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Electrophoresis of Bacteriophage T7 and T7 Capsids in Agarose Gels

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Agarose gel electrophoresis of the following was performed in 0.05 M sodium phosphate-0.001 M $MgCl_2$ (pH 7.4): (i) bacteriophage T7; (ii) a T7 precursor capsid (capsid I), isolated from T7-infected *Escherichia coli*, which has a thicker and less angular envelope than bacteriophage T7; (iii) a second capsid (capsid II), isolated from T7-infected *E. coli*, which has a bacteriophage-like envelope; and (iv) capsids (capsid IV) produced by temperature shock of bacteriophage T7. Bacteriophage T7 and all of the above capsids migrated towards the anode. In a 0.9% agarose gel, capsid I had an electrophoretic mobility of $9.1 \pm 0.4 \times 10^{-5}$ $cm^2/V \cdot s$; bacteriophage T7 migrated 0.31 ± 0.02 times as fast as capsid I. The mobilities of different preparations of capsid II varied in such gels: the fastest-migrating capsid II preparation was 0.51 ± 0.03 times as fast as capsid I and the slowest was 0.37 ± 0.02 times as fast as capsid I. Capsid IV with and without the phage tail migrated 0.29 ± 0.02 and 0.42 ± 0.02 times as fast as capsid I. The results of the extrapolation of bacteriophage and capsid mobilities to 0% agarose concentration indicated that the above differences in mobility are caused by differences in average surface charge density. To increase the accuracy of mobility comparisons and to increase the number of samples that could be simultaneously analyzed, multisample horizontal slab gels were used. Treatment with the ionic detergent sodium dodecyl sulfate converted capsid I to a capsid that migrated in the capsid II region during electrophoresis through agarose gels. In the electron microscope, most of the envelopes of these latter capsids resembled the capsid II envelope, but some envelope regions were thicker than the capsid II envelope.

Current evidence indicates that DNA enters a preassembled capsid (capsid I) during the in vivo assembly of bacteriophage T7 (8, 11, 14). In the electron microscope the envelopes of capsid I and bacteriophage T7 both appear roughly spherical, but the capsid I envelope appears less angular and thicker than the bacteriophage envelope (11). This greater thickness is probably caused by the presence of larger amounts of P9 (T7 proteins are referred to by P followed by the protein's gene number [14]) in the capsid I envelope than in the bacteriophage envelope (11). A capsid with an envelope that has a phage-like protein composition, angularity, and thickness (capsid II) has also been isolated from T7-infected cells; capsid I may convert to capsid II during an abortive or incomplete DNA packaging attempt (11, 14). Capsids I and II and bacteriophage T7 all have an internal, roughly cylindrical core with an axial hole (11). The capsid I and T7 phage cores appear to be attached to the capsid envelope, and DNA may enter capsid I through the axial hole. Capsids with envelopes resembling the envelope of capsid II in the electron microscope have been isolated from temperature-shocked bacteriophage T7; capsid IVA has no tail, and capsid IVB has a tail (11).

To determine the mechanism by which bacteriophage T7 DNA is packaged, the following data are useful: (i) physical characterization of bacteriophage T7 and T7 capsids; (ii) measurements of the amounts and time course of T7 capsids in cells infected with bacteriophage T7 and T7 mutants; (iii) induction in a cell-free system of the events occurring during DNA packaging. In the work described here, agarose gel electrophoresis of bacteriophage T7 and T7 capsids was developed as a technique to help obtain these data. Implications of the results in understanding the mechanism of T7 DNA packaging are discussed.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. Bacteriophage T7 was received from F. W. Studier. The host for T7 was *Escherichia coli* BB/1.

Media and buffers. Bacterial cultures were grown and infected in M9 medium (11). Tris/Mg buffer is 0.2 M NaCl-0.01 M Tris-chloride (pH 7.4)-0.001 M $MgCl_2$; for the purification and storage of radiolabeled bacte-

riophage T7 and capsids, 100 μg of boiled gelatin was included per ml (Tris/Mg/G buffer). Electrophoresis buffer is 0.05 M sodium phosphate (pH 7.4)–0.001 M $MgCl_2$.

Preparation and purification of protein-radiolabeled T7 bacteriophage and capsids. A stationary-phase bacterial culture in M9 medium was diluted into fresh M9 medium and was grown overnight at 30°C with aeration until there were 3×10^8 bacteria per ml. The culture was infected with bacteriophage T7 (multiplicity of infection, 10), and at 10 min after infection radioactive amino acids were added (either ^3H- or ^{14}C-labeled algal hydrolysate or [^{35}S]methionine was used at a final concentration of 20 to 40 μCi/ml, purchased from Schwarz-Mann or ICN). After spontaneous lysis (at 30 to 35 min after infection) the lysate was clarified by centrifuging at 10,000 rpm for 10 min, 4°C, in a Beckman JA-21 rotor. The lysate was then sedimented through a cesium chloride preformed step gradient containing 100 μg of gelatin per ml as previously described (11). Bacteriophage particles, which banded at a density of 1.51 g/ml, were collected and dialyzed into Tris/Mg/G buffer. Capsids I and II, which together banded in a single peak at 1.28 g/ml, were collected, dialyzed into Tris/Mg/G buffer, and then isolated by sedimentation through an 11.0-ml, 5 to 25% sucrose gradient supported by a 0.8-ml 62% sucrose shelf, all in Tris/Mg/G buffer (SW41 rotor, 25,000 rpm, 120 min, 18°C). Capsid I traversed 70 to 80% of the gradient and traveled 1.36 to 1.40 times as far as capsid II.

Preparation of unlabeled bacteriophage T7 and T7 capsids. Fifteen- or 30-liter lysates of T7-infected *E. coli* were prepared; T7 phage and capsids were purified as previously described (11), except that (i) the sucrose gradient used in the final step of the capsid purification had Tris/Mg buffer and (ii) the sucrose from this final step was not removed by dialysis.

Electrophoresis in agarose gels. Agarose (Seakem, ME) was dissolved in glass-distilled water by boiling. Buffer was then added by diluting 10-fold into the agarose solution, which was maintained at 80 to 90°C. Cylindrical gels were poured in glass tubes (6-mm ID, 8.5 cm long); horizontal slab gels (6 mm thick, 15.2 cm in the direction of the electrical field, 13.2 cm wide) were poured in an apparatus previously described (6). Samples in Tris/Mg or Tris/Mg/G buffer were diluted into 5 volumes of 2% sucrose–0.005 M sodium phosphate (pH 7.4)–0.001 M $MgCl_2$–400 μg of bromophenol blue per ml prior to layering on the gels. Samples can also be dialyzed into electrophoresis buffer before electrophoresis, but this usually resulted in the loss of 30 to 60% of a radiolabeled capsid sample on the dialysis tubing. The bottoms of cylindrical gels were covered with nylon mesh or dialysis tubing to prevent the agarose from falling out of the tube. The tubes were placed in a commercial electrophoresis apparatus and were filled with buffer; the samples were layered underneath the buffer. For horizontal slab gels, samples were placed in wells (2 by 6 mm) previously filled with electrophoresis buffer.

