INCORPORATION OF RADIOACTIVE SULFUR OF S\textsuperscript{35}-LABELLED L-CYSTINE AND L-METHIONINE INTO THE THIAZOLE MOIETY OF THIAMINE\textsuperscript{1}

HIROSHI KUMAOKA\textsuperscript{2}

Faculty of Pharmaceutical Science, School of Medicine, Hokkaido University, Sapporo

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In the previous paper (1), it was reported that sulfur-containing amino acids added to a medium stimulated the production of thiamine per unit weight of the cells by the growing cultures of Escherichia coli, and the possibility that the thiazole moiety of thiamine was synthesized via sulfur-containing amino acid was discussed.

This paper describes that radioactive sulfur of S\textsuperscript{35}-labelled L-cystine and L-methionine added to a medium is incorporated into the thiazole moiety of thiamine by the growing cultures of Escherichia coli.

EXPERIMENTAL

1. Organism and Medium
   Escherichia coli ATCC 9637 was used in this work. The medium employed in these experiments was that of Davis and Mingioli (2).

2. Growth
   The conditions of growth and the method of determination of cell weight were the same as described previously (1).

3. Isolation and Assay of Thiamine
   Extraction and hydrolysis of thiamine phosphates were performed as described previously (1). The extracts incubated with Takadiastase were poured onto a column (10×100 mm) of Dowex 50 x4 (Na\textsuperscript{+} type, 100–200 mesh). The column was then washed with 100 ml of distilled water. Thiamine was eluted from the column with 0.2 M citrate buffer, pH 6.5. The rate of the flow of the eluting solution through the column was about 1 ml per 3 minutes and the effluent was collected in 5-ml fractions. An aliquot was withdrawn from each eluate fraction for the detection of the thiamine-containing fraction with Lactobacillus fermenti. The thiamine-containing fractions (Fraction No. 41–45) from the column were pooled. An aliquot of this pooled solution was used for the microbiological assay of thiamine with Lactobacillus fermenti according to the method of Sarett and Cheldelin (3). The remainder was supplemented with 40 mg of non-radioactive thiamine as a carrier and the ethanolic solution of picric acid (150 mg) was added. The picrate of thiamine was separated by centri-

\textsuperscript{1} The abbreviation used: thiazole, 4-methyl-5-(2-hydroxyethyl) thiazole.
\textsuperscript{2} 髙岡 俊.
fugation, washed with a small amount of distilled water and of cold ether, dried in vacuo, and weighed.

4. Preparation of \( ^{35}\)S-Labelled L-Cystine and L-Methionine.

Radioactive yeast cell protein (5 g) prepared according to the procedure reported previously (4) was supplemented with non-radioactive L-cystine (150 mg) as a carrier, and hydrolyzed for 6 hours with 50 ml of 5 N HCl. The hydrolyzate was filtered from acid-insoluble humin and the filtrate was boiled for a few minutes with 150 mg of charcoal. After removal of charcoal, the solution was evaporated to a viscous residue under reduced pressure. The residue was taken up in 50 ml of water and adjusted to pH 4.5 with concentrated NaOH solution. After standing for 24 hours at \( ^{2}\), the crude L-cystine (103 mg) was filtered off. Pure L-cystine (51.5 mg) was obtained by repeated precipitation at isoelectric point and recrystallization from ethanol-water. The mother liquor from the L-cystine precipitation was subjected to the chromatography with chromatopile. L-Methionine fraction was eluted with distilled water. Non-radioactive L-methionine (300 mg) was added to the eluate as a carrier and the solvent was evaporated under reduced pressure to dryness. The residue was taken up in 10 ml of ethanol and adjusted to pH 5.0 with pyridine. After standing at \( ^{2}\) overnight, L-methionine precipitated was filtered off. Crude L-methionine was recrystallized (yield, 161 mg).

5. Estimation of Radioactivity.

The procedures for \( ^{35}\)S counting had been described previously.

