

SP02 Particles Mediating Transduction of a Plasmid Containing SP02 Cohesive Ends

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SP02 particles that mediate transduction of plasmid pPL1010, a 4.6-megadalton derivative of pUB110 containing an *EcoRI* endonuclease-generated fragment of SP02 deoxyribonucleic acid that spans the cohesive ends, exhibit three unusual features: the transducing particles have a lower buoyant density than infectious particles; the transduction of pPL1010 occurs at high efficiency; and the transducing activity of the particles is relatively resistant to ultraviolet irradiation when the recipient is recombination proficient. Evidence is presented which indicates that SP02(pPL1010) particles carry the plasmid predominantly as a linear multimer having a molecular mass comparable to that of infectious SP02 deoxyribonucleic acid (ca. 31 megadaltons). The plasmid monomers in the linear multimer appear oriented in the same polarity. The buoyant density difference between infectious and transducing particles appears to be due mainly to the buoyant density difference between pPL1010 (1.699 g/cm³) and SP02 deoxyribonucleic acid (1.702 gm/cm³).

ϕ 105 and SP02 are unrelated temperate bacteriophages which are infectious for strains of *Bacillus subtilis* (12). Both phages are incapable of transduction of several small drug resistance plasmids. Cloning various endonuclease fragments of either ϕ 105 or SP02 DNA into plasmid pUB110 or pCM194 renders the chimeric derivatives susceptible to transduction specifically by the phage whose DNA is in the chimera (16). The virus DNA fragments that render plasmids susceptible to transduction include fragments internal in the phage genome (e.g., ϕ 105 *EcoRI* fragments G, E, and I [17]) and the *EcoRI* DNA fragment that spans the SP02 cohesive ends (21). Transduction of plasmids containing inserts from internal positions within the phage genomes occurs at frequencies on the order of 10⁻⁵ to 10⁻⁸ transductants per plaque-forming unit. Transduction of plasmid pPL1010, which contains the *EcoRI* cohesive end fragment of SP02 DNA (1.6 megadaltons [Md]) inserted into pUB110 (16), occurs at a frequency of 10⁻² transductants per plaque-forming unit. The basis for transduction of plasmids containing internal fragments and the basis for transduction of pPL1010 may differ. In the present report, we describe properties of SP02(pPL1010) transducing particles and provide evidence indicating the molecular form in which pPL1010 is carried by the phage.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. *B. subtilis* 168 strains BR151 (*trpC2 metB10 lys-3*) and

BR151R (*trpC2 lys-3 recE4*) were employed (13). The bacteriophages used were ϕ 105, SP02, and the generalized transducing phage PBS1 (12). All plasmids used have been described in detail previously (16).

Growth media. Media used include tryptose-blood agar base (Difco Laboratories) and antibiotic medium 3 (Penassay broth; Difco Laboratories). When appropriate, the antibiotic chloramphenicol or neomycin sulfate was added to a final concentration of 10 μ g/ml. All incubations were at 37°C. Liquid cultures were grown with rotary shaking.

Plasmid analysis. Methods for plasmid isolation and electrophoresis of DNA through 1% agarose gels were performed as described earlier (15). Restriction enzymes and T4-induced DNA ligase were purchased from Bethesda Research Laboratories, Inc. and New England Biolabs, and each was used according to the specifications provided by the supplier. Southern transfers, nick translation, and hybridization were performed as detailed previously (16, 18).

Propagation of phages, transduction, and isolation of SP02(pPL1010) transducing particles. Phages were propagated and phage assays and transductions were performed as previously described (16). Isotopically labeled phage DNA was prepared by including 100 μ Ci of carrier-free ³²P (as H₃PO₄; New England Nuclear Corp.) per ml of the semisolid agar overlays. SP02(pPL1010) transducing particles have a lower buoyant density than infectious particles (16). Centrifugation of a crude SP02(pPL1010) lysate in CsCl with a Ti50 fixed-angle rotor resolved the transducing particles as a light density band (see Fig. 1). This band, referred to as enriched transducing particles, was removed, dialyzed against TM buffer (15), and extracted with phenol (4). The resulting DNA was then analyzed by gel electrophoresis and sucrose gradient centrifugation.

