Inhibitory Effect of Bacteriophage P22 Infection on Host Cell Deoxyribonuclease Activity

VANCE ISRAEL,¹ MARY WOODWORTH-GUTAI, AND MYRON LEVINE

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan 48104

Received for publication 26 November 1971

~101?
✗→ Infection of *Salmonella typhimurium* with phage P22 causes a decrease in the activity of host deoxyribonuclease which degrades single-stranded deoxyribonucleic acid (DNA). This decrease is reversed when the infecting phage is P22c⁺; it is not reversed if the infecting phage kills the cell. The decrease does not occur in infections with P22ts25.1 (which only adsorbs and injects DNA) or in infections of a lysogen by a nonvirulent phage. It does occur, however, after infections with other phages which are blocked in phage DNA synthesis. Inhibiting protein synthesis with chloramphenicol does not in itself cause the decrease in uninfected cells, but it does prevent infected cells from showing this effect.

Infections of *Salmonella typhimurium* with phage P22 cause profound changes in the metabolism of the host cell. Bacterial deoxyribonucleic acid (DNA) synthesis is inhibited (11, 12), and the synthesis of a number of bacterial proteins is markedly decreased (5). The pattern of bacterial DNA and protein synthesis depends on the type of phage used for infection. If the invading phage is wild type (c⁺), DNA and protein synthesis recover from their inhibition. In such infections, the cells survive and give rise to lysogenic progeny. When the invading phage carries a c₁ or c₂ mutation, the inhibitions persist and the vegetative growth cycle of the phage is initiated. This eventually leads to the lysis of all the cells and the release of mature phage particles.

In this paper, we describe the effect of phage infection on the level of host cell deoxyribonuclease activity which is specific for single-stranded DNA. The level of this activity shows a characteristic pattern which is dependent on the type of phage used for infection.

MATERIALS AND METHODS

Bacterial and phage strains. Strain 18 is a derivative of *S. typhimurium* LT-2 which has been cured of the prophage PB1 (15). This strain was used as background indicator for plating and for all experiments requiring a sensitive nonlysogenic cell. Strain DB103 (kindly provided by David Botstein) is strain 18 lysogenic for P22siebint3. Cells carrying the prophage bearing sie mutations do not exclude superinfecting phage but are still immune to phage P22 (10, 14). The int mutation plays no direct role in the experiments

described here (13; the *L* mutation in this paper has been renamed *int*).

Wild-type c⁺ phage P22 and the clear mutant c₁ have been previously described (7). *Ts12.1* and *ts18.1* are conditional lethal mutations which block phage DNA synthesis at 39 C, although the DNA of infecting phage carrying these mutations enters the P22 replication complex normally (9). The replication complex, also known as intermediate I, is a rapidly sedimenting association of phage DNA and various cellular constituents (1). All phage DNA synthesis takes place in intermediate I. *Ts25.1* is a mutation which prevents the association of parental phage DNA with intermediate I at restrictive temperatures (8). *Ts25.1* mutants carry out no known phage functions other than adsorption and injection (Hoffman and Levine, unpublished data). *Erf* mutants grow normally in *rec*⁺ hosts but not in *rec*⁻ hosts (2). In *rec*⁻ hosts they enter the replication complex and can undergo one round of DNA replication. *Ts3.1* and *ts5.1* mutants are both deficient in a late function; both act as tail donors in the in vitro assembly assay (6). *Ts19.1* mutants grow normally but cannot lyse the host cell (Botstein et al., unpublished data). *Vx* is one of the two mutations necessary for the *virB* type of virulence (3). *VirB* phages carry two mutations, *Vx* and *K5*, and can grow in a lysogen carrying a *sie*⁻ prophage, whereas a nonvirulent phage cannot. *Vx* and *K5* phages are not themselves virulent.

Media. All cultures were grown in supplemented M9 (7).

