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Localization and Functional Role of the *Pseudomonas* Bacteriophage 2 Depolymerase

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The adsorption apparatus of phage 2 consists of a symmetrical base plate of snowflake appearance, composed of six droplike spikes 7.0 to 7.5 nm in length with a maximum diameter of 4.5 to 5.0 nm. The spikes are attached by their narrow ends to a central ring 7.0 to 7.5 nm in diameter. Phage 2 depolymerase, a phage 2-induced hydrolytic enzyme, was found to be a structural protein of phage 2 or in close association with the base plate. Pdp_1, a phage 2 mutant, possesses a polypeptide that is antigenically similar to the depolymerase, but devoid of hydrolytic activity. This polypeptide was found to be located in the region of the base plate of pdp_1. Treatment of intact cells of strain BI with purified phage 2 depolymerase inhibited the adsorption of phage 2. When phage receptor-containing fractions of slime glycolipoprotein and lipopolysaccharide were hydrolyzed by the depolymerase, amino sugars were released, and the phage-inactivating activities of these fractions were lost. The depolymerase was also observed to induce the lysis of strain BI cells in hypotonic medium. The phage 2 depolymerase appears to play a role in adsorption and release of phage.

Bacteriophage 2 specifically infects and replicates on *Pseudomonas aeruginosa* BI. Adsorption to viable cells of strain BI has been observed to occur on the entire cell surface in typical tail-first arrangement (9). Phage 2 is believed to direct the synthesis of a depolymerase (5) that specifically hydrolyzes the glycolipoprotein, previously referred to as SPB, contained in the extracellular slime of strain BI, reducing the viscosity and releasing amino and neutral sugars (8). Experimental evidence supports the view that the depolymerase occurs in two different states: (i) free, and responsible for the spreading halo that surrounds phage 2 plaques on lawns of strain BI, and (ii) bound to the phage 2 particle, as indicated by the results of sedimentation in cesium chloride gradients. In these studies, despite exhaustive purification of the phage particles, the depolymerase activity was found to band with phage at a density of 1.49 to 1.51 g/ml (6). Although the depolymerase is known to be closely associated with the structured phage particle, its exact location has not been determined.

The possible functional role of the phage 2 depolymerase in the initial attachment of the phage to host cell is supported by experimental data (27). Analysis of several strains of *P. aeruginosa* indicated that only those strains containing substrate for the specific depolymerase carried by phage were capable of adsorbing phage. In these studies, two phage-associated depolymerases, differing in their specificities, were tested against various strains and mutants.

In the present study, a more precise localization of the phage 2 depolymerase is offered, and additional information has been assembled to provide a keener insight into the possible functional role of this enzyme in the life cycle of phage 2.

MATERIALS AND METHODS

Microorganisms. *Pseudomonas* phage 2, its mutant, pdp_1, and their host, *P. aeruginosa* BI, have been previously described (5, 8). The propagation and purification of phage were in accord with procedures detailed in a previous paper (13).

Extraction and purification of slime glycolipoprotein (GLP) and lipopolysaccharide (LPS). Detailed procedures concerning the preparation of GLP and LPS for use in these studies have been published previously (7-9).

Purification of phage 2 depolymerase. Phage 2 depolymerase was obtained and purified according to the method already described (4), with some modification. After precipitation and dialysis, the depolymerase was twice subjected to chromatography on Sephadex G-200 and once in Bio-Gel A-5m, eluting with 5 mM sodium phosphate buffer, pH 7.4. Finally, the enzyme was filtered seven times on the Amicon ultrafiltration system through a SM-300 membrane (exclusion limit, 300,000 molecular weight) to remove residual phage, and concentrated

through a PM-10 membrane in the same system. Homogeneity of the purified enzyme preparation was tested by immunodiffusion techniques. A single band was detected when purified or crude lysate preparations were reacted with rabbit antiserum prepared against purified enzyme, and also when the purified enzyme was reacted with antiserum prepared against whole phage particles. These criteria were used to indicate that purified enzyme preparations were devoid of detectable contaminating protein antigens.

