Bacteriophage P22 In Vitro DNA Packaging Monitored by Agarose Gel Electrophoresis: Rate of DNA Entry into Capsids

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Bacteriophage P22, like other double-stranded DNA bacteriophages, packages DNA in a preassembled, DNA-free procapsid. The P22 procapsid and P22 bacteriophage have been electrophoretically characterized; the procapsid has a negative average electrical surface charge density (σ) higher in magnitude than the negative σ of the mature bacteriophage. Dextrans, sucrose, and maltose were shown to have a dramatic stimulatory effect on the in vitro packaging of DNA by the P22 procapsid. However, sedoheptulose, smaller sugars, and smaller polyols did not stimulate in vitro P22 DNA packaging. These and other data suggest that an osmotic pressure difference across some particle, probably a capsid, stimulates P22 DNA packaging. After in vitro packaging was optimized by including dextran 40 in extracts, the entry kinetics of DNA into P22 capsids were measured. Packaged DNA was detected by: (i) DNA-specific staining of intact capsids after fractionation by agarose gel electrophoresis and (ii) agarose gel electrophoresis of DNase-resistant DNA after release of DNase-resistant DNA from capsids. It was found that the first DNA was packaged by 1.5 min after the start of incubation. The data further suggest that either P22 capsids with DNA partially packaged in vitro are too unstable to be detected by the above procedures or entry of DNA into the capsid occurs in less than 0.25 min.

All double-stranded DNA bacteriophages that have been studied package DNA in vivo in a preformed, DNA-free procapsid. For all sufficiently studied bacteriophages, the external surface of the procapsid differs from the external surface of the mature bacteriophage capsid in having a rounder shape and smaller radius (reviewed in references 8, 9, 22, and 39). In the case of bacteriophage T7, the procapsid was also shown to have a negative average electrical surface charge density (σ) higher in magnitude than the negative σ of the mature bacteriophage capsid (37, 38). For analysis of DNA packaging, it is desirable to have packaging as synchronous as possible and to maximize control of the compounds present during packaging. To help accomplish these goals, DNA is packaged in extracts of bacteriophage-infected cells (in vitro). DNA packaging in vitro has been achieved with bacteriophages φ29 (2–4), λ (1, 12), P22 (23, 24), P2 (25), T3 (11, 21), T7 (17–19, 27, 36), and T4 (5). In all of these studies, production of an infective particle was used as the assay for DNA packaging, and it was found that production of an infective particle required either ATP or some other ribonucleoside triphosphate. In the case of T7, a 10- to 50-fold stimulation of in vitro infectious particle assembly was observed in the

presence of dextrans and some smaller, related compounds (36).

To determine the rate at which DNA enters bacteriophage capsids in vitro and to determine the effects of added compounds on this rate, it is necessary to have an assay for DNA entry into capsids, independent of other assembly events needed for an infective particle. Thus far, only bacteriophages T3 and φ29 have been made to package DNA efficiently enough to assay physically for packaged DNA. In these studies (T3 [21]; φ29 [3, 4]), velocity sedimentation in sucrose gradients, sometimes after DNase treatment, was used to detect capsids with packaged DNA. However, no attempt was made to measure the rate of DNA entry. In addition, velocity sedimentation in sucrose gradients has the following limitations as an assay for DNA entry. (i) The state of the capsid (procapsid or its larger, more angular conversion product) is not reliably determined (4). (ii) DNA may empty from capsids during the assay. (iii) The cost (in time and materials) can become excessive during studies of entry rates.

A possible alternative DNA packaging assay for overcoming limitations (i) and (iii) above is DNA-specific staining of DNase-resistant DNA comigrating during agarose gel electrophoresis

with bacteriophage capsids (33, 37). However, there is no reason for believing that this procedure would assist in overcoming limitation (ii). To help overcome this limitation, agarose gel electrophoresis of DNase-resistant DNA after release from the capsid (no prefractionation) is a possible procedure. This latter procedure, unlike the former, can also be used to determine the length of partially packaged DNA.

Bacteriophage P22 has a linear, double-stranded DNA genome. P22 DNA has a nonrandom permutation of its ends (26, 42) and a molecular weight, determined by high-resolution agarose gel electrophoresis (30) of the intact genome, of $27.9 \pm 0.3 \times 10^6$ (P. Serwer and S. J. Hayes, unpublished data). P22 has a spherical procapsid (6, 7, 10, 15, 16) with an in vitro DNA packaging activity (23, 24) which is stable for months during storage (see Results). The P22 procapsid has an internal protein, p8 (P22 proteins are indicated by p, followed by the gene number of the protein [6]), that leaves the capsid during packaging (15). The P22 procapsid has, however, not yet been characterized electrophoretically, and in studies of in vitro DNA packaging, no assay but production of an infectious particle has been used for DNA packaging (23, 24). Therefore, in the present study, the P22 procapsid and mature bacteriophage were electrophoretically characterized, procedures for increasing the efficiency of in vitro packaging were developed, and the entry of DNA into capsids was observed as a function of time by using the two procedures of agarose gel electrophoresis described above. Implications of the results for understanding in vitro P22 DNA packaging are discussed.

