JOURNAL OF VIROLOGY, May 1982, p. 583–594
0022-538X/82/050583-12$02.00/0

Vol. 42, No. 2

Detection and Characterization of Agarose-Binding, Capsid-Like Particles Produced During Assembly of a Bacteriophage T7 Procapsid

PHILIP SERWER,* ROBERT H. WATSON, AND SHIRLEY J. HAYES

Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

Received 27 October 1981/Accepted 13 January 1982

It has previously been shown that: (i) during infection of its host, the DNA bacteriophage T7 assembles a DNA-free procapsid (capsid I), a capsid with an envelope differing physically and chemically from the capsid of the mature bacteriophage, and (ii) capsid I converts to a capsid (capsid II) with a bacteriophage-like envelope as it packages DNA. Lysates of phage T7-infected *Escherichia coli* contained a particle (AG particle) which copurified with capsid II during buoyant density sedimentation, velocity sedimentation, and solid support-free electrophoresis, but was distinguished from capsid II by its apparent diversity during electrophoresis in agarose gels. Treatment of AG particles with trypsin converted most of them to particles that comigrated with trypsin-treated capsid II during electrophoresis in agarose gels. Irreversible binding of AG particles to agarose gels was shown to contribute to the apparent diversity of AG particles during agarose gel electrophoresis. The results of quantitation of AG particles and of capsid I and capsid II in lysates of a nonpermissive host infected with T7 amber mutants suggested that, in spite of their capsid II-like properties, most AG particles were produced during assembly of capsid I and not during DNA packaging. The presence of AG particles in T7 lysates explains contradictions in previous data concerning the pathway of T7 assembly.

During attempts to understand the assembly of viruses, particles which are either precursors of the mature virus or breakdown products of such precursors (precursors and their breakdown products will both be referred to as assembly intermediates) are isolated, and the sequence of appearance of intermediates is determined. The accuracy with which a pathway is understood depends on the capacity of isolation techniques to discriminate the different assembly intermediates. The pathway cannot be accurately understood if particles from more than one position in the pathway copurify; for instance, if two copurifying intermediates are separated from each other in the sequence of assembly by a third intermediate that does not copurify with the other two, data obtained may not clearly indicate a sequence of appearance for the intermediates (Fig. 1).

During a previous study of the assembly of bacteriophage T7, it was shown by measurement of the kinetics of appearance of T7 capsids that a DNA-free procapsid (capsid I), a capsid with an envelope that has several physical properties different from those of the mature bacteriophage T7 envelope, is assembled and converts to a capsid with a bacteriophage-like envelope (capsid II) during the packaging of DNA (14; for a description of capsid I and capsid II, see Fig. 2); agarose gel electrophoresis was used to identify and quantitate capsids I and II in this study. In another study (9), sedimentation in sucrose gradients was used to detect and quantitate capsids I and II, and the kinetics of appearance of these two capsids did not indicate a clear precursor-product relationship between capsid I (referred to as prohead) and capsid II (referred to as empty head). As previously indicated (14, 18), some particles sedimenting as capsid II do not form a band of capsid II during electrophoresis in agarose gels; these particles appear to be electrophoretically diverse in agarose and will be referred to as agarose, or AG, particles.

It seemed possible that the AG particles that cosediment with capsid II in sucrose gradients are produced at a different point in the pathway of T7 assembly than particles migrating as capsid II in agarose gels and that this might be the cause of the failure of the data in reference 9 to reveal a precursor-product relationship between capsid I and capsid II. That capsid I-like, DNA-free procapsids of several other duplex DNA-containing bacteriophages (λ, T3, T4, and P22) are precursors of the capsid of their respective mature bacteriophage has previously been demonstrated (reviewed in references 2, 4, 7).

A → B → C → B' → D → → Mature Virus

FIG. 1. Possible source of confusion during analysis of pathways of viral assembly. Two intermediates, B and B', are separated in the assembly pathway by C. However, B and B' are not isolated from each other by the technique used for isolation and quantitation of the intermediates, although B and B' are both isolated from A and C. Measurements of the kinetics of appearance of intermediates may not yield clear precursor-product relationships between B, B', and C.

To determine the role of AG particles during T7 assembly, and to resolve the above mentioned difference in data, attempts were made to further characterize the AG particles and to determine whether AG particles accumulate in nonpermissive hosts infected with the various T7 amber mutants. Initially, these studies were restricted by the difficulty of recognizing and quantitating particles which are electrophoretically diverse. However, a procedure was discovered for converting most AG particles to particles electrophoretically uniform during electrophoresis in agarose gels, simplifying this study, the results of which are presented here.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. Wild-type bacteriophage T7 and T7 amber mutants (22) were received from F. W. Studier. The following amber mutants were used: gene 4-208; gene 5-28; gene 7-405; gene 8-11; gene 9-17; gene 10-13; gene 12-3; gene 13-149; gene 14-140; gene 15-31; gene 16-9; gene 17-290; gene 18-182; and gene 19-10. The host for 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'. A lysate of the nonpermissive host infected with an amber mutant will be referred to by the number of the mutant gene. Particles isolated from such lysates will also be referred to by the number of the mutant gene. For instance, capsid I isolated from a lysate of the nonpermissive host infected with a 14*am* mutant will be referred to as 14*am* capsid I.

Buffers and media. Standard/G buffer is 0.15 M NaCl, 0.05 M Tris-chloride, pH 7.4, 0.005 M EDTA, and 100 μg of gelatin per ml. Bacteria were grown in either M9 medium (12) or 2× LB broth (10). Two buffers were used for electrophoresis: Phos/Mg electrophoresis buffer was 0.05 M sodium phosphate, pH 7.4, and 0.001 M $MgCl_2$; Phos/EDTA electrophoresis buffer was 0.05 M sodium phosphate, pH 7.4, and 0.001 M EDTA. When preparing samples for electrophoresis in Phos/Mg electrophoresis buffer, the samples were diluted with Phos/Mg sample buffer: 0.005 M sodium phosphate, pH 7.4, 0.001 M $MgCl_2$, 400 μg of bromophenol blue per ml, 4% sucrose, and 100 μg of gelatin per ml. For electrophoresis in Phos/EDTA electrophoresis buffer, samples were diluted in Phos/EDTA sample buffer, a buffer identical to Phos/Mg sample buffer except that the $MgCl_2$ was replaced with 0.001 M EDTA. For the electrophoresis in agarose gels of known T7 capsids (see Results), use of Phos/EDTA electrophoresis buffer results in profiles indistinguishable from the profiles obtained with Phos/Mg electrophoresis buffer. However, Phos/Mg electrophoresis buffer is used in preference to Phos/EDTA electrophoresis buffer to prevent particles with packaged DNA from emptying their DNA; Phos/EDTA electrophoresis buffer is used in preference to Phos/Mg electrophoresis buffer to prevent DNases from digesting unpackaged DNA, possibly bound to capsids (17), after lysis of cells. The use of two different buffers for