Preparation of antisera. Each antigen preparation was emulsified in an equal volume of Freund incomplete adjuvant. White rabbits, 4 to 5 kg in body weight, were given weekly injections with 4 ml of antigen emulsion subcutaneously in the neck region behind the ears, for 2 to 4 consecutive weeks. One week after the final injection, the animals were test-bled from the marginal veins of the ears, and finally bled by cardiac puncture. The phage antigen consisted of purified phage at 2×10^{10} PFU/ml in 5 mM Tris buffer, pH 7.4. Serum neutralization rate constants (K values) were calculated as described by Adams (1). The purified enzyme solutions used for antigenic stimulation contained 85 μg of protein per ml in 5 mM sodium phosphate buffer, pH 7.4.

Immunodiffusion. Immunodiffusion was performed on glass slides and petri dishes overlayed with 1% Noble agar in barbital buffer, pH 8.6 (14).

Determination of enzyme activity. Hydrolytic activity of the phage depolymerase involved reaction mixtures containing 0.25 ml of slime (2.44 mg/ml) or 0.25 ml of LPS (2.18 mg/ml) in 0.2 M sodium phosphate buffer (pH 7.4) and 0.25 ml of enzyme solution (0.4 mg of protein/ml) in the same buffer. After incubation for different time intervals, the tubes were immersed in ice water to stop the reaction. Release of amino sugars was determined by the method of Belcher et al. (10).

Phage adsorption. Log-phase cultures were sedimented at 6,000 $\times$ *g* for 10 min and suspended in fresh Trypticase soy broth (TSB) at a concentration of 10^7 cells/ml. The cellular suspensions were mixed with an equal volume of phage containing 10^5 PFU/ml at 37 C. Aliquots were removed at various time intervals, diluted 1:10 in cold TSB, and immediately centrifuged at 6,000 $\times$ *g* for 10 min to sediment cells and adsorbed phage particles. The supernatant fluids were diluted and plated on wild-type strain BI to determine the number of unadsorbed or free phage particles. An adsorption medium composed of 5 mM Tris buffer (pH 7.4), supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate, was also used in place of TSB (27).

Phage inactivation. Phage 2 suspensions (10^5 PFU/ml) were mixed with the substances to be tested and incubated at 37 C; after the required time intervals, the mixtures were diluted in cold buffer and titrated to determine the number of remaining infective phage particles.

Effect of depolymerase on phage 2 adsorption to whole BI cells. Strain BI in the log phase of growth was centrifuged at 6,000 $\times$ *g* for 10 min at 4 C and resuspended in TSB to a concentration of 10^8 cells/ml. The reaction mixtures contained 1 ml of cells and 0.15 ml of enzyme solution (70 μg of protein/ml) in 0.2 M sodium phosphate buffer (pH 7.4). Controls were either devoid of cells or enzyme. The enzyme was added 20 min before phage in one case, and at the same time as phage in the other. After phage addition (10^5 PFU/ml), the mixtures were incubated 10 min at 37 C, and residual phage was titered as described above.

Effect of depolymerase on phage 2 inactivation by GLP and LPS. GLP and LPS solutions (200 μg/ml) in 5 mM Tris buffer (pH 7.4), supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate, were reacted for 60 min with equal volumes of enzyme (70 μg of protein/ml) in the same buffer. Controls contained buffer instead of enzyme solution. Phage 2 (10^5 PFU) was added to each tube and, after incubation for 15 min, samples were removed, and phage titers were determined.

Effect of depolymerase on intact bacterial cells. Four-hour log-phase cultures of *P. aeruginosa* BI were centrifuged at 3,000 $\times$ *g* for 10 min, washed twice in 5 mM sodium phosphate buffer (pH 7.4), and resuspended to half the original volume in the same buffer. For studies of lysis, 1-ml portions of the suspension of washed cells were diluted in 6 ml of sodium phosphate buffer and exposed to the lytic agents singly and in various combinations at the following concentrations: phage 2 depolymerase, 300 μg of protein per ml; egg white lysozyme, 350 μg/ml; EDTA, 320 μg/ml. Controls contained 1 ml of cell suspension in 6 ml of phosphate buffer. Lysis at room temperature was followed by measurement of optical density at 500 to 570 nm with a Klett-Summerson colorimeter.

