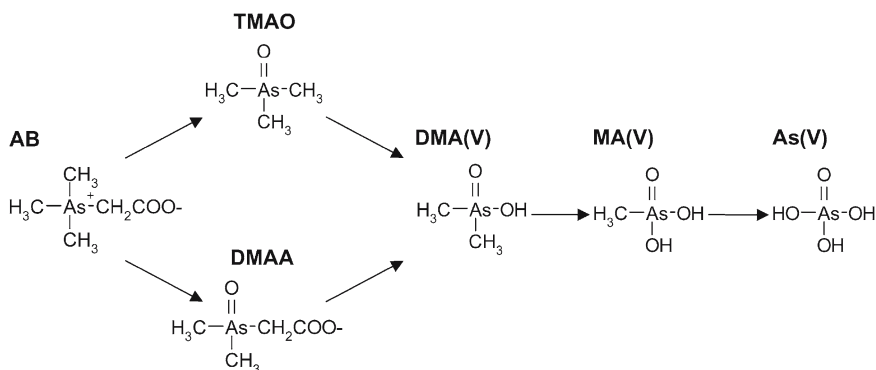


Fig. 5 Hypothesized degradation pathways of arsenobetaine

bacterial strains of the *Vibrio-Aeromonas* group isolated from coastal sediment by the culture enrichment method converted AB to DMA(V) under aerobic but not anaerobic conditions (Hanaoka et al. 1992). A microbial community isolated from the blue mussel (*Mytilus edulis*) converted AB to TMAO, MA(V), and DMA(V). Four AB-degrading bacterial strains (one of *Paenibacillus*, two of *Pseudomonas*, and one of *Aeromonas*) were isolated from this community to characterize the degradation pathway of AB (Jenkins et al. 2003). Degradation of AB to DMA(V) by each strain occurred after 21 d incubation. TMAO was detected during incubation with the microbial community, whereas DMAA but not TMAO was observed during incubation in the pure culture. One isolate further degraded DMAA to As(V) after 28 d incubation. Jenkins et al. (2003) assumed that the conversion of AB to DMAA would be a fortuitous reaction, whereas conversion of DMAA to DMA(V) would provide carbon or energy to the bacteria. Also, the degradation of AB was shown to be mediated intracellularly by *Paenibacillus* sp. (Jenkins et al. 2003). Devesa et al. (2005) observed that microbial communities from the hepatopancreas, tail, and remaining parts of the red swamp crayfish (*Procambarus clarkii*) degraded AB to TMAO, DMA(V), MA(V), and an unidentified arsenical. Interestingly, in the incubation experiments using either AC, TETRA, TMAO, DMA(V), or MA(V), only AC was converted to AB, but the other arsenicals were not transformed by these microbial communities. Five AB-degrading strains isolated from these microbial communities were all identified as *Pseudomonas putida* and were shown to degrade AB to DMA(V) and MA(V) in the incubation experiment (Devesa et al. 2005). Generally, microbial communities could degrade AB to inorganic arsenic, whereas most of the AB-degrading bacteria could not degrade AB completely by themselves (Hanaoka et al. 1992). Thus, degradation of AB to inorganic arsenic requires the cooperation of various microorganisms. The microbial community structure in AB degradation is important, because metabolites formed by the degradation are different among the sources of microbial communities (Hanaoka and Kaise 1999).

### **3 Distribution of Arsenic Species in the Tissues of Marine Mammals, Seabirds, and Sea Turtles**

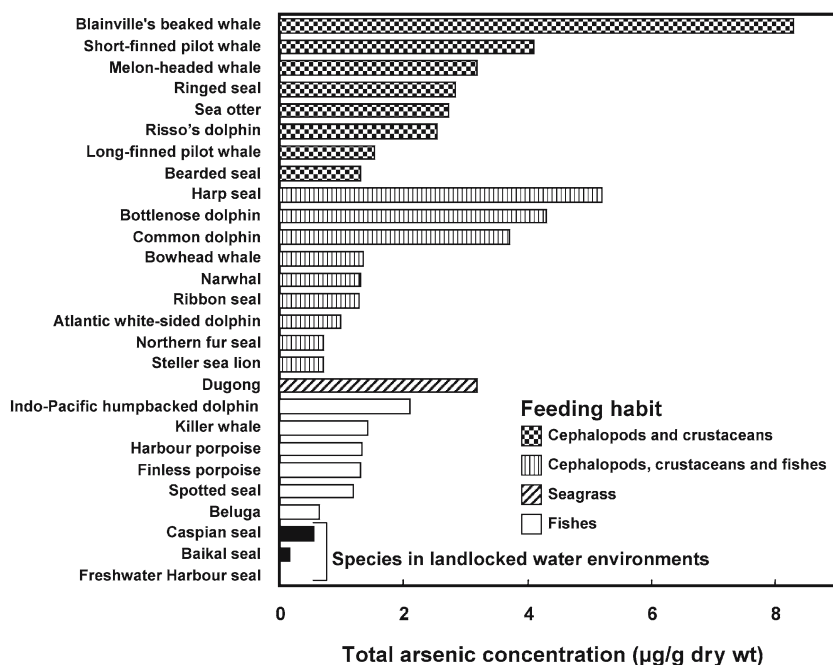
#### **3.1 Arsenic in Marine Mammals**

There are few studies that have examined the types of arsenic species in marine mammals, seabirds and sea turtles. Therefore, we have undertaken a detailed characterization of arsenic accumulation in such large marine animals.

Influences of feeding habits, age (or body size), and gender on the hepatic arsenic level in marine mammals were examined by analyzing in-house measurements of 16 species of marine mammals ( $n = 226$ ), as well as data from the literature (Kubota et al. 2001). The highest level of  $7.68 \mu\text{g g}^{-1}$  on a dry weight (dry wt) basis was observed in liver of the harp seal (*Pagophilus groenlandicus*); levels were

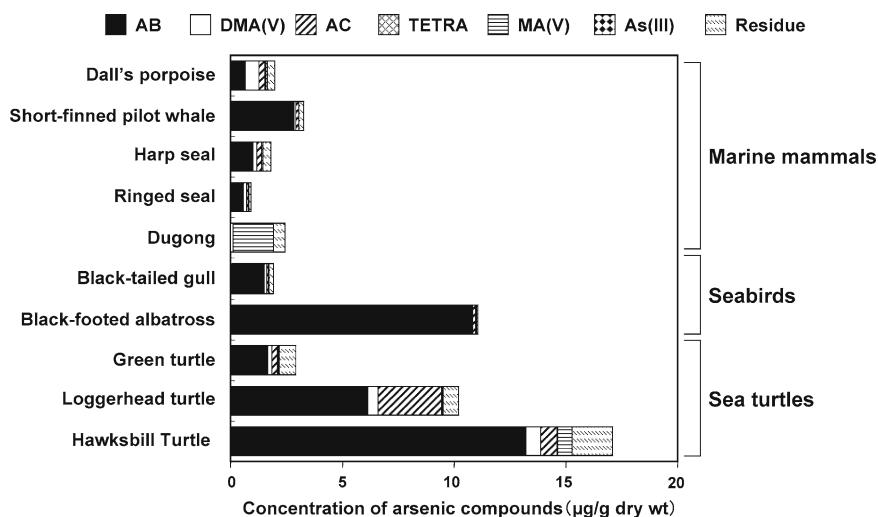
lower than those for animals at lower trophic levels (see Table 1). Hepatic levels were comparable between pinnipeds and cetaceans (Table 1). Influences of gender and age (or body size) on the arsenic level were not found (Kubota et al. 2001). The relatively low arsenic level in marine mammals is probably because arsenic is mainly present as AB, which has a short biological half-life in marine mammal tissues. However, hepatic levels in marine mammals vary by species and depend on feeding habits (Fig. 6); species feeding on cephalopods and crustaceans tend to contain higher arsenic concentrations than those feeding on fish, which is consistent with the pattern observed in prey organisms (Table 1). Generally, concentrations of other trace elements also vary with feeding habits. For example, marine mammals feeding on cephalopods show higher concentrations of cadmium and radioactive cesium, whereas animals feeding on fish exhibit higher mercury levels (Watanabe et al. 2002; Yoshitome et al. 2003).