UV irradiation of transducing lysates. ϕ 105, SP02, or PBS1 transducing lysates were centrifuged at 40,000 rpm for 2 h in a Ti50 rotor. The pellets were overlaid with 0.1 the volume of the original lysate by using 0.05 M Tris buffer containing 10^{-3} M $MgSO_4$ and 20 μ g of bovine serum albumin per ml. After sitting at 4°C for 20 h, the pellet was suspended and sterilized by filtration. The phage was diluted to approximately 10^8 to 10^9 plaque-forming units per ml in the sterile buffer. Five milliliters was placed in a sterile 25-ml glass petri dish. Irradiation of the phage was performed with a Sylvania germicidal UV light at a distance of 10 or 20 cm. The maximum irradiation time was 6 min. During irradiation, samples were periodically removed and subsequently assayed for infectious centers and transducing activity. All manipulations were performed in subdued light. The plates were incubated in a lightproof container. Under these conditions, the rates of inactivation of plaque-forming activity of ϕ 105, SP02, and PBS1 were nearly identical.

Sucrose gradient centrifugation. Neutral 5 to 20% sucrose gradients were prepared as previously described (4). Lambda phage DNA, labeled with [3H]thymidine, was taken to have a sedimentation velocity of 33.6 and a molecular weight of 31×10^6 (5). Lambda DNA and DNA from SP02 particles were heated at 65°C for 25 min before being applied to gradients. After centrifugation of the ^{32}P -labeled SP02 DNA and 3H -labeled lambda DNA (see figure legends), fractions were collected, precipitated with cold 5% trichloroacetic acid, and counted in a double-label mode.

Analytical ultracentrifugation. Equilibrium centrifugation of DNA in a model E analytical ultracentrifuge was performed as described earlier (14). PBS1 DNA ($\rho = 1.722$ g/cm 3) was the reference.

RESULTS

Effect of UV irradiation on transduction of cos-containing plasmids. Plasmid pPL1010, 4.6 Md, is transduced by SP02 particles at a frequency of about 1 transductant per 100 plaque-forming units (16). SP02 transducing particles carry pPL1010 in a form of higher molecular weight than monomeric pPL1010 (16). Since SP02(pPL1010) transducing particles have a lower buoyant density than infectious particles (Fig. 1), we suspected that the transducing particles carried pPL1010 as a multimer and that these transducing particles lacked an infectious viral genome. Arber (1) demonstrated that UV irradiation of a plasmid transducing lysate caused an immediate reduction in the ability of the phage to generate plasmid-containing transductants. This reduction, we presume, is due to the damage of plasmid genes essential for maintenance or expression in the recipient. We believed that if pPL1010 were carried as a multimer by SP02 particles, the transducing activity of SP02(pPL1010) lysates would be more resistant to UV irradiation than if the plasmid were carried as a monomer because of complementa-

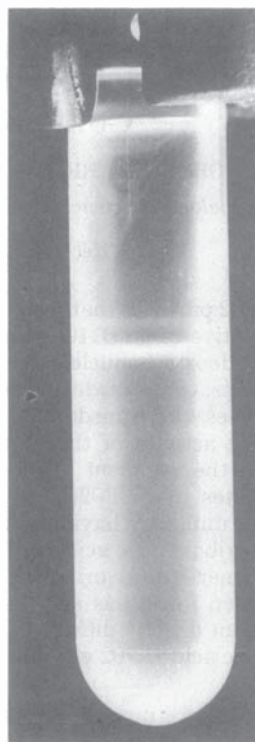


FIG. 1. Partial purification of SP02(pPL1010) transducing particles by equilibrium centrifugation in cesium chloride. SP02 particles (10^{12}) propagated on BR151(pPL1010) were mixed with 5 ml of a cesium chloride solution in 0.05 M TM buffer (pH 7.5). The initial average buoyant density was 1.5 g/cm 3 . Centrifugation was for 36 h in a Ti50 rotor at an rpm of 36,000. The upper and lower bands were removed with a syringe. The upper band contained 2×10^{10} plaque-forming units and 1.5×10^{10} transducing particles. The lower band contained 8×10^{11} plaque-forming units and 1.8×10^9 transducing particles.

