Short Communication

S-Sulfocysteine as a Source of the Sulfur Atom of Cephalosporin C

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Penicillin and cephalosporin antibiotics possess a sulfur atom, derived from cysteine, in the molecule. Cysteine is considered to be synthesized via either the sulfate reduction pathway or the reverse transsulfuration pathway. The sulfur of penicillin produced by *Penicillium chrysogenum* is derived efficiently via the sulfate reduction pathway from sulfate,\(^1,2\) while that of cephalosporin produced by *Cephalosporium acremonium* is derived preferentially via the reverse transsulfuration pathway from methionine.\(^3,4\)

In the course of strain improvement of *C. acremonium*, we found that the addition of thiosulfate or S-sulfocysteine, which is an intermediate in the conversion of inorganic sulfate to organic sulfur compounds, to the chemically defined medium was effective in the production of cephalosporin C (Table I). This finding suggests that the sulfur of cephalosporin C is derived via the sulfate reduction pathway, and that there are at least two routes for cysteine synthesis in *C. acremonium* as shown below.

Reverse transsulfuration pathway

Route 1; Methionine $\xrightarrow{\text{Serine}}$ Cysteine

Route 2; Thiosulfate $\xrightarrow{\text{S-sulfocysteine}}$ Cysteine

**Table I. Effect of Sulfur Compounds on Total Cephalosporin C Production**

<table>
<thead>
<tr>
<th>Sulfur source</th>
<th>T-CPC* (µg/ml)</th>
<th>Dry cell weight (mg/ml broth)</th>
<th>Specific T-CPC productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(A/B)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1180</td>
<td>5.96</td>
<td>198.0</td>
</tr>
<tr>
<td>L-Homoserine</td>
<td>692</td>
<td>6.52</td>
<td>106.1</td>
</tr>
<tr>
<td>L-Cystathionine</td>
<td>590</td>
<td>6.68</td>
<td>83.8</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>820</td>
<td>5.73</td>
<td>143.1</td>
</tr>
<tr>
<td>S-Sulfocysteine**</td>
<td>1620</td>
<td>6.47</td>
<td>250.4</td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$</td>
<td>1268</td>
<td>7.35</td>
<td>172.5</td>
</tr>
<tr>
<td>Na$_2$SO$_3$</td>
<td>792</td>
<td>6.45</td>
<td>122.8</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>804</td>
<td>8.00</td>
<td>100.5</td>
</tr>
</tbody>
</table>

* T-CPC (Total cephalosporin C) represents cephalosporin C, deacetylcephalosporin C and other cephem compounds susceptible to cephalosporinase, and is assayed by the enzyme method with cephalosporinase.\(^10\)

** S-sulfocysteine is prepared by the method of Segel and Johnson.\(^8\)

Medium used (g/liter): maltose 40, NH$_4$Cl 2, KNO$_3$ 5, KH$_2$PO$_4$ 0.1, CaCO$_3$ 5, MgCl$_2$ 6H$_2$O 0.1, L-serine 4, sulfur source 5, FeCl$_3$·nH$_2$O 0.01, ZnCl$_2$ 0.001, Na$_2$MoO$_4$·2H$_2$O 0.001 (pH 7.0).

Strain used: a high T-CPC producer No. 130-45.

Culture condition: 24°C, 240 rpm, 6 day-culture.
To test for the existence of route 2, the following experiment was carried out. We used a mutant, *C. acremonium 8650+/OAH-/SeMe*, kindly supplied by Ciba-Geigy Ltd. This mutant is characterized by: i) inability to utilize inorganic sulfur sources (8650+), ii) deficiency in homoserine-O-acetyltransferase EC 2.3.1.31 (OAH-), and iii) resistance to methane selenol (SeMe). Consequently, this mutant can not grow without methionine, homocysteine or cystathionine because of its inability to synthesize O-acetylhomoserine (OAH). In chemically defined medium containing S-sulfocysteine supplemented with a low level of methionine sufficient to support optimal growth but not antibiotic production, the mutant produced cephalosporin C in proportion to the amount of S-sulfocysteine added (Fig. 1).

Woodin and Segel reported that S-sulfocysteine was converted to cysteine by the cell-free extracts of *P. chrysogenum* and the driving force of the reaction was the NADPH-dependent reduction of oxidized glutathione by glutathione reductase (Reduced-NAD(P): oxidized glutathione oxidoreductase EC 1.6.4.2). We therefore determined the enzyme activity catalyzing cysteine synthesis from S-sulfocysteine by observing the decrease in absorbance at 340 nm based on the oxidation of NADPH. Figure 2 shows that the enzyme activity could be detected in the cell-free extracts of *C. acremonium* as well as *P. chrysogenum*.

Nakamura and Sato demonstrated that the enzyme preparation obtained from *Aspergillus nidulans* catalyzed the condensation of thiosulfate with serine to form S-sulfocysteine. Although the data were not shown in this paper, we have confirmed the presence of S-sulfocysteine in cells of *C. acremonium* according to reported methods.

These findings indicate that S-sulfocysteine plays an important role as the direct donor of sulfur in the biosynthesis of cephalosporin C.

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REFERENCES