Goessler et al. (1998) were among the first to report arsenic species in marine mammals. These authors found AB to be the predominant arsenical in all the liver samples of the ringed seal (*Pusa hispida*;  $n = 10$ ), bearded seal (*Erignathus barbatus*;  $n = 1$ ), pilot whale (*Globicephala melas*;  $n = 2$ ), and beluga (*Delphinapterus leucas*;  $n = 1$ ), accounting for 68%–98% of extractable arsenic. Arsenocholine and DMA(V) were also found in almost all samples, whereas MA(V) was detected only in 5 specimens. Because TETRA was detected in pinnipeds but not in cetaceans,



**Fig. 6** Influence of feeding habits on arsenic concentration in liver of marine mammals (Kubota et al. 2001)

these authors hypothesized that the presence or absence of TETRA reflects differences in metabolism between these two groups of marine mammals (Goessler et al. 1998). However, the limited number of samples used in their study (only 3 for cetaceans) made it difficult to draw a definitive conclusion about the origin of TETRA. Arsenic speciation analyses were performed in liver of Dall's porpoise (*Phocoenoides dalli*), short-finned pilot whale (*Globicephala macrorhynchus*), harp seal, ringed seal, and dugong, also by Kubota et al. (2002a, 2003a). Arsenobetaine was the dominant arsenical in all samples tested except in the dugong. Lower AB (42%) and higher DMA(V) percentages (38%) were found in the Dall's porpoise than in other species (Fig. 7). Although TETRA was not detected in cetaceans by Goessler et al. (1998), this arsenical was found in the Dall's porpoise and also in pinnipeds, the harp seal, and the ringed seal (Kubota et al. 2003a), indicating that TETRA is present in both cetaceans and pinnipeds. Interestingly, AB was present in only a trace amount in the dugong ( $n = 1$ ), with MA(V) being the major arsenical followed by DMA(V) (Kubota et al. 2003a). To confirm the generality of this unique composition of arsenicals in the dugong, arsenic speciation was conducted in four additional liver samples of the dugong (Kubota et al. 2003b); the animals did not contain AB at a detectable level and had appreciable amounts of MA(V) and smaller quantities of DMA(V) (Kubota et al. 2003b), which was in agreement with the result of Kubota et al. (2003a). These results can be attributed to the seagrass diet of the dugong. Although only limited data are available, it is believed that, in contrast to marine algae, seagrass might not contain arsenosugars (Shibata and Morita 2000). Because seagrass is phylogenetically related to terrestrial higher plants rather than to marine algae, it may contain principally MA(V) and DMA(V) as do terrestrial plants (Kuehnelt and Goessler 2003).



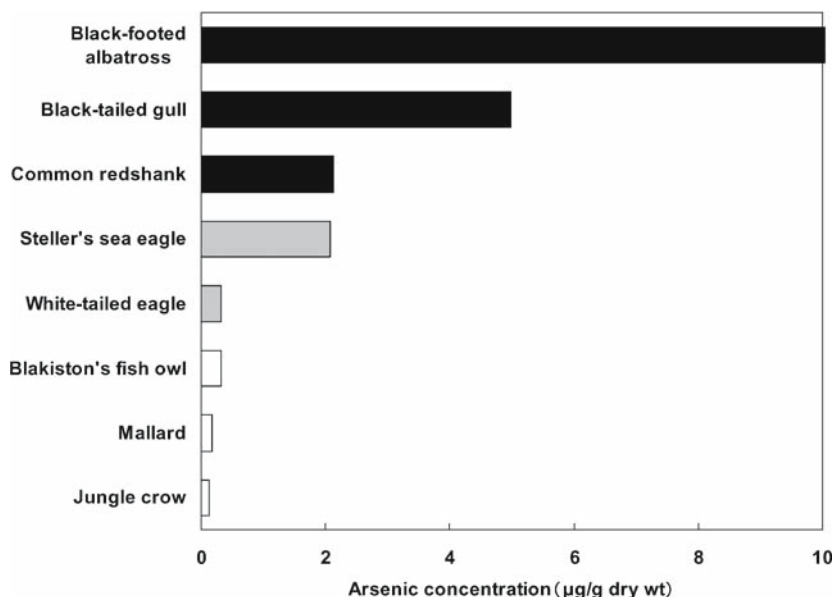
**Fig. 7** Arsenic species in liver of marine mammals, seabirds, and sea turtles (Kubota et al. 2003a; Fujihara et al. 2003)

Inorganic arsenic was not detected in the liver samples of marine mammals we examined. Sloth et al. (2005b) proposed a new method for determining inorganic arsenic in animal samples that probably successfully extracts As(III) bound to thiol groups of proteins. In their procedure, all inorganic arsenic was determined as As(V) by HPLC-ICP-MS after microwave-assisted alkaline digestion of the sample [oxidizing As(III) to As(V) in alkaline media]. Inorganic arsenic was not detected in the minke whale (*Balaenoptera acutorostrata*), harp seal, and hooded seal (*Cystophora cristata*), even though the aforementioned procedure was used (the tissue was not mentioned in the paper, but it was probably muscle; Sloth et al. 2005b).

Almost all studies on arsenic have focused on its speciation in the liver (the main metabolic organ) of marine mammals, but little is known about the distribution of arsenic species in other tissues. Ebisuda et al. (2002) analyzed arsenic species in liver, kidney, muscle, and gonad and total arsenic in blubber and hair of the ringed seal ( $n = 18$ ), and found that arsenic levels were highest in blubber, followed by liver and kidney, and lowest in muscle, gonad, and hair on a wet weight basis. Assuming that the respective tissue weight ratio of liver:kidney:muscle:blubber:hair is 10:1:100:200:5, about 90% of the arsenic burden of the five tissues is estimated to be present in ringed seal blubber. It is reported that the forms of arsenic differ between dogfish muscle and liver (Wahlen et al. 2004). In these samples, AB accounted for 96% of arsenic in muscle, and AB and DMA(V), respectively, accounted for 79% and 16% of arsenic in liver, suggesting differences in arsenic metabolism between the two tissues. However, there was no such difference in the ringed seal, where AB accounted for more than 70% of extractable arsenic in all the liver, kidney, muscle, and gonad (Ebisuda et al. 2002). It should be noted that lipid-soluble arsenicals prevail only in the blubber, accounting for about 90% of total arsenic (Ebisuda et al. 2002). Interestingly, AC was detected in all samples of liver, kidney, and gonad, but not in all muscle samples (Ebisuda et al. 2002). The predominant arsenical in the ringed seal was AB, followed by DMA(V) in stomach contents, but levels of both decreased after passing through the gastrointestinal tract whereas residual arsenic (nonextractable arsenic) increased. Arsenocholine and TMAO were also detected in stomach contents, and AB, DMA(V), and AC were present in tissues of the ringed seal, suggesting that AB, DMA(V), and AC were derived from the diet. In contrast, TMAO was detected in stomach contents but not in the tissues or contents of the intestine, whereas MA(V) was present in tissues but not in stomach or intestinal contents. These differences in arsenical forms between tissues of the ringed seal and contents of its stomach might be the result of metabolism by the ringed seal itself and/or metabolism by the intestinal bacteria it harbors.

