Presence of HgSe(tiemannite) in various tissues of the striped dolphin: evidence obtained by μ-XRF-XRD and XAFS analyses

Marine mammals accumulate mercury (Hg) in their tissues at a high concentration (up to > 10,000 μg/g dry weight) because of their high position in the marine food chain and their long lifespan [1,2]. In spite of the high concentration, no indication of Hg intoxication has been reported for marine mammals, and thus they seem to have the ability to tolerate high Hg concentrations. Many researchers have assumed that methyl Hg taken up from the diet should be demethylated to inorganic Hg and that the resultant inorganic Hg forms an equimolar HgSe compound (mineral name: tiemannite) mainly in the liver of marine mammals. Although Se might detoxify Hg by forming HgSe in tissues other than liver, very few studies have focused on HgSe in tissues other than the liver of marine mammal and no solid evidence as to the presence of HgSe has been reported. Could tiemannite be formed in the tissues of the striped dolphin other than liver? Thus, we explored HgSe granules in various tissues of the striped dolphin and tried to identify their chemical form nondestructively by utilizing a combined analytical system, μ-XRF-XRD and XAFS[3].

Seven tissues (liver, muscle, kidney, brain, lung, pancreas, and spleen) of the striped dolphin (*Stenella coeruleoalba*) were used in the present study. These samples were collected from a male striped dolphin stranded along the coast of Ehime Prefecture, Japan. For μ-XRF-XRD analysis, the tissues were embedded in OCT compound and cut into 25 μm frozen sections using a cryomicrotome.

The SR-μ-XRF-XRD analysis was carried out at beamline **BL37XU**. The monochromatic X-ray beam was focused by Fresnel zone plate (FZP) optics through an order-selecting aperture (OSA) onto a spot of 1.5 (V) × 1.5 (H) μm^2 in the sample. μ-XRD data were collected using the Deby Sherrer mode with an imaging plate (IP) placed behind the sample. The energy of incident X-rays was set to 15 keV (λ = 0.827 Å). The Hg-L_2-XAFS analysis for the non-extractable fraction from the nuclear and mitochondrial fraction of each tissue was also carried out at beamline **BL01B1** in the fluorescence mode by using a 19-element Ge solid state detector. The local structure of Hg was examined by analyzing XAFS data to determine whether Hg is detoxified by forming HgSe in tissues other than the liver of the dolphin.

The 2D elemental maps shown in Fig. 1 were obtained by μ-XRF imaging using an X-ray microbeam (13.4 keV). The normalized X-ray fluorescence intensities are scaled from red (maximum) to blue (minimum).

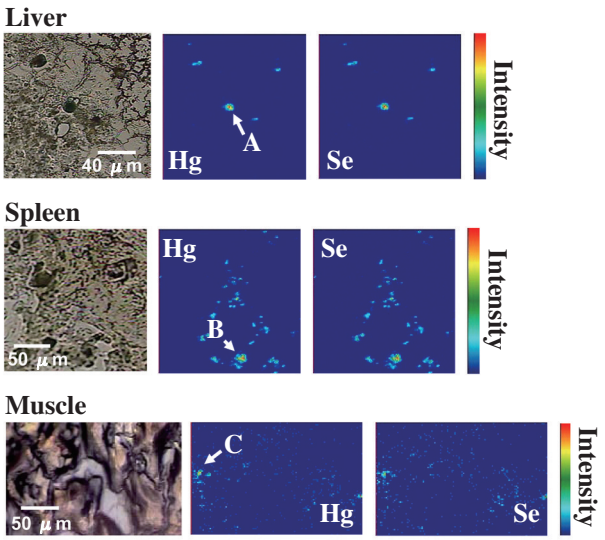

Fig. 1. Micrographs and 2D elemental maps of the liver, spleen and muscle of the striped dolphin. Pixel numbers: 100 (V) × 100 (H), 130 (V) × 130 (H), and 78 (V) × 120 (H) for liver, spleen, and muscle tissues, respectively.

Figure 1 shows the clear positive correlation between the distributions of Hg and Se, and this suggests that Hg forms a compound together with Se in these tissues. It seems that the concentrated points of Hg and Se in the liver and spleen also correspond well to the black granules (5 to 10 μm) in the optical micrograph given on the left side of Fig. 1.

The thin section samples were then subjected to μ-XRD analysis to identify the black granular materials. The XRD patterns were obtained for point A in the liver and point B in the spleen, as shown in Fig. 2. The smooth diffraction rings detected suggest that the biominerals are polycrystalline. The powder diffraction data are shown in Table 1 together with the reference PDF data of tiemannite. The diffraction data measured at points A and B were consistent with those of HgSe (tiemannite), confirming that the granules found in the liver and spleen sections were composed of HgSe

Table 1. A comparison of the diffraction data of point A (liver) and B (spleen) with that of HgSe

Point A (Liver)		Point B (Spleen)		HgSe; tiemannite [a]		
d (Å)	*I*	*d* (Å)	*I*	*d* (Å)	*I*	*hkl*
3.46	100	3.51	100	3.51	100	111
3.02	19			3.04	16	200
2.13	54	2.14	46	2.15	50	220
1.82	28	1.83	31	1.84	30	311

Relative intensity (*I*) is given in *hkl*: the indices of reflection.
[a] PDF file No. 08-0469.