Electron microscopy. Drops of specimen suspensions were placed on grids with carbon-coated Formvar film for 1 min, and excess material was blotted off. The samples were examined, after negative staining with 2% sodium phosphotungstate (pH 7.6), in an Hitachi HU-12 electron microscope at 75 kV.

RESULTS

Structure of phage 2 tail tip. To gain information on the structure(s) involved in the adsorption process, a study of the morphology of the phage particle, especially the tail tip, was undertaken.

The gross morphological characteristics of phage 2 have been described (9). This phage belongs to Bradley's group B (12), and possesses a head with hexagonal outline and a long, noncontractile tail. A close examination of the tail structure revealed finger-like projections or spikes at the distal end, as seen in Fig. 1A, that measured between 13 and 16 nm in length and 5.5 to 8 nm in diameter, or shorter spikes as seen in Fig. 1B. Phage 2 base plates were observed to have a snowflake appearance with radial symmetry, consisting of six drop-shaped spikes 7.0 to 7.5 nm in length with a maximum diameter of 4.5 to 5.0 nm, with the narrow ends oriented toward the center and attached to a central ring 7.0 to 7.5 nm in diameter (Fig. 1C).

Localization of the phage-associated depolymerase. Evidence has been presented indicating a close structural association of the depolymerase with the phage 2 particle (6), and the likelihood that the depolymerase functions in the initial attachment of phage (27). It therefore became of great importance to determine a more precise location of the enzyme on the phage particle.

If the phage-associated depolymerase is located on a region of the virus involved in the adsorptive process, then interaction of anti-depolymerase serum with the phage particles should render them incapable of initiating infection, and therefore inactive. Furthermore, under appropriate conditions, the interaction of phage 2 with anti-depolymerase serum might be easily visualized under the electron microscope, thus providing additional information concerning the exact location of the enzyme. Table 1 shows the results of the first experiment. GLP and LPS, known to contain phage receptors, were included in the experiment as positive controls since they readily inactivate phage 2. When phage 2 was mixed with purified GLP or LPS from strain BI, 85 and 97% inactivation occurred, respectively, after 10 min at 37 C. When the virus was reacted with antiserum prepared against purified phage particles, 100% inactivation took place. Likewise, antiserum prepared against purified depolymerase brought about complete inactivation of the phage, indicating that depolymerase is indeed associated with the virus particle, and essential to its infectivity.

When phage 2 was interacted with the anti-depolymerase serum, electron micrographs showed that the virus particles were bound together in a rosette arrangement, with the phage tails oriented toward the center (Fig. 2). In contrast, when phage 2 was reacted with antiserum prepared against purified phage 2 particles, agglutination of the virus occurred in a completely disorganized pattern. These results indicate that the polysaccharide depolymerase is located at the tip of the tail of phage 2, close to, or forming part of the base plate structure.

Pdp_1, a phage 2 depolymerase mutant. Pdp_1 is a halo-less mutant of phage 2 (Fig. 3A) that is able to infect and replicate on *P. aeruginosa* BI; however, depolymerase activity has not been observed to be associated with this mutant (5). The growth curve of pdp_1 was found to be simi-