### 3.2 Arsenic in Seabirds

Arsenic levels are higher in seabirds than terrestrial birds (Fig. 8), although there are few studies that define arsenic species in birds. Arsenic speciation in birds was first reported



**Fig. 8** Comparison of hepatic arsenic concentrations among birds from marine, coastal, and terrestrial environments (Kubota et al., unpublished results). *Filled, shaded, and open bars* correspond to birds from marine, coastal, and terrestrial environments, respectively

by Kubota et al. (2002b, 2003a). These authors reported average arsenic levels up to  $12.2 \mu\text{g g}^{-1}$  dry wt, and up to  $26.7 \mu\text{g g}^{-1}$  dry wt in liver of the black-footed albatross (*Phoebastria nigripes*,  $n = 5$ ), but only  $2.25 \mu\text{g g}^{-1}$  dry wt in liver of the black-tailed gull (*Larus crassirostris*,  $n = 5$ ) (see Fig. 7). The levels found in the black-tailed gull are comparable to those found in marine mammals (see Table 1). We also analyzed 24 additional liver samples of the black-footed albatross and found concentrations up to  $42 \mu\text{g g}^{-1}$  dry wt (Fujihara et al. 2004), which is comparable to those in marine animals at lower trophic levels (Table 1). Arsenobetaine predominated in liver of both the black-footed albatross and black-tailed gull, with DMA(V), AC, and TETRA also being detected (Fig. 7; Kubota et al. 2003a). Interestingly, a low level of AB ( $0.19 \mu\text{g g}^{-1}$  dry wt) was observed in one liver sample from a jungle crow (*Corvus macrorhynchos*) specimen, although arsenic was not detected in four other specimens (Kubota et al. 2003a). Low levels of AB were also reported in some other terrestrial birds (Koch et al. 2005). The jungle crow might have obtained AB by eating leftover marine fish and shellfish from garbage. Alternatively, because some terrestrial organisms have recently been reported to contain AB at trace amounts (Kuehnelt and Goessler 2003), AB might have originated in terrestrial organisms consumed by the jungle crow.

Tissue distribution of arsenicals in avian species has, so far, been reported only for the black-tailed gull (Kubota et al. 2002b) and black-footed albatross (Fujihara et al. 2004). Among the 13 tissues analyzed from the black-tailed gull, the concentration of arsenic was highest in liver (mean,  $1.59 \mu\text{g g}^{-1}$  dry wt), followed by kidney

(mean,  $1.17\mu\text{g g}^{-1}$  dry wt), and was lowest in feathers (mean,  $0.18\mu\text{g g}^{-1}$  dry wt), with AB predominating in all the tissues (75%–97% of extractable arsenic; arsenic speciation was not conducted for feathers) (Kubota et al. 2002b). In contrast, the arsenic level was highest in lung (mean,  $16\mu\text{g g}^{-1}$  dry wt) and muscle (mean,  $15\mu\text{g g}^{-1}$  dry wt), and lowest in bone (mean,  $<0.1\mu\text{g g}^{-1}$  dry wt) and feathers (mean,  $0.70\mu\text{g g}^{-1}$  dry wt), among the 17 tissues analyzed from the black-footed albatross (Fujihara et al. 2004). Similar to the black-footed albatross, marine animals at low trophic levels tend to accumulate arsenic in muscle. It is noteworthy that As(V) was detected in the muscle and testis of the black-footed albatross but that inorganic arsenic was not found in marine mammals.

The trophic transfer coefficient (TTC), defined as the ratio of the concentration in a consumer's body to the concentration in diet (stomach content) (Suedel et al. 1994), was found to be 1.0 for the black-footed albatross using arsenic levels of 17 tissues and diet (stomach content) (Fujihara et al. 2004). Because the TTC value is usually below unity for trace elements, other than those that are highly accumulative (e.g., mercury) (Anan et al. 2001), the black-footed albatross is believed to be very efficient in absorbing arsenic although arsenic does not biomagnify, thus leading to the levels reported (Fig. 8).

### 3.3 Arsenic in Sea Turtles

Few data are available on accumulation of arsenic or its species in sea turtles. In studies conducted in our laboratories (Saeki et al. 2000; Kubota et al. 2003a; Fujihara et al. 2003; Agusa et al. 2007), liver samples from the hawksbill turtle (*Eretmochelys imbricata*,  $n = 19$ ) showed the highest arsenic levels (mean,  $20.9\mu\text{g g}^{-1}$  dry wt), followed by the loggerhead turtle (*Caretta caretta*,  $n = 9$ ) (mean,  $9.0\mu\text{g g}^{-1}$  dry wt), and the lowest in the green turtle (*Chelonia mydas*,  $n = 34$ ) (mean,  $2.9\mu\text{g g}^{-1}$  dry wt). Although the dugong, which feeds on seagrass, exhibited relatively high arsenic concentrations in the liver (see Fig. 6), the carnivorous species (i.e., hawksbill and loggerhead turtles) tended to show higher arsenic levels than did herbivorous sea turtles (i.e., green turtle). This pattern in sea turtles is similar to that observed in other low-trophic-level marine animals such as mollusks (Cullen and Reimer 1989).

Edmonds et al. (1994) described the first characterization of arsenic species in sea turtles. Arsenobetaine, As(III), and AC accounted for 50%, 35%, and 15% of water-extractable arsenic, respectively, in liver of the leatherback turtle (*Dermochelys coriacea*), whereas methanol extracts of AB and AC were 80% and 20%, respectively. The relatively high percentage of AC and As(III) is characteristic of the leatherback turtle, but this does not occur in marine mammals and seabirds. In studies conducted in our laboratories, AB predominated in liver of the green turtle, loggerhead turtle, and hawksbill turtle (see Fig. 7). Interestingly, the loggerhead turtle showed a relatively high percentage of AC (30% of extractable arsenic species) (Fig. 7), which was similar to that of the leatherback turtle



(Edmonds et al. 1994). It is known that most AC is converted to AB and also to a small amount of lipid-soluble arsenicals when administered to marine animals (Edmonds and Francesconi 2003). The high percentage of AC in these two turtle species may originate in their diets, because they feed primarily on jellyfish (Bjorndal 1997), some species of which are known to contain high AC levels (Hanaoka et al. 2001a). Alternatively, it is assumed that these sea turtles have a low capacity to convert AC to AB. It is noteworthy that green turtles feeding on marine algae and seagrass (Bjorndal 1997) have high proportions of AB in their livers (Fig. 7). Similar to the green turtle, the luderick (*Girella tricuspidata*), a herbivorous fish species, contained 67% AB and 15% DMA(V) of total arsenic extractable from the liver (Kirby and Maher 2002). Arsenosugars predominate in marine algae, which comprise the primary diet of the green turtle (Francesconi and Kuehnelt 2004). In our studies, although arsenosugars have not been measured, no significant HPLC peak other than AB, DMA(V), and AC has been detected in the HPLC-ICP-MS analysis for the green turtle (Kubota et al. 2002a, 2003a). It is known that arsenosugars absorbed through the diet are converted primarily to DMA(V) and are then excreted in the urine by humans (Le et al. 2004) and sheep (Martin et al. 2005). In the green turtle, however, the percentage of DMA(V) was low (Fig. 7), despite possible uptake by this species of large amounts of arsenosugars from marine algae. There are three possible explanations for these discrepancies: first, AB may be synthesized from arsenosugars by the green turtle or by intestinal bacteria they harbor; second, AB absorbed from diet animals (jellyfish and zooplankton) may be efficiently retained [adult green turtles feed on small amount of jellyfish, and zooplankton is the chief diet of juvenile turtles (Bjorndal 1997)], whereas DMA(V) converted from arsenosugars may be rapidly excreted; and, third, the green turtles might efficiently retain in the body any AB gleaned from marine algae, although only a small amount of AB may be present in marine algae (Nischwitz and Pergantis 2005a).

High concentrations of arsenic were observed in the liver (up to 32.8 µg/g dry wt) and muscle (205 µg/g) of hawksbill turtles (Saeki et al. 2000). These turtles may have a peculiar mechanism for arsenic accumulation, because their main food source, sponges, have rather low arsenic levels, when compared to other low-trophic-level marine organisms (Saeki et al. 2000). Fujihara et al. (2004) summarized the distribution of arsenic in tissues of various marine animals and concluded that species with high arsenic levels (e.g., hawksbill turtle and the black-footed albatross) tend to accumulate AB in the muscle. Such accumulation is also characteristic of some fish (Shiomi et al. 1996; Amlund et al. 2006a,b). Agusa et al. (2007), in reviewing the literature for arsenic levels in various marine animals, found the ratio of arsenic concentration in muscle versus liver to be high in sea turtles (5.87). Generally, inorganic arsenic is retained in mammalian tissues whereas organoarsenicals are rapidly excreted in urine (Shiomi 1994). In contrast, AB and AC tend to accumulate in fish tissues (especially muscle) while inorganic arsenic, DMA(V), and TMAO are readily excreted (Shiomi et al. 1996; Amlund et al. 2006b).

