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# The Chemical Speciation and Behavior of Mercury and Selenium in the Insoluble Fraction of Striped Dolphin Liver

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**Abstract:** Most of the mercury and selenium exist in the insoluble fraction of dolphin liver. After the insoluble fraction was digested by alkaline protease in the presence of 1%SDS, approximately 50% of Hg and Se consisted in the supernatant and the others in the residue. Gel filtration chromatography of the hydrolysate showed that 96% of Hg and 87% of Se were combined with the high molecular weight proteins stably, which cannot be substituted by the complex reagents. Mercury and selenium in the residue were confirmed as HgSe crystal.

**Keywords:** Mercury, selenium, chemical speciation, dolphin liver.

As the top predators in the ocean, mammals are the end-points of mercury magnification in the marine food web. In the liver of dolphin, cetacean, seal, *et al*., the total concentration of Hg and Se reached an abnormal level[1]. Though a high proportion of the total concentration of Hg in contaminated marine fishes, which are the food for the mammals, exists as methylmercury[2], which is more toxic than others, most of mercury is inorganic in mammals' liver[3, 4]. These facts suggest that methylmercury is transformed into inorganic by the mammals, and the liver is the site of the transformation and accumulation. To clarify the mechanism of accumulation and antagonism between the toxicity of mercury and selenium, understanding the change and transfer of mercury speciation is a key. But researches have been dealing with the percentages of organic and inorganic, soluble and insoluble, and the speciation of soluble generally[4, 5]. A little was known about Hg and Se in the fraction of insoluble proteins. In the present paper, the chemical speciation and behavior of mercury and selenium in the insoluble fraction of striped dolphin liver were discussed and the fate of demethylation Hg was assumed.

## Experimental

Three striped dolphin (*Stenella Coeruleoalba*) liver samples (stored at -40℃) were obtained from the Ocean Research Institute of Tokyo University caught off the coast of Taichi located in Wakayama Prefecture, Japan. After the liver was homogenized and degreased in cold acetone and filtrated, the liver residue (Liver powder) was extracted by 0.2 mol/L ammonium acetate and 80% ethanol. The liver powder was digested by 10

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μg/mL alkaline protease solution (0.1 mol/L $NaHCO_3$-NaOH pH 11) at 50℃ and was centrifuged at 4000 rpm for 25 min to separate the supernatant and residue.

The supernatant (hydrolysate) was subjected to gel filtration Sephadex G-75 column, and the fractions of void volume were lyophilized. 50 mg of the lyophilized sample was dissolved in 2 mL 0.1 mol/L Tris-HCl (pH 8.3) and 10 mmol/L dithiothreitol (DTT), 0.01 mol/L sodium diethyl-dithiocarbamate (DDTC) was added. The mixture was incubated at 50℃ in nitrogen-saturated for 12 hr. 100 μL sodium iodoacetate solution (0.416 g/mL 1 mol/L NaOH pH 8.3) was added to the reaction mixture and incubated at 50℃ for 1 hr in nitrogen-saturated, then 1% 2-mercaptoethanol was add to cease the reaction and the mixture was subjected to Sephacryl S-300 column. 50 mg of the lyophilized sample was dissolved in 2 mL 12 mg/mL selenocysteine (reduced from selenocystine[6]) and the mixture was incubated at 50℃ in nitrogen-saturated for 42 hr. Then the products were subjected to Sephacryl S-300 column.

The concentrations of Hg and Se in all fractions were determined by cold AAS and graphite furnace AAS, respectively, after microwave digestion.

**Results and Discussion**

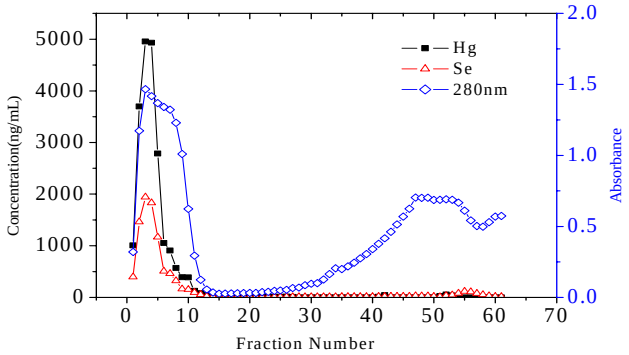
The ratios of Hg and Se, which were extracted by acetone, 80% ethanol and 0.2 mol/L ammonium acetate, were less than 10%. It shows more than 90% of Hg and Se exist in the insoluble fraction of the liver, and moreover most of Hg exists as inorganic[3, 4]. The concentrations of Hg and Se in a sample, which is reported in this paper, were 635.5 μg/g and 253.7 μg/g (dry weight), respectively, at 0.99 molar ratio of Hg to Se.

After the liver powder digested by alkaline protease in the presence of 1%SDS, 99% of the liver powder and approximately 50% of Hg and Se dissolved. As the liver powder was digested by the protease, the hydrolysate was suspected to consist mainly of low molecular weight (MW) peptides originated from the insoluble proteins. However, the elution profiles for proteins, Hg and Se on Sephadex G-75 column (**Figure1**) were totally different from what they were expected to be. Most of Hg (96.4%), Se (87.4%) and a portion of proteins appeared in the void volume ($V_0$), the MW of which is higher than 70,000 Da. The elution profiles resulted from the lyophilized sample of $V_0$ on the Sephadex G-75 column subjected to Sephacryl S-300 or Sephacryl S-500 column (1.2×60 cm) showed that the proteins containing Hg and Se appeared in the $V_0$ also.

