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Chemical nature of a methylmercury complex with a low molecular weight in the liver cytosol of rats exposed to methylmercury chloride

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Most of the mercury in the bile of rats exposed to methylmercury is found in two fractions, one bound to protein and the other bound to a small molecular compound [1–3]. The latter closely concerns the intestinal reabsorption of mercury excreted in bile [1, 3]. Several studies have been carried out to clarify the chemical nature of this mercury complex with a small molecular compound in the bile of the rat [1, 2, 4] and mouse [5]. A mercury fraction complexed with a small molecular compound, as well as a mercury fraction bound to protein, was also demonstrated in the cytosol of rat liver exposed to methylmercury [6]. A study of the chemical nature of the mercury complex with a low mol. wt within the liver cytosol might be valuable in understanding the transport mechanism of mercury from the liver cell to bile. We now present evidence that methylmercury glutathione is the predominant small molecular mercury compound within the liver cytosol.

Three female Wistar rats, weighing 190–200 g, were injected subcutaneously with methylmercury chloride (Wako Pure Chemicals, Osaka) at a dose of 2.5 mg, labeled with 125 μCi Me^{203} HgCl (930 mCi/m-mole, Radiochemical Centre, Amersham) per kg body wt. Methylmercury chloride was dissolved in 10 mM $NaHCO_3$–Na_2CO_3 buffer, pH 9.2. Two days after injection, the rats were killed after whole body perfusion under Nembutal anesthesia. The subsequent procedures were carried out at 0–4°C. Livers from three rats were pooled and homogenized with 3 vol of 0.32 M sucrose in 1 mM Tris–HCl buffer, pH 7.6. The homogenate was centrifuged for 10 min at 13,000 *g*, and the supernatant fraction was recentrifuged for 1.5 hr at

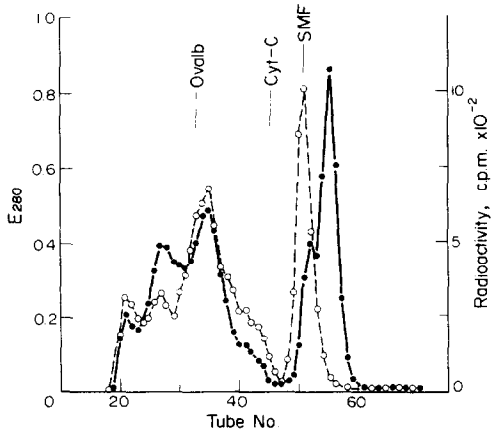

Fig. 1. Gel filtration on Ultrogel ACA-44 of liver cytosol prepared from rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μCi/Kg of Me^{203} HgCl for 2 days. Key: extinction at 280 nm (●——●); radioactivity, (○- - -○). Arrows indicate the position for ovalbumin (Ovalb), cytochrome *c* (Cyt-*c*) and the small molecular fraction (SMF).

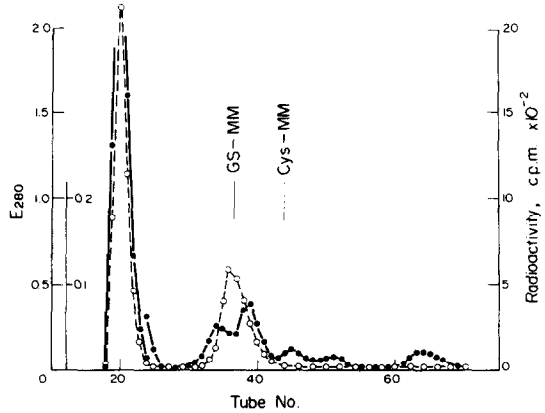

Fig. 2. Gel filtration on Sephadex G-25 of liver cytosol prepared from the rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μgCi/Kg of Me^{203} HgCl for 2 days. Key: extinction at 280 nm (●——●); radioactivity (○- - -○). The left scale of E_{280} represents the extinction of tubes 18–24, and the right scale of E_{280} is for the extinction of tubes 24–69. Arrows indicate the position for methylmercury glutathione (GS-MM) and methylmercury cysteine (Cys-MM).

