Utilization of 5-Bromouracil by Thymineless Bacteria

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ABSTRACT

Several thymineless *Escherichia coli* strains have been examined for their ability to replicate their deoxyribonucleic acid when bromouracil is substituted for thymine. The procedure we described was used to identify a thymineless strain with characteristics relatively favorable to its use in bromouracil labeling experiments. In addition, mutants with an "absolute" thymine requirement could be easily distinguished from one with a "leaky" thymine requirement.

Density labeling of bacterial deoxyribonucleic acid (DNA) with bromouracil (BU) has provided a method with which to study the effects of various physical and chemical environments on the replication of the bacterial chromosome. Many investigations have involved the use of *Escherichia coli* strain 15T¹ or its derivatives (2, 3, 6, 8). In some of these investigations, the study of bacterial chromosome replication has been undertaken after treatments which are known to induce prophage in lysogenic bacteria. It has been reported from several laboratories that *E. coli* 15 strains contain a presumably defective bacteriophage (4). Since the induction of phage would complicate the interpretation of results in many types of experiments, we have undertaken a search for other *E. coli* strains which could be of use in studies involving BU.

For a strain to be of value for such studies, it should meet the following criteria. (i) It must have an "absolute" requirement for thymine to ensure complete replacement of thymine by BU in newly synthesized DNA. (ii) The thymine requirement for growth in liquid medium should be satisfied by a thymine concentration of 2 µg/ml. This condition avoids the use in experiments of unnecessarily large amounts of radioactive thymine or BU. (iii) BU should substitute for thymine in sustaining DNA synthesis for at least one cycle of replication at a rate approaching that with thymine.

With these criteria in mind, several thymine-requiring (Thy⁻) strains of *E. coli* were tested for their ability to utilize BU in DNA synthesis.

A strain well suited for experiments utilizing BU was easily distinguished from several strains of less value. Moreover, a "leaky" Thy⁻ strain was easily detected, suggesting that the procedure used can simplify the search for a mutant with an "absolute" thymine requirement.

MATERIALS AND METHODS

Strains. The strains of *E. coli* used in this work are listed in Table 1. Strain 15T⁻(555-7) is a multiple amino acid-requiring derivative of 15T⁻, a Thy⁻ derivative of a Thy⁻ mutant isolated by Roepke (9). Ruth Hill kindly provided us with a tryptophan- and thymine-requiring mutant of strain B/r, which requires thymine at a concentration of at least 20 µg/ml. The Thy⁻ mutants of the other strains were derived by the method of Nishioka and Eisenstark (personal communication). This involved growing cells in appropriately supplemented minimal salts-glucose medium with the addition of 5 µg/ml of thymidine, 200 µg/ml of aminopterin, and 3 µg/ml of N-methyl-N’nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.). A 1-ml culture, lightly inoculated with stationary-phase cells, was shaken at 37 C. After 1 or 2 days, when the cultures became turbid, a loopful was streaked onto appropriately supplemented hard minimal agar containing 2 or 20 µg/ml of thymidine. After overnight incubation at 37 C, several colonies were picked, restreaked for purification, and marker-checked. Since Thy mutants derived by this method usually require more than 20 µg/ml of thymine for optimal growth (5; see also 10), in some cases, further selection was made for mutants with a low thymine requirement (designated Thy⁻, satisfied by 2 µg/ml thymine) by the procedure of Harrison (5). This involved selection of colonies growing farthest from the center of an agar plate where a drop of thymine (~100 µg) had been placed. Acceptable isolates could grow to stationary phase in medium containing 2 µg/ml of thymine.

Media and growth conditions. Cultures were grown...
at 37 C under forced aeration in minimal salts-glucose medium (1) containing appropriate supplements: thymine for growing inocula, 10 μg/ml; tryptophan, 14 μg/ml; arginine, 38 μg/ml; methionine, 50 μg/ml; and histidine, 100 μg/ml. To label cellular DNA with H3-radioactivity, cells were grown in H2-thymine (0.2 μCi/ml, 2 μg/ml). Density labeling was accomplished with C14-bromouracil (100 μg/ml), (Type 1.754 g/cc; or “new” strands at least once replicated (“heavy,” BU substituted for thymine in both strands), 1.800 g/cc. The extent of (“original”) DNA replicated is equivalent to the proportion of H2-thymine radioactivity having a density of 1.754 g/cc. The amount of DNA synthesis occurring during additional rounds of replication is related to the amount of C14-BU-labeled DNA fragments having a density of 1.800 g/cc. An exception to this classification will be noted in Results and Discussion.

RESULTS AND DISCUSSION

The procedure we adopted for testing the utilization of BU in DNA synthesis in several E. coli strains involved determining, after a 90-min incubation, the extent of replication of the DNA present at the time BU incubation commenced. Since the “original” DNA was uniformly labeled with H3-thymine, the extent of its replication was equivalent to the proportion of H3 radioactivity banding in a CsCl gradient at density 1.754 g/cc. DNA fragments of this density could arise by semiconservative replication (7) only if the substitution of BU for thymine was complete in the newly synthesized strand. Thus, the synthesis of truly “hybrid” DNA by a Thy- mutant during incubation with BU is deemed sufficient demonstration that the organism satisfies the first criterion stated in the introduction.

