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Polyacrylamide Gel Electrophoresis of Intact Bacteriophage T4D Particles

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A method for the electrophoresis of intact bacteriophage T4D particles through polyacrylamide gels has been developed. It was found that phage particles will migrate through dilute polyacrylamide gels (less than 2.1%) in the presence of a low concentration of $MgCl_2$. As few as 5×10^9 phage particles can be seen directly as a light-scattering band during the course of electrophoresis. The band can also be detected by scanning gels at 260 to 265 nm or by eluting viable phage particles from gel slices. A new mutant (*eph1*) has been identified on the basis of its decreased electrophoretic mobility compared with that of the wild type; mutant particles migrated 14% slower than the wild type particles at pH 8.3 and 35% slower at pH 5.0. The isoelectric points of both the wild type and *eph1* mutant were found to be between pH 4.0 and 5.0. Particles of T4 with different head lengths were also studied. Petite particles (heads 20% shorter than normal) migrated at the same rate as normal-size particles. Giant particles, heterogenous with respect to head length (two to nine times normal), migrated *faster* than normal-size particles as a diffuse band. This diffuseness was due to separation within the band of particles having mobilities ranging from 8 to 35% faster than those of normal-size particles. These observations extend the useful range of polyacrylamide gel electrophoresis to include much larger particles than have previously been studied, including most viruses.

Polyacrylamide gel electrophoresis has been used to separate a wide variety of macromolecules such as proteins, RNA, DNA, and cellular components such as ribosomes (23). It has also been used to separate and measure the isoelectric points of some small viruses (1, 10, 18, 19, 21, 22). These were spherical plant viruses or bacteriophages, all of which had a diameter of less than 30 nm and contained less than 4×10^6 daltons of DNA or RNA, with the exception of phage M13. The latter is 800 nm long but only 6 nm in diameter and contains 1.6×10^6 daltons of DNA (20).

This paper describes the electrophoresis of different types of *Escherichia coli* bacteriophage T4 particles through polyacrylamide gels. The dimensions of the head of a normal T4 particle are as follows: about 110 nm in length by 75 nm in diameter, with the tail being as long as the head (9, 16). It contains about 112×10^6 daltons of DNA (8, 13). Abnormal T4 particles (petite and giant) have been described which have normal tails and heads of normal diameter, but in which the length of the heads varies from 85 to 100 nm for petite particles and from 165 to more than 1,000 nm for giant particles (5, 6, 7, 9, 16, 24). In the present report, we demonstrate that normal and petite particles, as well as giant particles, will migrate through dilute polyacrylamide gels. Normal and petite particles migrate at the same rate but, unexpectedly, giant particles were found to migrate faster than normal-size particles. Also, a new mutation has been identified which gives rise to particles of normal size but with altered electrophoretic properties. The technique that we describe should be generally useful in identifying viral mutants with structural proteins altered in their net charge.

MATERIALS AND METHODS

Bacteriophage and bacteria. The media and methods used for the preparation and assay of the phage and bacteria were as described by Doermann et al. (6) except where noted. All phage and bacterial strains were obtained from A. H. Doermann. The T4 mutants *E920g* and *ptg19-80* map in gene 23 (7), which codes for the major head protein of T4, and these were used as sources of petite and giant particles. *E920g* lysates contain petite particles that are relatively homogeneous in size and of the smallest type known, but they do not contain giant particles (4, 6). Giants were obtained from *ptg19-80* lysates

and were separated from petite and normal-size particles by CsCl centrifugation as described below. An osmotic shock mutation, *os2*, was present in some of the mutants studied. Strains of *E. coli* used were CR63 and 011′ (both permissive for amber mutants) and B and S/6 (both restrictive for amber mutants). Strains CR63 and B were used for crosses and the preparation of phage stocks, and strains CR63, 011′, and S/6 were used for plaque assays. Strain 011′ was used in preference to CR63 to assay *ptg19-80* since plaques of *ptg19-80* on CR63 are very small (6).

Phage stocks. The lysis inhibition technique of Kellenberger as described by Doermann et al. (6) was used to prepare stocks. Stocks containing about 10^{11} phage particles per ml were dialyzed against 10 mM sodium borate buffer (pH 8.3) containing 5 mM $MgCl_2$ and were applied without further purification to polyacrylamide gels. Alternatively, infected bacteria were lysed directly in this buffer and the dialysis step was omitted.

