

The Lytic Enzyme of Bacteriophage PRD1 Is Associated with the Viral Membrane

Pia S. Rydman and Dennis H. Bamford*

Department of Biosciences and Institute of Biotechnology, Viikki Biocenter,
University of Helsinki, 00014 University of Helsinki, Finland

Received 23 July 2001/Accepted 26 September 2001

Bacteriophage PRD1 encodes two proteins (P7 and P15) that are associated with a muralytic activity. Protein P15 is a soluble β -1,4-*N*-acetylmuramidase that causes phage-induced host cell lysis. We demonstrate here that P15 is also a structural component of the PRD1 virion and that it is connected to the phage membrane. Small viral membrane proteins P20 and P22 modulate incorporation of P15 into the virion and may connect it to the phage membrane. The principal muralytic protein involved in PRD1 DNA entry seems to be the putative lytic transglycosylase protein P7, as the absence of protein P15 did not delay initiation of phage DNA replication in the virus-host system used. The incorporation of two different lytic enzymes into virions may reflect the broad host range of bacteriophage PRD1.

For successful initiation of infection, an incoming bacteriophage genome has to cross the barrier formed by the host cell wall. In some cases phage particles are known to contain lytic enzymes that are capable of producing local openings into the murein sacculus. In the case of enveloped double-stranded RNA (dsRNA) phage ϕ 6, the lytic endopeptidase, protein P5, is associated with the nucleocapsid surface (3, 11, 28, 38). In bacteriophage T4, the baseplate structure of the tail contains protein gp5, which has *N*-acetylmuramidase activity (29, 42). Amino acid sequence analyses have identified putative lytic transglycosylases among the structural proteins of bacteriophages T7, PRD1, and P1 (30, 33). The putative transglycosylase of phage T7, protein gp16, has recently been shown to increase the efficiency of phage genome translocation into the host cell (39).

Bacteriophage PRD1 is a lipid-containing dsDNA virus that infects gram-negative cells carrying a conjugative P, N, or W incompatibility group plasmid (44). The virion consists of an icosahedral protein capsid surrounding a membrane vesicle that encloses the linear dsDNA genome, which is 14,925 bp long (1, 7, 16). In contrast to other dsDNA phages that have a proteinaceous tail associated with the portal vertex, PRD1 particles have spike-penton structures consisting of the receptor-binding protein P2, the trimeric spike shaft protein P5, and the penton protein P31 at every vertex (13, 21, 48). The internal phage membrane consists of approximately equal amounts of phospholipids and phage-encoded proteins and is capable of transforming from a spherical vesicle into a tubular structure; it has been proposed that this tubular structure delivers the phage genome into the host cell cytoplasm (1, 34).

The PRD1 genome encodes two proteins with muramidase activity, a 1,4- β -*N*-acetylmuramidase (protein P15), which causes host cell lysis and liberation of progeny phages (12, 35), and protein P7, which carries a conserved transglycosylase

motif in its amino-terminal half (7, 30). Protein P7 has been shown to be active against peptidoglycan and has an accessory role in phage DNA entry (46). In this study we found that protein P15 is a structural component of the virion and that it is associated with the phage membrane, most probably via protein-protein interactions.

MATERIALS AND METHODS

Bacteria and phages. The bacterial strains, phages, and plasmids used in this study are listed in Tables 1 and 2 and 3, respectively. Cells were grown in Luria-Bertani medium (50), and when appropriate, chloramphenicol (25 μ g/ml) and/or kanamycin (25 μ g/ml) was added. For production of wild-type and mutant phage particles, *Salmonella enterica* DS88 cells were infected at a multiplicity of infection (MOI) of 6. When *Escherichia coli* HMS174(pJB21)(pLM2) cells were used as hosts, a MOI of 50 was employed. After lysis, viral particles were concentrated and purified in 5 to 20% sucrose rate zonal gradients, as previously described (5). DS88 and HMS174(pJB21)(pLM2) cells infected with phages carrying an amber mutation in the lytic enzyme (protein P15)-encoding gene *X'* (*sus174*, *sus232*, or *sus240*) were collected by centrifugation (Sorvall GS3 rotor, 3,600 rpm, 10 min, 4°C) 2 h postinfection, resuspended in 20 mM potassium phosphate buffer (pH 7.4) (using 1/80th of the original volume), and disrupted by passage through a 0.375-in. French press (300 lb/in²). The original volume was restored with buffer, and the viruses were concentrated and purified in rate zonal gradients as the wild-type virus was. Light-scattering zones formed by PRD1 particles were collected and either harvested by differential centrifugation, which resulted in 1 \times -purified virus preparations, or purified further in 20 to 70% sucrose equilibrium gradients and collected by centrifugation, which resulted in 2 \times -purified virus preparations (5, 6). The 1 \times -purified virus preparations or (in the case of mutant particles devoid of protein P15) 2 \times -purified virus preparations were purified further by ion-exchange chromatography with Sartorius D100 filter cartridges, as described by Walin et al. (52).

