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Role of Superinfecting Phage in Lysis Inhibition with Phage T4 in *Escherichia coli*

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ABSTRACT

RUTBERG, BLANKA (Karolinska Institutet, Stockholm, Sweden), AND LARS RUTBERG. Role of superinfecting phage in lysis inhibition with phage T4 in *Escherichia coli*. J. Bacteriol. **90**:891–894. 1965.—The ability of bacteriophage T4 to induce lysis inhibition upon superinfection was investigated after various treatments of the phage. This ability was found not to be a property of the external protein part of the phage, nor was it dependent on the functional and possibly structural integrity of the phage genetic material.

Lysis inhibition with T-even bacteriophage is characterized by a prolonged latent period and an increased burst size after superinfection with homologous phage of *Escherichia coli* primarily infected with wild-type phage (Doerman, 1948). The aim of the present investigation was to study what property of the superinfecting phage enables it to induce lysis inhibition.

MATERIALS AND METHODS

The following bacterial and phage strains were used: *E. coli* B, T4D wild type, T4D r73 (Edgar, 1958), T5, P2, and P2 Hy Dis (Cohen, 1959).

The standard experiment for demonstrating lysis inhibition was performed as follows. The bacteria were grown in nutrient broth (Difco) with 5 g of NaCl added per liter to a density of 5×10^7 bacteria per milliliter. The cells were centrifuged and resuspended in 0.5 volume of fresh broth. The bacteria were then infected at a multiplicity of infection sufficient to infect all the bacteria with at least one phage particle (primary infection). After 5 min, the bacteria were diluted 1:5 into broth containing the superinfecting phage or into plain broth. The latter sample served as a control. In both primary and secondary infection, unadsorbed phage was measured by centrifuging a diluted sample of the infected bacteria and determining the amount of free phage in the supernatant fluid. At 5 min after superinfection, the bacteria were diluted into anti-T4 serum ($K = 5$) to inactivate free phage. The bacteria were then diluted at least 1,000-fold into the final growth tubes. At intervals, the growth tubes were assayed for infectious centers on *E. coli* B by the top-layer agar method (Adams, 1959). All experiments were performed at 33 C. From the experimental results were calculated (i) the time at which a burst size of 10 was reached (burst-rate index) and (ii) the burst size. These two parameters, together with determinations of multiplicity of infection, are sufficient to characterize lysis inhibition within each experiment (Rutberg and Rutberg, 1964).

Ultraviolet (UV) irradiation was performed with a Philips 15-w UV lamp. The phage was suspended in phosphate buffer (*p*H 7.0) during irradiation, and was kept in petri dishes which were gently rocked during the irradiation. One hit corresponds to 11 sec of irradiation at the distance used.

X ray-inactivated phage was prepared by suspending phage in 10-times strength broth and irradiating them in a Picker Hot Pot Co^{60} source. The dose rate was 5,000 r per min. One hit corresponded to 8 min of exposure, which is in good agreement with previous data (Watson, 1950).

Ghost particles of T4 were prepared from purified high-titer stocks as described by Herriot and Barlow (1957*a*). The shocked phages were treated with deoxyribonuclease and were then subjected to alternate cycles of high- and low-speed centrifugation. Further purification of the ghosts was undertaken by centrifugation in a CsCl solution with a mean density of 1.32 in a Spinco SW 39 rotor at 36,000 rev/min at 10 C for 20 hr. The main bulk of the ghosts was found in a sharp band close to the top of the centrifuge tube. The band was collected with a Pasteur pipette inserted from the top. The identity of the band was assured by electron microscopy. The electron micrographs showed only ghost particles; 25 to 50% of these had contracted tail sheaths. Fewer than 10^{-7} infectious particles were found in the CsCl preparation, as compared with 2% in the "crude" preparation.

Endolysin was assayed as described by Sekiguchi and Cohen (1964). One unit of endolysin (EU) is defined as the amount of lytic activity causing a decrease in turbidity of the substrate of one scale unit per minute. Turbidity readings were performed in a Unicam colorimeter (Sp. 1300) with filter no. 5.