Cesium chloride gradients. Giant particles were separated from normal-size and petite particles by cesium chloride density centrifugation of *ptg19-80* lysates. To prevent inactivation of the phage particles by cesium chloride, the phage stocks were held at 45 C for 30 min before and for 30 min after addition of solid cesium chloride (16). The cesium chloride-phage mixture (30 ml of phage lysate plus 24 g of cesium chloride) was centrifuged at 20,000 rpm for 18 h at 24 C in a Beckman SW27 rotor. A band of giant particles (20 mm from the bottom of the 84-mm long gradient) was well separated from a band of normal and petite particles (65 mm from the bottom). Fractions (1 ml each) were collected from the bottom of the tube. The presence of giant particles in the lower band was confirmed by electron microscopy; the peak fractions contained less than 1% contamination with normal or petite particles. These fractions were pooled and dialyzed at 45 C against a solution of 10 mM sodium borate buffer (pH 8.3), 5 mM $MgCl_2$, and 3 M NaCl. NaCl was present to prevent osmotic shock. The concentration of NaCl was gradually lowered by dilution over a 6-h time period and finally eliminated completely. The band containing normal and petite particles was also collected and treated in the same way.

Assay for petite particles. The average length of DNA in petite particles was measured as described elsewhere (3, 15). The titer of petite particles was determined by their ability to complement *amB23* (17). This assay will detect about ⅔ of the *E920g* petite particles, because each particle contains only a random ⅔ of the genome. Therefore, only ⅔ of them contain the $amB23^+$ allele necessary for complementation.

Assay for giant particles. The *ptg19-80* giants can be distinguished from *ptg19-80* normal particles because the former are much more resistant to UV irradiation than the latter (6). Thus, at a fluence of 60 J/m^2, the average survival of giants used in these experiments was 10^{-1} compared to 3×10^{-7} for normal particles.

UV irradiation. UV irradiation was carried out with a General Electric 15-W germicidal lamp at a fluence of 2.0 J/m^2 per s (= 20 $ergs/mm^2$ per s) as measured by an International Light 254 UV dosimeter. Phage particles were suspended in phage buffer (7 g of Na_2HPO_4, 3 g of KH_2PO_4, and 4 g of NaCl per liter plus 1 mM $MgSO_4$) and irradiated in a petri dish with gentle stirring.

Preparation of gels. Acrylamide (BDH) and *N,N′*-methylene bisacrylamide (BDH) were purified by the method of Loening (14). The bisacrylamide concentration in all gels was 5% of the acrylamide concentration. The gel concentration is expressed in terms of the acrylamide concentration (weight/volume) only. Dilute gels (less than 2.5% acrylamide) were polymerized with 0.1% (vol/vol) tetramethylethylenediamine (TEMED) (adjusted to pH 7 with acetic acid) and 0.1% (wt/vol) freshly prepared ammonium persulfate. No other buffer was added to the gel mixture. Gels had a conductivity about 10% greater than that of the running buffer (see below).

Since completing the experiments described in this paper, we have successfully used nonpurified monomers (acrylamide [Baker] and *N,N′*-methylene bisacrylamide [Canalco]). It was necessary to reduce the concentration of both the TEMED and ammonium persulfate to 0.07 to 0.08% each (vol/vol and wt/vol, respectively) to obtain satisfactory gels. Lower concentrations of catalysts (0.05% of each) produced gels that were too stiff to permit phage migration.

The gel mixture was degassed at room temperature under vacuum for 1 min before the addition of ammonium persulfate. After a gentle mixing, the solution was pipetted into a vertical Plexiglas tube (6.25-mm ID by 90 mm long). In some experiments, the bottom 10 mm of the tube was first plugged with an 8% acrylamide gel to facilitate handling. In most experiments, the bottom of the tube was covered with a piece of dialysis membrane.

Gels were preequilibrated for 1 to 2 h at 10 V/cm to remove the polymerization catalysts and adjust the pH of the gel to that of the running buffer. During this period the gel surface became distorted slightly because of swelling. No significant improvement in the gel surface could be obtained by various alternative methods such as layering water on the surface of the gel mixture or by including glycerol in the gel.

Four volumes of phage suspension were mixed with 1 volume of (five-times-concentrated) sample buffer (see below), and 20 to 50 μl of this mixture was applied to each gel. Electrophoresis was carried out at 10 V/cm (about 3 mA per gel) at room temperature.

Electrophoresis buffers. Three running buffers were used for electrophoresis: (i) 50 mM sodium borate (pH 8.3)-5 mM $MgCl_2$, containing 22.4 g of boric acid, 11.9 g of sodium tetraborate decahydrate, and 1 g of $MgCl_2 \cdot 6H_2O$, per liter; (ii) 15 mM sodium acetate (pH 5.0)-2.5 mM $MgCl_2$; and (iii) 50 mM sodium acetate (pH 4.0)-3.5 mM $MgCl_2$. The magnesium ion concentration was varied to keep each of the buffers at the same conductivity. A low level of magnesium ion was found to be essential for phage to enter gels. Five-times-concentrated sample buffers, prepared without magnesium ion, contained 0.04% (wt/vol) bromophenol blue, 12.5% (vol/vol) glycerol,

and either 88 mM borate buffer, (pH 8.3), 50 mM acetate buffer (pH 5.0), or 90 mM acetate buffer (pH 4.0), depending on the particular running buffer used.