Isolation of the PRD1 membrane fraction. For isolation of PRD1-specific membrane vesicles devoid of the protein capsid, DS88 cells were infected with *sus636* mutant phage at an MOI of 10 (47). Vesicles were precipitated from the lysate with polyethylene glycol 6000 by using the standard phage purification method and were separated by isopycnic density flotation centrifugation in sucrose gradients as previously described (4, 22, 47). The light-scattering PRD1-specific membrane zone at a density of \sim 1.15 g/cm³ was collected and used in enzymatic assays as described below.

Membranes were obtained from 1 \times -purified wild-type PRD1 particles by treating the particles with 2 M guanidine hydrochloride (GuHCl) as described previously (1). Disrupted virus preparation (2 mg of protein) was layered on top of a linear 10 to 40% sucrose gradient (in 20 mM potassium phosphate buffer [pH 7.4] supplemented with 2 M GuHCl), and empty and DNA-filled membrane

* Corresponding author. Mailing address: Viikki Biocenter, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland. Phone: 358-9-19159100. Fax: 358-9-19159098. E-mail: dennis.bamford@helsinki.fi.

TABLE 1. Bacterial strains used in this study

Strain	Description	Reference or source
<i>Salmonella enterica</i> serovar Typhimurium strains		
DS88	SL5676(pLM2) standard non-suppressor host	5
DB7154(pLM2)	DB7100 <i>leuA414</i> (Am) <i>hisC527</i> (Am) <i>supD10</i> ; suppressor host for <i>sus44</i> , <i>sus148</i> , <i>sus170</i> , <i>sus471</i> , and <i>sus636</i>	53
DB7156(pLM2)	DB7100 <i>leuA414</i> (Am) <i>hisC527</i> (Am) <i>supF30</i> ; suppressor host for <i>sus174</i> , <i>sus232</i> , <i>sus234</i> , and <i>sus240</i>	53
PSA(pLM2)	<i>supE</i> ; suppressor host for <i>sus1</i> , <i>sus30</i> , and <i>sus42</i>	35
<i>Escherichia coli</i> K-12 strains		
DH5 α	Peptidoglycan source for zymograms and preparation of chloroform-treated cells for turbidimetric assays	50
HMS174(pJB21) (pLM2)	F ⁻ <i>recA hsdR</i> ; nonsuppressor host carrying wild-type gene <i>II</i> of PRD1; used in production of P2 ⁺ P15 ⁻ particles	14, 46
<i>Micrococcus lysodeikticus</i> ATCC 4698	Peptidoglycan source for zymograms	Sigma

vesicles were obtained by centrifugation (Sorvall TH641 rotor, 30,000 rpm, 5 h, 20°C). Light-scattering bands at densities of 1.14 and 1.17 g/cm³ corresponding to empty and DNA-filled membranes, respectively, were collected. In order to remove GuHCl from the samples, the samples were dialyzed against 2 liters of 20 mM potassium phosphate buffer (pH 7.4) for 15 h at 4°C before enzymatic assays were performed.

Protein contents in mutant infections and phage particles. Production of receptor-binding protein P2 in P15⁻ mutant (*sus174*, *sus232*, or *sus240*)-infected DS88 cells was analyzed as follows. One-milliliter samples were taken 30, 50, and 120 min postinfection, and cells were harvested and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western blotting with polyclonal P2 antiserum (21). Similarly, the protein compositions of purified P15⁻ (*sus174*, *sus232*, or *sus240*) mutant particles were analyzed by Western blotting with polyclonal P2 antiserum (21).

Enzymatic assays. Peptidoglycan sacculi of *E. coli* DH5 α and *Micrococcus lysodeikticus* were prepared as described by Hoyle and Beveridge (27) by boiling cells derived from 500-ml stationary-phase cultures in 4% SDS. The sacculi were washed with 2 M NaCl as described by Bernadsky et al. (9) to remove peptidoglycan-associated proteins, resuspended in 5 ml of Milli-Q water, and stored at -20°C until they were used. Zymogram analysis was used for detecting proteins with muralytic activity as previously described (9). SDS-polyacrylamide (14% polyacrylamide) gels (101 by 82 by 0.5 mm) were cast as described by Olkkonen and Bamford (43), except that an *E. coli* or *M. lysodeikticus* peptidoglycan preparation homogenized by sonication was added to a final concentration of 15% (vol/vol). Following electrophoresis, the gels were first rinsed and then soaked for 30 min in Milli-Q water and for 30 min in renaturation buffer (25 mM potassium phosphate buffer [pH 7.4], 0.1% Triton X-100) with gentle agitation at room temperature. The gels were transferred to fresh renaturation buffer and incubated for 48 to 72 h at 4°C. After incubation, zymograms were stained with 0.1% methylene blue in 0.01% KOH at 37°C for 1 h and destained with Milli-Q water.