Nuclease assay. This assay determines the amount of denatured ³H-DNA degraded by measuring the amount of ³H-thymidine made acid-soluble in a 30-min incubation period at 37 C. The reaction mixture (0.3 ml, except where otherwise indicated) contained 10 to 50 μ liters of sonically treated material, 0.2 μ g of denatured P22 ³H-DNA (about 10⁵ counts per min per μ g), MgCl₂ (0.01 M), and tris(hydroxymethyl)-

¹ Present address: Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Ga. 30902.

aminomethane (Tris)-hydrochloride, pH 9.0 (0.1 M). The reaction was stopped by the addition of 0.2 ml of cold calf thymus DNA (2.5 mg/ml) and 0.3 ml of cold 7% perchloric acid. This was centrifuged at 9,000 rev/min in a Sorvall SM-24 rotor, and the supernatant fluid was carefully poured off and saved. A 0.5-ml amount of the supernatant fluid was then counted with 15 ml of Bray's solution in a TriCarb liquid scintillation counter.

Preparation of labeled P22 DNA. Log-phase cells in supplemented M9 at a concentration of 4×10^8 cells/ml were infected at a multiplicity of 10 with P22c₁. At the time of infection, adenosine was added to a concentration of 200 μ g/ml, and ³H-thymidine (specific activity = 22.3 Ci/mmol) was added to a concentration of 20 μ Ci/ml. After lysis, bacterial debris was removed by low-speed centrifugation, and the phage were pelleted by centrifugation for 2 hr at $35,000 \times g$ in a Sorvall SS-34 rotor. After resuspension and another low-speed centrifugation, the phage were purified by CsCl density gradient centrifugation. The purified phage were disrupted by exposure to 1% Sarkosyl at 65 C for 5 min. The freed DNA was then precipitated with two volumes of 95% ethanol and suspended in $0.1 \times$ SSC (0.15 M NaCl and 0.015 M Na₃ citrate). The precipitation was repeated and the solution was brought to $1 \times$ SSC. DNA was denatured at a concentration of 20 μ g/ml by heating for 10 min in a boiling-water bath.

Preparation of sonically treated material. Cultures were started by diluting a fresh overnight culture 100-fold into M9CAA. The cells were grown with aeration to a concentration of 3×10^8 to 4×10^8 cells/ml and then infected with a multiplicity of 10 to 20 phage/cell. In the experiments involving temperature-sensitive mutants (Fig. 1, 3 and 4), 39 C was used as the nonpermissive temperature. The other experiments were performed at 37 C.

At various times before and after infection, 1 ml of the culture was pipetted rapidly into a 5-ml cellulose-nitrate tube and immersed in a dry ice-acetone bath. Each sample was individually thawed and then disrupted in a MSE sonic oscillator in two 30-sec bursts at about 1 amp in a salt water and ice bath.

When this sonically treated material is used in the nuclease assay, the amount of activity is linear with time for at least 1 hr (Fig. 7), and is roughly proportional to the amount of extract added over a limited range. The best response is found when the amount of extract used corresponds to about 1×10^7 to 2×10^7 cells. In the experiments shown in Fig. 2 to 5, the assays were done with both 20 μ liters and 50 μ liters, although only one is shown in each case. In all four experiments, the activity was approximately proportional to the amount of extract used.

RESULTS

Crude sonically treated material of *Salmonella typhimurium* contains nuclease activity which degrades single-stranded DNA. As shown in Fig. 1, this activity is proportional to the cell concentration through log phase and into standing phase of cell growth. If a culture is infected with phage

P22 at any time during log phase, there is a sharp drop in the amount of nuclease activity. No decrease in activity is seen when the infection is done during standing phase.

