Kinetics of Bacteriophage $\lambda$ Deoxyribonucleic Acid
Infection of Escherichia coli

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ABSTRACT

BARNHART, BENJAMIN J. (Los Alamos Scientific Laboratory, University of California, Los Alamos, N.M.). Kinetics of bacteriophage $\lambda$ deoxyribonucleic acid infection of Escherichia coli. J. Bacteriol. 90:1617–1623. 1965.—The kinetics of Escherichia coli K-12 infection by phage $\lambda$ deoxyribonucleic acid (DNA) were determined. An initial lag of 55 to 80 sec was found to be the time required for infecting DNA to become deoxyribonuclease-insensitive at 33 C. When cell-DNA interactions were stopped by washing away unbound DNA, the already bound DNA continued to infect the cell at rates described by linear kinetics with no apparent lag. Whereas the lag period was relatively insensitive to DNA and cell concentrations, both the lag and the subsequent linear portions of the rate curves were temperature-sensitive. Cell and DNA dose-response curves prescribed hyperbolic functions. Similarities between $\lambda$ DNA infection of E. coli and bacterial transformation systems are discussed.

A means of genetic transfer closely related to bacterial transformation is the infection of Escherichia coli with deoxyribonucleic acid (DNA) isolated from phage $\lambda$dg as described by Kaiser and Hogness (1960). Somewhat further removed from the usual notion of transformation is the infection of E. coli with DNA isolated from phage $\lambda$, resulting in death of the cell and release of progeny phage (Meyer et al., 1961; Kaiser, 1962). In the latter system, one observes a sequence of events which, on the surface, is identical to infection of bacteria by whole phage particles except for the manner in which the phage genome is introduced into the recipient cell. Infection by phage DNA may proceed via cellular mechanisms similar to those described for the infection of bacteria with transforming DNA.

The present report describes an investigation of the kinetics of infection of E. coli K-12 with DNA isolated from a clear mutant of phage $\lambda$. Helper phage-infected bacteria were used as competent cells according to the system described by Kaiser (1962).

MATERIALS AND METHODS

Media. Plating bacteria were grown in 1.0% tryptone broth (Difco) supplemented with 0.5% NaCl and 1 $\mu$g/ml of thiamine hydrochloride (TB, medium). Bacteria to be used as recipients for $\lambda$ DNA infection were grown in H medium (Kaiser, 1962) supplemented with 10 $\mu$g/ml of thiamine hydrochloride, 100 $\mu$g/ml of L-threonine, 100 $\mu$g/ml of L-leucine, and 1.75 mg/ml of glucose (H-supplemented medium). Soft agar consisted of 0.75% agar (Difco), 1.0% tryptone (Difco), and 0.25% NaCl (TA7 agar). Plating agar consisted of 1.2% agar (Difco), 1.0% tryptone (Difco), and 0.25% NaCl (TA12 agar).

Bacteria. E. coli K-12 strain C600 (Appleyard, 1954) was used as recipient for $\lambda$ DNA infection and as plating bacteria to titrate the bacteria infected by helper phage. E. coli K-12 C600 lysogenic for bacteriophage $\lambda^{+}$ was used as selective indicator in the plating of the DNA assay mixtures. These bacteria prevent plaque formation by phage of genotype $\lambda^{-}$ (helper phage) but permit plaque formation by phage of genotype $\lambda^{i}$ (infectious DNA).

The plating bacteria were grown at 37 C in TB medium to a density of $10^9$ cells per milliliter, sedimented, resuspended in 0.01 M MgSO$_4$, and stored at 4 C. The bacteria to be used as recipients for $\lambda$ DNA infection were grown at 37 C in H-supplemented medium to a density of $10^9$ cells per milliliter, sedimented, and resuspended in 0.01 M tris(hydroxymethyl)aminomethane (Tris) (pH 7.1) and 0.01 M MgSO$_4$ (TM) for helper phage infection.

Bacteriophage. Infectious DNA was prepared from a clear mutant of phage $\lambda$, $\lambda^{i}$c72 (Kaiser, 1957). Phage $\lambda^{i48}$sus$^{+}$sus$^{+}$ was used as helper phage. Although a double sus mutant was not essential in the experiments described here, it was necessary for another series of experiments to be reported subsequently. This phage was prepared by crossing $\lambda^{i48}$c$^{+}$sus$^{+}$ (Thomas, 1964) with $\lambda^{i}$sus$^{+}$e$^{+}$ (Thomas, 1964). The selected
double sus mutant was maintained in the lysogenic state. When helper phage were needed, a culture of C600 (λ44 sus-sus84) was induced with ultraviolet light from a 15-w General Electric germicidal lamp. The resulting phage were stored at 4 C in the TB1 medium in which they were prepared.