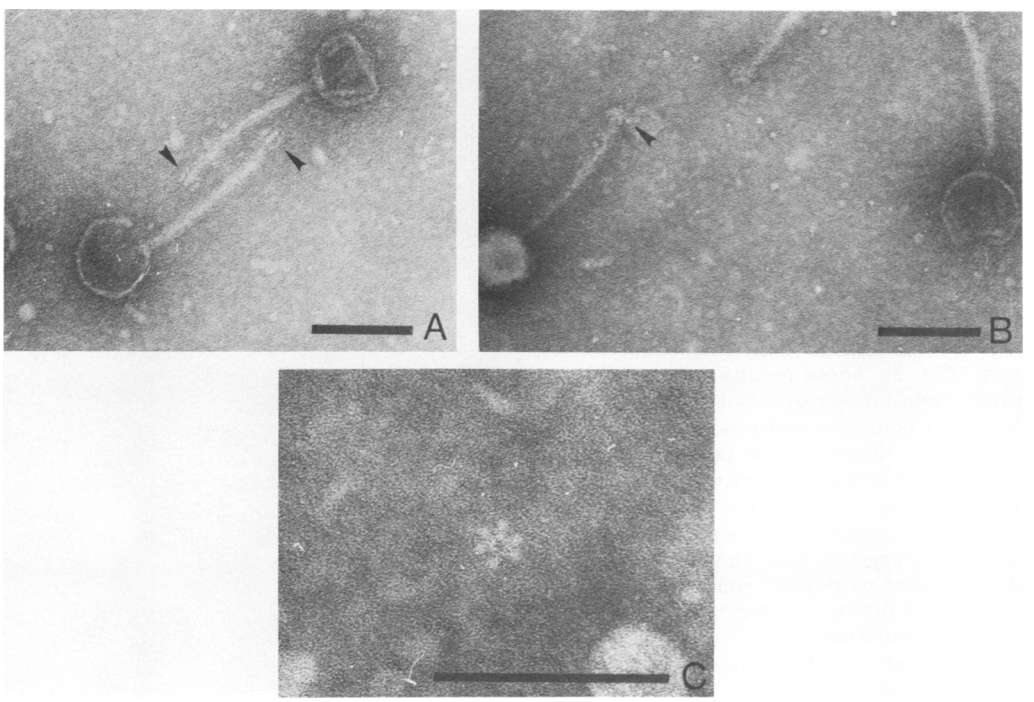

FIG. 1. *Pseudomonas phage 2. In (A) the arrows point to finger-like projections or spikes at the tail tip, and in (B) the arrow indicates the shorter spikes. The phage 2 base plate is shown in center of (C). Note the six drop-shaped spikes oriented toward the center. Bar represents 100 nm.*

TABLE 1. *Inactivation of phage 2 by anti-phage 2 and anti-depolymerase sera*

Medium	Phage input (PFU/ml)	Phage recovered (PFU/ml)	% Inactivation
Buffer[a]	1.3×10^4	1.3×10^4	0
TSB	1.3×10^4	1.3×10^4	0
GLP[b]	1.3×10^4	1.9×10^3	85
LPS[c]	1.3×10^4	3.8×10^2	97
Anti-phage 2 serum	1.3×10^4	0	100
Anti-depolymerase serum	1.3×10^4	0	100

[a] Tris, 5 mM, pH 7.4, supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate.
[b] Purified from *P. aeruginosa* BI (100 μg/ml).
[c] Purified from *P. aeruginosa* BI (100 μg/ml).

lar to that of phage 2, having a latent period of 55 min, a rise period of 20 to 25 min, and an average burst size of 10. The absence of halo around the plaques of this phage might be interpreted in two ways: (i) absence of depolymerase or (ii) the structural presence of depolymerase that lacks hydrolytic activity. Experiments were performed to examine these possibilities, and the results are presented in Table 2. Antiserum prepared against purified phage 2 was found to completely inactivate pdp_1, indicating a strong serological relatedness between phage 2 and its mutant. Antiserum prepared against the purified depolymerase similarly inactivated the mutant. This was taken as an indication that a cross-reacting polypeptide(s), antigenically similar to the depolymerase, was present in pdp_1 particles. Further examination under the electron microscope revealed that pdp_1 interacted with the anti-depolymerase, producing a tail-centered rosette arrangement (Fig. 3 B, C) similar to that observed when the wild-type phage 2 was reacted with anti-depolymerase serum (Fig. 2). These results suggest that the mutant, pdp_1, possesses tail region polypeptide(s) that is antigenically related to the wild-type phage 2 depolymerase polypeptide(s). Although enzymatically inactive, this polypeptide(s) appears to be in the same structural location as the depolymerase of phage 2.

It is of interest to note that GLP and LPS, although able to readily inactivate phage 2, showed no ability to inactivate pdp_1.

Effect of purified phage 2 depolymerase on cellular phage receptors. Since the possibility that phage 2 depolymerase may play a role in the adsorptive process has been considered (27), the effect of enzyme treatment on the ability of whole cells of strain BI to adsorb phage 2 was examined. It was observed that whereas 69% adsorption of phage 2 to BI cells occurred after 10 min of incubation, only 51% adsorption took place when enzyme was added simultaneously with the phage. Furthermore, when the cells were treated with the enzyme for 20 min before phage addition, no adsorption of phage occurred (Table 3).