MATERIALS AND METHODS

Strains. All bacterial strains used were derivatives of *Salmonella typhimurium* LT2 from the collection of D. Botstein (received from either D. Botstein or J. King). The permissive host for P22 amber mutants was DB7002; the nonpermissive host for amber mutants was DB7000. To make DNA donor extracts for in vitro DNA packaging, induction of strain DB7130, lysogenized with P22 carrying an amber mutation in gene 5 (major capsid protein), was used. This strain has been described previously (23). P22 carrying an amber mutation in gene 2 (referred to as 2*am*; mutant number H200) was received from J. King and has been described previously (6). This bacteriophage contained, in addition, clear-plaque mutation CI-7 and an amber mutation in gene 13 (lysis; mutant H101). Particles from lysates of the nonpermissive host infected with an amber mutant are referred to by the number of the mutant gene. Bacteriophage P22 with the CI-7 and H101 mutations (to be referred to as wild type) and 9⁻ (tail spikeless) P22 (7) were received in purified form from J. King; 17⁻ bacteriophage T7 (tail fiberless) was prepared as previously described (33).

Media and reagents. For preparation of capsids and

extracts, 2 × LB (28) (20 g of tryptone [Difco Laboratories], 10 g of yeast extract, and 5 g of NaCl in 1 liter of water) was used. Bacteriophages were stored in Tris-Mg buffer (0.2 M NaCl, 0.01 M Tris-Cl [pH 7.4], 0.001 M MgCl₂). Standard buffer was 0.15 M NaCl, 0.05 M Tris-Cl (pH 7.4), and 0.005 M EDTA. Electrophoresis buffer A was 0.05 M sodium phosphate (pH 7.4) and 0.001 M MgCl₂. Electrophoresis buffer B was 0.05 M sodium phosphate, (pH 7.4) and 0.001 M EDTA. Sample buffer A was 0.005 M sodium phosphate (pH 7.4), 0.001 M MgCl₂, 4% sucrose, and 400 μg of bromophenol blue per ml. Sample buffer B was the same as sample buffer A, with 0.001 M EDTA replacing 0.001 M MgCl₂. TSMB buffer used for in vitro assembly was 0.01 M Tris-Cl (pH 7.4) 0.06 M spermidine-hydrochloride, 0.2 M MgCl₂, and 0.03 M 2-mercaptoethanol. NET buffer was 0.1 M NaCl, 0.01 M Tris-Cl (pH 7.4) and 0.001 M EDTA. Agarose was purchased from Marine Colloids, Rockland, Maine. Unless otherwise indicated. ME-grade agarose was used. Dextrans were purchased from Pharmacia Fine Chemicals. The osmotic pressure (*P*) of sugar solutions was obtained by linear extrapolation of data (14).

Production and partial purification of procapsids. To prepare 2⁻ procapsids, a 1-liter, log-phase culture of *S. typhimurium* DB7000 was infected at $2 \times 10^8/cm^3$ at 37°C with 2*am* P22 (multiplicity of infection, 5). Aeration of the culture was continued at 37°C for 2 h. The infected cells were pelleted at 10,000 rpm and 4°C for 10 min in a Beckman JA-14 rotor. The pellet was suspended in 2 ml of standard buffer, and 67 μl of 1-mg/ml lysozyme in standard buffer was added. After incubation at 4°C for 20 min, 67 μl of 10% Brij 58 was added, and incubation was continued at 4°C for 20 min, followed by incubation for 60 min at room temperature (25 ± 3°C). At this time, at least 99% of the cells had lysed. After the addition of 10 μl of 1 M MgCl₂, the lysate was digested with 15 μl of 1-mg/ml DNase I (Millipore Corp.) at 30°C for 1 h, followed by 67 μl of 1-mg/ml boiled pancreatic RNase in 0.05 M Tris-Cl (pH 7.4) at room temperature for 15 min.

Capsids in the lysate were partially fractionated by being layered on a discontinuous cesium chloride gradient previously described (29) and centrifuged for 180 min at 40,000 rpm and 18°C in a Beckman SW41 rotor. The capsids (density ≃ 1.3 g/ml) were removed from the gradient through a punctured tube bottom, were diluted 1:1 with Tris-Mg buffer, and were clarified by centrifugation at 5,000 rpm and 4°C for 5 min in a Beckman J-21 rotor. The supernatant was brought to a volume of 5.2 cm³ and a density of 1.28 g/cm³ with cesium chloride and was subjected to buoyant density sedimentation at 40,000 rpm and 10°C for 20 h in a Beckman SW50.1 rotor. The capsid band was removed by pipetting from the top and was dialyzed against Tris-Mg buffer.

Production and purification of wild-type P22 bacteriophage. Lysates of wild-type P22-infected cells were prepared as described above. Bacteriophages were purified from these lysates as previously described for bacteriophage T7 (29).

