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Mercury speciation and selenium in toothed-whale muscles

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ABSTRACT

Mercury accumulates at high levels in marine mammal tissues. However, its speciation is poorly understood. The main goal of this investigation was to establish the relationships among mercury species and selenium (Se) concentrations in toothed-whale muscles at different mercury levels. The concentrations of total mercury (T-Hg), methylmercury (MeHg), inorganic mercury (I-Hg) and Se were determined in the muscles of four toothed-whale species: bottlenose dolphins ($n=31$), Risso's dolphins ($n=30$), striped dolphins ($n=29$), and short-finned pilot whales ($n=30$). In each species, the MeHg concentration increased with increasing T-Hg concentration, tending to reach a plateau. In contrast, the proportion of MeHg in T-Hg decreased from 90–100% to 20–40%. The levels of T-Hg and Se showed strong positive correlations. Se/I-Hg molar ratios rapidly decreased with the increase of I-Hg and reached almost 1 in all species. These results suggested that the demethylated MeHg immediately formed Se/I-Hg equimolar complex of mercury selenide (HgSe) in their muscles. In addition, an X-ray absorption fine structure analysis (XAFS) of a bottlenose dolphin muscle confirmed that the dominant chemical form of the Se/I-Hg equimolar complex was HgSe. HgSe was mainly localized in cells near the endomysium using electron probe microanalysis (EPMA). These results suggested that the demethylated MeHg finally deposits within muscle cells of bottlenose dolphin as an inert HgSe.

1. Introduction

Mercury is mainly released as elemental mercury into the atmosphere from natural and anthropogenic sources (UNEP, 2008). The elemental mercury emitted into the atmosphere is oxidized to divalent mercury, a portion of which is methylated to methylmercury (MeHg), which enters the aquatic food chain. In 2003, the Governing Council of the United Nations Environment Program (UNEP) started a global mercury assessment. In January 2012, the UNEP also agreed to develop a legally-binding global instrument on mercury for the global control of mercury pollution (UNEP, 2013), because the global mercury levels are estimated to be increasing after the industrial revolution, reflecting anthropogenic increases of mercury emissions (Dietz et al., 2009).

Toothed-whales form an infraorder of the artiodactyl suborder cetacean, including sperm whales, beaked whales, and dolphins, among others. Toothed-whales tend to be at the top of marine food chain and feed mainly on fishes, squids and crustaceans, and accumulate high levels of mercury in their tissues, including muscles (Andre et al., 1991; Endo et al., 2003). The mercury concentrations in the muscles of toothed-whales from Japanese market samples were reported to be 0.44–98.9 μg/g wet weight (Endo et al., 2003). Consequently, the continuous monitoring of the changes in mercury concentrations in stranded toothed-whales should be useful to predict global mercury contamination in the future. However, a better understanding of the mechanisms underlying high mercury accumulation in toothed-whales muscles is critical, before it can be used as a biomarker.

Selenium (Se) concentrations are also high in toothed-whale

Abbreviations: (T-Hg), total mercury; (MeHg), methylmercury; (I-Hg), inorganic mercury; (Se), selenium; (HgSe), mercury selenide; ICP-MS, inductively coupled plasma mass spectrometry; XAFS, X-ray absorption fine structure; EPMA, electron probe microanalysis; GC-ECD, gas chromatography with electron capture detection; XANES, X-ray near edge structure

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organs (Endo et al., 2005). Se has an extremely high affinity for mercury (Ralston et al., 2008), and the coexistence of mercury and Se was first recognized in marine mammals in an early study (Koeman et al., 1975). Se is an essential element that plays a key biological role as a component of antioxidative enzymes such as glutathione peroxidase and thioredoxin reductase (Tapiero et al., 2003). Additionally, increased evidence in experimental animals suggests that Se can counteract the toxicity of inorganic mercury (I-Hg) and MeHg (Chang and Suber, 1982; Ralston et al., 2008; Sakamoto et al., 2013). Some marine animals and bird species are known to demethylate MeHg and to accumulate mercury as immobile mercury/Se complex such as mercury selenide (HgSe) in their organs, especially the liver (Dietz et al., 1990; Koeman et al., 1975; Lailson-Brito et al., 2012; Nakazawa et al., 2011). Therefore, the role of Se is important to understand the mechanism of mercury tissue accumulation.

In previous investigations, much focus has been placed on the high total mercury (T-Hg) and MeHg concentrations in toothed-whales (Andre et al., 1991; Endo et al., 2003). However, recent studies have shown that toothed-whales accumulate inert forms of mercury in their organs (Lailson-Brito et al., 2012; Nakazawa et al., 2011). Therefore, it is important to study mercury speciation in tissues in order to understand the biology of mercury in toothed-whales. We hypothesize that MeHg is demethylated in the muscles of toothed-whales and the T-Hg keep increasing as an inert HgSe in them. Moreover, mercury speciation in the muscles may be an important public health concern, especially for those populations consuming toothed-whale meats (Nakamura et al., 2014).

In the present study, we conducted mercury speciation and Se analysis in the muscles of four species of toothed-whales. The mercury/Se complexes in the bottlenose dolphin muscle which had high T-Hg and I-Hg concentrations were measured using X-ray absorption fine structure (XAFS) analysis. An electron probe microanalysis (EPMA) was conducted to visualize the localizations of mercury and Se in the muscle fiber cells of bottlenose dolphins.

