Early Abortive Lysis by Phage BF23 in *Escherichia coli* K-12 Carrying the Colicin Ib Factor

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Received for publication 10 June 1968

Growth of phage BF23 was restricted in *Escherichia coli* K-12 strains carrying a colicin I factor (ColIb); most infected cells lysed early without producing progeny phages. Either addition of chloramphenicol before phage infection or ultraviolet irradiation of phage prevented early abortive lysis, an indication that certain phage functions are required for this phenomenon. Very little or no phage-induced lysozyme was synthesized in the infected ColI⁺ cells. This result suggests that early abortive lysis was not due to the lysozyme action. A small fraction (0.05) of BF23-infected ColI⁺ cells showed normal phage growth. This "escaped growth" may reflect the physiological state of the host bacteria rather than the heterogeneity of the infecting phage. Host-controlled modification was not observed. A phage mutant, BF23hI, able to grow on ColI⁺ cells, was isolated and was characterized to be recessive to the wild-type BF23 in its ability to undergo early abortive lysis. Among the T series phages, T5 induced early abortive lysis, and growth of T5 was restricted upon infection to ColI⁺ cells. These results and the other observations, including the occurrence of phenotypic mixing between BF23 and T5, suggest that these two phages are related to each other even though the receptor sites for BF23 and T5 are apparently different.

Since BF23, a virulent coliphage, and colicins of E group (including E1, E2, and E3) have a common receptor site on *Escherichia coli* strains, this phage has been conveniently used to examine the presence of the receptor site for E group colicins (8). During the course of studies on resistance of *E. coli* to colicin E and I groups, we found that growth of BF23 is restricted in bacteria producing colicin Ib. A similar observation has been reported by Strobel and Nomura (19).

When infected with BF23, most cells carrying colicin Ib factor (ColIb) are not able to produce phage particles and lyse rapidly before attaining the normal latent period measured in the system of *E. coli* noncolicinogenic for colicin Ib. In this paper, this phenomenon will be called "early abortive lysis." Phage T5 causes early abortive lysis of the host cells colicinogenic for colicin Ib, and T5 is presumably related to BF23, since phenotypic mixing results from mixed infection of those two phages.

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MATERIALS AND METHODS

Bacteria. The bacterial strains used were derived from *E. coli* K-12 W3110 (F⁻λ⁻) kindly supplied by J.-I. Tomizawa, National Institute of Health of Japan. To avoid complications that might be caused by absorption of colicin Ib in some of the experiments, this strain was made resistant to colicin Ib and then made colicinogenic by introducing a ColIb factor from *Salmonella typhimurium* LT2 *cys36* (ColIb) whose ColI factor was originally carried by *Shigella sonnei* P9 (18). The *E. coli* strain W3110 (ColIb) ColI⁻ thus obtained will be referred to as ColI⁺; the parental strain *E. coli* W3110 ColI⁻ will be called ColI⁻. The original strain W3110, sensitive to colicin Ib, was used as an indicator strain to detect colicin production.

Phage. Phage BF23 was kindly supplied by P. Frédéricq, University of Liege, Belgium (7). Coliphages of T series (T1 to T7) were obtained from J.-I. Tomizawa's collection.

Media. Brain heart infusion broth (BHI) powder was obtained from Nissui Seiyaku, Tokyo, Japan. BHI contained 35 g of the powder per liter. Penassay broth (Difco) was also used. Nutrient agar for plating (pH 7) contained, per liter: 1.5% agar, 10 g of meat extract, 10 g of polypepton (Daigo-eiyo), and 1.5 g of NaCl. Nutrient soft agar for overlays contained 0.75% of agar per liter. Tris(hydroxymethyl)-aminomethane (Tris)-glucose (TG) medium was

prepared according to the method of Echols et al. (5).

Standard phage techniques as described by Adams (2) were used with certain modifications. Colicinogeny was tested according to the method of Frédéricq (9). The turbidity of bacterial cultures, judged by the absorbancy at 660 nm, was measured with a Hitachi S-34 colorimeter.