Electrophoresis in metrizamide density gradients. Electrophoresis in metrizamide density gradients was performed by diluting one part of sample in Tris-Mg buffer with 1.2 parts of sample buffer A and by subjecting this mixture to electrophoresis with electrophoresis buffer A at 25°C as previously described (38).

For the larger preparations, a 1.0-cm (inner diameter) gel tube was used instead of the 0.6-cm tube used previously (38). As much as 20 mg of procapsid had been fractionated with a single 1-cm tube without obvious distortion caused by high concentrations. Dimerization of a T7 procapsid does not alter its solid support-free electrophoretic mobility (μ) (31), indicating that P22 procapsid aggregation would not alter its profile during electrophoresis in metrizamide density gradients.

Fractionation of capsids by agarose gel electrophoresis. To 35 μl of a sample was added 10 μl of sample buffer A. Of this mixture, 30 μl was layered in sample wells of a horizontal agarose gel cast in and submerged beneath electrophoresis buffer A. Electrophoresis was performed at 0.96 V/cm and at room temperature for 16 h as previously described (33). Gels were stained with ethidium bromide (DNA specific) after the packaged DNA was emptied (33). Subsequently, gels were stained with Coomassie brilliant blue (protein specific) (37).

Fractionation of DNase-resistant (packaged) DNA by agarose gel electrophoresis after release from capsids. Before releasing DNA from capsids for electrophoresis, it was necessary to inhibit DNases. This was done by adding a 0.06 volume of 0.2 M EDTA (pH 7.4) and, after 10 min at room temperature, adding a 0.09 volume of 10% Sarkosyl NL97 (Ciba-Geigy Corp.) in water. To release DNA from capsids, this mixture was incubated at 75°C for 15 min. Some samples were subsequently diluted in NET buffer. To 39 μl of the final mixture was added 10 μl of sample buffer B, and 30 μl of this mixture was layered in sample wells of a horizontal agarose gel cast in and submerged beneath electrophoresis buffer B, as described above. For high resolution of mature P22-sized DNAs, electrophoresis was conducted at 0.34 V/cm and at room temperature in a 0.25% gel for 3 to 4 days, as previously described (30). For all other DNA fractionations, electrophoresis was conducted at 1 V/cm and at room temperature for 16 h.

Measurement of solid support-free μ and related parameters. To determine the μ of a particle in the absence of a solid support (μ_0), μ values measured by agarose gel electrophoresis at 25.0°C were extrapolated to an agarose concentration of zero and corrected for electroosmosis, as previously described (34), by using either ME or HGT[P]agarose. From μ_0 and the particle radius (see Table 1), σ and total surface charge number (z; if R is the particle radius, $z = 4\Pi\sigma R^2$) were calculated as previously described (37).

Preparation of DNA donor extracts for in vitro assembly. *S. typhimurium* DB7130 was grown to 2×10^8/cm^3 in $2\times$ LB with aeration at 28°C. This culture was shifted to 38°C and incubated for an additional 90 min. The cells were pelleted at 10,000 rpm and 4°C for 10 min in a J-21 rotor and were suspended in a 1/200 volume of 0.05 M Tris-Cl (pH 7.4) containing a sufficient concentration of the indicated dextran, sugar, or polyol to give the indicated final concentration. The cells were slowly frozen in a -20°C freezer overnight and were thawed at 35°C. A 1/10 volume of 3-mg/ml lysozyme in 0.25 M Tris-Cl (pH 7.4) was added, and the mixture was incubated for 30 min on ice. Subsequently, a 1/10 volume of TSMB buffer and of 0.3 M ATP in 0.25 M Tris-Cl (pH 7.4) were added.

In vitro assembly. Unless 40% dextran was used, in vitro assembly was conducted by adding 15 μl of procapsid preparation to 15 μl of the DNA donor extract and by incubating at the indicated temperature for the indicated time. To terminate packaging, samples were transferred to an ice bath, and 2 μl of 1-mg/ml DNase I in water was added, followed by incubation at 30°C for 30 min. Termination of packaging with EDTA was also used in some experiments, but it did not affect the results. For packaging in 40% solutions of dextrans, 10 μl of procapsid preparation and 20 μl of extract were used as described above.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as previously described (41). A 10% gel was used, and proteins were detected by staining with Coomassie blue (29).

Quantitation of DNA packaged. To determine the amount of DNA packaged in capsids after agarose gel electrophoresis, the integrated intensity of ethidium bromide-stained bands was determined by scanning fluorimetry of the orange fluorescence of the bands. Fluorescence was excited by a short-wave UV light bulb in an Helena R and D scanning densitometer-fluorimeter. A Wratten 23A (orange) filter was placed over the entrance slits for the photomultiplier tube. To convert integrated intensities into amounts of DNA, the integrated intensities of bands formed by serially diluting a known concentration of 17$^-$ T7 were obtained in the same gel. From these data, a calibration curve was constructed for determining the amount of DNA from integrated intensities of the DNA packaged in vitro by P22. The amount of protein forming Coomassie blue-stained bands was determined by densitometry, performed with the above-mentioned densitometer.