(tiemannite). This is the first clear evidence of the presence of crystalline HgSe (tiemannite) in the spleen other than the liver. These results strongly suggest that Hg is also detoxified by forming HgSe in tissues other than the liver of the striped dolphin.

After the trypsin digestion of the non-extractable fraction from the nuclear and mitochondrial fraction, XAFS analysis was conducted to examine the chemical state of Hg in the seven tissues of the striped dolphin. The Hg L_2-edge XANES spectra of the tissues are shown in Figs. 3(a)–3(g), together with that of the reference samples for mercury compounds. It is found that all of the tissues of the striped dolphin showed similar XANES spectra, suggesting similar local structures around the Hg atoms in these samples. From the chemical shift, Hg was found to be present in the Hg(II) state in these tissues. Also, because of the similarity between the XANES spectra of the dolphin samples and that of the HgSe (tiemannite), the local structures around Hg atoms in the former samples seems to be the most similar to those of HgSe.

We then attempted to obtain the structural parameters for mercury-containing compounds in these tissues by EXAFS analysis. The fitting was conducted for the first strongest peak in the Fourier transform data of the k^3-weighted $\chi(k)$ values for Hg. The coordination numbers and bond lengths for the tissue samples and HgSe were calculated. For all the tissues examined, the Hg-Se bond length and the coordination number were similar (*ca.* 2.6 Å and *ca.* 4, respectively), and these values are consistent with those of tiemannite HgSe (2.63 Å and 4.0, respectively) confirming the presence of crystalline HgSe in the seven tissues.

The present results reinforce the importance of the detoxification role of Se in tissues other than the liver of marine mammals. The biosynthesis and storage of mercury selenide were mainly associated with the liver, but it was not exclusive to the liver. SR-μ-XRF-XRD measurement enables direct analysis of a biomineral at a high spatial resolution. The combination of these techniques would provide information valuable for determining other metal detoxification mechanisms and biomineralization processes in environmental samples [4].

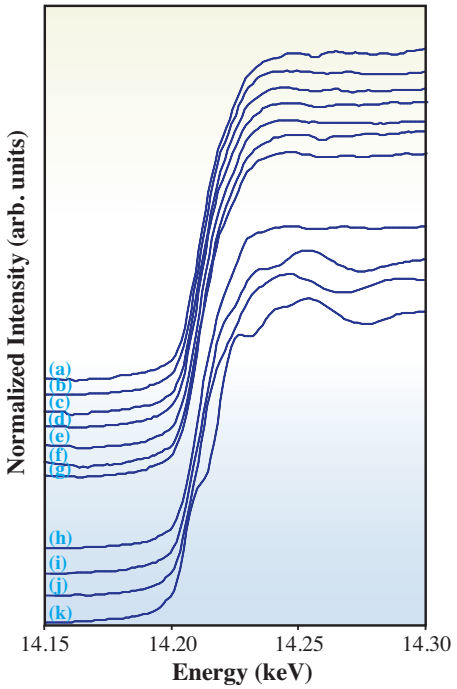

Fig. 3. Normalized Hg L_2-edge XANES spectra of the striped dolphin samples and the reference materials. **(a)** Brain, **(b)** muscle, **(c)** pancreas, **(d)** spleen, **(e)** lung, **(f)** kidney, **(g)** liver, **(h)** HgSe (tiemannite), **(i)** HgS (cinnabar), **(j)** *m*-HgS (metacinnabar), and **(k)** HgO.

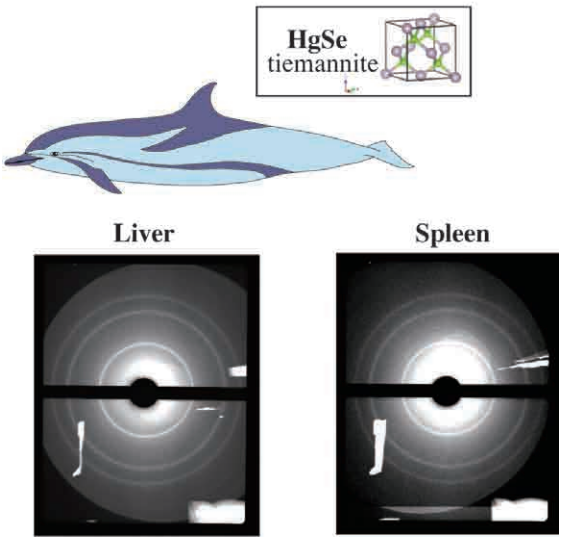

Fig. 2. μ-XRD patterns measured at point A in the liver and point B in the spleen as shown in Fig. 1.

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