Lysozyme activity was assayed by the method of Minagawa (14), with certain modifications. The lysozyme substrate was prepared as follows. A young culture (2×10^8 cells per ml) of strain W3110 grown in BHI at 37 C was harvested and washed twice with 0.85% saline and was stored at -20 C; after 1 week, it was lyophilized (E. A. Terzaghi and A. Tsugita, *personal communication*). Lysozyme samples were made as follows. An aerated culture of W3110, grown to 5×10^8 cells per ml in BHI at 37 C, was harvested, suspended in TG medium, and then infected with phage at a multiplicity of about 10, followed by incubation with aeration at 37 C. At desired times, chloramphenicol (60 μ g/ml) and NaCl (0.3 M, final concentration) were added to 2-ml samples, which were then placed in an ice bath. The crude extract of lysozyme was prepared by sonic treatment of the samples with a 10 kc/sec sonic disintegrator (Kubota KMS200). The reaction mixture for the assay of the phage-induced lysozyme contained, in 1 ml: 0.01 M Tris (pH 7.4), 0.05 ml of the lysozyme preparation, 0.26 mg of the lysozyme substrate, and 0.05 M NaCl. The mixture was incubated at room temperature, and the decrease in optical density at 350 nm was followed for 10 min by using a Beckman DUR spectrophotometer with an automatic recorder (Gilford model 2000). Under these conditions, the activity of enzyme is directly proportional to the amounts of lysozyme preparation and to the time of incubation.

RESULTS

Growth of BF23 on ColI⁺ cells. When a ColI⁺ strain was cross-streaked with a large number of BF23 (e.g., a loopful of 10^{11} particles/ml) on an agar plate, growth of the cells was completely inhibited at the intersection of the streaks without significant production of the progeny phage. However, such inhibition was not observed when a small number of the phage (e.g., a loopful of less than 10^9 particles/ml) were employed. Consequently, under the latter conditions ColI⁺ and a strain lacking the receptor site for BF23 were not distinguished.

The efficiency of plating (EOP) of BF23 on ColI⁺ cells was determined to be about 10^{-6} , compared to that on ColI⁻ cells. These results confirmed the results obtained by Strobel and Nomura (19). Figure 1 shows the results of the one-step growth experiments of BF23 on ColI⁻ and ColI⁺ cells. At time-zero, the number of infective centers obtained with ColI⁺ cells was about 5% of that with ColI⁻ cells, although more

FIG. 1. One-step growth curves of BF23 in ColI⁺ and ColI⁻ host. Aerated culture of ColI⁺ and ColI⁻ grown to 5×10^8 cells per ml in BHI at 37 C, were first reversibly poisoned by the addition of KCN to a final concentration of 4×10^{-3} M. The cultures were then infected with phage BF23 at a multiplicity of 0.1. After 10 min for adsorption, they were washed once with ice-cold broth, diluted 10^{-4} in prewarmed broth (zero-time), and further incubated at 37 C. At intervals, samples were removed and, after appropriate dilution, plated on ColI⁻. Solid lines represent the extracellular growth curves, and broken lines represent the intracellular growth curves.

than 90% of the input phages were adsorbed to the respective strains. The latent period in ColI⁺ cells was 35 min, slightly less than that in ColI⁻ cells (40 min). The average burst size per infective center was 60 in ColI⁺ cells and 200 in ColI⁻ cells. These results indicate that BF23 could produce progeny phages, to some extent, only in a minor fraction of a population of infected ColI⁺ cells.

The phages produced on ColI⁺ cells in a one-step growth cycle, however, could not grow normally upon the reinfection to ColI⁺ cells, and showed the same characteristics as those of the phage grown on ColI⁻ cells. These results indicate that no host-controlled modification (3) seems to take place. Thus, this "escaped growth" of phages in ColI⁺ cells may reflect certain physiological conditions of the host bacteria rather than a heterogeneity in the phage population.

