Occurrence of Transsulfuration in Synthesis of L-Homocysteine in an Extremely Thermophilic Bacterium, *Thermus thermophilus* HB8

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A cell extract of an extremely thermophilic bacterium, *Thermus thermophilus* HB8, cultured in a synthetic medium catalyzed cystathionine γ-synthesis with O-acetyl-L-homoserine and L-cysteine as substrates but not β-synthesis with L-homocysteine and L-serine (or O-acetyl-L-serine). The amounts of synthesized enzymes metabolizing sulfur-containing amino acids were estimated by determining their catalytic activities in cell extracts. The syntheses of cystathionine β-lyase (EC 4.4.1.8) and O-acetyl-L-serine sulphydrylase (EC 4.2.99.8) were markedly repressed by 1-methionine supplemented to the medium. L-Cysteine and glutathione, both at 0.5 mM, added to the medium as the sole sulfur source repressed the synthesis of O-acetylserine sulphydrylase by 55 and 73%, respectively, confirming that this enzyme functions as a cysteine synthase. Methionine employed at 1 to 5 mM in the same way derepressed the synthesis of O-acetylserine sulphydrylase 2.1- to 2.5-fold. A method for assaying a low concentration of sulfide (0.01 to 0.05 mM) liberated from homocysteine by determining cysteine synthesized with it in the presence of excess amounts of O-acetylserine and a purified preparation of the sulphydrylase was established. The extract of cells catalyzed the homocysteine γ-lyase reaction, with a specific activity of 5 to 7 nmol/min/mg of protein, but not the methionine γ-lyase reaction. These results suggested that cysteine was also synthesized under the conditions employed by the catalysis of O-acetylserine sulphydrylase using sulfur of homocysteine derived from methionine. Methionine inhibited O-acetylserine sulphydrylase markedly. The effects of sulfur sources added to the medium on the synthesis of O-acetylhomoserine sulphydrylase and the inhibition of the enzyme activity by methionine were mostly understood by assuming that the organism has two proteins having O-acetylhomoserine sulphydrylase activity, one of which is cystathionine γ-synthase. Although it has been reported that homocysteine is directly synthesized in *T. thermophilus* HB27 by the catalysis of O-acetylhomoserine sulphydrylase on the basis of genetic studies (T. Kosuge, D. Gao, and T. Hoshino, J. Biosci. Bioeng. 90:271–279, 2000), the results obtained in this study for the behaviors of related enzymes indicate that sulfur is first incorporated into cysteine and then transferred to homocysteine via cystathionine in *T. thermophilus* HB8.

Transsulfuration is the main reaction in microorganisms (30) and plants (11) to incorporate sulfur into a four-carbon amino acid, L-homocysteine, which is produced from L-cystathionine (CTT) through a reaction catalyzed by cystathionine β-lyase (EC 4.4.1.8). These organisms first synthesize L-cysteine with O-acetyl-L-serine (OAS) and sulfide and subsequently synthesize CTT with L-cysteine and L-homoserine by the catalysis of CTT γ-synthase (EC 4.2.99.9). This enzyme reacts with an activated form of L-homoserine: O-acetyl-L-homoserine (OAH) (fungi and some bacteria) (4, 15, 24), O-succinyl-L-homoserine (enteric and some other bacteria) (11, 13, 28), or O-phosphoryl-L-homoserine (plants) (9, 26). Thus, many organisms obtain homocysteine by incorporating sulfur that is first assimilated into cysteine, via CTT. However, a few microorganisms synthesize homocysteine directly through a replacement reaction of OAH with sulfide that is catalyzed by OAH sulphydrylase (EC 4.2.99.10) (6, 23, 34).

