Binding Metal Elements in Metallothionein Fraction from Liver of Rat Injected Various Metals

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Six elements, Cd, Co, Cu, Mn, Se and Zn, in the metallothionein fractions from liver of rat injected various metals (Cd, Co, Cu, Hg, Se and Zn) were analyzed by neutron activation analysis and were investigated metal binding of it. The concentrations of Cd and Zn were increased by administration of Cd and those of Co and Se were also increased by administration of the each element. On the contrary, the concentration of Cd and Cu were decreased by administration of Se. It may be considered that Co and Se are attached to metallothionein, if its protein is present and the presence of Se influence the binding of Cu and Cd to metallothionein, further it also influence Cd-thionein production.

Key Word: neutron activation analysis, rat liver, metallothionein

1. Introduction

Since metallothionein (MT) was first isolated from equine renal cortex by Margoshes and Vallee, many studies of this protein have been done. This protein has been characterized and several reviews were already published. It is well known that MT is synthesized in liver and kidney by certain metal ions, including Cd, Hg and Zn. The isolated protein contains the above metal elements. Such MT is one of the most interesting biological materials to study elements in organisms. In the previous paper, the binding metal elements in cytosol proteins of rat liver were determined. MT fractions was shown such a peculiar metal binding that Zn quantity in this fraction was increased significantly by Cd injection. Studies of MT have been thus far performed with regard to Cd toxicity and metabolism of essential elements such as Zn and Cu. Therefore, the study of binding metal elements on MT are little. The simultaneous multielemental analysis of Cd induced MT (Cd binding protein) by using neutron activation analysis has been only reported by Girardi, et al. In the present study, the binding metal elements in the MT fraction of rat liver injected various metals were investigated. Zn, Cu and Co as essential element and Cd, Hg and Se as toxic element were administrated singly or simultaneously in rats. The binding metals in the MT fraction isolated from rat liver were analyzed by neutron activation analysis and we have gained informations about metal binding of them.

2. Experimental

2-1 Preparation of metal solutions
The solutions of 0.1 mg Cd, Co, Cu, Hg, Se and Zn/ml were prepared by dissolving Cd, Co, Cu and Hg chloride, sodium selenate and Zn nitrate in isotonic saline solution for injection.

2-2 Preparation of the samples
3 male rats of Wistar strain weighing about 200 g were injected subcutaneously with 0.5 mg of each metal per kg body weight. Injections were made 6 times on alternate days. The rats were sacrificed by decapitation 48 hours after final injection. The livers of these rats were removed and cut into small strips. The liver strips were homogenized in 3 volumes of 0.25 M sucrose solution. The homogenate was ultracentrifuged at 105,000 g for 60 min at 4°C. The supernatant fraction (cytosol) was chromatographed on Sephadex G-75 (3× 90 cm) column using 0.01 M tris HCl buffer (pH 8.0). The
collected MT fraction was concentrated and washed 3 times with pure water by using ultrafiltration (10^5 pore size membrane filter). All samples were lyophilized and dried in an air oven at 85°C for 4 hours and several mg of each sample was packed in double clean polyethylene bags for neutron irradiation.

2.3 Neutron irradiation and gamma-ray spectrometry
NBS bovine liver (SRM-1577) were used as multielemental irradiation standards and synthetic standards were also used for the measurement of Mn, Cu and Cd.

The samples and standards were irradiated for one hour at the pneumatic irradiation facility of the Kyoto University Reactor with an estimated thermal neutron flux of $1.93 \times 10^{13} \text{n cm}^{-2} \text{s}^{-1}$. The irradiated samples and standards were counted with a coaxial Ge(Li) detector (FWHM 2.0 keV at 1.332 MeV) coupled to a 4096 channel pulse-height analyzer. The gamma activity was measured for 300, 1,000 and 10,000 seconds after a decay of 3~5 hours, 3 and 20~30 days, respectively.