Although about 5% of the ColI⁺ bacteria produced progeny phages, it was probably not sufficient for BF23 to form plaques on a lawn of ColI⁺ cells. Plaques formed on such a lawn at EOP of 10^{-6} contained phages which could multiply equally well both on ColI⁻ and ColI⁺ cells. The property of these phages did not depend on the previous host, and the phages were genetically stable. This phenomenon, therefore, led us to conclude that they were mutants, which were designated as BF23hI. The existence of this type of mutant was also reported by Strobel and Nomura (19).

Early abortive lysis. Exponentially growing cells of ColI⁺ and ColI⁻ strains in BHI broth medium ceased to increase in turbidity immediately after the addition of BF23 (Fig. 2). This was followed by loss in turbidity of the ColI⁺ culture at 15 min and of the ColI⁻ culture at 45 min after infection of phage. Direct observation with the phase-contrast microscope revealed that the onset of loss of turbidity coincided with that of lysis of the infected cells, an indication that most BF23-infected ColI⁺ cells lysed early without producing progeny phages. Early lysis of BF23-infected ColI⁺ cells, however, did not take place when the cells were incubated in a spheroplast-stabilizing medium (BHI supplemented with 20% sucrose). Under these conditions, the fraction of infected cells which permitted phage growth did not increase. Therefore, early rupture of the cell envelope does not seem to be the principal cause of the restriction of the phage growth in ColI⁺ cells.

Requirement of phage functions. Chloramphenicol (60 μ g/ml), when added to ColI⁺ cells before BF23 infection, prevented early abortive lysis, but it had no effect when added 15 min or later after infection. BF23 phage irradiated by ultraviolet light to a survival fraction of 10^{-4} killed ColI⁺ cells but did not induce early abortive lysis. These facts indicate that early abortive lysis was not induced merely by phage adsorption but requires certain phage-induced functions.

Early abortive lysis also occurred 15 min after phage infection when ColI⁺ culture was

FIG. 2. Early abortive lysis of a ColI⁺ culture infected with phage BF23. ColI⁺ or ColI⁻, grown to 5×10^8 cells per ml in BHI at 37 C, was added at zero-time by phage BF23 at a multiplicity of about 10 phages per bacterium. The turbidity was then followed during incubation at 37 C.

mixedly infected with BF23 and mutant phage BF23hI (Fig. 3). Thus, it may be concluded that wild-type phage is dominant over the mutant in determining early abortive lysis.

Lysis without detectable phage lysozyme. Since both restriction of growth of BF23 and early abortive lysis of phage-infected cells were associated, it might be asked whether early abortive lysis was due to the synthesis of phage-induced lysozyme. Figure 4 shows the results of the kinetics of lysozyme synthesis in infected cells. In ColI⁻ cells, lysozyme activity was first detected at 15 min after infection and shortly afterward increased at a linear rate for about 40 min. In contrast, synthesis of lysozyme in ColI⁺ cells was only a few per cent of that in the ColI⁻ cells. The small amount of lysozyme detected in infected ColI⁺ cells may reflect escaped growth in a few per cent of the cells. These observations suggest that early abortive lysis was not due to the action of the phage-induced lysozyme.

BF23 is a related phage to T5. The experiments described in the previous sections were mostly carried out by use of BHI medium. When Penassay broth was used without the addition of CaCl₂ (10^{-3} M), BF23 was unable to grow even on ColI⁻ cells and was unable to induce early abortive lysis in ColI⁺ cells. Under these experimental

FIG. 3. Dominance of wild-type BF23 over BF23hI in ability to give rise to early abortive lysis. ColI⁺ cells, grown to 5×10^8 cells per ml in BHI, were singly added at zero-time by either BF23 or BF23hI at a multiplicity of 10 or simultaneously added by both phages at a multiplicity of 10 each. Turbidity measurements were made during incubation at 37 C.