Chloroform treatment of *E. coli* DH5 α cells and the turbidimetric assay were performed as described by Caldentey and Bamford (11). Chloroform-treated cells were resuspended in 50 mM Tris-HCl (pH 7.2)-0.1% Triton X-100 to an absorbance at 450 nm (A_{450}) of about 0.450. Samples containing ~60 μ g of purified wild-type or mutant virus particles or the corresponding amount (major membrane-associated protein P11 of PRD1 was used for normalization) of virus-specific membrane vesicles were added to the cell suspension (800 μ l). Changes in turbidity (A_{450}) were monitored with a double-beam Cary 219 spectrophotometer by using cuvettes with an optical path length of 1 cm and a spectral bandwidth of 1 nm. Hen egg white lysozyme, used as a control in enzymatic assays, was purchased from Roche.

Phage adsorption and DNA replication assays. A phage adsorption test was performed by incubating 300 to 600 phage particles with 2×10^8 *Salmonella* cells grown to the optimal adsorption phase (2×10^9 CFU/ml) (31). The infection mixtures were incubated for 1, 4, and 8 min at 20°C, and the cells were collected by centrifugation (Heraeus Biofuge Pico centrifuge, 5,000 rpm, 3 min, 20°C). The numbers of nonadsorbed phage particles in the supernatant fractions were determined by plating on lawns of DB7156(pLM2) cells.

Replicating PRD1 DNA was labeled with [³H]thymidine by the method of Mosig and Colowick (40), except that nalidixic acid (40 μ g/ml) was added to inhibit host DNA replication (15). In the case of *sus232* mutant particles an MOI of 30 was employed, while for wild-type and *sus471* mutant infections an MOI of 15 was used. The amount of incorporated ³H was determined by scintillation counting.

Analytical methods. SDS-PAGE was performed as described by Olkkonen and Bamford (43). Western blotting was performed by transferring the proteins from the gels onto polyvinylidene difluoride membranes (Millipore), and the proteins were visualized with the ECL detection system (Amersham) by using horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins (Dako) as secondary antibodies. Protein concentrations were determined with Coomassie brilliant blue by using bovine serum albumin as a standard (10).

TABLE 2. Phages used in this study

PRD1 phage	Genotype	Phenotype ^a	Reference
Wild type	<i>wt</i>	Wild type	44
<i>sus1</i>	Amber mutation in gene <i>IX</i>	P9 ⁻	35
<i>sus30</i>	Amber mutation in gene <i>XX</i>	P20 ⁻ P6 ^R	35
<i>sus42</i>	Amber mutation in gene <i>XXII</i>	P22 ⁻ P6 ^R	35
<i>sus44</i>	Amber mutation in gene <i>XI</i>	P11 ⁻	35
<i>sus148</i>	Amber mutation in gene <i>XVIII</i>	P18 ⁻	35
<i>sus170</i>	Amber mutation in gene <i>II</i>	P2 ⁻	35
<i>sus174</i>	Amber mutation in gene <i>XV</i>	P15 ⁻ P2 ^R when propagated in nonsuppressor host, P15 ⁻ P2 ⁺ when protein P2 is provided in trans by complementation	35
<i>sus232</i>	Amber mutation in gene <i>XV</i>	P15 ⁻ P2 ^R when propagated in nonsuppressor host, P15 ⁻ P2 ⁺ when protein P2 is provided in trans by complementation	35
<i>sus234</i>	Amber mutation in gene <i>VII</i>	P7 ⁻ P14 ⁻	35
<i>sus240</i>	Amber mutation in gene <i>XV</i>	P15 ⁻ P2 ^R when propagated in nonsuppressor host, P15 ⁻ P2 ⁺ when protein P2 is provided in trans by complementation	35
<i>sus471</i>	Amber mutation in gene <i>II</i> , opal mutation in gene <i>VII</i>	P2 ⁻ P7 ⁻ when propagated in nonsuppressor host, P7 ⁻ when propagated in suppressor host	46
<i>sus636</i>	Amber mutation in gene <i>XXX</i>	Forms membrane vesicle intermediates containing membrane-associated phage proteins and a small amount of major capsid protein P3	47

^a A superscript R indicates that mutant particles contain reduced levels of the protein.

TABLE 3. Plasmids used in this study

Plasmid	Description	Replicon	Selective marker	Reference
pJB21	pSU18 Ω (PRD1 3128-4903) (gene II)	p15A	Cm ^r	46
pLM2	Encodes PRD1 receptor	IncP α	Km ^r	37
pSU18	Low-copy-number cloning vector	p15A	Cm ^r	8