Electrophoresis was done at room temperature (22 to 25°C); however, the temperature of horizontal slab gels using a field strength of 2.1 V/cm was 3 to 5°C above room temperature. The times and field strengths are described in the figure legends. During electrophoresis, buffer was circulated between the reservoirs at a rate of 50 to 200 ml/min. At the end of an electrophoresis, the pH values of buffer in both tanks of a horizontal slab gel were within 0.1 pH unit of the starting pH. The concentrations of bacteriophage T7 samples were determined by optical density (1); the concentrations of capsid samples were determined by the dye-binding method of Bradford (2).

Detection of bacteriophage and capsids in agarose gels. Radiolabeled bacteriophage T7 and capsids in cylindrical gels were detected by autoradiography after slicing the gels in a sagittal plane and drying under vacuum onto chromatography paper. Kodak Royal X-Omat film was used. Radiolabel in agarose slab gels can also be detected by autoradiography.

To detect unlabeled DNA, gels were soaked for 45 min in electrophoresis buffer containing 1 μg of ethidium bromide per ml. The gels were then illuminated with UV light and photographed as previously described (12). The presence of DNA is revealed by the ethidium bromide fluorescence enhancement induced by the DNA. DNA packaged inside bacteriophage T7 induces ethidium bromide fluorescence enhancement, although the amount is less than induced by unpackaged DNA (12).

To detect unlabeled protein, gels were stained in 0.05% Coomassie blue–10% acetic acid for 1.5 h at room temperature. The gels were destained by diffusion into 10% acetic acid at room temperature. Relative amounts of protein in capsid bands were determined by quantitative densitometry of the gels with a Helena Quick Scan Densitometer. Changes in gel dimensions during staining were less than 0.5%.

Elution from gels. To recover unlabeled capsids from agarose cylindrical gels, the gels were sliced in a sagittal plane, and one half of the gel was stained with Coomassie blue. Using the stained half of the gel as a template, the capsid regions of the unstained half were excised. Unlabeled bacteriophage T7 was recovered by observing light scattering from the bacteriophage band and excising the band region of the gel. Material was eluted from gel slices by one of two methods: (i) the gel slice was incubated with 20 to 50 μl of Tris/Mg buffer for 2 to 3 days at 4°C (diffusion elution); or (ii) the gel slice was placed in a dialysis bag with 100 to 500 μl of electrophoresis buffer, then placed in a chamber with two electrodes; after sufficient electrophoresis buffer was added to cover the dialysis tubing, the gel was subjected to electrophoresis (2.9 V/cm) for 2 to 3 h at 4°C (electrophoretic elution).

To recover radiolabeled bacteriophage or capsids from cylindrical gels, the gels were sliced into disks and the disks were subjected to diffusion elution into Tris/Mg/G buffer. The fractions containing radiolabel were determined by diluting portions of the eluates into toluene fluor containing Triton X-100. Recoveries of radiolabeled capsids I and II from capsid peak regions of 0.9% agarose gels were 30 to 55%.

Sucrose gradient sedimentation. For determination of the sedimentation rates of eluted capsids, capsid samples were layered on 4.8-ml, linear 5 to 25% sucrose gradients containing Tris/Mg/G buffer, poured over a 0.35-ml layer of sodium diatrizoate (density = 1.30 g/ml). The gradients were centrifuged in a Beckman SW50.1 rotor for 60 min at 28,000 rpm,

18°C, and were fractionated by tube puncture. Capsid I sedimented through 70 to 80% of the sucrose gradient.

Electron microscopy. Carbon support films for electron microscopy were prepared as previously described (11). A drop of sample was placed on a support film and was incubated for 1 min at room temperature. The film was washed with 4 drops of water followed by 2 drops of 1% sodium methyl phosphotungstate (pH 7.6) (7) (a gift of Glenn Williams). The film was dried with filter paper.

To determine the degree of aggregation of bacteriophage T7 or T7 capsids embedded in negative stain, particles separated by 5 nm or less were assumed to be attached to each other. Bacteriophages or capsids scored for their degree of aggregation were selected at random except that all those selected were more than two bacteriophage diameters from the edge of a stain droplet. This restriction was applied because aggregation induced by drying of the specimen appeared to occur most readily at stain droplet edges.

SDS-polyacrylamide gel electrophoresis of material in agarose cylindrical gels. A sample was subjected to electrophoresis in an agarose cylindrical gel, 3 mm in diameter. The gel was soaked in 1% sodium dodecyl sulfate (SDS)-0.05 M Tris-chloride (pH 6.8)-1% β-mercaptoethanol for 15 min at room temperature and was then dropped into a tube of the same buffer maintained at 100°C by boiling. The gel was boiled until it started to become transparent (for 0.9% gels this occurs at 20 to 35 s after the start of boiling). The gel was then chilled on ice, layered across a vertical SDS slab gel similar to those used previously (11), and sealed with agarose (the same percentage used in the tube gel) in the above buffer. A well was made in the agarose for a standard used to identify protein bands. Electrophoresis and staining of the SDS gel were performed as previously described (11).

Theoretical considerations. The electrophoretic mobility of a particle, u, is defined as V/E; V is the velocity of the particle in an electric field of strength E. The magnitude of u in buffer without agarose, u^0, for a spherical particle depends on (i) the average density of electrical charge per unit of surface area on the particle, σ; (ii) the radius of the particle, r; (iii) the viscosity, η, of the solution in which the particle is suspended; (iv) the Debye-Hückel constant, k, which is proportional to the square root of the ionic strength (the ionic strength of electrophoresis buffer is calculated to be 0.116). An approximate determination of u^0 in terms of the above parameters has been obtained (13) (this equation has been previously used in a study of bacteriophage T4 [5]):

$$u^0 = \frac{2\sigma r f(kr)}{3\eta(1 + kr)} \quad (1)$$

$f(kr)$ is a function that varies in value between 1.0 and 1.5 as kr varies from 0 to values above 100. An additional parameter of interest is the total number of electron equivalents of charge on a particle, z, calculated by multiplying σ by the surface area of the particle.