RESULTS AND DISCUSSION

In the experiments reported in Table I and II, the incorporation of radioactive sulfur of \( ^{35}\)-labelled L-cystine and L-methionine was shown. In the previous paper (1), it was reported that sulfur-containing amino acids added to a growing medium stimulated the production of thiamine by \( \text{Escherichia coli} \). These results confirm that the growing cells of \( \text{Escherichia coli} \) can utilize the sulfur of exogenous sulfur-containing amino acids to form thiazole. The specific radioactivity of \( ^{35}\)-labelled thiamine of the cells grown in a medium supplemented with \( ^{35}\)-labelled L-cystine was diluted to 19.8\%, compared with that of \( ^{35}\)-labelled L-cystine. With \( ^{35}\)-labelled L-methionine, the dilution of the specific radioactivity was 20.5\%.

Hitchcock and Walker (4) investigated the competition of \( ^{35}\)-containing compounds with radioactive inorganic sulfate on the biosynthesis of thiazole of thiamine by \( \text{Saccharomyces cerevisiae} \) and reported that as the source of sulfur for the

| Table I |

Incorporation of Radioactive Sulfur of \( ^{35}\)-Labelled L-Cystine into the Thiazole Moiety of Thiamine by the Growing Cultures of \( \text{Escherichia coli} \)

<table>
<thead>
<tr>
<th>Weight</th>
<th>( ^{35})S total activity</th>
<th>( ^{35})S specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>mg</td>
<td>cpm</td>
</tr>
<tr>
<td>Thiamine content of inoculum</td>
<td>5.1 \times 10^{-3}</td>
<td>3.79 \times 10^{7}</td>
</tr>
<tr>
<td>( ^{35})S-L-cystine added (per 1,000 ml)</td>
<td>100</td>
<td>5.2 \times 10^{5}</td>
</tr>
<tr>
<td>Resulting cells</td>
<td>1,040</td>
<td>9.1 \times 10^{4} (Dilution rate, 19.8%)</td>
</tr>
<tr>
<td>Total thiamine of cells</td>
<td>28.1 \times 10^{-3}</td>
<td>5.9 \times 10^{2}</td>
</tr>
</tbody>
</table>
Incorporation of Radioactive Sulfur of S\textsuperscript{35}-Labelled L-Methionine into the Thiazole Moiety of Thiamine by Growing Cultures of Escherichia coli

<table>
<thead>
<tr>
<th>Weight</th>
<th>S\textsuperscript{35} total activity</th>
<th>S\textsuperscript{35} specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>267 mg</td>
<td>5.7 x 10\textsuperscript{10}</td>
<td>2.0 x 10\textsuperscript{7}</td>
</tr>
<tr>
<td>100 cpm</td>
<td>2.0 x 10\textsuperscript{7}</td>
<td>2.98 x 10\textsuperscript{4}</td>
</tr>
<tr>
<td>1,130 cpm</td>
<td>1.9 x 10\textsuperscript{8}</td>
<td>6.1 x 10\textsuperscript{7}</td>
</tr>
<tr>
<td>29.3 x 10\textsuperscript{-8}</td>
<td>4.3 x 10\textsuperscript{2}</td>
<td>(Dilution rate, 20.5%)</td>
</tr>
</tbody>
</table>

biosynthesis of thiazole, the following compounds were utilized by \textit{Saccharomyces cerevisiae}. Very efficiently: thiazole. Efficiently: S-adenosylmethionine, glutathione, methionine, thiazolidine-4-carboxylic acid, 5'-deoxy-5'-methioadenosine. Fairly efficiently: \(\alpha\)-amino-\(\beta\)-(4-methylthiazole-5-yl)propionic acid. Inefficiently: cysteine, homocysteine. In the author's experiments, radioactive sulfur of S\textsuperscript{35}-labelled L-cystine as well as L-methionine was incorporated into thiazole by \textit{Escherichia coli}. On the other hand, it was reported that C\textsuperscript{14}-labelled formate was incorporated into the pyrimidine moiety of thiamine by \textit{Bacillus subtilis} (5) and \textit{Saccharomyces cerevisiae} (6), but that the radioactivity of carbon was absent in the thiazole moiety. These facts indicate that the biosynthetic reactions of thiazole do not involve one-carbon unit transfer system and that the methyl group of methionine is not utilized on the biosynthesis of thiazole. Further detailed studies should be worked out to decide the source of carbon atoms required for the biosynthesis of thiazole.

**SUMMARY**

Radioactive sulfur of S\textsuperscript{35}-labelled L-cystine and L-methionine added to a growing medium was incorporated into the thiazole of thiamine by the growing cultures of \textit{Escherichia coli}.

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**REFERENCES**