RESULTS

Electrophoresis of the P22 procapsid. To isolate the P22 procapsid, 2^- procapsids, prefractionated as described above, were subjected to electrophoresis in a metrizamide density gradient. To assay for procapsids after fractionation of this gradient, portions of each fraction were subjected to agarose gel electrophoresis. A band further from the origin of electrophoresis than the band formed by mature bacteriophage P22 was observed in fractions 21 through 25 of the metrizamide gradient (Fig. 1a). Equal portions of these and adjacent fractions were diluted into capsid-defective extracts for the assembly of infective P22. The titer of infective particles obtained was roughly proportional to the integrated intensities of the band in Fig. 1a for all fractions (data not shown; see also below). This observation suggests that the band of Fig. 1a is formed by the P22 procapsid, a conclusion confirmed by electron microscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7, 10, 16) of fractions in the gradient of Fig. 1 (data not shown).

In the preparation of Fig. 1, the distance migrated by procapsids in the agarose gel was independent of the metrizamide gradient frac-
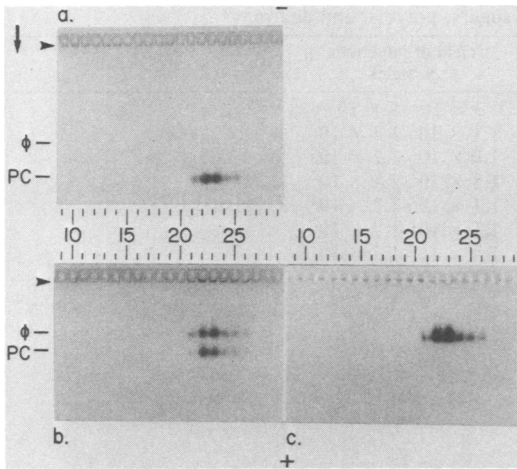
FIG. 1. Electrophoresis of the P22 procapsid in a metrizamide density gradient. A sample of 2^- P22 procapsids, prepared and prefractionated as described in the text, was subjected to electrophoresis in a metrizamide density gradient for 7 h. A portion of each fraction was subjected to agarose gel electrophoresis, followed by staining with Coomassie blue. Subsequently, an equal portion of each fraction was used for in vitro assembly at 35°C for 1 h in the presence of 20% dextran 40, as described in the text. After incubation, the reaction mixtures were subjected to agarose gel electrophoresis, followed by staining with ethidium bromide and Coomassie blue. (a) No incubation, staining with Coomassie blue. (b) Incubation, staining with Coomassie blue. (c) Incubation, staining with ethidium bromide (contrast reversed). The origins are indicated by arrowheads. Procapsids (PC) and bacteriophage (φ) are indicated. The direction of electrophoresis in the agarose gel is indicated by the arrow. The direction of electrophoresis in the metrizamide gradient is left to right. The voltage gradient for (b) and (c) dropped during electrophoresis because of a defective power supply. It is for this reason that the procapsid migration distance in (b) is less than it is in (a).

tion from which the procapsid was taken, ±2%. However, the distance migrated in agarose gels by 2^- procapsids from other preparations increased significantly with the distance migrated in the metrizamide gradient, up to 7%. A similar

heterogeneity of the T7 procapsid has been observed (19), but the cause of the heterogeneity is not known in either case. P22 procapsids, purified by electrophoresis in metrizamide density gradients, usually do not lose more than 25% of their DNA-packaging activity during storage for times up to 3 months at 4°C without dialysis of metrizamide (longer storage times have not been tried).

The μ of the P22 procapsid in metrizamide gradients is higher in magnitude than the μ of most negatively charged, contaminating host proteins. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, it was found that the electrophoresis shown in Fig. 1 separated the P22 procapsid from more than 95% of the contaminating host proteins. No host proteins (<2% of the amount of p5, the major P22 capsid protein [7]) were detected in the capsid-containing regions of the gradient of Fig. 1 (data not shown).

The μ_0, σ, and z of the P22 procapsid, phage P22, and 9^- P22 have been determined (Table 1). As previously found for bacteriophage T7 (37, 38), the P22 procapsid has a negative μ_0 higher in magnitude than the negative μ_0 of the mature bacteriophage. The magnitude of the 9^- P22 μ_0 was slightly, but significantly, higher than the magnitude of the P22 μ_0. This indicates a net positive external charge of p9 (tail spikes [7]). The difference in the μ_0 and sizes of procapsid and bacteriophage (Table 1) results in a difference in banding position during agarose gel electrophoresis. Agarose gel electrophoresis can be used to distinguish these two particles (Fig. 1). The μ of the mature P22 capsid is independent of the presence of packaged DNA (see below), as previously shown for T7 (37).