GLP and LPS from strain BI have been shown to contain receptors for phage 2 (9), and it was of interest to study the effect of the phage 2 depolymerase on GLP and LPS. It was observed that purified depolymerase had the ability to hydrolyze not only slime, as previously reported (8), but also LPS extracted from strain BI, inducing the release of amino sugars (Fig. 4). The effect of enzyme treatment on the ability of GLP and LPS to inactivate phage 2 was then determined. As shown in Table 4, when GLP and LPS were treated with the enzyme before addition of phage, they completely lost phage 2 inactivating activity. These experiments suggest that phage 2 receptors are either modified and/or destroyed by the action of the enzyme.

Depolymerase treatment of strain BI cells, suspended in a hypotonic medium, resulted in a

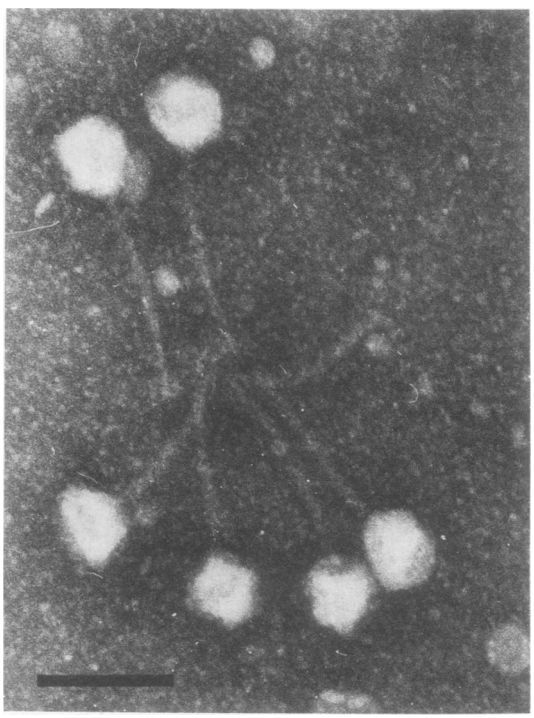

FIG. 2. *Bacteriophage 2 after interaction with anti-depolymerase serum. Note the rosette formed by the phage with their tails oriented toward the center. Bar represents 100 nm.*

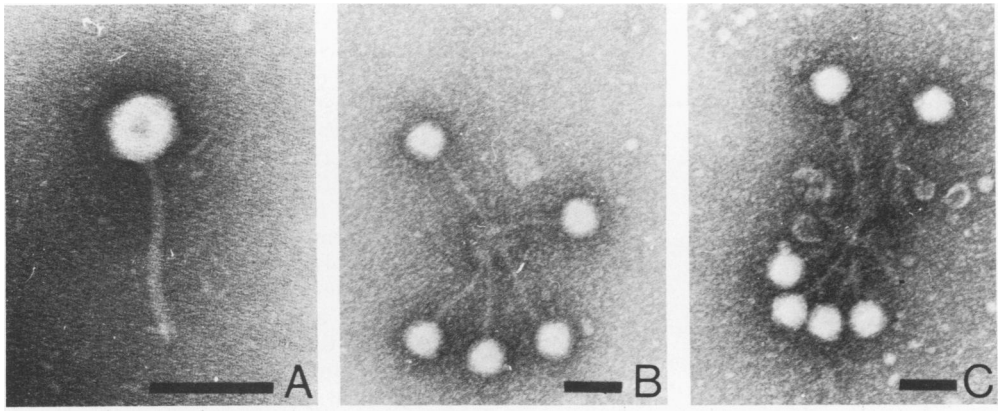

FIG. 3. *Phage 2 halo-less mutant pdp (A) after interaction with anti-depolymerase serum (B, C). Note rosette arrangement similar to that observed with wild-type phage 2. Bar represents 100 nm.*

TABLE 2. *Inactivation of phage mutant pdp$_1$ by anti-phage 2 and anti-depolymerase sera*

Medium	Phage input (PFU/ml)	Phage recovered (PFU/ml)	% Inactivation
Buffer[a]	1.95×10^5	1.95×10^5	0
GLP[b]	1.95×10^5	2×10^5	0
LPS[c]	1.95×10^5	2.08×10^5	0
Anti-phage 2 serum	1.95×10^5	0	100
Anti-depolymerase serum	1.95×10^5	0	100

[a] Tris, 5 mM, pH 7.4, supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate.
[b] Purified from *P. aeruginosa* BI (100 μg/ml).
[c] Purified from *P. aeruginosa* BI (100 μg/ml).

