Short Communication

*In Vitro* Peptidoglycan Synthesis Which is Very Sensitive to Cephalexin and Penicillin G in *Escherichia coli*: Presumable Septum-forming Reaction Sequence

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Biosynthetic reactions forming crosslinked peptidoglycan in *Escherichia coli* in which the step of crosslinkage formation is very sensitive to cephalexin and penicillin G could be demonstrated in a membrane preparation of *E. coli*. The cells of *E. coli* strain which lack activities of penicillin-binding protein (PBP)-1Bs\(^1\) and overproduce PBP-3 (about 10 times control) were used as the source of the membrane preparation. It was prepared by introducing colEl plasmid pLC 26-6\(^3\) into the mrc (PBP-1Bs\(^-\)) strain JST975srev6.\(^1\) The plasmid pLC 26-6 from the plasmid bank of Clarke and Carbon\(^3\) has been reported to involve a chromosomal part covering from leuA to ftsI, the structure gene of PBP-3.\(^4\) The cells at the late log phase of growth in a rich medium containing colicin El were gently sonicated in 0.05 M Tris-HCl buffer, pH 7.6, and the membrane fraction was obtained as described previously.\(^1\) The membrane fraction showed activity of synthesizing peptidoglycan from its nucleotide precursors, UDP-MurNAc-penta-peptide (L-Ala-D-Glu-m-[\(^1^4\)C]A2pm-D-Ala-D-Ala) and UDP-GlcNAc under reaction conditions described previously\(^1\) with slight modifications (Table I). About 1.5-3 times as much [\(^1^4\)C]-peptidoglycan was formed by the membrane preparations from cells overproducing PBP-3 as that by membrane preparations from the control PBP-1Bs\(^-\) cells producing a normal amount of PBP-3 (data not shown). The formed peptidoglycan was digested with lysozyme and the degree of crosslinking was measured as previously described.\(^1\) It was crosslinked to 6-10\% (Table I), which is considerably lower than the crosslinkage of the peptidoglycan formed by pure PBP-1Bs\(^+\) cells (15-24\%, reference\(^5\) and unpublished experiments) or by membrane preparations obtained from PBP-1Bs\(^+\) cells (16-30\%).\(^1,6\) The crosslinkage in the control experiment (membrane from PBP-1Bs\(^-\) 3\(^+\) cells) was 3.4-5.0\%.

The synthesis of the peptidoglycan was inhibited by the presence of 50 \(\mu\)g per ml enramycin (Takeda Chemical Industries Ltd., Osaka, previously called enduracidin),\(^7\) a potent inhibitor of lipid-cycle reactions.\(^8\) The result may suggest that the reaction proceeds through formation and utilization of lipid intermediates, MurNAc (pentapeptide)-PP-undecaprenol and GlcNAc-MurNAc (pentapeptide)-PP-undecaprenol.

**Table I. Peptidoglycan Synthesis in Membrane Preparations from *E. coli***

<table>
<thead>
<tr>
<th>Addition</th>
<th>Radioactivity (cpm)</th>
<th>Degree of crosslinkage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4,516</td>
<td>10.4</td>
</tr>
<tr>
<td>Enramycin, 50 (\mu)g per ml</td>
<td>0(^b)</td>
<td>—</td>
</tr>
<tr>
<td>Cephalexin, 10 (\mu)g per ml</td>
<td>3,342</td>
<td>5.7</td>
</tr>
<tr>
<td>Penicillin G, 0.3 (\mu)g per ml</td>
<td>3,084</td>
<td>7.3</td>
</tr>
<tr>
<td>Penicillin G, 3 (\mu)g per ml</td>
<td>3,680</td>
<td>5.3</td>
</tr>
</tbody>
</table>

\(^a\) [\(^1^4\)C]-Labeled substrate, UDP-MurNAc-L-Ala-D-Glu-\(m\)-\([\(^1^4\)C]A2pm-D-Ala-D-Ala (65,000 cpm per \(n\) mol) and 296 \(\mu\)g (as protein) membrane from strain JST975srev6/pLC26-6 were used. The reaction was carried out at 42\(^\circ\)C for 90 min. For other details of the reaction see ref. 1. About 1.5 times as much lipid intermediates accumulated in the presence of 50 \(\mu\)g per ml enramycin as in its absence (see ref. 7.)

Presence of cephalexin, apalacillin (data not shown) or penicillin G only slightly reduced...
the incorporation of $[14\text{C}]$-substrates into peptidoglycan but significantly affected the degree of crosslinking of the product at much lower concentrations than that required for inhibition of the crosslinking reaction due to PBP-1Bs enzyme. The ID$_{50}$ of crosslink formation was roughly estimated: 3~10 $\mu$g per ml for cephalixin, 0.2 $\mu$g per ml for apalcillin and 0.3~3 $\mu$g per ml for penicillin G. The ID$_{50}$ of the crosslink formation due to PBP-1Bs was previously measured: 1000 $\mu$g per ml for cephalixin, 3 $\mu$g per ml for apalcillin and 3 $\mu$g per ml for penicillin G. These antibiotics have affinities to bind to PBP-3 in E. coli and cause formation of long filament-shaped multinuclear cells in concentrations around their MICs$^{10,11}$. Probably the observed crosslinkage formation is due to PBP 3 and may be an important part of the septum-forming reaction sequence.

Demonstration of the enzyme activities using purified preparations of PBP-3 is under investigation. Further work is also necessary in order to demonstrate, whether the reaction for the formation of the glycan chain, the transglycosylase reaction, is the property of PBP-3, as it has been hypothesize for PBP-1Bs.$^5$ A study of the precise mechanism of the enzyme reactions is being undertaken.

Acknowledgment. Authors are very much obliged to Dr. K. Ueda in Kyoto University Medical School for providing strains from Clarke and Carbon's plasmid bank.$^2$ Cephalexin was obtained from Shionogi & Co., Osaka, and apalcillin from Sumitomo Chemical Industries, Co.

REFERENCES