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The Effect of Glucose on the Induction of Lambda Phage Formation by Mitomycin C

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SUMMARY

The effect of energy metabolism on the induction of λ phage development by mitomycin C was studied. If glucose is eliminated from the synthetic medium during exposure of *E. coli* K12 to mitomycin C, induction of phage development takes place more efficiently at a lower concentration of the drug than that in a normal medium. The effect of glucose deficiency on mitomycin C induction is also apparent on the addition of a bacteriostatic concentration of 2,4-dinitrophenol, potassium cyanide or sodium arsenate. This effect is not produced by inhibition of bacterial protein or ribonucleic acid synthesis. The results are discussed in terms of the relationship between mitomycin C induction and energy metabolism in bacterial cells.

INTRODUCTION

Recently, an antibiotic mitomycin C (MC) has received special attention because its effects on tumors and microorganisms are very similar to those of ultraviolet (UV) light. It has been reported that MC has a selective inhibitory action on the synthesis of deoxyribonucleic acid (DNA) in *Escherichia coli* (Shiba *et al.*, 1959; Reich *et al.*, 1960), but that the synthesis of DNA inhibited by MC is restored upon infection with phages T2, T3, T5 and P22 (Sekiguchi and Takagi, 1960; Levine, 1961). Phages λ and T3 inactivated by UV can be reactivated by infection on bacteria previously treated with UV or with MC (Otsuji and Okubo, 1960). It has also been found that induction of phage development in lysogenic bacteria is produced by exposing the cells to MC (Otsuji *et al.*, 1959).

It was reported that the metabolic disturbance produced in lysogenic bacteria by starvation resulting from insufficient nitrogen, carbon or energy sources before or after UV irradiation decreased the proportion of lysis in the bacterial population which occurred after a given latent period (Jacob, 1952; Borek, 1952). Thus the physiological state of the bacteria plays an important role in converting prophage into the vegetative state. This paper extends our previous work by analysis

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of the physiological factors during exposure of *E. coli* K12 to MC, which affect the induction of λ phage development in the bacteria.

MATERIALS AND METHODS

1. *Bacterial strains*

E. coli K12 wild type strain and its nutritional mutants (58-161, methionineless; 4506, adenineless), all of which are lysogenic for λ prophage, were used in these experiments. As plating indicators for phage λ, *E. coli* C600 or *E. coli* W3101 were used.

2. *Media*

All cultures, except the indicator strains for phage titration, were grown in a salts glucose synthetic medium (a medium containing 2.5 g $(NH_4)_2HPO_4$, 1.5 g KH_2PO_4, 0.1 g $MgSO_4.7H_2O$, 5 g NaCl and 3 g glucose in 1 liter of H_2O. Indicator strains for plating were grown on nutrient broth medium (10g meat extract, 10 g polypeptone, 1g NaCl in 1 liter of H_2O and pH 7.2 adjusted with NaOH). For titration of phage producing bacteria, the indicator strain was suspended in an AD solution containing 0.001 M phosphate buffer, 0.01 M $MgSO_4$ and 0.085 M NaCl.

3. *Method for assaying induced bacteria*

E. coli K12(λ) at the logarithmic phase of growth were exposed to MC for 10 minutes and then diluted with AD solution to dilutions of $1:10^5$ or 10^6. The diluted suspensions were plated on *E. coli* C600 or *E. coli* W3101 which were growth to the stationary phase at a concentration of about 10^9 cells per ml and were suspended at the same concentration in AD solution. At a dilution of 10^5 or 10^6, no plaques were produced by free phages. Plaque forming centers were assayed by the soft agar layer method (Adams, 1950).

4. *Chemicals*

The MC used in these experiments was supplied by Kyowa Hakko Kogyo Co. and chloramphenicol was obtained from Sankyo Co. Stock solutions of MC and chloramphenicol were made in salt solution and stored in a refrigerator.

RESULTS

The first experiment was to find whether the presence of glucose during exposure of *E. coli* to MC controls the ability to respond to MC induction. Cells harvested at the logarithmic phase of growth were suspended in salt solution and the suspension divided into two parts. To one part was added glucose at a concentration of 3 mg per ml and to the other sterile water. The cells after exposure to various concentrations of MC for 10 minutes, were immediately diluted and plated to count plaque former. The concentration of MC which gave maximal plaque formers was higher in the presence of glucose than in its absence (Fig. 1). This result indicates that elimination of glucose from the incubation medium results in more efficient inducation of phage formation at a low level of MC.

To see whether the effect produced by lack of glucose would be due to the metabolic disturbance produced by a deficiency of glucose utilization as an energy supply, the following experiment was carried out.