RESULTS

Small integral membrane proteins P20 and P22 modulate protein P15 incorporation into the virion. The particle-associated lytic activities of bacteriophage PRD1 were investigated by analyzing purified viruses in zymograms. Phage proteins separated by gel electrophoresis were renatured by incubating the gels in renaturation buffer for 2 to 3 days. Renatured muralytic proteins degraded the peptidoglycan substrate added to the gels and were detected as clear zones. Two such zones appeared when wild-type PRD1 particles were analyzed (Fig. 1a). The zone that moved more slowly was identified previously as protein P7, which has a conserved lytic transglycosylase domain in its N-terminal half (30, 46). The faster-moving zone, which had a molecular mass of ~ 15 kDa, was present in all of the other particles studied except those devoid of proteins P15 (*sus174*, *sus232*, and *sus240* mutants) and P20 (*sus30* mutant) (Fig. 1a and b). In addition, the signal caused by the faster-moving lytic activity was clearly decreased in P22-deficient particles (*sus42* mutant) when it was compared to the signal in wild-type virions and normalized to the signal caused by protein P7. The molecular mass of the faster-moving clear zone was found to correspond to that of protein P15 (17.3 kDa)

(45), the soluble lytic enzyme encoded by PRD1 (12, 35). Proteins P20 and P22 are integral membrane proteins that have molecular masses of 4.7 and 5.5 kDa, respectively, and it has been proposed that these proteins span the membrane once and have no recognizable lytic domains in their amino acid sequences (7, 36; J. K. H. Bamford, J. M. Grimes, D. I. Stuart, and D. H. Bamford, submitted for publication). Thus, we concluded that the appearance of the faster-moving clear zone in zymograms was due to the enzymatic activity of protein P15. The activity of PRD1 muramidases against gram-positive peptidoglycan isolated from *M. lysodeikticus* was also analyzed (Fig. 1c). Protein P7 was found to be inactive, while protein P15 degraded peptidoglycan obtained from *M. lysodeikticus* and caused the appearance of a clear zone in zymograms.

Muralytic activity of protein P15 is localized in the phage membrane. In addition to the zymogram analysis, the particle-associated lytic activities were probed by measuring the changes in A_{450} when purified phages were added to chloroform-treated *E. coli* cells. Figure 2a shows the decreases in absorbance when different amounts of the control hen egg white lysozyme were added to the substrate cells. Adding freshly purified wild-type particles did not result in a sharp decrease in the A_{450} . However, the activity of these particles could be significantly increased by partially disrupting the protein capsid by three cycles of freezing and thawing (Fig. 2a). Centrifugation and SDS-PAGE analysis demonstrated that this treatment led to dissociation of $\sim 10\%$ of the major capsid protein P3 and protein P6 from the virions, while all of the other proteins were still associated with the particles (data not shown). Turbidity measurements were performed with activated wild-type and mutant phage particles in order to analyze the muralytic activities of PRD1 particles with different protein

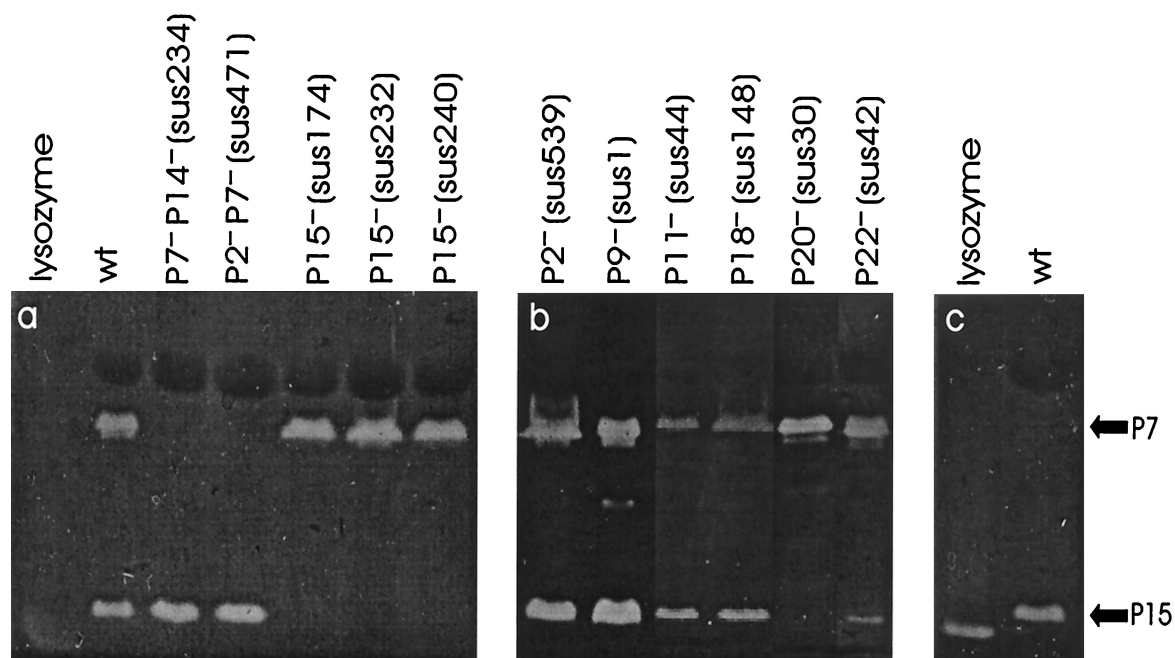


FIG. 1. Zymogram analysis of lytic activities associated with wild-type and mutant PRD1 particles with different protein compositions. (a and b) Zymograms supplemented with gram-negative *E. coli* peptidoglycan. (c) Zymogram supplemented with gram-positive *M. lysodeikticus* peptidoglycan. The positions of PRD1 proteins exhibiting muralytic activities (P7 and P15) are indicated by arrows. wt, wild type.

