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Head Maturation Pathway of Bacteriophages T4 and T2

III. Isolation and Characterization of Particles Produced by Mutants in Gene 17

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We have isolated and characterized two types of particles produced in comparable amounts by mutants in gene 17: the empty large particle and the empty small particle. Dimensions, morphology, stability, and protein composition of the empty large particle are very similar to those of the capsids or empty heads of mature phage. The other type of particle (empty small particle) is very similar in dimensions and stability to the prehead, but differs in that it is composed of processed proteins (gp23*, gp24*, IpIII*). Structural analysis has shown that the protein subunits of the empty small particles are arranged in an unexpanded type of lattice (11.2 to 11.3 nm), whereas the empty large particles have an expanded lattice (13 nm). The characterization of the empty small particle as being composed of cleaved proteins, but still unexpanded, shows that the expansion of the T4 head shell is not necessarily linked to the cleavage of the structural proteins.

During T4 head maturation, the membrane-associated τ-particle is the first observable precursor particle (6, 7, 20, 30) that undergoes a series of transformations leading to the mature head. These transformations include the processing of the proteins of the core (gp22 and internal proteins) and the shell (gp23, gp24) (10, 13, 16, 18), the expansion of the shell (7), the DNA filling (21, 22, 24), and the final steps of the assembly leading to the complete, stable virion.

Laemmli and Favre (20) proposed a maturation pathway in which the processing of the major head protein (gp23) precedes the DNA packaging of the capsid, and the cleavage of the core proteins was suggested to be linked to DNA packaging. In this pathway, it was not defined at which stage expansion occurs. In vitro experiments carried out with head-related particles (33) showed that the cleavage of the major protein of the shell always occurred together with expansion. Conditions of in vitro experiments are not necessarily the same as in vivo ones; thus, these observations do not demonstrate that these two processes are obligatorily linked; and their possible extrapolation to the in vivo situation is left open.

Mutants in genes 17 and 16 lead to lysates that contain what was assumed to be empty capsids that are composed of gp23* (20, 25). This particle was thought to be related to prohead II, a postulated intermediate in T4 head maturation (20). With temperature-sensitive mutants in gene 17, it was not possible to mature the particles accumulated under restrictive conditions into active phage after a shift to the permissive temperature (25; H. Wunderli, J. van den Broek, and E. Kellenberger, J. Supramol. Struct. "a," in press).

Thin sections of 16^-- or 17^--infected cells show empty particles of apparently two different species (R. T. Okinaka, Ph.D. thesis, UCLA, Los Angeles, 1971; Wunderli et al. [a], in press). By serial sectioning, these empty small particles (ESP) and empty large particles (ELP) were demonstrated to represent really two species and are not due to different levels of sectioning (23). In lysates, two types of particles are also recognizable in the micrographs of Luftig and Ganz (25) and then by Wunderli et al. (H. Wunderli, E. Couture, D. A. Vinçe, and E. Kellenberger, J. Supramol. Struct., "b," in press). The results obtained by correlating the amount of gp23* with the number of intracellular particles as counted in sections give evidence that both ESP and ELP are made of gp23* (H. Wunderli, Ph.D. thesis, Biozentrum der Universität, Basel).

These observations suggested that cleavage of gp23 might precede the enlargement of the particle.

In the present paper we report the isolation of ESP and ELP and demonstrate that both are composed of gp23*. Structural studies carried out on giant variants of these particles show that they represent two different characteristic

conformations of the basic lattice of T4 head-related shells.

MATERIALS AND METHODS

Bacteria and bacteriophage. *Escherichia coli* strain B^E was used as the nonpermissive strain, and *E. coli* CR63 was used as the permissive host for plating and producing stocks of conditional lethal mutants of phage T4. Two amber mutants in gene 17 were used: 17(*am*NG60) and 17(*am*N56). Phage λ and wild-type T4 empty capsids were a gift of R. van den Broek.

Growth conditions and radioactive labeling of infected bacteria. The experiments were performed in M9 media (6) supplemented with 1% Casamino Acids except in the case of continuous labeling where the amino acid concentration was 0.1%. *E. coli* B^E was grown to 4×10^8 cells per ml at 37°C with vigorous air bubbling. Cells were infected at a multiplicity of infection of 5 with the phage and superinfected 8 min later to inhibit lysis. The continuous labeling of the infected bacteria was performed by adding 1 mg of a ^{14}C-labeled amino acid mixture per ml of culture, which was obtained by diluting a ^{14}C-labeled amino acid mixture (Amersham CFB104) with cold Casamino Acids (Difco) to obtain a specific activity of 5 μCi/mg (Wunderli, Ph.D. thesis). For pulse-labeling, we followed the same procedure, adding the label 13 min after infection, but omitting the addition of Casamino Acids.

Isolation of particles accumulated in 17⁻-infected cells. *E. coli* B^E infected with 17(*am*NG60) or 17(*am*N56) were incubated at 37°C with aeration during 45 min and filtered through a Sartorius filter (0.45-μm pore size) with continued bubbling of air so as to maintain the oxygen supply to the culture. Particles were released from the retained cells by incubation of the filters with 1/20 of the original volume of triethanolamine buffer (75 mM triethanolamine [pH 8.0]-1 mM $MgSO_4$-100 mM NaCl-2 mM phenylmethylsulfonyl fluoride [PMSF]) saturated with chloroform. To cross-link the particles, 45 mM β-mercaptoethanol and 15 mM methyl-4-mercaptobutyrimidate (31) were added and incubated at 4°C during 15 min. To form the disulfide bond of the reagent imidate molecules, an excess of H_2O_2 (60 mM) was added and the mixture was incubated at 4°C for 30 min. After DNAse I (10 μg/ml) treatment and further incubation for 15 min, cell debris were eliminated by low-speed centrifugation, and the particles were concentrated by high-speed centrifugation (85,000 × *g*, 70 min). The particles were suspended in 1/20 of the original volume in 50 mM phosphate buffer at pH 6.5 containing 0.15 M NaCl and 2 mM PMSF and incubated with purified immunoglobulins from anti-hoc serum (kindly provided by U. Aebi) for 1 h at 4°C. The sera aggregate specifically the ELP, whereas the ESP remain in the supernatant (see below). The aggregated ELP were sedimented by low-speed centrifugation and further washed three times with phage buffer (0.15 M phosphate-0.07 M NaCl-0.001 M $MgSO_4$). ESP remaining in the anti-hoc-treated mixture were isolated by centrifugation on a linear sucrose gradient (10 to 30% sucrose, 75,000 × *g* for 65 min at 15°C). The recovery of ESP in the different steps of the isolation procedure was monitored by electron microscopy counts with agar filtration (15). From the peak of isolated particles, we recovered 50 to 70% of the particles initially present in the crude lysate.

To study the stability of the ESP, we isolated non-cross-linked particles by the same procedure, except that the triethanolamine buffer was substituted by phage buffer. Under these conditions the recovery of isolated particles was lower (about 30%).

Polyacrylamide gel electrophoresis. The gels were prepared by the system described by Laemmli (18). Gradient acrylamide gels were performed by superposing a sucrose gradient (0 to 20%). Autoradiography of gels was carried out as described by Fairbanks et al. (11), using Kodirex film (Kodak, Lausanne, Switzerland). Some proteins of the electropherograms were identified serologically by using the immunoreplicate technique of Showe et al. (29), with sera kindly provided by L. Onorato.