RESULTS AND DISCUSSION

The effect of varying the multiplicity and time of superinfection was studied first. The prolongation of the latent period is dependent on the time of addition of the superinfecting phage (Table 1). The burst size of the superinfected bacteria, however, is constant and is independent of the time of superinfection. Thus, mere prolongation of the latent period is not sufficient for a continued synthesis of phage (Streisinger, 1963). Varying the multiplicity of superinfection from 3.5 to 75 had no effect on the results.

TABLE 1. *Effect of time of superinfection on lysis inhibition*

Time of superinfection (min after the primary infection)	Burst rate index	Burst size	Effective multiplicity of infection	
			Primary infection	Secondary infection
—	37	61	7.3	—
5	56	126	7.3	10
10*	59	125	7.3	19
15*	67	120	7.3	10
20*	69	118	7.3	10
25*	85	115	7.3	8

* At 5 min, 96% of the input phage had adsorbed, which gives a multiplicity of infection of unadsorbed phage at this time of 0.35. Thus, about 30% of the bacteria may become superinfected before the second addition of phage. This does not detract, however, from the main point of the experiment, that the prolongation of the latent period depends on the time of addition of superinfecting phage and that there is no correlation between the prolongation of the latent period and the increase of the burst size.

In Table 2 are summarized experiments in which T4 phages inactivated with UV or ionizing irradiation, as well as ghost particles, were used in the superinfection. The superinfecting phage can receive more than 300 hits of UV and still give lysis inhibition. X ray-inactivated phage retains its ability to induce lysis inhibition after 40 hits. The prolongation of the latent period is less with such phage, whereas the burst size is much increased. It thus seems very unlikely that lysis inhibition is dependent on the integrity of a particular gene in the superinfecting phage. It has been suggested that ionizing radiation induces breaks in deoxyribonucleic acid (DNA) molecules (Bacq and Alexander, 1955). Physical continuity of the DNA of the superinfecting phage may, then, not be necessary for lysis inhibition to occur. However, experiments performed to measure the amount of DNA injected by phage inactivated with ionizing irradiation have not given any conclusive information regarding this point. An effect on the bacterial cell wall by the superinfecting phage is not the essential reaction in establishing lysis inhibition, as ghost particles, which have retained their ability to kill sensitive bacteria, do not give lysis inhibition. Herriot and Barlow (1957*b*) obtained slight lysis inhibition

TABLE 2. *Superinfection with T4 phages inactivated by UV or ionizing radiation and with ghost particles**

Treatment of superinfecting phage	Hits	Effective MOI (primary)	Total MOI (secondary)	Burst-rate index		Burst size	
				Primary	Secondary	Primary	Secondary
UV	10	5.9	6.2	37	49	61	113
UV	72	4.3	16	42	60	56	70
UV	330	3.5	17	42	58	19	40
X ray	10	1.7	22	36	41	71	163
X ray	30	1.7	22	36	41	71	162
X ray	39	6.1	33	40	43	109	145
X ray	47	6.1	33	40	44	109	127
X ray	140	3.9	80	33	33	200	214
Ghost 1	—	9.2	62	40	39	114	124
Ghost 2	—	0.1	6.5†	35	34	32	38
Ghost 2	—	0.4	61	34	34	77	80

* The killing ability of T4 showed a sensitivity to the ionizing irradiation employed which was 1/6.6 of the sensitivity of the plaque-forming ability. This is roughly ½ the sensitivity reported by Watson (1950). The difference is probably due to the different sources of radiation employed. MOI is multiplicity of infection. Ghost 1 refers to ghosts not purified in CsCl; Ghost 2 refers to ghosts purified in CsCl.

† In this experiment, adsorption of the ghosts was measured as follows. The bacteria were primarily infected at low MOI, and the fraction of surviving bacteria was measured. The ghosts were then added in the superinfection. After 5 min of adsorption of the ghosts, the number of surviving bacteria was determined. From these results, the "killing" MOI was calculated. The figures for total MOI given for the other ghost experiments refer to "killing" MOI.

