Methionine Synthesis from 5'-S-Methylthioadenosine

RESOLUTION OF ENZYME ACTIVITIES AND IDENTIFICATION OF 1-PHOSPHO-5-S-METHYLTORIBULOSE*

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5'-S-Methylthioadenosine is converted to methionine in mammalian systems, microorganisms and plants. 5'-S-Methylthioadenosine is first converted to 1-phospho-5-S-methylthiobofuranoside (1-PMTR) which is then converted to 2-keto-4-S-methylthiobutyrate, the precursor of methionine. We have now investigated the conversion of 1-PMTR to the keto acid. This conversion requires at least three protein fractions designated A, B, and C. Fraction A catalyzes an isomerization of 1-PMTR to form 1-phospho-5-S-methylthiobulose. The identification of this compound is based in part on the products obtained after NaIO₄ oxidation, i.e. S-methylthioacetaldehyde, formate, and phosphoglycolic acid. When fractions A and B are added to 1-PMTR, two additional compounds, designated II and III, were detected. No O₂ consumption was presumed in the formation of compounds II and III. These compounds are, therefore, at the oxidation state of 5-S-methylthiobulose. Compound II is phosphorylated as evidenced by its electrophoretic behavior before and after alkaline phosphatase treatment. Addition of fraction C to compounds II and III leads to O₂ consumption and to the conversion of these compounds to 2-keto-4-S-methylthiobutyrate. Thus, compounds II and III are precursors of the keto acid. These compounds have not been fully characterized.

5'-S-Methylthioadenosine is derived from S-adenosylmethionine through the action of the spermine and spermidine synthase (1, 2) and S-adenosylmethionine hydrolase (3-6). It has been established that 5'-S-methylthioadenosine undergoes phosphorylation in mammalian tissues, to produce adenine and 5-S-methylthiobulose (20-23), which is then converted into methionine (25). In bacteria (16) as well as in rat liver extracts, 2-keto-4-S-methylthiobutyrate is an intermediate in the conversion of 1-PMTR to methionine (26, 27). In the mammalian system, oxygen is consumed, and formic acid is released as 2-keto-4-S-methylthiobutyrate is formed from 1-PMTR (27). 5-S-Methylthiobulose and 5-S-methylthiobulonic acid are not intermediates (26). We have further examined the conversion of 1-PMTR to 2-keto-4-S-methylthiobutyrate in rat liver homogenates, and the results are described in this communication.

MATERIALS AND METHODS²

See next page.

RESULTS

When rat liver homogenate was fractioned as described in the miniprint, three protein fractions (denoted A, B, and C) were isolated which were required to convert 1-PMTR to 2-keto-4-S-methylthiobutyrate. The reaction was assayed by measuring O₂ uptake and by chromatography. Little O₂ consumption was detected and little ketoacid was formed when any one of the protein fractions was omitted (Table I). A small amount of 2-keto-4-S-methylthiobutyrate was formed in the presence of fraction A + B. We believe this was due to contamination of fraction B by fraction C.

When 1-PMTR was incubated with fraction A, it was converted to a new product, compound I, which could be assayed by a reducing sugar method (28). In a typical experiment, 3.6 mm 1-PMTR was incubated with 0.24 mg of fraction A in standard buffer in a final volume of 100 µl. After 50 min at 37 °C, the reaction was stopped by the addition of 100 µl of 95% ethanol, and protein was removed by centrifugation. The reducing sugar assay indicated the presence of 0.22 mmol of reducing sugar, showing that 61% 1-PMTR had been converted to compound I. A positive reducing sugar test was dependent upon the presence of both 1-PMTR and fraction A. Examination of compound I derived from 1-[5-methyl-³H] PMTR by high voltage paper electrophoresis and in system A indicated that the compound co-migrated with 1-PMTR. Treatment of compound I with 0.01 N HCl for 10 min at 100 °C does not affect the compound, whereas 1-PMTR (24) is hydrolyzed to 5-S-methylthiobulose and orthophosphate as judged by high voltage paper electrophoresis at pH 3.5, and chromatography in system A. Treatment of compound I with

² Portions of this paper (including "Materials and Methods," Figs. 1 and 2, and Tables III and IV are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 8CIC-7, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
alkaline phosphatase changed its chromatographic properties. Hydrolyzed compound I migrated with an alkaline phosphatase changed its chromatographic properties. The compound was generated from 1-[5-methyl-3H]PMTR. After isolation and purification, it was reduced with NaBH₄ and then oxidized with NaIO₄. The results are summarized in Table II. The data show that NaIO₄ oxidation generates two compounds. One of these contains all results are summarized in Table I. The oxidation generates two compounds. One of these contains all reducing sugar derived from 1-PMTR.