Detection of packaged DNA after in vitro assembly. To assay physically for the entry of P22 DNA into capsids, DNA-specific staining of capsids fractionated by agarose gel electrophoresis might be used (see above). To test this procedure, in vitro assembly, optimized as described below, was performed with portions of fractions of the metrizamide gradient shown in Fig. 1. Assembly was followed by agarose gel

TABLE 1. μ_0, radius, σ, and z

Structure	Radius (nm)	$-\mu_0$ (cm²/V · s × 10^{-4})[a]	$-\sigma$ (ESU/cm² × 10^3)[a]	$-z$ (× 10^2)[a]
P22	31.4 ± 0.2[b]	1.47 ± 0.08	4.8_6	12.5
9^- P22	31.4 ± 0.2[c]	1.52 ± 0.08[d]	5.0_3	13.0
Procapsid	29.6 ± 1.0[b]	1.72 ± 0.08	5.6_9	13.0

[a] μ_0, σ, and z were determined as described in the text.
[b] Radii were previously determined by low-angle, X-ray scattering (10). ESU, Electrostatic charge units.
[c] The radius is assumed to be the same as the radius of P22.
[d] That the μ_0 of 9^- P22 is significantly higher in magnitude than the μ_0 of P22 has been shown by coelectrophoresis in the same slab gels. Differences in μ_0's are determined with an accuracy higher than the accuracy of μ_0 determination.

TABLE 2. Effects on assembly of sugars, polyols, and dextrans[a]

Compound	% Present	Yield of infectious particles	P (atm)
None added		3×10^1–5×10^1	
Ethylene glycol	5, 10, 20, 40	1.1×10^2–1.3×10^2	
Glycerol	5, 10, 20, 40	1.0×10^2–1.2×10^2	
Ribitol	5, 10, 20, 40	1.8×10^2–2.1×10^2	
Sorbitol	5, 10, 20, 40	1.1×10^2–1.2×10^2	
Inositol	5, 10, 20, 40	1.5×10^2–1.9×10^2	
Methyl glucopyranoside	5, 10, 20, 40	1.4×10^2–1.7×10^2	
Mannitol	5, 10, 20, 40	1.1×10^2–1.2×10^2	6.5, 13.1
Glucose	5, 10, 20, 40	2.1×10^2–2.2×10^2	6.6, 13.2, 29.2, 74.8
Sedoheptulose	5, 10, 20, 40	1.3×10^2–1.5×10^2	
Maltose	5	5.3×10^6	
	10	8.4×10^6	
	20	1.16×10^7	
	40	1.20×10^7	
Sucrose	5	6.7×10^6	3.9
	10	1.08×10^7	7.7
	20	1.28×10^7	16.1
	40	1.27×10^7	41.1
Dextran 10	5	1.5×10^7	
	10	2.4×10^7	
	20	2.8×10^7	
	40	2.5×10^7	
Dextran 40	5	1.6×10^7	
	10	2.0×10^7	
	20	2.8×10^7	
	40	2.6×10^7	

[a] In vitro assembly was conducted for 60 min, as described in the text, in the presence of the indicated concentration of the indicated compound.

electrophoresis and staining for DNA (Fig. 1c) and protein (Fig. 1b). The procapsid-containing fractions of Fig. 1a reacted to form bacerio-phage-migrating particles which stained with ethidium bromide, specific for DNA (Fig. 1c). Conversion of 40 to 50% of the procapsids to bacteriophage-migrating capsids occurred (Fig. 1b). Thus, as expected, the procedure used in Figs. 1b and c successfully monitored the packaging of DNA.

Effects of dextrans, sugars, and polyols. Dextrans, some sugars, and some polyols stimulate in vitro T7 DNA packaging (36). To increase in vitro DNA packaging efficiencies and to help determine mechanisms of DNA packaging, the assembly-promoting capabilities of dextrans and several sugars and polyols were determined. Sedoheptulose, glucose, sorbitol, mannitol, and smaller polyols were all ineffective in stimulating the formation of infective particles (Table 2). These compounds were also ineffective in stimulating either production of particles with packaged DNA or conversion of the procapsid to a bacteriophage-like capsid; no detectable conver-

sion occurred (data not shown). In contrast, sucrose, maltose, dextran 10, dextran 40, and dextran 500 all stimulated (i) production of infective P22 (Table 2), (ii) production of particles with packaged DNA (data not shown), and (iii) conversion of the procapsid to a bacteriophage-like capsid (data not shown). Stimulation occurred only when the compound added was a disaccharide or larger compound. Smaller, related compounds were not stimulatory.