FIG. 4. Synthesis of phage lysozyme in BF23-infected cells. An aerated culture of ColI⁺ or ColI⁻, grown to 5×10^8 per ml in 40 ml of BHI, was harvested, suspended in the same amount of TG medium, and infected by phage BF23 at a phage-bacterium ratio of 10, followed by incubation at 37 C with aeration. At intervals, 2-ml portions were removed and were used for measuring both the turbidity and the phage-induced lysozyme activity. Broken lines represent turbidity of cultures, and solid lines represent lysozyme activity.

conditions, adsorption of the phage to the host cells was normal. The requirement of CaCl₂ for the growth of BF23 suggested that this phage might have properties similar to T5 (1, 10, 12, 13). Thus, several experiments were carried out in the T5 system and are summarized as follows. Growth of T5 was restricted on ColI⁺ cells, and EOP of T5 on ColI⁺ cells was about 10^{-6} compared to that on ColI⁺ cells. T5 was also able to induce early abortive lysis in ColI⁺ cells. Furthermore, when BF23 and T5 were simultaneously infected to ColI⁻ cells, about 15% of the progeny formed had the phenotype of one parent and the genotype of the other (Table 1). This result may be explained by the phenotypic mixing between BF23 and T5. However, it should be noted that the receptor site of *E. coli* for BF23 is apparently different from that of T5, which is common to the receptor site for the colicin E group. The other T series phages were neither restricted by ColI⁺ cells in their multiplication nor did they induce early abortive lysis.

DISCUSSION

When BF23 were infected to ColI⁺ cells, growth of the phage was restricted, and the most infected cells started to lyse during the first 15 min. This phenomenon was named "early abor-

TABLE 1. Phenotypic mixing between phages BF23 and T5^a

Indicator strain	No. of plaque-forming units/ml	Phage types able to produce plaques ^b
W3110	4.2×10^7	(BF23)BF23 and (T5)BF23
W3110 BF23-r	6.3×10^6	(T5)BF23
W3110 T5-r	3.6×10^7	(BF23)BF23

^a A phage lysate was obtained after infecting W3110 (grown in BHI to 5×10^8 cells per ml at 37 C) simultaneously with phages BF23 and T5, each at an input phage-bacterium ratio of 10. The lysate obtained was added to W3110 T5-r (grown in BHI at 37 C to 5×10^8 cells per ml and then poisoned reversibly by the addition of KCN at a final concentration of 4×10^{-3} M), at an input phage-bacterium ratio of 0.2. After 10 min of adsorption at 37 C, the culture was washed twice with cold BHI and, after appropriate dilution, was plated on three different indicators: W3110, W3110 BF23-r, and W3110 T5-r. The resulting plaques were counted after overnight incubation at 37 C. Under these conditions, the number of free phages measured after chloroform treatment was 10^4 .

^b (T5)BF23 indicates a phage particle having the genome of phage T5 and the host range adsorption specificity of phage BF23.

tive lysis" in this paper. One-step growth experiments showed that about 5% of the infected ColI⁺ cells were capable of producing progeny phages whose properties were similar to that of phages grown on ColI⁻ cells. EOP on ColI⁺ strain was about 10⁻⁶ compared to that on ColI⁻ cells. This low EOP may be explained by the assumption that the fraction of the cells producing the progeny phages would be decreased during the process of reinfection to form plaques on the lawn of the cells.

Strobel and Nomura (19) have reported that the injection of BF23 deoxyribonucleic acid (DNA) into ColI⁺ cells is normal and that the injected phage DNA is not degraded into acid-soluble components. Under these conditions, no phage DNA was newly synthesized. They have also shown that BF23 degrades the host cell DNA at the same extent in both ColI⁻ and ColI⁺ cells. We confirmed this observation. Infection in the presence of chloramphenicol or by heavily ultraviolet-irradiated phages did not induce early lysis of ColI⁺ cells. These observations, collectively taken into account, suggest that certain phage functions are required for the expression of early lysis.