tion among the subunits of the multimer or through recombination among the subunits in the transductants, or both. Exposure of an SP02(pPL1010) transducing lysate to UV irradiation sufficient to reduce the infectious titer 99.9% caused only a 50% reduction in transducing activity when a recombination-proficient transduction recipient was used and a 99.9% reduction in transducing activity when a recombination-deficient transduction recipient was used (Fig. 2). PBS1 transduction of pPL1010 occurred at a frequency of 10^{-7} transductants per plaque-forming unit. UV irradiation of a PBS1(pPL1010) transducing lysate inactivated transducing activity by more than 99.9% under conditions that caused only a 50% reduction in SP02(pPL1010) transduction. This result was consistent with our view that pPL1010 is carried

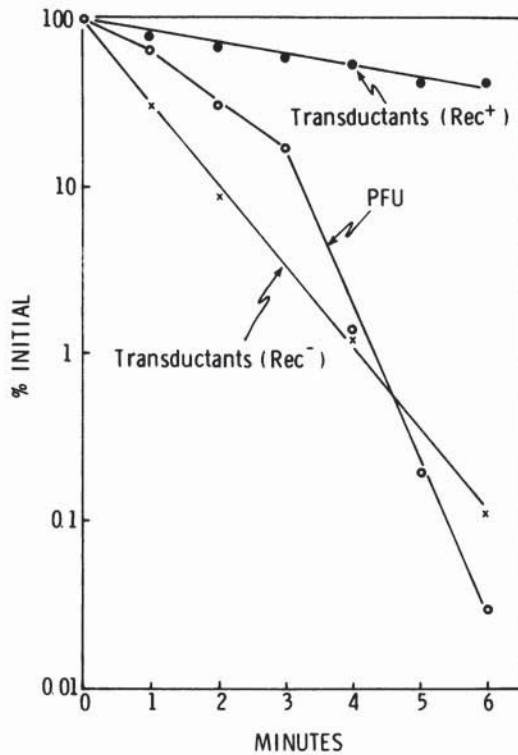


FIG. 2. Inactivation of plaque-forming activity and transducing activity of an SP02(pPL1010) transducing lysate by UV irradiation. The initial plaque-forming activity of the lysate was 1.4×10^8 plaque-forming units (PFU) per ml when assayed on BR151 or BR151R. The initial transducing activity was 1.1×10^6 transductants per ml with BR151 as the recipient and 1.3×10^6 transductants per ml with BR151R as the recipient. Irradiation was performed at a distance of 20 cm.

as a multimer in SP02 particles, but the plasmid is probably carried as a monomer by a generalized transducing phage such as PBS1.

The resistance of the transducing activity of SP02(pPL1010) particles to inactivation appeared to be unique to this plasmid because the transducing activity of $\phi 105$ (pPL1010), SP02(pPL1012), and SP02(pPL1011) lysates (16) was inactivated by UV irradiation at approximately the same rate as or more rapidly than the inactivation of infectious centers. Thus, the transducing activity of these lysates was much more sensitive to irradiation than SP02(pPL1010) lysates. Plasmid pPL1010 itself did not appear to mediate radiation resistance, since the irradiation sensitivity of $\phi 105$ transduction of pPL1006 was not altered regardless of whether or not the BR151 recipient harbored pPL1010 (data not shown). Moreover, BR151 and BR151(pPL1010) appeared equally sensitive

to killing by UV irradiation (data not shown).

To test whether the resistance of SP02(pPL1010) transducing activity to UV irradiation was specifically related only to the presence of *cos* in the plasmid or whether the recognition of *cos* during packaging by SP02 was important, we constructed a *cos* plasmid that could be transduced by both $\phi 105$ and SP02. pPL1017 (4.6 Md) was generated by excising a 0.6-Md *Eco*RI fragment from the 1.62-Md $\phi 105$ immunity fragment in pPL1004 (16) and inserting into this site the 1.6-Md SP02 *cos* fragment. pPL1017 was transduced by $\phi 105$ and SP02 at the respective frequencies of 10^{-5} and 10^{-2} transductants per plaque-forming unit. The transduction of pPL1017 by $\phi 105$ was much more sensitive to UV irradiation than the transduction of pPL1017 by SP02 (Fig. 3). It is therefore likely that the physical state of the plasmid which determines radiation resistance is a function of the *cos* region and the recognition of this region by SP02.