UV scanning. Gels were transferred from the Plexiglas tubes into a Gilford gel-scanning trough (5 mm wide by 100 mm long) that was partially filled with 50% (vol/vol) ethanol. The ethanol solution was used in preference to water because its lower density permitted the gel to sink more readily and lie flat on the bottom of the trough. Gels were scanned at 260 or 265 nm in a Gilford model 240 spectrophotometer.

Gel slicing. A gel slicer, described previously (2), was used with slight modification in the gel handling procedure. A 1.9% acrylamide gel was cast in a tube plugged with an 8% gel. After electrophoresis, the gel tube was introduced directly into the slicer to minimize the problem of handling a very soft gel. Alternatively, the gel was frozen and then it could be sliced without difficulty.

Electron microscopy. Electron microscopy was used to determine the relative distribution of phage particles with different head lengths. Specimens were prepared by simply allowing phage to adhere to grids, a method that has given quantitatively similar results for the distribution of giant head lengths (F. A. Eiserling, personal communication) to the more complicated agar filtration procedure (11). Copper grids (400 mesh), coated with carbon and collodion, were floated on a drop of phage suspension for 1 min. Grids were washed twice by being floated on water, drained, and then negatively stained by floating on a drop of 2% (wt/vol) uranyl acetate for 1 min. Uranyl acetate was drained and the grid was air dried. Head lengths of phage particles were measured at a total magnification of $\times$20,000 for giants and $\times$160,000 for normal-size particles. Magnification was calibrated with latex spheres (diameter of 109 $\pm$ 2.7 nm; Fullam, Inc.) mixed with the phage suspension or by a carbon grating (Fullam, Inc., no. 1002); both calibration standards gave similar results.

RESULTS

Acrylamide-bisacrylamide monomers can be polymerized to form gels at a very wide range of concentrations. At low concentrations, soft gels are formed that have relatively large pore sizes. Such gels have been used to separate small viruses (1, 10, 18, 19, 21, 22). We have investigated the possibility that gels could be formed with sufficiently large pore sizes to permit particles as large as intact bacteriophage T4 (minimum head dimension 75 nm) to enter. In preliminary experiments, the minimum concentration of monomers at which gelation would occur was found to be about 1.7 to 1.8%, but such gels were too soft to handle. At 1.85% and above, gels were firm enough to support a sample. Gels made from 1.85 to 2.2% acrylamide were tested to determine their suitability for the electrophoresis of phage T4. Samples containing 8×10^9 T4 particles were applied and a band, readily observable by oblique lighting against a dark background, migrated towards the anode (Fig. 1B). In 1.85% to 2.0% gels, the band migrated at about 10% of the rate of the bromophenol blue dye marker. In 2.1 and 2.2% gels, the band failed to penetrate more than 1 to 2 mm into the gel. Since T4 can readily be visualized as a light-scattering band in CsCl gradients, it seemed likely that the bands observed in acrylamide gels contained phage particles. A gel electrophoresed without added phage is shown in Fig. 1A for comparison.

To verify that phage particles were, in fact, migrating through the gels coincident with the light-scattering band, electrophoresis in a 1.9% gel supported with a nylon mesh was continued until the band had traversed the entire length of the gel (25 mm) and entered the lower reservoir. The lower reservoir was sampled periodically for phage. Infectious particles were detected at the same time as the light-scattering band was seen to pass out of the gel. The experiment demonstrated that infectious phage migrated electrophoretically through a 1.9% gel close to, or coincident with, the light-scattering band.

Further confirmation of the nature of the band was obtained in two ways: (i) by scanning a gel at 265 and 350 nm, and (ii) by elution of phage from gel slices. In the first experiment, electrophoresis was continued until the light-scattering band had moved 19 mm from the surface. A peak of phage DNA was detected by scanning at 265 nm; it was coincident with the light-scattering band detected at 350 nm and with the band seen by eye (data not shown). In the second experiment, a similar gel was sliced and the phage were eluted in phage buffer for 3 days at 4 C. A single peak of infectious phage was found coincident with those slices that could be seen to contain light-scattering material (Fig. 2). Recovery was 33% of the viable phage originally added to the gel. These experiments demonstrate that intact phage T4 particles can migrate through a gel and be detected by light scattering, UV absorption and recovery of viable phage.