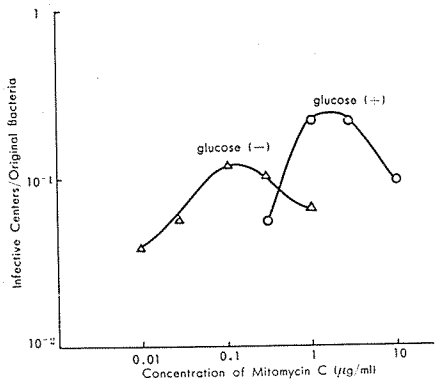

Fig. 1. Effect of Glucose on the Induction of Phage Development by Mitomycin C

E. coli K 12 at a logarithmic phase of growth were exposed to various concentrations of MC with or without glucose (3 mg/ml). After 10 minutes at 37° C aliquots were plated as infective centers (corresponding to induced bacteria).

Cells at a logarithmic phase of growth were exposed to MC in a salt glucose synthetic medium to which 2,4-dinitrophenol was added. After 10 minutes exposure of the cells, the incubation medium was diluted with salt solution and assayed for plaque formers. A concentration of 10^{-3} M of 2,4-dinitrophenol inhibited bacterial growth, but did no kill the bacteria. Therefore, the 2,4-dinitrophenol treated cells show the same degree of colony formers as non-treated cells.

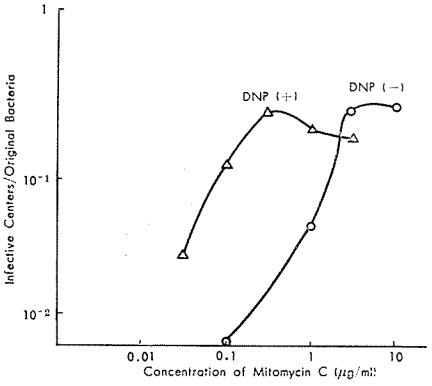

Fig. 2. Effect of 2,4-dinitrophenol on Induction of Phage Development by Mitomycin C

E. coli K12 at a logarithmic phase of growth were collected by centrifugation and divided into two portions. One was suspended in sterile water and the other was preincubated for 5 minutes with 2,4-dinitrophenol at a final concentration of 10^{-3} M. Then the former was incubated with MC in a salts glucose synthetic medium and the latter was incubated in the same medium containing MC and 2,4-dinitrophenol at 10^{-3} M. After 10 minutes plating for plaque former was carried out.

As seen in Fig. 2, this agent was found to be effective in increasing the susceptibility of cells to induction by MC. In the presence of 2,4-dinitrophenol a concentration of 0.1 μg per ml of MC causes a conversion of 12 per cent of the bacterial population into phage formers, whereas only 1.1 per cent was induced by the same concentration of MC in the absence of 2,4-dinitrophenol. It can be concluded from the data that addition of 2,4-dinitrophenol to the glucose containing medium has the same effect as lack of glucose in the incubation medium.

The same type of experiments were performed with an inhibitor of the energy supply, and the results were identical with those obtained with 2,4-dinitrophenol. Potassium cyanide at a concentration of 10^{-2} M or sodium arsenate at a concentration of 10^{-1} M had no effect on the colony forming ability of cells, but inhibit bacterial growth. As is apparent in Fig. 3, λ phage producing bacteria are induced at a lower concentration of MC if potassium cyanide is present than in its absence. The result obtained with sodium arsenate is essentially the same as that with 2,4-dinitrophenol or potassium cyanide.

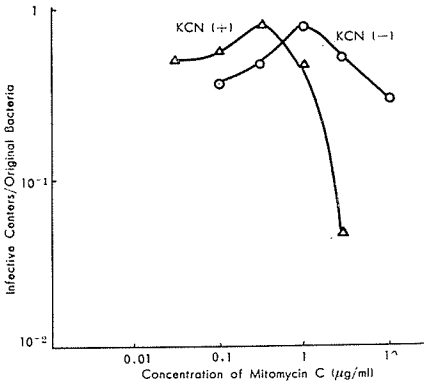

Fig. 3. Effect of Potassium Cyanide on Induction of Phage Development by Mitomycin C
The treatment of *E. coli* K12 with MC and potassium cyanide at a final concentration of 10^{-2} M was similar to that described in figure 2. After incubation with the drug for 10 minutes, the bacteria were diluted into AD solution to a 1 : 10 dilution and further incubation with shaking for 30 minutes was continued. Then aliqnots were plated for plaque formers.

To see whether the effect produced by blocking the energy supply could also be produced by other treatments which inhibit protein and ribonucleic acid synthesis of *E. coli*, the following experiments were carried out. In the first place, *E. coli* K12 at a logarithmic phase of growth were exposed to 20 μg per ml of chloramphenicol. At this concentraion protein synthesis of bacteria is suffciently inhibited. The cells were then incubated with various concentration of MC. At the concentration tested, chloramphenicol did not affect the effciency of induction by MC. In the second set of experiments a methionine requiring mutant

of *E. coli* K12 was used. Cells grown in a salt glucose synthetic medium supplemented with 50 μg per ml of methionine were harvested and starved for 60 minutes in a minimal medium. The cells were divided into two parts. To one part was added methionine at a final concentration of 50 μg per ml and to the other was sterile water. The cells were exposed to MC for 10 minutes and then the plaque forming centers were counted. Again it was found that methionine starvation did not affect the inducing ability of MC. In the third set of experiments, an adenine requiring mutant *E. coli* K12, grown in a salt glucose synthetic medium supplemented with adenine (50 μg per ml), was starved for 60 minutes. Then the cells were exposed to MC in a salts glucose synthetic medium with or without adenine. Elimination of adenine from the incubation medium also had no effect. These results are illustrated in Table 1.

