THE EFFECT OF LYSOGENIC INDUCTION WITH MITOMYCIN C ON THE DNA AND DNA POLYMERASE OF ESCHERICHIA COLI K12λ

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The temperate phage λ contains DNA which is very similar to that of its host cell Escherichia coli K12 as shown by base composition and nearest neighbor sequences (1). The ability of λ to form a lysogenic relationship and attach its genome to the bacterial chromosome is presumably related to this similarity of the DNA of the host cell and the phage. In contrast, the DNA of the lethal phage T_2 (which cannot show a temperate response) is markedly different from that of E. coli (1). Furthermore, infection of E. coli B with T_2 leads to the formation of large amounts of a new T_2-DNA polymerase which is different from that found in the uninfected cell (2).

We have now compared the DNA polymerase in normal E. coli K12λ with the DNA polymerase found in cells 60 minutes after induction of λ phage formation with mitomycin C. In both cases, before and after induction, the enzymes have been purified 7500-fold over the crude extract, and both fractionated in the same manner during identical purification procedures. The normal and induced DNA polymerases show identical pH curves, Mg^{++} requirements, and heat inactivation curves. The details of the purification and a comparison of the properties of the enzymes will be the subject of a future publication.

In addition to other similarities, the DNA polymerases from normal and lysogenically induced cells utilize various DNA primers with the same relative abilities. This communication will show that the DNA obtained from E. coli K12λ cells which have been lysogenically induced with mitomycin C is a relatively poor primer for DNA polymerase but functions in a normal manner with RNA polymerase.

DNA was isolated from E. coli K12λ, normal or induced with mitomycin C, by the method of Lehman (3). Calf thymus DNA was obtained from the Worthington Biochemical Corporation, Freehold, N. J. Phage λ was produced by mitomycin C induction of E. coli K12λ (4) and purified by low and high speed centrifugation followed by banding in CsCl solution (5). DNA was prepared from λ phage by treatment with phenol (6). DNA polymerase was assayed by a modification of the procedure of Lehman, Bessman, Simms, and Kornberg (7) using tritiated deoxynucleotide triphosphates as substrates with collection of the DNA polymerase product on teflon coated glass fiber paper [1]. RNA polymerase assays were carried out as previously described using an enzyme from E. coli B which had been purified 150-fold (DEAE-fraction) (8) [2]. Radioactive samples were counted in a Packard Liquid Scintillation Spectrometer, Series 314-F.

Table I shows the ability of various DNA's to prime DNA polymerase. The DNA polymerase was obtained from E. coli K12λ cells which were exposed to 1 μg/ml of mitomycin C for 60 minutes in tryptone broth at 37°, and had been purified 7500-fold. Lysis of such cells under these conditions occurs in about 100 minutes with the formation of 100 λ phage per cell. In these experiments λ DNA was approximately 4-fold better as a primer for DNA polymerase than was K12λ DNA. However, DNA isolated from mitomycin-treated cells (Mc-DNA), after various time intervals, was only about one-third as good a primer for DNA polymerase as was normal K12λ DNA. Furthermore, Mc-DNA had less than 10% of the priming activity of λ DNA under comparable conditions. It should be noted that mitomycin C has no effect on the DNA polymerase reaction in vitro [1]. The ability of the various DNA's to act as primers over a wide concentration range is shown in Fig. 1. The difference in priming abilities of the various DNA's extends over a 10-fold range of DNA concentration. Though Fig. 1 shows the results obtained with Mc-DNA isolated

[1] W. E. Pricer, Jr., and A. Weissbach, unpublished.

[2] We are indebted to Miss Norma Zabriskie and Dr. Marshall Nirenberg for performing the RNA polymerase assay.

TABLE I. Utilization of various primers by DNA and RNA polymerase

DNA primer	Incorporation of substrate in mμmoles	
	DNA polymerase*	RNA polymerase**
None	<0.02	<0.05
K12λ	0.39	0.82
λ	1.6	0.72
Mc-5	0.13	
Mc-15	0.11	0.86
Mc-30	0.16	0.83
Mc-45	0.15	0.86
Mc-60	0.11	
Calf thymus DNA	0.37	0.98

* Incubations contained 30 μmoles of Tris-ethanolamine buffer, pH 8.75, 1 μmole of $MgCl_2$, 0.3 μmole of mercaptoethanol, 0.25 μmole of EDTA, 25 units of enzyme, 12 mμmoles of H^3-deoxynucleotide triphosphate containing 3.6×10^4 cpm, and 10 mμmoles (as phosphate) of DNA primer in a total volume of 0.30 ml. Incubations were carried out at 37° for 20 minutes. One unit of enzyme is defined as that amount which will incorporate 1 mμmole of substrate per hr. with the above conditions with a standard amount of heated crude extract as primer.