Electrophoresis of structural protein mutants of T4. We have employed our technique of electrophoresis in dilute polyacrylamide gels to determine whether among a number of amber mutants (grown on the *suI*$^+$ host CR63) and missense mutants of gene 7 (baseplate protein), gene 23 (the major head protein), and gene 24 (head protein) any were altered in their electrophoretic mobility. Mutants examined were *amB23* (gene 7), *amB66* (gene 7), *amB17* (gene 23), *amE509* (gene 23), *E920g* (gene 23), *os2* (gene 24), and double mutants of *os2* (gene 24) with *E920g* and *ptg19-80* (gene 23). The

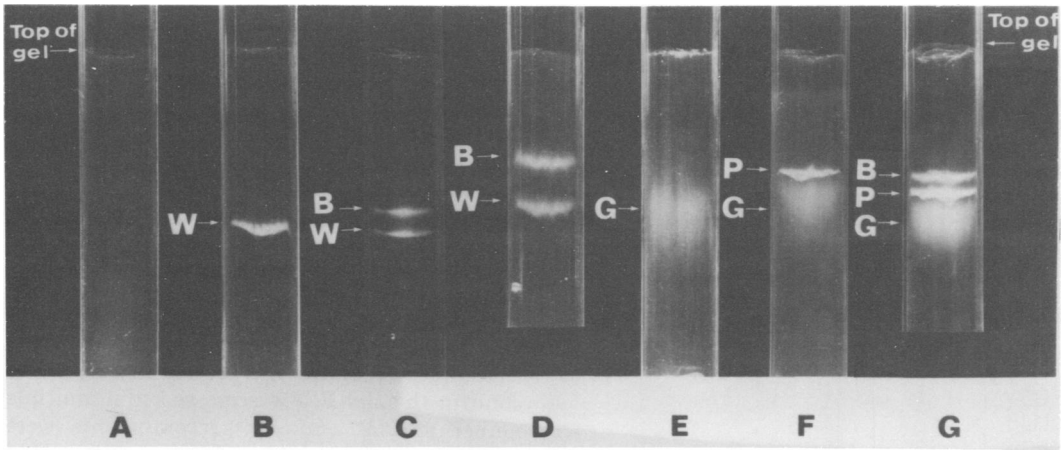

FIG. 1. *Electrophoresis of intact T4 particles in polyacrylamide gels. In all figures, unless otherwise stated, the gel concentration is 1.9% acrylamide, the pH is 8.3, and the direction of migration is from the cathode (top) to anode (bottom). All photographs were taken directly in the tubes by light-scattering, with a slide projector as the light source. The tubes are made of Plexiglas and small scratches scatter light. The imperfections seen in the photographs are almost entirely on the surface of the tubes and not within the gels. In this figure the number of phage particles of type applied to the gels was: 7.8 × 10⁹, wild type; 7.4 × 10⁹, amB23; 9.0 × 10⁸, ptg19-80 giants; 5.5 × 10⁸, ptg19-80 normal-size particles; and 5.9 × 10⁹, am⁺ ptg19-80 petite particles. The phage applied to the gels and electrophoresis times are: (A) no phage added, 100 min; (B) wild type, 150 min; (C) amB23 and wild type, 150 min; (D) amB23 and wild type, 300 min, pH 5.0; (E) ptg19-80 giants, 100 min; (F) ptg19-80 giants, normal-size and petite particles, 100 min; (G) amB23, and ptg19-80 giant, normal-size and petite particles, 120 min. Abbreviations used in this figure are: B, amB23; G, giants; P, ptg19-80 normal-size and petite particles; W, wild type.*

E920gos2 stock contained about 1.5 times as many petite particles, with an average genome size of 70.9%, as normal-size particles. The *ptg19-80os2* stock contained about 10 times as many petite particles as normal-size particles; giant particles had been removed by CsCl density centrifugation. Light-scattering bands of phage were visualized directly. The mobility of each mutant phage was compared to that of wild type by electrophoresing a mixture of the two on a gel at pH 8.3 in borate buffer (Table 1). The band of wild-type phage was identified by reference to its mobility on a separate gel. In all cases, phage migrated through the gel as a single sharp band, over a distance of at least 30 mm, with the exception of *amB23* particles. The latter separated from wild-type particles; they migrated as a sharp band with a relative mobility of 0.86 (Table 1 and Fig. 1C). The stocks of *E920g* and *ptg19-80* contained both normal-size and petite particles, but no separation was observed between either of these and wild-type particles. Since *E920gos2* petite particles had heads that were 20% shorter than normal, separation from normal-size particles was anticipated due to a reduced frictional coefficient. We presume that the lack of separation was due to a reduced net charge which offset the expected increased mobility of petite particles. The separation of *amB23* particles from other normal-size and petite particles is likely due to a reduction in their net charge.

If *amB23* particles have a lower net negative charge, then it is probable that the relative charge difference between them and wild-type particles would be enhanced by decreasing the pH of the electrophoresis buffer to a value closer to their isoelectric points. To test this possibility, we examined wild-type and *amB23* particles on a gel at pH 5.0. As expected, the separation of the two was markedly increased (Fig. 1D and Table 1). Thus, acetate buffer at pH 5.0 is appreciably superior to borate buffer at pH 8.3 for maximizing the separation of the two types of phage particles. There was considerable variation in the rates of migration of phage particles in different batches of gels, but very little difference in the relative mobility of *amB23* compared to that of wild type (compare the standard deviation for absolute mobility and relative mobility in Table 1).