If, to prevent convection, electrophoresis is done in the presence of a supporting gel, the gel may affect u by (i) forming channels with a pore size small enough to slow particles down because of multiple collisions with gel fibers (sieving); if the channels are narrow enough, migration may be totally inhibited; and (ii) adsorbing particles by chemical interaction. The latter effect will tend to spread out electrophoretic zones and is therefore undesirable.

It has been assumed that u^0 can be obtained by extrapolating u measured in agarose gels of decreasing agarose concentration to an agarose concentration of 0. Values of u are readily measured on horizontal slab gels; E is measured by monitoring the voltage drop across two points on the gel. However, for 0.5% and less concentrated agarose gels it is extremely difficult to stain slab gels without gel breakage; 0.9% slab gels were used for most measurements. Also, bacteriophage T7 and capsid IVB bands, for unknown reasons, were three to five times broader in slab gels than they were in cylindrical gels. Therefore, extrapolation to 0% agarose concentration was performed by subjecting a sample to electrophoresis on agarose cylindrical gels (which are less fragile than slab gels) with agarose percentages of 0.3, 0.5, 0.9, 1.5, 2.0, and 2.5% (all gels were run simultaneously in the same apparatus). The distance migrated by the sample in each gel, divided by the distance migrated in the 0.9% gel (referred to as c), was extrapolated to 0% agarose (referred to as c^0), and u^0 was calculated:

$$u^0 = u \cdot c^0 \quad (2)$$

Determinations of c were made at E values of 5.8 V/cm; determinations of u were made at 2.1 V/cm. Values of u measured for capsids I and II and bacteriophage T7 were independent of E ±10% between 1.8 and 6.8 V/cm in 0.9% gels; u for capsids I and II was also independent of time between 100 and 529 min at 2.1 V/cm. However, for unknown reasons, u for T7 phage was an increasing function of time using the conditions of Fig. 2, for times above 140 min.

Values of c decreased linearly as a function of agarose concentration, A, below an A of 2.0%. By including ethidium bromide in the tube gel before and during electrophoresis, phage T7 was stained without having to remove the gel from the glass tube, and data taken down to 0.1% agarose were still linear, although $-(dc/dA)$ was 1.5 times as high in the presence of ethidium bromide as in its absence [$-(dc/dA) = 0.48$ to 0.56 without ethidium bromide].

Assuming that the pore size of agarose gels decreases as A increases, $-(dc/dA)$ should be an increasing function of r for spherical particles. Using bacteriophages T7 and R17 and tail-free bacteriophage T5 full capsids (12), this has been shown qualitatively to be the case (P. Serwer, unpublished data).

Direct determinations of u have been made only for capsids I and II. For other structures the ratio of the distance migrated to the distance migrated by capsid I, $R(CI)$, in the same gel was used to quantitate mobility.

RESULTS

Electrophoresis of bacteriophage T7. Samples that contained 60, 30, 15, 6, and 3 μg of unlabeled bacteriophage T7 were each mixed with ^{35}S-labeled bacteriophage T7 and subjected to electrophoresis through 0.9% cylindrical gels.

After ethidium bromide staining, two bands, both migrating toward the anode, were revealed on the gels with 60 to 15 μg of bacteriophage (Fig. 1b, gels 1 to 3). Coomassie blue, however, stained only the slower band (not shown). This indicates that the faster band was T7 DNA (released from disrupted bacteriophages) and that the slower band had T7 DNA and capsid protein. To determine whether DNA from the slower band was packaged two experiments were performed.

(i) Experiment 1. A 15-μg bacteriophage T7 sample was diluted for electrophoresis as described in Materials and Methods and was then subjected to electrophoresis with and without DNase I digestion (50 μg/ml, 30 min, 30°C). The DNase eliminated the faster band but had no detectable effect on the slower band (Fig. 1b, gel 7); the undigested control is in Fig. 1b, gel 6. (A comparatively small quantity of DNase-resistant, fluorescent material migrated slightly ahead of the major bacteriophage band in this bacteriophage preparation, a different preparation from the one used in Fig. 1b, gels 1 to 6; the properties of the minor bacteriophage band have not yet been further investigated.) In addition, a cylindrical gel was soaked in DNase I (20 μg/ml, 1.0 h, 30°C) after electrophoresis and before electrophoresis in a second dimension on a horizontal slab gel. This did not eliminate the slower band during electrophoresis in the second dimension, though the faster band was eliminated (demonstrating that material in the gel was accessible to the DNase). These data indi-

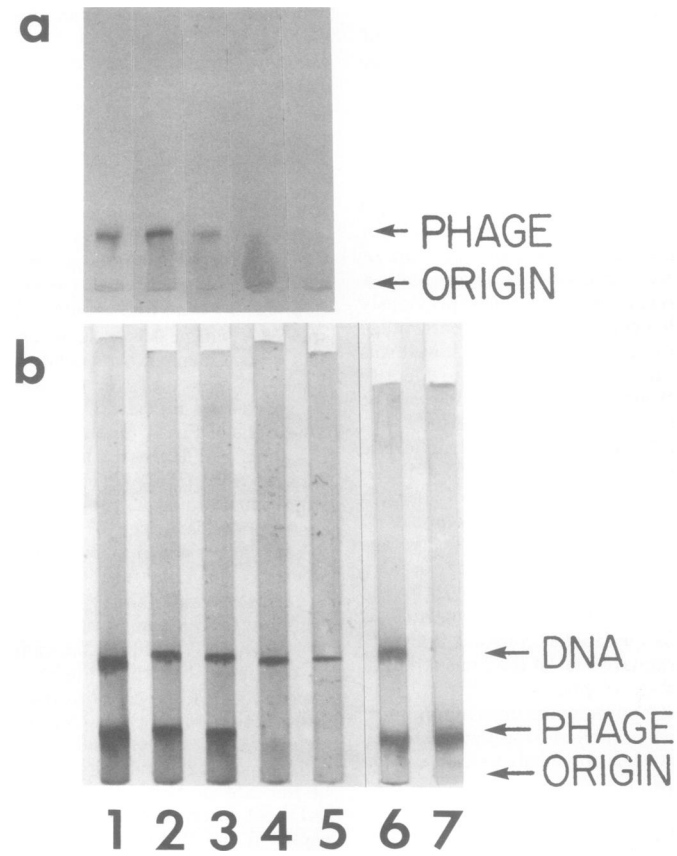

FIG. 1. *Electrophoresis of bacteriophage T7 as a function of bacteriophage concentration and treatment with DNase. Samples containing ^{35}S-labeled bacteriophage T7 (1,500 cpm, <0.01 μg) were added to 60, 30, 15, 6, and 3 μg of unlabeled T7 phage in a total volume of 10 μl. These mixtures were diluted and subjected to electrophoresis through 0.9% cylindrical gels as described in the text (6.8 V/cm, 142 min). In a separate experiment, 15 μg of bacteriophage was subjected to electrophoresis in a 0.9% cylindrical gel with and without the DNase treatment described in the text. The gels were stained with ethidium bromide, and gels with ^{35}S-labeled bacteriophage were subsequently sliced and subjected to autoradiography. (a) Autoradiograms. (b) Ethidium bromide-stained gels. Samples: (1) 60 μg; (2) 30 μg; (3) 15 μg; (4) 6 μg; (5) 3 μg; (6) control for DNase; (7) DNase. The anode is at the top.*

cate that DNA in the slower band was packaged inside the capsid before and after the cylindrical gel electrophoresis.

