Thymine Starvation and Single-Strand Breaks in Chromosomal Deoxyribonucleic acid of Escherichia coli

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Received for publication 13 July 1970

Single-strand breaks, as measured by the McGrath and Williams procedure, occur in chromosomal deoxyribonucleic acid of Escherichia coli cells during thymine starvation.

When bacteria are prevented from synthesizing thymidylate, colony-forming ability decreases (3) and some physical properties of the deoxyribonucleic acid (DNA) change. DNA of Escherichia coli 15 T- decreases in substrate activity for DNA methylases (8) and for ribonucleic acid polymerase (13). Menningmann and Szybalski showed that Bacillus subtilis DNA decreases in viscosity and increases in sensitivity to shear during thymine starvation, and they suggested that single-strand breaks might be responsible (15). Freifelder and Maaløe (7) reported that no single-strand breaks could be detected by sedimentation velocity studies of DNA purified from E. coli cells that had undergone thymineless death; the resolution limit was fewer than one break per 10 million molecular weight. However, thymine starvation of E. coli strains which harbor episomes causes the production of single-strand breaks in the episome, as indicated by loss of the covalent circles measured by zone centrifugation in alkaline sucrose gradients (6). In this communication, I report that single-strand breaks are formed in chromosomal DNA of E. coli during thymine starvation and can be detected by the McGrath and Williams procedure (14).

E. coli K-12 thy- cells were grown in [3H]-thymine to label DNA uniformly and were then shifted to minimal medium lacking thymine. At intervals the cells were collected, converted to spheroplasts, lysed on the surface of alkaline sucrose gradients, and centrifuged (14). During thymine starvation, the molecular weight of the single strands decreased (Fig. 1), as indicated by the shift of radioactivity toward the meniscus.

As originally used (14), the McGrath and Williams procedure underestimated the number of X-ray-induced single-strand breaks, primarily because the molecular weights calculated were approximately equivalent to weight-average molecular weight calculated from sedimentation velocity data. minced DNA homogenates (20) were layered onto a 5-ml (pH 12.0) sucrose gradient (14) and stirred gently with a pin (17). The gradients were centrifuged for 2 hr at 30,000 rev/min at 20°C in a Spinco SW50.1 rotor. About 44 two-drop fractions were collected on paper discs and counted (14). This thy- strain is similar to other thy- strains in the following respects: (i) thymineless death occurs with the usual kinetics during thymine starvation (3); (ii) cells elongate during thymine starvation; and (iii) cells undergo readdition of thymine to thymine-starved AX116 (λ) [reference 10]. AX116 apparently does not harbor a defective prophage (21).
lacellular weights (4, 5). However, when modified by the calculation of number-average molecular weights, the McGrath and Williams technique is widely used (4, 11, 17, 20). The number-average molecular weights previously reported (e.g., references 4, 11) were in the same range as those reported here.

The number-average molecular weight of the DNA single-strands (1, 14, 17, 19) can be used to calculate the approximate number of single-strand breaks per genome during thymine starvation (Table 1). Single-strand breaks were formed

<table>
<thead>
<tr>
<th>Period of starvation (min)</th>
<th>Fraction survivors</th>
<th>Number-average molecular weighta</th>
<th>Approximate single-strand breaks per genomeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.03 X 10^10</td>
<td>27</td>
</tr>
<tr>
<td>60</td>
<td>0.90</td>
<td>0.74 X 10^10</td>
<td>38</td>
</tr>
<tr>
<td>120</td>
<td>0.49</td>
<td>0.54 X 10^9</td>
<td>52</td>
</tr>
</tbody>
</table>

*Calculated for fractions 7 through 37 which contained more than 90% of the total radioactivity.

*Based on Cairns' (2) maximum value of 2.8 X 10^10 daltons per native genome.

at the rate of about 0.21 per genome per minute of thymineless incubation. Freifelder predicted that single-strand breaks would be formed in E. coli chromosomal DNA at a rate of about one break per genome per minute because the rate of loss of episodes (resulting from single-strand breaks) was proportional to the episode molecular weight (6). Since there is the possibility of aggregation of DNA strands during centrifugation by the McGrath and Williams procedure, the estimate of 0.21 single-strand break per genome per min might be considered a minimum value. Nonetheless, this procedure should be useful in studies of thymine starvation effects on DNA of a wide variety of organisms, e.g., Lactobacillus (16), Mycoplasma (18), or amethopterin-treated L1210 mouse leukemia cells (12).

This investigation was supported by Public Health Service grant AI 08286 from the National Institute of Allergy and Infectious Diseases and by American Cancer Society grant E-578.

Nawal A. Shafiq provided very capable technical assistance.

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LITERATURE CITED