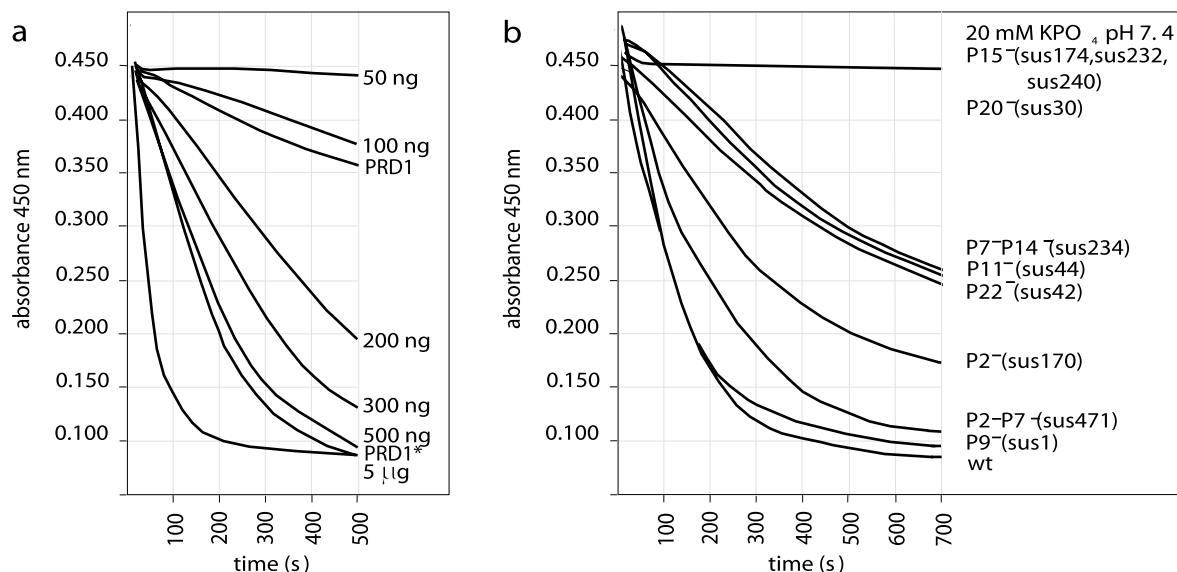


FIG. 2. Lytic activities as determined by turbidity measurements. (a) Control assay in which different amounts of hen egg white lysozyme were added to a suspension of chloroform-treated substrate cells. For comparison, assay mixtures containing freshly purified wild-type particles (PRD1, 60 μ g of protein) and particles activated by freezing and thawing three times (PRD1*, 60 μ g of protein) were included. (b) Corresponding assay performed with wild-type and mutant PRD1 particles activated by freezing and thawing three times. In this assay 1 \times - or 2 \times -purified virus particles (60 μ g of protein in approximately 15 μ l) were added to substrate cells (in an 800- μ l mixture). As a control, 15 μ l of 20 mM potassium phosphate buffer (pH 7.4) was added to the assay mixture. wt, wild type.

compositions (Fig. 2b). The absence of protein P7 did not have a detectable effect on the muramidase activity. No decrease in the A_{450} was detected when P15⁻ (*sus174*, *sus232*, or *sus240*) or P20⁻ (*sus30*) mutant particles were added to the assay mixture. Decreases in lytic activity were associated with mutants in which a membrane protein (P14 or P22) or a major membrane-associated protein (P11) was missing. The DNA contents of viral particles did not affect their muralytic properties as the empty P9⁻ (*sus1*) mutant particles were found to be as active as wild-type virions.

In order to determine the location of protein P15 in the virion more precisely, phage membranes were isolated. PRD1-specific membranes devoid of DNA were obtained from *sus636* mutant-infected preparations in which protein capsids were not assembled. Disruption of wild-type viruses with GuHCl yielded both empty and DNA-filled membrane vesicles. Zymogram analysis revealed the presence of proteins P7 and P15 in both DNA-filled and empty phage membranes (Fig. 3a). When turbidity was measured, only the DNA-filled membranes caused a decrease in A_{450} when they were added to chloroform-treated cells (Fig. 3b). In this case the activity detected was \sim 50% of the activity associated with similar amounts of wild-type virions, most probably due to the GuHCl treatment.