(ii) Experiment 2. Material from the bacteriophage band was observed by electron microscopy after electrophoretic elution from a 0.9% agarose gel. Of the capsids observed, 94% (244 counted) appeared full of DNA, further demonstrating that particles producing this band had packaged DNA. The bacteriophage recovery, measured by optical density at 260 nm, was 14%. Infectivity per unit of optical density at 260 nm was lower by a factor of 0.48 than it was prior to electrophoresis (4.0×10^{11} PFU per optical density unit at 260 nm before electrophoresis).

For a bacteriophage amount of 6 μg, the bacteriophage band became diffused and slowed (Fig. 1b, gel 4); for 3 μg of bacteriophage, no band was visible, although there was sufficient material to observe a band if it existed (gel 5). The ^{35}S-labeled bacteriophage behaved similarly (Fig. 1a), further indicating that the loss of the bacteriophage band as the concentration was lowered was not the result of inadequate detection. The cause of this phenomenon is not kown; two possible causes are (i) adsorption of bacteriophage T7 to sites on the gel matrix that became saturated as the bacteriophage concentration was increased and (ii) destabilization and disruption of bacteriophage T7 at the lower concentrations.

There was no detectable dependence of the distance migrated by the bacteriophage on phage amount between 15 and 60 μg, indicating that changes in reversible aggregation were not altering bacteriophage T7 mobility in this range of phage amounts. To determine whether the phage were irreversibly aggregated during electrophoresis, material recovered by electrophoretic elution was prepared for electron microscopy less than 15 min after electrophoretic elution. Eighty-five percent (292 counted) of the bacteriophage particles observed were more than 5 nm from a second particle; 14% were in dimers, 1% were in trimers, and no larger aggregates were observed. This indicates that most bacteriophage were not irreversibly aggregated. However, it is still possible that some aggregates were present during electrophoresis and disaggregated during elution or preparation for electron microscopy. Bacteriophage T7 prior to electrophoresis had a significantly greater tendency to appear aggregated than after elution from agarose gels.

Electrophoresis of capsid I. Capsid I was mixed with bacteriophage T7 and subjected to electrophoresis in a 0.9% agarose cylindrical gel; the gel was stained with ethidium bromide and subsequently with Coomassie blue. The DNA and phage bands were observed with the ethidium bromide stain (Fig. 2a). After the Coomassie stain (Fig. 2b) the phage band was observed, as were two additional bands, both of which came from the capsid I preparation. Independent experiments showed that no material from the capsid I preparation migrated with the phage band (see Fig. 4). Bacteriophage T7 migrated 0.31 ± 0.02 times as fast as the major capsid I band (capsid I in Fig. 2b) and 0.87 ± 0.03 times as fast as the minor capsid I band (capsid I′ in Fig. 2b).

To determine whether material in the capsid I and I′ bands had the known properties of T7 capsids, the following experiments were performed.

(i) Experiment 1. ^{14}C label was diffusion eluted from the capsid I region of a preparative 0.9% cylindrical gel and was cosedimented with ^{3}H-labeled capsid I in a sucrose gradient; the eluted ^{14}C label from the capsid I band sedimented in a single peak 9 to 11% slower relative to the ^{3}H-labeled capsid I than ^{14}C-labeled capsid I that was not subjected to electrophoresis. ^{14}C label eluted from the capsid I′ region of the same gel sedimented in a single peak 6 to 8% slower than ^{14}C-labeled capsid II. This indicates that the major and minor bands both contain capsids; capsids in the capsid I band probably had envelopes resembling the capsid I envelope before electrophoresis, although some alteration

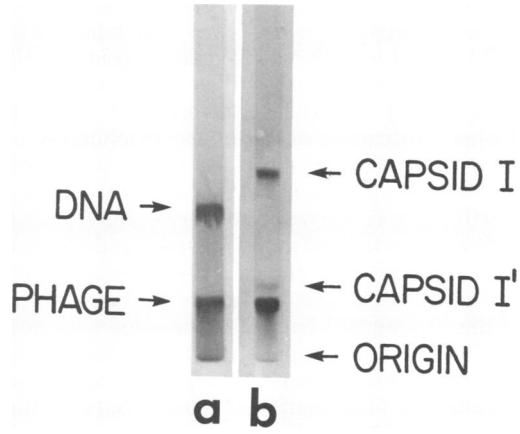

FIG. 2. *Electrophoresis of capsid I and bacteriophage T7. A mixture containing 1.5 μg of capsid I and 30 μg of T7 phage (15 μg of phage protein) in a 10-μl volume was diluted and subjected to electrophoresis through a 0.9% cylindrical agarose gel as described in the text (6.8 V/cm, 140 min). The gel was stained with ethidium bromide and was subsequently stained with Coomassie blue. (a) Ethidium bromide stain. (b) Coomassie blue stain. The anode is at the top.*

has occurred during electrophoresis; capsids in the capsid I′ band probably had capsid II-like envelopes.

(ii) Experiment 2. Unlabeled capsids were diffusion eluted from the capsid I region of a preparative 0.9% agarose gel and were observed in the electron microscope. Of the capsids in the eluate, 91% (248 counted) had envelopes with the thickness and roundness of capsid I; 88% of the capsids with a capsid I-like envelope had the internal core of capsid I. (An electron micrograph of capsid I is in Fig. 3a). The remaining 9% of the capsids had envelopes resembling the envelope of capsid II (a micrograph of capsid II is in Fig. 3b). Of capsids in an eluate of the capsid I′ region, 99% (247 counted) had envelopes with the thickness and angularity of capsid II (a micrograph of capsid I′ after electrophoresis is in Fig. 3c). This supports the conclusions drawn from the sedimentation studies of eluted capsids I and I′. Because the capsids in the capsid I band had envelopes resembling the capsid I envelope before electrophoresis, these capsids should also have amounts of P9 comparable to the amounts found in capsid I before electrophoresis (36 ± 2% of the amount of P10). This has been shown to be the case using the SDS gel electrophoresis technique described in Materials and Methods.

