Properties of Thymineless Strains of Bacillus megaterium

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Both Bacillus megaterium KM: T\(^{-}\) R\(_1\), a strain partially resistant to thymineless death, and strain KM: T\(^{-}\), the parent strain, can satisfy their thymine requirement with either thymidine, 5-methyldeoxycytidine, or 5-methyluridine. Neither strain can use 5-methylcytosine, 5-hydroxymethylcytosine, 5-hydroxymethyluracil, or 5-aminouracil for this purpose. Strain KM: T\(^{-}\) R\(_1\) requires as little as 0.01 mm thymine for maximum growth, whereas strain KM: T\(^{-}\) requires 0.10 to 0.20 mm thymine. Lysogenic KM: T\(^{-}\) R\(_1\) dies more rapidly in the presence of mitomycin C than the corresponding phage-sensitive strain. Unexpectedly, the lysogenic strain was found to be less sensitive to thymineless death than the phage-sensitive strain. Lysogenic KM: T\(^{-}\) R\(_1\) is induced by exposure to mitomycin C and by thymineless incubation. It is concluded that thymineless death occurs by a mechanism which is unrelated to phage induction and that a major lethal effect of mitomycin C is probably a consequence of phage induction.

Previous studies with thymineless strains of Bacillus megaterium KM have shown that both lysogenic and phage-sensitive derivatives undergo similar rates of thymineless death, despite the fact that lysogenic strains are induced by thymine deprivation (5, 6). Since there is no evidence that phage-sensitive strains of B. megaterium carry any inducible elements (prophage or probacteriocins), it was concluded that thymineless death occurs via mechanisms unrelated to phage induction (6). Recent experiments by Donachie and Hobbs (2) with inducible and noninducible strains of Escherichia coli 15 T\(^{-}\) have led to a similar conclusion. In contrast to these findings, Sicard (8) and Sicard and Devoret (9) have reported that strains of E. coli, which carry either prophage or colicinogenic factors, die more rapidly under thymineless conditions than do the corresponding non-inducible strains. The induction of defective prophage has been proposed as the cause of thymineless death in both E. coli (4) and Bacillus subtilis (7).

B. megaterium KM: T\(^{-}\) R\(_1\), is a strain partially resistant to thymineless death (10). In an attempt to understand the mechanism of this partial resistance, growth studies were performed with both strain KM: T\(^{-}\) R\(_1\), and the parent strain (KM: T\(^{-}\)). They were compared with respect to the thymine concentration that would support maximum growth and the pyrimidine derivatives that would replace thymine in the growth medium. In addition, parallel studies were carried out with lysogenized and phage-sensitive derivatives of KM: T\(^{-}\) R\(_1\), to determine if the presence of a temperate phage would alter the sensitivity of this strain to either thymineless conditions or to mitomycin C. It was important to determine if lysogenized KM: T\(^{-}\) R\(_1\) could be induced by the above conditions.

MATERIALS AND METHODS

Thymineless strains of B. megaterium KM were grown at 37 °C in the thymidine-supplemented basal medium previously described (6). In most experiments, exponentially growing cells were harvested by centrifugation at 0 °C, washed twice with the non-supplemented basal medium, and suspended in the appropriately supplemented medium. Viable cell counts and free phage titers were determined as described previously (5), using a soft-agar overlay technique with Tryptose-yeast extract-agar. All compounds were commercial samples of analytical grade. Mitomycin C was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Strain KM: T\(^{-}\) has been described (10). Strain KM: T\(^{-}\) R\(_1\) also requires thymine for growth, but is

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RESULTS AND DISCUSSION

Strains KM:T⁻ and KM:T⁻ R₁ were found to use thymine or thymidine with equal efficiency. As can be seen in Table 1, strain KM:T⁻ R₁ dies much more slowly in the absence of thymine than does strain KM:T⁻. Furthermore, strain KM:T⁻ R₁ was found to grow optimally in the presence of low levels of thymine (0.01 mM) (not shown). Thymine (0.01 mM) is growth-limiting for strain KM:T⁻ and results in some thymineless death during a 4-hr incubation (Table 1). Strain KM:T⁻ requires 0.10 to 0.20 mM thymine for optimal growth (not shown). Thymineless strains of E. coli have also been found to vary with respect to the level of thymine required for optimal growth (3). It can also be seen (Table 1) that both strain KM:T⁻ R₁ and strain KM:T⁻ can efficiently utilize either 5-methyluridine or 5-methyldexoycytidine (5-MDC) in place of thymine. Extracts of B. megaterium rapidly convert 5-MDC to thymidine (J. T. Wachsmann, unpublished data). As has been found for E. coli 15T⁻ (1), neither strain can use 5-methylcytosine in place of thymine, even when the concentration is raised to 0.5 mM. The following pyrimidines at a concentration of 0.5 mM each did not support the growth of either strain in the absence of thymine: 5-hydroxymethylcytosine, 5-hydroxymethyluracil, and 5-aminouracil.

