Molec. gen. Genet. 147, 195–202 (1976)

MGG

Growth of Coliphage BF23 on Rough Strains of *Salmonella typhimurium*: The *bfe* Locus

T. Mojica-a* and E. Garcia**
Cell Biochemistry, Radiobiology Department, C.E.N./S.C.K. 2400 Mol, Belgium

Summary. Coliphage BF23 develops in *Salmonella typhimurium* rough strains. The phage is neither restricted nor modified by *S. typhimurium*. The growth patterns of the phage were slightly different in *S. typhimurium* than in *Escherichia coli*, although phage propagated on *S. typhimurium* is identical to the phage propagated in *E. coli* by several criteria used. Mutants of *S. typhimurium* resistant to BF23 were isolated and found to map (by P22- and Pl-mediated transduction) in the same position as *bfe* mutants of *E. coli*. The order of genes was: *metB* – *argC* – *bfe* – *rif* – *purD* – *metA*.

Phage BF23 does not form plaques on smooth *S. typhimurium* strains, since the phage fails to adsorb irreversibly to smooth cells. Nevertheless, on solid agar, the phage prevents growth of many (but not all) smooth strains. Moreover, UV- and alkali-inactivated phage BF23, although unable to form plaques on sensitive hosts, retains the ability to prevent growth of the host on solid medium. This ability is sensitive to protease and resistant to DNAse and RNase. Heat treatment of the phage causes rapid loss of the cell-growth-preventing-ability whereas the ability to form plaques is lost much more slowly. These results lead to a proposal that phage BF23 virions carry a colicin-like factor that kills sensitive cells.

Introduction

Bacteriophage BF23 was isolated by Fredericq (1949) who used it to detect *Escherichia coli* mutants resistant to colicins of group E. Sensitivity of *E. coli* strains to phage BF23 and to colicins E_1, E_2 and E_3 requires the expression of the bfe^+ locus, that maps at min. 77.5 (Buxton, 1971; Jasper, et al., 1972), close to the *argCBH* cluster on the *E. coli* genetic map (Taylor and Trotter, 1972).

Bacteriophage BF23 is closely related to phage T5 in a number of characteristics: they have similar gross morphology and physiological patterns of infection, they undergo phenotypic mixing and genetic recombination with one another and both are restricted in their growth in cells of *E. coli* that carry colicinogenic factor Ib (see Mizobuchi et al., 1971).

The host range of bacteriophages is normally limited to a single bacterial species or at most to very few related species. This limitation is the result of one or both of two general barriers: an adsorption barrier (the phage fails to recognize adsorption sites on the cell surface), and a second barrier that interferes with normal phage development. The latter barrier can be expressed at several levels and/or in several fashions. For example, a very common barrier is that of nuclease-mediated restriction (see: Arber, 1974 for review).

Since the barriers can, at least in theory, be circumvented by mutation (and/or recombination) either of the bacterium and/or of the phage, systems with extended host range can be useful in the study of certain problems of host-phage relationships.

Several systems of host range extension have been reported (e.g. Baron, et al., 1970; Gemski, et al., 1972; Ornellas and Stocker, 1974) and used for particular purposes (Colson and Colson, 1972; Enomoto and Stocker, 1974; Mojica-a, 1975).

The present report deals with the behavior of phage BF23 in *Salmonella typhimurium* strains.

Materials and Methods

Bacterial Strains

The *S. typhimurium* strains used in this study were derivatives of strain LT2 and are described in Table 1. The following *E. coli* strains were used: K175 (Scott, 1968) was kindly supplied by Dr.

* Dr. T. Mojica-a, Department of Biological Sciences, Hunter College, 695 Park Avenue, New York, N.Y.
** Dr. E. Garcia, Instituto "Jaime Ferran" de Microbiologia, C.S.I.C., Joaquin Costa 32, Madrid-6, Spain

Table 1. *S. typhimurium* strains

Strain	Description	Reference/Source
LT2	wild type	B.N. Ames
SL3684	*galE503 hut*$^+$	Ornellas and Stocker (1974)
TB206	*thi A thy*	M. Bagdasarian
MA70	*metA metE trpB val str-r* $r_{LT}^- m_{LT}^+ r_S^- m_S^+$	Strain 4420 of Colson and Colson (1972)
73	MA70 *galE1585*	(A)
135	*ilvA38 purD55 galE1597*	(A)
176	TB206 *galE1601*	(A)
203	TB206/F110	(B)
209	MA176/F110	(B)
210	*metB92*	K. Sanderson
211	MA210 *galE1602*	(A)
214	*argC133* (deletion)	K. Sanderson
217	MA176 *bfe2*	(C)
222	MA217/F110	(B)
227	SL3684 *bfe4*	(C)
236	MA210 *bfe3 rif751 galE1605*	(A), (C), (D)
254	*metA222 trpE2 hisF1009 strA201 xyl-1 ilvA99 pyrE231 malB galE1603*	(A), From strain SA572 of K. Sanderson
264	MA214 *galE1604*	(A)

(A) Isolated as FO resistant as previously described (Mojica-a, 1975)
(B) Transfer of episome F110 with KLF10 as donor
(C) Isolated as resistant to phage BF23 (see: Materials and Methods)
(D) Isolated as resistant to rifampicin (see: Materials and Methods)