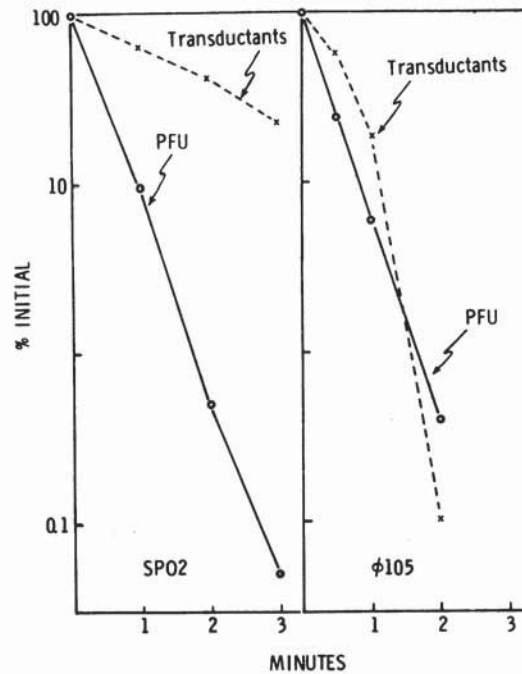


FIG. 3. Inactivation of plaque-forming activity and transducing activity of SP02(pPL1017) and $\phi 105$ (pPL1017) transducing lysates. The SP02(pPL1017) lysate had an initial titer of 2×10^8 plaque-forming units (PFU) per ml and generated 6.8×10^5 transductants per ml with BR151 as the indicator. The $\phi 105$ (pPL1017) lysate had an initial titer of 2.4×10^9 plaque-forming units per ml with BR151 as the indicator. The transducing activity of the lysate, 3.7×10^6 /ml, was assayed on BR151($\phi 105$). Irradiation was at a distance of 10 cm.

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Analysis of DNA from SP02 and enriched SP02(pPL1010) transducing particles by gel electrophoresis, velocity centrifugation, and equilibrium centrifugation. DNA from SP02 infectious particles has been previously reported to consist of primarily linear molecules having cohesive termini (7). The molecular mass of SP02 DNA has been determined as 26 Md by electron microscopy (7) and 23 Md by adding the molecular masses of restriction fragments (21). We began determining an approximate molecular weight for SP02 DNA by two methods. Lambda DNA, DNA from infectious (nontransducing) SP02 particles, and DNA from enriched SP02(pPL1010) transducing particles were heated at 65°C for 25 min, rapidly chilled, and electrophoresed through a 1% agarose gel (Fig. 4). DNA from both types of SP02 particles was resolved into two bands, designated A and B (Fig. 4). The majority of SP02 DNA was in band A, which migrated slightly slower than the linear form of λ DNA. It is likely that band A represents the linear form of SP02 DNA. The molecular weight of band A appeared slightly greater than the molecular weight of λ DNA. We suspect that band B consists of the open circular form of SP02 DNA. SP02 DNA migrating in a gel to a position expected for the covalently closed form was seen only once among several preparations examined and represented a very minor component. On the basis of the electrophoresis experiments, it was not possible to distinguish DNA isolated from enriched trans-

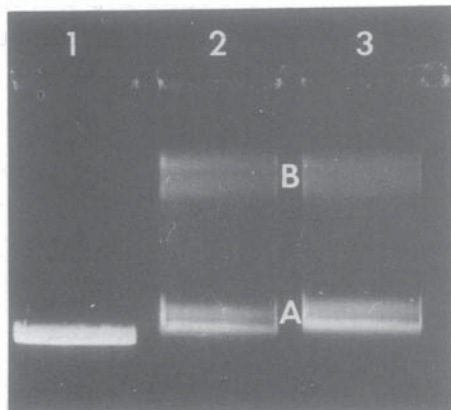


FIG. 4. Agarose gel electrophoresis of λ DNA, DNA from infectious SP02 particles, and DNA from enriched transducing SP02(pPL1010) transducing particles. Each DNA species (ca. 2 μ g) was heated at 65°C for 20 min and then rapidly chilled. Well 1, λ DNA; well 2, DNA from enriched transducing particles; well 3, DNA from infectious (nontransducing) SP02 particles. Electrophoresis was at 36 V for 27 h at room temperature.

ducing particles from DNA isolated from SP02 infectious particles.