The smaller, nonstimulatory compounds used above did not inhibit stimulation of infectious particle assembly by dextran 40 (Table 3). This observation suggests that these smaller compounds do not irreversibly inactivate an extract component necessary for DNA packaging. The above-mentioned compounds that did stimulate assembly are chemically closely related to the compounds that did not stimulate assembly. Therefore, it is unlikely that binding of stimulatory compounds to an extract component accessible to all compounds is the cause of the stimulation. Concentrations of mannitol and glucose sufficient to lower P to levels stimulatory for
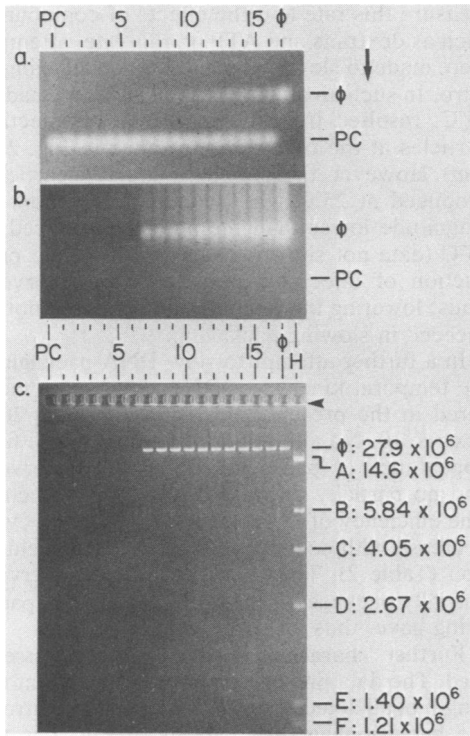
FIG. 2. DNA entry as a function of time. Several 15-µl samples (≈8 µg) of 2⁻ P22 procapsids, isolated as described in the legend to Fig. 1, were diluted with DNA donor extracts and incubated at 35°C in the presence of 20% dextran 40, as described in the text. At the indicated times after incubation was begun, the reaction was terminated by chilling and the addition of DNase. After the addition of 10 µl of sample buffer A, 30 µl of each sample was subjected to agarose gel electrophoresis for fractionation of undenatured particles. Of the remainder, 5 µl, diluted as indicated below, was treated to inhibit DNase and release DNA from capsids (denatured) before electrophoresis in a 0.90% agarose gel. (a) Undenatured, stained with Coomassie brilliant blue (contrast reversed). (b) Undenatured, stained with ethidium bromide. (c) Denatured (DNA released), stained with ethidium bromide. The times of incubation (in minutes) and the yield of infective particles (PFU × 10⁶/ml, background subtracted) were (time, yield): (1) 0, 0; (2) 0.25, 0; (3) 0.50, 0; (4) 0.75, 0; (5) 1.0, 0; (6) 1.25, 0; (7) 1.50, 0; (8) 1.75, 0; (9) 2.0, 7.5; (10) 2.25, 10.1; (11) 3.0, 12.0 (12) 5.0, 15.0; (13) 10.0, 22.0; (14) 15.0, 24.0; (15) 25.0, 24.1; (16) 60.0, 24.5. On (a) and (b), the lane marked φ has mature P22 assembled in vivo (16 µg); the lane marked PC has 2⁻ proheads, unreacted (≈8 µg). On (c), the lane marked H has a HindIII digest of bacteriophage λ DNA, and the lane marked φ has mature P22 DNA from bacteriophages assembled in vivo. The molecular weights of the HindIII fragments, obtained from reference 43, are indicated. For (c), the samples were diluted as follows in NET buffer before electrophoresis: (1) through (6), no dilution; (7) and (8), 1:10; (9) and (10), 1:12.5; (11) 1:16; (12) through (16), 1:20. The

TABLE 3. Effects on assembly of mixing dextran 40 with smaller compounds[a]

Compound	Yield of infectious particles ($\times 10^7$)
None	2.6
Ethylene glycol	2.4
Glycerol	2.5
Ribitol	2.6
Sorbitol	2.7
Inositol	2.7
Methyl glucopyranoside	2.5
Mannitol	2.5
Glucose	2.8

[a] In vitro assembly was conducted for 60 min at 35°C, as described in the text, in the presence of 20% dextran and 20% of the indicated compound.

sucrose were nonstimulatory (Table 2). Therefore, it is unlikely that lowering P is a sufficient cause for stimulation. Because of its apparent dependence on compound size, stimulation probably depends on the nonpenetration by the compound of some particle, probably a capsid.

The efficiency of infective particle formation increased when the sucrose or dextran concentration was increased from 5 to 20% but did not further increase at 40% of either sucrose or dextrans (Table 2). Sucrose present in the extract originally used for in vitro P22 DNA packaging (24) was apparently necessary for the packaging observed. This was not discussed previously (24). The maximum efficiency has been obtained with dextrans 10 and 40 (Table 2).

Temporal kinetics of DNA entry. When DNA entry was observed as a function of time after the initiation of in vitro assembly, DNA packaged in capsids fractionated by agarose gel electrophoresis first appeared at 1.5 min (Fig. 2b, lane 7). The amount of capsid-associated DNA increased from 1.5 to 5.0 min (Fig. 2, lanes 7 through 14), and all packaged DNA was in bacteriophage-like capsids, not procapsids. The procapsid began to convert to a bacteriophage-migrating capsid at 1.5 min (Fig. 2a, lane 7), the same time that packaged DNA began to appear and 0.5 min before infectious P22 began to appear (legend to Fig. 2). The appearance of infectious P22 at 2 min is in agreement with data previously obtained (24).

