An infective deoxyribonucleic acid from bacteriophage ϕX174

It has recently been demonstrated that bacteriophage øX174 contains an unusual DNA which is composed of a single polynucleotide strand[1]. A nucleic acid of this type is of special interest because enzymic studies have suggested that double-stranded DNA is converted to a single-stranded condition during the course of duplication[2]. The purpose of the present investigation was to devise a method of estimation of the activity of øX174 DNA.

øX174 used in these experiments was generously furnished by Dr. I. TESSMAN of Purdue University. The phage was propagated on *Escherichia coli* strain C in a glycerol–casamino acid medium[3] and purified by differential centrifugation at 6,000 rev./min and 37,000 rev./min. Spheroplasts of *E. coli* and other bacterial strains were prepared by lysozyme treatment[4].

The DNA was extracted from øX174 by suspending the phage in 0.1 *M* NaCl–0.01 *M* phosphate buffer, pH 7.0, at a concentration of 10^{11}–10^{12} phages/ml, and heating at 90° for 10 min. After addition of NaCl to give a concentration of 1 *M*, the solution was shaken with an equal volume of a chloroform–octanol (8:1) mixture to remove protein. The extraction procedures with chloroform were repeated until no gelatinous interface could be observed (at least four times). Ethanol (2 vol.) was added to the aqueous solution and the appearing fibrous precipitate was collected, dissolved in a small volume of 1 *M* NaCl, and re-precipitated with ethanol. DNA thus obtained was dissolved in a sterilized medium.

This preparation of DNA was found to produce many infective phages when incubated with bacterial spheroplasts in a hypertonic sucrose medium. The properties of the preparation differ from those of normal øX174 in the following respects.

(1) The preparation could infect spheroplasts but not normal cells. In addition, spheroplasts of bacteria originally resistant to øX174 (*e.g. E. coli* B, *E. coli* K-12 and

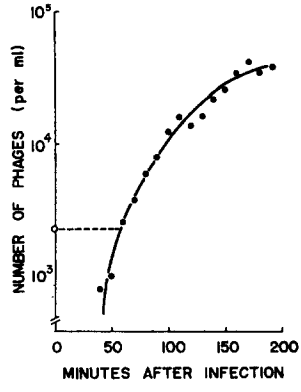

Fig. 1. Production of infective phages by spheroplasts incubated with øX174 DNA. Spheroplasts of *E. coli* B ($2 \cdot 10^8$/ml) and øX174 DNA (2 μg/ml) were incubated 37° in a medium containing (per liter) 2.5 g $(NH_4)_2HPO_4$, 1.5 g KH_2PO_4, 5 g NaCl, 3 g sodium glutamate, 3 g glucose, 0.1 g $MgSO_4 \cdot 7H_2O$, and 200 g sucrose, at pH 7.2. Aliquots of the mixture were taken at intervals as shown, diluted with saline, and plated on agar layer with a culture of *E. coli* C. Number of infective centers at zero time, indicated by open circle, was determined by plating an aliquot of the mixture on 20% sucrose-agar with *E. coli* C.

Abbreviation: DNA, deoxyribonucleic acid.

Aerobacter aerogenes 1033) were also infected by the preparation. Thus, in the subsequent experiments, spheroplasts from the resistant strain were used in order to exclude the possibility that the cells remaining after lysozyme treatment were reinfected with any active phages produced by the infected spheroplasts. Fig. 1 shows the formation of infective phages from the DNA preparation in spheroplasts of *E. coli* B.

(2) Infectivity of the preparation was completely destroyed by a brief treatment with DNA-ase, whereas such treatment did not impair the infectivity of intact phage. The results are shown in Table I.

TABLE I

ACTION OF ENZYMES ON THE INFECTIVITY OF ϕX174 DNA

Assay technique as described in Fig. 1.

Treatment of DNA	*Host*	*No. of phages produced*
None	*E. coli* B spheroplast	18,820
DNAase (1 μg/ml)*	*E. coli* B spheroplast	0
Trypsin (100 μg/ml)**	*E. coli* B spheroplast	18,640
None	*E. coli* B normal cell	0
None	*E. coli* C normal cell	0

* Incubated with crystalline DNAase (Sigma Chemical Co.) in 0.02 *M* $MgCl_2$–0.1 *M* tris-(hydroxymethyl)aminomethane buffer, pH 7.2 for 60 min at 37°.

** Incubated with crystalline trypsin (Sigma Chemical Co.) in 0.05 *M* phosphate buffer, pH 7.5 for 60 min at 37°; the enzyme action was stopped by the addition of soybean trypsin inhibitor prior to mixing with spheroplasts.

(3) The preparation had an ultraviolet-absorption spectrum characteristic of nucleic acid and did not contain any detectable amount of protein. It maintained its proper infectivity even after being treated with a high level of trypsin, indicating a distinct difference from the spheroplast-infecting agent from T2 phage[5–8].

Thus evidence was obtained that the preparation of DNA from øX174 possessed the ability to produce infective phage. The characterization of the preparation is now in progress, since the possibility that a minor component in the preparation is required to infection has not yet been completely excluded.

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After this manuscript was submitted we were informed that HOFSCHNEIDER[9] had obtained similar results with a DNA agent prepared by the phenol method.

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