J.R. Scott and was used as the indicator strain for phages BF23 and P1. CM21 ($r_k^- m_k^-$) was strain 921 described by Arber and Wauters-Willems (1970), generously supplied by Prof. W. Arber. CM45 was a *bfe* derivative of CM21, isolated as resistant to phage BF23 and colicin E2 simultaneously. Strain KLF10 (Low, 1972) used as the source of F-prime 110 was generously sent by Dr. B. Bachmann. Strains K53, CA42 and 185 m4 (E3)b/23, that carry col E_1, col E_2 and col E_3 factors, respectively, were received from Prof. P. Fredericq and used as sources of colicins E_1, E_2 and E_3.

Phage Strains

Bacteriophages F0, P22 and P1CM *clr-100* were described previously (Mojica-a, 1975). Bacteriophage BF23 was from the collection of Dr. M. Mergeay. All phage strains were passed three times through single plaque on the proper host strain before preparing high-titer lysates.

Media

LB broth (LB) contained, per liter of distilled water, 10 g Difco Bacto Tryptone, 5 g Difco Yeast Extract, 5 g NaCl, and was adjusted to pH 7.0 with 1 M NaOH. LA and LSA were LB with 1.5% and 0.6% Difco Bacto Agar, respectively. LArif was LA with 50 μg/ml of rifampicin (Sigma). Minimal salts medium was described previously (Mojica-a, 1975).

Construction of Strains

S. typhimurium strains with mutations in the *galE* gene (isolated as resistant to phage F0) are sensitive to and unable to grow on galactose, sensitive to phages C21 and P1 and resistant to phages F0 and P22; the phage sensitivity phenotype is partially reversible in the presence of galactose and glucose (Mojica-a, 1975). Strains resistant to rifampicin (*rif*) were isolated on LArif plates and strains resistant to BF23 (*bfe*) were obtained as survivors to BF23 propagated on strain K175. The frequency of spontaneous *rif* and *bfe* mutants was of the order of 10^{-7}.

Episome F110 was transferred from *E. coli* to *S. typhimurium*, or vice versa, by mixing donor and recipient (*ratio 1:4*) in minimal salts medium supplemented with donor and recipient requirements and with 50 μg/ml L-methionine. Under these conditions the yield of F-ductants was at least 100-fold higher than in LB or without methionine. Intraspecific transfer of F110 was done by cross-streaking donor and recipient strains on selective plates.

Episome F110 (Low, 1972) mobilizes the *E. coli* chromosomal fragment from *metA*$^+$ to *rha*$^+$ (min 80 – min 77). In many crosses the *malB* locus (and other chromosomal markers) was not transferred. Thus this episome had lost the *malB* locus (Low, 1972). Markers immediately counterclockwise of *rha*$^+$ were not tested.

Phage Methods

In general, phage methods used in this study were as described by Eisenstark (1967). One-step-growth experiments were performed as described by Mizobuchi and McCorquodale (1974), except that the medium used was LB.

Phage sensitivity of bacterial strains was tested by spotting a drop of high-titer lysate (at least 10^9 phage particles) on a lawn of bacteria on LA plates.

Titers of BF23 were determined by mixing 0.2 ml of indicator grown to log phase with 0.1 ml of $CaCl_2$ (5×10^{-3}M) and 0.1 ml of phage dilution (in LB broth). Mixture was left at room temperature for 30 min and overlaid with 2.5 ml LSA.

Phage BF23 was UV-inactivated in 10 ml volumes, placed in plastic Petri dishes, with a Quartzlampen Ges Hanau germicidal lamp at a distance of 40 cm. At suitable time intervals, samples were removed and titered on K175 (survival) and spotted on bacterial lawns to check for the ability to prevent bacterial growth on solid medium.

Sensitivity to colicins E_1, E_2 and E_3 was tested by spotting a drop of cell-free colicin preparation on the test strain.

Transduction Crosses

Phage P22- and P1-mediated transduction crosses were performed as described previously (Mojica-a, 1975). The *bfe* marker was always unselected, and was tested by suspending recombinant colonies on a drop of sterile saline and cross-streaking with phage BF23 propagated on K175. The *rif* marker was usually on the donor, since it was observed that *rif* strains are poorly transducible.

Purification and Equilibrium Density Gradient Centrifugation of Phage

High-titer lysates of BF23 were purified by differential centrifugation followed by two bandings in CsCl preformed density gradients

(Thomas and Abelson, 1967). Equilibrium density gradient centrifugation of whole phage was done at 35,000 rpm for 18 hr in preformed gradients in a swinging bucket rotor.

Determination of the Buoyant Density of Phage DNA

^{3}H-Thymine labelled (Shinozawa, 1973) phenol extracted DNA (Thomas and Abelson, 1967) from purified phage BF23 was centrifuged to equilibrim in CsCl and Cs_2SO_4 as described by Szybalski and Szybalski (1971). Radioactivity was counted in a Packard Scintillation counter.