Isoelectric point of T4. In preliminary experiments, no band of phage particles (either wild type or *amB23*) could be detected if electrophoresis was carried out in acetate buffer at pH 4.0. This suggested that T4 particles have an isoelectric point above pH 4 and that they migrate towards the cathode at this pH. To confirm this,

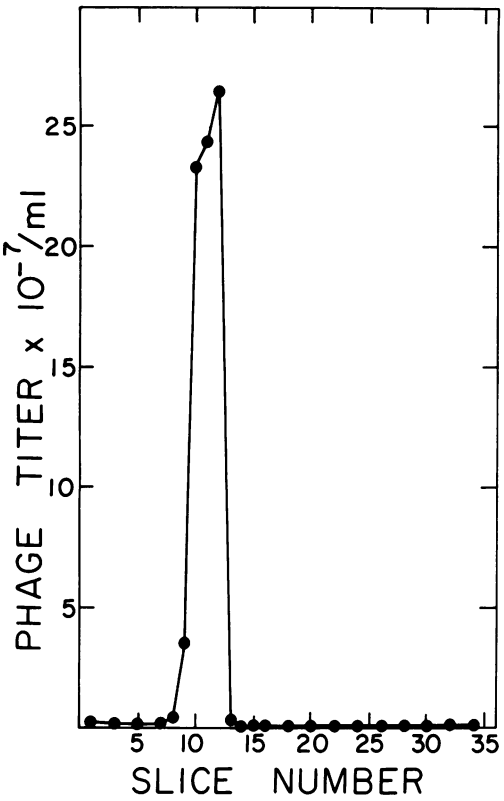

FIG. 2. *Confirmation that T4 particles migrate through polyacrylamide gels as a visible light-scattering band. The gel contained 5.5 × 10^9 wild-type particles and electrophoresis was continued for 80 min until the band of light-scattering material had migrated 17 mm. The gel was sliced into 1.4-mm thick slices, and each slice was eluted for 3 days in 2 ml of phage buffer. Light-scattering material was seen in slices 10, 11, and 12. Recovery of phage from slices 9, 10, 11, and 12 was 33% of that added to the gel.*

wild-type and *amB23* particles were applied to a gel at pH 5.0. After the wild-type phage band had migrated 20 mm, buffer in the upper and lower reservoirs was replaced with acetate buffer (pH 4.0) and electrophoresis was continued. Both phage bands continued to migrate towards the anode for 30 min; then both bands reversed their direction of migration and moved towards the cathode at a slower rate (about 1 mm/h). Presumably, the phage particles developed a net positive charge as the pH in the gel dropped from 5 to 4 and they began migrating in the reverse direction. This result is consistent with an isoelectric point between pH 4 and 5 for both wild-type and *amB23* particles; a more accurate determination of their isoelectric points would require isoelectric focussing.

Evidence that a mutation different from amB23 is responsible for the altered electrophoretic property of amB23 particles. The *amB23* mutation maps in gene 7, which codes for a protein of the tail baseplate. Each phage particle contains only 6 to 24 molecules of this protein (12), and it seemed unlikely that such a small amount of protein could have any significant effect on the net charge of a phage particle. To determine whether *amB23* carries a second mutation responsible for the electrophoretic difference between it and wild type, the electrophoretic mobilities of five revertants of *amB23* to *amB23*$^+$ were tested. All five were found to migrate at the same rate as *amB23*, indicating that a second mutation is present in *amB23*. To confirm this, *amB23* was crossed to a multiple amber mutant and *am*$^+$ recombinants were selected. The multiple amber mutant carried mutations in genes *e* (*lys882*), 8 (*N132*), 24 (*E355*), 31 (*N54*), 32 (*A453*), 52 (*H17*), 56 (*E51*), and 46 (*N130*), which are distributed fairly uniformly around the chromosome. Thus, *am*$^+$ recombinants would inherit *amB23*$^+$ from the multi-amber parent but most of their genetic material from the *amB23* parent. Unless the electrophoretic mutation was closely linked to *amB23*, most of the *am*$^+$ recombinants should carry the electrophoretic mutation. Four *am*$^+$ recombinants were tested and all migrated at the same rate as *amB23*. Therefore, *amB23* carries a second mutation which affects its electrophoretic mobility. This mutation we propose to designate *eph1*.

