Binding of Bacteriophage P22 Tail Parts to Cells

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Purified base-plate parts of bacteriophage P22 can bind to the host cell, *Salmonella typhimurium*. Although the reaction is reversible, a stable equilibrium is not formed between bound and unbound base-plate parts. This is because the binding sites on the cell, presumably the O antigens, are destroyed. The destruction of binding sites does not kill the cells, and, in fact, the binding sites are soon regenerated. The site-destroying activity reacts with P22 heads to make active phage and with antiserum made against purified phage. Therefore site-destroying activity is a characteristic of the base-plate parts and not some contaminant of the preparation.

The large deoxyribonucleic acid (DNA) viruses, such as the T phages, P22, and lambda, all adsorb to their host by means of a tail, or base plate, which is distinct from the head. The structures employed for this purpose vary enormously from phage to phage in terms of size, design, and efficiency.

One of the simplest of all adsorbing structures is the base plate (also referred to as a tail) of phage P22. This phage apparently consists only of a head containing DNA, a short neck, and the small base plate. The base plate is composed of six identical spikes which are arranged symmetrically around the neck. Each spike is a protein of approximately 2×10^5 daltons. Thus only one protein species is responsible for converting an inactive head, which cannot adsorb, into an active phage which can adsorb and inject its DNA (1).

The "smooth specific" phages of the *Salmonellae* are known to attach to the O-antigenic side chains of the cell envelope lipopolysaccharide (5). In this report we show that isolated base-plate parts of phage P22, a smooth specific phage, can attach reversibly to *Salmonella typhimurium* in a way which results in the destruction of the P22 receptor sites. Presumably the O antigen is destroyed as well. A preliminary report of this work has been presented (J. V. Israel et al., Bacteriol. Proc., p. 211, 1971).

MATERIALS AND METHODS

Bacterial and phage strains. Strain 18 is a derivative of *S. typhimurium* LT-2 which has been cured of the prophage PBI (6). This strain is nonpermissive for

phage carrying an amber mutation; it was used in all the tail-part binding experiments reported here. Strain 192 carries the amber suppressor *su⁺527.1* and the auxotrophic marker *hisC527* (4). It was used as permissive host for platings of phage carrying an amber mutation.

P22^{c+} and the clear mutant P22_{c₁} have been previously described (2). P22-3-*am*N102 carries an amber mutation in gene 3. It is deficient in head production but produces a normal amount of free tail parts under nonpermissive conditions. P22_{ts9.1c₁} is a late mutant which grows normally at 25°C but makes normal heads and no tails at 39°C.

Media. All cultures were grown in supplemented M9 medium (3). Buffered saline (BS) is 0.85% (w/v) NaCl in 0.0067 M potassium phosphate buffer, pH 7.0. BS-CN is buffered saline with 0.01 M NaCN. Phage were plated on nutrient agar plates using soft agar for top layers (2). Dilutions for plating were made in dilution broth (0.85% NaCl, 0.1% Difco nutrient broth). Assay buffer is 0.01 M NaCN and 0.03 M potassium phosphate, pH 6.0.

Adsorption. P22^{c+} was used for all adsorption experiments. Experiments were begun by diluting phage into a cell suspension prewarmed to 37°C. From time to time samples were removed and diluted 100-fold into dilution broth and shaken with a few drops of chloroform to destroy infected cells. Other details are given in the text.

Tail purification. Strain 18 cells were grown to a concentration of 2×10^8 to 3×10^8 cells/ml and infected at a multiplicity of 10 with P22-3-*am*N102. After lysis, cell debris was removed by low-speed centrifugation. Ammonium sulfate was added with stirring at 0°C to 70% saturation, and the mixture was allowed to stir for about 20 min more. The precipitate was collected by centrifugation and resuspended in one hundredth of the original volume of BS. This was dialyzed overnight at 4°C against 0.03 M acetate buffer, pH 5.0. The precipitate was removed by centrifugation, and the supernatant was heated at 79°C for 15 min. The precipitate was again removed by centrifugation, and the supernatant was layered in 2-

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ml samples on 30 ml of 5 to 20% sucrose gradients containing 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.0. This was centrifuged for 22 hr at 20°C in a Spinco SW25 rotor at 25,000 rev/min. The gradient was collected in 1-ml samples, and the peak tubes were pooled and dialyzed against 0.03 M phosphate buffer, pH 6.0. This method results in tails which are free of contaminating phage particles. Recovery is on the order of 25%; specific activity was about 10^{14} tails/mg.