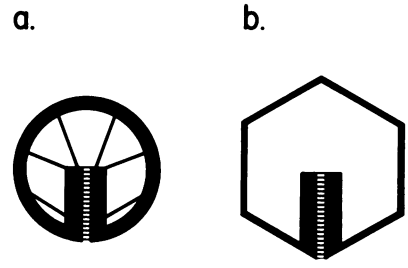

FIG. 2. Structures of capsid I and capsid II. The structures of: (a) capsid I and (b) capsid II are drawn in projection. In electron micrographs of negatively stained specimens, the envelope of capsid I appears to be that of a sphere which has been flattened (flattening probably occurs during negative staining); capsid II appears to be a polygon (presumably icosahedral) circumscribed around a sphere, also flattened (12). The most abundant protein in the envelopes of capsids I and II is P10 (T7 proteins are indicated by P followed by the number of the protein's gene, as determined in reference 23). By both electron microscopy (12) and low-angle X-ray scattering (21a), the envelope of capsid I is thicker than the envelope of capsid II, presumably because the envelope of capsid I has larger amounts of P9 than the envelope of capsid II (12); the results of X-ray scattering also indicate that the mean outer radius of capsid I is 8% smaller than the mean outer radius of capsid II. An internal, cylindrical core of capsid I appears in electron micrographs to be attached at its base to the envelope of the capsid and to have fibers connecting it to the envelope; the core of capsid I also appears to have an axial hole through which DNA may enter the capsid during DNA packaging (12, 13). The proteins of the core are P8, P14, P15, P16, and possibly P13 (12); locations of these proteins in the core are not known, although it has been proposed that the envelope-proximal part of the core is P8, the envelope distal part of the core is P15, and the fibers are P14 or P16 (13). Some particles of capsid II also have the cylindrical region of the internal core (the core appears to have disintegrated in particles of capsid II missing the core) (12, 14). Though fibers have never been observed connecting the capsid II core to its envelope, the fibers are so difficult to observe (13) that it cannot be said for certain whether they are present in capsid II. Further fractionation of capsid II and the positions of capsid I and the various forms of capsid II in the T7 DNA packaging pathway have previously been described (14, 17).

electrophoresis results from the interfacing of experiments presented here with the isolation and characterization of intact bacteriophages and particles in the DNA packaging pathway of T7 (17; P. Serwer and S. J. Hayes, *in* R. C. Allen and P. Arnaud, ed., *Electrophoresis '81*, in press).

Stocks of amber mutants. Stocks of amber mutants were grown in 2× LB broth, clarified by centrifugation, and concentrated by centrifugation, as previously described (11). Stocks used for preparing radiolabeled lysates were further purified by sedimentation in a cesium chloride step gradient (see below). Stocks used for preparing nonradiolabeled lysates were used without further purification.

Radiolabeling and artificial lysis of T7-infected *E. coli*. Log-phase cultures of *E. coli* BB/1 were infected at 4×10^8/ml with either phage T7 or a T7 amber mutant, using a multiplicity of 15. Cultures were labeled from 14 to 24 min after infection with 10 μCi of either ^{3}H- or ^{14}C-algal hydrolysate per ml (ICN or Schwarz/Mann); the cultures were chilled and lysed with the nonionic detergent Brij 58, as previously described (11).

Fractionation of radiolabeled lysates. Fractionation of radiolabeled lysates by sedimentation through a biphasic gradient of sucrose and metrizamide (to be referred to as a sucrose-metrizamide gradient) was performed as previously described (14). Further fractionation of material copurifying in sucrose-metrizamide gradients with phage T7 (see Fig. 1 of reference 14) was performed by diluting the sample by at least a factor of 1.5 with standard/G buffer (final volume, 1 ml) and then layering the diluted sample on a discontinuous gradient of cesium chloride in 0.01 M Tris-chloride, pH 7.4, 0.001 M $MgCl_2$, and 100 μg of gelatin per ml with layers having the following volumes and densities: 0.90 ml; 1.717 g/ml; 1.00 ml; 1.499 g/ml; 1.40 ml; 1.393 g/ml; 0.90 ml; and 1.288 g/ml. The gradient was centrifuged at 40,000 rpm at 20°C for 1 h and was fractionated from the bottom by puncture of the centrifuge tube.

^{14}C-labeled AG particles (see Results) stick to borosilicate glass tubes used for collection of gradients (see also reference 13). To prevent this sticking, glass tubes used to collect gradients were incubated for at least 16 h in a saturated atmosphere of Prosil 28 Organosilane concentrate (PCR Research Chemicals, Inc.), a procedure which eliminated detectable sticking of AG particles for a period of at least 6 months.

Electrophoresis in agarose slab gels. Samples were diluted in a sample buffer as described in the figure legends and were layered in the sample wells of a horizontal ME agarose (Marine Colloids, Rockland, Maine) gel submerged beneath 4 to 6 mm of buffer; the apparatus holding the gel has previously been described (15). To reduce discontinuities in the buffer at the origin of electrophoresis (and therefore to increase the homogeneity of bands formed by capsids), the samples were allowed to remain in the sample wells for 1.5 h before electrophoresis was started at 0.96 ± 0.08 V/cm, room temperature (25 ± 3°C), for the time indicated. Buffer was circulated over the top of the gel at 50 ml/min, starting at 1 h after the start of electrophoresis. After electrophoresis, the gels were dried and subjected to autoradiography.

Two-dimensional electrophoresis in agarose gels. A sample with a final volume of 12 μl, prepared by diluting particles in standard/G buffer with 2 parts of Phos/EDTA sample buffer, was layered on a cylindrical agarose gel in a glass 100-μl micropipette, 1 mm in inner diameter. Dialysis tubing was placed over the bottom of the micropipette (to prevent the gel from falling out) and was held on the pipette with a piece of rubber tubing. The micropipette was placed and held in a cylindrical glass tube, 6 mm in diameter, by inserting the pipette into a hole in a rubber stopper at the top of the tube. The 6-mm glass tube was then inserted in a commercial electrophoresis apparatus, and the sample was subjected to electrophoresis at 3.3 V/cm for 6 h in the buffer indicated. Electrophoresis was performed overnight and was terminated with a timer. The next morning, a horizontal slab gel of agarose was prepared, as described in the previous section, and the septa between some sample wells were removed to make room for cylindrical gels. The cylindrical gels were layered at the origin of slab gels (usually two cylindrical gels were used per slab); samples serving as markers were layered in sample wells left intact, and electrophoresis was performed for 18 h as described in the previous section. After electrophoresis, ^{14}C-labeled particles in the slab gel were detected by fluorography (6).

Densitometry. The amount of ^{14}C in selected regions of gels was determined by densitometric scanning of autoradiograms (an R and D densitometer of Helena Laboratories, Inc., Beaumont, Tex., was used) and converting areas thus obtained to counts per minute, using an empirically obtained calibration factor.

Digestion with trypsin. A 1/10 volume of 20 mg of trypsin per ml (Millipore Corp; tolylsulfonyl phenylalanyl chloromethyl ketone) in water (dissolved no less than 1 h before use and kept on ice) was diluted into a sample, and this mixture was incubated at 30°C for 1 h. After incubation, a 1/10 volume of 0.001 M *p*-nitrophenyl-*p'*-guanidinobenzoate hydrochloride (an inhibitor of trypsin [3]) in dimethyl sulfoxide was added to this mixture and was allowed to sit at room temperature for at least 20 min. In some experiments, a control digestion was performed as above with trypsin inhibited before digestion (20 min, room temperature) with a 1/10 volume of 0.01 M *p*-nitrophenyl-*p'*-guanidinobenzoate hydrochloride in dimethyl sulfoxide.