Thin sections of isolated particles. For thin sectioning, the ESP were fixed with 4-methyl mercaptobutyrimidate and isolated as described above, except that the final sucrose gradient was substituted by high-speed centrifugation (75,000 × *g* for 70 min at 15°C). The particles were suspended in phage buffer, fixed with 1% OsO_4, and embedded and sectioned as described previously (17). For the preparation of ELP, 8 M urea was added to a mixture of partially purified ESP and ELP that were not treated with the cross-linker. After 2 h of incubation, ESP were destroyed and ELP were concentrated by high-speed centrifugation. After this step, the procedure was the same as described above for the ESP. The length of ESP and ELP was calculated in the following way: as the particles were not oriented and also partially deformed in the pellets, only particles sectioned along the long axis were considered (23). About 30 particles were measured in each case.

Production of giants. To produce 17⁻-derived giant particles, we followed the procedure described by Cummings et al. (9). 17⁻-infected cells were treated by adding 100 μg of L-canavanine sulfate per ml at 10 min after infection. At 25 min the culture was chased with 400 μg of L-arginine per ml. After the chase, cells were incubated at 37°C for 100 min. Cells were filtered on Sartorius membrane filters (0.45-μm pore size) and suspended in 1/20 of the original volume, lysed, and fixed as described above for the 17⁻ particles. The giants were partially purified by removal of cell debris by low-speed centrifugation and concentration of the giants by centrifugation at 31,000 × *g* for 20 min.

Electron microscopy. Electron microscopy of lysates and suspensions of isolated particles was carried out by applying a drop of the sample to carbon-coated collodion film on 200-mesh copper grids. The samples were then negatively stained with a neutral 2% sodium phosphotungstate solution and examined with a Phillips 300 electron microscope.

For immunomicroscopy (35), particles were first deposited on the supporting film and then fixed with 1% glutaraldehyde for 30 s. After washing off the fixative, the samples were incubated for 1 h at room temperature with the appropriate dilution of the an-

tiserum. After the serum was washed off thoroughly, samples were negatively stained and examined as described above.

Image processing of electron micrographs. Selected areas of the specimens were optically diffracted by the method of Aebi et al. (3) and Steven et al. (33). The windowed areas of giant particles contained approximately 200 unit-cells and were rectangular; only when important diffraction spots lay near the axes was a diamond-shaped window used to rotate the axes away from the spots.

The magnification of the specimens was calibrated relative to an assumed 3.8-nm spacing of the extended T4 tail sheath as described previously (2); this allows a comparison of our data with those previously given for lattices of T4 head-related particles.

RESULTS

Particles in 17⁻ lysates. When lysing cells infected with mutants in gene 17, we found two types of particles (Fig. 1). The one has the

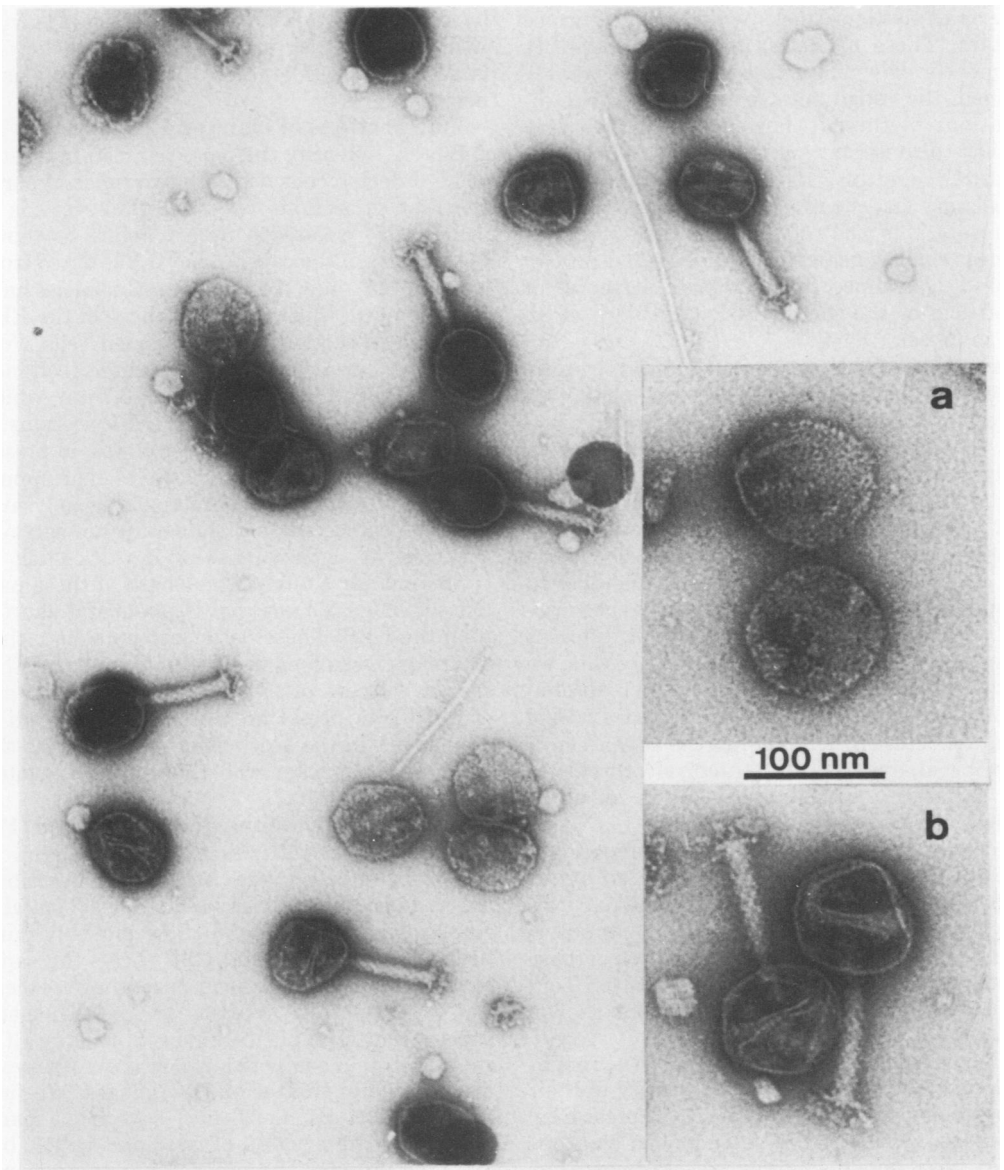

FIG. 1. *Electron micrographs of gene 17⁻ particles. 17⁻-infected cells were concentrated and lysed, and the particles were fixed as described in the text. The sample was prepared for microscopy by the agar filtration method and stained with sodium phosphotungstate, pH 7. (a) Higher magnification of the rough disks; (b) higher magnification of the normal-looking empty capsids.*

dimensions and negative-staining behavior of the normal empty T4 capsids, that is, characteristic foldings and some retention of stain inside. Based on these similarities, together with those observed on sectioned empty capsids and 17⁻ ELP (23; Wunderli et al. [a], in press), we tentatively assumed that the ELP found in sections appear as capside-like particles in the lysates. Below we will definitely establish the identity of these particles. On negatively stained micrographs the other particles found in 17⁻ lysates appear as flattened disks with a rough surface texture. These particles, as well as the ELP, frequently show tails attached. When negatively stained, the rough surface texture and the dimensions of the 17⁻ flattened disks are very similar to those of τ-particles (19). In sections, τ-particles and ESP have the same dimensions, suggesting that the rough disks in negative stain represent the ESP of sections. The apparent large size of the negatively stained 17⁻ disks can easily be explained by a complete flattening of a particle of the size of ESP (Wunderli et al. [b], in press).

