A SIMPLE AND RAPID METHOD FOR PREPARATION OF $^{203}$Hg-
labeled methylmercury from $^{203}$HgCl$_2$ AND METHYLCO-
BALAMIN

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A simple and rapid method for preparation of $^{203}$Hg-labeled methylmercuric chloride (CH$_3$$^{203}$HgCl) from $^{203}$HgCl$_2$ and methylcobalamin is described. More than 99% of $^{203}$Hg was methylated with methylcobalamin 0.01 N HCl during 1 h. CH$_3$$^{203}$HgCl formed during the reaction was rapidly and completely separated from cobalamin and unreacted $^{203}$HgCl$_2$ by CM-Sephadex C-25 minicolumn. The low cost procedure for preparation of CH$_3$$^{203}$HgCl can be completed within 2 h and yields an inorganic $^{203}$Hg-free CH$_3$$^{203}$HgCl which is useful in methylmercury toxicology.

Keywords — $^{203}$Hg-labeled methylmercury; synthesis; isolation; methylation; methylcobalamin

INTRODUCTION

Many toxicological studies on metabolism of methylmercury are performed using $^{203}$Hg-labeled methylmercury compound. However, commercial $^{203}$Hg-labeled methylmercuric chloride (CH$_3$$^{203}$HgCl) is expensive compared to $^{203}$Hg-labeled mercuric chloride ($^{203}$HgCl$_2$) and contaminated with inorganic mercury. Moreover, it has come to be difficult to purchase a CH$_3$$^{203}$HgCl sample of high specific activity because of recent closing of one of the major suppliers from furnishing it. We describe here a simple rapid, and safe method for synthesis of CH$_3$$^{203}$HgCl from commercially available and highly radioactive $^{203}$HgCl$_2$ and methylcobalamin, and its isolation from the reaction mixture using CM-Sephadex C-25 column.

MATERIALS AND METHODS

Reagents — $^{203}$HgCl$_2$ in HCl solution (pH 1—2) was purchased from Amersham, Int. Corp. Methylcobalamin was obtained from Sigma Chemical Co. and dissolved in 0.01 N HCl solution to be a concentration of 25—50 mM just before use.

Methylation of $^{203}$Hg$^{2+}$ — To a solution (1 ml) containing $^{203}$HgCl$_2$ (the amount of $^{203}$HgCl$_2$ is less than 5 μmol), was added 5 molar equivalent of methylcobalamin and the volume of the mixture was adjusted to 2 ml with 0.01 N HCl. The mixture was kept for 1 h at room temperature in the dark.

Isolation of Methylmercury — The reaction mixture was applied on CM-Sephadex C-25 column (5 × 60 mm) equilibrated with 20 mM Tris-HCl buffer (pH 7.6). Excess buffer was drained off the top of the column before the application of the reaction mixture. The column was eluted with the same buffer at a flow rate of about 1 ml/min and 2 ml each of the eluate was fractionated in a tube.

Concentration of Methylmercury — Pooled fractions containing CH$_3$$^{203}$HgCl eluted from the separation column were applied on a different size of CM-Sephadex C-25 column (20 × 25 mm) equilibrated with 20 mM Tris-HCl buffer (pH 7.6). After the application, the column was washed with 20 ml of same buffer and eluted with 0.9% NaCl solution and 2 ml each of the eluate was fractionated in a tube.
**Determination of Cobalamin** — Elution profiles of cobalamins (methylcobalamin and aquocobalamin) from the column were monitored by measuring the absorbance at 265 nm. Methylcobalamin is decomposed into aquocobalamin after release of the methyl group. The two types of cobalamins show similar extinction coefficient at 265 nm.

**Determination of Organic Mercury** — Organic mercury was extracted from the reaction mixture with 5-volume of benzene. The benzene extraction was repeated 4 or 5 times. Radioactivities in the benzene (organic mercury) and in aqueous layer (inorganic mercury) were measured by Aloka Auto well gamma system.

**Thin-Layer Chromatography** — The benzene soluble organic mercury formed by the reaction of $\text{CH}_3\text{HgCl}_2$ and methylcobalamin was identified by thin-layer chromatography according to the method of Imura et al. The radioactive spots were scraped off the silica gel plate and the radioactivity was measured.