Results

S. typhimurium Strains are Sensitive to Phage BF23 and Unable to Support Phage Growth

In the course of experiments involving deletion formation of F-primes it was observed that *S. typhimurium* strains bearing F110 were sensitive to phage BF23. When the wild-type (LT2) as well as several other *S. typhimurium* strains were spot-tested, they were also found to be sensitive to the phage (Table 2). This sensitivity was lost by mutation at the *bfe* locus (see below, mapping), and was restored by introduction of F110 from *E. coli* to resistant cells (e.g. strain MA222). Thus, the *E. coli bfe*$^+$ locus is expressed in *S. typhimurium*. The same strains were tested for sensitivity to colicins E_1, E_2 and E_3 (not shown), but they were found to be not sensitive, under conditions where the corresponding *E. coli* strains were sensitive.

Some *arg* strains of *S. typhimurium* were found to be naturally resistant to BF23. Transduction to *arg*$^+$ with phage P22 propagated on strain LT2 restored the BF23 sensitivity. Thus, it is possible that the resistant strains were double mutants.

When phage BF23 was assayed on strain LT2 by a standard method, it came as a surprise that, although the strain was sensitive to the phage, it did not allow plaque formation. The efficiency of plating was less than 10^{-9} (Table 2). Moreover, all smooth *S. typhimurium* strains were found to be unable to support growth of BF23, even when the *E. coli bfe*$^+$ locus was present on the F110 episome (e.g. strain MA203). A distinct possibility was that phage BF23 was very efficiently restricted in *S. typhimurium*. This was however not the case, since strain MA70, deficient in the two known restriction systems of *S. typhimurium* (Colson and Colson, 1972), was also sensitive to BF23 and unable to support phage growth.

Table 2. Sensitivity of S. *typhimurium* and *E. coli* strains to phage BF23

Strain	Relevant phenotype	Lysis[a] by: BF23 or BF23-B_1 or BF23-B_5	Efficiency of plating of		
			BF23[c]	BF23-B_1	BF23-B_5
S. typhimurium					
LT2	smooth	+	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$
SL3684	galE[b]	+	0.6	1.0	0.2
MA227	galE bfe	−	$<10^{-10}$	$<10^{-10}$	$<10^{-10}$
TB206	smooth	+	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$
MA203	smooth/F110	+	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$
MA176	galE	+	0.8	1.1	0.5
MA209	galE/F110	+	0.7	0.9	0.8
MA217	galE bfe	−	$<10^{-10}$	$<10^{-10}$	$<10^{-10}$
MA222	galE bfe/F110	+	0.6	1.2	0.4
E. coli					
K175		+	1.0	0.9	1.0
CM21	r_k^- m_k^-	+	1.8	1.5	0.6
CM45	r_k^- m_k^- bfe	−	$<10^{-10}$	$<10^{-10}$	$<10^{-10}$

[a] + Lysis; − no visible lysis. BF23-B_1 was derived by cloning BF23 on SL3684. BF23-B_5 was derived by cloning BF23-B_1 on K175

[b] Strains with mutations in the *galE* gene are phenotypically rough

[c] BF23 was always propagated on strain K175

Phage BF23 Plates on "rough" S. typhimurium Strains

Mutations in the *galE* gene make *S. typhimurium* strains phenotypically rough (Ornellas and Stocker, 1974; Mojica-a, 1975). As seen in Table 2, strains carrying mutations in the *galE* gene were able to support growth of BF23 with efficiencies nearing those in *E. coli*. Thus, BF23 is not restricted by *S. typhimurium*. This conclusion is strengthened by the observation that BF23 and two derivative phage stocks (BF23-B_1 obtained by propagating BF23 on strain SL3684 and BF23-B_5 obtained by propagating BF23-B_1 on K175) plated with similar efficiencies on *E. coli* strains and *S. typhimurium galE* derivatives (Table 2).

Since phage stocks BF23-B_1 and BF23-B_5 showed the same properties as BF23, it was concluded that successive passages of BF23 through *S. typhimurium* and *E. coli* did not alter the biological properties of the phage.

S. typhimurium mutants resistant to phage P22 are rough (Wilkinson, Gemski and Stocker, 1972). We tested 199 independently isolated phage P22-resistant mutants of strain LT2 and all supported growth of BF23 with efficiencies ranging from 0.1 to 1.2. Thus, other *S. typhimurium* rough mutants, in addition to *galE*, are able to support growth of BF23.

Phage BF23 Kills Sensitive Cells by Two Different Mechanisms

The behavior of BF23 on smooth and rough *S. typhimurium* strains described above raised two questions; namely: what is the biological barrier that prevents development of BF23 in sensitive smooth strains and what is the nature of the growth-preventing activity of BF23 detectable on sensitive smooth strains? An adsorption kinetics experiment (Fig. 1) answered the first question; BF23 does not adsorb to *S. typhimurium* smooth cells. On the other hand, BF23 adsorbs very efficiently to K175 (rate of adsorption 5×10^{-10} for m.o.i. 1.0) and more slowly, and less efficiently, to SL3684 (rate of adsorption 1.4×10^{-10} for m.o.i. 1.0). Adsorption at other m.o.i. gave similar results.