Head purification. Cells were grown to a concentration of 2×10^8 cells per ml at 39°C and infected at a multiplicity of 10 with P22ts9.1c₁. This results in a yield of about 3×10^{10} heads/ml and 3×10^7 phages/ml. The heads were freed from contaminating phage as previously described (1).

Tail assay. The head-tail reaction was carried out at 37°C for 2 hr. The reaction mixture contained 3×10^{10} ts9 heads (active heads/active phage = 10^7) and from 10^9 to 10^{10} tails. Volume was brought to 1 ml with assay buffer, and all dilutions were made with assay buffer. Tail titers were calculated according to the formula: $\log \phi = 3.3 \log T - 2.3 \log H$, where ϕ = plaque-forming units (PFU), H = heads, and T = tails (1).

Sonic treatment. Cells were resuspended at a concentration of 2×10^9 to 4×10^9 cells/ml in 1 ml of assay buffer or BS and cooled in a salt water-ice bath. The cells were disrupted by two 30-sec bursts at about 1 amp. The temperature was kept below 7°C during sonic treatment.

Note on terminology. One tail (base plate) means the number of tail parts (base-plate parts) required to saturate one head. Similarly, one phage equivalent of tail parts is that amount of tail parts required to saturate one head.

RESULTS

Free unattached tail parts of phage P22 can bind to host cells as demonstrated by the following simple experiment. Free tail parts (10^{10} phage equivalents/ml) were incubated with log-phase *S. typhimurium* or *Escherichia coli* (10^9 cells/ml). After a 10-min incubation at 37°C, low-speed centrifugation removed about 99% of the tails from the *S. typhimurium* culture, whereas very few tails were removed by the *E. coli* cells. Furthermore, almost all of the tails could be recovered from the *Salmonella* pellet after sonic treatment (Table 1). Thus the tails bind to cells with some of the specificity of the P22 phage particle.

The tail parts also interfere with the adsorption of normal phage particles. When phage were incubated for 5 min with log-phase cells in the presence of tail parts (100 phage equivalents/cell), only 50% of the phage were adsorbed. In a control without tails over 90% of the phage adsorbed (Fig. 1). These results suggest that the binding site of the free tails is the same as that of the whole phage.

TABLE 1. Binding of free tail parts to *S. typhimurium* and *E. coli*^a

Tails in	<i>E. coli</i>	<i>S. typhimurium</i>
Supernatant	8×10^{10}	6×10^8
Pellet	3×10^8	6×10^{10}

^a The reaction mixture contained 10^9 cells/ml and 10^{10} tails/ml in a volume of 10 ml. Incubation was for 10 min at 37°C, after which each culture was centrifuged for 10 min at $10,000 \times g$. The pellet was sonically treated (see Materials and Methods), and both supernatant and sonically treated pellet were assayed for tails.

FIG. 1. Adsorption of phage to cells in the presence and absence of tail parts. Adsorption was carried out on log-phase cells (2×10^8 cells/ml) in the presence of 0.01 M NaCN. At time zero, tail parts were added to a concentration of 2×10^{10} phage equivalents per ml. Symbols: ○, with tails; ●, without tails.

In an attempt to extend the analogy between the binding of free tails and the binding of phage, we performed a saturation experiment to see if the number of tails which could be bound to a cell was comparable to the number of phage. Various amounts of tails were incubated with 10 ml of cells at a concentration of 10^9 cells/ml, the cultures were centrifuged, and the number of tails in the supernatant solution and the sonically treated pellet were measured. It is clear from Fig. 2 that the number of bound tails reaches a plateau at 10^{10} /ml which corresponds to about 10 phage equivalents per cell. In other experiments we have been able to bind as many as 20 tails per cell, but at no time have we been able to approach the number of mature phage which can adsorb to a cell. The number of particles which can attach to one bacterium seems to vary somewhat depending on the physiological state of the cell. However, we can say with certainty that one cell can bind upwards of 1,000 phage (data not shown).

