Abortive Infection of *Shigella dysenteriae* P2 by T2 Bacteriophage

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We have investigated some of the biochemical events that accompany the abortive infection by T2 of *Shigella dysenteriae* lysogenized with the temperate phage P2. After infection with T2, protein and RNA synthesis continued for 3 to 5 min. The virus-induced enzyme, deoxycytidylate hydroxymethylase was produced in reduced amounts (15% of normal), and the extent of deoxyribonucleic acid (DNA) synthesis was 0.1% of that found with a nonlysogenic strain. Measurements of the production of acid-soluble fragments and sedimentation analyses failed to detect enzymatic degradation of the infecting viral DNA which could be specifically related to the presence of the prophage P2. Each interaction between T2 and a bacterium resulted in the death of the cell. This observation is consistent with results obtained with other types of bacteria which show that only when a nucleolytic attack occurs on T2 DNA does the cell have an increased capacity to survive after adsorption of T2.

When *Shigella dysenteriae* is lysogenized with P2 phage, it is unable to support infection by T-even phage (4, 18), although the virulent phage adsorbs to and kills the lysogen (18). It is not understood what factors interfere with the infectious process and the purpose of the present paper is to describe the intracellular events occurring when SH(P2) is infected with T2 phage.

Abortive infections of enteric bacteria by T-even phage have been reported for only a few other systems. Abortive infections occur when *Escherichia coli* strain K-12 (λ) is infected by T4rII (3), when *E. coli* B is infected by non-glucosylated phage (13, 24), and when *E. coli* W is infected by T2 (6, 19, 25). For each system, the disruption of phage deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis occurs at a characteristic stage in the replication cycle. In the case of K-12 (λ) infected with T4rII, synthesis continues for 12 min with the formation of a normal complement of early enzymes, some DNA, and a low level of the late protein, lysozyme (11, 23). In the case of nonglucosylated phage restriction, the disruption of phage polymer synthesis occurs earlier than in rII restriction. Only approximately 10 to 20% of the normal level of early enzymes was synthesized, and no phage DNA was made (14, 15). In this case, approximately 50% of the parental DNA was degraded to acid-soluble fragments (9, 14), and it was observed that the nonglucosylated phage did not kill *E. coli* B as efficiently as normal

phage (15). The infection of strain W by T2 is characterized by an abrupt, early cessation of all phage macromolecule synthesis (25), and two nucleolytic activities which degrade the infecting phage DNA. Initially, there was a rapid degradation of 30 to 50% of the phage DNA molecules to acid-soluble fragments, followed by a double-stranded endonuclease attack on the remaining intact molecules (25). It was also found that T2 did not kill the bacteria with the same efficiency as it killed a permissive host (25).

A comparison of events occurring after T2 infection of SH(P2) with events occurring after infection of *E. coli* strain W is particularly relevant, since recent work has shown that strain W carries a prophage which is morphologically (21) and antigenically related to P2 (12) and which is responsible in part for the restriction of T2 by strain W (21). Therefore, a description of the events occurring after SH(P2) infection by T2 was undertaken with the hope that correlations with other abortive T-phage infections would result in some insight into the mechanism by which prophages interfere with virulent phage infection.

MATERIALS AND METHODS

Bacterial strains, growth conditions and phage preparations. *Shigella dysenteriae* strains 15 and 36 were those previously used by Lederberg (18) and were reobtained from Erich Six. Strain 36 is lysogenic for the temperate bacteriophage P2 and is referred to in

this paper as SH(P2). Strain 15 is nonlysogenic. Bacteria were grown in Brain Heart Infusion (BHI) broth (Difco) with aeration at 37 C. Growth was measured in a Klett colorimeter fitted with a 660 filter. Viable counts were performed on BHI plates solidified with 1.5% agar. Standard techniques were used for the preparation of phage stocks and the assay of T2 (1).

When chloramphenicol was present, it was added 2 min before phage infection at 100 μ g/ml. The antibiotic was removed from the culture by centrifugation and resuspension in fresh medium.

Acid solubilization of the parental phage DNA. The preparation of radioactive T2 and the measurement of acid solubilization were performed as described previously (25).

