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Fibrous Projections From the Core of a Bacteriophage T7 Procapsid

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A cylindrical core previously demonstrated in a bacteriophage T7 procapsid (capsid I) has been further examined by electron microscopy. Fibrous extensions of the core have been observed; these fibers appear to connect the core to the capsid I envelope. After infection of a nonpermissive host with bacteriophage T7 amber mutant in any gene coding for a core protein, the resulting lysates contained more noncapsid assemblies of capsid envelope protein than did wild-type lysates; these assemblies had a mass two to at least 500 times greater than the mass of capsid I. This suggests that the internal core and fibers assist the assembly of subunits in the envelope of capsid I.

Key words: bacteriophage T7, procapsid core, electron microscopy, mutant lysates

Protein subunits in the envelopes of the capsids of spherical viruses are arranged in equivalent or quasi-equivalent icosahedral lattices [1, 2]. Interactions between envelope subunits and the viral nucleic acid are sufficient to direct "in vitro" assembly of the envelopes of some of the smaller RNA plant and bacterial viruses (180 envelope subunits) [3–5]. However, nonenvelope proteins are necessary for the efficient "in vivo" assembly of the envelopes of the larger bacteriophages ($\geqslant$420 envelope subunits) including λ [6, 7], P22 [8], T4 [9], and T7 [10] (see also Results below). The evidence also indicates that these latter bacteriophages assemble a DNA-free procapsid and that the procapsid subsequently packages DNA (reviewed in Casjens and King [11]). Thus, interactions with DNA probably do not assist procapsid assembly.

The procapsid of bacteriophage T7 (capsid I) has an internal cylinder (core) with an axial hole; the core is attached at one end to the capsid I envelope. This core is also present in bacteriophage T7 [12]. Bacteriophage T7 with an amber mutation in any core protein gene is deficient in capsid I envelope assembly when grown in a nonpermissive host [10] (see also Results) (capsid I is referred to as prohead in Roeder and Sadowski [10]), indicating that the core assists assembly of the capsid I envelope. In the present communication ultrastructural aspects of the core-envelope interaction have been investigated and additional data concerning the phenotypes of T7 core protein-deficient mutants have been obtained.

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METHODS

Bacteriophage and Bacterial Strains

Bacteriophage T7 and T7 amber mutants [13] were received from Dr. F. W. Studier. The following amber mutants were used: gene 8-*am*11; gene 14-*am*140; gene 15-*am*31; gene 16-*am*9. Mutants will be referred to by their gene number followed by *am*. The host for wild-type bacteriophage T7 and the nonpermissive host for T7 amber mutants was Escherichia coli BB/1. The permissive host for amber mutants was E coli 0-11′. Lysates of E coli BB/1 infected with a T7 amber mutant will be referred to by the name of the mutant (ie, an 8 *am*-infected E coli BB/1 lysate will be referred to as an 8 *am* lysate).

Concentration of Protein Aggregates From Lysates and Purification of Capsid I

Capsid I was precipitated from wild-type T7 lysates using carbowax 6000 and was purified as previously described [12]. For the electron microscopic observation of capsids and other protein aggregates in mutant lysates an aerated, log-phase, 6-liter culture of E coli BB/1 (3×10^8 bacteria per milliliter) in M9 medium [12] was infected at 30.0°C with a bacteriophage T7 amber mutant (multiplicity of infection, 15). When lysis occurred (30–45 min after infection), the capsids and other protein aggregates were twice precipitated with Carbowax 6000 and were clarified by low-speed centrifugation as previously described [12].

Electron Microscopy

Support films for electron microscopy were prepared as previously described [12]. A 2- to 5-μl drop of sample was placed on a support film and was incubated at room temperature for 1.0 min. The film was washed with four drops of water and then two drops of sodium methyl phosphotungstate, pH 7.6 [14]. Excess stain was removed from the film with a Kimwipe and the stain was allowed to dry.

Glutaraldehyde Fixation

Capsid I was fixed with glutaraldehyde by diluting three parts of sample into one part of 12.5% glutaraldehyde (TAAB), 0.5 M sodium phosphate, pH 7.4; this mixture was incubated at room temperature for 5 min. Some capsid I samples were immediately dialyzed against 0.2 M NaCl, 0.01 M sodium phosphate, pH 7.4, 0.001 M $MgCl_2$; others were stored at 4°C for times as great as 6 months before dialysis. No significant difference in the properties of capsid I particles (see Results) resulted from the different fixation times. All micrographs shown are of capsid I particles fixed for 6 months at 4°C.