As(III) was detected at low levels in two of five green turtle liver samples and one of five loggerhead turtle liver samples (Kubota et al. 2003a). According to Agusa

et al. (2007), As(III) was detected in all examined tissues of green and hawksbill turtles. Remarkably, high levels of As(III) were found in spleen of the hawksbill turtle ( $2.83 \mu\text{g g}^{-1}$  dry wt; Agusa et al. 2008). As(III) comprised 35% of water-extractable arsenic in the liver of the leatherback turtle (Edmonds et al. 1994). Storelli and Marcotrigiano (2000) analyzed organic and inorganic arsenic levels in the loggerhead turtle and found that inorganic arsenic comprised 3% and 11% of total arsenic in the muscle and liver, respectively. Although inorganic arsenic was not detected in liver of the hawksbill turtle by Fujihara et al. (2003), sea turtles generally have higher levels of inorganic arsenic than do marine mammals and seabirds.

A strong positive correlation was observed between AB and total arsenic concentrations in the liver of seabirds, sea turtles, and marine mammals (see Fig. 4). However, some differences exist in AB accumulation among species. Arsenobetaine was not detected in the dugong (not included in Fig. 4). Kubota et al. (2003a) reported that the proportion of AB increased with total arsenic concentration in marine mammals, seabirds, and sea turtles. Loggerhead turtles have a high arsenic level (mean,  $11.2 \mu\text{g g}^{-1}$  dry wt; Fig. 7) and would, therefore, be expected to have high proportion of AB; however, the value was relatively low (mean, 54.0%). The low proportion of AB was attributed to high levels of AC in the loggerhead turtle (Fig. 7).

### **3.4 Toxicological Significance of Arsenic in Marine Mammals, Seabirds, and Sea Turtles**

In general, inorganic arsenic is more toxic than organic arsenic (Shiomi 1994). Because AB is the dominant form in most marine mammals, seabirds, and sea turtles, risk to these marine animals may be rather low despite retention of high concentrations in their tissues. However, the more toxic inorganic form was detected in some specimens of sea turtles and seabirds. Inorganic arsenic acts as a carcinogen by forming certain reactive oxygen species (Kitchin 2001; Kitchin and Ahmad 2003; Hei and Filipic 2004). Oxidative damage to DNA is indeed reported in humans exposed to inorganic arsenic through contaminated groundwater (Feng et al. 2001; Basu et al. 2005; Kubota et al. 2006). However, oxidative stress induced by arsenic has not received much attention in marine organisms. Furthermore, arsenic has recently been accused of being a potent endocrine disruptor (Darbre 2006). Stoica et al. (2000) showed that As(III) activated the estrogen receptor- $\alpha$  (ER- $\alpha$ ) through formation of a high-affinity complex with the hormone-binding domain of the receptor in human breast cancer cells. Bodwell et al. (2004, 2006) revealed that at very low levels As(III) stimulated transcription [mediated by glucocorticoid receptors (GR), progesterone receptors, and mineralocorticoid receptors of humans and rats), whereas at slightly higher but not cytotoxic concentrations, inhibition of transcription was observed. Waalkes et al. (2004) reported that exposure of inorganic arsenic can cause overexpression of ER- $\alpha$  through its promoter region hypomethylation in mice and humans. According to Stanton et al. (2006) and Shaw et al. (2007), inorganic arsenic may act as an endocrine disruptor

in killifish; inorganic arsenic inhibits the ability of killifish to adapt to increased salinity by altering GR-mediated posttranscriptional steps that regulate cystic fibrosis transmembrane regulator (CFTR) protein abundance. Furthermore, some organoarsenicals such as DMA(V) and DMA(III), as well as inorganic arsenic, show carcinogenic action, probably by inducing oxidative stress (Kitchin 2001; Kitchin and Ahmad 2003; Hei and Filipic 2004). DMA(V) may be a carcinogen and tumor promoter in some experimental animals (Yamanaka et al. 2004). Presumably, dimethylarsenic peroxide may act as a tumor promoter and the dimethylarsenic radical and dimethylarsenic peroxy radical act as tumor-initiating factors, all of which seem to be metabolites of DMA(V) (Yamanaka et al. 2004). In HeLa S3 cells, As(III) induced oxidative DNA damage at  $0.075\text{ }\mu\text{g ml}^{-1}$ , MA(III) and DMA(III) at  $7.5\text{ }\mu\text{g ml}^{-1}$ , and MA(V) and DMA(V) at  $750\text{ }\mu\text{g ml}^{-1}$  (Schwerdtle et al. 2003). Because inorganic arsenic exerts adverse effects at low levels, its risk should be assessed in marine animals in which As(III) and As(V) are found. However, MA(V) and DMA(V) have not been found at levels that could adversely affect marine mammals, seabirds, and sea turtles (Fig. 7), but effects of their chronic exposure remain uncertain. Highly toxic MA(III) and DMA(III) have not been detected in marine organisms.

Arsenobetaine is known to be scarcely metabolized in animals, but small amounts of TMAO, TETRA, MA(V), DMA(V), As(V), and As(III) were detected in the urine of rats administered orally with AB (Yoshida et al. 2001). Excreted forms in the rat may result from degradation of AB by intestinal bacteria. Hence, effects of degradation products of AB, especially toxic inorganic arsenic, should be evaluated in marine animals known to absorb large amounts of AB from the organisms they consume.

Mammal species vary considerably in their capacity to methylate arsenic (Aposhian 1997; Vahter 1999); inorganic arsenic is methylated to MA(V) and DMA(V) in most mammalian species, but some species, such as the marmoset monkey and the chimpanzee, have low or no methylation capacity. In humans, genetic polymorphisms are known to affect arsenic biotransformation (Aposhian and Aposhian 2006), but no such information is available for marine organisms. For the future, information is needed on the metabolism of various forms of arsenic, not only in marine mammals, seabirds, and sea turtles but also in other marine animals and algae.

### 3.5 Newly Identified Arsenicals

Recently, various new arsenicals have been identified in tissues of marine mammals and other animals. Geiszinger et al. (2002) detected trimethylarsoniopropionate (TMAP) in muscle, liver, kidney, and lung of the sperm whale (*Physeter catodon*). The concentrations of TMAP were considerably lower than those of AB, but higher than those of DMA(V) and AC, and accounted for 3%–5% of total arsenicals found. Sloth et al. (2005a) detected DMAA, DMAP, and DMAE in the liver of hooded seal and DMAE in the kidney of harp seal. Mancini et al. (2006) identified a novel polyarsenic compound (arsenicin A;  $\text{C}_3\text{H}_6\text{As}_4\text{O}_3$ ) in the marine sponge *Echinochalina*

*bargibanti* from the coast of New Caledonia. Recently, various thio-arsenicals were also identified in the urine of sheep feeding on marine algae and humans exposed to arsenic from groundwater. In addition, 2-dimethylarsinothioyl acetic acid  $[(\text{CH}_3)_2\text{As}(=\text{S})\text{CH}_2\text{COOH}]$  was detected in urine of wild sheep feeding on brown kelp (*Laminaria hyperborea*, *L. digitata*, etc.), which was the first identification of a thio-arsenical in mammals (Hansen et al. 2004a). Thio-dimethylarsinate  $[(\text{CH}_3)_2\text{As}(=\text{S})\text{OH}]$ ; thio-DMA(V) was also identified in urine of sheep (Hansen et al. 2003, 2004b). It is reported that humans convert arsenosugars to thio-arsenicals such as thio-DMAE and thio-DMAA (Raml et al. 2005). Furthermore, thio-DMA and thio-methylarsonate  $[\text{CH}_3\text{As}(=\text{S})(\text{OH})_2]$ ; thio-MA(V) were identified in urine of humans exposed to inorganic arsenic in groundwater in Bangladesh (Raml et al. 2007). The sulfur of these thio-arsenicals is thought to be derived from  $\text{H}_2\text{S}$ , produced by sulfate-reducing bacteria in the gastrointestinal tract (Conklin et al. 2006) and released from cysteine degradation within cells (Hansen et al. 2004c). It is assumed that the oxo (As-O) and thio (As-S) forms have been readily interconverted (Raml et al. 2005). Surprisingly, Raab et al. (2007) identified a complex between thio-DMA(V) and glutathione in shoots of cabbage (*Brassica oleracea*) exposed to DMA(V), even though this is not a trivalent arsenic compound, suggesting that pentavalent arsinothioyl species may interact with proteins. We have not examined whether TMAP, DMAA, DMAP, and DMAE are present in other marine mammals, seabirds, and sea turtles because the corresponding standard compounds necessary for HPLC-ICP-MS analysis are unavailable. However, an unidentified arsenical, with behavior on HPLC similar to that of TMAP, was found in extracts from various marine mammals, seabirds, and sea turtles (Ebisuda et al. 2002; Kubota et al. 2003a, 2005). Also, thio-arsenicals, a new group of arsenic species, could be present in marine mammals, seabirds, and sea turtles, although they were not identified in the studies we conducted. We used ion-exchange columns for separation of arsenic species, but this method is not suitable for analysis of thio-arsenicals (Raml et al. 2006). Instead, a reverse-phase HPLC method would enable analysis of thio-arsenicals (Raml et al. 2006); thus, prospects for analyzing thio-arsenicals in marine mammals, seabirds, and sea turtles look promising.