Isolation of the two species of empty particles produced in 17⁻-infected cells. We have found that the ESP can easily be lost if the 17⁻-infected cells are centrifuged. Apparently, secondary effects are induced in centrifugation (e.g., trapping, centrifugation force, or lack of oxygenation). To achieve concentration together with better preservation, we filtered the cells under aerobic conditions (see above). In spite of this, we still observed a great variability in the ratio of ESP to ELP in different experiments. To obtain reproducible results, we used cross-linking with methyl-4-mercaptobutyrimidate (31), which fixes the particles during isolation. This cross-linker has the advantage of allowing analysis of the structural proteins of the isolated particles because its disulfide bridges can be cleaved by reduction.

According to the procedures given above, cultures of 17⁻-infected cells, concentrated by filtration of the cells, were lysed and the particles were cross-linked with methyl-4-mercaptobutyrimidate. Sucrose gradient centrifugation of these lysates gives a broad peak at about 340S containing both ELP and ESP (data not shown, similar to that shown in Fig. 3).

The characteristics of ESP (small size, roughness, instability) suggest the possibility of their being unexpanded, like τ-particles, whereas ELP are expanded (larger size, more stable) and thus would have bound hoc and soc proteins, thus being similar to capsids. To test this hypothesis, we reacted a mixture of ESP and ELP with anti-hoc serum (Fig. 2). It is evident that the ELP react strongly with anti-hoc serum, whereas ESP do not, similarly to other unexpanded particles (τ-particles, coarse polyheads).

When we reacted a mixture of partially purified ESP and ELP with purified immunoglobulin from an anti-hoc serum, the ELP were precipitated, whereas the ESP remained in the supernatant. These particles could be further purified in sucrose gradients, where they gave a peak at about 330S (Fig. 3), which contained more than 90% of ESP (Fig. 4). In the mixture of ESP and ELP, we could easily destroy ESP by dissociation with urea (see above), leaving ELP intact, which subsequently could be isolated by sucrose gradient centrifugation as a peak at 350S.

Thin sections of isolated 17⁻ particles. To definitely indentify the particles seen in lysates of 17⁻-infected cells with the two types of particles seen in sections, we made thin sections of pelleted 17⁻ isolated particles. Figure 5a shows the sections of the capsid-like ELP isolated from lysates of 17⁻-infected cells. These particles have the same wall thickness as do those of the ELP observed in sections of 17⁻-infected cells. Furthermore, our measurements of these sectioned particles gave a length of 96.7 ± 2.7 nm, which agrees with the data given for ELP sections of 17⁻-infected cells (Wunderli et al. [a], in press).

On the other hand, the particles that appear as rough disks in electron micrographs of lysates of 17⁻-infected cells are shown in thin sections (Fig. 5b) as particles having a thick shell of approximately 6.0 nm. The length of these particles is 83.6 ± 2.3 nm, that is, about 15% shorter than the ELP. These data clearly indicate that the rough disks seen in 17⁻-lysates and the ESP seen in sections of 17⁻-infected cells (Wunderli et al. [a], in press) are the same, although it seems that in the sections of infected cells the ESP have a thicker wall than do the isolated particles.

Structural proteins of the purified 17⁻ particles. The particles that accumulated in 17⁻-infected cells were isolated as described above and subjected to sodium dodecyl sulfate-gel electrophoresis (Fig. 6). The partially purified mixture of ELP and ESP shows the same proteins as are found in mature phage, except wac protein, which remains in the high-speed supernatant. The purified, normal-looking ELP show a protein composition that is qualitatively comparable to that of mature phages. We find gp23*, gp24*, B1, gp20, hoc, soc, and a band running in the position of gp12 and IpIII*, besides tail proteins and other minor components that are also present in the T4 phage. hoc and gp24* were identified by the immunoreplicate technique with the corresponding antisera (29).

