Bacteriophage P22 Capsids with a Subgenome Length of Packaged DNA

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Received 11 July 1983/Accepted 5 October 1983

Bacteriophage P22 assembles a DNA-free procapsid that subsequently packages P22 DNA. To study the packaging of bacteriophage P22 DNA, attempts were made to isolate P22 capsids with a subgenome length of packaged DNA. With the use of cesium chloride buoyant density sedimentation and agarose gel electrophoresis, the following capsids with a subgenome length of packaged DNA were isolated and characterized: (i) a capsid with the solid-support-free electrophoretic mobility and radius of the DNA-free P22 procapsid; (ii) a capsid with the solid-support-free electrophoretic mobility and radius of the mature P22 bacteriophage; and (iii) a capsid with a solid-support-free electrophoretic mobility and possibly a radius intermediate to those of the procapsid and bacteriophage.

During the assembly of all studied double-stranded DNA bacteriophages (including P22, λ , T3, T4, T7, P2, and ϕ 29), DNA-free procapsids are assembled and subsequently package DNA. During packaging of DNA, the procapsids expand and become more angular (the DNA packaging-associated conversion product will be referred to as a bacteriophage-like capsid) (reviewed in references 5 and 7). In the case of bacteriophages P22, T7, T3, and ϕ II, measurements of electrophoretic mobility (μ) during agarose gel electrophoresis have revealed that the solid-support-free μ (μ_0) of the procapsid is negative and decreases in magnitude during the conversion to a bacteriophage-like capsid (6, 16; P. Serwer, R. H. Watson, S. J. Hayes, and J. L. Allen, J. Mol. Biol., in press). Fractionation by agarose gel electrophoresis is thus far the most rigorous way of identifying P22 procapsids and has been used to monitor P22 DNA packaging performed in cell extracts (in vitro) (6). During this latter study, no capsid with a subgenome length of packaged DNA (to be referred to as an spDNA-capsid) was detected; the reason for this appeared to be either (i) DNA entry into capsids too rapid (in <0.25 min) to accumulate sufficient quantities of spDNA-capsids for detection or (ii) instability of spDNA-capsids. During other studies of P22 assembly (1, 2, 4, 8-10), spDNA-capsids have also not been observed. Of the capsids observed in these previous studies, only DNA-free procapsids, DNA-free bacteriophage-like capsids, and bacteriophage-like capsids with a mature length of DNA have been observed (mature P22 DNA has a molecular weight of 27.9×10^6 [Serwer et al., in press] and has a restricted permutation of its ends [17]). No capsid with an outer shell structure intermediate to the structures of the procapsid and mature bacteriophage capsid outer shells has been observed (a capsid with such an intermediate structure will be referred to as an intermediate capsid). It also appears that no intermediate capsid has been isolated during studies of any other double-stranded DNA bacteriophage (reviewed in references 5 and 7).

For understanding the mechanisms of bacteriophage DNA packaging and the accompanying capsid transformations, it is desirable to isolate and characterize spDNA-capsids and intermediate capsids. It is possible for a capsid to be an spDNA-capsid and an intermediate capsid. In preliminary studies, we fractionated lysates of P22-infected *Salmonella*

typhimurium by buoyant density sedimentation in cesium chloride density gradients and observed, by agarose gel electrophoresis, particles that had some of the properties expected of spDNA-capsids. Therefore, the properties of these particles were investigated, and the results are presented here.

Sedimentation of a lysate of P22-infected *S. typhimurium* through a cesium chloride step gradient and observation of the gradient contents by light scattering revealed a bacteriophage band and a continuum of other material at lower densities (Fig. 1a). Particles from the step gradient with an average density of 1.37 g/ml were subjected to buoyant density sedimentation in a cesium chloride density gradient (the region of the step gradient used is indicated in Fig. 1a). The result was a comparatively broad profile of light-scattering material with densities between 1.27 and 1.38 g/ml (Fig. 1b).