TABLE 3. *Effect of depolymerase on adsorption of phage 2 to viable cells of P. aeruginosa BI*

Medium	Phage added (PFU/ml)	Phage recovered (PFU/ml)	% Adsorption
TSB + buffer[a]	2.56×10^5	2.56×10^5	0
TSB + depolymerase	2.56×10^5	2.60×10^5	0
Cells[b] + buffer	2.56×10^5	0.80×10^5	69
Cells + depolymerase (at zero time)	2.56×10^5	1.25×10^5	51
Cells + depolymerase (at −20 min)	2.56×10^5	2.56×10^5	0

[a] Tris, 5 mM, pH 7.4, supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate.
[b] *P. aeruginosa* BI (10^8 cells/ml).

reduction of turbidity, indicating cellular lysis. Similar lytic activity was observed in the presence of EDTA, lysozyme, or both (Fig. 5). Thus, the phage 2 receptors appear to be intimately related to essential structural components of the cell wall, and the hydrolytic activity of the phage 2 depolymerase is capable of disrupting this integrity of the cell envelope.

DISCUSSION

Phage 2 has been previously described as possessing a knoblike structure at the distal end of its tail, and observations have also been made of tail spikes in the region of interaction of the tail tips with slime GLP from strain BI (9). In the present work, it was possible not only to corroborate the presence of these spikes, but also to provide information on the structural detail of the phage 2 base plate.

It is of interest to note that the phage spikes were observed in forms that suggest the possibility of two states: extended and retracted. The physiological implication of this dimorphism is not understood. However, it may explain the earlier reports of a knoblike structure at the tail tip of phage 2. On the other hand, the differences observed might also be accounted for by the fact that the specimens were viewed in different planes, resulting in different two-dimensional projections of the same type of structure. Isolated base plates were observed as a symmetrical snowflake structure. Although in the present study only one structural state of the isolated base plates was observed, the possibility of alternative states is not disregarded. Electron microscope examinations of other systems, such as T-even coliphages, have suggested that basal plates may exist in two states (2, 17, 20, 25, 26, 28). In the intact T-even phage particles with extended tail sheath, the base plates occur as fine hexagonal structures with a maximum diameter of 31 to 40 nm; with contraction of the sheath, the appearance of the basal plates changes from hexagons to six-pointed stars with a maximal diameter of 50 to 60 nm.

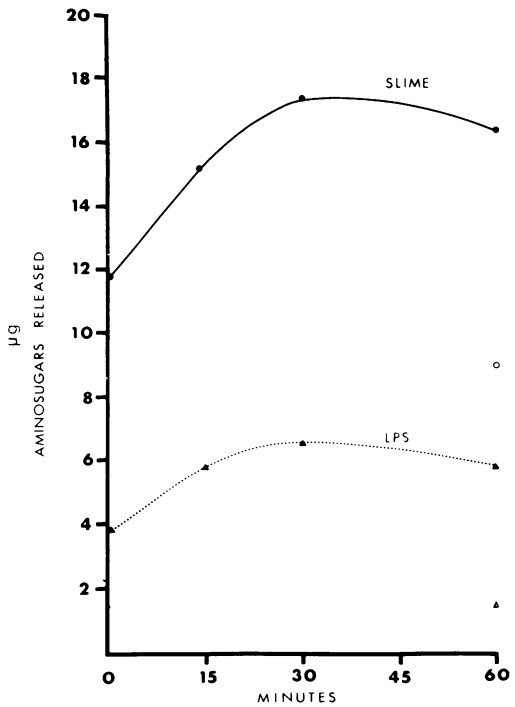

FIG. 4. *Release of amino sugars from P. aeruginosa BI slime GLP (0.61 mg) (●) and LPS (0.55 mg) (▲) during treatment with phage 2 depolymerase (0.1 mg). Controls of slime GLP (○) and LPS (△) without addition of depolymerase are also shown.*