FIG. 2. Cell-tail binding as a function of tail concentration. Varying amounts of tails were incubated with 10 ml of log-phase cells (10^9 cells/ml) for 5 min at 37 C. The cells were sedimented and the pellet was sonically treated and both the sonically treated pellet and supernatant were assayed for tails. The titer of bound tails was determined from the sonically treated centrifuged pellet, but is represented here as the concentration of bound tails before centrifugation. The total represents the sum of free and bound tail concentrations; in all cases recovery was close to 100%.

There is thus an obvious discrepancy between the number of free tails and the number of mature phage which can be bound to a single cell. This seems puzzling in view of the efficiency with which tails are able to prevent the adsorption of mature phage to cells. We believe that at least a partial solution to this problem is provided by two further discoveries about the nature of the cell-tail reaction. These are (i) the reaction is reversible and (ii) the reaction causes the destruction of the tail binding sites.

To demonstrate reversibility, log-phase cells were saturated with tail parts and then centrifuged to separate the cell-tail complexes from unbound tail parts. The cell-tail complexes were then suspended at cell concentrations ranging from 2×10^8 to 5×10^8 cells/ml. After a brief incubation at 37 C each culture was centrifuged again and the sonically treated pellet and supernatant were assayed for tails. As shown in Table 2, the number of tails bound depended on the cell concentration; as the dilution factor was increased, the number of tails bound per cell was decreased. Since all the cells started with the same number of tails bound, it is clear that tails can detach themselves from the cell surface.

Although the reaction is reversible, an inspection of the preceding data shows that it cannot

be explained in terms of a conventional equilibrium formulation. For example, in Table 1 and the first two points of Fig. 2 the vast majority of tails are bound. But in Table 2, the majority of tails are free even at high cell concentrations. Obviously, it is impossible to calculate one equilibrium constant which would explain all the data. These considerations led us to test the idea that the tails can destroy their own binding sites.

If the reaction is reversible and the tails can destroy binding sites, then the number of bound tails should decrease with time. That this occurs is demonstrated by the experiment shown in Fig. 3. In this experiment tails were simply mixed with cells, and the number of bound and free tails was measured as a function of time. It is clear that the number of bound tails decreases and the number of free tails increases. (Control experiments show that resuspending cells in BS-CN for 2 hr does not impair the cells' ability to bind tails.) We interpret these results as follows: the tails react quickly with the cells; a certain fraction of the bound tails then detach; of the tails which detach, a certain fraction (possibly all of them) leave behind a destroyed site; the tails which are in solution now have fewer sites with which to bind. This process continues until virtually all of the sites are destroyed.

An alternative explanation of the results shown in Fig. 3 is that the sites remain intact but the free tails lose their ability to bind to cells. This interpretation is strengthened by the fact that recoveries in the latter part of the experiment are poor compared to the early part of the experiment. We therefore tried to demonstrate site-destroying activity (SDA) of the tails in a different way.

TABLE 2. Cell-tail binding as a function of cell concentration^a

Dilution factor	Tails recovered ^b		
	Free	Bound	Total
Undiluted	3.1×10^{10}	1.3×10^{10}	4.4×10^{10}
3	4.2×10^{10}	9.3×10^9	5.1×10^{10}
10	4.3×10^{10}	2.6×10^9	4.6×10^{10}
30	4.3×10^{10}	1.4×10^9	4.4×10^{10}

^a Log-phase cells (4×10^8 cells/ml) were made 0.01 M with NaCN, and tails were added at a multiplicity of about 150. After a 10-min incubation at 37 C, the cells were centrifuged and resuspended at one-tenth the original volume in BS-CN. One-milliliter samples were removed and diluted in BS-CN as shown. After another 10-min incubation at 37 C, each culture was centrifuged and assayed as in Table 1.

^b Numbers shown are total tails recovered, not concentrations.

FIG. 3. Cell-tail binding as a function of time. Log-phase cells were centrifuged and resuspended at a concentration of 10^9 cells/ml in BS-CN. Tails were added at a multiplicity of 20, and bound and free tails were assayed at intervals thereafter.