3. Results and Discussion
6 elements such as Cd, Co, Cu, Mn, Se and Zn in the each sample were analyzed. The results are given in Table 1 and 2 as the obtained value by the injection of single element and that of 2 or 6 elements, respectively. The concentration ratios of the elements in the MT fraction from the liver of rats injected each element to those of non-treated rats are given in Fig. 1 and 2.

On the discussion of metal binding in the MT fraction, it should be noted that MT production occurs by injected metals (Cd, Cu, Zn and Hg). When MT is induced by injected metals, metal composition in MT varies according to their ability of MT production. Cd injection is a typical case of this alteration and its phenomenon have already described in the previous paper. On the other hand, if MT is not induced by injected metals, an increase of

<table>
<thead>
<tr>
<th>Detected element</th>
<th>Injected elements</th>
<th>Non-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Co</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Cu</td>
<td>3.2 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Mn</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>Se</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>6.5 ± 0.6</td>
<td>6.5 ± 0.6</td>
</tr>
</tbody>
</table>

* ND: Not detected

Table 2 Elemental concentrations in the metallothionein fraction from liver of rats treated simultaneously with 2 or 6 kinds of elements (µg/g)

<table>
<thead>
<tr>
<th>Detected element</th>
<th>Injected elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd–Cu</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>Cd–Co</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>Cd–Se</td>
<td>9.8 ± 1.0</td>
</tr>
<tr>
<td>Cd–Zn</td>
<td>10.9 ± 1.1</td>
</tr>
<tr>
<td>Zn–Cu</td>
<td>12.0 ± 1.2</td>
</tr>
<tr>
<td>Hg–Se</td>
<td>13.1 ± 1.3</td>
</tr>
<tr>
<td>All*</td>
<td>14.2 ± 1.4</td>
</tr>
</tbody>
</table>

* All: Contain same amounts of Cd, Co, Cu, Hg, Se and Zn  ** ND: Not detected
all: Contain same amounts of Cd, Co, Cu, Hg, Se and Zn

Fig. 1 The ratios of elemental concentration in the metallothionein fraction from liver of rats treated with each element to that in non-treated rats. The height of columns and vertical lines represent mean values and standard deviations, respectively. Broken lines represent elemental concentration in the metallothionein fraction from liver of non-treated rats.

Fig. 2 The ratios of elemental concentration in the metallothionein fraction from liver of rats treated simultaneously with 2 or 6 kinds of elements to that in non-treated rats. The height of columns and vertical lines represent mean values and standard deviations, respectively. Broken lines represent elemental concentration in the metallothionein fraction from liver of non-treated rats.

their concentrations indicates that injected metals attach to basal MT. Their metals also influence on the binding of another metals to MT.

The following evidence was observed from the analytical results obtained from rats injected single element:

(1) The concentrations of Co and Se were increased to 7.4 and 1.5 times, respectively, by administration of Co and Se (not induce MT).

(2) The concentrations of Cu and Zn were not increased by administration of Cu and Zn (induce MT).

(3) The concentrations of Cd and Cu were decreased to 27% and 40%, respectively, by
administration of Se.
The following conclusion may be clear from the above facts and the analytical results obtained by simultaneous injection of 2 or 6 elements.

(1) Co and Se were attached to MT, if MT is present. The binding amounts of their metals were increased by simultaneous injection with Cd because of MT was produced by Cd injected.

(2) From metal composition of MT fractions, Cu-, Zn-thionein was assumed as basal MT.

(3) The binding of Cu to MT was inhibited by the presence of Se. This is the reason for the decrease in the concentration of Cu by simultaneous injection of Hg and Se. Further, the concentration of Cd was decreased by simultaneous injections of Cd and Se, and 6 elements. Therefore, it can be considered that the presence of Se also inhibit the binding of Cd to MT. Further, it influences the production of Cd-thionein. It is because the concentration of Zn in the MT fractions injected Cd was increased by Cd-thionein production\(^7\) but in the case of simultaneous injection of Cd and Se, it was decreased.

The effects of Se on metal binding of MT and MT production are particularly interesting, therefore, further study on this problem should be needed.

References