** Incubations contained 20 μmoles Tris buffer, pH 7.9, 2.0 μmoles of $MgCl_2$, 0.5 μmole of $MnCl_2$, 6 μmoles mercaptoethanol, 24 mμmoles of ATP, CTP, and GTP, 27 mμmoles of C^{14}-UTP containing 6.9×10^4 cpm, and 20 μg of enzyme in 0.50 ml. Incubations were carried out for 20 minutes at 37°. 10 mμmoles of DNA was used as primer except for calf thymus DNA incubations which contained approximately 400 mμmoles of DNA.

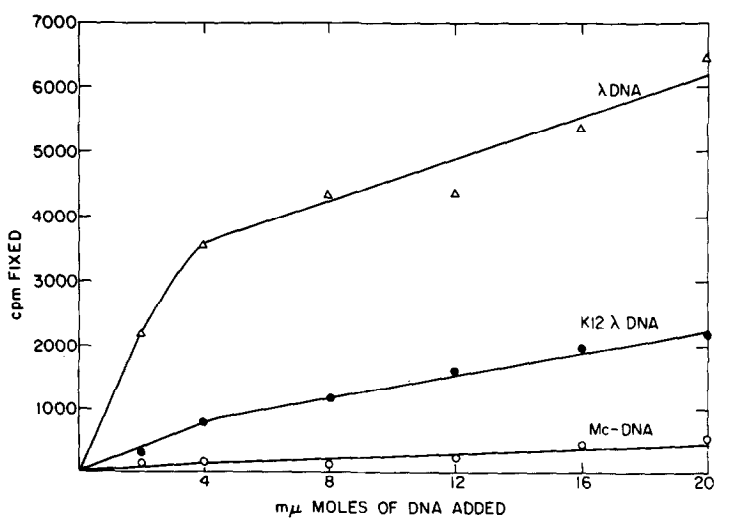

Fig. 1. Effect of primer concentration on incorporation of substrate by DNA polymerase. The incubations were performed as described in Table I.

from the cell 15 minutes after exposure to mitomycin C, the DNA's obtained from cells exposed to mitomycin C for 5, 30, 45, and 60 minutes, respectively, act in an identical manner over this concentration range. When DNA polymerase (7500-fold purified) from normal cells was substituted for the DNA polymerase from induced cells, the same results shown in Fig. 1 or Table I were obtained. In both Fig. 1 and Table I the concentration of DNA primer is limiting and the data represent rates rather than limits of incorporation of deoxynucleoside triphosphate into DNA.

Though the Mc-DNA is a poor primer for DNA polymerase, it is still an effective primer for RNA polymerase. As shown in Table I, the various Mc--DNA's are as good for priming RNA polymerase as are λ or K12λ DNA.

The data presented here suggest that exposure of E. coli K12λ cells to mitomycin C at concentrations which produce lysogenic induction and maximal phage yield, results in a rapid alteration (within 5 minutes) of the DNA in the cell. This lesion apparently makes the cellular DNA a much poorer primer for DNA polymerase, but does not affect its ability to prime for RNA polymerase. It would therefore seem that the DNA in such an induced cell could not be replicated well, but could still be used for RNA synthesis. It has been previously reported that mitomycin C stops DNA synthesis in bacterial cells, but does not affect RNA or protein synthesis (9). Our results would offer an explanation for this. The Mc-DNA, obtained from K12λ, still retains some priming ability for DNA polymerase, in vitro. Whether this DNA can be fully replicated by DNA polymerase is under study. It is pertinent that ultraviolet light, an effective lysogenic inducer, has been shown, in vitro, to lower the ability of DNA to serve as primer for calf thymus DNA polymerase (10). Recently, Iyer and Szybalski have shown that exposure of Bacillus subtilis or Escherichia coli B cells to inhibitory concentrations of mitomycin C results in the formation of a damaged or "cross-linked" DNA (11).

Since most lysogenic inducing agents (12) seem to affect DNA in various ways, the data reported here support the view that such inducing agents

damage the bacterial DNA so that it becomes relatively unavailable for replication. In the lysogenic cell exposed to inducing agents, only the undamaged prophage would be replicated, leading eventually to the formation of mature phage and cell lysis.

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