The capsid I′ band was present in all preparations made thus far, even in preparations that did not have a capsid II peak during sucrose gradient sedimentation (the amount is 5 to 15% of the capsid I band). However, radiolabeled capsid I preparations had some radiolabel (10 to 15% of the total) that trailed the capsid I peak, and this may be capsid I′. It is possible that some capsid I particles converted to capsid I′ during purification or during electrophoresis.

To test the effect of capsid I concentration on the mobility of capsid I, ^{14}C-labeled capsid I (<0.01 μg) was subjected to electrophoresis on a 0.9% agarose slab gel in the presence and absence of 5 μg of unlabeled capsid I; the gel was stained and autoradiographed. The position and width of the ^{14}C-capsid I band was the same with and without the added unlabeled capsid I, indicating that (i) sample concentration does not affect the appearance of a sharp band, as it does in the case of T7 phage, and (ii) reversible aggregation does not affect the value of *u*. The distances from the origin of the stained and autoradiographed capsid I bands were the same ±5%.

There was no ethidium bromide fluorescence in the capsid I region of the gel of Fig. 2a; this was the case if up to 15 μg of capsid I was used and the capsids were disrupted after electrophoresis and prior to ethidium bromide staining by fixing the gel in 10% acetic acid for 45 min Because 0.05 μg of DNA can be detected, this means that no more than 0.3% of the mass of capsid I is DNA.

Electrophoresis of capsids II and IV. Because the capsid I′ envelope has a capsid II-like electron microscope appearance, it was expected that capsid II would have the same electrophoretic mobility as capsid I′. Surprisingly, it was found that different capsid II preparations had different mobilities. Electrophoresis of unlabeled capsid I and three different preparations of unlabeled capsid II was performed on a 0.9% agarose horizontal slab gel stained with Coomassie blue. The three capsid II preparations migrated at different rates (Fig. 4, slots b to d); the slowest (slot c) and fastest (slot d) had $R(CI)$ values of 0.37 ± 0.02 and 0.57 ± 0.03. If the fastest and slowest preparations were mixed 1 h before electrophoresis, both capsid II bands were observed (Fig. 4, slot e), indicating that the difference in mobility was not caused by a factor that exchanged before or during electrophoresis. Capsid I preparations (two unlabeled, one ^{14}C labeled) have, thus far, all had the same mobility.

The capsid I profile had a band migrating 0.85 times as far as capsid I (arrow in Fig. 4a). This band was not present 5 days after completion of this capsid I preparation, but was present 11 days after preparation and later. For further characterization, see below.

To test the intactness of capsid II after electrophoresis, ^{35}S-labeled capsid II was diffusion eluted after electrophoresis in a 0.9% tube gel and was sedimented in a sucrose gradient. No alteration (<1%) in the sedimentation rate of capsid II was observed. To test the effect of sample concentration on mobility, ^{14}C-labeled capsid II (<0.01 μg) was subjected to electrophoresis on a 0.9% agarose slab gel with and without 2.0 μg of unlabeled capsid II. The distance migrated by, the intensity of, and the band width of the ^{14}C-labeled capsid II, measured from an autoradiogram, were the same with and without the unlabeled capsid II. Thus reversible aggregation does not affect *u*.

To help determine the cause of the mobility differences of different capsid II preparations, the preparations in slots c and d of Fig. 4 were observed by electron microscopy. The envelope thickness of both preparations was 2 to 3 nm and no systematic difference in evelope shape or size was noted. The ages of the capsid II preparations in slots b, c, and d of Fig. 4 were 31.7, 7.8, and 0.8 months at the time of electrophoresis. The mobility differences are therefore not correlated with age.

SDS-polyacrylamide gel electrophoresis of the three capsid preparations revealed that three proteins were present in the faster-migrating