Portions of samples from the experiment (Fig. 2a and b) were also treated with DNase and subjected to agarose gel electrophoresis after

origin of electrophoresis in (c) is indicated with an arrowhead; the origin of electrophoresis in (a) and (b) is not shown. The direction of electrophoresis is indicated with an arrow.

disruption of capsids and release of DNA as described above. It was known from previous experiments that the amount of mature P22-sized DNA became large enough at the later times to cause the band distortions described previously (30) if amounts of lysate necessary to detect smaller DNA fragments at the earlier times were used. Thus, as described in the legend to Fig. 2, the amount of sample used in Fig. 2c was decreased with the time at which the sample was taken. A band at the position of DNA from mature bacteriophage P22 first appeared at 1.5 min after the start of the incubation (Fig. 2c, lane 7). DNA forming this band also comigrated with mature P22 DNA during the procedure of comparatively high-resolution agarose gel electrophoresis described above, indicating a molecular weight differing by no more than 2% from the molecular weight of mature P22 DNA (data not shown).

The sample taken at 1.25 min after infection had no detectable DNA either shorter than or the same length as mature P22 DNA (Fig. 2c, lane 6), even though the amount of the 1.25-min sample used was 10 times the amount of the 1.50-min sample used. This was true for a 1.8% agarose gel as well as for the 0.9% gel used in Fig. 2c (data not shown). In addition, no evidence of capsid-associated DNA was found at 1.25 min (Fig. 2b, lane 6). The failure to observe partially packaged DNA at 1.25 min indicates that either (i) entry of DNA into capsids occurs in less than 0.25 min, (ii) partially packaged DNA empties from capsids before completion of the agarose gel electrophoresis in Fig. 2b and before the completion of the DNase digestion performed for Fig. 2c, or (iii) DNases enter capsids with partially packaged DNA and digest this DNA; endogenous DNases would do this in the experiment of Fig. 2b. Although the correct alternative has not been rigorously determined, possibilities (ii) and (iii) seem less likely than (i) for the following reasons. The conditions of in vitro packaging are designed to stabilize the packaged state of DNA, and dextrans have a dramatic stabilizing effect on the packaged state of P22 DNA (36). Therefore, it seems unlikely that partially packaged DNA emptied from capsids before the completion of DNase digestion performed for Fig. 1c. The data presented above suggest capsid impermeability to disaccharides during DNA packaging (see also the discussion below). Thus, larger molecules such as DNases probably also could not enter the capsid during packaging.

Attempts to slow DNA packaging. The results in the previous section suggest that the in vitro rate of DNA entry into a P22 capsid is too high to be measured by the procedures used here. To measure this rate and the effects of compounds such as dextrans and ATP on this rate, attempts were made to slow down P22 DNA packaging in vitro. In such an attempt, use of 25°C, instead of 35°C, resulted in first appearance of infective particles at the time of appearance in Fig. 2 (2 min). However, the amount of infective particles produced at 25°C was two to three orders of magnitude lower than the amount produced at 35°C (data not shown). At 5 and 15°C, no production of infectious particles was observed. Thus, lowering the temperature appeared not to succeed in slowing packaging.

In a further attempt to slow DNA packaging, the temporal kinetics of DNA entry were measured in the presence of 2, 3, 5, 10, and 20% dextran 10. No alteration in the time of the first appearance of packaged DNA was observed, and no partially packaged DNA was detected. The efficiency of DNA packaging did, however, decrease with decreasing dextran 10 concentration (Table 2). Thus, all attempts at observing partially packaged DNA by slowing DNA packaging have, thus far, been unsuccessful.

Further characterization of particles assembled. The data presented above indicate that the length of all detectable DNA packaged in vitro in the bacteriophage-like capsids of Fig. 1 is the same as the length of mature P22 DNA. Quantitative densitometry and fluorimetry, performed as described above, revealed that the ratio of packaged DNA to capsid protein is less for particles forming the band in Fig. 1b and c than for purified bacteriophage P22. At the completion of packaging (15 min after starting), the ratio of packaged DNA to capsid protein for particles assembled in vitro had a value 0.8 times its value for purified bacteriophage P22 assembled in vivo. These observations indicate that 20% of the bacteriophage-like capsids did not package DNA. Because packaging was no longer occurring at 15 min, these "empty" bacteriophage-like capsids are abortive end products. The comigration of empty and DNA-containing capsids during agarose gel electrophoresis has also been observed for bacteriophage T7 (37).

From quantitative densitometry of ethidium bromide-stained bands (Fig. 1c), it was also found that the number of infectious particles per packaged DNA is 1×10^{-4} to 3×10^{-4}. This ratio is 3×10^{-1} to 4×10^{-1} for P22 assembled in vivo. The reason for the comparatively large number of uninfective particles with packaged DNA formed in vitro is not known. However, two possible reasons have been eliminated. The first reason is packaging of host DNA. HindIII restriction enzyme digests of DNA packaged in vitro are indistinguishable from HindIII digests of DNA packaged in vivo. The second reason is

breaks in single DNA strands. Most strands have the length of the strands of DNA packaged in vivo, determined by electrophoresis in the alkaline agarose gels described previously (20) (data not shown).