Enzyme assays. Cells grown to 3.5×10^8 cells/ml were infected with three phages per cell. Samples of the infected culture were collected for enzyme assays 30 min after phage infection or 30 min after removal of the chloramphenicol. For the nonlysogenic strain SH, 10-ml samples were taken; for SH(P2), 50-ml samples were taken. The bacterial cultures were chilled and centrifuged, and the bacteria were suspended in 1 ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris), pH 7.4, containing 0.01 M dithiothreitol. The cells were sonically treated for 10 sec in a sonic oscillator (MSE.) Deoxycytidylate hydroxymethylase (dHMP) was assayed by the method of Pizer and Cohen (20). One unit of enzyme activity equals 1 nmole of dHMP formed under standard assay conditions.

Isotope incorporation data. The conversion of isotopically labeled precursors into an acid-precipitable form was followed by pipetting 0.1-ml portions of infected culture to 2.4-cm filter paper discs (Whatman no. 3 MM) suspended on steel pins (5). The discs were immediately immersed in a beaker of cold 5% trichloroacetic acid. After all samples were collected, they were washed twice with 5% trichloroacetic acid and once with acetone, dried in air, and counted by the method described by Frankel (8).

Because labeling of DNA with radioactive thymine was impractical with these strains, DNA synthesis was measured by adding ^3H -uracil at a concentration of 5 $\mu\text{C}/\text{ml}$, 4 min after infection. Samples (1 ml) were removed into 1 ml 10% trichloroacetic acid and the precipitate was collected by centrifugation. The pellet was washed with ether, dissolved in 3 ml of 0.5 M KOH, and incubated at 37 C for 16 hr to hydrolyze RNA. The solution was acidified with 0.1 ml of 10 N HCl, and then 50% trichloroacetic acid was added to give a final concentration of 5%. The samples were filtered through membrane filters (Millipore Corp., Bedford, Mass.), washed, and counted in a liquid scintillation counter.

Preparation of extracts and sucrose gradient analysis of lysates. Bacteria were grown to about 3×10^8 cells per ml and infected at the indicated multiplicities with ^{32}P -labeled T2. At the desired times 2-ml portions of the infected culture were chilled, centrifuged, and resuspended in 1 ml of lysing medium. The lysing procedure and the sucrose gradient centrifugation analyses were performed as previously described (25).

RESULTS

The first series of experiments compare the biochemical events following infection of permissive nonlysogenic *Shigella* with the events occurring following SH(P2) infection. The purpose of this comparison was to determine at what point in the infectious process restriction occurred.

Synthesis of phage DNA. To compare DNA synthesis by infected SH with the synthesis by infected SH(P2), ^3H -uracil was added to each culture 4 min after infection. After hydrolyzing the RNA, we determined the kinetics of incorporation of ^3H -uracil into acid precipitable material (Fig. 1). It was concluded from this experiment that the radioactive pyrimidine incorporated into DNA after infection of SH(P2) was less than 0.3% of that incorporated by SH under comparable conditions of infection. It was considered probable that the small amount of DNA synthesis by infected SH(P2) occurred in those cells which yielded phage (0.1%). This experiment did not exclude the possibility that some unlabeled phage DNA was synthesized from internal DNA precursor pools made available by the breakdown of host DNA.

Synthesis of early enzymes. Since little, if any, phage DNA was synthesized by infected SH(P2), it was of interest to determine whether any phage-directed functions occurred. The level of the phage enzyme dHMP was compared in infected SH and SH(P2). Extracts were prepared 30 min after phage infection, by which time protein synthesis had stopped (*see below*). It was found that infected SH(P2) made approximately 15% of the amount of this early enzyme

FIG. 1. Incorporation of ^3H -uracil into DNA. Bacteria at 4×10^8 cells per ml were infected with an input multiplicity of 5.5 phage per cell. The bacterial survivors were 0.5%. Symbols: ●, SH. △, SH(P2).