If the tails do destroy sites, it should be possible to show the effect of SDA even after the bulk of the tails have been removed from the cells. To accomplish this, log-phase cells were exposed to a high multiplicity of tails (about 200), and, after a 15-min incubation at 37 C, they were centrifuged, washed, centrifuged again, and resuspended at a concentration of 4×10^8 cells/ml in supplemented M9. This treatment resulted in the removal of 99% of the tails. These cells and a culture which had not been exposed to tails were then allowed to grow normally and were tested from time to time for the ability to adsorb phage (Fig. 4 and Table 3).

At time zero (immediately after resuspension in growth medium) the tail-treated cells were completely refractory to phage particles ($K = 0$), whereas the control culture adsorbed phage at a normal rate ($K = 0.76$). This shows unequivocally that sites have been destroyed although it does not rule out the possibility that a small number of the tails were also damaged in the process.

After 90 min had elapsed, the concentration of both cultures had increased to about 2×10^9 cells/ml, and the tail-treated cells had recovered some ability to adsorb phage. At this point the K of the treated culture had not quite returned to the value found for the control culture at time

zero (Table 3). Thus, although the tail treatment does not kill the cells or slow their growth rate, apparently the ability to synthesize new phage receptor sites is somewhat impaired (*see below*).

Although the tails used in the experiments described here were quite pure, it is possible that the SDA is a property of some cellular contami-

FIG. 4. Destruction and recovery of binding sites. Log-phase cells (4×10^8 cells/ml) were made 0.01 M in NaCN and incubated for 15 min at 37 C, with tails at a multiplicity of about 200 phage equivalents per cell. The same treatment was also given to a culture not exposed to tails. Both cultures were centrifuged, washed in an equal volume of BS, centrifuged again, and resuspended at a concentration of 4×10^8 cells/ml in supplemented M9. At 0, 45, and 90 min, 1-ml samples were withdrawn and made 0.01 M in NaCN, and the capacity of cells to adsorb phage was determined. At the end of 90 min, the titer of both cultures was about 2.2×10^9 cells/ml.

TABLE 3. Recovery of phage-adsorbing capacity by tail damaged cells^a

Min after resuspension in growth medium	K value ^b	
	Cells exposed to tails	Control cells
0	0	0.76 (3) ^c
45	0.25 (4)	1.78 (2)
90	0.58 (4)	3.82 (1)

^a K values shown are calculated from the data of Fig. 4.

^b K values are based on the equation $d\phi/dt = K\phi$, where t = time in minutes and ϕ = free phage. Using this formulation, K is directly proportional to the number of cells, or, more precisely, to the number of receptor sites.

^c The numbers in parentheses are the number of points on which the calculation for the K value is based. All four points were not always used because we have noted that phage disappearance consistently deviates from first-order kinetics after about 99% of the phage have adsorbed.

nant which co-purified with them. To rule out this possibility, we attempted to inactivate the tails in two different ways to see if this would also inactivate SDA.

The first method was to inactivate the tails with antiphage serum and then to determine the presence or absence of SDA by the method used in Fig. 4. When tails were incubated overnight with antiphage serum at a final K of 20, all of the SDA was destroyed. Tails subjected to the same treatment without serum retained their SDA (Fig. 5). Since the antiphage serum was made against CsCl-purified phage, it was deemed highly unlikely that it would contain significant activity against some contaminant of the tail preparation. The experiment is marred somewhat because the cells exposed to antiserum alone apparently retained enough antiphage activity to inactivate 50% of the adsorbing phage after 7 min. However, the rate of disappearance of phage was at least five times greater from adsorption to cells than it was from the action of any residual antiserum. Therefore, it is fair to conclude that antiphage serum inactivated the SDA.

The other method of inactivating SDA was to first saturate a tail preparation with heads and then centrifuge out the newly assembled phage particles. When this was done, the tail preparation retained essentially no SDA. The control tail preparation was completely active when given the same treatment without heads (Fig. 6). Since SDA can be destroyed by antiphage serum and can bind phage heads, the conclusion that SDA and tails are the same is inescapable.

DISCUSSION

Free P22 tail parts bind reversibly to host cells by a process which results in the eventual destruction of the receptor sites on the host. The destruction of these sites does not kill the host; in fact, no discernible change in the growth rate has been observed even after 100% destruction of the receptor sites.