The purified ESP show basically the same

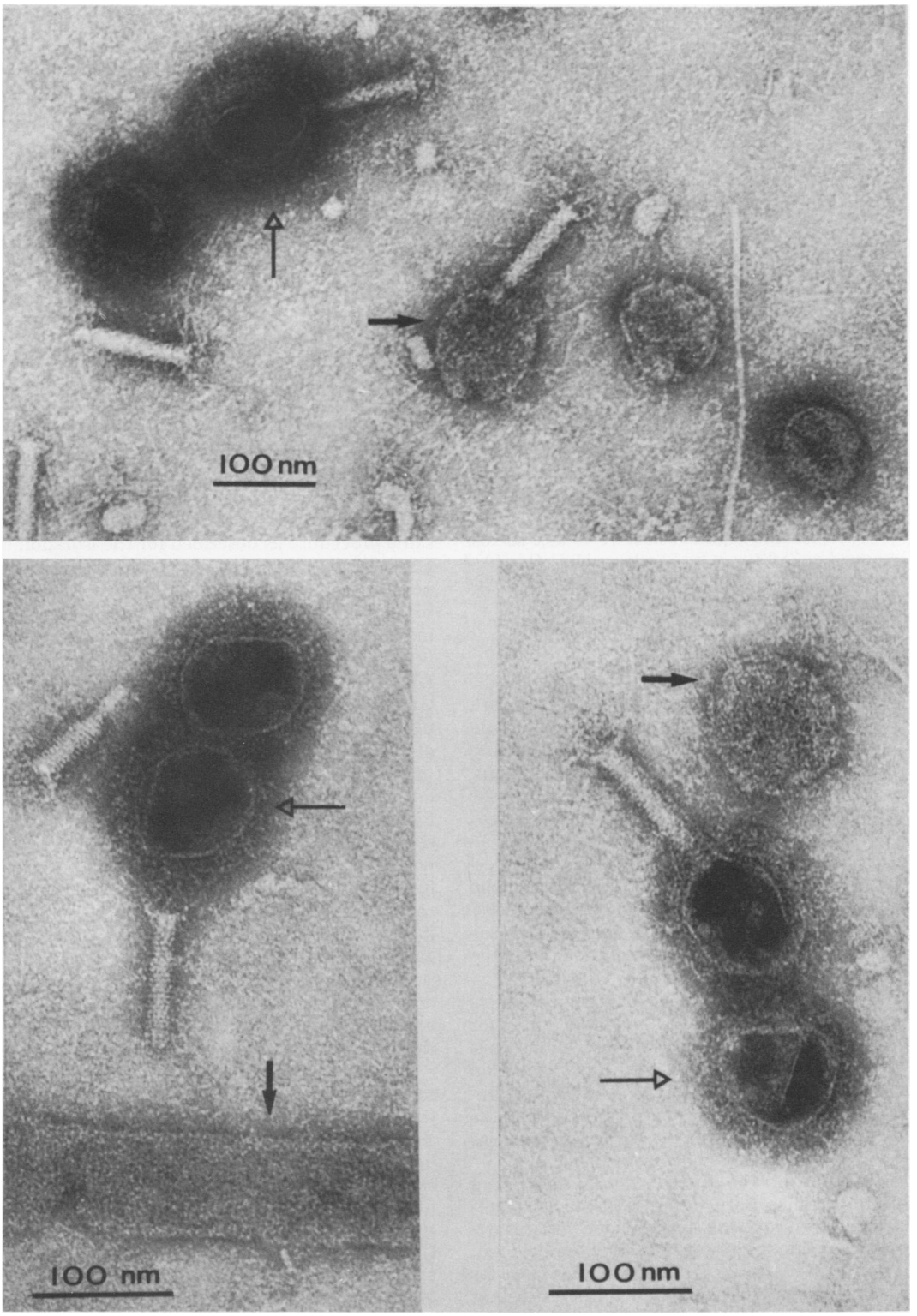

FIG. 2. *Immunomicroscopy of 17⁻ particles reacted with anti-hoc serum. A partially purified mixture of ESP and ELP was reacted with anti-hoc serum as described in the text and negatively stained. Black arrows show the unreacted rough disks (ESP) and in the lower left corner an unexpanded "rough" polyhead that also did not react with the serum. Arrows with open heads show the ELP that reacted with the antibodies, which are seen as a fuzzy halo surrounding the particles.*

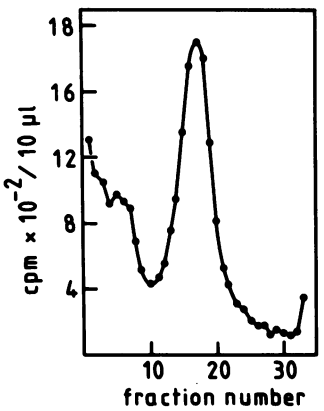

FIG. 3. *Sucrose gradient purification of 17⁻ ESP. A mixture of ESP and ELP, continuously labeled and partially purified as described in the text, was depleted of ELP by antibody precipitation of these particles with anti-hoc purified immunoglobulin. After elimination of the ELP, the supernatant was subjected to centrifugation in a 10 to 30% sucrose gradient at 75,000 × g for 65 min at 15°C. One 10-µl portion of each fraction was analyzed for radioactivity. To estimate the sedimentation coefficient of the particles, we used as markers phage λ (410S) and tailless T4 empty capsids (300S).*

protein pattern as do capsids (gp23*, gp24*, IpIII*, etc.), but, as expected, they do not contain hoc and soc. The absence of hoc was also confirmed with the immunoreplicate technique. A protein band sometimes appears in the gel near the position of hoc. This protein is derived from the product of gene 23 by undergoing an artifactual cleavage during the isolation procedure, as is shown by the reaction of this band with anti-gp23* serum in the immunoreplicate. To avoid as much as possible this artifact, the whole isolation procedure was carried out in the presence of a protease inhibitor (PMSF).

We have never found uncleaved gene products in the isolated 17⁻ particles: neither gp22 nor uncleaved IpIII has been detected in the gels. The band running in the gp12/gp23 position does not react with anti-gp23 serum in the immunoreplicate, strongly suggesting the complete absence of gp23 in the head-related particles that accumulated in 17⁻-infected cells.

Stability of 17⁻ particles in denaturing agents. It has been described that T4 head-related particles with unexpanded lattices (τ-particles, coarse polyheads) are rapidly dissociated in denaturing agents, whereas particles with expanded lattices are much more resistant to this treatment (33). To test the behavior of ESP and ELP under these conditions, we isolated these particles from 17⁻ lysates without cross-linking them.

The isolated particles were tested with 4 M urea (pH 7) or with 1% sodium dodecyl sulfate at room temperature. Under these conditions, ESP were dissociated (Fig. 7), following the behavior of unexpanded particles. The ELP were resistant to both treatments (data not shown), again demonstrating the similarities of this particle to the normal empty capsids.

Structure of giants derived from 17⁻ particles. To confirm the above-described observations that suggested an unexpanded lattice for the ESP produced in 17⁻-infected cells, we studied directly the structure of the ESP by optical diffraction of electron micrographs. Because of its small size, the ESP is not very convenient for this technique. We thus produced giant particles by canavanine pulse treatment of 17⁻-infected cells by the procedure described by Cummings et al. (see above). The cells were concentrated on membrane filters and lysed by the same procedure as described for the isolation of ESP. To get some idea about the protein composition of the giants, the cells were pulse-labeled with ^{14}C-amino acids during the canavanine pulse. According to Cummings and Bolin (8), under these conditions the labeled proteins are incorporated preferentially into giants. Giants partially purified by differential centrifugation (see above) showed clearly the presence of the major head protein in the cleaved form (data not shown). In electron micrographs of negatively stained preparations of lysates, we found two types of giants (Fig. 8a): one has the smooth surface and folding characteristics of the normal, empty giants previously described (1, 4, 5, 34), and we thus assume that this giant is representative of the ELP accumulated in 17⁻-infected cells. The diffraction pattern of this giant particle shows (Fig. 9a) the hexagonal lattice typical of T4 giants, with prominent 7th order spots and with a lattice constant of 12.95 nm, indicating clearly an expanded type of lattice (33).

The other type of giant (Fig. 9b) has the same rough surface and flattening characteristics of the ESP. Diffraction patterns of this type of giant show dominant 2nd and 5th orders in a hexagonal array with a 11.23-nm lattice constant, which is the same as that determined for unexpanded lattices of the T4 head-related coarse polyheads (32) and giant τ-particles (A. C. Steven, personal communication). The different parameters of the two types of 17⁻-derived giants are given in Table 1. The tubular parts of both giants undergo the same degree of flattening, allowing comparison of their widths. It is evident that the ESP-derived giant is about