Figures 2 and 3 show the effect of infection on host activity in more detail. In Fig. 2, a wild-type, or c⁺, phage was used. When sensitive cells were infected with high multiplicities of P22c⁺, there was a delay in division of about one generation, but all the cells survived and produced lysogenic progeny. The nuclease activity increased slightly in the first 2 min after such an infection, but then fell off sharply, reaching a minimum between 5 and 10 min after infection. The amount of activity at this point is somewhat variable, ranging from 25 to 50% of the activity at the time of infection. Following this decrease, the activity rose rapidly until 60 min after infection when the levels of the infected and uninfected cultures were nearly equal. The inhibition of nuclease activity is thus reversible when the infecting phage is P22c⁺ and the infected cells survive.

If the infecting phage kill the cell, a different

FIG. 1. Effect of phage infection on nuclease activity against denatured DNA at various stages of cell growth. Strain 18 was grown as described in Methods. At cell concentrations of 3×10^7 , 4×10^8 , 2.5×10^9 , and 5×10^9 per ml, 5 ml-portions were infected with P22ts12.1c₁ at multiplicities of 27, 10, 6.4, and 6.7, respectively. The amount of sonically treated material assayed varied from 50 μ liters early in the experiments to 10 μ liters late in the experiments. The activity plotted is normalized to the expected value for 10 μ liters. (●) Nuclease activity from uninfected cells, (○) nuclease activity from infected cells, (◐) viable cell count.

FIG. 2. Effect of $P22c^+$ infection on nuclease activity against denatured DNA. The nonlysogenic strain 18 was used as host. Cells at a concentration of 3×10^8 per ml were infected at 0 minutes; multiplicity of infection = 25. Fifty microliters of sonically treated material was assayed.

FIG. 3. Effect of $P22ts12.1c_1$ infection on nuclease activity against denatured DNA. Multiplicity of infection = 20; other conditions as in Fig. 2.

pattern of nuclease activity is seen (Fig. 3). As in Fig. 2, there is a transient rise in activity followed by a sharp decrease. But in this case the activity increased only slightly after the initial drop. This late gradual rise is variable and is sometimes totally absent. Phages which fail to show the reversal of inhibition include those carrying the following mutations: c_1 , c_2 , and c_1 in combination with erf , $ts12.1$, $ts18.1$, $ts3.1$, $ts5.1$ and $ts19.1$. Presumably any other phage which kills the host will give a similar pattern. Since gene 12 and 18 functions are both necessary for DNA synthesis (9), it is clear that phage DNA synthesis is not required for this effect.

The decrease in nuclease activity is not seen in infections with $P22ts25.1c_1$ (Fig. 4). Gene 25 acts earlier than any other gene known for P22, and phage carrying a mutation in this gene perform no known functions other than adsorption and injection at high temperature.

If a lysogen is infected, the situation is quite similar to an infection of a sensitive cell with a $ts25.1$ mutant. Phage can adsorb and inject normally into the lysogen, but the DNA is repressed and cannot be replicated. The results shown in Fig. 5 demonstrate that the nuclease activity of the cell is not disturbed when a lysogen is infected by $P22c_1$. The drop seen after infection of the

FIG. 4. Effect of $P22ts25.1c_1$ infection on nuclease activity against denatured DNA. Multiplicity of infection = 11; other conditions as in Fig. 2.

FIG. 5. Effect of phage infection on nuclease activity against denatured DNA of a lysogen. The lysogenic strain DB103 was used as host; multiplicity of infection (MOI) of $P22c_1$ = 20; MOI of $P22Vx$ = 9; 20 μ liters of extract was assayed.

lysogen with P22Vx is a control to show that there is nothing wrong with the lysogenic cell.

The experiments with P22ts25.1 and the lysogen suggest that some phage function(s) must be expressed before the drop in nuclease activity can occur. They also rule out the possibility that the decrease results merely from increasing the amount of DNA in the extract.

