Degradation of Enteric Bacterial Deoxyribonucleic Acid by the *Escherichia coli* B Restriction Endonuclease

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The deoxyribonucleic acid of five different genera of enteric microorganisms was shown to be degraded by the *Escherichia coli* B restriction endonuclease.

In conjugation experiments between bacteria having different restriction and modification specificities, the results are anomalous in two ways (1, 3, 5–7). First, the frequency of recovery of marker recombinants or recovery of exconjugants with established episomes is significantly reduced. Secondly, the linkage of one marker to another is drastically affected. This was interpreted in terms of the F– recipient cell having a restriction endonuclease which attacked the unmodified male deoxyribonucleic acid (DNA) upon entry. Recently this interpretation was supported by the demonstration that the DNA of an rB– *Escherichia coli* B mutant was degraded in vitro by a purified preparation of the *E. coli* B restriction endonuclease (8).

To establish the general idea that DNA transferred between bacteria of different restriction and modification specificities was subject to degradation by this mechanism, we examined the in vitro degradation of isotopically labeled DNA by the *E. coli* B restriction endonuclease. Tritium or 32P-labeled DNA extracted from *E. coli* rB+ mB+, *E. coli* rB– mB+, *Salmonella typhimurium*, *Shigella flexneri*, *Aerobacter aerogenes*, *Proteus mirabilis*, and an untyped representative of the *Arizona* group were used as substrates for the *E. coli* B restriction endonuclease. Each DNA preparation was tested for degradation in the presence of unmodified *E. coli* B DNA (i.e., DNA extracted from an *E. coli* rB+ mB+ mutant) and in the presence of modified *E. coli* B DNA. Degradation was determined by zone centrifugation in linear sucrose gradients (Fig. 1).

The results (Table 1) show that the median molecular weights of the DNA preparations treated with the B restriction endonuclease were considerably altered by the enzyme (with the

![Fraction of gradient from top](http://jb.asm.org/)

**Fig. 1.** Shigella flexneri DNA (3H) and *E. coli* B r–m– DNA (32P) were incubated with (A) and without (B) the *E. coli* B restriction endonuclease for 30 min at 37°C. The reaction mixture consisted of the following in a total volume of 200 μl: tris(hydroxyethyl)aminomethane buffer (pH 7.6), 18 μmoles; S. flexneri DNA, 1 μg; *E. coli* B r–m– DNA, 1 μg; 50 units of *E. coli* B restriction endonuclease (8). The reaction was stopped by the addition of 0.01 ml of a 20% sodium dodecyl sulfate-0.1 M ethylenediaminetetraacetate solution. The entire volume was layered on a 5 to 20% linear sucrose gradient (13.0 ml total volume in 0.5 by 1.4 cm cellulose nitrate tubes) and centrifuged (Beckman L2-65B preparative ultracentrifuge) in an SW40 rotor at 40,000 rev/min 10°C, for 210 min. The gradients were fractionated and the 32P and 3H contents were determined as described previously (8). Treatment of the DNA with 100 units of *E. coli* B restriction endonuclease did not measurably alter the sedimentation patterns. Bacteriophage λ DNA was used as marker DNA for estimation of the molecular weights according to the Burgi-Hershey formula (4).
TABLE 1. *In vivo* degradation of isotopically labeled DNA by the *E. coli* B restriction endonuclease

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Molecular weight (\times 10^6) (amu)</th>
<th>Minus restriction endonuclease B</th>
<th>Plus restriction endonuclease B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> B r-m+</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> B r-m</td>
<td>28</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>95</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>46</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td><em>Aerobacter aerogenes</em></td>
<td>48</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>54</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td><em>Arizona</em></td>
<td>115</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

* The estimated molecular weights of the DNA preparations were calculated from zone sedimentation data using phage X DNA as a marker (see Fig. 1); amu-atomic mass unit.

By exception of the modified *E. coli* B DNA). We conclude that these enteric representatives do not have the *E. coli* B modification specificity, and the DNA transferred from any one of these strains to *E. coli* B would be degraded in vivo as well. Apparently there are a large number of different restriction and modification specificities found in different *E. coli* strains, *Salmonella*, etc. (2), and we would predict that the DNA transferred (bacterial, episomal, or viral) between two nonidentical enteric bacterial cells would very likely be degraded if the recipient cell had a restriction and modification mechanism.

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**LITERATURE CITED**