Preparation of infectious DNA. The preparation, and purification of phage and the subsequent preparation of λ DNA by aqueous phenol extraction were patterned after Kaiser and Hogness (1960) and Kaiser (1962). A 24-liter portion, in 12-liter portions, of E. coli K-12 C600 was grown at 37 C in TB1 medium to late log phase. Phage λC72 was added at a final multiplicity of 0.4, and the mixture was aerated with bubbling air until maximal lysis occurred (6.0 X 10^10 phage per milliliter). The mixture was cleared of unlysed cells, cell debris, etc., by continuous centrifugation at 4 C in a Servall high-speed centrifuge at 12,000 X g for 2 hr. The phage were concentrated from the lysate by slowly adding, with stirring, 250 g of (NH4)2SO4 per liter of lysate, and allowing a precipitate to settle out for 48 hr at 4 C. The precipitate was separated from the supernatant fluid by continuous centrifugation at 4 C in the Servall at 12,000 X g for 2 hr. The pellet was resuspended in 100 ml of 0.01 M MgSO4-0.01 M potassium phosphate buffer (pH 7.1) and dialyzed for 48 hr with agitation against 4.5 liters of the same solution. Two dialyzed preparations were treated with 2 μg/ml of pancreatic ribonuclease (Worthington Biochemical Corp., Freehold, N.J.) for 2 hr at 40 C. After centrifuging for 20 min at 12,000 X g, the phage were concentrated by centrifuging for 3 hr at 21,000 X g in a Spinco model L ultracentrifuge and resuspended in 20 ml of 0.01 M MgSO4-0.01 M potassium phosphate buffer (pH 7.1). After overnight dialysis in the cold, the ribonuclease treatment, the low-speed high-speed centrifugation, and the dialysis procedure were repeated.

The DNA was prepared according to Kaiser and Hogness (1960) with 85% liquid phenol (Fisher Scientific Co., Pittsburgh, Pa.), equilibrated with 0.01 M Tris (pH 9.0) at 6 C. All operations were performed at 5 C. The resulting preparation contained not more than 5% RNA as determined by the Meijbaum (1939) procedure. The DNA was frozen in an ethyl alcohol-Dry Ice mixture and stored at -65 C with no loss in biological activity over a period of 6 months.

After the DNA was used and after being thawed for 10 min at 37 C, it was heated for 10 min at the subcritical temperature of 72 C and rapidly cooled to prepare linear molecules from aggregated and folded molecules (Hershey, Burgi, and Ingraham, 1963; Hershey and Burgi, 1965). DNA treated in this manner gave significantly higher transformation of infection than untreated material.

Infectivity assay. The biological assay for λ DNA was similar to the procedures described by Kaiser (1962) and by Radding and Kaiser (1963).

E. coli K-12 strain C600 was grown overnight in H-supplemented medium. The bacteria were diluted 1:50 into fresh H-supplemented medium and were grown to a density of 10^8 cells per milliliter, sedimented, and resuspended in an equal volume of TM. To 1.0 ml of cells in TM was added 0.2 ml of a stock of 2 X 10^4 helper phage per milliliter, and the mixture was incubated for 15 min at 37 C. The phage-infected bacteria were centrifuged at 4 C and resuspended in 0.3 ml of cold 0.01 M Tris (pH 7.1), 0.01 M MgSO4, and 0.01 M CaCl2 (TCM). A fraction (0.1 ml) of λC72 DNA in TCM was added to the chilled competent bacteria to give a final DNA concentration of 4 μg/ml, and the mixture was incubated at 33 C. At the desired times, 0.05 ml of 1 mg/ml pancreatic deoxyribonuclease was added, and, after an additional 2 min of incubation, the cells were appropriately diluted and plated on E. coli K-12 C600 (434) to assay for plaque-forming units and on E. coli K-12 C600 to assay for total competent cells (helper phage-infected).

In all experiments except the last one reported in this paper, the cell-DNA interaction was terminated by the addition of deoxyribonuclease as indicated above.

Termination of DNA adsorption by washing. After the bacteria had been phage-infected and incubated with DNA as described above, the adsorption of DNA was terminated by the addition of one of two solutions: (i) cold TCM containing 0.3 μg/ml pancreatic deoxyribonuclease or (ii) cold 0.3 M NaCl. At preset times, 1.0 ml of solution (i) or solution (ii) was pipetted into the 0.4-m reaction mixture of cells and DNA. The cells were immediately collected by filtration on 0.3-μm Millipore filters (type PH) and washed one time with 2 ml of cold 0.3 M NaCl. The filter disc was placed in a 12-ml beaker and agitated on a reciprocal-action shaker at 33 C to resuspend the cells. This procedure allowed 60 to 80% recovery of the competent cells. It is for this reason that results from the washing experiment (Fig. 6) are presented as the ratio of infected units to competent cells.