Table 1 Effect of Various Treatments of *E. coli* K12 on Induction of Phage Development by mitomycin C

Bacteria	Addition (μg/ml)		Glucose (mg/ml)	Concentration of MC (μg/ml) 0.01	0.03	0.1	0.3	1.0	3.0	10.0
K12	CM**	0	3.0	—	5.5*	9.1	15.0	30.0	24.0	8.5
		20	3.0	—	5.2	8.3	9.7	23.0	21.0	15.0
58-161		0	3.0	3.8	—	3.0	2.7	8.0	12.0	—
	Met.**	50	3.0	3.9	—	2.8	5.4	8.0	8.7	—
		0	0	7.4	17.0	23.0	16.0	—	—	
		50	0	4.3	13.0	20.0	15.0	—	—	
4506	Ad**	0	3.0	0.8	1.0	1.5	2.0	6.0	—	10.0
		50	3.0	0.2	—	1.3	1.3	9.0	—	11.0

* The number shows $\frac{\text{induced bacteria}}{\text{original number of bacteria}} \times 10^2$

** CM : chloramphenicol, Met : methionine, Ad : Adenine

DISCUSSION

It is apparent from Figure 1 that conversion of prophage into its vegetative form by MC occurs more effectively in the absence of glucose than in its presence, when the concentration of MC is low. The result shows that glucose inhibits the ability of MC to convert the prophage into the vegetative state. The addition of a bacteriostatic concentration of 2,4-dinitrophenol (Fig. 2), cyanide (Fig. 3) or arsenate to the glucose containing medium induces a high rate of phage multiplication at lower concentration of MC. These results indicate that induction of phage development by MC in a lysogenic strain of *E. coli* has some relations to the energy metabolism of bacterial cells. However, there is no relationship

between the MC induction and protein or RNA synthesis, because inhibition of protein or RNA synthesis with chloramphenicol or nutritional requiring mutants of the bacteria has no effect on induction of phage development (Table 1).

At the present time the mechanism of action of glucose on MC induction is not known, but the result is of interest in connection with the effect of glucose on enzyme formation, because there are several reports indicating that enzyme formation is inhibited by the presence of glucose (Neidhardt and Magasanik, 1957; Pardee *et al.*, 1959; Freundlich and Lichstein, 1960). This inhibitory effect of glucose on enzyme formation has been discussed on the basis of a repressor model, suggesting a metabolite from glucose may act as a precursor of internally synthesized repressor of enzyme synthesis (Neidhardt and Magasanik, 1957; Pardee *et al.*, 1959). Therefore, one possible explanation for the inhibitory action of glucose on MC induction may be that the repressor for phage multiplication is stabilized only under conditions when there are sufficient energy sources, because Jacob and Campbell (1959) showed that there are repressors in lysogenic bacteria which constrain phage multiplication. According to this hypothesis it may be supposed that the repressor is easily inactivated by MC, so that the induction of phage formation takes place at a low level of drug, if the energy source is removed from the incubation medium.

However, there is an alternative explanation for the present study. One can suppose that when energy supplying systems are actively operating in bacterial cells, MC itself is inactivated in some manner or can not penetrate into the bacterial cells, so that the efficiency of MC induction in lysogenic bacteria decreases. We have not as yet sufficient data to give a difinte explanation for this glucose effect. These striking effects of energy metabolism are observed not only in experiments on the induction of phage formation in the lysogenic strain of *E. coli* K12, but also in experiments on the bacteriocidal action of MC on *E. coli* B. The killing of *E. coli* B by MC is inhibited by addition of glucose, the effect of which is decreased by a bacteriostatic concentration of 2,4-dinitrophenol, cyanide or arsenate (Otsuji, unpublished data).

The data obtained in the analysis of the physiological factors affecting MC induction are worthy of further discussion in connection with the report of Bertani (1957). He found that the frequency of lysogenization of *E. coli* C by phages P2 and P22 could be increased by the addition of chloramphenicol or 5-hydroxyuridine to the infected bacteria, but the addition of bacteriostatic concentrations of cyanide, azide or dinitrophenol did not increase the frequency of lysogenization. These results apper to be opposite to those obtained in the present experiments on MC induction. The difference between these two experiments is of interest because lysogenization and induction are opposite phenomena, that is, the former is the integration of phage chromosome to a specific site on the bacterial chromosome, while the latter is the initiation of development of the temperate phage from the

prophage state. The significance of the effect of energy metabolism and protein synthesis on lysogenization and induction of the temperate phage has not been assessed, but its study will provide a useful approach to investigation of the properties of lysogenic bacteria.

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