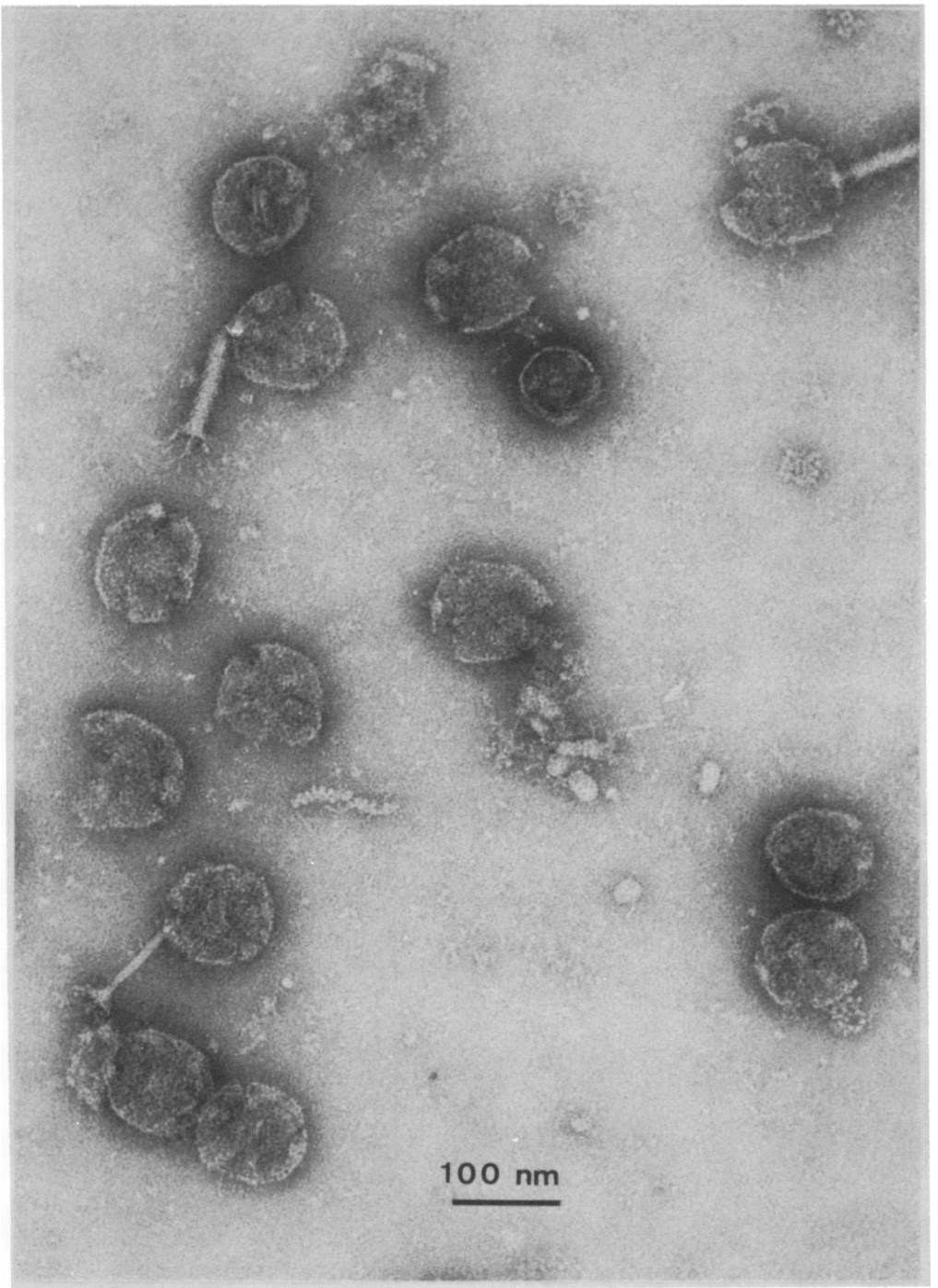

FIG. 4. *Electron micrograph of isolated 17⁻ ESP. ESP isolated by sucrose gradient centrifugation, as described in the text, were dialyzed against phage buffer (see text) and prepared for electron microscopy by negative staining. About 90% of the particles observed show the typical rough surface texture and complete flattening associated with frequent ruptures.*

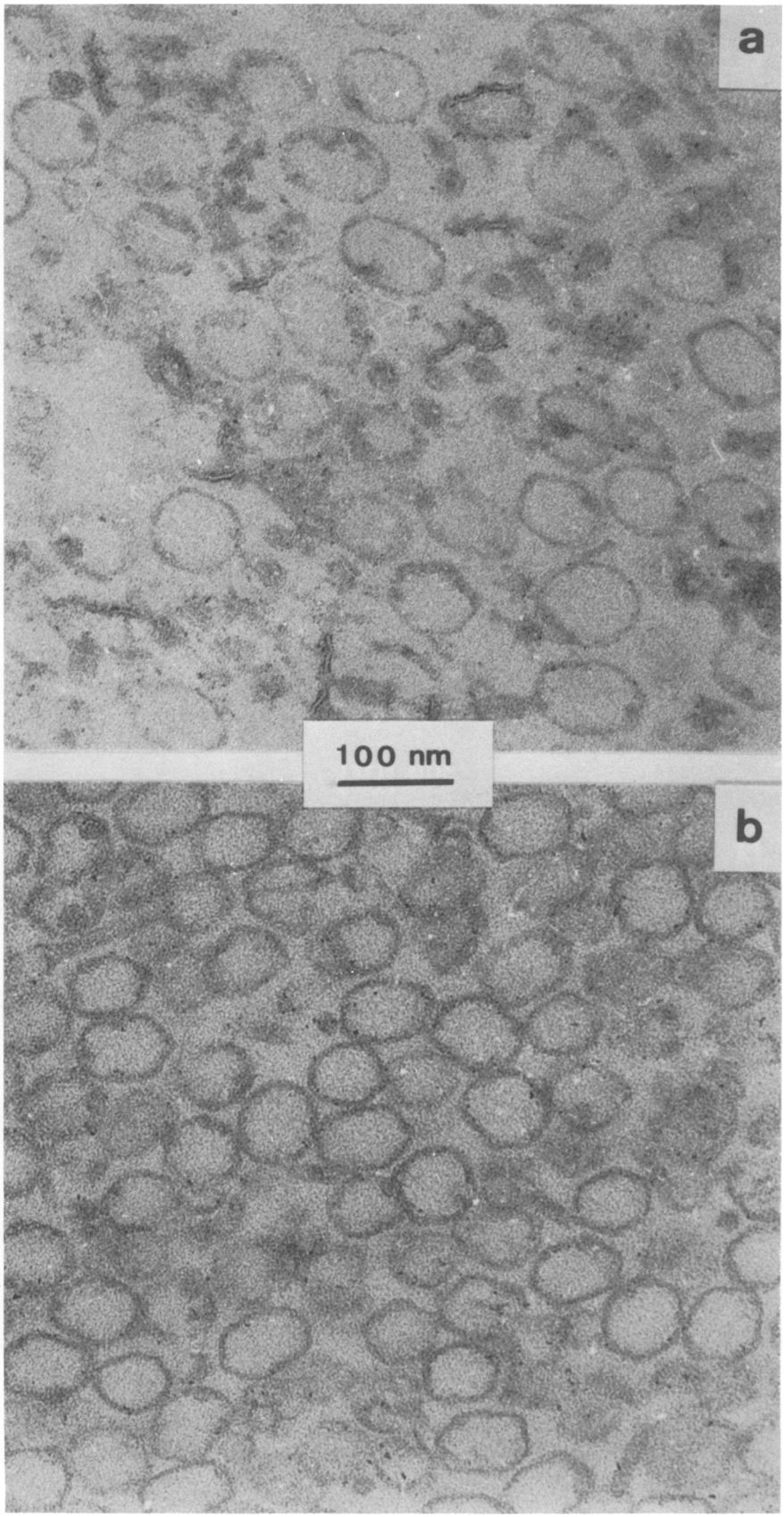

FIG. 5. *Thin sections of 17⁻ particles. The particles were fixed, isolated, and partly purified as described in the text. Pellets of the particles were fixed, dehydrated, embedded, and sectioned as described elsewhere (17). (a) Purified ELP; (b) purified ESP.*