Little is known about sulfur metabolism and the related enzymes of bacteria living under extreme conditions such as alkaline pH or high temperature. Some archaea have been reported to synthesize cysteine from methionine through reversed transsulfuration from homocysteine to cysteine (36), but an OAS sulphydrylase (EC 4.2.99.8) has been purified from an archaeon and characterized in detail, suggesting that the enzyme is functional as a cysteine synthase (1). Thus, two groups of archaea can be distinguished with respect to cysteine synthesis. This is supported by the result of genome analysis (16) showing that some archaea have genes homologous to the OAS sulphydrylase gene of enteric bacteria, while others do not. Recently, we have found that an alkaliophilic bacterium produces a very large amount of OAS sulphydrylase, the specific activity of which is very low compared with that of other microorganisms (31). Its extreme stability to heat and alkaline pHs is also notable.

We have recently found activities of CTT γ-synthase, CTT β-lyase, and OAH sulphydrylase in a cell extract of an extremely thermophilic bacterium, *Thermus thermophilus* HB8, in addition to a very high OAS sulphydrylase activity (35). On the other hand, Kosuge et al. (17, 18) have reported that *T. thermophilus* HB27 synthesizes homocysteine through direct sulphydrylation of OAH and that transsulfuration is not func-
tional, as reported for *Saccharomyces cerevisiae* (6) and *Brevibacterium flavum* (23), by demonstrating that mutant strains deficient in a gene homologous to *S. cerevisiae* MET17 require homocysteine but not CTT to grow. In order to determine which pathway is physiologically active for the synthesis of homocysteine in *T. thermophilus* HB8, transsulfuration or direct sulfhydrylation of OAH, we examined the activities of enzymes involved in the pathway leading to methionine in extracts of cells fed with various sulfur sources, and we estimated the amounts of the enzymes synthesized. In this paper, we will argue that transsulfuration is functional in *T. thermophilus* HB8 when sulfate is available and that sulfur of homocysteine derived from methionine, probably through adenosylmethionine and adenosylhomocysteine (30), can be liberated by being catalyzed by homocysteine γ-lyase and then used to synthesize cysteine by the catalysis of OAS sulfhydrylase in the presence of methionine as a sole sulfur source.

**MATERIALS AND METHODS**

**Chemicals.** OAS and OAH were synthesized according to the method of Nagai and Flavin (21). CTT and S-adenosyl-L-methionine were products of Sigma (St. Louis, Mo.). All other chemicals were of the highest quality available.

The organism and culture. Cells of *T. thermophilus* HB8 (22) were cultured at 65°C for 23 h in 3-liter flasks containing 1 liter of a synthetic medium (31), the pH of which was adjusted to 7.5. The medium contained ammonium sulfate as the sulfur source. Shaking of the culture was carried out in an incubator (Bio-Shaker BR-3000 L; TAITEC, Tokyo, Japan) at a shaking speed of 90 strokes min⁻¹. In experiments using a smaller culture size, cells were shaken at the same temperature for 24 h in 500-ml flasks containing 100 ml of the medium at speed 7 (Eyela Shaker III; Tokyo Rikakiki, Tokyo, Japan). The culture medium was inoculated with 0.1% volume of a fully grown seed culture. S-Adenosyl-L-methionine, L-cysteine hydrochloride, and glutathione were added to the heat-sterilized medium after filtration through a filter (0.2-μm pore size). L-Methionine and L-phenylalanine were dissolved in the medium before heat sterilization. Cells were collected by centrifugation at 12,000 × g for 20 min. The precipitated cells were washed with 50 mM Tris-hydrochloride buffer (pH 7.8) containing 0.03 M NaCl and kept at −20°C until use. Adenosylmethionine added in the culture medium was ascertained to be stable by subjecting the medium to high-temperature liquid chromatography (TSKgel ODS-80TM column; Tosoh) for up to 25 h under the conditions for the culture in the absence of cells, and approximately 10% of the amount employed remained stable after shaking for 25 h in the presence of cells.

Extraction. To obtain cell extracts, approximately 2 g of wet cells was suspended in 10 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 1 mM 4-(2-aminoethyl)benzenesulfonfyl fluoride hydrochloride, and 0.2 mM pyridoxal 5'-phosphate. The same ratio of the wet weight of cells to the volume of the solution was applied to all samples of cells obtained in the two types of culture. The suspensions were subjected to three cycles of sonication in an ultrasonicator (Model W-225; Heat Systems-Ultrasonic, Inc., New York, N.Y.) as described previously (31). The homogenates were centrifuged at 12,000 × g for 20 min, and the supernatant fractions, after mixing with the same volume of 0.2 M potassium phosphate buffer (pH 7.8) containing 0.2 mM pyridoxal 5'-phosphate and 50% glycerol, were kept at −20°C until use.