Results

Kinetics of infection at different phage-cell concentrations. The rate of λ DNA infection increased until it reached a maximum before gradually decreasing (Fig. 1). At a constant DNA concentration of 0.1 μg/ml, the lag period extended from 60 sec at 2.5 X 10^5 to 8.7 X 10^7 competent cells per milliliter to 80 sec at 2.2 X 10^7 competent cells per milliliter. These data show that the lag period was not greatly affected by the concentration of cells capable of reacting with DNA. The rates of infection described by the linear portions of the curves were markedly influenced by variations in cell concentration.

Kinetics of infection at different DNA concentrations. The data presented in Fig. 2 show that at a constant cell concentration of 10^8 per milliliter.
PHAGE \( \lambda \) DNA INFECTION KINETICS

The initial lag in the kinetics of infection was not markedly affected by a large variation in the DNA concentration. The rates of infection described by the linear portions of the curves were, however, sensitive to changes in this parameter and indicated that between 0.5 and 4 \( \mu \)g of DNA per ml the system was saturated by an excess of DNA.

**DNA dose-response curves.** If more than one molecule of DNA were required to bring about an infection, a sigmoidal curve would result from a plot of the numbers of infected units as a function of DNA concentration. That this was clearly not the case with the system reported here can be seen in Fig. 3. The rate of infection was a linear function of DNA concentration, with no apparent lag before approaching a region in which the number of infected units was independent of DNA concentration. Saturation of the cells with DNA occurred at 1.0 to 1.5 \( \mu \)g of DNA per ml for \( 1.2 \times 10^9 \) competent cells per milliliter and at approximately 0.1 \( \mu \)g of DNA per ml for \( 10^8 \) competent cells. In each case, the multiplicity of infection at saturation was very near to \( 10^{-9} \) \( \mu \)g or 20 molecules of DNA applied per competent cell, i.e., assuming a molecular weight for \( \lambda \) DNA of \( 33 \times 10^6 \) (MacHattie and Thomas, 1964). As expected, the plateau value decreased by a factor of 10 when the concentration of competent cells was reduced 10-fold.

**Cell dose-response curves.** The data in Fig. 4 show that, before approaching a plateau value, the rate of infection increased linearly as a function of the concentration of cells capable of incorporating DNA. The plateau value was in direct proportion to the DNA concentrations, as

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**Fig. 1.** Kinetics of \( \lambda \) DNA infection (38 C) at different competent cell concentrations.

**Fig. 2.** Kinetics of \( \lambda \) DNA infection (38 C) at different DNA concentrations.
one would predict from the previous experiment. These results indicate that a single competent cell reacting with a single molecule of DNA is sufficient for an infective event to occur.

**Effect of temperature.** The results presented in Fig. 5 show that the rate and the extent of infection are temperature-dependent. A $Q_{10} = 4$ is obtained from the linear portions of the rate curves when the data are plotted as percentages of maximal infected units as a function of time (i.e., the maximal value at 28°C is one-half that at 33°C). The apparent energy of activation was calculated to be 45 to 50 kcal per mole of DNA. When the temperature was reduced from 33 to 28°C, the lag period increased from 56 to 88 sec, a 1.6-fold increase. The decrease in rate between 28 and 23°C is disproportional relative to that between 33 and 28°C and suggests that a step in the infection process has a critical temperature which lies within that range. Experiments with $\text{P}^{32}$ labeled λ DNA will determine temperature effects specifically on the initial steps leading to infection (i.e., adsorption and loss of deoxyribonuclease sensitivity).

**Characterization of the lag period.** It was shown by Levine and Strauss (1965) that the lag in the kinetics of *Bacillus subtilis* transformation was the result of terminating the transformation with
deoxyribonuclease. When the cell-DNA reaction was terminated by a washing procedure, the lag was no longer apparent and the kinetics were linear. In Fig. 6 it can be seen that, when infection was stopped by washing with 0.3 M NaCl to remove λ DNA which had not yet become irreversibly associated with the cells, the kinetics are linear and no lag occurs. These results indicate that DNA becomes firmly associated with the cells very soon after infection and that the 55- to 80-sec lag period is the time required for the λ marker to become deoxyribonuclease-insensitive.