RESULTS

In a previous study electron micrographs of unfixed capsid I were obtained after negative staining with sodium phosphotungstate, pH 7.6 [12]. In an attempt to obtain improved electron micrographs, capsid I particles were fixed with glutaraldehyde and were prepared for electron microscopy using sodium methyl phosphotungstate, pH 7.6, as the negative stain. This stain has better spreading properties [14] and less grain than sodium phosphotungstate. Of 321 capsid I particles observed at random, 71 (22%) appeared to have at least one fiber connecting the internal cylindrical core to the capsid envelope (Fig. 1). Of particles with fibers, 77% were viewed at right angles to the core axis; in the

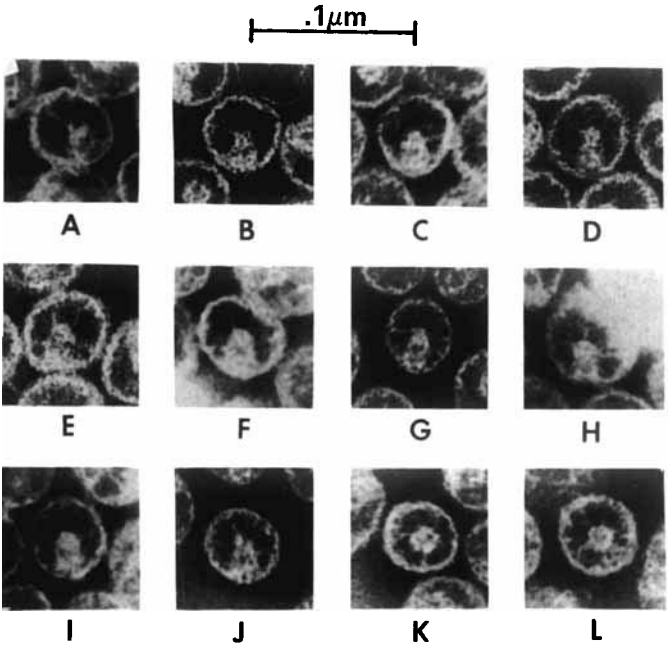

Fig. 1. Capsid I particles with an internal fiber. Capsid I was fixed with glutaraldehyde and prepared for electron microscopy as described in Methods. Particles with an internal fiber are shown.

interior of these particles the following types and numbers of fibers were observed: a) A single fiber was observed in 45% of the particles (Fig. 1A–C); this fiber was usually attached to the end of the core distal to the point of contact with the envelope; b) two to four fibers, with a variable degree of clarity, were observed in the remaining 55% of the particles; some particles had a fiber at the envelope proximal end of the core (Fig. 1D) and 18% had two roughly parallel fibers; these fibers were either parallel or perpendicular to the core axis (Fig. 1E–H, J). Particles viewed parallel to the core axis with fibers connecting the capsid I core to its envelope (23% of the fiber-containing particles) are in Figure 1K–L.

The variation in the number of fibers observed in capsid I particles may be caused by alterations in the fibers induced before or during staining. If it is assumed that capsid I particles have a unique number and configuration of fibers, there must be at least four envelope-distal fibers (two parallel fibers for each direction; at least two directions), and at least one envelope-proximal fiber.

Unfixed capsid I particles in micrographs taken of sodium phosphotungstate-stained specimens [12] were also examined for the presence of internal fibers. Of 280 randomly selected particles, 14 (5%) appeared to have at least one internal fiber. The fibers were not previously noticed in these specimens because of the comparatively small number of particles with fibers and because the fibers were usually not as clearly defined as the fibers in Figure 1.

Mutant Lysates

The proteins associated with the core in capsid I are P8, P14, P15, P16 [10, 12]. (T7 proteins are labeled by P followed by the protein's gene number [15].) In a previous report [10] it was found that 8 *am*, 14 *am*, 15 *am*, and 16 *am* lysates, fractionated by sucrose gra-

dient velocity sedimentation, had more misassembled envelope protein than wild-type lysates. Forty percent of the assemblies of capsid protein were larger and of a different shape than capsids (electron micrographs presented in Roeder and Sadowski [10] suggest that the misassembled envelopes were 2–5 times as massive as the capsid I envelope). However, after sucrose gradient sedimentation of radiolabeled assemblies of capsid proteins from 14 *am*, 15 *am*, or 16 *am* T7 lysates, it has been found that the misassembled capsid protein had a greater tendency to adhere to containers (borosilicate glass) than T7 capsids.* To block this loss of protein in mutant lysates, assembled proteins were concentrated 2,000 × with Carbowax immediately after lysis. Carbowax precipitates, prepared and clarified as described in Methods, were observed by electron microscopy.