## 4 Maternal Transfer of Arsenic Species

### 4.1 Maternal Transfer of Arsenic in Marine Mammals

Very few studies have been performed on the maternal transfer of arsenic species in marine mammals, seabirds, and sea turtles. Previously, it was reported that inorganic arsenic would pass through mammalian placentas but that organic arsenic would not (Morton and Dunnette 1994). However, women exposed to inorganic arsenic from drinking water contained mainly DMA(V) in cord blood, demonstrating placental transfer of some organoarsenicals in humans (Concha et al. 1998). Meador et al. (1993) detected arsenic in brain, liver, and kidney of fetal pilot

whales, confirming placental transfer of arsenic in marine mammals. However, the arsenic species was not determined in either the mothers or fetuses. Kubota et al. (2005) studied maternal transfer of arsenic to the fetus of the Dall's porpoise. Analytes included liver, kidney, muscle, and blubber of both mother and fetus. Arsenobetaine, DMA(V), AC, and MA(V) were found in liver, kidney, and muscle of the fetus (arsenic speciation was not conducted in the blubber), and the arsenical composition was similar to that of the mother, suggesting that these arsenicals can transfer from mother to fetus. However, the arsenic level in the fetus was less than one-half of that in the mother. In the fetal Dall's porpoise, 25.2% and 59.0% of total arsenic burden was distributed in blubber and muscle, respectively, whereas 59.6% and 33.5% of the burden was distributed in blubber and muscle of the mother, respectively. This difference reflects the low placental transferability of lipid-soluble arsenicals from the mother's blubber (Ebisuda et al. 2002, 2003). It is reported that hydrophobic chemicals are much less transferable from mother to fetus in these marine mammals (Tanabe et al. 1982).

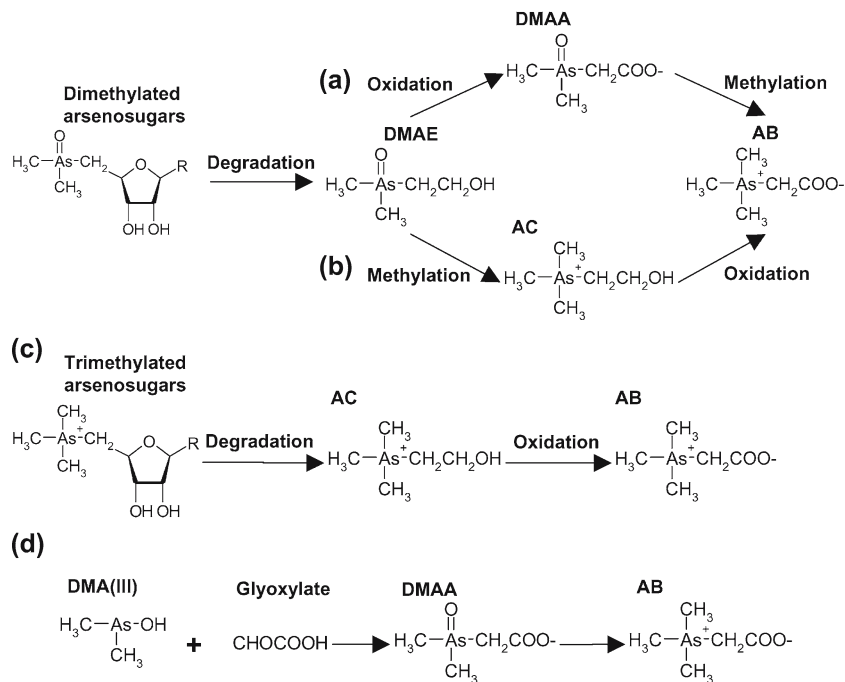
## **4.2 Maternal Transfer of Arsenic in Seabirds**

Limited information is available on the maternal transfer of arsenicals to bird eggs. To our knowledge, only one study on arsenic species in bird eggs has been reported; DMA(V) and As(III) but not AB were detected in eggs of the spoonbill (*Platalea leucorodia*), although arsenic speciation was not conducted for the mother bird (Gómez-Ariza et al. 2000). Kubota et al. (2002b) studied the maternal transfer of arsenicals to eggs of the black-tailed gull. Arsenic composition in the eggs was similar to that in tissues of the mother bird, with AB being predominant, followed by DMA(V). However, the arsenic level in eggs was low compared to that in the mother bird. The eggs weighed 32% of the body weight of the mother black-tailed gull, but the percentage of arsenic in eggs was only 11% of that existing in the mother. For the black-tailed gull (Agusa et al. 2005), the transfer rate of arsenic from mother to eggs was comparable to that of vanadium, chromium, and antimony, which are generally less transferable in birds. Arsenic was detected in eggs of sea turtles (Lam et al. 2006), and, thus, is confirmed to transfer to eggs. However, as far as we know, no studies exist on the nature of arsenic species in sea turtle eggs.

## **5 Arsenobetaine: Accumulation Mechanism and Origin**

### **5.1 Origin and Synthetic Pathway of Arsenobetaine**

The origin and synthetic pathways followed by AB are controversial. Principally, four pathways for synthesis of AB have been proposed (Fig. 9). In the first two, AB is transformed from dimethylated arsenosugars through DMAA or AC (Fig. 9a,b).



**Fig. 9** Hypothesized synthetic pathways of arsenobetaine

In the third, AB is converted from trimethylated arsenosugars (Fig. 9c). Finally, it is postulated that AB is synthesized from DMA(III) and 2-oxo acids, glyoxylate (Fig. 9d) and pyruvate, a similar pathway as exists for amino acid biosynthesis. DMAE and DMAA, at low levels, were observed in several marine animals and marine algae (Sloth et al. 2005a). The existence of these two forms supports the concept that a pathway exists from dimethylated arsenosugars (Fig. 9a,b) to AB. Despite the natural occurrence of trimethylated arsenosugars in marine organisms, their very low concentrations do not account for the presence of AB at a high concentration in marine animals (Edmonds and Francesconi 2003). However, relatively high concentrations of a trimethylated arsenosugar (2'3'-dihydroxypropyl 5-deoxy-5-trimethyl arsonioriboside) were detected in abalone (*Haliotis rubra*) from New South Wales, Australia. This arsenosugar accounted for 28% ( $5 \mu\text{g g}^{-1}$  dry wt) of all arsenicals in intestinal tissue and 0.9% ( $0.4 \mu\text{g g}^{-1}$  dry wt) of the total in muscle (Kirby et al. 2005). Hence, the trimethylated arsenosugar might contribute to synthesis of AB in this marine animal. The pathway that produces AB in deep-sea organisms and some terrestrial ones that are not dependent on marine algae is unclear (Edmonds and Francesconi 2003). A pathway (Fig. 9d) starting from DMA(III), recently proposed by Edmonds (2000), could explain the presence of AB in these animals and also some other arsenicals found in marine organisms (Edmonds and Francesconi 2003). For example, DMAA could be synthesized in this pathway,

although DMAE, an arsenical occasionally found in marine organisms, is not part of this pathway. Furthermore, DMAP and TMAP, which are found in various marine animals and algae, could be synthesized from oxaloacetate instead of glyoxylate (Fig. 9d). The major pathway leading to production of AB may vary with location, organism, and ecosystem. To be sure, a comprehensive understanding of this topic requires further research studies.