To probe for capsids in the gradient (Fig. 1b), fractions of this gradient were dialyzed and subjected to agarose gel electrophoresis, followed by staining for DNA with ethidium bromide and for protein with Coomassie blue. Throughout the gradient were found particles that formed sharp bands and that stained with ethidium bromide (Fig. 2a) and Coomassie blue (Fig. 2b). Bands were formed by particles comigrating with the P22 procapsid (PC in Fig. 2) and with the mature P22 bacteriophage (ϕ in Fig. 2). In addition, a band formed by particles that migrated more rapidly than P22 bacteriophage and less rapidly than the P22 procapsid was observed (Int in Fig. 2). These latter particles are potentially intermediate capsids.

Most of the protein migrating as procapsid in Fig. 2b (PC band) had a density of 1.31 ± 0.02 g/ml, a density indistinguishable from the density of previously isolated (6), DNA-free procapsids (fractions 4 to 6, Fig. 2). However, some protein forming PC bands in Fig. 2b had higher densities, up to 1.39 g/ml (fractions 7 to 15). A peak of ethidium bromide-staining, procapsid-migrating particles was found at a density of 1.36 g/ml (fractions 13 and 14). The ratio of ethidium bromide staining intensity to Coomassie blue staining intensity is at least two orders of magnitude higher for procapsid-migrating particles in fractions 13 and 14 than it is for procapsid-migrating particles in fractions 4 to 6. Thus, it is concluded that the former procapsid-migrating particles have ethidium bromide-staining material other than protein. That this ethidium bromide-staining material is DNA is

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FIG. 1. Fractionation of a P22 lysate. A 6-liter log-phase culture of *S. typhimurium* DB7000 was grown at 37°C to 2×10^8 bacteria per ml. This culture was infected at a multiplicity of 5 with P22 bacteriophage containing mutations CI-7 (clear plaque) and HI101 (lysis defective). Bacteriophage and host are described in (10) and were received from J. King. After being incubated at 37°C for 60 min, the infected cells were pelleted at 10,000 rpm at 4°C for 10 min in a Beckman JA-14 rotor. The pellet was suspended in and brought to 20 ml with 0.15 M NaCl–0.05 M Tris-chloride (pH 7.4)–0.005 M EDTA. The infected cells were then lysed with lysozyme and Brij 58 as previously described (6). After the addition of 1.0 ml of 1 M $MgCl_2$, the lysate was digested with 30 μ l of 1-mg/ml DNase I (Millipore Corp.) in water at 30°C for 16 h. The lysate was then fractionated by being layered on a cesium chloride step gradient as previously described (11) and centrifuged for 180 min at 33,000 rpm at 18°C in a Beckman SW41 rotor (the lysate was distributed to four identical gradients). After fractionation of this gradient by letting it drip through a punctured tube bottom, the fraction with a density of 1.37 g/ml was diluted with an equal volume of 0.2 M NaCl–0.01 M Tris-chloride (pH 7.4)–0.001 M $MgCl_2$ (Tris-magnesium buffer) and was clarified by centrifugation at 5,000 rpm for 10 min in a Beckman J21 rotor. After clarification, the sample was brought to a density of 1.34 g/ml with CsCl in 0.01 M Tris-chloride (pH 7.4)–0.001 M $MgCl_2$ and centrifuged at 40,000 rpm at 10°C for 20 h in a Beckman SW50.1 rotor (buoyant density sedimentation). After buoyant density sedimentation, fractions were collected by pipetting from the top of the gradient and were dialyzed against Tris-magnesium buffer. Photographs of light scattered from particles in both gradients were taken by using illumination from the bottom. (a) Step gradient; (b) gradient from the buoyant density sedimentation. The fraction number and density (ρ) (densities were determined from refractive indexes [11]) are indicated.

indicated by its sensitivity to DNase I and not to pancreatic RNase after release from capsid-migrating particles (data not shown). This DNA must be either packaged or otherwise protected from DNase because it is insensitive to the DNase I used during preparation (see legend to Fig. 1) and also to DNase I used after preparation of the particles in fractions 13 and 14 of Fig. 2 (data not shown). It is assumed below that this DNA is packaged.