Although the nature of our experimental design

FIG. 5. Destruction of SDA with antiphage serum. The following solutions (final volume, 1 ml) were incubated for 10 hr at 45°C: 10¹² tails, antiphage serum (K = 20) (●); 10¹² tails (○); antiphage serum (■); BS-CN (▲). A 0.5-ml amount of each of the above was incubated with 9.5 ml of log-phase cells (5 × 10⁸ cells/ml) for 30 min at 37°C. The cultures were then treated and tested for the capacity to adsorb phage as described for Fig. 4. In the antiserum control (■), phage disappearance was followed by assaying for PFU without CHCl₃, so that both free phage and infected cells could form plaques. Since the phage-to-cell ratio was low (about 0.1), the decrease in PFU must be attributed to residual antiphage serum.

FIG. 6. Destruction of SDA by binding to heads and centrifugation. Tails (3×10^{11}) were reacted overnight at 25 C with 4.5×10^{11} heads in BS-CN (final volume = 2.0 ml). One control tube lacked heads, the other lacked both heads and tails. The contents of each tube was then centrifuged for 2 hr at 17,000 rev/min in a Sorvall SM24 rotor. This sedimented 99% of the particles formed in the tube with heads and tails. A 1-ml amount of each supernatant was then added to 3 ml of cells at a concentration of 9×10^8 cells/ml. Each culture was treated as described for Fig. 4 and tested for the capacity to adsorb phage.

makes it difficult to quantitate the rate at which site destruction occurs, it is possible to make a minimum estimate. In experiments similar to that shown in Fig. 4, we have found that a multiplicity of 17 tails per cell was sufficient to destroy all binding sites in 30 min. This would correspond to a minimum rate of about two sites per tail per minute, or 0.67 site per tail part per minute, assuming 1,000 binding sites per cell and 6 tail parts per tail.

The results of Fig. 4 and Table 3 suggest that

the synthesis of new receptor sites is inhibited by the destruction of the old sites. At time zero the control culture showed a K of 0.76, and one would expect that value to be achieved by the tail-damaged culture after one generation if the synthesis of new sites were unimpaired. In fact after two generations the damaged culture had a K of only 0.58. It is conceivable that the slow recovery is the result of damage caused by the tail parts to the O antigen-synthesizing machinery, but we consider this unlikely. There are, however, at least three other ways to explain the data. (i) It is possible that a few tails which remained in the culture were able to continue destroying sites. Before this can be ruled out rigorously, we will need more quantitative data on the rate of site destruction. (ii) Perhaps after the O antigen is destroyed, fragments of it remain loosely bound to the cell wall, thereby preventing phage adsorption by steric hindrance. (iii) Another possibility is that the damaged O antigen is never regenerated as such, which is plausible since it has been shown that the antigen is synthesized at its proximal end (5). If the phage actually recognize the O antigen polymers in groups of six, for example, one can visualize how one or two damaged polymers could interfere with phage adsorption even if they were surrounded by perfectly normal newly synthesized O antigen. Although we favor the latter hypothesis, we cannot yet rule out the other two.

The binding of phage to cells is apparently not a reversible process. Nevertheless, it is possible that the phage bind to cells and destroy the binding sites in the same manner that tail parts do. This is a plausible idea if a tail part can remain bound to a site it has destroyed, since the binding constant of the phage would be expected to be greater than that of one tail part by a power of six. However, comparisons are difficult without further information, since it is possible that the phage particle is further stabilized by subsequent steps in the process of infection.

As yet we know little about the destruction of the sites. It would be particularly interesting to know whether every time a tail part detaches from a cell it leaves behind a destroyed site or whether this only happens a certain fraction of the time. This question could be answered if one possessed a sufficiently sensitive method for quantitating binding sites. A related question is whether site destruction is the direct result of binding or whether these are separable phenomena. Still another question is whether detachment is the result of site destruction; perhaps tails can remain bound with a certain probability to sites they have destroyed even though they cannot reattach once they are released.

Recent work using an entirely different approach has demonstrated that the tail of $\phi 15$ destroys its attachment site on *Salmonella annatum* (A. Wright, *personal communication*). Site destruction is thus very likely an important step in the early stages of infection in all smooth specific phages.

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