In order to fractionate a cell extract, cells (4 g of wet weight) harvested from 6 liters of the synthetic medium were subjected to sonication as described above, and 41 ml of extract was obtained. This was fractionated with ammonium sulfate as described previously (31). The reaction mixture comprised of 0.1 M potassium phosphate buffer (pH 7.8) containing 0.2 mM pyridoxal 5'-phosphate, 10 mM L-cysteine hydrochloride, 10 mM dithiothreitol (DTT), 1 mM CuCl₂, 10 mM OAH, and an appropriate amount of protein. The reaction was stopped by the addition of 0.2 ml of 30% trichloroacetic acid (TCA), and the product was treated on a small column of Dowex 50-8× H⁺ and then oxidized with performic acid as described previously (33). Electrophoresis of the solution was carried out to confirm the synthesis of CTT as described previously (33) (also see the legend to Fig. 2). The CTT-β-synthese (EC 4.2.1.12) reaction was carried out as described above for the γ-synthese reaction, except for the substrates employed, which were replaced by 10 mM L-homocysteine and 10 mM OAS (or l-serine).

**RESULTS**

Confimation of CTT γ-synthesis. Synthesis of CTT in cells cultured in synthetic medium was ascertained after a CTT γ-synthese reaction was carried out with all enzyme preparations (cell extract, ASF I, ASF II, and ASF III) acting as the enzyme. Among the three ammonium sulfate-precipitated fractions, ASF III was the most active (data not shown). Typical results of electrophoresis of the reaction products obtained by the catalysis of this fraction are shown in Fig. 2. It is evident that the amount of CTT synthesized increased as the reaction time increased. The amount of homocysteic acid (oxidized product of homocysteine with performic acid) also increased with reaction time, suggesting that CTT-β-synthese was also contained in the ASF III fraction. The results also show that *O*-succinyl-L-homoserine can replace OAH to a slight extent.

Regulation of enzyme synthesis by l-methionine and S-adenosyl-L-methionine. Cells were cultured in synthetic medium supplemented with sulfur-containing amino acids as follows: none, 0.1 mM L-methionine, 1 mM l-methionine, and 0.5 mM S-adenosyl-L-methionine. The extracts obtained were sub-
Regulation of synthesis of the two sulfhydrylases by L-cysteine and the related compounds. In order to check for possible regulation of the two sulfhydrylases by cysteine and its related compounds, cells were cultured in 100 ml of the medium from which ammonium sulfate was omitted. A sole sulfur source was employed in each medium as follows: ammonium sulfate (control), 0.5 mM L-cysteine, 0.5 mM glutathionine, and 0.5 mM L-djenkolic acid. Amounts of protein and the two sulfhydrylases determined for the extracts are summarized in Table 2.

No clear difference in the wet weights of cells was found in any of the cultures, but the protein amount in the extract of cells cultured with L-djenkolic acid was considerably lower than those of others.

Specific contents of enzymes in the extracts of cells cultured in synthetic medium were significantly higher than those seen in Table 1, particularly that of OAH sulfhydrylase. The reason for this is unclear at present, but the results were reproducible. In a separate experiment in which cells were cultured in medium of various volumes (50 to 200 ml) contained in 500-ml flasks, it was found that both the specific activity and the total amount of the enzyme synthesized were highest when they were grown in 100 ml of the medium. It seems, therefore, that production of this enzyme is significantly affected by the degree of shaking (affecting contact of cells with nutrients, oxygen supply, and so on). However, the difference between the 1-liter and 100-ml cultures would not interfere with comparing the enzyme syntheses in cells fed with different sulfur sources in each culture type. Synthesis of OAS sulfhydrylase was significantly repressed by cysteine and glutathionine, directly confirming that the enzyme functions to synthesize cysteine in this organism. Derepression of the synthesis of the same enzyme by djenkolic acid (by 47%), as reported for *Salmonella enterica* serovar Typhimurium (20), also supported the same role of the enzyme, because djenkolic acid supplies cysteine very slowly.

