EFFECT OF MITOMYCIN C ON THE SYNTHESIS OF INDUCED β-GALACTOSIDASE IN E. COLI.

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It has been firmly established that the addition of mitomycin C (MC) to E. coli does not affect the synthesis of protein and RNA for a considerable period of time (Shiba, et.al., 1959) and that the bacteria grow into long filamentous forms (Reich, et.al., 1961). This cytoplasmic growth takes place without concomitant DNA synthesis and is in fact accompanied by DNA degradation (Nakata, et.al., 1961) and dispersion of nuclear material in the cell (Reich, et.al., 1961). The apparently "normal" cytoplasmic growth suggests that many, or most, of the genes involved in the synthesis of cytoplasmic materials under the experimental conditions have remained intact and have continued their expression. If this were true, then the DNA degradation must take place at the expense of other genes which are not involved in such synthetic activities and which would probably be dormant under the experimental conditions. In order to test this hypothesis, we have examined the effect of MC on the β-thiomethylgalactoside (TMG) induced synthesis of β-galactosidase. Some of the findings are reported here.

Materials and Methods

E. coli B were grown at room temperature ($\sim 23^{\circ}C$) in 0.2% glycerol-salt medium. Log phase cells (cell density approximately 5×10^{8}/ml) were used for the experiments. β-galactosidase was induced by 10^{-3}M. TMG. MC was used at a final concentration of 10μg/ml unless otherwise stated. Enzyme assay was performed on toluene treated cells according to the method of Pardee, et. al. (1959) with the exception that the color developed

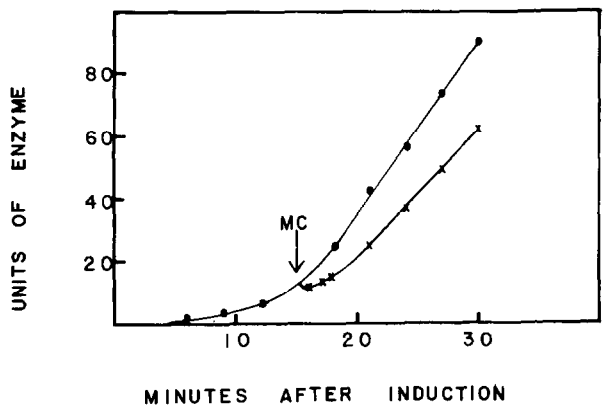
was read in a Klett colorimeter. Enzyme units are defined as the change in Klett reading per minute per 2 ml of the bacteria mixed with another 0.4 ml of reagents and buffer.

Results and Discussion

If the cells were first induced for β-galactosidase by TMG, the subsequent addition of MC led to a temporary halt of β-galactosidase synthesis lasting 2-3 minutes. (In separate experiments, it was shown that protein synthesis continued steadily during this time.) After this, there was resumption of enzyme synthesis at slightly reduced rate. (Figure 1). In some, but not all, experiments, there appeared to be a temporary dip in total enzyme activity. The explanation of this phenomenon is unknown except that it was not due to the direct inhibition of the enzyme by MC. If MC treated cells were treated a second time with this antibiotic, no interruption of enzyme synthesis was noticed. The use of MC at 30μg/ml instead of 10μg/ml gave identical results.

FIGURE 1

Effect of Mitomycin C on β-Galactosidase Synthesis in Pre-induced Cells



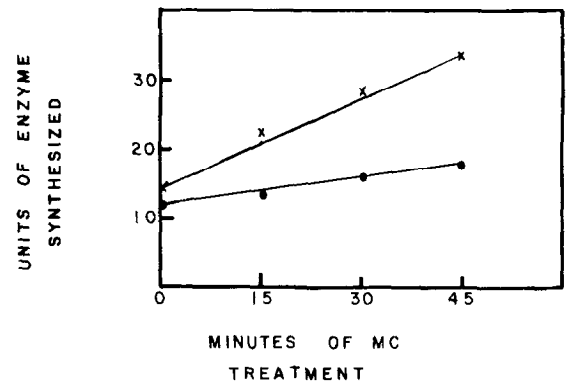
Cells were induced with TMG at 0 minute. Circles represent control cells. Crosses represent mitomycin C treated cells.

In other experiments, cells were pre-treated with MC for different periods of time before their induction with TMG. The rate of enzyme

synthesis was then compared to that in control cells which were not treated with mitomycin C. (Figure 2). Here the amount of enzyme synthesized in 25 minutes after the addition of TMG was plotted against the duration of MC treatment prior to the addition of TMG. As can be seen in the upper curve, the amount of enzyme synthesized in aliquots of untreated cells increased with time. This is to be expected as these bacteria were growing normally. When MC and TMG were added simultaneously (0 minute pretreatment with MC), the amount of enzyme synthesized in MC-treated cells was only slightly less than that in normal control cells. With increased duration of pretreatment with MC, the difference between normal control cells and MC-treated cells increased. In separate experiments, it has been established that the total amount of protein synthesized by the MC-treated cells and by the normal control cells were identical throughout the duration of the experiment. Thus, with increased duration of MC treatment in the absence of the inducer, the ratio of enzyme synthesis to protein synthesis decreased. In other words, when compared to normal bacteria the MC treatment in the absence of TMG caused partial inhibition of β-galactosidase synthesis without any effect on overall protein synthesis.

FIGURE 2

Induced β-Galactosidase Synthesis
in Cells Pretreated with Mitomycin C



One half of a culture of E. coli B in log phase growth was treated with Mitomycin C at 0 minute. Aliquots of this were induced with TMG at 0, 15, 30 and 45 minutes and the amounts of β-galactosidase synthesized in 25 minutes determined (represented by circles). Control cells (crosses) were induced with TMG at 0, 15, 30 and 45 minutes in the absence of Mitomycin C.

These results are in agreement with the hypothesis of selective destructi of dormant genes in MC-treated bacteria. Assuming a steady random destruction of such genes, one would expect that progressively lenghtening MC treatment would lead to more cells with damaged β-galactosidase gene and thus reduced ability of the bacterial population to synthesize the enzyme. However, the results do not exclude the alternative possibility that MC treatment leads to a selective partial inhibition of β-galactosidase synthesis in each and all cells and that the longer the MC treatment, the greater is the inhibition. It is hoped that experimental determination of β-galactosidase level in single cells will provide a definitive answer for the results discussed here.

Summary

Addition of mitomycin C to E. coli B which had been induced for β-galactosidase by β-thiomethylgalactoside led to a temporary halt (2-3 minutes) of β-galactosidase synthesis followed by synthesis of this enzyme at slightly reduced rate. When the cells were pre-treated with mitomycin C and then induced by β-thiomethylgalactoside, the relative rate of induced β-galactosidase synthesis in treated versus normal cells was found to decrease with increasing duration of mitomycin C pretreatment even though the rate of overall protein synthesis was identical.

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