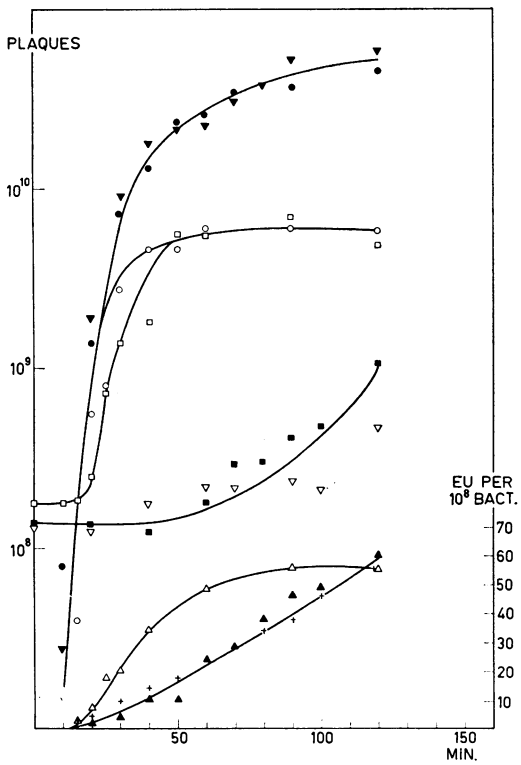

FIG. 1. *Escherichia coli B (B-6) was grown in broth at 33 C with aeration to a density of about 1.5 × 10^{8} bacteria per milliliter. The bacteria were then infected with phage T4 (effective multiplicity of infection was about 8 in all experiments). In the lysis-inhibited samples, the superinfecting phage (ordinary T4) was added after 15 min (multiplicity of infection was about 12 in all experiments). At intervals, the cultures were assayed for infectious centers. Samples were also diluted into chilled broth containing a few drops of chloroform, shaken, and stored in the cold until assayed for endolysin content. All assays were performed within 24 hr of the beginning of the experiment. The assays were performed with chloroform-treated B-6 as substrate as described in Materials and Methods. The chloroform-treated samples were also assayed for their content of plaque-formers. Symbols: □, infectious center control; ○, infectious center control after shaking with chloroform; △, lysozyme units control; ■ and ▽, infectious centers superinfected samples (the different symbols refer to different experiments), ● and ▼, infectious centers superinfected samples after shaking with chloroform; ▲ and +, lysozyme units superinfected samples.*

upon superinfection with ghosts. This effect was probably due to residual viable phage in the ghost preparation. Furthermore, X ray-inactivated phage, which has lost its killing ability, still retains its ability to give lysis inhibition upon superinfection (Table 2; Watson, 1950). Most likely, the superinfecting phage must be able to inject some component into the bacterium to give lysis inhibition. A number of experiments have been performed with phages T5, P2, or P2 Hy Dis as superinfecting phages, in bacteria primarily infected with T4 phage. A slight prolongation of the latent period was seen in most of these experiments, but lysis inhibition, as defined above, was never obtained. Thus, the ability of T-even phage to give lysis inhibition would either reside in the specific nature of the DNA of these phages or in some non-DNA material contained within the phage.

The endolysin content of lysis-inhibited and noninhibited bacteria was measured (Fig. 1). Endolysin activity appeared in the two samples at the same time, but the rate of increase of activity was slower in the superinfected sample. That the chloroform treatment employed breaks open the cells is evident from the curves for free phage in the chloroform-treated samples. Thus, superinfection interferes with endolysin production or activity, or both, in the infected bacteria.

Lysis inhibition with T-even phage is significantly more pronounced in a concentrated bacterial culture as compared with a diluted sample of the same culture (Doerman, 1948; see also Table 2 and Fig. 1). This is due to repeated superinfections occurring in the concentrated culture (Rutberg, *unpublished data*). In the diluted culture, the increase in the burst size is independent of the time of addition of the superinfecting phage, whereas the latent period is proportional to this time. This suggests that lysis inhibition is a composite of at least two events, prevention of lysis and continued synthesis of phage. Prevention of lysis is a necessary but not sufficient prerequisite for continued phage production (Streisinger, 1963).

It is suggested that, upon superinfection of T-even phage-infected bacteria with homologous phage, some compound is introduced into the bacteria which alters the course of infection for a limited period of time. In view of the present ideas on the timing and regulation of phage synthesis in infected bacteria (Champe, 1963), it is tempting to relate such an effect to the as yet hypothetical regulating substances which are thought to appear in the phage-infected cell (Sköld and Buchanan, 1964).

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