The increase in the amount of OAH sulfhydrylase (by 40 to 90%) caused by the use of cysteine and glutathione cannot be explained clearly at present. This will be discussed later, together with the behavior of the enzyme mentioned above.

Inhibition of enzyme activities by end products. To check the inhibitory effects of the end product(s) on the four enzymes, reactions were carried out in the presence of methionine and adenosylmethionine added at various concentrations. Table 3 summarizes the results obtained for reactions carried out with the extract of cells grown in synthetic medium as enzymes. CTT β-lyase activity was not inhibited by either methionine or by adenosylmethionine. On the other hand, the two sulfhydrylases and CTT γ-lyase activities were all inhibited by L-methionine, with OAH sulfhydrylase being less sensitive. Similar behaviors of enzyme activities were observed when extracts of cells grown in variously supplemented media (as shown in Table 1) were employed as enzymes (data not shown). Adenosylmethionine did not significantly affect any enzymes in the reaction mixtures under the conditions employed.
Cysteine synthesis with homocysteine and OAS as substrates. When cells were cultured in the presence of methionine as a sole sulfur source, they grew well and the amount of OAS sulfhydrylase in the extract increased to twice as much as the value obtained with synthetic medium alone or more (Table 1). The fact that the OAS sulfhydrylase synthesis was evidently derepressed suggested that the cells synthesized a certain amount of cysteine by direct sulfhydrylation of OAS with sulfide and not by γ-elimination of CTT as mentioned above, although CTT γ-lyase activity also increased slightly.

The mechanism by which the cell withdrew sulfide from methionine is a very attractive subject to study. We therefore compared γ-elimination activities with methionine, homocysteine, and CTT as substrates and with extracts of cells cultured in synthetic medium as the enzyme. As a result, no activity with methionine as the substrate was detected, suggesting that sulfide was not produced from methionine.

FIG. 2. Confirmation of the presence of CTT in the product of a CTT γ-synthase reaction catalyzed by the extract of cells of *T. thermophilus* HB8. The CTT γ-synthase reaction was carried out with OAH and L-cysteine as substrates at 50°C for 0, 10, 20, and 30 min (as indicated) in 1 ml of the reaction mixture comprised of 0.1 M potassium phosphate buffer (pH 7.8), containing 0.2 mM pyridoxal 5′-phosphate, 10 mM L-cysteine hydrochloride, 10 mM DTT, 1 mM CuCl₂, 10 mM OAH, and the enzyme. A portion (containing 0.58 mg of protein) of an ammonium sulfate (55 to 80% saturation)-precipitated fraction (ASF III) was employed as the enzyme. The reaction was stopped at the indicated time by the addition of 0.2 ml of 30% TCA solution. The mixture was applied to a small column of Dowex 50-8×1.5 cm. After the column was washed with 2 N HCl, a CTT-containing fraction was eluted with 6 N ammonia water. The eluate was subjected to centrifugal evaporation at 40°C, and the dried materials were oxidized with performic acid. Samples finally obtained were subjected to high-voltage paper electrophoresis at pH 1.8 (formic acid-acetic acid buffer) for 90 min at 2,000 V as described previously (33). After electrophoresis, the paper was air dried and then was immersed in hydrochloride, 10 mM DTT, 1 mM CuCl₂, 10 mM OAH, and the enzyme. Accordingly, they were considered to be derivatives of CTT produced under the conditions employed. Symbols: OSH/20, OAH was seen in addition to spots of CTT were also seen when authentic CTT was dissolved in the reaction mixture without other amino acids and the ninhydrin was dissolved (0.1%). After being dried as described above, the paper was heated by ironing to develop color. Three other spots given; CTT, authentic CTT dissolved in distilled water.