The particles migrating more slowly than procapsids and more rapidly than bacteriophage (Int band in Fig. 2) had a distribution in the cesium chloride gradient similar to the distribution of particles forming the PC band. Particles forming the Int band also had packaged DNA. The particles migrating as P22 bacteriophage (ϕ band in Fig. 2) were found at densities varying from 1.27 to 1.43 g/ml and had packaged DNA.

When portions of single fractions of the gradient (Fig. 1b) were subjected to a second buoyant density sedimentation in a cesium chloride density gradient, capsids were again found to be spread throughout the gradient. That the gradient of Fig. 1b had been centrifuged for a time sufficient to band

unaggregated capsids in sharp peaks is indicated by the sharp peaks of particles forming the PC band in Fig. 2 (i.e., fractions 4 to 6 and 13 and 14). These observations suggest that capsids with and without DNA are aggregating and disaggregating either with each other or with other particles after buoyant density sedimentation or during sedimentation or both, thus changing the density at which they band. The particles in Fig. 2 that (i) stain with both ethidium bromide and Coomassie blue and (ii) are arrested at the origin of electrophoresis (indicated by the arrowheads) are probably capsid aggregates, supporting the hypothesis that capsids are aggregating (antibody-aggregated particles of bacteriophage T7 behave similarly [15]). Other aggregates may not have entered the gel and, if so, would not have been detected in Fig. 2.

Ethidium bromide-staining particles forming the Int and PC bands (Fig. 2) were also found immediately below the fraction of the step gradient (Fig. 1a) used for the gradient in

FIG. 2. Agarose gel electrophoresis of particles with densities of 1.26 to 1.43 g/ml. A 13- μ l portion of each (dialyzed) fraction of Fig. 1b was diluted with 17 μ l of 0.005 M sodium phosphate (pH 7.4)–0.001 M $MgCl_2$ –4% sucrose–400 μ g of bromophenol blue per ml. Of this mixture, 20 μ l was layered in sample wells and subjected to agarose gel electrophoresis at 0.96 V/cm at room temperature ($25 \pm 3^\circ$ C) for 16 h, as previously described (13). After electrophoresis, the gel was stained in the electrophoresis buffer with 1 μ g of ethidium bromide per ml for 2 h at room temperature. A photograph of the stained gel was taken, as previously described (13). Subsequently, the gel was treated with acetic acid to release packaged DNA from capsids; the gel was then neutralized and restained with ethidium bromide as previously described (13). After a second photograph, the gel was stained with Coomassie blue and photographed, as previously described (16). (a) Ethidium bromide stain, no acetic acid treatment. (b) Coomassie blue stain (contrast reversed). (c) Ethidium bromide stain after acetic acid treatment. Lanes 1 to 17 contain samples from fractions 1 to 17 of Fig. 1b, lane ϕ contains purified bacteriophage P22 (6), and lane PC contains electrophoretically purified P22 DNA-free procapsid (6). The arrow indicates the direction of electrophoresis; the arrowheads indicate the origin of electrophoresis.

Fig. 1b but were not found in other regions of the step gradient. The integrated intensity of ethidium bromide-stained PC and Int bands indicates that less than 0.01% of the total packaged DNA is in particles forming these bands. However, additional such particles may not have been observed because of their presence in aggregates.

Electron microscopy of material in fractions such as those used for the gel in Fig. 2 revealed spherical capsids and a variety of unidentified particles, probably fragments of the host (not shown). By electron microscopy, it was not possible to discriminate from each other the particles forming the three bands observed by agarose gel electrophoresis in Fig. 2.