Separation of giant particles from normal-size particles. Lysates of *ptg19-80* contain, in addition to petite and normal-size particles, some particles with much longer heads (6). These were purified by CsCl density gradient centrifugation, and the distribution of head lengths, as determined by electron microscopy, is shown in Fig. 3. The length of the heads was 622 ± 141 nm (± standard deviation) compared to 118 ± 9 nm for normal heads. The population of giant particles was heterogeneous with respect to the length of their heads; wild-type phage heads were much more homogeneous and are likely monodisperse. Giant particles (3 × 10^8) were mixed with *ptg19-80* normal-size and petite particles (about 5 × 10^8 and 5 × 10^9, respectively) and electrophoresed at pH 8.3 in a 1.9% gel. Two bands were seen (Fig. 1F); the faster-migrating, diffuse band contained giant particles (Fig. 1E). Particles in the diffuse band had mobilities ranging from 108 to 135% that of normal-size or petite particles (Table 1). This experiment shows that giant phage particles can enter 1.9% gels and secondly that they run *faster* than do normal-size and petite particles. This is the reverse of what would be expected on the basis of the known sieving properties of

TABLE 1. *Mobility of intact T4 particles in gels at pH 8.3 and 5.0*[a]

Phage particles	Relative mobility		Absolute mobility (mm/h)	
	pH 8.3	pH 5.0	pH 8.3	pH 5.0
Wild type	1.00	1.00	6.4 ± 1.7	4.5 ± 1.8
ptg19-80 (23) *os2* (24) petite and normal	1.00	1.00		
E920g (23) *os2* and *E920g* petite and normal	1.00			
os2 (24), *amB17* (23), *amE509* (23) and *amB66* (7)	1.00			
amB23 (7)	0.86 ± 0.01	0.65 ± 0.06	5.5 ± 1.5	3.1 ± 1.5
ptg19-80os2 giants, diffuse band, range	1.08 to 1.35			

[a] Mobilities of intact phage particles were determined in 1.9% polyacrylamide gels at pH 8.3 and 5.0 with a voltage gradient of 10 V/cm. Relative mobility of 1.00 indicates no visible separation into two bands during electrophoresis of a mixture of mutant and wild-type particles. The ratio of am^+ petite particles to normal-size particles in the stock of *ptg19-80os2* obtained from a CsCl gradient was 10:1. In the *E920gos2* lysate, this ratio was 1.1:1, and the petite particles contained an average of 70.9% of the T4 genome. Mobility of *amB23* relative to wild type was determined in five separate gels at pH 8.3 and in four gels at pH 5.0. The mean and standard deviation are shown. The absolute mobility of wild type and mutant *amB23* in these experiments varied more than did their relative mobilities; this is indicated by their standard deviations. Numbers in parentheses are the gene numbers.

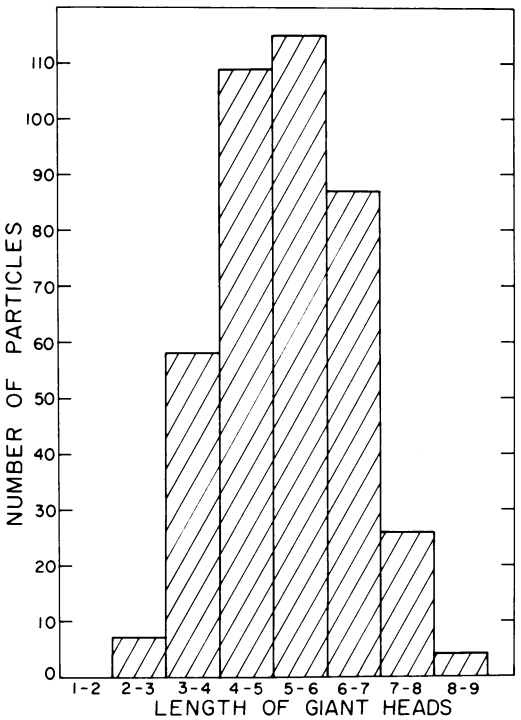

FIG. 3. *Length distribution of giant heads purified by a cesium chloride gradient. The length of giant heads as determined by electron microscopy is expressed as multiples of the normal head length.*

polyacrylamide gels. The band of giant phage became progressively broader during electrophoresis (Fig. 4A through D), in contrast to a band of normal or petite particles which remains relatively sharp. The increasing band width may be due to partial separation of heterogeneous particles. (Size heterogeneity of giant particles has been observed by electron microscopy [Fig. 3].) This hypothesis was tested by the refocussing method of Talens et al. (23). If there is partial resolution of different particles, then reversal of the field should cause resharpening of the band as migration proceeds in the reverse direction. On the other hand, if band spreading is due to an electrophoretic artifact, then sharpening should not occur and the band width should continue to increase. The result of this experiment is shown in Fig. 4. Complete refocussing was seen to occur, indicating that the increase in band width was due to separation of particles that differ in their electrophoretic mobility.