Comparative studies were performed on the effects of both thymine starvation and of mitomycin C on the viability of strain KM:T⁻ R₁ (phage sensitive) and its lysogenic derivative. As can be seen in Fig. 1, the viable count of the phage-sensitive strain decreases even more rapidly and to a greater extent under thymineless conditions than does the viable count of the lysogenic strain. This is in contrast to previous findings with phage-sensitive and lysogenic derivatives of strain KM:T⁻, both of which die at the same exponential rate, and to the same extent, under thymineless conditions (6). We have no explanation for this strain difference, but feel that it may be related to the low thymine requirement and partial resistance of strain KM:T⁻ R₁ to thymineless death. The important point is that the lysogenic strain does not undergo a more rapid rate of thymineless death than the phage-sensitive strain.

Thymine starvation of lysogenic KM:T⁻ R₁ results in phage induction which is similar to that previously described for lysogenic KM:T⁻ (5). When cells of either lysogenic strain (10⁷ colony-forming units/ml) are subjected to 2 hr of thymine starvation, followed by 2 hr of incubation with thymine, maximum free phage titers varying from 2 x 10⁹ to 4 x 10⁹ per ml were obtained. The use of free phage titers to determine the extent of induction assumes that different strains have similar average burst sizes. Therefore, the strain partially resistant to thymineless death (KM:T⁻ R₁) is as readily induced by thymine deprivation as is lysogenic KM:T⁻.

Figure 2 shows the comparative sensitivities of the KM:T⁻ R₁ derivatives to mitomycin C. It is apparent that in contrast to thymineless

| Strain | Time (hr) | None⁺ | Thymine⁺ | 5-Methylcytosine⁺ | 5-Methyluridine⁺ | 5-Methyldexoxyuridine⁺ |
|--------|-----------|-------|----------|------------------|-----------------|----------------------|
| KM:T⁻  | 0         | 4.9 x 10⁷ | 4.9 x 10⁷ | 4.9 x 10⁷ | 4.9 x 10⁷ | 4.9 x 10⁷ |
|        | 2         | 4.4 x 10⁸ | 7.1 x 10⁷ | 5.0 x 10⁷ | 4.9 x 10⁷ | 1.1 x 10⁸ |
|        | 4         | 5.8 x 10⁸ | 1.8 x 10⁷ | 5.1 x 10⁷ | 4.7 x 10⁷ | 3.1 x 10⁷ |
| KM:T⁻ R₁ | 0        | 3.8 x 10⁷ | 3.8 x 10⁷ | 3.8 x 10⁷ | 3.8 x 10⁷ | 3.8 x 10⁷ |
|         | 2         | 4.2 x 10⁷ | 2.4 x 10⁸ | 4.1 x 10⁷ | 1.8 x 10⁸ | 1.9 x 10⁸ |
|         | 4         | 5.3 x 10⁸ | 3.8 x 10⁸ | 5.8 x 10⁷ | 3.0 x 10⁸ | 3.9 x 10⁸ |

⁺ Exponentially growing cells were harvested and washed as described in Materials and Methods. Cells were suspended in the basal medium with the above supplements at a final concentration of 0.01 mM each.

⁺ Supplement to basal medium.
conditions, exposure to mitomycin C results in a much more rapid rate of death for lysogenic KM:\textsuperscript{T-}R\textsubscript{1} than for the phage-sensitive strain. In addition, the free phage titer was found to increase from about 10\textsuperscript{8} per ml to about 5 \times 10\textsuperscript{8} per ml, a level which is several hundredfold greater than that found during normal exponential growth. Therefore, we conclude that lysogenic KM:\textsuperscript{T-}R\textsubscript{1} is induced by mitomycin C. It is probable that one of the major lethal effects of mitomycin C action is a consequence of phage induction.

These data further substantiate the previous conclusion that thymineless death occurs by a mechanism which is unrelated to, and probably more rapid than, phage induction (6); also, that lysogenic cells are much more sensitive to the lethal action of mitomycin C than are phage-sensitive cells.

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