The fact that only the tail tips of phage 2 particles interacted with antiserum prepared against purified depolymerase, with the concomitant loss of phage infectivity, suggests that this enzyme is located in close association with or is one of the structural components of the base plate. This observation indicates, once again, the possible importance of the enzyme in the infectious process. Localization of phage-associated enzymes at the tail tip of other phages is well documented in the literature (11, 22, 23, 29–31). It thus appears very certain that the phage 2-depolymerase exists in two states: free, and bound to the phage particle as one of the structural proteins of the base plate (6, 8). The relatedness of the free and bound depolymerase was determined by their serological cross-reactivity, since antiserum prepared against the purified free enzyme reacted with the phage-associated enzyme.

If the depolymerase activity plays a role in the attachment of the wild-type phage 2 to BI cells, then pdp_1, a mutant devoid of depolymerase activity, should be incapable of adsorption or show a lower rate of adsorption, as reflected by longer latent and rise periods than those of phage 2. However, the same values, 55 min for the latent periods and 20 to 25 min for the rise periods, were observed with both phages. These results suggest that active enzyme is not essential in the adsorption process. Nevertheless, the presence of structural enzyme in an inactive form might still be involved in the adsorptive process. This hypothesis was supported by the fact that the mutant phage, pdp_1, although devoid of depolymerase activity, reacted with anti-depolymerase serum to produce a tail-centered rosette arrangement similar to that observed with the wild-type phage 2. These results clearly indicated the presence of wild-type depolymerase cross-reacting polypeptides in the tail tip of pdp_1, which, although devoid of enzymatic activity, might retain the ability to recognize complementary receptors and allow phage to attach to the cell surface.

In this connection, it should be mentioned that coliphage T4D possesses an enzyme, dihydrofolate reductase, as one of the structural components of the base plate, and that this enzyme has been shown to play a role in the adsorption process. Two mutants of T4D, neither of which induces the production of active dihydrofolate reductase, are fully infective. These mutants induce the production of a protein that resembles the enzyme in that it binds to the substrate, but does not reduce free dihydrofolate. Kozloff et al. indicated that the protein may serve a structural rather than an enzymatic role in the phage tail plate (24). This hypothesis has also been proposed by Dawes and Goldberg (18), who stated that the dihydrofolate reductase plays no enzymatic role in the virion, but is structurally important during the early stages of phage adsorption. A similar explanation may apply to the role of phage 2 depolymerase in the adsorption process,

TABLE 4. *Effect of depolymerase on phage 2 inactivation by purified GLP and LPS from P. aeruginosa BI*

Medium	Phage input (PFU/ml)	Phage recovered (PFU/ml)	% Inactivation
Buffer[a]	6.75×10^5	6.75×10^5	0
Buffer + depolymerase	6.75×10^5	6.80×10^5	0
GLP[b] + buffer	6.75×10^5	1.24×10^5	82
GLP + depolymerase	6.75×10^5	6.8×10^5	0
LPS[c] + buffer	6.57×10^5	1.75×10^3	99.8
LPS + depolymerase	6.75×10^5	6.9×10^5	0

[a] Tris, 5 mM, pH 7.4, supplemented with 0.1 M sodium chloride and 0.01 M magnesium sulfate.
[b] Purified from *P. aeruginosa* BI.
[c] Purified from *P. aeruginosa* BI.