32 P-labeled DNA isolated from infectious SP02 particles or enriched transducing particles was sedimented through 5 to 20% neutral sucrose gradients. In different preparations, 70 to 90% of the total 32 P-labeled DNA precisely cosedimented with a 3 H-labeled λ DNA standard. This material, we believe, represented the linear form of SP02 infectious or transducing DNA, and we assigned this species a molecular weight of 31×10^6 (5). The remainder of the 32 P-labeled SP02 DNA sedimented slightly faster at the approximate position expected for the open circular form of a 31-Md molecule (8). We were unable to detect any covalently closed circular DNA forms in one preparation of infectious DNA and one preparation of transducing DNA by sedimentation analysis in alkaline sucrose gradients or by centrifugation in cesium chloride-ethidium bromide gradients. It is likely that the SP02 DNA that cosedimented with λ DNA corresponds to band A detected in gel electrophoresis. Band A was resolvable from λ DNA in gel electrophoresis but not in the sucrose gradients. At present, we do not know whether this is due to the high resolving power of the gel system or to some unknown feature of SP02 DNA. It is likely that the presumed open circular form of SP02 DNA detected in sucrose gradients corresponds to band B in the agarose gel electrophoresis experiments.

To determine the amount of pPL1010 DNA in the enriched transducing particles, we sought a restriction endonuclease that would selectively cleave the plasmid but not the SP02 genome. SP02 DNA is not sensitive to *Bam*HI endonuclease, whereas pPL1010 has one *Bam*HI site located in the vector portion of the plasmid (16). 32 P-labeled DNA from the enriched transducing particles was digested with *Bam*HI and centrifuged with 3 H-labeled λ DNA through a 5 to 20% sucrose gradient. More than 90% of the 32 P-labeled DNA recovered from the gradient sedimented at about 17S, the expected position for the linear monomeric form of pPL1010 (19). Only 10% of the 32 P-labeled DNA cosedimented with λ DNA. This suggested that the majority of the DNA from the enriched transducing particles was pPL1010 and not the SP02 genome. This result appeared inconsistent with the fact that the enriched transducing phage band in a CsCl gradient generally was found to contain about equal numbers of infectious particles and transducing particles (Fig. 1). However, as shown below, the DNA from the enriched transducing particle band did contain significantly more DNA having the buoyant density of

pPL1010 than DNA having the buoyant density of SP02 DNA. Thus, it seems likely that a fraction of SP02 particles containing pPL1010 did not score as transductants in our assay.

The buoyant densities of pUB110 ($\rho = 1.697$ g/cm³), pPL1010 ($\rho = 1.699$ g/cm³), and SP02 DNA ($\rho = 1.702$ g/cm³) were determined by centrifugation of 2 μ g of each species with PBS1 DNA ($\rho = 1.722$ g/cm³) for 24 h in CsCl in a model E analytical ultracentrifuge. DNAs extracted from the upper (enriched transducing) band and lower (plaque-forming) band and isolated from a preparative CsCl gradient (Fig. 1) were centrifuged to equilibrium with PBS1 DNA (Fig. 5). DNA from the enriched transducing particles contained two DNA species having buoyant densities of 1.699 g/cm³ (buoyant density of pPL1010) and 1.702 g/cm³ (buoyant density of SP02 DNA). The former species appeared in a larger quantity than the latter. DNA from the lower band in a preparative CsCl gradient (plaque-forming band) contained predominantly the latter species (Fig. 5).