Adsorption of BF23 was repeated using ^{3}H-labelled phage. Radioactivity remaining in the supernatant as well as TCA-insoluble radioactivity present inside the cells was measured. Results of these experiments (not shown) were identical to those shown in Figure 1. Namely, with strain LT2 roughly 100% of the label was found in the supernatant while with strain K175; the amount of free label decreased very rapidly.

Thus, BF23 does not develop in *S. typhimurium* smooth strains due to an adsorption barrier.

The nature of the growth-preventing effect was then investigated by infecting liquid cultures of cells in exponential growth phase. Three host strains were infected with BF23, previously propagated on K175,

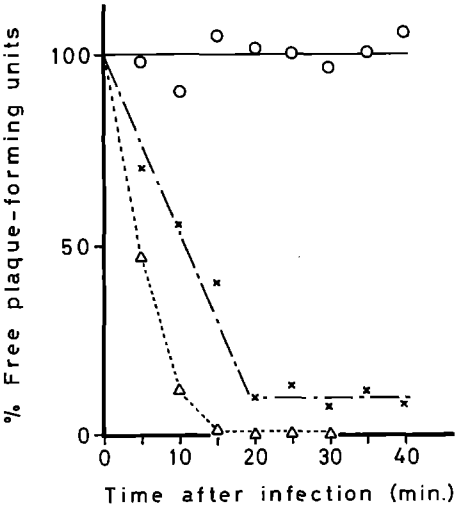

Fig. 1. Kinetics of adsorption of BF23 to bacterial cells. Exponentially growing cells were infected with about 0.5 phage particles per cell in the presence of 25 μg/ml of Chloramphenicol. At the indicated times, aliquots were removed, spun down in the cold and the supernatant titered on K175. The bacterial hosts were: *S. typhimurium* LT2 (0); SL3684 (X) and K175 (△)

at m.o.i. of 1.0, 10 and 100. The results of this experiment are shown in Figure 2.

Growth of *S. typhimurium* strain LT2 as measured by increasing $O.D._{660}$ was not affected by BF23 regardless of the m.o.i. used (Fig. 2A). This was rather surprising since the same cultures were sensitive to BF23 by spot tests. The viability (colony forming ability) of the culture as compared to the uninfected control forty minutes after infection was reduced only very slightly: 5–7% at m.o.i. 1.0; 15–25% at m.o.i. 10; and 40–50% at m.o.i. 100. Such small reductions would have little or no effect on the O.D. of the culture. Phage BF23 (as detected on K175) was not released and in all experiments nearly 100% of phage input was recovered from the cultures. The effect of phage BF23 on the viability of LT2 was more pronounced (about 2-fold greater) in static cultures than in shaken cultures. Thus, it seems clear that BF23 can affect *S. typhimurium* smooth strains in liquid medium, although the effect is not as dramatic as that seen on solid plates. We do not understand the reason for this difference.

Results shown in Figure 2B indicate that BF23 affects the growth patterns of SL3684 at the three m.o.i. tested, although the effects were not as dramatic as those on K175 (Fig. 2C). At m.o.i. of 1.0 lysis of SL3684 was only partial and began very late after infection (viability was reduced by more than 70%), while lysis of K175 was more widespread and began sooner (viability was reduced by at least 80%). At m.o.i. of 10, lysis of SL3684 began relatively soon after infection but was very gradual; in contrast, lysis of K175 took place very rapidly (in both cases viability was reduced by more than 97%). At m.o.i. of 100, lysis of SL3684 began soon after infection and the decrease of O.D. was very sharp; on the other hand, K175 stopped growing almost immediately (within 2 minutes) and the culture remained static for nearly 2 h before lysis started. This bacteriostatic effect was probably due to effects of the phage from without. Phage production began concomitantly with lysis, both in K175 and in SL3684 (except for m.o.i. 100). The total number of viable phage particles released decreased remarkably with increase in m.o.i. At m.o.i. 100 the increase in titer was 2–4 fold, at m.o.i. 10 the increase in phage titer was about 12-fold, while at m.o.i. 1.0 the increase in phage titer was of the order of 100-fold. At high m.o.i. (10 and 100) possibly fewer cells are able to release phage; the rest of the cells are probably rendered inviable (and unable to release phage) by effects from without. Differences in phage adsorption between SL3684 and K175 (Fig. 1) might account partially for the differences recorded in Fig. 2. On the other hand, as shown below (see One-step-growth of BF23) there might be