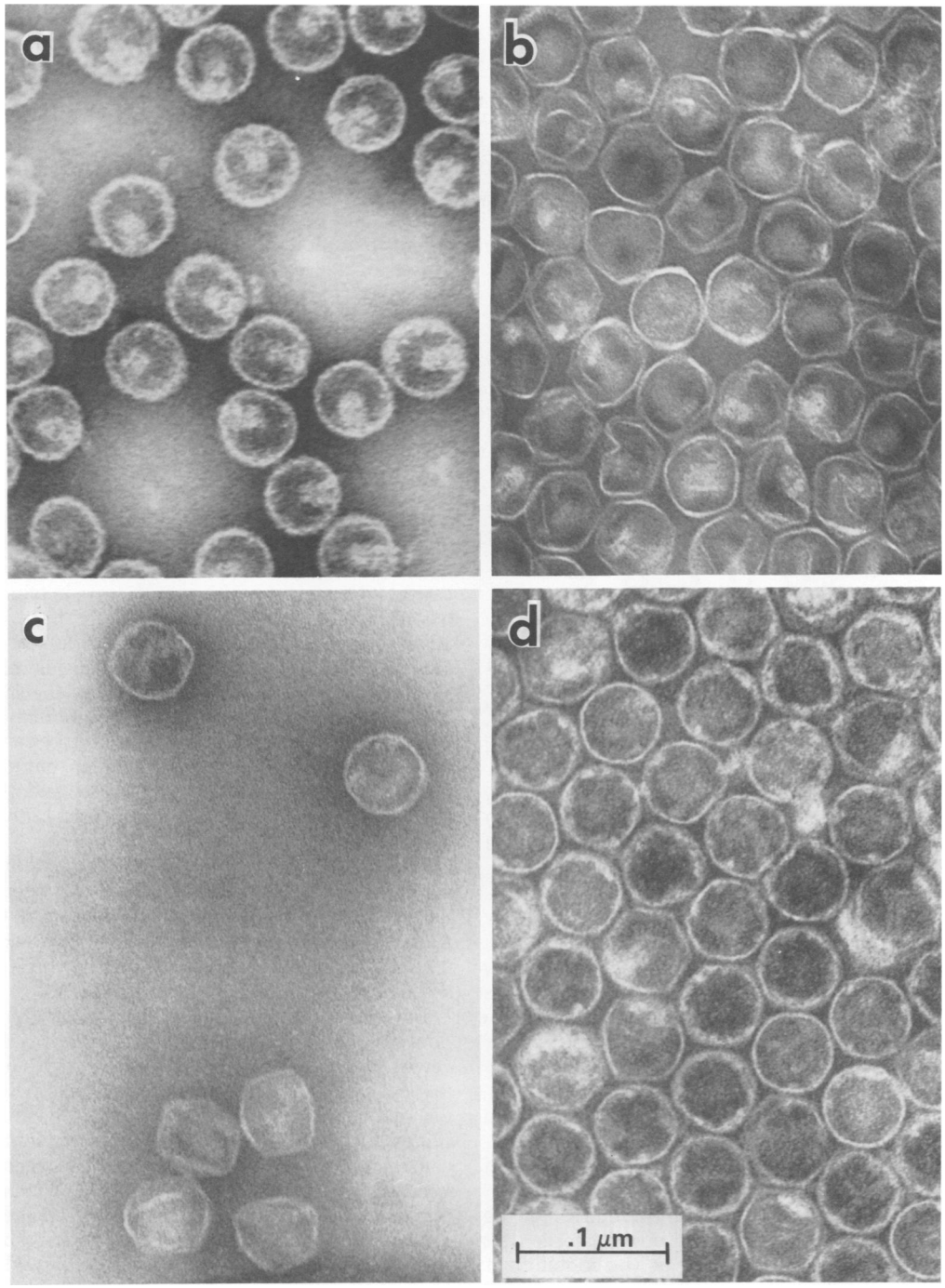

FIG. 3. *Electron microscopy of T7 capsids. Samples were prepared for electron microscopy as described in the text. (a) Capsid I. (b) Capsid II. (c) Capsid I' diffusion eluted from an agarose gel after electrophoresis. (d) Capsid I treated with 0.025% SDS as described in the legend to Fig. 5.*

capsid II preparations in slots b and d of Fig. 4, but were not present in the capsid II preparations in slot c (the amounts present by mass were 3 to 9% of the amount of P10): (i) P9; (ii) protein Q (11), for which a gene has not yet been assigned; and (iii) a protein that migrates 6%

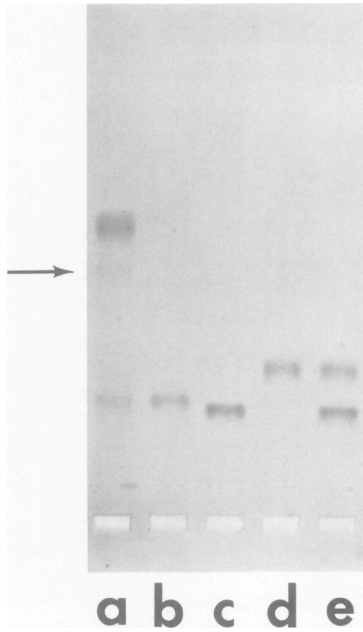

FIG. 4. *Electrophoresis of capsid II. The following samples were prepared in a 10-μl volume: capsid I (5 μg), capsid II (0.7 to 0.9 μg) from three different preparations (separate samples), and a mixture of the second and third capsid II preparations incubated for 1 h at room temperature. The samples were diluted and subjected to electrophoresis in a 0.9% agarose slab gel as described in the text (2 V/cm, 420 min). The gel was stained with Coomassie blue. (a) Capsid I. (b) to (d) Three different capsid II preparations. (e) A mixture of capsid II preparations from slots (c) and (d). The anode is at the top.*

further than P10 on SDS gels; this protein may be the same as a comparatively minor protein previously designated P10 (14). Both P9 and Q are probably envelope proteins (11), and it is therefore possible that their presence caused an alteration of the surface charge or diameter of capsid II. Proteins Q and P9 are also missing from some bacteriophage T7 preparations (11). However, thus far, the maximum difference in mobility between two bacteriophage T7 preparations is only 5% (three preparations with and three without P9 and Q have been tested).

The observation of different mobilities for the different capsid II preparations suggests that some factor which determines the mobility of capsid II varies from one preparaion to another. All preparations of capsid II were grown from the same bacteriophage inoculum, indicating that genetic variation is not the cause of the capsid II variation. The above factor is not known.

The values of $R(CI)$ of capsids IVA and IVB have been determined on 0.9% agarose slab and cylindrical gels. Capsids IVA (tail$^-$) and IVB (tail$^+$) have $R(CI)$ values of 0.42 ± 0.02 and 0.29 ± 0.02 (data not shown).

Effect of aggregation on c^0, u, and u^0: a capsid I dimer. Aggregates of bacteriophage T7 or T7 capsids have a larger diameter than the monomer and therefore during electrophoresis should be retarded by sieving to a greater extent than monomers [i.e., $-(dc/dA)$ should be larger for aggregates than for monomers]. The value of $-(dc/dA)$ for the band marked with an arrow in Fig. 4 was measured to be 0.65 ± 0.02 (with A between 0.9 and 2.0%), 1.22 times as high as $-(dc/dA)$ determined for the capsid I band in the same gels. This suggests that the material forming the band marked with an arrow in Fig. 4 is larger than capsid I and may be an aggregate of capsid I particles. After diffusion elution of material from the gel region marked with an arrow in Fig. 4, 70% of the capsids observed in the electron microscope had capsid I-like envelopes; 42% of the capsids were in capsid I dimers; 5% of the capsids were in trimers (some capsids in trimers had bacteriophage-like envelopes); and larger aggregates were not observed (394 capsids observed). Of capsids diffusion eluted from the capsid I region of the same gel, 91% (202 counted) were capsid I monomers. These data suggest that the band marked with an arrow is formed by a capsid I dimer that does not dissociate during electrophoresis and that the capsid I band is formed by a capsid I monomer.

When $R(CI)$ of the capsid I dimer was measured for the series of gel concentrations used to determine c^0s (Materials and Methods), $R(CI)$ linearly approached 0.98 ± 0.03 as A approached 0. Thus, u^0 measurements for capsid I are not significantly affected by dimerization.

For capsids I and II the observation that u does not depend on concentration indicates that reversible aggregation does not affect u (though it is possible that some aggregation occurs). The disappearance of the band formed by bacteriophage T7 below 15 μg of bacteriophage might be caused by the reversible formation of aggregates necessary for bacteriophage stability. Bacteriophage T7 has a diameter 15% larger than capsid I (Table 1), and therefore $-(dc/dA)$ for a bacteriophage dimer or larger aggregate should be at least 0.65. The values of $-(dc/dA)$ for bacteriophage T7 from three different experiments were 0.48, 0.54, and 0.56, suggesting that bacteriophage T7 is migrating at least to some extent as a monomer. Even if aggregation were modifying values of u for bacteriophage T7, the equality of u^0s for the capsid I monomer and dimer

suggests that the difference in shape between a monomer and dimer does not cause a difference in u^0. Thus, u^0 for bacteriophage T7 should not be affected by aggregation unless aggregation selectively blocks one class of charged groups on the bacteriophage surface.