Evidence that pPL1010 is carried by SP02 predominantly as a linear multimer. Nearly 90% of the DNA isolated from enriched transducing particles appeared to be pPL1010, as evidenced by the *Bam*HI digestion experiment and the buoyant density analyses. Therefore, pPL1010 must be carried by transducing particles in a form that is indistinguishable from SP02 infectious DNA by gel electrophoresis or velocity centrifugation. We thought the plasmid was carried by transducing particles as a linear multimer and not as a recombinant between plasmid and the infectious SP02 genome because of the resistance of the plasmid transducing activity to UV irradiation and the detection of a major DNA species from transducing particles which had a buoyant density identical to that of pPL1010 DNA. Digestion of DNA from transducing particles with a restriction enzyme that specifically cuts pPL1010 but not infectious SP02 DNA (e.g., *Bam*HI) or with an enzyme that cleaves infectious SP02 DNA but not pPL1010 (e.g., *Sst*I) was therefore tested. Agarose gel electrophoresis of SP02 (pPL1010) DNA digested to near completion with *Bam*HI generated three distinct species having molecular masses of 4.6, 3.3 and 1.3 Md (Fig. 6). These three species hybridized with nick-translated pUB110; hence, each represented a portion of pPL1010. Since the DNA was not completely digested, the position of minor partially digested products in the autoradiogram could still be detected (Fig. 6). If pPL1010 were carried by transducing particles predominantly as a circle, then digestion with *Bam*HI should have gener-

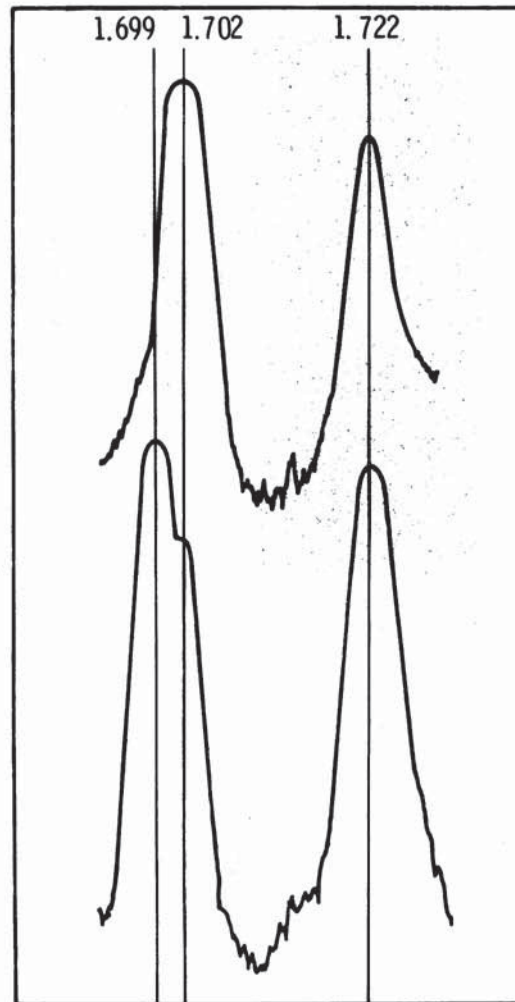


FIG. 5. CsCl equilibrium centrifugation of DNA from the enriched plaque-forming particles in a model E analytical ultracentrifuge. Two micrograms of DNA from the upper band in Fig. 1 (lower tracing, this figure) and 2 μ g of DNA from the lower band in Fig. 1 (upper tracing, this figure) were centrifuged to equilibrium (44,000 rpm for 24 h) with 2 μ g of PBS1 DNA ($\rho = 1.722$ g/cm³).

ated only the monomeric linear form of pPL1010 (4.6 Md). Since two additional bands were detected, 3.3 and 1.3 Md, it is likely that pPL1010 is carried as a multimeric linear, and the 3.3 and 1.3 Md fragments represent the *Bam*HI-generated ends of the linear. Only three species were detected in the range of 1.3 to 4.6 Md. Therefore, the polarity of the monomeric subunits in the multimer must be the same.

pPL1010 contains no *Sst*I-sensitive sites, but the enzyme digested SP02 infectious DNA into seven fragments having molecular masses of

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