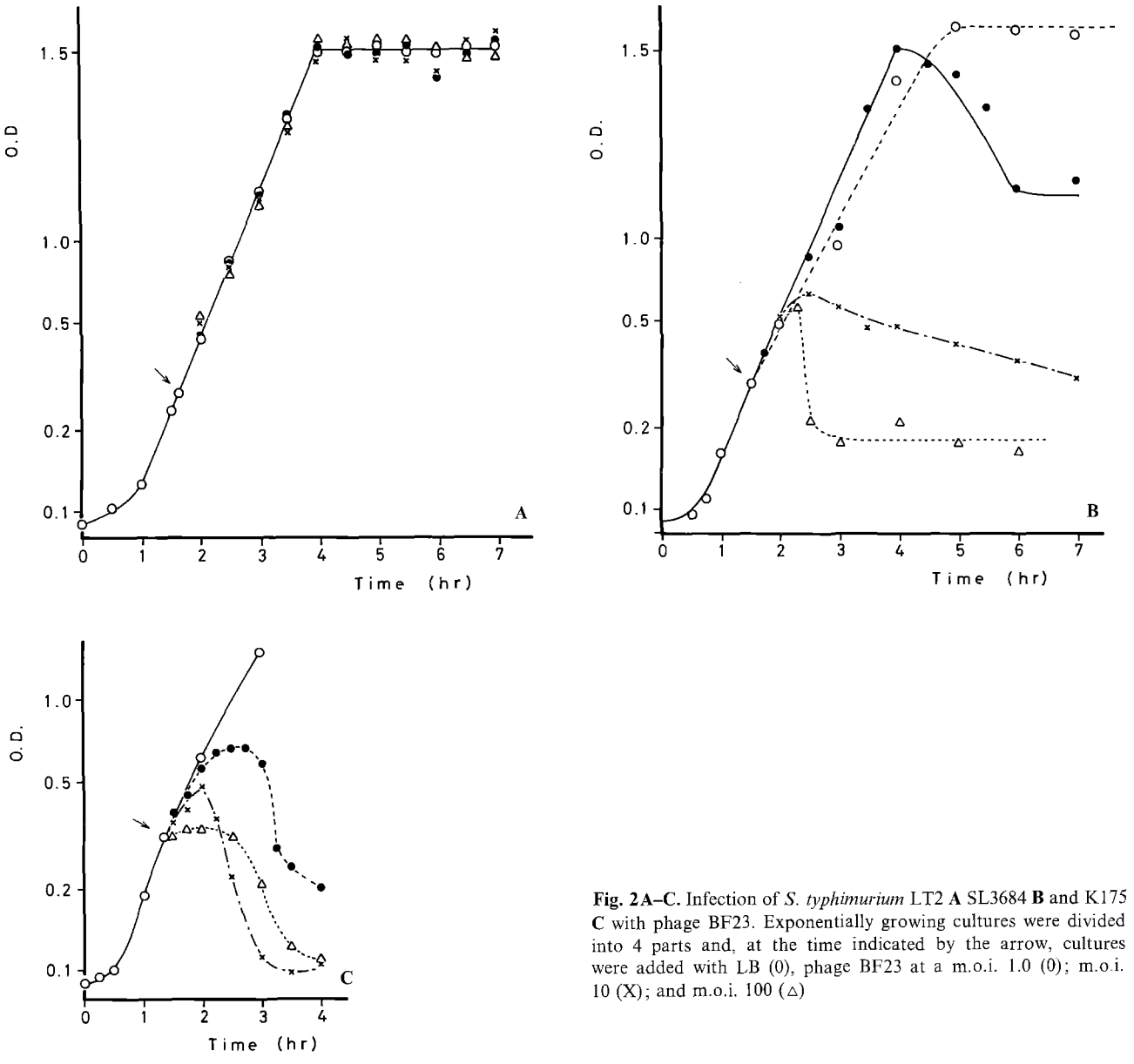

Fig. 2A–C. Infection of *S. typhimurium* LT2 **A** SL3684 **B** and K175 **C** with phage BF23. Exponentially growing cultures were divided into 4 parts and, at the time indicated by the arrow, cultures were added with LB (0), phage BF23 at a m.o.i. 1.0 (0); m.o.i. 10 (X); and m.o.i. 100 (△)

some inherent differences between the two bacterial hosts. The experiments described in Fig. 2A strengthened the conclusion that BF23 does not adsorb to *S. typhimurium* LT2 and thus the sensitivity of the cells is not the results of lytic growth but rather it is an effect from without that does not inactivate the phage and does not seem to lyse the bacteria.

High-titer BF23 lysates (4×10^{11} plaque forming units (p.f.u./ml) on K175 were UV-irradiated to 5×10^{-10} survival (2×10^{2} p.f.u./ml). At various times of irradiation, samples were removed and a) titered on K175, b) spotted on bacterial lawns (bacterial strains of Table 2) and c) heated (at 80 °C) for 60 sec and immediately spotted on bacterial lawns. All *bfe*$^+$ strains, whether smooth or rough were sensitive to UV-inactivated phage, at all stages of the inactivation experiment, and the spots were similar to those produced by the nonirradiated phage. The phage samples that were UV-irradiated and heated failed to lyse the lawns of *S. typhimurium* smooth strains. The first 2 samples (4×10^{-2} and 1.6×10^{-4} survival) heated did lyse rough strains (K175, SL3684, etc.). Moreover a high-titer phage lysate was heated at 98 °C for varied lengths of time. After 60 seconds, the titer dropped from 4×10^{11} p.f.u./ml to 10^{8} p.f.u./ml. A sample of this treated phage was able to lyse only rough strains (K175, SL3684, etc.) but not smooth strains. An estimation of the amount of phage re-

quired to lyse a lawn indicated that a least 5×10^7 p.f.u. are necessary to produce visible lysis of strain LT2, while 10^4 p.f.u. produced complete lysis of a lawn of K175. Lower amounts of phage resulted in individual plaques.