Electrophoresis in density gradients of metrizamide. Electrophoresis in density gradients of metrizamide was performed at 25°C for 8.5 h, as previously described (19). From theoretical considerations (21), it has been calculated that during solid support-free electrophoresis the electrophoretic mobility of spherical particles the size of T7 capsids is determined primarily by the average electrical surface charge density on the particle.

Buoyant density sedimentation. Buoyant density sedimentations in density gradients of cesium chloride and in density gradients of metrizamide were performed as previously described (14).

Electron microscopy. Samples were prepared for electron microscopy by negative staining with 1% sodium phosphotungstate, pH 7.6, as previously described (13).

SDS-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed as previously described (24); a linear 10 to 16% acrylamide gradient was used. ^{14}C-labeled proteins were detected by fluorography (1).

RESULTS

Detection of AG particles. Characterization and quantitation of AG particles in lysates of wild-type T7-infected *E. coli* initially encountered two obstructions: particles which are electrophoretically diverse cannot be accurately quantitated; and the AG particles are contaminated with capsid II after both sedimentation in sucrose gradients and electrophoresis in agarose gels (14, 18). These obstructions were overcome when it was discovered that: (i) digestion with trypsin converted some of the AG particles to particles that comigrated with capsid II during electrophoresis in agarose gels, and (ii) in ^{14}C-radiolabeled lysates of a nonpermissive host infected with some T7 amber mutants, the ratio of the amount of ^{14}C in AG particles to the amount of ^{14}C in capsids I and II was higher than in wild-type lysates (see Table 1). Data from which these conclusions were drawn are shown in Fig. 3 for capsids from a 15*am* lysate, a lysate enriched for AG particles.

In Fig. 3a, fractions of a sucrose-metrizamide gradient used to fractionate a ^{14}C-labeled, 15*am* lysate were pooled in pairs and subjected to electrophoresis in a slab gel of agarose. Particles of capsid I and capsid II in the gradient of Fig. 3a formed bands in the gel, and the sedimentation profile of each capsid was revealed by the intensity of its band as a function of the fraction number of the gradient. Also present in the gradient of Fig. 3a are AG particles, particles which were found continuously distributed between the origin of electrophoresis and a position roughly 1.2 times as far from the origin as capsid I, and were, therefore, more diverse in electrophoretic mobility than capsid I and capsid II. In some experiments the AG particles were found continuously distributed in a region less extensive than in Fig. 3a, but always extended from the origin to a position roughly 0.6 times as far from the origin as capsid I. The AG particles appear to form a peak in the gradient of Fig. 3a at the position of capsid II (vertical arrowhead), suggesting a relationship of these particles to capsid II.

The samples of Fig. 3a were also subjected to electrophoresis after digestion with trypsin (Fig. 3b). Trypsin reduced the amount of ^{14}C in AG particles and increased the amount of ^{14}C in particles forming the band of capsid II in the agarose gel. In independent experiments it has been shown that the intensity of the trypsin-induced component of the capsid II band does not vary as a function of the concentration of trypsin used between 0.05 and 5 mg of trypsin per ml (data not shown). The intensity of the capsid II band was not increased if preinhibited trypsin (Materials and Methods) or DNase I (0.1 to 0.5 mg/ml) was used for digestion (see Fig. 7), indicating that it was the proteolytic activity of trypsin, not a nonspecific effect of trypsin, that was causing the increased intensity of the capsid II band after digestion with trypsin. In most AG particle-enriched lysates, roughly 60 to 80% of ^{14}C-labeled AG particles were converted by trypsin to particles comigrating with capsid II during agarose gel electrophoresis. This trypsin-induced conversion of most AG particles facilitated their identification and quantitation (see also Fig. 7).

After trypsinization, the distance migrated by capsid I in the agarose gel of Fig. 3 increased by 6%, and the band formed by capsid I became sharper (the total amount of ^{14}C in the band formed by capsid I was not changed). A 6 to 12% increase in distance migrated also occurred with capsid I from wild-type or any of the mutant lysates of Table 1. That capsid I was intact after treatment with trypsin was indicated by the finding that such treatment did not affect the rate of sedimentation of capsid I in sucrose gradients ($\pm 5\%$).

Physical properties of AG particles. The data presented in the previous section suggest that AG particles are related to capsid II in structure. To further test this possibility, ^{14}C-labeled 15*am* AG particles from the capsid II region of a sucrose-metrizamide gradient were subjected to the following procedures of fractionation, together with ^{3}H-labeled capsid II from the capsid II region of a sucrose-metrizamide gradient of a wild-type T7 lysate, the latter used as a marker: buoyant density sedimentation in a cesium chloride density gradient, buoyant density sedimentation in a metrizamide density gradient, and electrophoresis in a metrizamide density gradient. After all of these procedures, the AG particles copurified with capsid II, the higher-density (1.28 g/ml) subfraction of capsid II (14) during buoyant density sedimentation in a metrizamide gradient; the data for electrophoresis in a metrizamide density gradient are in Fig. 4. After recovery from all of the above gradients, the 15*am* AG particles retained their apparent diversity during electrophoresis in agarose gels and their partial convertibility by trypsin to particles comigrating with capsid II during electrophoresis in agarose gels (data not shown).

The copurification of capsid II and AG particles during buoyant density sedimentation indicated that AG particles probably were not bound to vesicles of host membrane (see reference 14). The comigration of AG particles with capsid II during electrophoresis in metrizamide density gradients supported this conclusion and indicated that AG particles had the same average electrical surface charge density as capsid II, further indicating that AG particles had enve-