Point are phosphoglycolic acid and S-methylthioacetaldehyde. These compounds appear to be formed in equimolar amounts. These results confirm that S-methylthioacetaldehyde was added to compound I, obtained from 1-[5-methyl-3H]PMTR, and NaIO₄ was then added to the reaction mixture. At the end of the reaction, the mixture was subjected to bulb to bulb distillation. An aliquot was examined by HPLC (system G).

Radioactive material present in the distillate co-chromatographed with phosphoethylene glycol. This reaction sequence was carried out in systems E and F. All of the radioactivity co-chromatographed with S-methylthioacetaldehyde isolated by HPLC was 70% of that expected from the amount of radioactivity in compound I and the amount of unlabeled S-methylthioacetaldehyde added. To another aliquot of the reaction mixture, S-methylthioethanol was added, followed by NaBH₄. The reaction mixture was vacuum distilled. Of the radioactivity originally in compound I, 60% was recovered in the distillate. The distillate was then examined by HPLC (system G). All of the radioactivity co-chromatographed with S-methylthioethanol. These results confirm that S-methylthioacetaldehyde is formed when compound I is oxidized with NaIO₄.

Compound I was then generated from 1-[72P]PMTR and was oxidized with NaIO₄. An aliquot of this reaction mixture was examined by high voltage electrophoresis (system D) and chromatography in systems E and F. All of the 32P initially present in compound I co-chromatographed with phosphoglycolic acid. The NaIO₄ cleavage products identified at this point are phosphoglycolic acid and S-methylthioacetaldehyde. Since compound I is most likely a derivative of a five-carbon sugar, an additional one-carbon compound must be present. In order to identify this compound, another aliquot of oxidized 32P-labeled compound I was acidified to pH 1 with H₂PO₄ and was subjected to bulb to bulb distillation. With the formate dehydrogenase assay (30), it was established that the distillate contained formic acid. The quantity of formic acid measured corresponded to 85% of the amount of compound I oxidized, assuming that 1 mol of compound I yields 1 mol of formic acid.

Compound I, upon oxidation with NaIO₄, yields formate, phosphoglycolic acid, and S-methylthioacetaldehyde. These compounds appear to be formed in equimolar amounts. These results and other results presented establish that compound I is most probably 1-phospho-5-S-methylthiobulose. The structure of compound I and its reaction with NaIO₄ is shown in Equation 1.

\[
\text{CH}_2\text{S-CH}_{2}\text{CHO} + \text{HCOOH} + \text{OHC-CH}_2\text{OPO}_4^* \xrightarrow{\text{NaIO}_4} \text{CH}_2\text{S-CH}_{2}\text{CH(OH)}_2\text{CO-CH}_2\text{OPO}_4^* \quad (1)
\]

\[R^*\text{ values for all compounds are given in Table IV (miniprint).}\]

### Table I

<table>
<thead>
<tr>
<th>Protein fraction added</th>
<th>O₂ consumed** % of maximal</th>
<th>2-Keto-4-S-methylthiobutyrate % of recovered radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;5</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>&lt;5</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>&lt;5</td>
<td>0</td>
</tr>
<tr>
<td>A + B</td>
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<td>19</td>
</tr>
<tr>
<td>A + B + C</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>A + C</td>
<td>&lt;10</td>
<td>11</td>
</tr>
<tr>
<td>B + C</td>
<td>&lt;5</td>
<td>0</td>
</tr>
</tbody>
</table>

*100% O₂ consumption assumes 1 mol of O₂ consumed per mol of 2-keto-4-S-methylthiobutyrate formed (27).