Decreased amounts of receptor-binding protein P2 in P15⁻ mutant particles can be restored to wild-type levels by complementation in trans. SDS-PAGE and Western blot analysis showed that in P15⁻ mutant particles the amount of receptor-binding protein P2 was approximately 10% of the amount found in wild-type virions (P2^R P15⁻) (Fig. 4). Also, the specific infectivities obtained for P2^R P15⁻ mutant particles (6.5×10^9 , 1.1×10^9 , and 1.3×10^9 PFU/ μ g of protein in 2 \times -purified virus preparations for *sus174*, *sus232*, and *sus240* mutants, respectively) were approximately 10% of the specific

infectivity of wild-type particles ($\sim 1.0 \times 10^{10}$ PFU/ μ g of protein in a 2 \times -purified virus preparation) (6).

In order to determine whether the reduction in the amount of protein P2 in P15⁻ mutant particles was due to a decrease in the amount of protein P2 in mutant-infected cells or to an

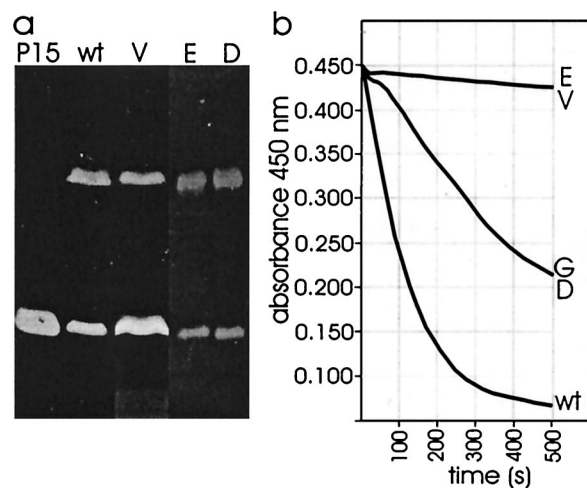


FIG. 3. Zymogram analysis (a) and turbidity measurement (b) of lytic activities associated with isolated PRD1 membrane fractions. In both cases the major membrane-associated protein P11 was used to normalize the amounts of virus particles versus membranes used in the assays. P15, partially purified protein P15 (prepared as described by Caldentey et al. [12]); wt, wild-type PRD1; G, wild-type virions disrupted with 2 M GuHCl; V, empty phage-specific membranes isolated from *sus636* mutant infections; E and D, empty and DNA-filled membranes obtained from wild-type particles disrupted with GuHCl, respectively.

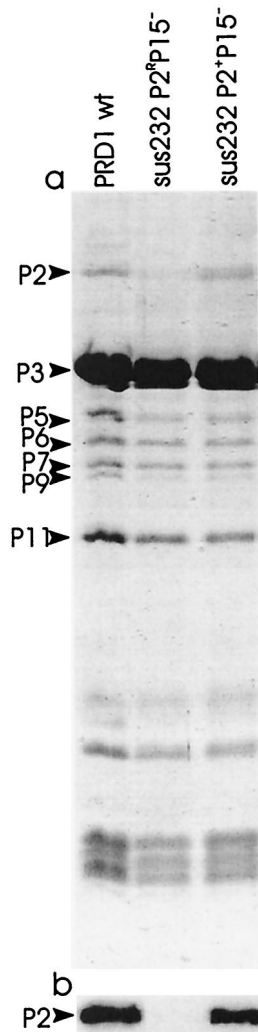


FIG. 4. SDS-PAGE analysis (a) and immunological detection (b) of protein P2 from ion-exchange chromatography-purified wild-type and P15⁻ mutant PRD1 particles. The positions of some phage proteins are indicated by arrows.

inability to assemble protein P2 into P15⁻ mutant particles, we analyzed the production of protein P2 during wild-type and mutant infections by SDS-PAGE and Western blotting with P2 antiserum. In wild-type infections, P2 was detected in samples taken 30 min postinfection or later. Similarly, in P15⁻ mutant infections, protein P2 was detected at the same times, but the levels were significantly lower.

PRD1 particles containing wild-type amounts of protein P2 but devoid of protein P15 (P2⁺ P15⁻) could be obtained by providing protein P2 in *trans*. *sus232* mutant phages were propagated in *E. coli* HMS174(pJB21)(pLM2) carrying wild-type gene *II* cloned in the pSU18 vector. Incorporation of protein P2 into mutant particles was verified by SDS-PAGE and by Western blotting (Fig. 4). The specific infectivity obtained for P2⁺ P15⁻ particles was 6.5×10^9 PFU/ μ g of protein, which was $\sim 50\%$ of the specific infectivity of ion-exchange chromatography-purified wild-type viruses (1.8×10^{10} PFU/ μ g of protein) (52).