Identification of multiple phage bands separated by gel electrophoresis. To confirm previous visual observations about the relative mobilities of different phage types, we sliced a gel containing a mixture of *amB23* and *ptg19-80os2*, and viable phage eluted from the gel were distinguished as described below. In this experiment, three light-scattering bands were seen directly (Fig. 1G), and three absorbance peaks (A, B, C) and a shoulder (BI) could be detected (Fig. 5A). The mixture applied to the gel contained normal-size *amB23* and petite, normal-size, and giant *ptg19-80os2* particles. The gel was sliced and phage were eluted with 2 ml of phage buffer at 4 C for 20 days. Elution was slow and incomplete (2.9% of giants and 12.5%

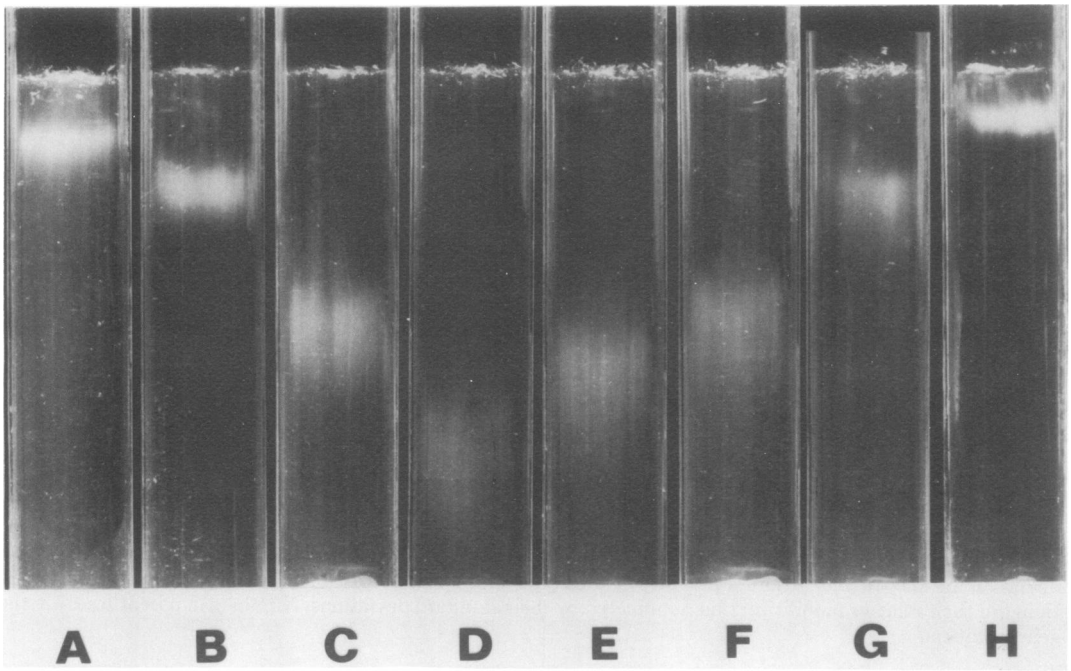

FIG. 4. *Refocussing of a band of giant particles. A gel was loaded with 9.0 × 10⁸ ptg19-80 giant particles, and photographs were taken at intervals during electrophoresis. At 150 min the polarity was reversed. (A) 27 min; (B) 50 min; (C) 100 min; (D) 150 min; (E) 180 min; (F) 200 min; (G) 250 min; (H) 280 min.*

of normal-size particles), but different phage types could readily be assayed and distinguished as follows. The *ptg19-80* giant and normal particles can form plaques on both *E. coli* 011′ and S/6, whereas *amB23* particles can form plaques only on 011′. Thus *ptg19-80* giant and normal particles can be assayed on S/6, and the difference in numbers of plaques on 011′ and S/6 is equal to the number of *amB23* particles. This difference could be measured reasonably accurately as the *amB23* particles were present in 10-fold excess over the *ptg19-80* plaque-forming particles. The *ptg19-80* giants could be distinguished from *ptg19-80* normal particles as the former give an average survival of 10^{-1} compared with 3×10^{-7} for normal particles at of 60 J/m². To calculate the number of giants eluting from each gel slice, it was assumed that their average survival in each eluant was the same (i.e., 10^{-1} at 60 J/m²). If separation based on size was occurring, then the titer of eluants containing smaller giants would be underestimated, and the titer of eluants containing larger giants would be overestimated, since resistance of giants to UV irradiation increases with size (Childs, manuscript in preparation). The *ptg19-80* petite particles can be assayed by a complementation test (see above).

The four different phage types were measured, and their distribution in the gel is shown in Fig. 5B. *ptg19-80* giant particles migrated most rapidly, *amB23* particles migrated least rapidly, and *ptg19-80* petite and normal-size particles migrated together as an intermediate band. This experiment confirms the order of migration of the different phage types, which was deduced earlier from a comparison of the migration of light-scattering bands. Although the middle peak of Fig. 5A appears to show a partial resolution of two components, BI and B (seen also in another experiment), two components in peak B of Fig. 5B have not been resolved. Peak C (giants) is not as well resolved in the sliced gel (Fig. 5B) as compared with the scanned gel (Fig. 5A). This may be due to lower recovery from the gel of the fastest migrating giants.

