Note
Growth-dependent Topological Alteration of Plasmid DNA in Escherichia coli Topoisomerase Mutants

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DNA topology in the cell is modulated by a balance of topoisomerase actions.1,2 There have been reported some effects of topoisomerase mutations on DNA topology. Shishido et al.3,4 have reported that the plasmid DNA in knotted form accumulates in Escherichia coli gyrase mutant strains about 6 to 10 times more than in wild type strains. Huang and Chen5,6 have found that the unusual structural component of plasmid DNA termed “supercondensed structure” is formed in E. coli strain SD108 (topA^−, gyrB^225) but not in the strain having wild-type gyrase. The gyrase mutation seems to be critical for formation of the unusual topology. In previous works, however, the possibility of topological alteration of plasmid DNA during growth stages has never been mentioned. Here we report the growth-dependent alteration of plasmid topology and generation of unusual structure on plasmid DNA in various E. coli strains.

The bacterial strains used are listed in Table I. They were transformed with the plasmid pUC18 and cultured in 150 ml of ampicillin-containing L-broth (1% peptone/0.5% yeast extract/0.5% NaCl/0.05 mg/ml ampicillin) at 37°C with shaking. Growth profiles of each transformant were monitored by a type U-3210 spectrophotometer (Hitachi). Figure 1 shows growth profiles of each strain. The strains JTT1 (wild type), SD7 (topA^10, gyrB^226), and DM800 (top^−, gyrB^225) grew almost identically. The plasmid DNAs in bacterial native superhelix were isolated at various growth stages indicated in Fig. 1 by a rapid-boiling method7,8 followed by RNease A treatment to remove RNAs. Superhelicity of the plasmid DNAs isolated was examined by agarose gel electrophoresis with chloroquine (Fig. 2). As reported by Pruss,9 the plasmid DNAs in topoisomerase I-deficient strains SD7 and DM800 were less supercoiled and the heterogeneity in the linking number of plasmid DNAs was larger than those in the wild type strain JTT1. In strains JTT1 and SD7, increase of superhelix was observed while the cells were growing, and partial relaxation of plasmid DNAs occurred after the culture reached stationary phase. In contrast, the superhelicity of plasmid DNAs was almost constant in the strain DM800 throughout the culture. Supercoiling of plasmid DNA can be affected by transcription of the genes carried on the plasmid.10 We had found that β-lactamase encoded on the pUC18 plasmid was expressed in E. coli transformants most efficiently at the late-log phase or at the beginning of stationary phase.11 At such stages, the plasmid DNAs were most supercoiled (Fig. 2) and the plasmid copy number increased.12 This may reflect that the transcription of the β-lactamase gene and replication of plasmid DNA introduces the supercoiling of template DNA.

We have also found faster-migrating molecules in agarose gel electrophoresis appearing at a particular growing stage in each strain. At a stage when the plasmid DNAs were most supercoiled, the unknown structural materials migrated faster in agarose gel electrophoresis than usual superhelices in the strains JTT1 (Fig. 2, lanes 3). In the case of strain SD7, a very faint band can be observed at the same position (lane 9). These molecules were also observed in the strain DM800 even though the superhelicity of plasmid DNA isolated from this strain were almost constant (lane 14). The electrophoretic mobilities of these molecules were similar to that of the “supercondensed structure” described by Huang and Chen5 which cannot be unwound by chloroquine, but we have observed this type of structure (hereafter, called the CS-like molecules) in not only the gyrase mutants (SD7 and DM800) but also in a wild-type strain (JTT1). Furthermore, the CS-like molecules observed here might consist of two different forms. In Fig. 2, the CS-like molecules gave broad bands on the agarose gel, probably due to low resolution of separation. But when electrophoresed under low voltage and using the agarose gel with a thinner

<table>
<thead>
<tr>
<th>Strain</th>
<th>CGSC No.</th>
<th>Relevant genotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>JTT1</td>
<td>6334</td>
<td>top^−, gyr^+</td>
<td>Pruss et al.²⁰</td>
</tr>
<tr>
<td>SD7</td>
<td>6335</td>
<td>topA^10, gyrB^226</td>
<td>Pruss et al.²⁰</td>
</tr>
<tr>
<td>DM800</td>
<td>6322</td>
<td>Δtop-cys, gyrB^225</td>
<td>Pruss et al.²⁰</td>
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Fig. 1. Growth Profiles of Escherichia coli Transformants.

Growth of E. coli strains harboring pUC18 was monitored by measuring absorbance at 450 nm. Cells were harvested at the times indicated by arabic numbers with triangles, and used for the DNA analysis. Absorbance values at 24 hr of panels A and B have not been measured. A, strain JTT1; B, strain SD7; C, strain DM800.

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Fig. 2. Topoisomer Analysis of Plasmid DNA with Bacterial Native Superhelicity.

Plasmid DNAs were fractionated on 1% agarose gel in the presence of 6 μg/ml chloroquine in TAE buffer (40 mM Tris-acetate/5 mM sodium acetate/1 mM EDTA, pH 7.6), transferred to a Hybond-N membrane (Amersham), then probed by the digoxigenin-labeled DNA (Boehringer Mannheim). The EcoRI-PstI fragment of pBR322 was used for the probe which contained a part of the β-lactamase gene. The lane numbers correspond to the sampling times indicated in Fig. 1. R and RD represent relaxed molecule and relaxed dimer molecule, respectively. Arrows indicate the unknown structural materials (the CS-like molecules).

well, they were clearly separated into two bands (Fig. 3), and these two components of the CS-like molecules migrated equally under the presence of 10 μg/ml chloroquine. Although Huang and Chen have concluded that generation of the supercondensed structure is caused by a mutation on the gyrase gene which decreases the gyrase activity, we consider that formation of the CS-like molecules observed here may relate to supercoiling of the plasmid DNA. This is supported by the fact that the CS-like molecules appeared when plasmids were most supercoiled and disappeared after overnight cultivation in accordance with partial relaxation of plasmid DNA in the strains JTT1 and SD7. Independent analyses reproducibly showed that even though the CS-like molecules had appeared at earlier stages such as log phase and the yield of CS-like molecules varied in a few experiments, they cannot be observed in the overnight-cultured cells. In conclusion, the CS-like molecules may be generated when supercoiling of plasmid DNA is instigated during growth.

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References