2. Materials and methods

2.1. Features of the toothed-whales used for this study

The four species of adult toothed-whales, with almost equal numbers of males and females, bottlenose dolphins ($n=31$; 15 males and 16 females), Risso's dolphins ($n=31$; 16 males and 15 females), striped dolphins ($n=29$; 15 males and 14 females) and short-finned pilot whales ($n=30$; 15 males and 15 females) were caught for commercial and scientific purposes under the appropriate permission at the cost of Taiji Town, Wakayama Prefecture, Japan in the 2007/2008 winter season. We chose the species and sizes of the adult whales which are commonly consumed by the local populations. Body length and sex data were recorded for all specimens. The lengths (mean $\pm$ SD) of bottlenose dolphins were 290 ± 5.4 cm for males and 281 ± 5.7 cm for females, those for Risso's dolphins were 277 ± 7.5 cm for males and 267 ± 8.1 cm for males, those for striped dolphins were 231 ± 4.7 cm for males and 218 ± 5.9 cm for males, and for short-finned pilot whales the lengths were 402 ± 66 cm for males and 347 ± 11 cm for females. The former three species showed comparatively uniform length. However, the sizes of the short-finned pilot whales, especially males, varied over a wide range, because they swam in groups, making it difficult to sample specific sizes. The approximately 100 g of muscles from the central dorsal part of the body were preserved at -20 °C for further scientific analysis. T-Hg, MeHg, and Se were measured in the muscles of four toothed-whale species: bottlenose dolphins ($n=31$), Risso's dolphins ($n=31$), striped dolphins ($n=29$) and short-finned pilot whales ($n=30$). XAFS and EPMA measurements were conducted on the muscles which showed the first and 2nd highest T-Hg concentrations, because of the limited resolution powers of these analyses.

2.2. Chemical analyses of T-Hg, MeHg, and Se

T-Hg concentrations in approximately 20 mg of wet weight samples were determined by cold vapor atomic absorption spectrophotometry according to a previously described method (Akagi et al., 2000). The method involved sample digestion with HNO_3, $HClO_4$, and H_2SO_4, followed by reduction to Hg^0 by $SnCl_2$. T-Hg in standard reference material, DORM-2 (Dogfish Muscle; National Research Council, Ottawa, Canada), was measured as quality control, and the measured results always fell within the certified range of 4.64 ± 0.26 μg/g. MeHg concentrations in the muscle samples (approximately 20 mg) were determined by gas chromatography with electron capture detection (GC-ECD) according to a previously described method (Akagi et al., 2000). Briefly, the method involved sample digestion with KOH–ethanol. After extraction with dithizone–toluene, MeHg was back-extracted with a slightly alkaline sodium sulfide solution. MeHg was re-extracted with a small portion of dithizone–toluene. The extract was then washed with NaOH solution to remove the excess dithizone and analyzed by GC-ECD (Yanaco G8600; Yanaco, Tokyo, Japan). The precision and accuracy of the MeHg measurements were repeatedly verified by inter-laboratory calibration exercises, including analyses of standard reference materials such as IAEA-085, 086, and 142 (Horvat et al., 1997). Dogfish muscle DORM-2 was also used as the standard reference material for MeHg determinations in the muscle samples. The measured results always fell within the certified range of 4.47 ± 0.32 μg/g. Se concentrations were measured using an inductively coupled plasma mass spectrometer equipped with a collision cell (Agilent 7500ce; Agilent Technologies, Santa Clara, CA) by IDEA Consultants, Inc. (Shizuoka, Japan). NIST 1577 (Bovine Liver; Gaithersburg, USA) was used. The obtained results fell within the certified range of 0.73 ± 0.06 μg/g. Inorganic mercury (I-Hg) was calculated as T-Hg minus MeHg.

2.3. XAFS measurements

Measurements of the XAFS spectra at the mercury LIII-edge and Se K-edge were conducted at BL01B1 of SPring-8 (SPring-8 Facility, Hyogo, Japan). The bottlenose dolphin muscle with the second-highest T-Hg concentration (T-Hg: 62.5 μg/g wet weight; MeHg: 8.4 μg/g wet weight; I-Hg: 54.1 μg/g wet weight; Se: 17.4 μg/g wet weight) was used for analysis. Approximately 1 g wet weight sample and reference materials were placed in an oxygen-impermeable film just before the XAFS measurements. All measurements were carried out at room temperature under ambient air conditions. The X-ray near edge structure (XANES) spectra of the reference materials were determined in the transmission mode, while the muscle sample was measured in the fluorescence mode. All samples were positioned at 45° to the incident beam in the fluorescence mode. The incident X-ray was tuned with a Si (111) monochromator. Energy calibration was performed by assigning the first peak of HgO as 12.286 keV and the peak top of $NaHSeO_3$ as 12.655 keV. Incident and transmitted intensities were measured by ionization chambers in the transmission mode, while fluorescent X-rays were determined by a 19-element silicon semiconductor detector.

The XANES spectra of HgO, α-HgS, and HgSe were measured as the reference materials for mercury, while those of $CaSeO_4 \cdot 2H_2O$, $CaSeO_3$, SeS_2, and HgSe were measured as the reference materials for Se. Background removal, normalization, and linear curve fitting (LCF) analysis were performed using REX