Determination of c^0, u^0, and σ. The measured differences in u among bacteriophage T7 and T7 capsids can be caused by differences in u^0, and therefore r or σ (equation 1), and/or differences in c^0 (equation 2). Assuming the r values in Table 1 and using equation 1, variation in u^0 at constant σ among capsids I, II, and IVB and bacteriophage T7 is calculated to be <0.005%. The observed differences in u (Table 1) must, therefore, result from differences in c^0 or σ. The values of c^0 and σ were determined.

For capsid I and the capsid II preparation in slot b of Fig. 4, u was measured directly in a 0.9% agarose slab gel as described above. Values of u for other capsids and bacteriophage T7 were measured from their $R(CI)$ values; c^0s for bacteriophage T7, capsid I, capsid II, and capsids IV were measured as described above (Table 1). No significant difference in c^0 among bacteriophage T7 and the T7 capsids was found. Thus the observed differences in u were caused by differences in σ.

Values of c^0 were used to calculate u^0 with equation 2 and then σ with equation 1 (Table 1). Most of the error in σ arises from uncertain knowledge of η resulting from imprecise temperature control. Relative values of u, u^0, and σ do not depend on η and have an error of ±4%. For instance, the difference in u^0 between capsids IVA and IVB is significant and suggests that the exterior portion of the T7 tail is positively charged.

Values of c^0 for the capsid II preparations in slots c and d of Fig. 4 could not be determined because sufficient material was not available. Because capsids from all capsid II preparations appeared to be the same size in the electron microscope (50 ± 2.5 nm, edge to edge), we have assumed that c^0 is the same for all capsid II preparations, and that variations in u among the capsid II preparations are caused by variations in σ.

Treatment of capsid I with SDS. If capsid I is a precursor to the capsid of mature T7 phage in vivo, it should be possible to convert capsid I to a capsid with a bacteriophage-like envelope. This type of transition has previously been observed after treatment of the precursor capsid of bacteriophage P22 with the ionic detergent SDS (3). To test the effect of SDS on capsid I, capsid I was subjected to electrophoresis after treatment with 0.01, 0.025, 0.05, and 0.1% SDS in Tris/Mg buffer at 30.0°C (Fig. 5, slots a to d). The sample treated with an SDS concentration of 0.01% (slot a) had the same profile as an untreated sample (not shown), but the profile of the sample treated with 0.025% SDS (slot b) had all detectable protein in the capsid II region. This capsid II-like conversion product band became more diffuse as the SDS concentration was increased to 0.05 and 0.1% (slots c and d). All SDS concentrations used had no detectable effect on the mobility of capsid II.

Electron microscopy of capsid I after treatment with 0.025% SDS revealed capsids (Fig. 3d), none of which had the internal core of capsid I (500 observed). Most of the envelopes of the 0.025% SDS-treated capsid I particles were 2 to 3 nm thick, equal to the envelope thickness of capsid II and bacteriophage T7. However, some envelopes had regions that were thicker, suggesting that some protein, probably P9, associated with the capsid I envelope had only been partially removed from the envelope. The cap-

TABLE 1. *Determination of c^0, u^0, σ, and z*[a]

Structure[b]	r (cm × 10^{-6})	$-u$ ($cm^2/V \cdot s \times 10^{-5}$)	c^0	$-u^0$ ($cm^2/V \cdot s \times 10^{-5}$)	$-\sigma$ (ESU per $cm^2 \times 10^3$)	$-z$ ($\times 10^2$)
Bacteriophage T7	3.01	2.8 ± 0.1	1.43 ± 0.04	4.0 ± 0.2	1.3 ± 0.1	3.1 ± 0.3
Capsid I	2.61	9.1 ± 0.4	1.43 ± 0.04	13.0 ± 0.6	4.3 ± 0.4	7.6 ± 0.7
Capsid II	2.84	3.6 ± 0.2	1.46 ± 0.04	5.2 ± 0.2	1.7 ± 0.2	3.6 ± 0.4
Capsid IVA	[c]	3.8 ± 0.2	1.49 ± 0.04	5.7 ± 0.2	1.9 ± 0.2	
Capsid IVB	2.89	2.6 ± 0.1	1.50 ± 0.04	3.9 ± 0.2	1.3 ± 0.1	2.8 ± 0.3

[a] Values of the outer radius, r, for bacteriophage T7 and T7 capsids were determined by low-angle X-ray scattering (J. E. Ruark, P. Serwer, M. J. Ross, and R. M. Stroud, submitted for publication). Determination of u, c^0, u^0, and σ was performed as described in the text. Values of z were obtained by multiplying σ by $4\pi r^2$ and dividing by 4.80×10^{-10}, the charge of one electron in electrostatic charge units (ESU). The temperature was 26 ± 3°C.

[b] Capsid I, Precursor capsid. Capsid II, Slot b of Fig. 5; probable conversion product of capsid I in vivo. Capsid IVA, $Tail^-$, from temperature-shocked bacteriophage T7. Capsid IVB, $Tail^+$, from temperature-shocked bacteriophage T7.

[c] The radius of capsid IVA has not been determined.

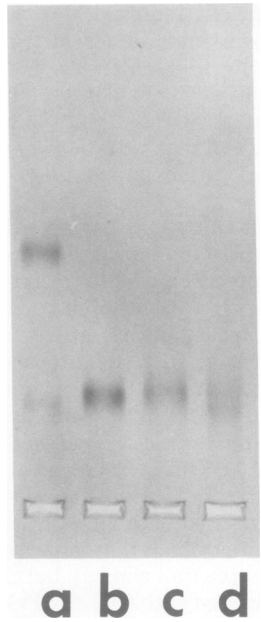

FIG. 5. *Treatment of capsid I with SDS. To 2.5 μl of a 2.0-mg/ml solution of capsid I in Tris/Mg buffer was added 2.5 μl of Tris/Mg buffer containing SDS at 0.02, 0.05, 0.10, or 0.20%. These mixtures were incubated at 30.0°C for 1.0 h and were diluted for electrophoresis as described in the text. The samples were subjected to electrophoresis in a 0.9% agarose slab gel (2 V/cm, 337 min) and were then stained with Coomassie blue. Final SDS concentration: (a) 0.01%; (b) 0.025%; (c) 0.05%; (d) 0.1%. The anode is at the top.*

sids also had a tendency to form symmetrical two- and three-dimensional arrays, a phenomenon never before observed with bacteriophage T7 or any T7 capsid.