Although the mechanism by which the growth of *S. typhimurium* smooth strains is prevented was not clarified by these experiments, it seemed clear that BF23 was able to exert two different activities on sensitive cells. One was lytic growth and the second one was exerted from without and readily detectable with smooth cells. This second activity is possibly carried out by a protein(s) on the phage. Further evidence that a proteins(s) is involved comes from the observation that high titer purified phage degraded with 2M NaOH to final 0.2 M NaOH, as described by Baylor and Roslansky (1970), loses completely the plaque forming ability but retains the ability to kill sensitive cells. This activity is resistant to DNase and RNase and sensitive to protease. A conventional assay revealed no lysozyme activity, although the sensitivity of our assay was very low (at least 0.2 μg/ml pure egg white lysozyme were required.). Attempts to separate the killing activity from the phage by a variety of methods (e.g. column chromatography) were unsuccessful.

The experiments described strongly suggested that a protein factor(s) is involved in the growth-preventing activity.

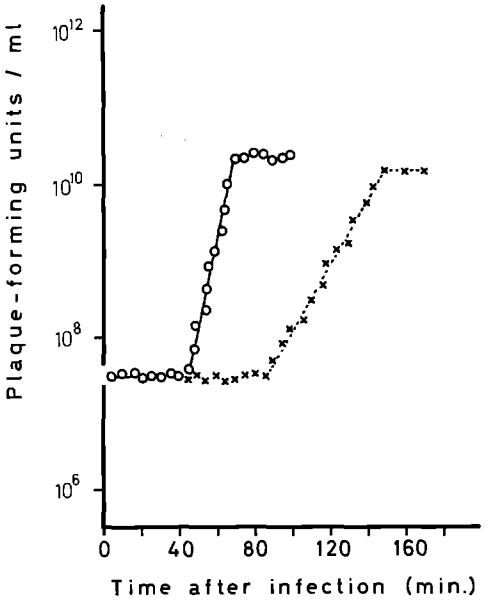

Fig. 3. One-step-growth curves of BF23 in K175 (0) and SL3684 (X). The m.o.i.'s were approximately 0.1. The average burst sizes were 145 in K175 and 130 in SL3684

One-Step Growth Curves of B. 23

Because of the differences in the effect of BF23 on growing cells of SL3684 and K175 (Figs. 2B and 2C), it seemed important to understand the physiology of BF23 infection in *galE* strains of *S. typhimurium;* consequently a one-step growth experiment on strain SL3684 was performed (Fig. 3). The results of the one-step growth experiment of BF23 on K175 closely resembled those reported in another *E. coli* strain (Mizobuchi and McCorquodale, 1974). Two differences between the *S. typhimurium* and *E. coli* host are evident from Figure 3: (a) the eclipse period in the *S. typhimurium* host was almost twice as long (85 min versus 45 min) as that on the *E. coli* host. (b) The bursting period was also longer in SL3684 (60 min) than in K175 (about 25 min); the expansion in the bursting period was probably due to less efficient phage adsorption. Despite the differences in eclipse period, the average burst sizes were very similar, however: 130 in SL3684 and 145 in K175.

Phage BF23 Propagated in S. typhimurium is Identical to the Phage Propagated in E. coli.

The differences observed in the behaviour of BF23 in *S. typhimurium* with respect to *E. coli* could be due to metabolic differences of the hosts, that would result in different phage progeny. Accordingly, both BF23 and BF23-B_1 were analyzed by a variety of techniques:

a) The buoyant density of the phage DNA in CsCl ($\rho = 1.700$ gr/ml), was identical for both phages.

b) The buoyant density of the whole phage in CsCl density gradients ($\rho = 1.536$ gr/ml) was also identical for both phages.

c) Phage BF23 DNA does not contain unusual bases, that could be absent (or made at slower rates) in *S. typhimurium,* since the buoyant density ($\rho = 1.423$ gr/ml) in Cs_2SO_4 density gradients, corresponded to the same G+C content as determined by CsCl density gradient centrifugation (Szybalski and Szybalski, 1971).

d) Anti-BF23 antiserum inactivated BF23 and BF23-B_1 with essentially identical neutralization constants ($k = 251$ for BF23; $k = 257$ for BF23-B_1), thus indicating that the two phage stocks are immunologically identical.

e) Electron microscopic observations indicated no detectable differences between the two phage stocks (C. Ascaso, personal communication).

It is concluded that if there are any differences between the two phage stocks, these cannot be detected with the techniques employed.