Arsenobetaine has been detected in marine animals but not in marine algae (Francesconi and Edmonds 1993), so it is presumed that synthesis of AB is ascribed to metabolic capacities of the animals and their intestinal bacteria. Involvement of bacteria in AB synthesis has been reported by several researchers. Ritchie et al. (2004) observed that AB was synthesized from DMAA and the methyl donor *S*-adenosylmethionine by lysed-cell extracts of *Pseudomonas fluorescens* A (NCIMB 13944) isolated from the blue mussel. Interestingly, this bacterium was known to degrade AB to DMAA (Jenkins et al. 2003), so the reaction in both directions might be catalyzed by a methyltransferase (Ritchie et al. 2004). The results of Ritchie et al. (2004) also suggest a direct involvement by bacteria in synthesis of AB within marine animals. In 2005, Nischwitz and Pergantis (2005a) identified and quantified AB in marine algae, by HPLC-ES-MS/MS, for the first time. In this study, commercially available brown algal powder, and fresh green, brown, and red algae, which were carefully washed to remove epifauna and contaminants from the surface, were employed; visible epifauna with body size >0.1 mm were fully removed, especially from transparent green algae. Furthermore, extraction was performed by a mild procedure using only deionized water, and methanol and sonication were avoided to prevent arsenic transformation. Analysis under these conditions revealed that AB accounted for 7.5% of extracted arsenic in green algae and 0.25%–1.3% in other algal samples. These authors also indicate that the chromatographic peak of AB present in trace amounts cannot be separated chromatographically from the larger peaks of the major arsenicals (i.e., arsenosugars) in marine algae, and therefore the presence of AB could not be confirmed in marine algae by HPLC-ICP-MS analysis (Nischwitz and Pergantis 2005a). It should be noted that the HPLC-ES-MS/MS enables analysis of arsenicals co-eluting from the HPLC column, in contrast to HPLC-ICP-MS. These results cast doubt on the general assumption that AB is not present in marine algae and the belief that this arsenical is either synthesized or accumulated only in marine animals. Therefore, further studies are needed to confirm these findings.

## 5.2 Accumulation Mechanism of Arsenobetaine in Marine Animals

It has been pointed out that high levels of AB in marine animals may be related to the salinity of seawater. Organisms are known to utilize various osmolytes, low molecular weight osmotically active solutes, to adapt to osmotic stress. Glycine betaine [GB,  $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COO}^-$ ] is the nitrogen analogue of AB (Shibata et al. 1992) and behaves as an osmolyte in marine animals at lower trophic levels (Yancey

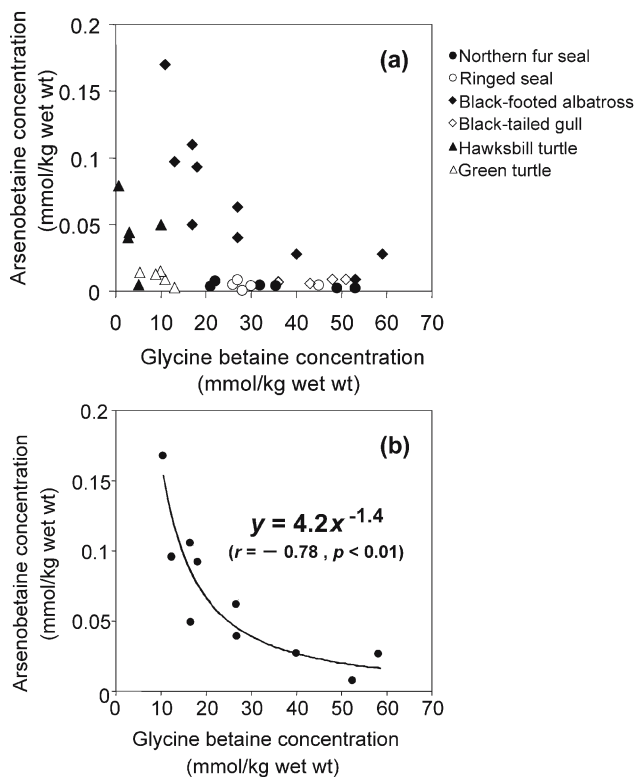


et al. 1982), in mammals (Burg et al. 1997), and in birds (Lien et al. 1993). It is suggested that once AB is synthesized in the marine food chain, it may be taken up into cells through the same route that absorbs GB and thereby behave as GB does in the cells (Shibata et al. 1992; Shibata and Morita 2000). Indeed, it has been reported that neither GB nor AB is bound to macromolecules (e.g., proteins) (Vahter et al. 1983).

Arsenobetaine was not detected in the bivalve, *Corbicula japonica*, which lives in a low-salinity estuary (Shibata and Morita 1992). This result suggests the possibility that AB was not accumulated in the bivalve because osmolytes are not necessary in a low-salinity environment. It was also shown that the blue mussel accumulated AB efficiently from seawater, but the accumulation decreased in the presence of GB in seawater (Gailer et al. 1995). Clowes and Francesconi (2004) reported that AB levels increased in the blue mussel when the animals were maintained at high salinity. When the blue mussel that had been maintained at high salinity was transferred to low-salinity seawater, AB levels decreased in the gill but not in other tissues (Clowes and Francesconi 2004). These results suggest that AB behaves as an osmolyte in the blue mussel and that the gill responds sooner to osmotic changes than did other tissues. Also, for herring (*Clupea harengus*), cod (*Gadus morhua*), and flounder (*Platichthys flesus*), total arsenic concentrations in muscle were correlated with salinity at locations where the fish were collected, which may be because arsenic levels (probably AB) in fish or their diet animals reflected the ambient salinity (Larsen and Francesconi 2003). Although controversial, Amlund and Berntssen (2004), after studying the retention capacity of AB in seawater- and freshwater-adapted Atlantic salmon (*Salmo salar*), found no significant difference between such groups, despite the AB level in muscle of seawater-adapted wild salmon being more than 10 fold that of freshwater-adapted wild salmon. Thus, the high AB level of seawater-adapted wild salmon might be caused by the AB level in diet rather than an adaptation to salinity. On the other hand, the bacterium *Escherichia coli* (Pichereau et al. 1997) and Madin Darby canine kidney (MDCK) cells (Randall et al. 1996) absorbed AB and GB in response to osmotic stress, although the uptake rate of AB was lower than that of GB (Randall et al. 1996). Furthermore, it was shown that AB was efficiently absorbed through two GB transporters, ProP and ProU, in *Escherichia coli* (Randall et al. 1995). An alternative explanation for high concentrations of AB in marine animals is that AB might be largely distributed in cellular organelles. Vahter et al. (1983) reported that urinary excretion of AB was slower in rabbits, which have more AB in cellular organelles, than in mice or rats. The relationship between AB accumulation and its subcellular distribution has not yet been examined in wildlife.

To gain insight into the mechanisms of the high AB accumulation, we determined subcellular distribution of arsenic and the relationship between AB and GB concentrations in livers of the northern fur seal (*Callorhinus ursinus*), ringed seal, black-footed albatross, black-tailed gull, hawksbill turtle, and green turtle (Fujihara et al. 2003). Results indicated that arsenic levels were not related to the subcellular distribution in these marine animals. However, a significant negative correlation was observed between AB and GB concentrations for all animals examined (Fig. 10a); a strong negative correlation was observed, especially for the black-footed albatross





**Fig. 10** Relationship between arsenobetaine and glycine betaine concentrations in liver of marine mammals, seabirds, and sea turtles (Fujihara et al. 2003)

(Fig. 10b). These results were contrary to our expectation; we had assumed that GB might increase with increasing AB levels in the animals, but, instead, a negative correlation was observed (Fig. 10). It is assumed that AB and GB are both taken up and efficiently retained when osmolyte content (e.g., GB) is insufficient in these animals or in their food supply. If true, this condition would lead to a negative correlation between AB and GB levels in the marine animals. It is likely that the contribution of AB to osmoregulation was low because the AB level was remarkably lower than that of GB (Fig. 10).