A kinetic study of synthesis of phage-induced lysozyme in BF23-infected ColI⁺ showed that very little lysozyme was detectable. This fact may exclude the possibility that early lysis is due to the synthesis of unusually large amounts of the enzyme at the early stage of the infection process. However, our data cannot completely rule out the possibility that if lysozyme produced by phage is really responsible for early lysis, the cell wall of phage-infected ColI⁺ cells must be so modified as to be more susceptible to the action of lysozyme. Consequently, lower amounts of lysozyme are still sufficient to produce osmotic fragility of the cells. This possibility may be examined when a BF23 mutant lacking lysozyme is isolated. Conversely, early lysis can also be explained in terms of phage-induced changes in the host cell surface. An early change in the surface of infected cells with T2 and T4 has been demonstrated, and in this case, protein synthesis is required (17). Such change may also occur as a normal process of BF23 infection, not only in ColI⁺ cells but also in ColI⁻ cells, and leads to cell lysis unless a certain function(s) is expressed to restore such a change. If the restoring function(s) is blocked in ColI⁺ cells, infection of BF23 to the cells would lead to lysis at an earlier stage of the infection process. However, many other explanations are also possible at present; thus, further experiments are needed to clarify the mechanism.

Although early lysis of the BF-23-infected ColI⁺ cells is a phenomenon associated with the

restriction of growth of phage, it is not a principal mechanism of the restriction of phage growth since infected ColI⁺ cells do not permit phage growth even under conditions preventing early lysis. The fact of the absence of host-controlled modification indicates that the restriction mechanism operated in this system may be different from that demonstrated in the system of phage λ -*E. coli* K-12 lysogenic for phage P1 (3, 4). On the other hand, it is of value to note that the restriction of BF23 on ColI⁺ cells may be analogous to that of T4rII mutants on K-12 lysogenic for phage λ . In the case of the latter, it has been suggested that the immunity substance of λ represses growth of the T4rII mutants (20). Similarly, we have assumed that a certain repressor(s) of ColIb factor specifically interacts with BF23. In this regard, the existence of a genetic regulation system in colicinogenic bacteria, comparable to that of temperate phages, has been pointed out (15, 16). In a population of ColI⁺ bacteria, a small fraction (0.05) of cells permit growth of BF23. This level of escaped repression or "transmission" is somewhat higher than that of T4rII in λ lysogens (0.001 to 0.005). Thus, one may speculate that the transmission coefficients reflect the strictness of the repression system of the replicon involved. In autonomous replicons, self-repression may be somewhat loose, fluctuating according to the stage of the cellular cycle of the host. In temperate phages, however, complete repression may operate full-time in the lysogenic conditions. Since the expression of a certain function(s) of BF23 is required for early abortive lysis, the repression of the phage by the ColI factor cannot be complete.

Although the receptor sites on the host cells for T5 and BF23 are different from each other, several lines of evidence indicate that these two phages have many common properties: that is, early abortive lysis upon infection to ColI⁺ cells; requirement of Ca⁺⁺ for the growth; and the existence of phenotypic mixing between both phages. Furthermore, electron microscopic observation of BF23 has revealed that this phage is very similar in its morphology to T5 (*unpublished data*). A large number of temperature-sensitive and amber mutants of T5 have been isolated and characterized genetically to some extent (6, 11). Thus, application of these mutants will give more promising results for the eventual understanding of the mechanism of the early abortive lysis.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the Jane Coffin Child Memorial Fund for Medical Research (Project no. 188).

We are deeply indebted to Junichi Tomizawa and

David Shub, National Institute of Health of Japan, for their advice and critical discussions, and also to Teiji Minagawa, Kyoto University, for his aid in the measurement of lysozyme activity. We express our gratitude to Royston C. Clowes, Southwest Center for Advanced Studies, Dallas, Tex., for critical reading of the manuscript and to Humihiko Ono, Tokyo Metropolitan University, for his encouragement.

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