To further characterize the DNA-containing particles forming the three bands in Fig. 2, it was assumed that these particles are spherical, and a previously described (12) procedure of agarose gel sieving was used to determine the particle radius (R) for particles with a density of 1.37 g/ml. In addition, μ_0 extrapolated to an agarose concentration of 0 was obtained and was corrected for electro-osmosis (14) to obtain μ_0 . The R of particles forming the PC band was 12 $\pm$ 4% smaller than the R of particles forming the ϕ band; the μ_0 of particles forming the PC band was 16 $\pm$ 2% larger in magnitude than the μ_0 of particles forming the ϕ band (Table 1). These differences are also characteristics of P22 (with a mature length of DNA) and its DNA-free procapsid (4, 6, 12). In addition, the R s and μ_0 s of Table 1 are equal within experimental error to values previously obtained (6, 12) for mature P22 bacteriophage and its DNA-free procapsid. (Experimental errors for values of μ_0 are, however, greater than experimental errors for measured differences in μ_0 [Table 1].) Thus (as suggested by the data in Fig. 2), by these additional criteria, the PC band is formed by a (packaged DNA-containing) P22 procapsid. In previous studies (1, 2, 4, 6, 8–10), procapsids with packaged DNA have not been detected. The previous failure to observe such particles is probably caused, at least in part, by the comparatively small amount of these particles in lysates (see above). However, with the exception of a previous study (6), the procedures used for isolating and detecting capsids were probably not adequate to detect such particles. In the case of bacteriophage λ , spDNA-procapsids have been isolated (B. Hohn, personal communication).

The Int band of Fig. 2 is formed by particles that have a μ_0 significantly different from and intermediate to the μ_0 s of particles forming the PC and ϕ bands (Table 1). This is also a characteristic of tail-spikeless bacteriophage P22 (6). However, the μ_0 of particles forming the Int band is significantly higher in magnitude than the μ_0 of tail-spikeless P22, as determined by coelectrophoresis in the same gel (data not shown). Thus, the particles forming the Int band differ from tail-spikeless bacteriophages (also see below). The R of the particles forming the Int band was also intermediate to the R s of particles forming the PC and ϕ bands, though the differences involved were at the reliability limits of the data (Table 1). Thus, the data presented here suggest that particles forming the Int band of Fig. 2 are intermediate capsids. Investigations are being performed to further test this possibility.

To help determine whether spDNA-capsids are among the capsids forming the three bands in Fig. 2a and b, the gel of Fig. 2a and b was treated with acetic acid to release packaged DNA and then was restained with ethidium bromide (Fig. 2c). If a mature length of packaged DNA is present in a particle, this treatment results in an increase in staining intensity (compare Fig. 2a, ϕ lane and Fig. 2c, ϕ

TABLE 1. μ_0 and R of capsids

Particle ^a	$-\mu_0^b$ (cm ² /V · s $\times 10^{-4}$)	R^c (nm)
PC	1.91	26.5 $\pm$ 1.1
Int	1.76	29.0 $\pm$ 1.2
ϕ	1.61	30.2 $\pm$ 1.2

^a Labeled as in Fig. 2. The sample used was prepared as described in the legend to Fig. 2b, density = 1.37 g/ml.

^b Determined as described in (14), using HGT[P] agarose (14). Values are accurate $\pm 0.09 \times 10^{-4}$ cm²/V · s. Because values of μ_0 were obtained on a mixture of the three particles, differences in μ_0 s are more accurate (± 0.02 cm²/V · s) than μ_0 s.

^c Determined as described in (12), using 17⁻T7 (R = 30.1 nm; see [12]) as a standard mixed in with the three particles analyzed in the same gel.

lane), as previously shown for bacteriophage T7 (13). However, no detectable increase in staining intensity was observed for the particles forming either the PC or Int bands in Fig. 2a and c, fractions 1 to 17. This observation suggests that the particles forming these bands in Fig. 2 have less than a mature length of packaged DNA (and are, therefore, spDNA-capsids). In contrast, some increase in the intensity of the ϕ band in fractions 1 to 17 of Fig. 2 was observed after treatment with acetic acid. This suggests that at least some capsids forming the ϕ band have more packaged DNA than capsids forming the PC and Int bands.