synthesized by infected SH (Table 1, column A). Since the dHMP, which is only synthesized after infection was found in infected SH(P2), we conclude that the phage DNA was injected and that the infected cell had the capacity to begin synthesis of viral proteins. An experiment was designed to test whether this limited capacity for synthesis would be lost if phage protein synthesis was delayed. When protein synthesis was blocked during the first 10 min of infection by the addition of chloramphenicol, the infected SH(P2) retained some capacity to make dHMP after the inhibitor was removed (Table 1, column B). Although there was a drop in the level of enzyme made after the cells were removed from the chloramphenicol, the per cent of decrease in final level was similar in infected SH and infected SH(P2; Table 1, column B). This experiment indicated that the limited capacity for viral enzyme synthesis by infected SH(P2) was not lost if protein synthesis was inhibited during the first 10 min of infection. Later in this paper the significance of this observation is related to a proposed mechanism of restriction.

Synthesis of macromolecules as measured by isotope incorporation. The previous experiments indicated that only a limited amount of functional phage protein was made in T2-infected SH(P2). One explanation for this observation was an early cessation of all phage macromolecule synthesis. To test this possibility, incorporation of isotope into macromolecules was measured. It was found that the incorporation of ^{14}C -leucine and ^3H -uracil into acid-precipitable material stopped abruptly early after infection. To compare the relative incorporation of the two compounds as a function of time, both were added to the same culture, and the data were expressed as a percentage of the maximum incorporation observed (Fig. 2). It can be seen that the incorporation of both isotopes ceased at approximately the same time. Since very little ^{14}C -uracil was incorporated

during a pulse given at 20 to 25 min after infection (Fig. 2), the plateau in net incorporation was not caused by turnover of the RNA. Fig. 2 shows that approximately 25% of the RNA was unstable, with the remainder being acid-precipitable for as long as 30 min after infection. The nature of this stable RNA is unclear; some of it may be host RNA. Under these conditions, isotope incorporation by infected SH was linear for at least 30 min. These studies on the biochemical events after T2 infection of SH(P2) indicate that there is an early cessation of all phage macromolecule syntheses.

Fate of the infecting phage DNA. Destruction of the infecting phage DNA was one explanation for the cessation of phage synthesis. To test this possibility, the kinetics of acid solubilization of ^{32}P -labeled phage DNA by infected SH and SH(P2) were compared. Figure 3 shows that the amount of phage DNA which was degraded to acid-soluble fragments was similar for the lysogenic and nonlysogenic strains.

It was possible, however, that the phage DNA was attacked by a nuclease present in SH(P2) which produced DNA fragments too large to be soluble in acid. To test this possibility, the infecting ^{32}P -labeled phage DNA was analyzed by sucrose gradient centrifugation. SH(P2) was infected with ^{32}P -labeled phage, centrifuged to remove unadsorbed phage, and then lysed at 7 and 15 min after infection. ^3H -thymidine-labeled marker phage DNA was added before lysis. The lysates were layered on neutral sucrose

TABLE 1. dCMP hydroxymethylase activity^a

Strain	Enzyme units/10 ml culture	
	A No chloramphenicol	B Chloramphenicol removed at 10 min after infection
SH.....	930	460
SH(P2).....	154	77

^a Preparation of infected cell extracts and the assay of enzyme activity has been detailed above. Extracts were prepared 30 min after infection or removal of the cells from chloramphenicol.

FIG. 2. Isotope incorporation into acid precipitable material by infected SH(P2). Relative incorporation of ^3H -uracil and ^{14}C -isoleucine into acid-precipitable material expressed as percentage of maximum incorporation. Bacteria at 5×10^8 per ml were infected with input multiplicity of 5 phage per cell. ^{14}C -isoleucine ($2 \mu\text{C}$ per ml) and ^3H -uracil ($5 \mu\text{C}$ per ml) were added immediately after infection. Samples (0.1 ml) were removed at the times indicated. 100% incorporation is ^3H -uracil = 404 counts/min. ^{14}C -isoleucine = 240 counts/min.

FIG. 3. Acid solubilization of T2 DNA. Breakdown of infecting phage DNA by strains SH and SH(P2). Bacteria were grown to a concentration of 3×10^8 per ml and infected with 3 phage per cell. ●, SH. ○, SH(P2). The dashed line shows the extent that T2 DNA was converted to acid-soluble fragments by *E. coli* W. The procedure used was the same as described previously (25).

gradients, and the sedimentation profile of the acid-precipitable radioactivity was determined. As late as 15 min after infection most of the infecting phage DNA sedimented like marker DNA (Fig. 4).