Viral DNA replication is not delayed in the absence of protein P15. The ability of *sus232* P2⁺ P15⁻ mutant particles, as well as P2^R P15⁻ mutant particles, to bind to a host cell surface was determined by an adsorption assay (Fig. 5a). This analysis showed that P2^R P15⁻ mutant particles bind to the host cells approximately 50% less efficiently than the wild-type viruses bind. However, the level of adsorption of P2⁺ P15⁻ mutant particles was the wild-type level. Phage DNA replication was studied by monitoring the incorporation of [³H]thymidine into infected cells. *sus471* mutant particles devoid of the putative lytic transglycosylase protein P7 were used as a control because in *sus471* mutant infections the initiation of DNA replication is known to be delayed and asynchronous (46). In order to compensate for the reduction in adsorption, the MOI used in P2⁺ P15⁻ and P2^R P15⁻ mutant infections was twice the MOI used in P7⁻ (*sus471*) mutant and wild-type phage infections. In P2^R

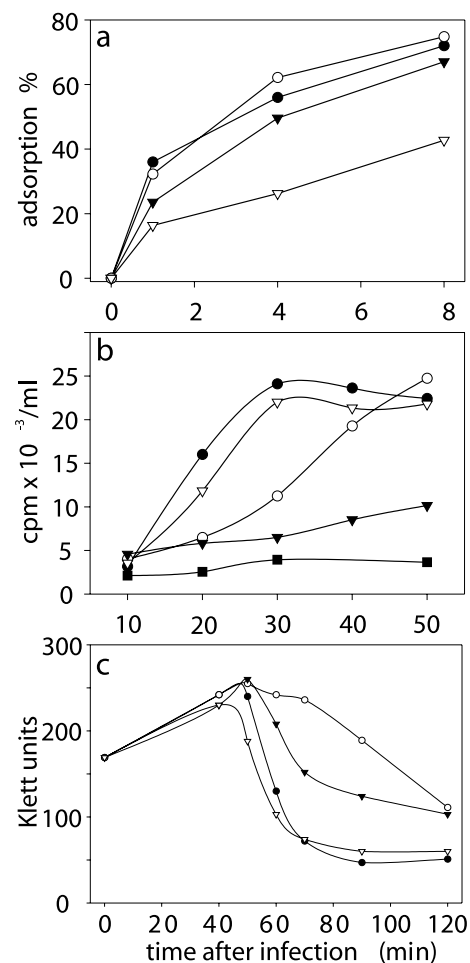


FIG. 5. (a) Adsorption of wild-type and mutant particles to DB7156(pLM2) cells. Symbols: \circ , wild-type PRD1; \bullet , P7⁻ mutant (*sus471*); \blacktriangledown , P2⁺ P15⁻ mutant (*sus232*, protein P2 provided in *trans*); ∇ , P2^R P15⁻ mutant (*sus232*). (b) [³H]thymidine incorporation into replicating DNA in DB7156(pLM2) cells treated with nalidixic acid. Nalidixic acid inhibits host DNA replication by inactivating DNA gyrase. PRD1 DNA replication is not affected. \blacksquare , DB7156(pLM2) cells not infected with PRD1. Other symbols as described above for panel a. (c) One-step growth curves for wild-type and mutant PRD1 phages in a suppressor host. Symbols as described above for panel a.

P15⁻ mutant infections the absence of protein P15 had no effect on the initiation or the rate of phage DNA replication (Fig. 5b). In P2⁺ P15⁻ mutant infections the level of viral DNA synthesis was substantially decreased. Accordingly, host cell lysis was delayed and incomplete, as shown by the one-step growth curves in Fig. 5c.

DISCUSSION

The host cell lysis induced by bacteriophage PRD1 is caused by the muralytic activity of protein P15 (35). Previously, recombinant protein P15 was purified and characterized, and it was found to be a soluble 1,4-β-N-acetylmuramidase with strong lytic activity against gram-negative peptidoglycan (12). In this study we found that it is also a structural component of the PRD1 virion.

Several lines of evidence suggest that protein P15 is associated with the internal phage membrane. Freezing and thawing purified phage particles three times led to partial dissociation of the protein capsid surrounding the membrane. Simultaneously, an approximately fivefold increase in particle-associated lytic activity was detected by turbidity measurements. When isolated PRD1 membranes were studied, the presence of protein P15 in empty and DNA-filled phage membranes could be detected by zymogram analysis. In turbidity assays only membranes containing the phage genome were associated with muralytic activity. Based on these results, we propose that the proper (spherical) conformation of the membrane vesicle is critical for positioning protein P15 correctly in order to bind and cleave the peptidoglycan substrate. The proper conformation can be maintained by supporting the membrane vesicle either internally (with the phage genome in DNA-filled membranes) or externally (with the protein capsid in empty sus1 mutant particles). The release of DNA from PRD1 particles is associated with formation of a tubular membrane structure that is inactive in the turbidity assay. In zymogram analysis proteins are separated by electrophoresis before they are re-natured, and the presence of P15 in membrane vesicles devoid of DNA is revealed. The missing (P20⁻ particles) or considerably reduced (P22⁻ particles) P15 activities in mutants devoid of two small integral membrane proteins indicate that these proteins are involved in positioning P15 in the virus particle or are responsible for providing the phage membrane integrity needed for proper binding or activity of P15. As the absence of proteins P20 and P22 results in DNA leakage from virions (36), we envision that these proteins, as well as protein P15, are located at or near the vertex. This is also consistent with protein P15 lytic enzyme activity.