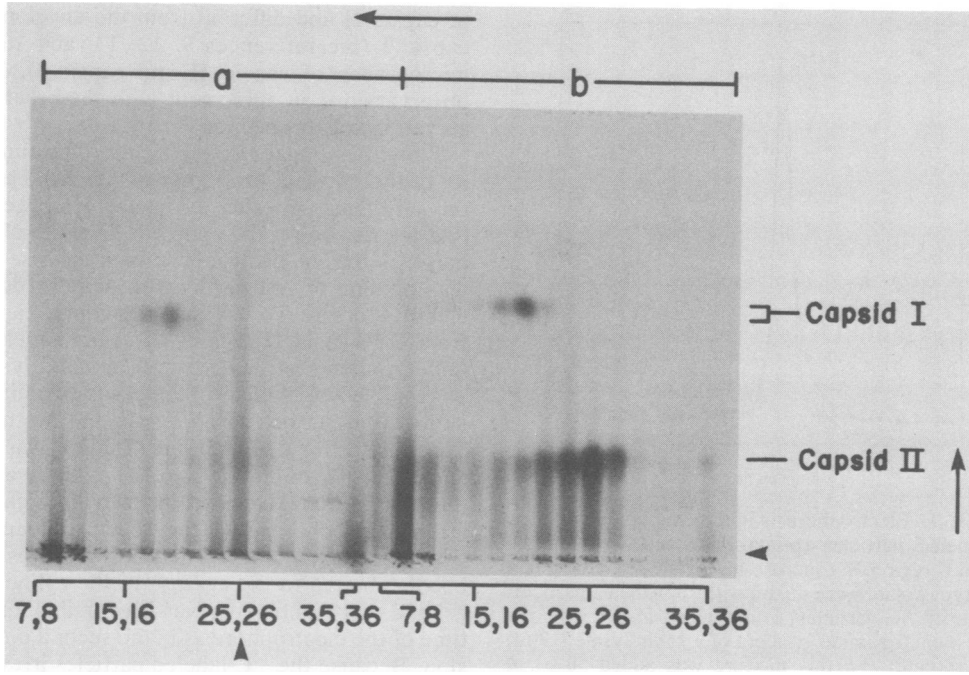

FIG. 3. Agarose gel electrophoresis of 15*am* T7 capsids fractionated by sedimentation. A 5-ml culture of *E. coli* BB/1 was infected by 15*am* T7, labeled with ^{14}C-amino acids, chilled, lysed, and sedimented in a sucrose-metrizamide gradient, as described in Materials and Methods. Neighboring fractions were pooled in pairs, 3 μl from each fraction, and were digested with trypsin; a second set of the same samples was prepared without trypsinization. To each trypsinized and untrypsinized sample was added 20 μl of Phos/Mg sample buffer; 20 μl of these mixtures was then subjected to electrophoresis in a 0.9% agarose slab gel in Phos-Mg electrophoresis buffer for 16 h as described in Materials and Methods. (a) Untrypsinized samples; (b) trypsinized samples. The fractions of the sucrose-metrizamide gradient are indicated. Samples from the first six fractions were not subjected to electrophoresis because most of the ^{14}C in these fractions is in bacteriophage T7, a particle that does not form a sharp band during agarose gel electrophoresis unless higher concentrations of sample than those used here are used (16). Samples from the last four fractions of the gradient were also not subjected to electrophoresis because these fractions contain unincorporated amino acids and slowly sedimenting proteins, neither of which is sieved sufficiently to prevent diffusion from obscuring identifiable bands (see also reference 18). Fractions 9 and 10 contain *E. coli* membranes and other particles sedimenting to the interface of sucrose and metrizamide (14).

lopes with a capsid II-like (rather than a capsid I-like) structure (16, 19).

To help determine the size of particles, the extent to which they were sieved by agarose gels was measured (8, 15a). After electrophoresis in 0.3, 0.5, 0.9, and 1.5% agarose gels (Fig. 5 a–d, respectively), the sieving of trypsinized AG particles from a 4*am* lysate (see Table 1) was determined by measuring the ratio of the distance from the origin of the band formed by trypsinized AG particles (channel 1 of Fig. 5) to the distance from the origin of the band formed by trypsinized capsid II (channel 3 of Fig. 5). In all of these gels, this ratio was 1.00 ± 0.04. This indicated that capsid II and those trypsinized AG particles forming the band in Fig. 5 were sieved to the same extent by the gel, further indicating that capsid II and these latter AG particles were roughly the same size and shape; capsid II has already been shown to be roughly spherical (Fig. 2). Assuming that capsid II and trypsinized AG particles forming the band in Fig. 5 are both spherical, the absence of differential sieving of these two particles indicates that they have the same radius ± 8% (15a).

To complete the physical characterization of AG particles, we attempted to observe them by electron microscopy. To obtain negatively stained specimens with an adequate number of particles, use of lysates 200- to 1,000-fold more concentrated than the lysate used in Fig. 3 is desirable (12). When capsids in lysates enriched for AG particles (8*am* and 15*am* lysates) were concentrated by a factor of 200 to 1,000 by using either precipitation after lysis with Carbowax 6000 (12) or chilling and pelleting of the infected cultures in their growth medium before lysis, the number of capsid II-sedimenting, capsid-like particles observed by electron microscopy was one to two orders of magnitude lower than the number of such particles observed in similar preparations from wild-type lysates. If the

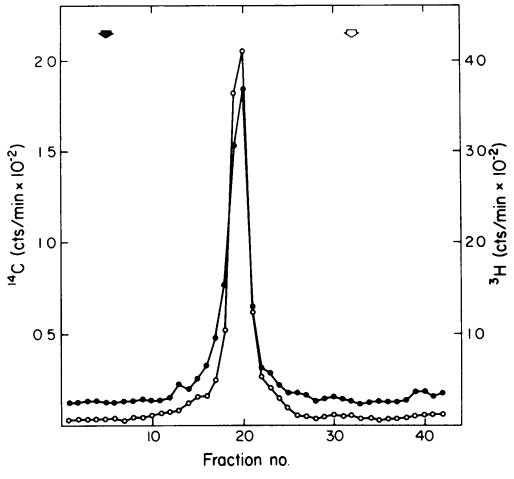

FIG. 4. Electrophoresis in a metrizamide gradient. ^{3}H-labeled particles (primarily capsid II) from the capsid II region of a sucrose-metrizamide gradient of a wild-type lysate were mixed with ^{14}C-labeled particles (primarily AG particles) from the capsid II region of a sucrose-metrizamide gradient of a 15*am* lysate (19 μl, final volume). To this mixture was added 50 μl of Phos/EDTA sample buffer, and the entire amount of this latter mixture was subjected to electrophoresis in a gradient of metrizamide, as described in the text. The closed arrow indicates the origin of electrophoresis; the open arrow indicates the position of bromophenol blue. The anode is at the right.

amount of AG particles and capsid II in lysates is roughly proportional to the amount of ^{14}C entering these capsids, the amount of AG particles in 8*am* and 15*am* lysates is roughly the same as the amount of capsid II in wild-type lysates. Thus, the data above indicate a preferential loss of AG particles during or after concentration. After mixing ^{14}C-labeled AG particles and capsid II with such concentrated lysates (before sedimentation of the concentrated lysates for isolating AG particles), it was found that AG particles, but no or comparatively little capsid II, pelleted at comparatively low speeds (10,000 rpm, 10 min, Beckman J21 rotor). Postlysis conversion of AG particles to more rapidly sedimenting particles (possibly by adherence to another particle, such as a fragment of host membrane), lost during clarification of concentrated lysates, was, therefore, presumably the reason for the low recovery of AG particles from concentrated lysates. Thus, electron microscopy could be reliably performed only on more dilute preparations such as the one in Fig. 3.

AG particles isolated by sedimentation of a 5*am* lysate (see Table 1), as described in Materials and Methods, were observed by electron microscopy. Of 83 capsids observed, all had envelopes indistinguishable from the envelope of capsid II and different from the envelope of capsid I (see references 9, 12, 13, and 16 for micrographs of capsid II and capsid I). This observation is in agreement with the other observations of this section.