### 5.3 Arsenobetaine in Freshwater and Terrestrial Environments

Arsenic concentrations in seals from freshwater environments, the Baikal seal (*Pusa sibirica*) (Kubota et al. 2001) from Russia, and the harbor seal (*Phoca vitulina*) from northern Quebec in Canada (Langlois and Langis 1995), were lower

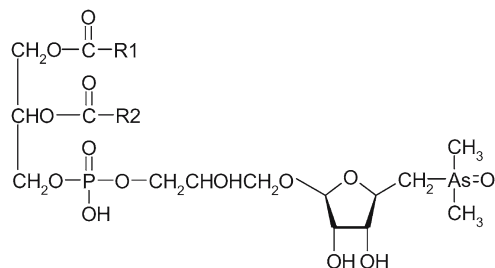
than those in seals from marine environments (see Fig. 6), suggesting that these freshwater species are exposed to low AB concentrations in their food supply because of the low salinity of freshwater habitats. It is frequently reported that AB is not a dominant arsenical in freshwater animals, although arsenosugars dominate in both freshwater and marine algae (Francesconi and Kuehnelt 2002). Nondetectable or very low concentrations of AB was observed in freshwater animals from the River Danube in Hungary (Schaeffer et al. 2006). Jankong et al. (2007) reported relatively low accumulation of AB in tissues, especially liver, of four species of freshwater fish collected from highly arsenic-contaminated ponds (550 and 990  $\mu\text{g L}^{-1}$ ). Soeroes et al. (2005) described the absence of AB in the common carp (*Cyprinus carpio*) from lakes in Hungary. Low concentrations of AB in freshwater fish may reflect the low salinity of their ambient environment. However, some freshwater fish species contain predominantly AB (Shibata and Morita 2000; Francesconi and Kuehnelt 2002). Šlejkovec et al. (2004) suggest that composition of arsenic species is different among freshwater fish species; AB predominates especially in species of salmonids even though they inhabit freshwater environments all through their life. Increasing evidence suggests that AB is present also in various terrestrial organisms (Kuehnelt and Goessler 2003) such as mushrooms (Kuehnelt et al. 1997a), earthworms (Geiszinger et al. 1998), and ants (Kuehnelt et al. 1997b), although the levels are remarkably low. Future elucidation of the origin, behavior, and function of AB, not only in the marine ecosystem, but also in freshwater and terrestrial ecosystems, is necessary.

## 6 Lipid-Soluble Arsenic

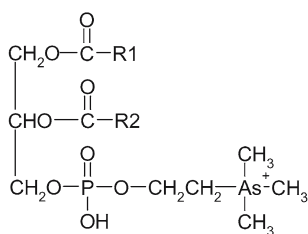
### 6.1 *Lipid-Soluble Arsenicals in Marine Organisms at Low Trophic Levels*

It is well known that various marine organisms contain lipid-soluble arsenic. The ascidian (*Halocynthia roretzi*), the turban shell (*Turbo cornutus*), the short-necked clam (*Tapes japonica*) (Shinagawa et al. 1983), the red sea urchin (*Pseudocentrotus depressus*), the abalone (*Haliotis diversicolor supertexta*), the three-line grunt (*Parapristipoma trilineatum*), and the Japanese surfperch (*Neoditrema ransonneti*) (Kaise et al. 1988) all showed relatively high concentrations of lipid-soluble arsenic, although the levels were lower than those of water-soluble arsenic. A phosphatidylarsenosugar was identified for the first time in a brown alga (*Undaria pinnatifida*) as a lipid-soluble arsenical in 1988 (Fig. 11; Morita and Shibata 1988). Phosphatidylarsenocholine (Fig. 11), a phosphatidylcholine analogue, was also identified in the digestive gland of the Western rock lobster (Edmonds et al. 1992). Because direct introduction of organic solvents, used for extraction of lipid-soluble arsenicals, into ICP-MS is generally problematic, lipid-soluble arsenicals in marine organisms are poorly studied. Until now, only water-soluble arsenicals

## Phosphatidylarsenosugar



### Phosphatidylarsenocholine



**Fig. 11** Lipid-soluble arsenicals identified in marine organisms

released from the lipid-soluble arsenicals have been studied after alkaline or acid digestion. Unfortunately, these analyses cannot provide direct information on the structure of lipid-soluble arsenicals.

In the star spotted shark (*Mustelus manazo*), almost all lipid-soluble arsenic was detected in the polar lipid fraction. Lipid-soluble arsenic levels in this fraction were particularly high in liver, gallbladder, and kidney (Hanaoka et al. 1999). Lipid-soluble arsenic in the shark was fractionated into alkali (0.027 M NaOH)-stable and alkali-labile portions, with the former predominating in liver and gallbladder, and the latter in kidney (Hanaoka et al. 1999). Thus, the results suggest that the nature of lipid-soluble arsenicals varies among tissues of the shark. Altogether, four arsenicals were detected in the alkali-labile fraction of 12 shark tissues, with one of these arsenicals predominating in kidney, spleen, and brain (Hanaoka et al. 2001b). After digestion with 6 M HCl of three arsenicals detected in the alkali-labile fraction, AC or DMA(V) was released from two of three of these arsenicals; HCl failed to digest the third arsenical. Hydrolysis of the alkali-stable fraction with saturated Ba(OH)<sub>2</sub> gave primarily DMA(V) for liver, and DMA(V) and AC for muscle, skin, stomach, and intestine (Hanaoka et al. 2001b). Therefore, at least six lipid-soluble arsenicals exist in and contain precursors of DMA and AC in the star spotted shark. In contrast, water-soluble arsenicals were not released by alkaline hydrolysis (1 M NaOH), but DMA(V) was detected after acid digestion (conc. HNO<sub>3</sub>) in fish oil (Kohlmeyer et al. 2005). Lipid-soluble arsenicals were only detected in the polar lipid fraction of

fish oil, where neutral lipids constituted more than 90% of the total (Kohlmeyer et al. 2005); this is consistent with the distribution of lipid-soluble arsenicals in the star spotted shark (Hanaoka et al. 1999).

## 6.2 *Lipid-Soluble Arsenicals in Marine Mammals*

As far as we know, no information was available on lipid-soluble arsenicals in marine mammals, seabirds, and sea turtles before we conducted studies on the blubber of marine mammals. Lipid-soluble arsenic was found in ringed seal liver, kidney, muscle, and gonad tissues, but proportions were very low (Ebisuda et al. 2002). In contrast, lipid-soluble arsenic accounted for about 90% of arsenic in ring seal blubber (Ebisuda et al. 2002). Hence, lipid-soluble arsenicals in the ringed seal blubber were characterized using the method of Edmonds et al. (1992). Results showed at least two lipid-soluble arsenicals: one was tetraethylammonium hydroxide (TEAH) hydrolyzable, and the other was TEAH stable but NaOH labile. Both these released DMA(V) after hydrolysis (Ebisuda et al. 2003). Thus, it is suggested that marine mammals accumulate arsenic mostly in blubber as DMA-containing lipid-soluble arsenicals. Structural identification of these lipid-soluble arsenicals will be critical to understanding how arsenic is metabolized in marine mammals. Insights into how DMA(V) is incorporated into lipid-soluble arsenicals in marine animals would also be useful, considering the genotoxic potential of DMA(V).