DISCUSSION

The P22 procapsid, like the T7 procapsid, was found to have a negative μ_0 higher in magnitude than the negative μ_0 of the mature bacteriophage capsid. The capsids of the mature bacteriophages T7 and P22 are different serologically (35), and the arrangements of genes in the genomes of these two bacteriophages are different (cf. references 6 and 40). Thus, it is unlikely that the above similarity is explained by common ancestry and may be a response to a selective evolutionary pressure experienced independently by T7 and P22. Analysis of the μ_0's of other bacteriophages and their procapsids is currently being performed.

Agarose gel electrophoresis appears to be the most reliable and efficient procedure for determining whether or not a P22 capsid is in the procapsid state during or after in vitro DNA packaging. Velocity sedimentation is of limited value for this purpose (see the discussion for ϕ29 in reference 4). Determination of the presence of or absence of p8 is also not reliable. Some T7 capsids that have bacteriophage capsid-like outer envelopes as seen by electron microscopy and agarose gel electrophoresis contain T7 p9 (32), the counterpart to P22 p8; the p9 apparently had been dislodged from its normal position in the procapsid. Electron microscopy requires a comparatively large amount of time. In addition, electron microscopy depends on accurate subjective appraisal of the shape of capsids and absence of selective washing of capsids from grids. These conditions can sometimes be difficult to achieve.

To assay for packaged DNA during in vitro assembly, agarose gel electrophoresis of (i) DNA packaged in intact capsids and (ii) DNase-resistant DNA released from capsids were used. The data indicate that it takes 1.5 min for achievement of all steps in the initiation of packaging and the entry into a capsid of the first DNA to be packaged. If capsids with partially packaged DNA are stable during the procedures of analysis used here, the data further suggest that entry into the capsid of the first DNA to be packaged occurs between 1.25 and 1.50 min after the start of assembly. No information was obtained concerning events occurring before 1.25 min, although these presumably include binding of the procapsid to DNA. The conversion of the P22 procapsid to a bacteriophage-like capsid occurred at a time indistin-

guishable from the time of appearance of a mature length of packaged DNA. Because the procapsid expands during this transition and because the procapsid (before expansion) is probably not big enough to package all of the P22 DNA (9), it is likely that the P22 procapsid expands to a bacteriophage-like capsid before completion of DNA packaging. Data obtained with T4 (13), ϕ29 (4), and T7 (32) further indicate that this conversion occurs before most DNA is packaged. If so, the temporal coincidence of the P22 capsid conversion and the appearance of a mature length of packaged DNA is further evidence that entry of the first DNA packaged occurs in a time span less than the difference in sampling times, 0.25 min. Attempts to slow DNA packaging to better measure the entry rate have, thus far, been unsuccessful. These results are in contrast to results in a study of bacteriophage ϕ29 in which partially packaged DNA was detected by velocity sedimentation (3, 4). Because the procedure shown in Fig. 2c involved no fractionation during which DNA could empty from P22 capsids, it is concluded that either entry of P22 DNA into capsids occurs more rapidly than entry of ϕ29 DNA or loss of partially packaged P22 DNA (by the mechanisms described above) occurs to a greater extent than loss of partially packaged ϕ29 DNA. As discussed above, the former alternative is probably correct.

P22 assembly, like T7 assembly, was found to be stimulated by sucrose and dextrans in extracts. Maltose was also stimulatory, but sedoheptulose and all smaller sugars and polyols did not stimulate packaging. The correlation of stimulatory effect with compound size suggests that nonpenetration of some particle, probably a capsid, is required for stimulatory activity. In contrast to the results obtained here with P22, sorbitol and glucose (but not glycerol) stimulated production of infective bacteriophage T7 (36). This latter observation suggests that the smallest hole in the T7 "nonpenetrated" particle is smaller than the smallest hole in the P22 "nonpenetrated" particle. The stimulation by dextran of T7 infective particle formation decreases above 14% dextran 10 (36). This is a second difference in the results obtained with T7 and P22 (Table 2).

The nonstimulatory compounds glycerol and ribitol were previously shown to stabilize packaged DNA in mature P22 (36). This observation suggests that stabilization of packaged DNA (after entry into a capsid) by the stimulatory compounds (possibly to facilitate tail addition) is not a sufficient explanation of the stimulatory effect. That is, at least some stimulation occurs during or before entry of DNA into the capsid. This stimulation could result from the require-

ment for an osmotic pressure across capsids to assist DNA entry (32). Because the in vitro entry rate of P22 DNA into capsids could not be measured here, it has not yet been possible to test the effect of external osmotic pressure on the DNA entry rate.

ACKNOWLEDGMENTS

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