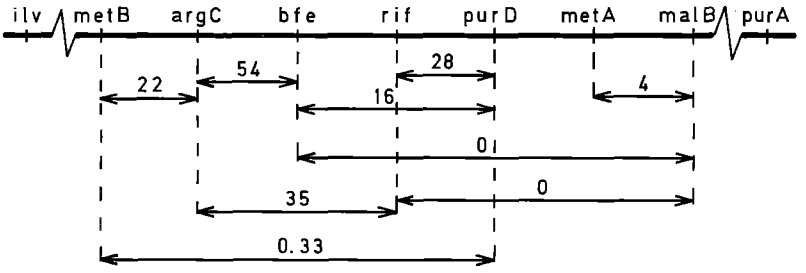

Fig. 4. Summary of P1-mediated transduction data in the *metB-argC-bfe-purD* region of the *S. typhimurium* linkage group. The numbers indicate frequencies of cotransduction. A number zero means < 1/600

The bfe Locus of S. typhimurium has the Same Chromosomal Location as the bfe Locus of E. coli

In order to map the *bfe* locus in *S. typhimurium* a series of P1-mediated transduction crosses were performed (Fig. 4). The *bfe* locus was found to be 54% cotransducible with *argC* and 16% cotransducible with *purD*. Three-point transduction crosses indicated that the most likely order of genes was: *metB, argC, bfe, rif, purD, metA*. The *bfe* marker in those crosses was obtained in strains with mutations in the *galE* gene, able to support phage growth. The possibility that BF23 recognizes a different site on the cell surface of smooth strains was considered. This was not the case; since 100 independently isolated BF23-resistant mutants of strain MA214 (*argC133*, smooth; unable to support phage growth) were transduced to $argC^+$ with P22; 50 recombinants were analyzed from each cross. In all cases linkage *argC-bfe* was found. Some arg^- smooth strains were found to be "naturally" resistant to BF23, these strains could be transduced to arg^+ and to bfe^+ with characteristic frequencies by P22 prepared on strain LT2.

Thus, mutants of smooth strains whose growth is not prevented by BF23 map in the *bfe* locus, indicating that normal infection as well as the growth-preventing activity recognize the same site on the cell surface; namely, the product of the bfe^+ gene.

Discussion

The observations described in this paper can be summarized as follows: i) *S. typhimurium* smooth strains are sensitive to phage BF23 but are unable to support phage growth since the phage fails to adsorb to smooth cells. ii) Phage BF23 adsorbs to an develops in *S. typhimurium* strains with mutations in the *galE* gene (rough). iii) Phage BF23 is neither restricted nor modified by either *E. coli* K12 or *S. typhimurium* LT2. iv) UV-inactivated phage BF23 is able to kill sensitive cells; heat inactivated phage losses this killing ability very rapidly, however. v) Mutations that render *S. typhimurium* (smooth and rough) resistant to phage BF23 map in the same position as the *bfe* mutations of *E. coli* and thus are called *bfe*.

The aspects of the behaviour of phage BF23 are accounted for if phage BF23 virions contain two different activities: one is lytic growth and the other, recognizable with smooth cells or with UV-inactivated phage, acts from without. Both mechanisms recognize the product of the bfe^+ gene. The second mechanism is probably due to a protein factor. A strong possibility is that the second mechanism is identical or very similar to the activity of T-even phage ghosts recently reviewed by Duckworth (1970). On the other hand, its is possible that "whole" ghosts are not required for activity either in BF23 or in T-even phages but that a colicin-like factor exists on the virion that would account for the second growth-preventing activity. Preliminary attempts to separate this killing factor from virions have been unsuccessful.

The BF23 receptor on the cell-surface has not been chemically defined. The receptor for T5 (a phage very similar to BF23: Mizobuchi et al., 1971) seems to reside in the lipoprotein layer (Weidel, 1958; cited by Lindberg, 1973). It is possible that the BF23 receptor lies in the same layer. The observations that mutants (*galE*) lacking the O-specific side chain and part of the core polysaccharide (see Lindberg, 1973 for review) are able to adsorb the phage do not necessarily mean that the receptor is on the lipopolysaccharide. This notion conflicts somewhat with a recent proposal (Bhattacharyya et al., 1970), but is consistent with the observation that mutations in the *bfe* locus do not affect the sensitivity to several smooth and rough specific *Salmonella* phages. Should the receptor be on the lipopolysaccharide, a further assumption would be required, namely, the product of the *bfe* gene is recognized only by the growth-preventing factor, in which case this factor would be absolutely required for phage adsorption.

It is rather surprising that BF23 is not restricted by *S. typhimurium* since the similar phage T7 is sepa-

rated from *S. typhimurium* by a dual barrier, an adsorption barrier and a restriction barrier (Brunovskis and Burns, 1973). Lack of restriction of BF23 means that the only barrier that separates BF23 from *S. typhimurium* is in adsorption. It is possible, however, that host gene product(s) contribute to normal BF23 development in *E. coli* K12 and that this gene product(s) is absent from *S. typhimurium*. If this is the case, this hypothetical gene product(s) is not absolutely required. Alternatively, *S. typhimurium* gene product(s) "retards" the development of BF23.

Recently, mutants of *E. coli* unable to support multiplication of BF23 (Shinozawa, 1973) have been described. The product of this gene could either be absent from or different in *S. typhimurium*, such a difference would explain the differences in behaviour of BF23 reported here.

Acknowledgments. We are very grateful to: Prof. L. Ledoux for his generous hospitality. Drs. C. Godard, M. Mergeay and R.B. Middleton for their critical comments on the manuscript. Prof. W. Arber, P. Fredericq and B. Bachman for sending bacterial strains. Dr. C. Ascaso for communicating freely electron microscopic observations. J. Gerits for indispensable technical assistance and P. Charles for his help with the Cs_2SO_4 centrifugations.