### Table 1. Comparison of enzyme contents in the extracts of *T. thermophilus* HB8 cells cultured in synthetic medium supplemented with L-methionine or S-adenosyl-L-methionine

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Protein (mg)/cells (g, wet wt)</th>
<th>OAS SHase</th>
<th>OAH SHase</th>
<th>CTT β-lyase</th>
<th>CTT γ-lyase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OAS SHase (U/mg of protein)</td>
<td>OAH SHase (U/mg of protein)</td>
<td>CTT β-lyase (milliU/mg of protein)</td>
<td>CTT γ-lyase (milliU/mg of protein)</td>
<td></td>
</tr>
<tr>
<td>SY</td>
<td>184/2.4</td>
<td>1.31 (± 0.29)</td>
<td>1.004 (± 0.011)</td>
<td>9.21 (± 1.58)</td>
<td>1.59 (± 0.35)</td>
</tr>
<tr>
<td>SY + 0.1 mM Met</td>
<td>174/2.5</td>
<td>0.63 (± 0.17)</td>
<td>0.025 (± 0.007)</td>
<td>4.92 (± 0.71)</td>
<td>0.82 (± 0.16)</td>
</tr>
<tr>
<td>SY + 1 mM Met</td>
<td>187/2.3</td>
<td>1.03 (± 0.07)</td>
<td>0.020 (± 0.005)</td>
<td>2.05 (± 0.39)</td>
<td>1.27 (± 0.25)</td>
</tr>
<tr>
<td>SY + 0.5 mM SAM</td>
<td>331/3.1</td>
<td>1.68 (± 0.42)</td>
<td>0.055 (± 0.013)</td>
<td>2.70 (± 0.60)</td>
<td>1.16 (± 0.24)</td>
</tr>
<tr>
<td>SY (without SO₄²⁻) + 1 mM Met</td>
<td>219/2.6</td>
<td>2.74 (± 0.35)</td>
<td>0.031 (± 0.012)</td>
<td>4.52 (± 0.91)</td>
<td>2.17 (± 0.45)</td>
</tr>
<tr>
<td>SY (without SO₄²⁻) + 5 mM Met</td>
<td>254/2.8</td>
<td>3.28 (± 0.43)</td>
<td>0.044 (± 0.010)</td>
<td>4.63 (± 0.39)</td>
<td>2.00 (± 0.05)</td>
</tr>
</tbody>
</table>

* Cells were cultured at 65°C for 23 h in 1 liter of the culture medium supplemented with L-methionine or S-adenosyl-L-methionine. Average values of three determinations are shown for all enzyme contents.

** Amounts are relative to the results for synthetic medium alone.

* SY, synthetic medium.
sulfur of methionine was utilized to synthesize cysteine by the presence of the cell extract. This result strongly suggested was synthesized with homocysteine and OAS as substrates in reaction (data not shown). It was thus demonstrated that cysteine 5 to 7 nmol/min/mg of protein.

In order to further concentrate fraction (ASF III) of the extract of cells cultured in thase reaction by the catalysis of an ammonium sulfate-con- tained fraction in the CTT metabolism. As described in Materials and Methods, it was found that an extract related to CTT metabolism. As described in Results, evidence was given for the function of transsulfuration in the cell to synthesize homocysteine. What was most evident was repres- sion of the synthesis of CTT β-lyase by the end products given to the culture medium, both together with sulfate and as a sole sulfur source (Table 1), strongly suggesting that transsulfuration from cysteine to homocysteine is functional in the pathway of methionine biosynthesis in this organism. The synthesis of OAS sulfhydrylase was also repressed when methionine was added to the medium in the presence of sulfate, supporting the notion that sulfur of cysteine is used to synthesize homocysteine via CTT. The amount of OAS sulfhydrylase increased in the cell when the end product was employed as a sole sulfur source (Table 1). The behavior of the enzyme was considered to be a result of the adaptation of cells to the absence of a sufficient concentration of sulfide with which the organism synthesizes a required amount of cysteine. The inhibitory effect of methionine on OAS sulfhydrylase activity (Table 3) also indicated that this enzyme was responsible for the synthesis of methionine. CTT β-lyase activity was quite insensitive to the