The elution profiles on Sephacryl S-300 of the lyophilized sample reaction with DTT and DDTC (**Figure2**) showed that the MW of some proteins was reduced after treatment with DTT due to that –S-S- was reduced to –SH. However, Hg and Se can hardly be determined in this portion of proteins. If Hg exists in the high MW proteins as protein-S-Hg- or protein-Se-Hg-, substitution reaction may occur between the compounds and DTT, selenocysteine and DDTC, which acted as complex reagents for $Hg^{2+}$. Whereas Hg did not appear in the low MW fractions on the column, and it was centralized in the higher MW proteins when the proteins was treated by selenocysteine on the Sephacryl S-300 column.

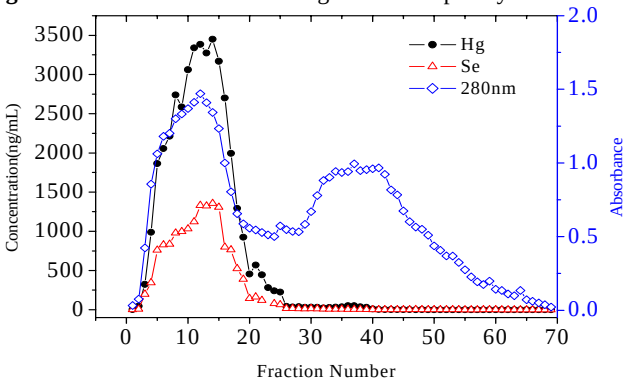
The above results indicate that most of Hg and Se combined with the high MW proteins (over the separation upper limit of the gel resin: $1.5\times10^6$ Da) stably, and Se plays an important role in the formation of the complexes of Hg, Se and proteins. Such

**Figure 1** Gel-filtration chromatogram on a Sephadex G-75 column




The supernatant extracted from the liver powder by alkaline protease in the presence of 1%SDS was subjected to the column (1.2×60 cm). Eluent: 0.1 mol/L $NH_4Ac$-$NH_3$ (pH 9)

**Figure 2** Gel-filtration chromatogram on a Sephacryl S-300 column



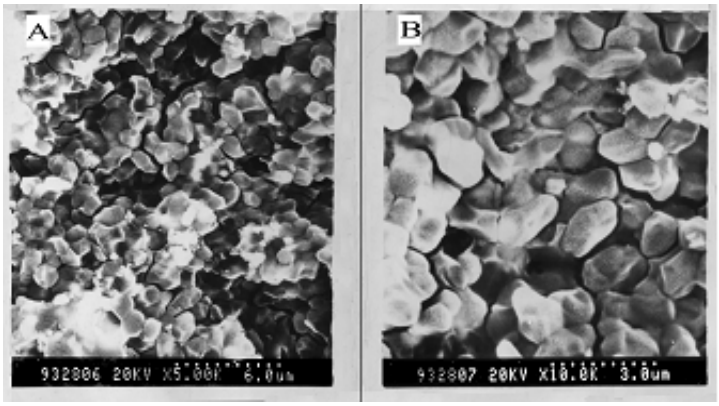

The fractions of void volume on Sephadex G-75 were lyophilized and the mixture of it reacted with DDT and DDTC was subjected to the column (1.2×60 cm). Eluent: 0.1 mol/L $NH_4Ac$-$NH_3$ (pH 9)

high MW of proteins containing Hg and Se were discovered firstly.

Under the scanning electron microscope (Hitachi X-650, Japan), the residue appeared as irregular tiny particles measuring approximately 2 μm in diameter (**Figure 3**, A×5000, B×10000). X-ray energy dispersion spectrometer analysis (EDAX PV-9100, America) of the granules revealed the presence of Hg and Se only with equal atomic percentage. The diffraction parameter (X'Pert pro MPD, Philips, Holland) is similar to the standard crystal of HgSe. Accordingly, though sulfur has a high affinity to $Hg^{2+}$ and has high content in liver proteins, HgS or Hg ($S_xSe_{1-x}$) can be ruled out. These results strongly suggest that the particles stored in the liver of dolphin, which cannot be digested by alkaline protease, consist of pure tiemannite. The fact that there is not formation of sulfide shows the unique and vital function of selenium in the mineralization of mercury.

According to the opinion of Wood *et al.*[7], the detoxification of methylmercury poisoned animals by selenium can be explained transformation of methyl group out of the mercury cycle into the selenium cycle. Since HgSe is non-biodegradable, Martoja and Berry8 suggested that the crystallization of tiemannite should be the final stage of a

**Figure 3** SEM photos of the residue showing HgSe granules




The liver powder was digested by alkaline protease in the presence of 1%SDS five times and was centrifuged at 4000 rpm for 25 min

detoxification process, and the fate of demethylated mercury is to form HgSe. According to our research, the demethylated mercury is more likely to be combined with high MW proteins and fixed. When the high MW proteins were decomposed by catabolism, Hg and Se form tiemannite. In the procedure of demethylation, diversion of Hg to high MW proteins and final formation of HgSe, selenium exerts a significant and unique effect.

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