To further test the possibility that spDNA-capsids are among particles forming the bands of Fig. 2, particles forming these bands (density, 1.37 g/ml) were mixed with tail-fiberless bacteriophage T7 (used as an internal standard; referred to as 17⁻T7) and fractionated by electrophoresis in an agarose slab gel. After the section of the gel traversed by the sample was excised, DNA was released from capsids in the piece of gel, and the gel was subsequently subjected to electrophoresis in a second dimension through a 0.9% agarose slab gel. The DNA released from particles forming the PC band migrated more rapidly than DNA released from 17⁻T7 (the molecular weight of T7 DNA is 26.4×10^6 [3]) (Fig. 3). Similarly, the DNA released in the region of the Int band migrated more rapidly than T7 DNA (Fig. 3). Some DNA released in the region of the ϕ band migrated more rapidly than T7 DNA, and some did not. It is concluded, therefore, that all detectable DNA-containing (ethidium bromide-staining) particles forming the Int and PC bands and some such particles forming the ϕ band in Fig. 3 are spDNA-capsids. The length of DNA in spDNA-capsids, crudely estimated from the position of *Hind*III restriction enzyme fragments of bacteriophage λ DNA (Fig. 3), varied from 0.1 to 1.0 times the length of mature P22 DNA. The spDNA capsids forming the Int and ϕ bands in Fig. 1 may have been included among the empty heads in previous studies (1, 2, 4, 5, 8, 9).

The spDNA-capsids detected here might be either degradation products of particles in the P22 assembly pathway (assembly intermediates) or abortive end products of assembly. The appropriate data for determining the role of spDNA-capsids have not yet been obtained, in part because of the comparatively small amount, physical heterogeneity, and possible aggregation of spDNA-capsids. If the spDNA-capsids are assembly intermediates, their stability and comparatively small amount suggest that the failure to observe spDNA-capsids during P22 DNA packaging in vitro (6) was not caused by instability of spDNA capsids. If so, then the data in (6) indicate that P22 DNA packaging occurs too rapidly to readily determine the role in DNA packaging of

FIG. 3. Agarose gel electrophoresis of the DNA in particles forming the PC, Int, and ϕ bands. A sample containing particles forming the three bands in Fig. 2 (density = 1.37 g/ml) and 17-T7 (used as a standard) was subjected to electrophoresis, as described in the legend to Fig. 2. Subsequently, the region of the slab gel traversed by particles in the sample was excised. To release packaged DNA from capsids, the excised piece of gel was soaked at 75°C for 15 min in a buffer containing 0.1 M NaCl–0.01 M Tris-chloride (pH 7.4)–0.001 M EDTA–0.1% Sarkosyl NL97 (Ciba-Geigy Corp.). Before being soaked, the gel was perforated several times with a safety pin to facilitate access of the buffer to the interior of the gel. After the DNA was released from capsids, the excised gel was placed in a trench at the electrophoresis origin of a 0.9% slab gel in 0.05 M sodium phosphate (pH 7.4)–0.001 M EDTA. The trench was formed by removing some of the septa between sample wells formed as for the experiment shown in Fig. 2. After placing molecular weight markers in sample wells whose septa had not been removed, the slab gel was subjected to electrophoresis at 1.0 V/cm at room temperature for 16 h in the above buffer as previously described (13). Subsequently, this slab gel was stained with 1 μ g of ethidium bromide per ml and photographed. In addition, an identical sample was subjected to electrophoresis in the first dimension only and was then stained with ethidium bromide and Coomassie blue. (a) First dimension gel, stained with Coomassie blue. (b) First dimension gel, stained with ethidium bromide. (c) Second dimension slab gel. The origins of electrophoresis in the first dimension are indicated by the vertical arrowhead; the origins of electrophoresis in the second dimension are indicated by the horizontal arrowhead. The direction of electrophoresis in the first dimension is indicated by the horizontal arrow; the direction of electrophoresis in the second dimension is indicated by the vertical arrow. DNA molecular weight markers are a restriction endonuclease *Hind*III digest of mature bacteriophage λ DNA (left) and mature P22 DNA (right) (6). Molecular weights ($\times 10^6$) of DNA markers are indicated at the left.

either the particles isolated here or other potential DNA packaging intermediates. To make this determination, it would, therefore, probably be necessary to either slow P22 in vitro DNA packaging or use a DNA packaging assay with

time resolution greater than the time resolution of previously used DNA packaging assays.

For secretarial assistance, we thank Donna Scoggins. For facilities used for electron microscopy, we thank the Anatomy Department of The University of Texas Health Science Center at San Antonio.

This research was supported by Public Health Service grants AI 16117 and GM 24365 from the National Institutes of Health and grant AQ-764 from the Robert A. Welch Foundation.

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