Concentrated 8 *am*, 14 *am*, 15 *am*, and 16 *am* lysates were examined. In all of these lysates were observed tubular structures (polycapsids) that were open in at least one location and that had an envelope thickness of 2.0–3.0 nm. This is the same thickness as that of the bacteriophage T7 envelope [16] and a significantly smaller thickness than that of the capsid I envelope (4.0–7.0 nm; from electron micrographs). Some polycapsids had a mass 2–5 times the mass of a T7 capsid, but most of the assembled capsid protein in 8 *am*, 14 *am*, 15 *am*, and 16 *am* lysates was assembled in polycapsids that had a mass at least 50 times the mass of capsid I, and some polycapsids had a mass at least 500 times the mass of capsid I; a polycapsid from an 8 *am* lysate is in Figure 2. The size of these polycapsids is greater by one to two orders of magnitude than the size of polycapsid-like material previously shown [10]. The polycapsids sometimes had pieces of host membrane attached; the membranes varied in size from comparatively small vesicles (white arrows in Fig. 2) to pieces of membrane 10–50 times as large as the vesicles (not shown). Stacks of subunits with roughly the same width as the capsid I core were also observed (black arrows in Fig. 2); these may be partially assembled cores. In negatively stained preparations of concentrated 8 *am*, 14 *am*, 15 *am*, and 16 *am* lysates no less than 95% of the mass of assembled envelope protein was in polycapsids. In wild-type T7 lysates no less than 60% of the mass of assembled envelope protein was in capsids; roughly half of the capsids were capsid I (other T7 capsids observed in lysates are described in Serwer [12]). More precise estimates of polycapsid amounts will be made when high-yield techniques for further purifying polycapsids are developed.

DISCUSSION

The internal fiber of capsid I is probably not made of DNA because DNA synthesis is not required for the assembly of capsid I [15]. Of the possible core proteins (P8, P15, P14, P16) it is unlikely that P8 and P15 are in the fiber because of evidence indicating that P8 and P15 are in the cylindrical part of the core [12]. Remaining are P14 (MW = 18,000) and P16 (MW = 150,000) [15]. Assuming an average amino acid molecular weight of 120 daltons and assuming that the internal radius of the envelope of capsid I is 20.0 nm (determined from electron micrographs), the rise per residue of P14 is 0.13 nm if a single molecule of P14 spans the distance from the center of capsid I to the inner edge of the capsid I envelope. This is 13% less than the rise per residue of an α helix, indicating that the P14 molecule should appear 0.5–0.6 nm in diameter, probably too thin to have been observed by negative-stain electron microscopy. At least three P14 molecules would be necessary to

*A week was sufficient time to adhere 95.0–99.5% of ^{14}C in 80S–200S particles sedimented from the mutant lysates; detectable amounts of T7 capsids were not lost during this time period. The buffer used in the sucrose gradients was 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.4, 0.005 M EDTA, 100 μg/ml gelatin (P. Serwer, unpublished data).