The infecting phage DNA was also characterized by denaturing the DNA and then sedimenting the separated strands in alkaline sucrose. This technique would reveal any single-stranded breaks which might be present in the DNA and which would not be apparent in the double-stranded structure. There were, however, difficulties in interpreting alkaline sedimentation experiments. Nucleases which normally appear after phage infection (16) could degrade the DNA. This difficulty was avoided by infecting the cells in the presence of chloramphenicol to prevent phage enzymes from being synthesized. A second complication, namely single stranded breaks due to isotope decay, was minimized by using the phage within 24 hr after it was prepared. Nevertheless, some fragmentation due to decay could not be avoided and it was necessary, in each experiment, to analyze a sample of the ^{32}P -labeled phage DNA. To perform the experiment, chloramphenicol was added to SH(P2) 2 min before the cells were infected with ^{32}P -labeled phage. The infected cells were centrifuged to remove unadsorbed phage and then lysed at 7 min after infection. Low specific activity ^3H -thymidine-labeled phage DNA ($4 \mu\text{C}/\mu\text{g}$) was added before centrifugation to mark the sedimentation position of normal strands. The same lysates were analyzed on alkaline and on neutral sucrose gradients. On neutral sucrose, the DNA sedimented like the marker DNA, as was the case

when chloramphenicol was not present. On alkaline sucrose, however, most of the ^{32}P -labeled DNA was fragmented, indicating that the double stranded structure contained single-stranded breaks (Fig. 5b). Figure 5a shows that relatively few of the single-stranded breaks were present in the phage prior to infection. When the nonlysogenized strain SH was analyzed in a similar manner, the infecting phage DNA showed a similar sedimentation pattern. The DNA sedimented like marker DNA on neutral sucrose, but was fragmented when analyzed on alkaline sucrose (Fig. 5c). These experiments indicate that some endonucleolytic attack on the infecting phage DNA was found; however, there was no detectable difference in nucleolytic attack by infected SH and infected SH(P2).

Efficiency of killing the host. The cessation of macromolecular synthesis soon after SH(P2) was

FIG. 4. Neutral sucrose gradient analyses. The culture of SH(P2) was grown to 3×10^8 cells per ml and infected with T2 at a multiplicity of infection of 1.9 phage per cell. ^{32}P -labeled parental DNA, ●; ^3H -labeled marker phage DNA, ○. (A) At 7 min after infection, per cent recovery ^3H = 112%, ^{32}P = 85%. (B) At 15 min after infection, per cent recovery ^3H = 98% and ^{32}P = 97%; (C) 30 min after infection, per cent recovery ^3H = 91%, ^{32}P = 82%.

FIG. 5. Alkaline sucrose gradient analyses. ^{32}P -labeled parental DNA ($\bullet$); ^3H -labeled marker phage DNA ($\circ$). (A) Phage alone, per cent recovery $^3\text{H} = 102\%$; $^{32}\text{P} = 80.1\%$; (B) 7 min lysate of infected SH(P2), per cent recovery, $^3\text{H} = 101\%$; $^{32}\text{P} = 94\%$; and (C) 7-min lysate of infected SH, per cent recovery, $^3\text{H} = 84\%$, $^{32}\text{P} = 80\%$.

infected with T2 was similar to the situation found when nonglucosylated phage infected *E. coli* B (9, 14, 15) and when T2 infected *E. coli* strain W (25). In the latter two cases it was also observed that the bacteria were not killed with the same efficiency as during a normal infection (15, 25). A "normal" infection occurs when each viral-bacterial interaction leads to the killing of the cell (1). The work of Lederberg (Ph.D. dissertation, University of Illinois, Urbana, 1955) indicated that the killing of SH(P2) by T2 was not

abnormal. We compared the number of surviving bacteria as a function of multiplicity of infection for SH(P2) and *E. coli* strains W and B. For SH(P2) and *E. coli* B, the killing followed the predictions of the Poisson distribution (1) indicating that each virus-cell interaction killed the bacterial cell (Fig. 6). These results indicate that SH(P2) infection differs from the restriction found when T2 infects *E. coli* W in the ability of the host to survive infection.