Two-dimensional electrophoresis. The similarity of the physical properties of AG particles to the physical properties of capsid II suggested that the reason for the apparent diversity of AG particles during electrophoresis in agarose gels was binding of AG particles to agarose during electrophoresis. To test this possibility, capsids from the capsid II regions of sucrose-metrizamide gradients of AG particle-enriched lysates were subjected to electrophoresis in two dimensions, as described in Materials and Methods. The result using a 14*am* lysate (Table 1) was that almost all ^{14}C migrating into the cylindrical gel (first dimension) remained in the cylindrical gel after electrophoresis in the second dimension (Fig. 6b), demonstrating that AG particles had become bound to the agarose of the cylindrical gel and that this binding was irreversible for the time of the electrophoresis in the second dimension. Because the ^{14}C-labeled particles irreversibly bound to the agarose of the cylindrical gel had penetrated this gel during electrophoresis in the first dimension, the irreversible adsorption did not occur until the ^{14}C-labeled AG particles migrated some distance through the cylindrical gel (0 to 2 cm in Fig. 6b). Results indistinguishable from those in Fig. 6b were obtained with 5*am*, 8*am*, 14*am*, 15*am*, and 16*am* AG particles (not shown). A two-dimensional electrophoresis of ^{14}C-labeled particles from the capsid II region of a sucrose-metrizamide gradient of a wild-type T7 lysate is shown in Fig. 6a (the gel used for the second dimension was the same as the gel in Fig. 6b). In this sample, most ^{14}C was present in particles that comigrated with capsid II in both the first and the second dimensions, although some of the ^{14}C appeared to be in particles irreversibly bound to the cylindrical gel used for the first dimension. This latter observation indicated that AG particles were present in wild-type lysates.

For comparison, Fig. 6c shows a two-dimensional electrophoresis of particles from the capsid I region of a sucrose-metrizamide gradient of a wild-type T7 lysate. The proportion of ^{14}C adsorbed to the cylindrical gel used for the first dimension was smaller for the sample of capsid I than for the sample of capsid II, and most ^{14}C migrated as capsid I in the first and second dimensions. The band of capsid I in Fig. 6c is elliptical and has its longer axis pointing toward the origin of electrophoresis. This indicates that particles of capsid I did not all have the same mobility; i.e., the slower-migrating particles during electrophoresis in the first dimension were

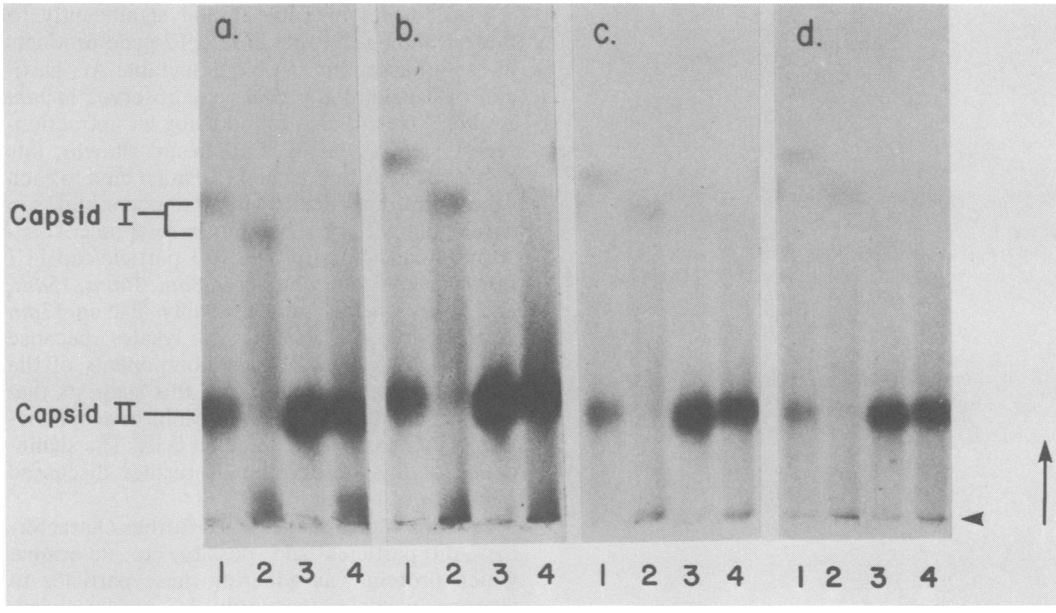

FIG. 5. Electrophoresis as a function of the concentration of agarose. A 60-μl sample of ^{14}C-labeled particles (primarily AG particles) from the capsid II-sedimenting region of a sucrose-metrizamide gradient of a 4*am* lysate was digested with trypsin, as described in the text; ^{14}C-labeled particles (primarily capsid II) from the capsid II-sedimenting region of a sucrose-metrizamide gradient of a wild-type lysate were prepared in the same way. To the resulting digested samples and also to the same samples undigested was added 90 μl of Phos/Mg sample buffer, and 25 μl of each mix was layered in the sample wells of agarose slab gels with (a) 0.3%, (b) 0.5%, (c) 0.9%, and (d) 1.5% agarose. Electrophoresis was performed in Phos/Mg electrophoresis buffer, as described in the text, for the following times, respectively: 11.6, 13, 16, and 25 h. The channels contain the following samples: (1) 4*am* particles, trypsinized; (2) 4*am* particles, untrypsinized; (3) wild-type particles, trypsinized; (4) wild-type particles, untrypsinized. The vertical arrow indicates the direction of electrophoresis; the arrowhead indicates the origin of electrophoresis. Some particles of capsid I contaminated the preparation of 4*am* particles. The increase in intensity of bands with decrease in agarose concentration results from a decrease in quenching of film exposure with decrease in agarose concentration.

also slower migrating during electrophoresis in the second dimension (see also reference 25).

Capsids in lysates of mutants. Even though AG particles resembled capsid II in all characteristics thus far determined, with the exception of affinity for agarose gels, it is possible, as suggested in the beginning of this paper, that AG particles are not DNA packaging intermediates like capsid II, and may even be capsid I assembly intermediates. Because infection of a nonpermissive host with a 5*am* mutant (no DNA synthesized; 23) results in the production of capsid I, but no detectable capsid II (14; Fig. 7b), it is likely that DNA packaging does not initiate during such an infection. Therefore, if AG particles are present in 5*am* lysates, these AG particles would most probably not be DNA packaging intermediates. That AG particles are found in 5*am* lysates is demonstrated in Fig. 7, in which are shown profiles of a ^{14}C-labeled, unfractionated 5*am* lysate subjected to agarose gel electrophoresis after treatment with trypsin (Fig. 7a) and after treatment with preinhibited trypsin (Fig. 7b). Thus, it is likely that AG particles are not DNA packaging intermediates and probably are produced at an earlier point in the T7 assembly pathway than capsid II.