One possible explanation for these results is that the nuclease (or nucleases) in question is unstable and that the decrease results simply from normal decay of activity following phage-induced inhibition of cellular protein synthesis. This idea was tested by inhibiting protein synthesis in infected and uninfected cells with chloramphenicol. As shown in Fig. 6, chloramphenicol at a concentration of 100 μ g/ml did not cause a decrease of nuclease activity in the uninfected culture, indicating that inhibition of protein synthesis is not sufficient to cause the decrease. However, the same concentration of chloramphenicol was sufficient to prevent the infected culture from showing

FIG. 6. Effect of chloramphenicol on nuclease activity against denatured DNA in infected and uninfected cells. Upper graph: an infection of strain 18 with P22c₁ under standard conditions. Multiplicity of infection = 10. Lower graph: conditions as in upper graph except that chloramphenicol was added to a concentration of 100 g/ml 2 min before infection.

the characteristic decrease. This suggests that some phage protein(s) must be synthesized if the drop is to occur.

All of the foregoing data are consistent with the idea that phage P22 codes for a protein which reversibly inactivates host nuclease activity. However, attempts to detect a soluble inhibitor with mixing experiments have been negative (Fig. 7). Mixing extracts from infected and uninfected cultures gives almost exactly the results expected if the activities were completely additive.

Although effecting an inhibition *in vitro* is not yet possible, it is quite simple to reverse the inhibition *in vitro* by dialyzing extracts overnight against Tris-ethylenediaminetetraacetic acid (EDTA) buffer. As shown in Table 1, dialysis

FIG. 7. Mixing experiment. Strain 18 was infected with P22c₁; multiplicity of infection = 10. Samples were removed for sonic treatment immediately prior to and 10 min after infection. In this experiment the reaction mixture was 1.5 ml. Each tube contained 0.5 ml of extract. One tube contained 0.5 ml of uninfected extract (●); a second contained 0.5 ml of infected extract (○); and a third contained 0.25 ml of each (□). From time to time 0.2-ml samples were removed from each tube and assayed for soluble radioactivity, as described in Methods. The fourth curve (△) represents the arithmetic mean of the count from tubes with infected and uninfected extract.

TABLE 1. *Effect of dialysis on nuclease activity^a*

Determinations	Nuclease activity (counts/min)	
	Infected	Uninfected
Undialyzed.....	277	554
Dialyzed.....	1,671	1,516

^a Five milliliters of sonically treated material from infected and uninfected cells were prepared as in Fig. 7. One-half of each sonic extract was dialyzed overnight at 0 C against 0.02 M Tris, pH 9.0, and 0.001 M EDTA. The other half was stored at 0 C for an equal length of time.

markedly increases the activity of both infected and uninfected sonicates. After dialysis the levels are almost the same, whereas before dialysis the infected culture had only one-half the activity of the uninfected culture.

DISCUSSION

Infection of *S. typhimurium* with phage P22 causes a sharp decrease in the amount of host nuclease activity specific for single-stranded DNA. Whether one or more than one nuclease is involved is not clear. The decrease is not seen if the host cell carries a P22 prophage or if the infecting phage carries a mutation in gene 25. It occurs in all other mutant infections tested, including infections with *ts12* and *ts18* mutants which are deficient in phage DNA replication. Therefore, it is clear that injection of parental phage DNA is not sufficient to cause the decrease and that replication of parental DNA is not necessary to cause the decrease.

The inhibition can be reversed in vivo if a c^+ phage is used, and it can be reversed in vitro by dialysis. These findings suggest that the phage does not code for a protease which specifically destroys the host nuclease. They do not rule out the possibility that there is a phage-coded protein produced which reversibly inactivates the host enzyme. The existence of such a protein is consistent with the findings that chloramphenicol prevents the inhibition in infected cells and that at least one phage function must be expressed before the inhibition can occur. The failure of the mixing experiment to show an inhibition of the uninfected extract could be explained by assuming that the number of inhibitor molecules is less than the number of nuclease molecules.

It is also possible that as a consequence of phage infection the intracellular concentration of some small molecule is increased and that this molecule can bind reversibly to the nuclease and inhibit it. If this is true, it should be possible to

isolate such a molecule from the dialysate of an infected culture. This would not be a simple matter, since dialyzing uninfected extracts also increases their activity.