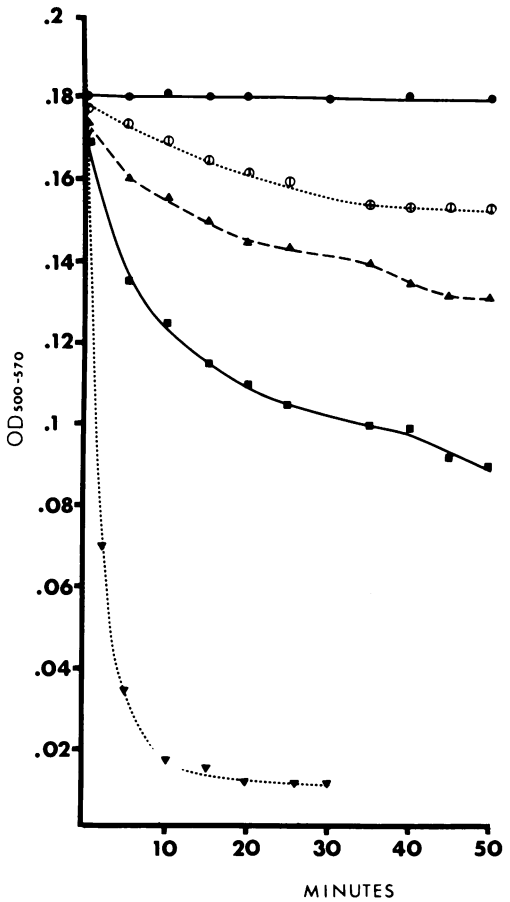

FIG. 5. *Changes in optical density of P. aeruginosa BI suspensions during treatment with 5 mM sodium phosphate buffer, pH 7.4 (●), depolymerase (300 μg/ml) (⦶), lysozyme (350 μg/ml) (▲), EDTA (320 μg/ml) (■), and EDTA (320 μg/ml) plus lysozyme (350 μg/ml) (▼).*

thereby clarifying the fact that pdp_1 is fully infective. Alternatively, the phage 2 depolymerase may possess a bifunctional activity, involving the ability to hydrolyze the slime GLP and LPS and to recognize and attach to the phage receptors present in slime GLP and LPS.

It is of interest to note that whereas wild-type phage 2 is irreversibly inactivated by slime GLP and LPS, the mutant phage pdp_1 is not inactivated by these cellular components. It is possible that the mutant depolymerase carried by pdp_1 has more stringent requirements for recognition of receptors in purified slime GLP and LPS, or that purification of GLP and LPS induces modifications in the tertiary structure of these substances which limit or modify the availability of the receptors. Another possibility is that secondary receptors may be necessary for the inactivation of pdp_1, and these are either missing or hidden in the purified slime and LPS. Alternatively, the free and bound enzymes, although serologically similar, may have a different mechanism of interaction with the phage receptors.

Purified phage 2 depolymerase was found to hydrolyze not only slime GLP from strain BI, as previously reported (8), but also LPS from the same strain. This hydrolytic activity resulted in the release of amino sugars and destroyed the phage-inactivating activity of GLP and LPS. Furthermore, cells of strain BI treated with the enzyme lost the ability to adsorb phage 2. These results indicate that the substrate for the enzyme is present in LPS as well as in GLP. This is of great significance since these two cell fractions possess the functional receptors for phage 2. The enzyme is able to destroy the receptor activity of GLP and LPS, not only in vitro, but also in situ. Thus, the free depolymerase produced in excess during the process of phage replication may protect newly formed progeny from inactivation by GLP and LPS that is present on the surface of the cell.

Gram-negative bacteria contain several surface layers of different composition and physical nature (15). Each of these layers presents a mechanical barrier that might impede the free release of mature phages from infected cells. Phage release may be accomplished by the production of specific enzymes that disrupt the structural integrity of these layers. The peptidoglycan layer is usually considered to be responsible for maintaining the rigidity of the cell in the majority of gram-negative bacteria. However, in *P. aeruginosa* the situation seems somewhat different. Although some lesions were observed after treatment of cells with lysozyme, the cell wall structure remained intact (19). This indicates that peptidoglycan is not solely responsible for the rigidity of the cell. Furthermore, if the outer membrane is disrupted by treatment with Tris buffer, EDTA, or a combination of both, cell lysis ensues (3, 16, 19, 21, 33). As reported in the present work, treatment of BI cells with phage 2 depolymerase in hypotonic media induced cell lysis. Under natural conditions, depolymerase activity may facilitate the release of phage particles by eliminating a portion of the physical barriers represented by the structural layers of the cell envelope. In addition, progeny phage 2 may be protected from the receptors present in LPS and GLP which are hydrolyzed or possibly blocked by the depolymerase. Thus, the phage 2 depolymerase appears to play a role in two important steps of the phage life cycle: adsorption and release.

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