To probe the role of the T7 proteins that appear in capsids (P7-P19; 23) in the production of AG particles, ^{14}C-labeled lysates of the nonpermissive host infected with T7 amber mutant in genes 7-19 were fractionated as in the experiment of Fig. 3. In each lysate, the amounts of ^{14}C in capsid I, capsid II, and those AG particles converted by trypsin to particles migrating as capsid II during agarose gel electrophoresis were determined; the AG particle/capsid I and the capsid II/capsid I ratios are given in Table 1. These ratios were determined by agarose gel electrophoresis after prefractionation in sucrose-metrizamide gradients (Fig. 3), rather than by analysis of unfractionated lysates (Fig. 7), because particles with packaged DNA and tails are found in some mutant lysates (9, 23), and

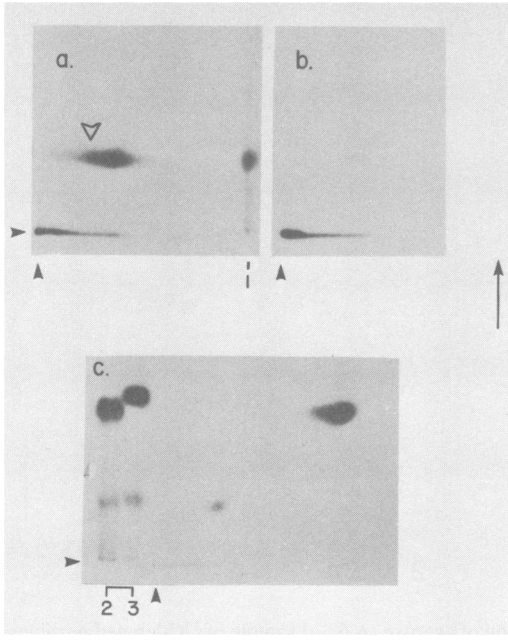

FIG. 6. Electrophoresis in two dimensions. The following ^{14}C-labeled particles, all isolated by sedimentation in sucrose-metrizamide gradients, were subjected to electrophoresis in two dimensions, as described in Materials and Methods: (a) particles (primarily capsid II) from the capsid II region of a sucrose-metrizamide gradient of a wild-type lysate (400 cpm); (b) particles (primarily AG particles) from the capsid II region of a sucrose-metrizamide gradient of a 14*am* lysate (400 cpm); (c) particles from the capsid I region of a sucrose-metrizamide gradient of a wild-type lysate (700 cpm). (a) and (b) are different regions of the same slab gel used for electrophoresis in the second dimension. The arrowheads indicate the positions of the origins of the cylindrical gels used for electrophoresis in the first dimension, and the horizontal arrowheads also indicate the direction of electrophoresis in the first dimension; the arrow indicates the direction of electrophoresis in the second dimension. Markers are: (1) capsid II; (2) capsid I; (3) trypsinized capsid I. The open arrowhead in (a) indicates the position of particles migrating more slowly than capsid II in the first dimension, but migrating at the same rate as capsid II in the second dimension; this phenomenon is probably caused by discontinuity of buffer at the origin of electrophoresis in the first dimension.

these particles migrate in a comparatively broad band overlapping the position of capsid II (16), thereby interfering with quantitation of AG particles and capsid II. During studies in which the data for Table 1 were obtained, no particles other than capsid I and capsid II, indistinguishable by sieving from wild-type capsid I and capsid II (14, 15a), were observed (i.e., radii were the same as for wildtype capsids ± 8%; 15a).

Two results in Table 1 add significantly to understanding the role of late T7 gene products in capsid assembly. (i) No detectable AG particles or capsid of any kind were observed in 9*am* lysates, a result also found using an unfractionated lysate, as in Fig. 7 (data not shown); this result suggests that P9 and P10 must bind to each other for any assembly of P10 to occur and is in agreement with observations in reference 9 concerning capsid I. (ii) The AG particle/capsid I ratio is significantly higher in 8*am*, 14*am*, 15*am*, and 16*am* lysates, and probably also in 13*am* lysates, than it is in wild-type lysates; because P8, P14, P15, and P16 are components of the internal core of capsid I (12), this suggests that absence of a functional core results in accumulation of AG particles in infected cells. The significance of this observation is further discussed below.

Proteins of AG particles. To further characterize AG particles and possibly to determine which protein was adhering these particles to agarose, particles (primarily AG particles) isolated from the capsid II region of sucrose-metri-

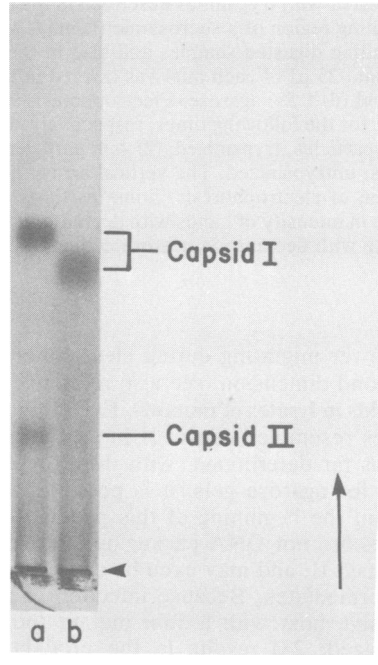

FIG. 7. Agarose gel electrophoresis of an unfractionated 5*am* lysate. A 5-ml culture of 5*am* T7-infected *E. coli* was labeled with ^{14}C-amino acids and lysed as described in the text. A 2-μl sample of the lysate was diluted with 11 μl of standard/G buffer and was digested with trypsin; a second sample was similarly treated with preinhibited trypsin. Both of these mixtures were diluted with 17 μl of Phos/Mg sample buffer, and 20 μl was subjected to agarose gel electrophoresis in Phos/Mg electrophoresis buffer, as described in the text. (a) Trypsinized; (b) untrypsinized.

TABLE 1. Presence of AG particles and capsid II in T7 mutant lysates

Mutant gene	AG particles[a]	Capsid II[b]	Function or location of gene product during assembly[c]
4	+	−	DNA synthesis
5	+	−	DNA synthesis
7	+	+	Not known
8	++	−	Core
9	—[d]	—[d]	Envelope of capsid I
10	—[d]	—[d]	Envelope of all capsids
12	+	+	Tail
13	—[e]	+	Interior, possibly core
14	++	±	Core
15	++	±	Core
16	++	±	Core
17	+	+	Tail
18	+	−	DNA packaging (minor protein)
19	+	±	DNA packaging (minor protein)

[a] The amount of ^{14}C cosedimenting with capsid II (in sucrose-metrizamide gradients) and forming a band of capsid II during agarose gel electrophoresis after, but not before, digestion with trypsin. −, None detectable; +, 0.2 to 0.9 times the amount in capsid I; ++, three to six times the amount in capsid I.

[b] The amount of ^{14}C cosedimenting with capsid II (in sucrose-metrizamide gradients) and forming a band of capsid II during agarose gel electrophoresis before digestion with trypsin. −, None detectable or trace amount; ±, 0.05 to 0.2 times the amount in capsid I; +, 0.4 to 0.9 times the amount in capsid I.

[c] From references 9, 12, and 23.

[d] No capsids or AG particles were detected.