A third hypothesis involves the P22 replication complex, or intermediate I (1, 8). This replication complex is a rapidly sedimenting association of parental phage DNA, newly synthesized phage DNA, and various cellular constituents. Phage DNA replication takes place in intermediate I. Infecting phage DNA normally associates with intermediate I early in the infection, but this association does not occur in infections with P22 *ts25.1* at nonpermissive temperatures or in infections of a lysogen. Thus, the two situations in which DNA association cannot be demonstrated are also the two situations where the drop in nuclease activity does not occur. It is possible that the nuclease becomes bound to intermediate I in a normal infection, and that this renders it more difficult to detect with our assay. At the present time, we are attempting to overcome the technical difficulties which have thus far prevented us from testing this hypothesis directly.

In addition to the effect on host nuclease activity described here, infections leading to lysogeny cause a number of other changes, all of which are reversed in surviving bacteria. These include the inhibition of cell division (13), inhibition of host DNA synthesis (11, 12), inhibition of some host protein synthesis (5), and prevention of induction of at least one inducible enzyme (4). It seems likely that further investigation will add to this list. There is clearly much to be learned about how the invading phage exerts this pervasive control over the host cell.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant NIH-1-P01-GM-15419-05 from the Institute for General Medical Sciences.

LITERATURE CITED

1. Botstein, D. 1968. Synthesis and maturation of phage P22 DNA. I. Identification of intermediates. *J. Mol. Biol.* 34:621-641.
2. Botstein, D., and M. J. Matz. 1970. A recombination function essential to the growth of bacteriophage P22. *J. Mol. Biol.* 59:417-440.
3. Bronson, M. J., and M. Levine. 1971. Virulent mutants of phage P22. I. Isolation and genetic analysis. *J. Virol.* 7:559-568.
4. Chakravorty, M. 1970. Induction and repression of L-arabinose isomerase in bacteriophage-infected *Salmonella typhimurium*. *J. Virol.* 5:541-547.
5. Cohen, L. W., and M. Levine. 1966. Detection of proteins synthesized during the establishment of lysogeny with phage P22. *Virology* 28:208-213.
6. Israel, V., T. F. Anderson, and M. Levine. 1967. *In vitro* morphogenesis of phage P22 from heads and base plate parts. *Proc. Nat. Acad. Sci. U.S.A.* 57:284-291.

7. Levine, M. 1957. Mutations in the temperate phage P22 and lysogeny in *Salmonella*. *Virology* 3:22-41.
8. Levine, M., M. Chakravorty, and M. J. Bronson. 1970. Control of the replication complex of bacteriophage P22. *J. Virol.* 6:400-405.
9. Levine, M., and C. Schott. 1971. Mutations of phage P22 affecting bacteriophage DNA synthesis and lysogenization. *J. Mol. Biol.* 62:53-64.
10. Rao, R. H. 1968. Bacteriophage P22 controlled exclusion in *Salmonella typhimurium*. *J. Mol. Biol.* 35:607-622.
11. Smith, H. O., and M. Levine. 1964. Two sequential repressions of DNA synthesis in the establishment of lysogeny by phage P22 and its mutants. *Proc. Nat. Acad. Sci. U.S.A.* 52:356-363.
12. Smith, H. O., and M. Levine. 1965. The synthesis of phage and host DNA in the establishment of lysogeny. *Virology* 25:585-590.
13. Smith, H., and M. Levine. 1967. A phage P22 gene controlling integration of prophage. *Virology* 31:207-216.
14. Walsh, J., and G. G. Meynell. 1967. The isolation of non-excluding mutants of phage P22. *J. Gen. Virol.* 1:581-582.
15. Zinder, N. D. 1958. Lysogenization and superinfection immunity in *Salmonella*. *Virology* 5:291-326.