**DISCUSSION**

These data show that 55 to 80 sec are required for the immunity region of a bound molecule of λ DNA to become deoxyribonuclease-insensitive. The infection process is temperature-dependent with a Q10 = 4 and an apparent activation energy of 45 to 50 kcal per mole of DNA. This indicates that at least one energy-requiring step is involved in the infection process. It will be shown in a subsequent publication (Barnhart, in preparation) that λ DNA infection is sensitive to inhibition by uncouplers of oxidative phosphorylation. These results are similar to those reported for penetration of transforming DNA into bacteria (Fox and Hotchkiss, 1957; Young and Spizizen, 1963; Barnhart and Herriott, 1963), an infection process which apparently involves highly ordered, energy-requiring systems. Although there is an analogy with bacterial transforming systems, a conclusion on the mechanism of λ DNA infection must await additional information; especially important may be the role of the helper phage.

The kinetic data showed that, although the duration of the lag period was relatively insensitive to cell and DNA concentrations, the linear portions of the rate curves were greatly affected by variations in these parameters. These results indicated that the rate-limiting factor contributing to the lag was not the time required for the initial cell-DNA interaction. The cells reacted rapidly with DNA, but the subsequent infection process was relatively slow. Determination of the time required for deoxyribonuclease insensitivity of widely separated markers on infectious λ DNA would provide support for the notion that DNA infects the cell in a linear, end-to-end manner after attachment of one end of the DNA molecule to the cell.

The cell and DNA dose-response curves prescribed hyperbolic rather than sigmoidal functions. The linearity of the initial portions of these rate saturation curves indicated that a single molecule of DNA penetrating a single cell was sufficient to result in an infection. The plateau values of λ-infected units were low in these dose-response experiments of short cell-DNA exposure times. However, plateau values of 10^3 to 3 × 10^3 were obtained when 10^8 competent cells per milliliter were exposed to excess DNA until completion of infection occurred. This represented a frequency of 1 to 3 × 10^-4 infections per competent cell and is in close agreement with the value of 4 × 10^-4 calculated from the data of Radding and Kaiser (1963) for infection with unbroken molecules of λ DNA.

The infection kinetics of λ DNA were found to be dependent on the method used to terminate the interaction of cells with unbound DNA. When deoxyribonuclease was added to the system before the necessary complement of phage genetic markers had become deoxyribonuclease-insensitive, an infective event was prevented. If, however, unbound DNA was removed by washing, the already bound DNA could continue to infect the cell. Termination of cellular binding of DNA by washing resulted in linear kinetics of

**FIG. 6. Dependence of λ DNA infection kinetics (55 C) on the method used to terminate cell-DNA interaction.**
infection which passed through the origin of the plot. This showed that only the DNA which was already penetrating the cell at the time of washing resulted in infection. It was observed in the course of these experiments that washing performed at room temperature or delayed washing resulted in linear kinetics which extrapolated to a point on the ordinate of the plot, indicating that transformants were present at zero time. However, control tubes showed that no \( ^{32}P \)-infected units were obtained in the absence of cellular exposure to DNA. The infection kinetics of transforming DNA into \( B. subtilis \) were reported by Levine and Strauss (1965) to be dependent on the method used to terminate cell-DNA interaction. These investigators found a lag of 1.0 to 1.6 min at 37 \( ^\circ \)C when termination was effected with deoxyribonuclease, and linear kinetics were obtained with no apparent lag when a washing procedure was used. Stuy and Stern (1964) reported a 4- to 5-sec requirement for the penetration of homologous transforming DNA into competent \( H. influenzae \).

The penetration of a cell by a \( \lambda \) DNA molecule, the dimensions of which are many times the length of the cell, favors a mechanism involving infection by an end attachment of the DNA molecule to the cell. If DNA infects the cell in a linear fashion, the infection time for the entire length of the \( \lambda \) genome, based on the location of the immunity region (Kaiser, 1957), would be increased by a factor of 3/2 to 3/1 depending on the polarity of infection. However, since at this time there is no evidence which precludes other infection configurations, clarification of this point must await additional information. It is apparent from the results presented in this report that \( \lambda \) DNA infection of \( E. coli \) K-12 has a temperature-sensitive, rate-limiting step which renders the infecting material deoxyribonuclease-insensitive.

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Addendum in Proof

Additional experiments with DNA concentrations in the linear region of the dose-response curve, 0.01 to 0.25 \( \mu \)g of DNA/ml, and extended incubation times to 140 sec confirm the finding that the lag time is independent of DNA concentration.

Kaiser and Inman (J. Mol. Biol. 13:78, 1965) recently reported that in preparations of fragmented \( \lambda \) DNA only the terminal pieces, which have cohesive sites, are capable of infecting competent bacteria. This finding supports an infection mechanism involving terminal attachment of the DNA molecule to the cell, followed by a lengthwise infection process. In addition, they showed that when the two ends of a molecule have cohered the DNA has a lowered biological activity which can be augmented 20-fold by relatively mild heating and rapid cooling. Similar findings are reported in this paper.

LITERATURE CITED