Figure 1 shows that E. coli C thy-321 is such an organism. After 90 min of incubation in the presence of BU, a symmetrical H2-labeled peak having a density of 1.754 g/cc was observed. Profiles obtained from the first five strains listed in Table 1 were similar to that shown in Fig. 1, showing a
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symmetrical band at truly "hybrid" density; thus, it is concluded that the thymine requirement of each of these strains is "absolute."

Also to be noted in Fig. 1 is the small amount of H\(^3\) radioactivity which banded within the peak of "heavy" DNA of density 1.800 g/cc. This was also observed to a variable extent with the other five strains. Usually a definite peak was not observed at 1.800 g/cc, but rather a shoulder of H\(^3\) radioactivity was seen, which extended from the "hybrid" peak toward the middle of the "heavy" DNA band. We assume that such "heavy" H\(^3\)-labeled DNA arose from the transient utilization of an H\(^3\)-thymine pool present at the moment of transfer to BU medium. This would allow the synthesis of "hybrid" DNA containing BU and thymine in the newly replicated strand. Subsequent replication of this strand would yield "heavy" DNA having a density dependent on its thymine content.

E. coli K-12 Hfr P4X met-161 thy-88 exhibited the characteristics shown in Fig. 2: (i) the hybrid DNA had a density less than 1.754 g/cc, (ii) the hybrid DNA was not symmetrical, but rather was skewed toward the "light" band, with an increased ratio of H\(^3\) to C\(^14\) radioactivity within the "hybrid" band in the lower density fractions. This DNA density profile (Fig. 2) suggested that the mutant is "leaky" Thy", an observation which was supported by other means (Suit, unpublished data). Synthesis of "lighter-hybrid" DNA would be expected if some thymine-nondependent synthesis took place (as in the case of a mutant with a "leaky" thymine requirement) or if an endogenous supply of thymine was available to compete with exogenously supplied BU for incorporation into DNA. The latter case could be transient, be-

![Fig. 1. DNA density profile of H\(^3\)-thymine-labeled Escherichia coli strain C thy-321 after 90 min of incubation in C\(^14\)-BU.](image)

![Fig. 2. DNA density profile of H\(^3\)-thymine-labeled Escherichia coli strain K-12 Hfr P4X thy-88 met-161 after 90 min of incubation in C\(^14\)-BU.](image)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth doubling time</th>
<th>DNA density distribution</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>H(^3)&quot;heavy&quot;</td>
</tr>
<tr>
<td>15T(^-) (555-7) thy try arg met...</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>B/t thy try........................</td>
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<td>33</td>
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<tr>
<td>B(^-t) thy met....................</td>
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<tr>
<td>K-12 F(^-) thy-334 his-323......</td>
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<tr>
<td>K-12 Hfr G6 thy-156 his-323.....</td>
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<td>21</td>
</tr>
<tr>
<td>C thy-321..........................</td>
<td>55</td>
<td>35</td>
</tr>
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* Determined by turbidimetric measurements at 420 and 660 mu.
cause of an intracellular pool of thymine present at the time of transfer to BU medium, or continuous, perhaps from continuous DNA breakdown and reutilization of the resulting thymine.

All of the strains tested meet the second criterion (that the thymine requirement for growth in liquid medium be satisfied by 2 μg/ml), except *E. coli* K-12 Hfr P4X met-161 thy-88, since they were selected for the ability to grow with a low concentration of thymine. A thymine concentration of 2 μg/ml or less will support their growth to a normal stationary-phase cell density.

Table 2 summarizes data obtained with the six "absolute" thymine-requiring organisms. Presented are the growth doubling times, and the percentage of H3 "hybrid" and C14 "heavy" DNA observed after 90 min of growth in C14-BU. Since the 90-min incubation should have allowed sufficient time for the complete replication of "original" DNA (conversion to hybrid) if the rate of DNA synthesis in BU was nearly the same as that in thymine (criterion iii), failure to complete the replication of "original" DNA in 90 min could reflect either a decreased rate of synthesis in BU or a toxic effect from BU, causing a premature termination of DNA synthesis, or both. *E. coli* C thy-321 showed near-total replication of the "original" DNA, whereas strains with similar growth rates did not (e.g., *E. coli* B/r thy try, Bn−1 thy met, and K-12 F− thy-334 his-323). Thus *E. coli* C thy-321 appeared to display the most normal rate of DNA synthesis in BU.

Since C14 "heavy" DNA should be synthesized only after a cycle of replication has been completed, the presence of C14 "heavy" DNA, at a time when a large portion of the "original" DNA remains unreplicated, may reflect heterogeneity among chromosomes with respect to rate of DNA synthesis in the presence of BU. More complex explanations are possible, but our data do not provide information concerning them.

For our purposes, it is sufficient to conclude that BU has a variable effect on DNA synthesis in Thy− strains of *E. coli*. This effect could be to reduce the rate of synthesis in all chromosomes or prevent certain chromosomes or chromosome regions from replicating. As judged by the results of our tests on the strains studied, *E. coli* C thy-321 has the most favorable characteristics for use in BU density labeling studies.

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Theresa Barbee performed the isolation of *E. coli* C thy-321 (Thy−).

**Literature Cited**