After electrophoresis of capsid I treated with 0.025% SDS, material in an agarose cylindrical gel was denatured and subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods (Fig. 6). The following observations were made. (i) No detectable P9 (<10% of the amount of capsid I) was found in the central region of the capsid II-like conversion product peak (arrow c in Fig. 6); however, some P9 was detected in the leading and trailing regions of this peak. Possibly the capsid II-like conversion product is heterogeneous with respect to P9 content. (ii) The amount of core protein P16 in all regions of the capsid peak was the same relative to P10 as in capsid I; core proteins P8 and P15 were, however, partially removed from the capsid peak. Apparently some core proteins remained in the capsid envelope, although the core structure was disrupted. (iii) Some capsid I was not converted to a capsid II-like capsid (arrow d in Fig. 6). (iv) Most P9 and P8 released from capsid I migrated to the bottom of the agarose gel, and most P15 released from capsid I stayed near the origin of the agarose gel.

DISCUSSION

The data presented indicate that σ for capsid I is two to three times σ for capsids II and IV and bacteriophage T7, resulting in a clean electrophoretic separation of capsid I from the other capsids. The observations that no more than 0.3% of the capsid I mass is DNA (Results) and that capsid I isolated from lysates of a T7 gene 5 mutant (DNA synthesis negative) (14) has the same mobility as capsid I from a wild-type T7 lysate (data not shown) indicate that the comparatively high surface charge of capsid I is not caused by DNA bound to capsid I.

At least two functions for the relatively high σ of capsid I can be imagined: (i) increased bound water resulting from the increased density of negative charge on protein subunits (4) may serve as a lubricant or a vacuum seal (10) for the rearrangement of envelope subunits that occurs during conversion of capsid I to the capsid of bacteriophage T7, and (ii) decreased interaction with DNA resulting from increased electrostatic repulsion between capsid I and DNA may prevent nonspecific aggregation of DNA with capsids; such aggregation occurs between capsids IV and DNA (11). If the latter is true, some mechanism for bypassing this repulsion must exist to package DNA. A possibility is that capsid I has an external projection, possibly positively charged, that protrudes beyond the negatively charged surface of capsid I and binds DNA or binds a protein attached to DNA. An external projection at the base of the capsid I core is sometimes observed (11). It seems possible that a projection is initially inside the capsid envelope, but is extended when the capsid receives an external signal.

The constituents of T7-infected *E. coli* lysates have in the past been analyzed by zone sedimentation in sucrose gradients after lysis with nonionic detergents (8, 9). The results of preliminary experiments (Serwer, unpublished data) indicate that agarose gel electrophoresis is another useful technique for analyzing the contents of lysates, with or without prefractionation by sedimentation.

The SDS-induced conversion of capsid I to a particle with a phage-like envelope suggests that such a conversion may occur in vivo. This supports the hypothesis that capsid I is a precursor to the capsid of T7 phage. The slab gel system used here can be used to screen for conditions more closely resembling in vivo conditions that

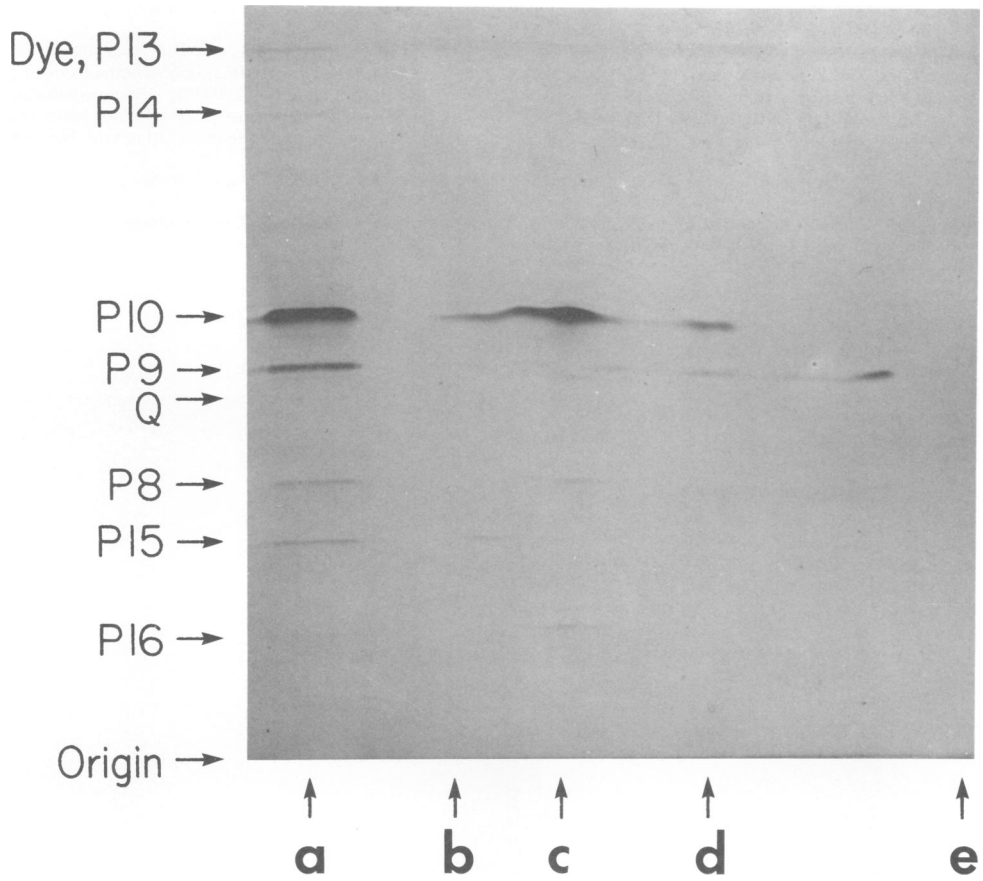

FIG. 6. *SDS-polyacrylamide gel electrophoresis in a second dimension after agarose gel electrophoresis of 0.025% SDS-treated capsid I. To 5.0 μl of a 2.0-mg/ml solution of capsid I in Tris/Mg buffer was added 5.0 μl of 0.05% SDS in Tris/Mg buffer; this mixture was incubated at 30.0°C for 1.0 h. After electrophoresis in a cylindrical 0.9% agarose gel (6.8 V/cm, 140 min), the sample was denatured and subjected to SDS-polyacrylamide gel electrophoresis in a second dimension as described in the text. The bottom 25% of the separating portion of the SDS gel was 17% acrylamide, and the top 75% was 13% acrylamide. A 20-μg sample of capsid I that had not been subjected to agarose gel electrophoresis was also on the SDS gel (vertical arrow a). The other vertical arrows indicate: (b) the origin of the agarose cylindrical gel; (c) the capsid II-like conversion product of 0.025% SDS-treated capsid I; (d) capsid I particles not converted by the SDS to a capsid II-like particle; and (e) the bottom of the agarose cylindrical gel.*

will induce transformations occurring during T7 DNA packaging and capsid morphogenesis.

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