References

Arber, W.: DNA modification and restriction. Progr. Nucl. Acid Res. Mol. Biol. **14**, 1–37 (1974)

Arber, W., Wauters-Willems, D.: Host specificity of DNA produced by *Escherichia coli* XII. The two restriction and modification systems of strain 15T. Molec. gen. Genet. **108**, 203–217 (1970)

Baron, L.S., Penido, E., Ryman, I.R., Falkow, S.: Behavior of coliphage lambda in hydrids between *Escherichia coli* and *Salmonella*. J. Bact. **102**, 221–233 (1970)

Baylor, M.B., Roslansky, P.F.: Gel electrophoresis of the head proteins of T-even phage. Virology **40**, 251–259 (1970)

Bhattacharyya, P., Wendt, L., Whitney, E., Silver, S.: Colicin-tolerant mutants of *Escherichia coli:* Resistance of membranes to colicin E1. Science **168**, 998–1000 (1970)

Brunovskis, I., Burns, R.O.: Growth of coliphage T7 in *Samonella typhimurium*. J. Virol. **11**, 621–629 (1973)

Buxton, R.S.: Genetic analysis of *Escherichia coli* K12 mutants resistant to bacteriophage BF23 and the E-group colicins. Molec. gen. Genet. **113**, 154–156 (1971)

Colson, A.M., Colson, C.: Expression of the *Escherichia coli* K, B and P1 DNA host specificities in *Salmonella typhimurium*. J. gen. Microbiol. **70**, 123–128 (1972)

Duckworth, D.H.: Biological activity of bacteriophage ghosts and "take-over" of host functions by bacteriophage. Bact. Rev. **34**, 344–363 (1970)

Eisenstark, A.: Bacteriophage techniques, pp. 449–524. In: Methods in virology, Vol. 1, ed. by K. Maramorosch and H. Koprowski, New York: Academic Press 1967

Enomoto, M., Stocker, B.A.D.: Transduction by phage Plkc in *Salmonella typhimurium*. Virology **60**, 503–514 (1974)

Fredericq, P.: Sur la resistance croisee entre colicine E et bacteriophage II. C. R. Soc. Biol. **143**, 1011–1013 (1949)

Gemski, P., Jr., Alexeichik, J.A., Baron, L.S.: Behavior of coliphage lambda in *Shigella flexneri* 2a. J. Virol. **19**, 668–674 (1972)

Jasper, P., Whitney, E., Silver, S.: Genetic locus determining resistance to phage BF23 and colicins E1, E2 and E3 in *Escherichia coli*. Genet. Res. (Camb.) **19**, 305–312 (1972)

Lindberg, A.A.: Bacteriophage receptors. Ann. Rev. Microbiol. **27**, 205–241 (1973)

Low, K.B.: *Escherichia coli* K12 F-prime factors, old and new. Bact. Rev. **36**, 587–607 (1972)

Mizobuchi, K., McCorquodale, D.J.: Abortive infection by bacteriophage BF23 due to colicin Ib factor. II. Involvement or prearly proteins. J. molec. Biol. **85**, 67–74 (1974)

Mizobuchi, K., Anderson, G.C., McCorquodale, D.J.: Abortive infection by bacteriophage BF23 due to the colicin Ib factor: I: Genetic studies of nonrestricted and amber mutants of bacteriophage BF23. Genetics **68**, 323–340 (1971)

Mojica-a, T.: Transduction by phage P1CM *clr-100* in *Salmonella typhimurium*. Molec. gen. Genet. **138**, 113–126 (1975)

Ornellas, E.P., Stocker, B.A.D.: Relation of lipopolysaccharide character to P1 sensitivity in *Salmonella typhimurium*. Virology **60**, 491–502 (1974)

Scott, J.R.: Genetic studies of bacteriophage P1. Virology **36**, 564–574 (1968)

Shinozawa, T.: A mutant of *Escherichia coli* K12 unable to support the multiplicacion of bacteriophage BF23. Virology **54**, 427–440 (1973)

Smith, H.O., Levine, M.: A phage P22 controlling integration of prophage. Virology **31**, 297–216 (1967)

Strobel, M., Nomura, M.: Restriction of the growth of bacteriophage BF23 by a colicin I (Col I-Pa) factor. Virology **28**, 763–764 (1966)

Szybalski, W., Szybalski, E.H.: Equilibrium density gradient centrifugation. In: G.L. Cantoni and D.R.Davies (eds.), Procedures in nucleic acids research, Vol. 2, p. 311–354. New York: Harper and Row 1971

Taylor, A.L., Trotter, C.D.: Linkage map of *Escherichia coli* strain K12. Bact. Rev. **36**, 504–524 (1972)

Thomas, C.A., Abelson, J.: The isolation and characterization of DNA from bacteriophage. In: G.L. Catoni and D.R. Davies (eds.) Procedures in nucleic acid research, Vol. 1, pp. 553–561. New York: Harper and Row 1967

Wilkinson, R.G., Gemski, P., Stocker, B.A.D.: Non-smooth mutants of *salmonella typhimurium:* Differentiation by phage sensitivity and genetic mapping. J. gen. Microbiol. **70**, 527–554 (1972)

Communicated by W. Arber

Received January 28, 1976/Revised April 13, 1976