**RESULTS**

$^{203}$Hg-labeled organic mercury (extractable with benzene) was observed after 1 h reaction of $^{203}$HgCl$_2$ (1 μmol) and methylcobalamin (1, 2 or 5 μmol) in 0.01 N HCl solution (Table I). When $^{203}$HgCl$_2$ and methylcobalamin were added at a molar ratio of 1:5, the yield of benzene extractable organic $^{203}$Hg-compound was almost 100% (Table I). The organic $^{203}$Hg-compound formed was then identified as CH$_3$$^{203}$HgCl by thin-layer chromatography. Formation of $^{203}$Hg-dimethylmercury was not observed.

CH$_3$$^{203}$HgCl formed by the reaction of 1 μmol $^{203}$HgCl$_2$ and 5 μmol methylcobalamin in 2 ml of 0.01 N HCl was separated from $^{203}$HgCl$_2$ and cobalamins by CM-Sephadex C-25 column chromatography using 20 mM Tris-HCl buffer (pH 7.6) as an eluting buffer. The overall yield of CH$_3$$^{203}$HgCl from $^{203}$HgCl$_2$ was 99%. The separation capacity of the column (5 × 60 mm) was shown to be enough for application of a reaction mixture of 5 μmol $^{203}$HgCl$_2$ and 25 μmol methylcobalamin (Fig. 1). Each fraction containing CH$_3$$^{203}$HgCl showed a neutral pH values. Use of 10 mM sodium carbonate buffer (pH 9.2), 50 mM Tris-HCl buffer (pH 9.2) or distilled water for the equilibration of the column and for the elution was not suitable for good separation of CH$_3$$^{203}$HgCl from cobalamins. Lowering the concentration (less than 10 mM) of Tris-HCl buffer resulted in a broadening of the peak of CH$_3$$^{203}$HgCl.

To concentrate the CH$_3$$^{203}$HgCl peak fraction and remove cobalamins, if contaminated in a trace amount, the pooled fractions (fraction No. 15–30 in Fig. 1) was applied on a CM-Sephadex C-25 column (20 × 25 mm), which adsorbed CH$_3$$^{203}$HgCl dissolved in 20 mM Tris-HCl but not cobalamins. The CH$_3$$^{203}$HgCl was eluted from the column with 0.9% NaCl solution into 2 to 3 tubes (4–6 ml fraction). Recovery of CH$_3$$^{203}$HgCl in the rechromatography was 99%.

**TABLE I. Formation of CH$_3$$^{203}$HgCl from $^{203}$HgCl$_2$ in the Presence of Methylcobalamin in 0.01 N HCl**

<table>
<thead>
<tr>
<th>Compound added</th>
<th>$^{203}$Hg$^{a,b}$ (cpm)</th>
<th>CH$_3$$^{203}$HgCl formed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{203}$HgCl$_2$ (μmol)</td>
<td>Me[Co] (μmol)</td>
<td>Inorganic</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>135967</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>56690</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>492</td>
</tr>
</tbody>
</table>

* a) Amounts of benzene soluble (methyl) and insoluble (inorganic) $^{203}$Hg after reaction of $^{203}$HgCl$_2$ and methylcobalamin (Me[Co]) in 0.01 N HCl for 1 h.
DISCUSSION

Chemical transmethylation from methylcobalamin to inorganic mercury in neutral pH solution has been known, and monomethylmercury and dimethylmercury were identified as reaction products. Imura et al. reported that these two products were formed in different ratio depending on the molar ratio of the reactants and the reaction time, and dimethylmercury was obtained in good yield when HgCl₂ and methylcobalamin were mixed at a molar ratio of 1:2. Dimethylmercury formed during the reaction was converted into methylmercuric chloride by the addition of HCl after the reaction. In the present study 0.01 N HCl was chosen as a medium for the reaction of methylcobalamin and HgCl₂, in which the reaction proceeded at a remarkably high rate and gave only CH₃HgCl as a sole reaction product regardless of the molar ratio of HgCl₂ and methylcobalamin added.

For removal of methylcobalamin, aquacobalamin (a reaction product) and HgCl₂ from the reaction mixture, CM-Sephadex C-25 minicolumn (5 × 60 mm) was adopted. It was reported that CH₃HgCl is eluted through CM-

![Graph](https://example.com/graph)

**FIG. 1. Separation of CH₃HgCl from Cobalamin and Unreacted HgCl₂ Using CM-Sephadex C-25 Minicolumn**

Sample was taken from the reaction mixture 1 h after reaction of 5 μmol HgCl₂ and 25 μmol methylcobalamin.

REFERENCES

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