**DISCUSSION**

CTT was ascertained to be synthesized in the CTT γ-syn- thase reaction by the catalysis of an ammonium sulfate-con- nitent in the CTT pathway (Table 3) also indicated that this enzyme was responsible for the synthesis of methionine. CTT β-lyase activity was quite insensitive to the

![Table 2](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Protein (mg)/cells (g, wet wt)</th>
<th>OAS sulfhydrylase</th>
<th>OAH sulfhydrylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SY</td>
<td>32.6/0.49</td>
<td>4.91 (± 1.24)</td>
<td>0.482 (± 0.076)</td>
</tr>
<tr>
<td>SY (without SO₄²⁻) + 0.5 mM l-cysteine hydrochloride</td>
<td>29.5/0.55</td>
<td>2.16 (± 0.44)</td>
<td>0.670 (± 0.131)</td>
</tr>
<tr>
<td>SY (without SO₄²⁻) + 0.5 mM glutathione (reduced form)</td>
<td>27.4/0.40</td>
<td>1.35 (± 0.39)</td>
<td>0.919 (± 0.113)</td>
</tr>
<tr>
<td>SY (without SO₄²⁻) + 0.5 mM l-djenkolic acid</td>
<td>18.2/0.41</td>
<td>7.20 (± 1.15)</td>
<td>0.523 (± 0.120)</td>
</tr>
</tbody>
</table>

*Cells were cultured at 65°C for 24 h in 100 ml of synthetic medium supplemented as shown. Average values of three determinations are shown for all enzyme contents.

**TABLE 3**

<table>
<thead>
<tr>
<th>Inhibitor and concentration (mM)</th>
<th>OAS SHase⁰</th>
<th>OAH SHase</th>
<th>CTT β-lyase</th>
<th>CTT γ-lyase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sp act (pmol/min/mg of protein) (± SD)</td>
<td>Ratio⁰</td>
<td>Sp act (pmol/min/mg of protein) (± SD)</td>
<td>Ratio⁰</td>
</tr>
<tr>
<td>None</td>
<td>1.20 (± 0.25)</td>
<td>1</td>
<td>0.046 (± 0.009)</td>
<td>1</td>
</tr>
<tr>
<td>l-methionine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.05 (± 0.14)</td>
<td>0.87</td>
<td>0.042 (± 0.004)</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>0.68 (± 0.09)</td>
<td>0.57</td>
<td>0.037 (± 0.007)</td>
<td>0.79</td>
</tr>
<tr>
<td>10</td>
<td>0.43 (± 0.07)</td>
<td>0.36</td>
<td>0.030 (± 0.005)</td>
<td>0.65</td>
</tr>
<tr>
<td>S-adenosyl-l-methionine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.97 (± 0.47)</td>
<td>1.22</td>
<td>0.045 (± 0.009)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*The extract was obtained from cells cultured in 1 liter of synthetic medium as described in Table 1. Average values of three determinations for all enzyme activities are shown.

Shase, sulphydrylase.

*Amounts are relative to the results without inhibitors.
end product inhibition described above. Since the repression of synthesis of this enzyme was very sensitive to both methionine and adenosylmethionine (Table 1), insensitivity of the synthesized enzyme to the end product(s) in the reaction would be negligible for the cell to regulate the metabolism at this step.

Serine sulfhydrylases of S. cerevisiae (29), Aspergillus nidulans (25), and animal liver (3, 32) have been reported to catalyze CTT β-synthesis as well. Therefore, the possibility that OAS sulfhydrylase synthesized in T. thermophilus cells cultured with methionine as a sole sulfur source (Table 1) catalyzed a CTT β-synthase reaction to make reversed transsulfuration functional was examined. However, no CTT was synthesized with methionine as a sole sulfur source (Table 1) catalyzed by the T. thermophilus CTT.