DISCUSSION

We have presented evidence that the abortive infection of SH(P2) by T2 is characterized by (i) an abrupt, early cessation of all phage macromolecular synthesis; (ii) no degradation of the infecting phage DNA which can be related specifically to restriction, and (iii) no increase in the capacity of the cells to survive infection. These observations may be compared with the characteristics of the abortive infection of *E. coli* strain W by T2. In both cases, there was an early cessation of macromolecular synthesis, and the restriction was dependent on the presence of prophages which are antigenically related (12). The systems differ in the extent to which the viral DNA is degraded. The result with SH(P2) demonstrates that restriction can occur in the absence of nuclease activity greater than that found after

FIG. 6. Bacteria surviving infection as a function of virus multiplicity. $\square$ Experimental points obtained with strain SH(P2), $\bullet$ Experimental points obtained with *E. coli* strain B, $\circ$ Experimental points obtained with *E. coli* strain W.

infection of SH. The presence of intact viral DNA with the cell after acid solubilization was completed made it unlikely that nucleases were the primary cause of the abortive infection of strain W (25). The present work, where a similar pattern of early restriction is found without nuclease activity, supports the view that the nucleases are not responsible for the restriction by strain W.

Another major difference between the two systems was the efficiency with which T2 killed the host. The curves describing the per cent survivors versus multiplicity of infection followed the prediction of the Poisson distribution for SH(P2), indicating that the bacteria were killed by a single interaction with the phage. Similar curves obtained with *E. coli* strain W indicated that this strain had an increased capacity to survive infection with T2. Two explanations for this increased survival were considered. (i) A phage function needed to efficiently kill the bacteria did not have a chance to appear during strain W infection; (ii) the rapid degradation of 30 to 50% of the infecting phage by strain W destroyed the killing ability of those phage. The normal killing of SH(P2) despite a very early cessation of phage macromolecule synthesis indicates that the second hypothesis is more likely. Additional correlations between nucleolytic activity and increased survival have been made with cured and relysogenized strains of *E. coli* W in an accompanying paper (26) and for infection of *E. coli* B by nonglucosylated T2 (15).

The data presented does not suggest a mechanism to explain how lysogenization with P2 establishes a restriction on T2. Fields (7) has also observed the early cessation of macromolecular synthesis after T2 infection of SH(P2) and has found experimental evidence that the permeability of the cell towards galactosides and adenosine triphosphate (ATP) is impaired. She hypothesized that damage to the cytoplasmic membrane leads to the cessation of macromolecule synthesis. There is evidence that virulent phage normally damage the cytoplasmic membrane while injecting their DNA but possess the capacity to repair this damage (2, 10, 22, 23). The presence of P2 as a prophage could result in an altered membrane which the infecting phage more readily damages or is unable to repair. As a consequence of a damaged membrane, the cell could leak metabolites or be unable to concentrate nutrients from the medium. In either case, as a consequence of depletion of substrates, macromolecular synthesis would soon stop. The observation that, in the presence of chloramphenicol, SH(P2) had not lost its capacity to synthesize deoxycytidylate

hydroxymethylase 10 min after infection, whereas protein synthesis, if allowed to occur, was finished by 5 min, suggests that the infected cell has a limited capacity for synthesis rather than a limited time in which syntheses can proceed. Since leakage of intracellular pools would have occurred during treatment with chloramphenicol, the observation that infected SH(P2) retained the capacity for macromolecule synthesis after the chloramphenicol treatment is some evidence against leakage of metabolites having occurred. The observation is more compatible with membrane damage affecting the capacity of the cell to concentrate nutrients. On the other hand, Field's data show that ATP leaks from SH(P2) after infection (7).

Finally, this work shows endonucleolytic attack on T2 DNA by the permissive strain SH when chloramphenicol is present throughout infection. This endonucleolytic attack does not occur when *E. coli* B is infected under similar conditions (17). Preliminary experiments indicate that infected SH will still produce phage if the chloramphenicol is removed 10 min after infection.

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