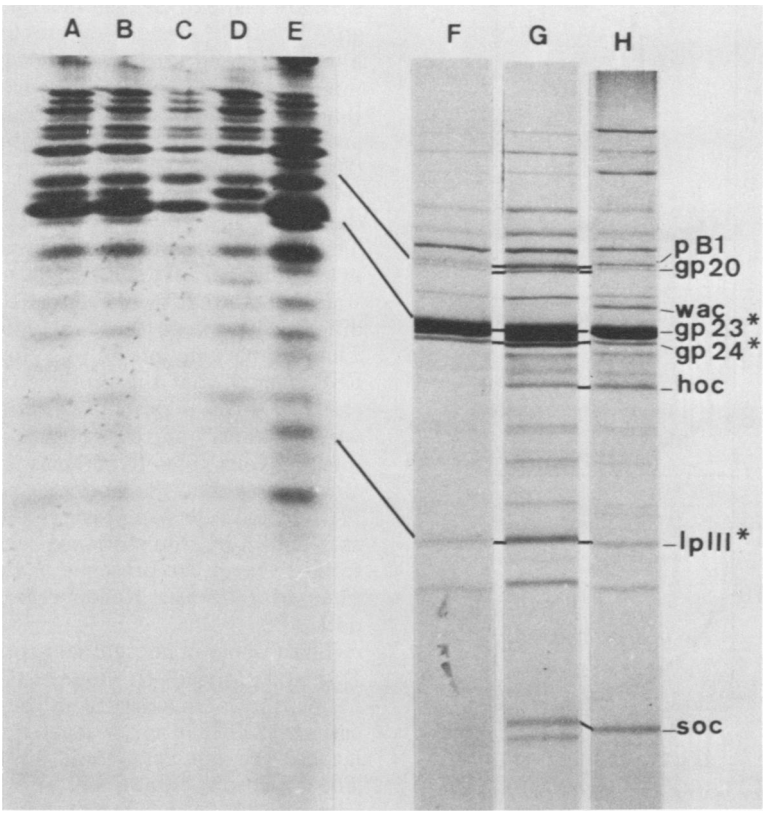

FIG. 6. *Electrophoretic analysis of isolated 17⁻ particles. Comparative samples of (A) material concentrated by filtration on membrane filters, (B) supernatant, and (C) cell debris fraction of a low-speed centrifugation and (D) high-speed supernatant were subjected to electrophoresis, together with a sample of a partially purified mixture of ESP and ELP in 15% acrylamide gel (see text). To study the structural proteins of the isolated particles, we used a high-resolution 10 to 15% acrylamide gradient gel (see text), (F) isolated ESP, (G) isolated ELP, and (H) sucrose gradient-purified T4 phage.*

15% less wide than the ELP-derived giant or the ghosted T4 giant (1). This difference of width corresponds to that of the ratios of the two lattice constants. The pitch angles and the corresponding (u,v) coordinates [14° and (15,5), respectively] are the same for both of these giants.

To extrapolate these findings on giants to the related particles, we selected micrographs of isolated ESP that were well enough preserved to give diffraction patterns (Fig. 9c). We found that they are consistent with the unexpanded hexagonal lattice found in the giants: five good diffractograms of small particles show a lattice constant of 11.3 nm, which corresponds very well with the value of 11.2 nm for the ESP-derived giants, thus confirming the unexpanded nature of the ESP lattice.

DISCUSSION

Mutants in gene 17 accumulate under restrictive conditions of growth two types of particles as defined by electron microscopy. One, ELP, is similar to normal T4 ghosts or empty capsids, both in sections and by negative staining. This particle is made of cleaved head proteins (gp23*, gp24*, IpIII*) and in addition contains hoc and soc proteins. These data strongly suggest that the ELP has the same basic structure as does the normal empty capsid found after DNase treatment of tailless heads.

The other particle found in 17⁻-infected cells is the ESP. Thin sections of 17⁻-infected cells (Wunderli et al. [a], in press) or of purified ESP show that they have the same shape and dimensions as do τ-particles; ESP are about 15% smaller in linear dimensions than normal, empty capsids and have a thicker shell. Electron microscopy of negatively stained 17⁻ lysates shows that the ESP, when adhered to the supporting film, appear as rough disks that are completely flattened. The apparent larger size of ESP as compared with ELP or normal capsids is related to the flattening behavior of these two particles.

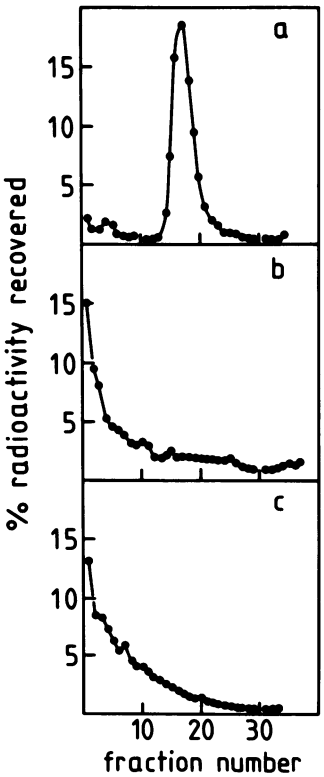

FIG. 7. *Treatment of 17⁻ ESP with denaturing agents. Sedimentation profiles after sucrose gradient centrifugations under conditions described in the legend to Fig. 3 of (a) sucrose gradient-isolated ESP, pulse-labeled with ^{14}C-amino acids as described in the text; (b) isolated ESP treated with 4 M urea for 2 h and dialyzed against phage buffer before sucrose gradient centrifugation; and (c) isolated ESP treated with 1% sodium dodecyl sulfate for 1 h at room temperature. Gradients were collected and counted directly. To make the gradients comparable, the radioactivity in each fraction is expressed as the percentage of the total counts recovered in each gradient.*

The tubular part of giant variants of ESP and ELP undergo the same complete flattening; we have found indeed that the width of the ESP-derived giant is about 15% smaller than the width of the ELP-derived giant. The latter is, in turn, the same as that of ghosted T4 giants (1).

Previous experiments showed that the stable capsid-like particles isolated from 17⁻-infected cells are made of the cleaved major head protein (gp23*) (20, 25). In addition, the comparison of the amount of gp23* contained per 17⁻-infected cells with the number of particles per cell found in sections of the same cells suggested that not only the stable capsid-like ELP but also the ESP are made of gp23* (Wunderli, Ph.D. thesis).

We have now demonstrated that the isolated ESP are made of cleaved head proteins (gp23*, gp24*, IpIII*), besides others (gp20, B1). We have not found uncleaved core proteins (gp22, internal proteins) in the isolated ESP, suggesting that the particle has been almost completely processed by the T4 prehead protease (T4ppase) (28). When observed in sections of 17⁻-infected cells, the ESP have a thicker "shell" than do the τ-particles (Wunderli et al. [a], in press). This is related either to the presence of IpIII* in the inner part of the ESP shell or to a different stain uptake that would reflect some structural difference between the two types of particles. The variable amounts of IpIII* found in isolated ESP suggest that the proteins of the core are easily lost; this is probably also the reason why we see "empty" particles in thin sections of 17⁻-infected cells. This hypothesis is supported by our finding that the particles accumulated in 17⁻-infected cells can preserve internal content, as is shown by thin sections prepared from material fixed in the presence of high concentrations of salts (E. Kellenberger, unpublished data).

The absence of hoc and soc proteins in isolated ESP and preheads (L. Onorato, B. Stirmer, and M. K. Showe, manuscript in preparation) indicates that gp23 or gp23* located in unexpanded lattices are not competent for binding them. These two dispensable proteins have been reported to be T4 specific (14) and are only found as part of expanded shells.