[e] 1.2 to 1.6 times as much ^{14}C as present in capsid I was found in two separate experiments.

zamide gradients of AG particle-enriched lysates (8*am*, 13*am*, 14*am*, 15*am*, 16*am*; at least 20 times as much ^{14}C in AG particles as in capsid II present in all but the 13*am* lysate; Table 1) were subjected to SDS-polyacrylamide gel electrophoresis with and without digestion with trypsin. For comparison, particles (primarily capsid II) from the capsid II region of a sucrose-metrizamide gradient of a wild-type lysate were also thus treated. The most intense band in all samples was formed by P10 (T7 proteins are indicated by P followed by the number of the protein's gene, determined in reference 23) (Fig. 8), the most abundant protein in the envelope of the capsid of bacteriophage T7 (9, 12, 23). Only a comparatively small amount or no ^{14}C was present in P9, a protein whose molar amount in the envelope of capsid I is 0.27 times the molar amount of P10 (14). All of the AG particles of Fig. 8 also had protein Q, a capsid envelope protein whose gene has not yet been identified (12). The percentage of ^{14}C in P8, P13, P14, P15, and P16, all internal proteins of capsid I (12), varied with the source of AG particles, and in all cases the protein coded for by the mutant gene was absent (Fig. 8, channels a, c, e, g, and i). In addition, bands formed by the following proteins of the core were either missing or reduced in intensity by at least a factor of 10 in the samples from lysates of bacteriophages mutant in the genes indicated: P8, gene 14; P13, genes 8, 14, 15, and 16; P14, genes 8, 13, 15, and 16; P15, genes 8, and 16; P16, genes 8 and 14 (an exposure three times as long as the exposure used for Fig. 8 was used for these determinations). Quantitation of the proteins in these bands could not be performed because the specific activity of ^{14}C in each of the proteins is not known and could vary. The apparent dependence of the presence of some core proteins in AG particles on the presence of other core proteins could result from binding of these proteins to each other during assembly of the core and has previously been reported for other T7 capsids (9). Components of the T7 tail, P12 and P17 (12, 23), were also present in detectable amounts in the 8*am*, 13*am*, 14*am*, 16*am*, and wild-type samples of capsid II-sedimenting material in Fig. 8 (slots a, c, e, i, and k, respectively) (the longer exposure was also used for this determination).

After treatment of the 8*am*, 14*am*, 15*am*, and 16*am* capsid II-sedimenting particles with trypsin, a series of bands (indicated by the horizontal arrows in Fig. 8) either appeared or increased in magnitude; P8, when present, was digested (Fig. 8, slots b, d, f, h, and j). The bands indicated by an asterisk were not visible after digestion of the wild-type capsid II (Fig. 8, slot l), indicating that the peptides forming these bands were released from AG particles and not capsid II. The starred bands were also in the profile of the trypsinized 13*am* sample, supporting the conclusion that 13*am* lysates were enriched for AG particles; this conclusion is not as clearly indicated by the data in Table 1. In Fig. 8, slots b and f, the amount of ^{14}C in the trypsin-produced, starred peptides was more than could have been released from any protein but P10. Therefore, we conclude that at least the starred, trypsin-released peptides in Fig. 8 were released from P10. Because P10 is exposed on the exterior of T7 capsids, it is possible that the trypsin-digestible molecules of P10 were the components of AG particles that bound agarose gels.

DISCUSSION

The data presented here indicate that AG particles, although inseparable from capsid II by buoyant density sedimentation, velocity sedi-

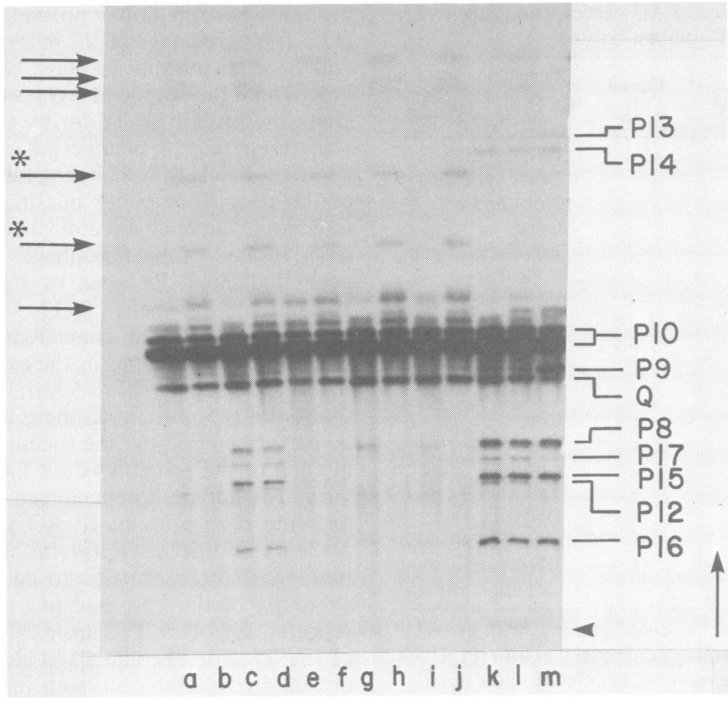

FIG. 8. SDS-polyacrylamide gel electrophoresis. The following ^{14}C-labeled lysates were fractionated by sedimentation in a sucrose-metrizamide gradient, as described in the text, and particles from the capsid II regions of these gradients were subjected to SDS-polyacrylamide gel electrophoresis with and without trypsinization, as described in the text: (a) 8*am* untrypsinized; (b) 8*am*, trypsinized; (c) 13*am*, untrypsinized; (d) 13*am*, trypsinized; (e) 14*am*, untrypsinized; (f) 14*am*, trypsinized; (g) 15*am*, untrypsinized; (h) 15*am*, trypsinized; (i) 16*am*, untrypsinized; (j) 16*am*, trypsinized; (k) wild-type, untrypsinized; (l) wild-type, trypsinized. In channel m is a profile of capsid I, untrypsinized. Each sample contains 950 cpm of ^{14}C. The origin of electrophoresis is indicated by the arrowhead; the direction of electrophoresis is indicated by the arrow.

mentation, or density gradient electrophoresis, are produced at a different, probably earlier, point in the T7 assembly pathway than capsid II. The only property by which AG particles and capsid II are distinguished from each other (thus far) is affinity for agarose gels during electrophoresis. Therefore, the use in reference 9 of velocity sedimentation for the quantitation of capsids results in the treatment of AG particles and capsid II as one particle. A possible reason that no clear precursor-product relationship between capsid I and capsid II was found in reference 9 can, therefore, be visualized by using Fig. 1 if it is assumed that intermediate B is an AG particle, C is capsid I, and B′ is capsid II. Failure to distinguish B from B′ during measurements of the kinetics of appearance of intermediates could result in failure to observe a precursor-product relationship between C and the mixture of B and B′.

In reference 9 and in a second study (23), it was reported that no capsid-like particles copurifying with capsid II were present in 5*am* lysates (buoyant density sedimentation of Carbowax-concentrated lysates in cesium chloride density gradients was used to detect capsids I and II in reference 23; F. W. Studier, personal communication), not the case in Results. The apparent absence of 5*am* AG particles in reference 23 is probably caused by the conversion of these particles to a more rapidly sedimenting form during or after concentration of lysates, a phenomenon observed in the present study (Results). Examination of Fig. 8 of reference 9, a figure which shows the sedimentation profile of capsids from 5*am*, 18*am*, and 19*am* lysates, reveals a comparatively small amount of radiolabeled particles sedimenting at the position of capsid II, particles (not mentioned in the text) which are probably AG particles. The AG particles observed may be an underestimate of the AG particles present in the lysate because precautions were not taken to avoid adherence of AG particles to storage vessels.