Proteins P2 and P15 are translated from the same mRNA (20), and the reduced amounts of protein P2 in P15⁻ mutant-infected cells and the resulting particles are probably due to the polar effects of the amber mutation in gene *XV*. However, the polarity-caused P2 deficiency could be bypassed by providing P2 *in trans*. Although such viruses (P2⁺ P15⁻) had normal receptor-binding efficiency, they were compromised in terms of initiating infection. This implies either that in the absence of P15 protein P2 does not properly function in signaling initiation of the DNA delivery process or that P15 somehow is part of the signal transduction pathway. This also supports the idea that P15 is located at the vertex.

The lytic transglycosylase activity exhibited by protein P7 was previously shown to have an accessory role in PRD1 DNA entry (46). In the absence of this activity phage DNA replication and host cell lysis are both delayed and asynchronous, although the activity is not absolutely essential for infectivity. This could be due to the presence of another lytic enzyme in the virion. However, in the absence of protein P15 no delay in phage DNA replication was detected, suggesting that protein P7 is the main muralytic enzyme involved in bacteriophage PRD1 DNA entry into *Salmonella* cells. As our attempts to isolate P7⁻ P15⁻ double mutants have been unsuccessful so far, we do not know whether the lytic activities of proteins P7 and P15 can replace each other or whether they are dispensable for PRD1 DNA entry. *E. coli* cells are known to contain at least six different lytic transglycosylases located at the outer membrane or in the periplasmic space (17–19, 25, 26, 32, 51), and it has been proposed that these enzymes function in enlargement and division of the murein sacculus (23–25). We suppose that it would be advantageous for the infecting bacteriophage to use a lytic transglycosylase activity to locally digest the peptidoglycan layer as the enzymatic machinery needed to repair the damage would be readily available in the host cell. As a result, the host cell integrity needed for production of progeny phages would be maintained.

Bacteriophage PRD1 can infect a great variety of gram-negative hosts, including *Escherichia*, *Pseudomonas*, *Salmonella*, *Proteus*, *Vibrio*, and *Acinetobacter* species (44). It is the type virus of the family *Tectiviridae*, which also contains phages that infect gram-positive *Bacillus* species (AP50 and φNS11) (2, 41, 49). Interestingly, in zymograms protein P15 of PRD1 was able to degrade peptidoglycan isolated from the gram-positive *M. lysodeikticus*. We propose that the presence of two lytic enzymes in PRD1 might be a common feature in tectiviruses that infect gram-negative and gram-positive hosts; i.e., the tools for penetrating both types of cell walls are always available. However, it remains to be seen how closely related tectiviruses that infect gram-negative and gram-positive hosts are at the genome sequence level.

ACKNOWLEDGMENTS

We thank Marja-Leena Perälä for her excellent technical assistance. Helsinki Graduate School in Biotechnology and Molecular Biology is acknowledged for a fellowship to P.S.R. This work was supported by research grants 162993, 164298, and 168694 [Finnish Centre of Excellence Programme (2000-2005)] to D.H.B. from the Academy of Finland.

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ERRATA

The Lytic Enzyme of Bacteriophage PRD1 Is Associated with the Viral Membrane

Pia S. Rydman and Dennis H. Bamford

Department of Biosciences and Institute of Biotechnology, Viikki Biocenter, University of Helsinki, 00014 University of Helsinki, Finland

Volume 184, no. 1, p. 104–110, 2002. Page 108, legend to Fig. 5, line 2: “○” should read “●” and “●” should read “○.”

The High-Molecular-Weight Cytochrome *c* Cyc2 of *Acidithiobacillus ferrooxidans* Is an Outer Membrane Protein

Andrés Yarzábal, Gaël Brasseur, Jeanine Ratouchniak, Karen Lund, Danielle Lemesle-Meunier, John A. DeMoss, and Violaine Bonnefoy

Laboratoire de Chimie Bactérienne and Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS, IBSM, Marseille, France

Volume 184, no. 1, p. 313–317, 2002. Page 316, column 1, line 32: “Cyc2 rusticyanin” should read “Cyc2 → rusticyanin.”

Quantification of Expression of *Staphylococcus epidermidis* Housekeeping Genes with Taqman Quantitative PCR during In Vitro Growth and under Different Conditions

S. J. Vandecasteele, W. E. Peetermans, R. Merckx, and J. Van Eldere

Infectious Diseases Research Group, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, and Departments of General Internal Medicine and Microbiology and Immunology, University Hospital Leuven, Leuven, Belgium

Volume 183, no. 24, p. 7094–7101, 2001. Page 7095, Table 1, the *gmk* probe should read as follows: ATGCGTTGTTTCATAT TTTTAGCGCCTCCA.

Page 7095, Table 1, the 16S rRNA gene reverse primer should read as follows: CTTCGACGGCTAGCTCCAAAT.

Page 7095, column 2, lines 19 and 20: “900 nmol of each primer, and a 200-nmol probe” should read “900 nmol of each primer per liter, and a 200-nmol/liter probe.”