Isolated ESP, but not ELP, are destroyed in 4 M urea or 1% sodium dodecyl sulfate at room temperature. Again, this behavior is characteristic of unexpanded lattices (τ-particles, "coarse" polyheads) (33), whereas the expanded normal capsids, as well as the 17⁻ ELP, are resistant to these treatments.

By optical diffraction we reached independent evidence that the lattice of ESP is indeed unexpanded and has a lattice constant of 11.3 nm. Despite their similarity, τ-particles and ESP are fundamentally different because τ-particles are composed of gp23 whereas the ESP have gp23*. We thus conclude that cleavage of the major head protein does not provide resistance to the shell, but that an expansion is also required for increasing the stability of the particle toward protein-denaturing agents.

To further study the lattice of ESP by image processing of electron micrographs, we have constructed giant variants of these particles by canavanine treatment (9) of 17⁻-infected cultures. In lysates, we find predominantly two main different types of giants. One is similar to the empty normal T4 giants described previously (1, 4, 5, 34); it has an expanded lattice (12.95 ± 0.14 nm), and the diffraction patterns, as well

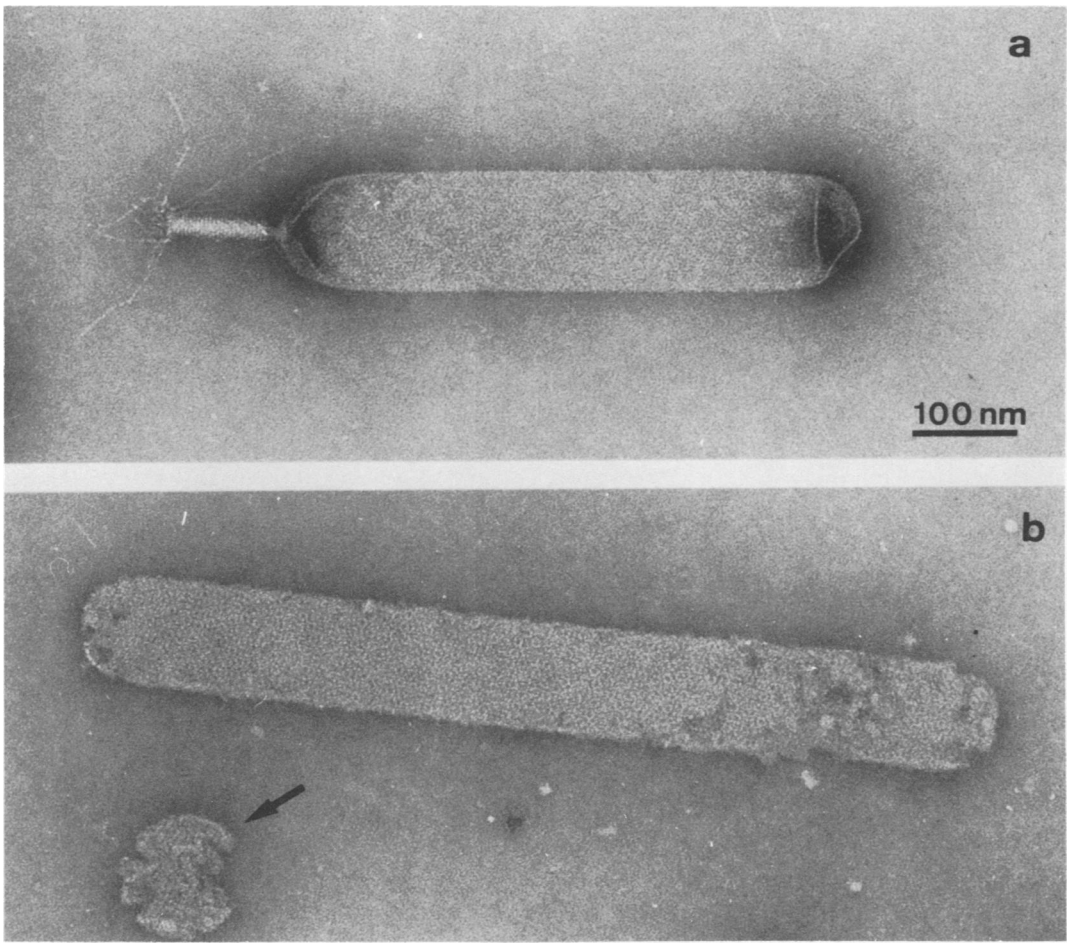

FIG. 8. *Electron micrographs of giant particles induced in 17⁻-infected cells. 17⁻-infected cells were treated with canavanine and chased with arginine by the method of Cummings et al. (9) as described in the text. The lysate was prepared and negatively stained as described in the text. (a) ELP-derived giant (note both the typical foldings and stain retention in the less flattened caps and the complete flattening of the cylindrical part); (b) ESP-derived giant (note the rough surface texture and few ruptures). Arrow shows an ESP for comparative purposes.*

as the reconstructions obtained (J. L. Carrascosa and A. C. Stevens, manuscript in preparation), show a (6+6+1)-type capsomere, that is, with hoc and soc bound. Considering these characteristics, as well as the flattening behavior in negative staining, we infer that these giants are elongated ELP. The other class of giants found in 17⁻ lysates has the characteristics and the rough surface texture peculiar to ESP. The diffraction patterns of these giants show that the p6 lattice in which the protomers are arranged is unexpanded (11.23 ± 0.11 nm). They are significantly different from the diffraction pattern of the giants derived from τ-particles, in which the major head protein is uncleaved (gp23). A more detailed comparison of both types of lattices is presently under study (Carrascosa and Steven, manuscript in preparation).

Laemmli et al. (19) have described a type of polyhead that is composed of the cleaved major head protein (gp23*) but that has an unexpanded ("anchored") lattice. Our studies on the giant variant of ESP show a close structural relationship with anchored polyheads, suggesting that both are apparently representatives of the "cleaved but anchored" lattice. The existence in 17⁻ of ESP that are composed of cleaved proteins (gp23*, gp24*, IpIII*) and that are still unexpanded demonstrates that expansion is not necessarily linked to cleavage. In certain temperature-sensitive mutants in gene 23, Onorato and Showe (Experientia, in press) have recently found that expansion of the prehead-related shell may occur without previous cleavage of