Analyzing CTT α-sulfhydrylase activity in the presence of a purified preparation (Y. Mizuno and S. Yamagata, unpublished data) of the OAS sulfhydrylase of this organism. The cell extracts were also not able to catalyze α-synthesis of CTT in the presence or absence of CuCl₂. These findings suggested that the increase in CTT γ-lyase activity was meaningless in regard to supplying the cell with cysteine. Thus, it was considered that OAS sulfhydrylase functioned to synthesize cysteine in the absence of sulfate as well, with OAS and sulfide liberated from homocysteine derived from incorporated methionine, probably through adenosylmethionine and adenosylhomocysteine (30).

To determine low activity of homocysteine γ-lyase, establishment of a new assay method was needed, because the method employed for the determination of γ-lyase activities with CTT and methionine described in Materials and Methods produced incorrect values when homocysteine was employed as the substrate. The reason for this was considered to be that homocysteine and pyridoxal 5'-phosphate formed thiazolidine (5), which has an absorption at 340 nm that interfered with the correct observation of the change in the consumption of NADH. Chemical determination of sulfide liberated from homocysteine by the use of ethylene blue (27) also failed to enable accurate determination of the activity. This might be due to the instability of sulfide liberated during incubation under the conditions employed. Therefore, we prepared a reaction mixture for the detection of the activity of homocysteine γ-lyase in which the sulfide produced was measured enzymatically (Fig. 1).

The synthesis of OAH sulfhydrylase appeared to be affected by the presence of methionine in the culture medium (Table 1), and the activity was inhibited by methionine to some extent (Table 3). These facts suggested that the enzyme plays a role in the pathway of methionine synthesis. However, it is difficult to consider that the enzyme functions as a direct homocysteine synthase under ordinary conditions of the cell. First, the presence of two pathways for the same purpose would interfere with exact regulation. Second, the increase of OAH sulfhydrylase activity in the cells fed with cysteine (or glutathione) as a sole sulfur source (Table 2) is very difficult to understand, if we assume that the enzyme functions as a homocysteine synthase.

Two types of enzyme are known to contribute to OAH sulfhydrylase activity. One is an OAH sulfhydrylase lacking CTT γ-synthase activity of B. flavum that has no CTT γ-synthase (23), and the other is a CTT γ-synthase of Bacillus sphaericus (12), which also has an activity of OAH sulfhydrylase that is not functional to directly synthesize homocysteine in the cell. The increase in the synthesis of OAH sulfhydrylase with abundant cysteine (or glutathione) in the medium is, therefore, considered to be a result of substrate induction of CTT γ-synthase. However, it must be noted that the organism might have two proteins, each having OAH sulfhydrylase activity (see below), as reported for Neurospora crassa (15), in which the role of OAH sulfhydrylase is unclear. The ambiguous behaviors (including less susceptibility to end product inhibition) of the OAH sulfhydrylase we observed support the presence of two enzymes, one of which is sensitive and the other of which is insensitive to inhibition by methionine.

CTT γ-synthase of Salmonella serovar Typhimurium (13) has been described to catalyze not only γ-replacement with O-succinylhomoserine as a substrate but also γ-elimination with O-succinylhomoserine, OAH, or CTT as a substrate, with reactivity descending in that order. If the CTT γ-lyase activity we observed is also another activity of CTT γ-synthase of T. thermophilus, it is apparent that the enzyme synthesis was repressed in the cell affected by methionine and adenosylmethionine added to the synthetic medium (Table 1) and that the activity was inhibited by methionine (Table 3). However, we also found that the CTT γ-lyase synthesis was enhanced slightly in the presence of methionine without sulfate in the medium (Table 3). The reason for this is not known at present.

This organism has been found to have two genes homolo-
sylmethionine remained stable in the culture medium. Therefore, the second gene is considered, at present, to encode CTT incorporating the amino acid. The absence of sulfur, implying that the organism is not able to utilize sulfur directly as a sulfur source was not different from that of the cells cultured in the presence of sulfur, which has been built up by enzyme recruitment. J. Bacteriol. 170:104–112.

Acknowledgments

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References