## 6.3 *Promising Analytical Methods for Lipid-Soluble Arsenicals*

Miyajima et al. (1988) purified and analyzed, with nuclear magnetic resonance (NMR), a lipid-soluble arsenical from the tiger shark (*Galeocerdo cuvier*) without digestion that revealed the presence of  $(\text{CH}_3)_2\text{As}(\text{O})\text{CH}_2^-$  in this arsenical. Devalla and Feldmann (2003) characterized lipid-soluble arsenicals by an enzymatic hydrolytic method. Water-soluble arsenicals, released by phospholipase D treatment of lipid-soluble ones from a marine alga, was mainly an arsenosugar. In contrast, DMA(V) and MA(V) were the major arsenicals released from lipid-soluble arsenicals in kidney, and muscle, and DMA(V) from lipid-soluble ones in feces of marine algae-eating sheep. Also, the digestion of the lipid-soluble arsenicals by phospholipase D indicated that the released arsenicals were bound not to the simple lipid (comprising 91% of total lipid) but to a complex lipid (e.g., phospholipid and sphingolipid) present in sheep tissues. Importantly, limited numbers of water-soluble arsenicals released from lipid-soluble arsenicals may result from binding of an arsenical moiety to various lipid-soluble species (Schmeisser et al. 2006a). Ninh et al. (2007) suggested the possible presence of two DMA(V)-containing lipid-soluble arsenicals, phosphatidyl dimethylarsinic acid and DMA(V)-containing sphingomyelin, in the

Japanese flying squid (*Todarodes pacificus*) using a combination of chemical and enzymatic hydrolysis techniques.

Little is known about the toxicity of lipid-soluble arsenicals in organisms (Francesconi 2005). In humans, after metabolism, DMA(V) and trace amounts of oxo-DMAP, thio-DMAP, oxo-DMAB, and thio-DMAB were excreted in the urine (Schmeisser et al. 2006a,b). The toxicological evaluation of lipid-soluble arsenicals and their metabolites will be the subject of future research.

Because it has been difficult to directly analyze for lipid-soluble arsenicals by HPLC-ICP-MS, very little progress has been made with lipid-soluble arsenicals in contrast to water-soluble arsenicals. Very recently, it became feasible to analyze for lipid-soluble arsenicals directly by HPLC-ICP-MS (Schmeisser et al. 2005). These authors found that problems associated with the introduction of organic solvent to the plasma were considerably reduced by using a low column flow rate, a cooled spray chamber ( $-5^{\circ}\text{C}$ ), and addition of oxygen directly to the plasma (20% in argon). The authors applied this method to successfully observe the presence of at least 10 lipid-soluble arsenicals in fish oil. This method would be useful to characterize lipid-soluble arsenicals in marine mammals, seabirds, and sea turtles.

## 7 Future Areas of Study

More than 30 arsenicals have been identified in marine environments (Francesconi and Kuehnelt 2004), and it is expected that more will be identified as analytical techniques advance. For example, 15 unknown trace arsenicals, which were not detected by conventional methods such as HPLC-ICP-MS and HPLC-hydride generation (HG)-ICP-MS, were found in human urine using LC-high-efficiency photooxidation (HEPO)-HG-ICP-MS (Nakazato and Tao 2006). The development of such new analytical techniques is important because such tools improve our understanding of arsenics behavior in geochemical cycles, ecosystems, organisms, and transformation pathways, as well as their toxicity. If progress is to be made, convenient methods of synthesis for standard compounds of recently identified arsenicals and commercial production thereof are badly needed. As recently identified arsenicals (e.g., DMAA and TMAP) have been quantified in some CRMs such as DORM2 (dogfish muscle) and BCR CRM627 (tuna fish tissue) (Sloth et al. 2003), these CRMs can be utilized to evaluate the accuracy of methods for these arsenicals.

Studies on the interaction between arsenic and other elements in marine organisms are also necessary. It is well known that mercury is detoxified through binding to selenium and/or sulfur in marine mammals and seabirds (Shibata et al. 1992; Ng et al. 2001; Arai et al. 2004; Ikemoto et al. 2004). Similarly, interaction between arsenic and selenium and/or sulfur may also occur (Gailer 2007). A metabolite containing glutathione (GSH) and equimolar amounts of As and Se,  $[(\text{GS})_2\text{AsSe}]^-$ , was identified in bile from rabbits injected with selenite  $[\text{Se(IV)}]$  and As(III) (Gailer et al. 2000). This metabolite is thought to be synthesized in hepatocytes (Gailer et al. 2002a) and erythrocytes (Manley et al. 2006) with high endogenous

concentrations of GSH. Furthermore,  $[(\text{CH}_3)_2\text{AsSe}_2]^-$  can be synthesized chemically by reacting DMA(V) with GSH and Se(IV) (Gailer et al. 2002b). Even though these metabolites are present in marine mammals, seabirds, and sea turtles, the levels may be low. Small granules (diameter about 3 nm) of  $\text{As}_2\text{Se}$  were observed in the kidney of rats injected with inorganic arsenic and selenium (Berry and Galle 1994), and thus it will be interesting to examine whether such granules are present in marine animals. Also, Kanaki and Pergantis (2007) recently synthesized seleno-arsenosugars and seleno-DMA(V) by reacting arsenosugars and DMA(V) with  $\text{H}_2\text{Se}$ , respectively; these reaction products are unstable and, if present, are not expected to occur in organisms at significant levels.

DMA(V), which is widely distributed in marine animals, was thought to be a metabolite formed during the detoxification of inorganic arsenic; however, the metabolites of the methylation process for inorganic arsenic (see Fig. 3) include MA(III) and DMA(III), which have recently been reported to be highly toxic; hence, this process is no longer regarded as one of detoxification for inorganic arsenic. The possible carcinogenicity of DMA(V) should induce additional interest in conducting safety evaluations on marine animals. Furthermore, arsenosugars, which are generally considered to be practically non-toxic, exhibit genotoxicity by forming reactive oxygen species when present as trivalent arsenicals (Andrewes et al. 2004). Such trivalent arsenosugars are readily formed by the reaction of pentavalent arsenosugars with thiols (Andrewes et al. 2004). Additional evaluations of the *in vivo* toxicity of arsenosugars are therefore needed.

Nonextractable arsenic in the tissues of marine organisms should be studied. For example, an average of  $0.73 \mu\text{g g}^{-1}$  dry wt of arsenic was present as nonextractable residue after extraction with methanol:water (9:1 v/v) in the liver of green turtles (Kubota et al. 2003a). This residue constituted about 25% of total arsenic. Thus, nonextractable arsenic cannot be ignored if the complete pattern of arsenic metabolism in marine organisms is to be understood. Arsenic-binding proteins are known to exist in experimental animals, and such proteins may be involved in the transformation and detoxification of arsenic (Aposhian and Aposhian 2006). For example, it was reported that most of the As(III) added to rat liver cytosol became protein bound, and this binding affected the extent of its subsequent methylation (Styblo et al. 1996; Styblo and Thomas 1997). Trivalent methylated arsenicals, MA(III) and DMA(III), also bind to proteins (Naranmandura et al. 2006). Further work is needed to clarify the role arsenic-bound proteins play in the metabolism of arsenic by marine mammals, seabirds, and sea turtles.

## 8 Summary

Although there have been numerous studies on arsenic in low-trophic-level marine organisms, few studies exist on arsenic in marine mammals, seabirds, and sea turtles. Studies on arsenic species and their concentrations in these animals are needed to

evaluate their possible health effects and to deepen our understanding of how arsenic behaves and cycles in marine ecosystems. Most arsenic in the livers of marine mammals, seabirds, and sea turtles is AB, but this form is absent or occurs at surprisingly low levels in the dugong. Although arsenic levels were low in marine mammals, some seabirds, and some sea turtles, the black-footed albatross and hawksbill and loggerhead turtles showed high concentrations, comparable to those in marine organisms at low trophic levels. Hence, these animals may have a specific mechanism for accumulating arsenic. Osmoregulation in these animals may play a role in the high accumulation of AB. Highly toxic inorganic arsenic is found in some seabirds and sea turtles, and some evidence suggests it may act as an endocrine disruptor, requiring new and more detailed studies for confirmation. Furthermore, DMA(V) and arsenosugars, which are commonly found in marine animals and marine algae, respectively, might pose risks to highly exposed animals because of their tendency to form reactive oxygen species. In marine mammals, arsenic is thought to be mainly stored in blubber as lipid-soluble arsenicals. Because marine mammals occupy the top levels of their food chain, work to characterize the lipid-soluble arsenicals and how they cycle in marine ecosystems is needed. These lipid-soluble arsenicals have DMA precursors, the exact structures of which remain to be determined. Because many more arsenicals are assumed to be present in the marine environment, further advances in analytical capabilities can and will provide useful future information on the transformation and cycling of arsenic in the marine environment.

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