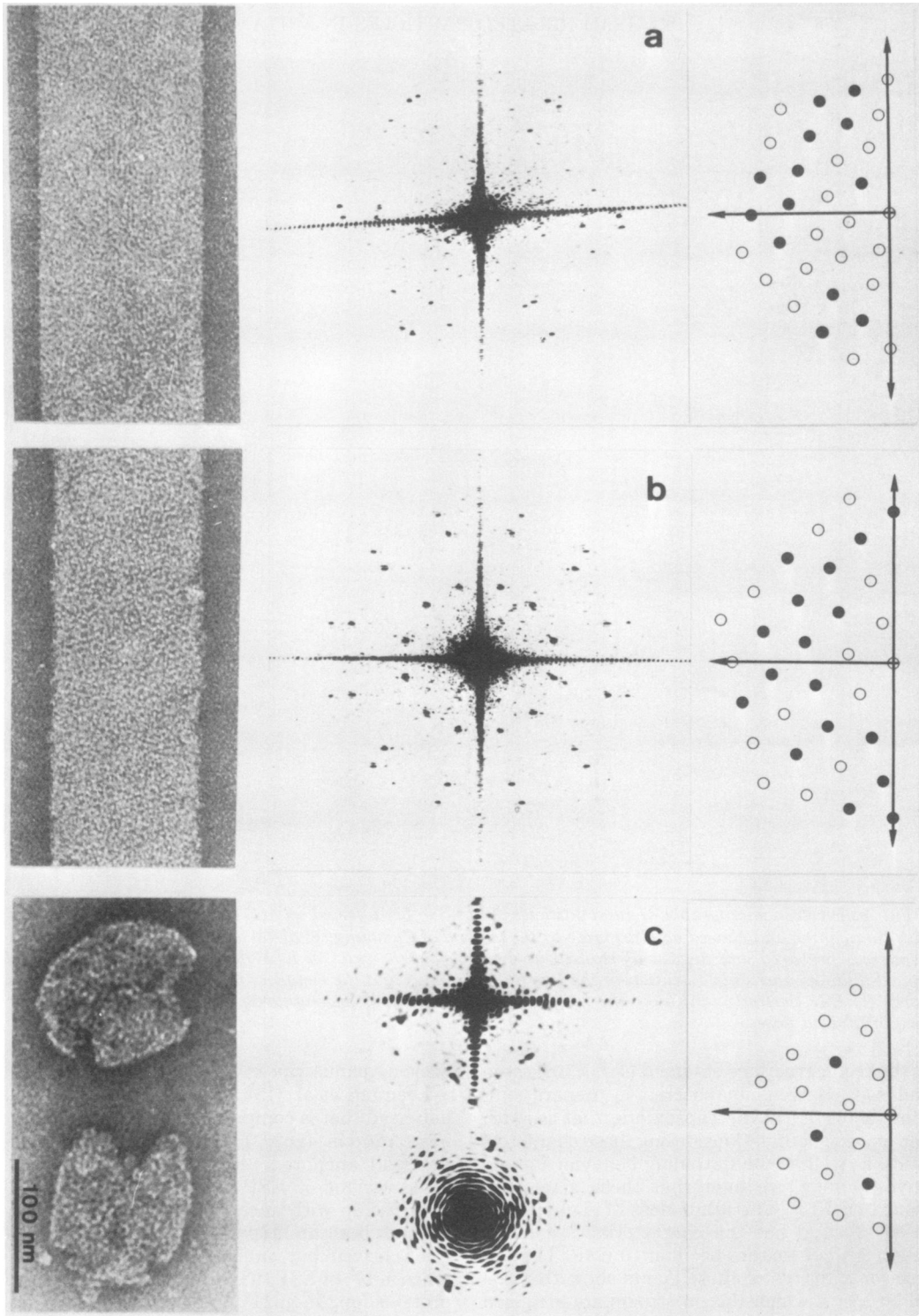

FIG. 9. *Electron micrographs and diffraction patterns of 17⁻ particles. Horizontal line (a) shows from left to right a high-magnification picture of an ELP-derived giant, its diffraction pattern, and the corresponding schematic index of the diffraction pattern generated by one of the faces of the flattened particle. Observed diffraction spots are shown as solid circles. Line (b) shows the same for an ESP-derived giant. Line (c) shows a micrograph of isolated ESP with their diffraction patterns obtained with two different windows. Note that the lattices indexed in (b) and (c) are basically identical.*

TABLE 1. *Structural parameters of giants obtained by canavanine treatment of 17⁻-infected cells*

Type[a]	Orders[b]	Lattice constant (nm)[c]	Pitch angle[d]	Width (nm)	u,v coordinates[d]
ESP derived	2nd, 5th	11.23 ± 0.11	14°	98.9 ± 4.0	15,5
ELP derived	7th	12.95 ± 0.14	14°	115.0 ± 2.0	15,5

[a] The numbers of ESP- and ELP-derived giants studied were 25 and 15, respectively.
[b] Predominant orders in the diffraction pattern (33).
[c] Calibrated by assuming the meridional reflections of optically diffracted T4 tail sheaths to be due to a 3.8-nm periodicity (also see the text). Averages and standard deviations are given.
[d] For definition, see Bijlenga et al. (5).

the major head protein (gp23). This result supports the idea that shell expansion can only be triggered when the subunits assume a specific conformation. This conformation might be a consequence of either cleavage or of an abnormal structure of the protein produced by the temperature-sensitive mutant.

ESP and ELP have been found in cells infected with mutants in genes 17 and 16 or with the triple mutant 43⁻.30⁻.46⁻ (Wunderli et al. [a] and [b], in press). Minagawa has reported recently (26) that the product of gene 17 is related to a nuclease activity associated with the T4 capsid. In cells infected with mutants in either genes 17 or 16, the DNA maturation is halted (12), whereas the triple mutant 43⁻.30⁻.46⁻ cannot synthesize DNA (27). The observation that ESP accumulate under conditions in which vegetative phage DNA is either absent or possibly not competent for packaging suggests the possibility that this type of particle might be related to some intermediate of the T4 head maturation pathway. Previous results indicated that 17⁻ particles are not capable of maturing into infective phage (20, 25; Wunderli et al. [a], in press). As we will discuss now, these data are, however, not sufficient to exclude the precursor role of similar "cleaved but unexpanded" particles in T4 head maturation. We propose the hypothesis that the empty 17⁻ ESP are derived by some aberrant modifications from a true intermediate in T4 head maturation, probably as a consequence of DNA not being available or competent for packaging. The intermediate would undergo some small transformations leading to abortiveness of the particle. Our hypothesis is in agreement with the following facts. (i) Preliminary experiments have shown that when grown at restrictive conditions some temperature-sensitive mutants in gene 17 accumulate empty particles which, after shift to a permissive temperature, can give rise to DNA-filled heads observable in thin sections, but not to a corresponding amount of infective phages (E. Kellenberger and J. van den Broek, personal communication). (ii) In cells infected with T4D⁺ and treated with 9-aminoacridine (a drug that is known to bind strongly to DNA, thus rendering it probably unable to be packaged normally), we find an accumulation of particles (ε-particles), which are very closely related to 17⁻ ESP and which can be matured in vivo to DNA containing morphologically intact phages (C. Schärli and E. Kellenberger, Experientia, in press). (iii) The isolated 17⁻ ESP can be expanded in vitro into particles resembling closely normal empty capsids (J. L. Carrascosa, submitted for publication).

Although shown to be capable of maturation in the case of recovery after acridine treatment, the cleaved but unexpanded ε-particle does not necessarily need to also occur in the normal pathway.

Our data are, however, consistent with a T4 head maturation pathway in which the prehead (τ-particle) is cleaved and gives rise to the ε-particle. This particle might be related to the prohead II in the pathway of Laemmli and Favre (20), which was believed to be an empty expanded capsid. According to our results, it would still be unexpanded. The expansion of this precursor particle might be triggered by the onset of DNA packaging, as suggested by the fact that partially filled particles are always expanded (Wunderli et al. [a], in press). To be consistent, this would request proving that ELP are the consequence of an artifact. If this is not verified, we have to search for another, still unknown, mechanism for initiating in vivo expansion.

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