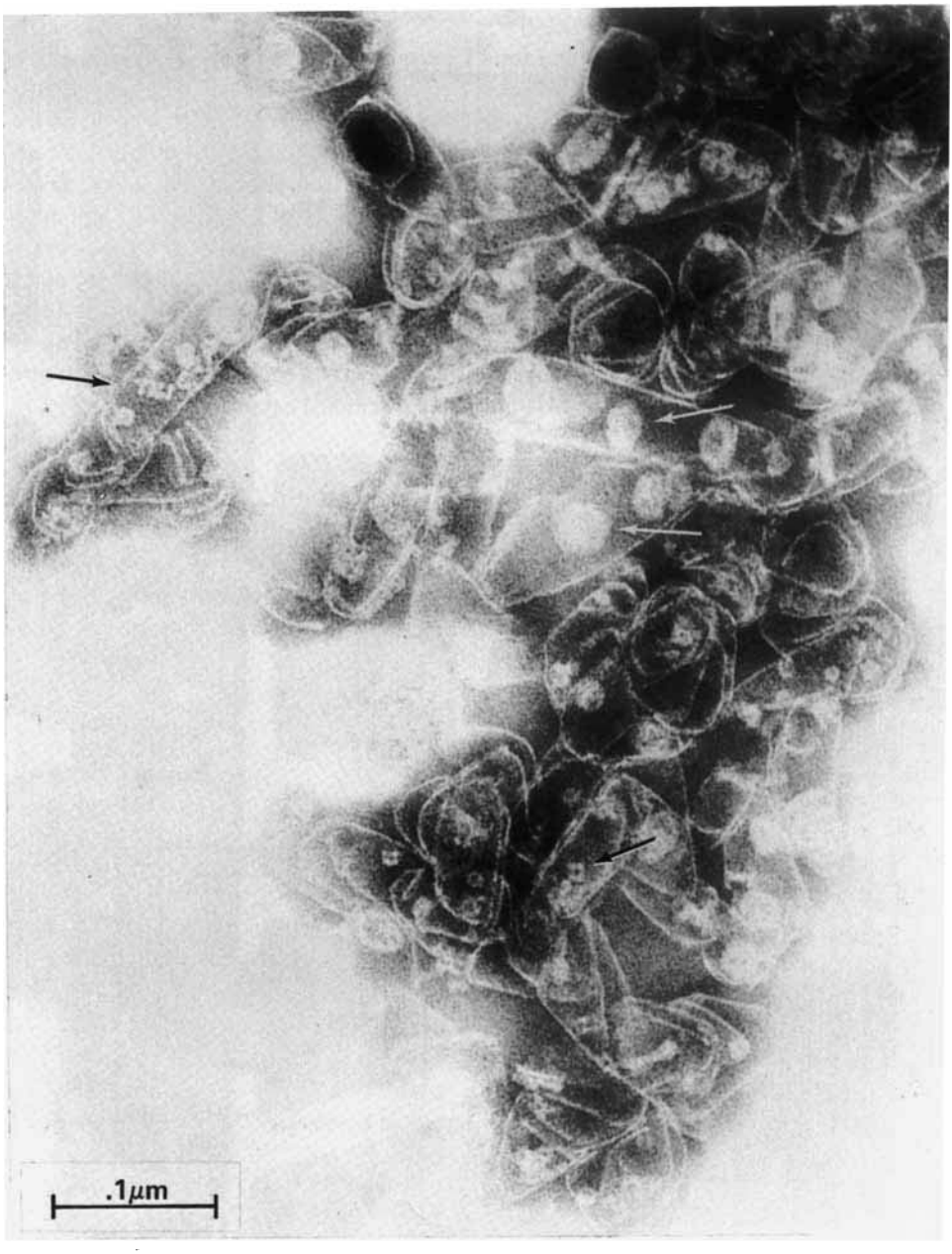

Fig. 2. A T7 polycapsid from an 8 *am* lysate. An 8 *am* lysate was prepared as described in Methods. A clarified carbowax precipitate of the lysate was negatively stained and observed by electron microscopy as described in Methods.

form the fiber observed in the electron microscope. If P16 forms the fiber, the rise per residue is 0.016 nm and a single protein molecule would produce a fiber thick enough to be observed by electron microscopy. A protein fiber with a rise per residue of 0.016 nm would, however, have to be supercoiled or folded.

The occurrence of a deficiency in capsid I assembly when bacteriophage T7 core protein-deficient amber mutants infect a nonpermissive host indicates that the core assists the assembly of the capsid I envelope. Because the cylindrical part of the core contacts the envelope at only one point, parts of the envelope distal to this contact point must interact with the core either a) indirectly, using a signal propagated from the core along the capsid envelope, or b) directly, by contact with the fibrous extensions of the core. The data presented here indicate that possibility (b) occurs; possibility (a) may also occur. The fibers might assist capsid I envelope assembly by binding envelope subunits at a unique distance from some point within the core; this point eventually becomes the envelope center. Testing of this hypothesis, and development of more detailed hypotheses concerning the dynamics of capsid I envelope assembly, require a more extensive knowledge of the structure of the capsid I interior, and the isolation of partially assembled capsid I precursors.

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