### Table II

<table>
<thead>
<tr>
<th>Compound and treatment</th>
<th>Distance traveled toward cathode (cm)</th>
<th>Radioactivity</th>
<th>Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthophosphate</td>
<td></td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>1-[5-methyl-3H]PMTR</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>NaBH₄</td>
<td></td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>NaBH₄, NaIO₄</td>
<td></td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>NaBH₄, NaIO₄, alkali Phosphate</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>NaBH₄, NaIO₄, alkali Phosphate</td>
<td></td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Reduction of 1-phospho-5-S-methylthiobulose with NaBH₄ followed by NaIO₄ oxidation should produce phosphoglycolaldehyde. This reaction sequence was carried out in order to confirm the identification of compound I. To facilitate explanation of reaction products [3H]NaBH₄ was used. An aliquot was examined by high voltage electrophoresis. All radioactivity co-migrated with phosphoglycolaldehyde (system D). The sample was then reduced with NaBH₄ and chromatographed in system D. More than 85% of the radioactivity co-chromatographed with phosphoethylene glycol. After exposure to alkaline phosphate, the compound co-chromatographed with ethylene glycol on HPLC (system G) and (system A). These experiments are consistent with the expected formation of phosphoglycolaldehyde and therefore confirm the identification of compound I as 1-phospho-5-S-methylthiobulose.

An experiment was then carried out to establish that compound I is an intermediate in the synthesis of 2-keto-4-S-methylthiobutyrate from 1-PMTR. Compound I was generated from fraction A and 1-[5-methyl-3H]PMTR, and subsequently isolated. It was then incubated with fractions B and C under standard assay conditions. Typically, fractions B and C were present at 4.4 and 15.2 mg/ml, respectively, and compound I was 1 mM in a final volume of 50 μl. The reaction was carried out at 37 °C for 40 min, and the reaction was stopped with 1 volume of 95% ethanol. After centrifugation, aliquots of the supernatant fluid were chromatographed in system A and subjected to high voltage paper electrophoresis.
at pH 3.5. By elution of the chromatogram, it was determined that 35 nmol of 2-keto-4-S-methylthiobutyrate were generated. This corresponds to ~76% of compound I added. Furthermore, no 2-keto-4-S-methylthiobutyrate was formed when 1-PMTR was exposed to fractions B and C without prior exposure to fraction A. These results establish that 1-phospho-5-S-methylthioribulose, (compound I) is converted to 2-keto-4-S-methylthiobutyrate by fractions B and C.

Next, the effect of fractions A and B on 1-PMTR was examined. The reaction was carried out under conditions of Table I. After completion of the reaction, the mixture was examined by TLC (system A). The reaction mixture was found to contain 1-PMTR or compound I. Fraction C (3.8 mg in 75 µl) was then added to both reaction mixtures. Upon addition of fraction C, O2 consumption started. After 10 min, 53 nmol of O2 were consumed, equivalent to approximately 15% of 1-PMTR present. An aliquot of the other reaction mixture was examined by TLC (system A). This showed that all of the 1-PMTR originally added was converted to compounds II and III. Their formation does not require O2 consumption. However, the conversion of either compounds II or III to 2-keto-4-S-methylthiobutyrate requires O2.

To further characterize compounds II and III, the compounds were examined by chromatography in system A before and after treatment with alkaline phosphatase. This shifted the mobility of compound II from an RF of 0.45 to 0.95. The dephosphorylated compound was not 5-S-methylthiobiose (RF = 0.70). Compound III was not sensitive to alkaline phosphatase treatment and is a neutral compound as judged by high voltage electrophoresis, pH 3.5. Thus, compound II is a phosphorylated compound, while compound III probably does not contain a phosphoryl group.

CONCLUSION

We have established that an enzyme exists in rat liver homogenates which converts 1-PMTR to 1-phospho-5-S-methylthioribulose. The latter compound is an intermediate in the conversion of 1-PMTR to 2-keto-4-S-methylthiobutyrate and eventually methionine. At least three proteins (A, B, and C) and two additional intermediates (compounds II and III) are involved in the conversion of 1-PMTR to the keto acid precursor of methionine. At this stage, the structure of compounds II and III is unknown. We have established that compound II is a phosphate ester and that compound III must be at the oxidation state of ribose since the conversion of 1-PMTR to compounds II and III does not involve net oxygen consumption. We previously proposed a mechanism for the conversion of 1-PMTR to methionine, which involved oxidation of 1-PMTR as the initial step, and subsequent elimination of orthophosphate (27). This is incorrect since we had not anticipated that 1-phospho-5-S-methylthioribulose is an intermediate. However, it seems likely that compound III is one of the intermediates which we had originally proposed.

REFERENCES

Methionine synthesis from 5'-S-Methylthioadenosine. Resolution of enzyme activities and identification of 1-phospho-5-S methylthioribulose.

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