After concentration of capsid-like particles from lysates of T7-infected *E. coli*, using

Carbowax 6000 as a concentrating agent, tubular forms of P10 (polycapsids) that have a capsid II-like envelope and that vary in size from roughly twice the size of capsid II to many times larger than capsid II have been observed (13). The smaller polycapsids may be identical to some of the AG particles that are not converted by trypsin to a particle comigrating with capsid II during agarose gel electrophoresis. The larger polycapsids sediment more rapidly than capsids I or II (S values greater than 200), and many do not enter the agarose gels used here for electrophoresis (P. Serwer, R. H. Watson, and S. J. Hayes, unpublished data). For the following reasons it is likely that those AG particles converted by trypsin to particles comigrating with capsid II during electrophoresis in agarose gels are structurally different from polycapsids: (i) in electron micrographs there is in AG particles no apparent deviation from the polygonal structure of capsid II (Results), a deviation observed in electron micrographs of polycapsids (13); (ii) by sieving of trypsinized AG particles comigrating with capsid II, these particles, unlike polycapsids, are the same size as capsid II (Results); trypsin does not detectably change the shape of polycapsids (P. Serwer and S. J. Hayes, unpublished data), indicating that trypsin could not have converted polycapsids to particles migrating as capsid II during agarose gel electrophoresis, as it does with AG particles. Because of the conversion of AG particles to more rapidly sedimenting particles during procedures of concentration identical to those used to isolate polycapsids (Results), it is possible that at least some AG particles convert to polycapsids during this concentration.

The data presented in Results indicate that AG particles are formed independently of capsid II, an observation explainable by assuming that AG particles are an intermediate in capsid I assembly. However, another possibility to consider is that capsid I converts to an AG particle during an abortive attempt to package DNA. For instance, the occurrence of AG particles in *5am* lysates might be explained by the attempt and failure of capsid I to package unreplicated parental DNA; the failure to package could result because unreplicated DNA is not concatemeric (a review of replicative and postreplicative forms of T7 DNA is in reference 5). However, if AG particles were formed during an unsuccessful attempt by capsid I to package DNA, removal of the DNA-binding capacity of capsid I should prevent the formation of AG particles. Because no capsid I converts to capsid II during *8am* infections (Table 1), in spite of the production of concatemeric DNA (5), it is likely (but not necessary) that *8am* capsid I has no capacity for binding DNA. That removal of P8 from capsid I should stop capsid I from binding DNA is also suggested by the following: (i) P8 is located at the core-envelope junction of capsid I, the region of this capsid at which DNA binding is likely to occur during packaging (12); and (ii) *8am* capsid I does not have P19, a second protein needed for binding DNA (9). Because it is likely that *8am* capsid I cannot bind DNA, the proposal that AG particles are produced during an abortive attempt to package DNA predicts that AG particles are present in reduced amounts in an *8am* lysate; however, AG particles are present in increased relative amounts in an *8am* lysate (Table 1). Thus, although it is still possible that some AG particles are produced during DNA packaging, possibly abortive, it is likely that most or all AG particles are produced during assembly of capsid I. This is confirmed in the accompanying communication (20), in which data are presented indicating that AG particles are intermediates in capsid I assembly (not abortive by-products of assembly).

ACKNOWLEDGMENTS

For technical assistance we thank Elena S. T. Moreno. For secretarial assistance we thank Brenda Moylan. For helpful comments we thank P. M. Horowitz, N. C. Robinson, and F. W. Studier.

Support was received from the National Institutes of Health (Public Health Service grants AI 16117 and GM 24365) and from the Robert A. Welch Foundation (grant AQ-764).

LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Casjens, S., and J. King.** 1957. Virus assembly. Annu. Rev. Biochem. **44**:555–611.
3. **Chase, T., Jr., and E. Shaw.** 1967. p-Nitrophenyl-p'-guanidinobenzoate HCl: a new active site titrant for trypsin. Biochem. Biophys. Res. Commun. **29**:508–514.
4. **Earnshaw, W. C., and S. Casjens.** 1980. DNA packaging by the double-stranded DNA bacteriophages. Cell **21**:319–331.
5. **Krüger, D. H., and C. Schroeder.** 1981. Bacteriophage T3 and bacteriophage T7 virus-host cell interactions. Microbiol. Rev. **45**:9–51.
6. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–341.
7. **Murialdo, H., and A. Becker.** 1978. Head morphogenesis of complex double-stranded deoxyribonucleic acid bacteriophages. Microbiol. Rev. **42**:529–576.
8. **Rodbard, D., and A. Chrambach.** 1970. Unified theory for gel electrophoresis and gel filtration. Proc. Natl. Acad. Sci. U.S.A. **65**:970–977.
9. **Roeder, G. S., and P. D. Sadowski.** 1977. Bacteriophage T7 morphogenesis: phage-related particles in cells infected with wild-type and mutant phage. Virology **76**:263–285.
10. **Sadowski, P. D., A. McGeer, and A. Becker.** 1974. Terminal cross-linking of DNA catalyzed by an enzyme system containing DNA ligase, DNA polymerase, and exonuclease of bacteriophage T7. Can. J. Biochem. **52**:525–535.
11. **Serwer, P.** 1974. Fast sedimenting bacteriophage T7 DNA from T7-infected *Escherichia coli*. Virology **59**:70–88.
12. **Serwer, P.** 1976. Internal proteins of bacteriophage T7. J. Mol. Biol. **107**:271–291.

13. **Serwer, P.** 1979. Fibrous projections from the core of a bacteriophage T7 procapsid. J. Supramol. Struct. **11**:321–326.
14. **Serwer, P.** 1980. A metrizamide-impermeable capsid in the DNA packaging pathway of bacteriophage T7. J. Mol. Biol. **138**:65–91.
15. **Serwer, P.** 1980. A technique for electrophoresis in multiple-concentration agarose gels. Anal. Biochem. **101**:154–159.
15a. **Serwer, P., and S. J. Hayes.** 1981. Sieving of spherical viruses and related particles during electrophoresis in gels of agarose, p. 237–243. *In* R. C. Allen and P. Arnaud (ed.), Electrophoresis '81. Walter de Gruyter, Berlin.
16. **Serwer, P., and M. E. Pichler.** 1978. Electrophoresis of bacteriophage T7 and T7 capsids in agarose gels. J. Virol. **28**:917–928.
17. **Serwer, P., and R. H. Watson.** 1981. Capsid-DNA complexes in the DNA packaging pathway of bacteriophage T7: characterization of capsids bound to monomeric and concatemeric DNA. Virology **108**:164–176.
18. **Serwer, P., and R. H. Watson.** 1981. Detection of viral capsid-DNA complexes, p. 231–238. *In* M. S. Dubow (ed.), Bacteriophage assembly, Alan R. Liss, New York.
19. **Serwer, P., and R. H. Watson.** 1981. Electrophoresis in density gradients of metrizamide. Anal. Biochem. **114**:342–348.
20. **Serwer, P., and R. H. Watson.** 1982. Function of an internal bacteriophage T7 core during assembly of a T7 procapsid. J. Virol. **42**:595–601.
21. **Shaw, D. J.** 1969. Electrophoresis. Academic Press, Inc., London.
21a. **Stroud, R. M., P. Serwer, and M. J. Ross.** 1981. Assembly of bacteriophage T7: dimensions of the bacteriophage and its capsids. Biophys. J. **36**:743–757.
22. **Studier, F. W.** 1969. The genetics and physiology of bacteriophage T7. Virology **39**:562–574.
23. **Studier, F. W.** 1972. Bacteriophage T7. Science **176**:367–376.
24. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
25. **Wieme, R. J.** 1965. Agar gel electrophoresis. Elsevier/North-Holland Biomedical Press, Amsterdam.