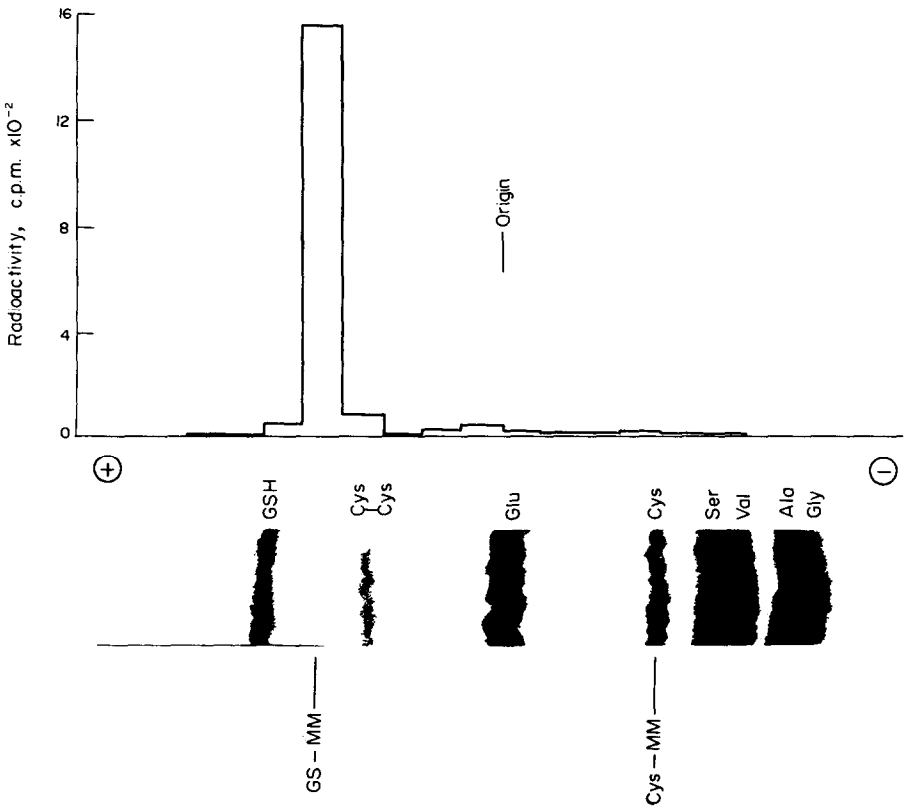

Fig. 3. High-voltage electrophoresis of deproteinized cytosol of liver from rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μCi/Kg of Me^{203} HgCl for 2 days. The liver cytosol was prepared as described in the text, except that distilled water was used as a homogenizing medium. Six vol of a mixture of methanol and acetone (3:1) was immediately added to the cytosol. After standing overnight at −20°, the insoluble material was removed by centrifugation for 20 min at 15,000 *g*. The supernatant fraction was concentrated to one-tenth of its original volume under reduced pressure and then extracted with chloroform–methanol (4:1). The loss of radioactivity caused by this treatment was about 15 per cent. When this extraction was omitted, turbidity appeared in the course of further concentration. The supernatant fraction was then concentrated to about 0.15 ml. The electrophoretic run was at pH 3.6 (acetic acid–pyridine–water, 10:1:89 by volume) for 50 min at 100 V/cm. A mixture containing eight species of marker compounds, as indicated in the figure (GSH, glutathione), was run in parallel with the sample of interest. The position of methylmercury glutathione (GS–MM) and methylmercury cysteine (Cys–MM) was determined in a separate experiment by running a mixture of Me^{203} HgCl and glutathione or cysteine in parallel with marker compounds. Marker compounds were developed with a 0.5% (w/v) ninhydrin in acetone. A strip containing the sample was cut off, dried without heating and cut into segments to analyze the radioactivity. The radioactivity was recovered almost completely. About 87 per cent of the recovered radioactivity was located in the position of GS–MM.

100,000 *g*. Two ml of the cytosol thus obtained was subjected to gel filtration on either Ultrogel ACA-44 (2.5 × 40 cm) or Sephadex G-25 (2.5 × 40 cm) columns with an elution buffer of 0.01 M Tris-HCl, pH 8.2, containing 0.1 M NaCl. Fractions of 3.8 ml were collected at a flow rate of 20 ml/hr. The radioactivity of each fraction eluted from the column was determined in an NaI crystal well-type scintillation counter (Aloka JDC-601, Tokyo).

Figure 1 shows that about 24 per cent of the mercury in the cytosol was located in the small molecular fraction (tubes 47–55). The mercury in this fraction was almost completely transferred into the benzene phase by repeated benzene extractions of this fraction, which was acidified to 2 N with HCl. As for the higher molecular weight fraction (tubes 20–45), about 6.5 per cent of the mercury remained in the aqueous phase after the acid benzene extraction. The mercury extracted into benzene existed exclusively in the form of methylmercury, as identified by gas chromatography (Shimazu GC-4BMPE, Kyoto), which was performed according to the method of Sumino [7]. A similar elution pattern was also obtained with a different dosage schedule of methylmercury; that is, three rats were injected every 3 days with 2.5 mg/kg of methylmercury chloride for a total of nine times and then injected with 125 μCi/kg of Me^{203} HgCl 2 days before death. In this case, about 37 per cent of the radioactivity in the cytosol was found in the small molecular fraction. An addition of Me^{203} HgCl (about 70,000 cpm) to liver cytosol which was freshly prepared from non-injected rats also exhibited a similar elution pattern, in which about 21 per cent of the radioactivity was found in the small molecular fraction of the cytosol.

To clarify the chemical form of the small molecular compound bound to methylmercury, liver cytosol prepared from rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μCi/Kg of Me^{203} HgCl for 2 days was immediately applied to a column of Sephadex G-25. The result, as shown in Fig. 2, revealed that methylmercury

is bound to glutathione. This was further demonstrated, as shown in Fig. 3, by using high-voltage paper electrophoresis, according to the method of Michl [8].

Thus, the present work indicates that methylmercury glutathione is the predominant mercury compound with a low mol. wt in the liver cytosol of rats exposed to methylmercury chloride.

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Department of Biochemistry,
Faculty of Science,
Niigata University,
Igarashi, Niigata, Japan

SABURO OMATA
KENJI SAKIMURA
TETSUAKI ISHII
HIROSHI SUGANO

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