DISCUSSION

The experiments described in this report have demonstrated for the first time that one of the largest known virus particles can migrate electrophoretically through dilute polyacrylamide gels. The minimum head dimension of T4 phage particles is 75 nm, more than twice that of the largest virus previously studied by polyacrylamide gel electrophoresis (Turnip yellow mottle virus [19]). Thus, polyacrylamide gels can prob-

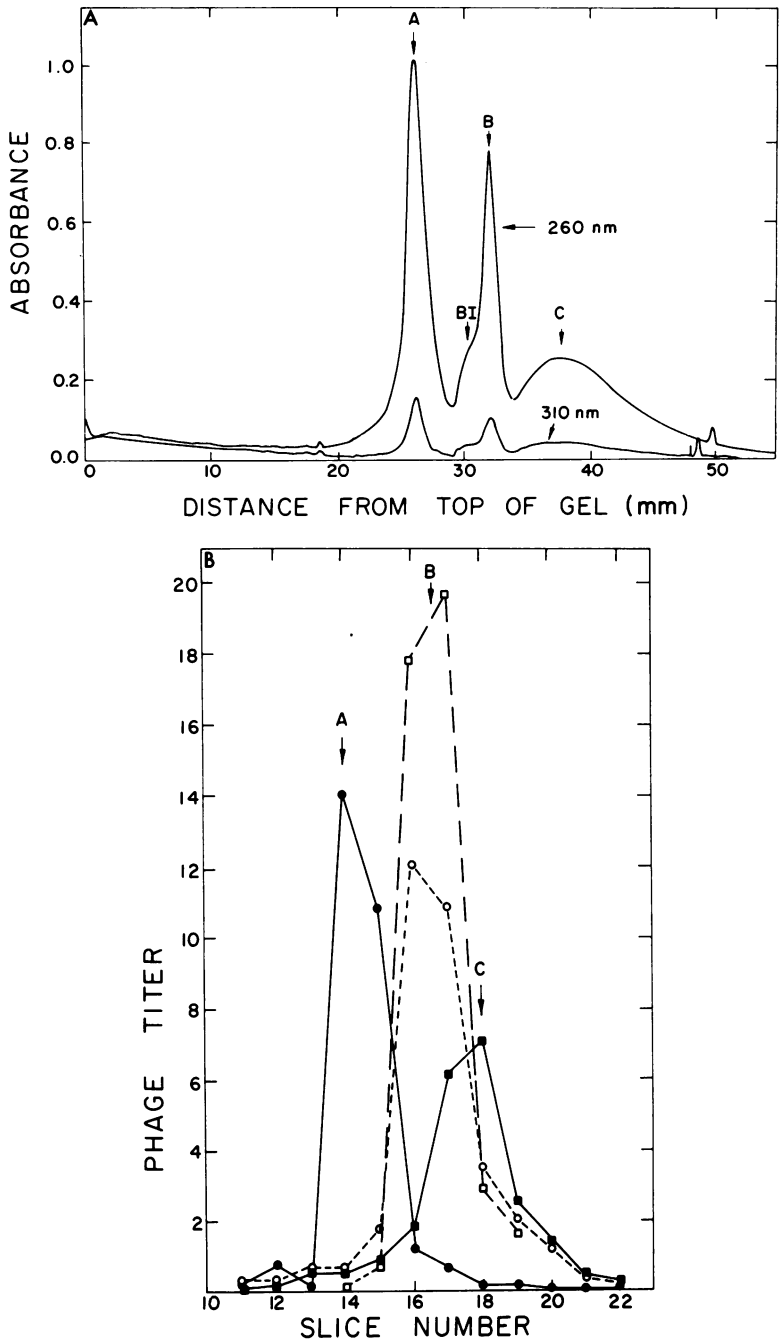

FIG. 5. *Analysis of multiple phage types within a single gel. Each gel was loaded with 6.3 × 10⁹ amB23 particles, 5.1 × 10⁹ am⁺ petite ptg19-80 particles, 4.7 × 10⁸ normal-size ptg19-80 particles, and 3.3 × 10⁸ giant ptg19-80 particles, and peak C is giant ptg19-80 particles (see text and compare with Fig. 5B). (B) Assay for Scan of gel at 260 nm and 310 nm. Peak A is amB23 particles, peaks BI and B are normal-size and petite ptg19-80 particles, and peak C is giant ptg19-80 particles (see text and compare with Fig. 6B). (B) Assay for phage eluted from gel slices. Gel slices were about 2 mm thick and were eluted with 2 ml of buffer. The scale on the ordinate is ×10⁷ per ml (of eluant) for amB23 particles, ×10⁶ per ml for ptg19-80 normal-size particles, ×10⁷ per ml for ptg19-80 am⁺ petite particles, and ×2 × 10⁵ per ml ptg19-80 giants. Symbols: ●, amB23; □, am⁺ petite particles; ○, ptg19-80 normal-size particles; ■, ptg19-80 giants. Recovery of viable phage was 8.9% for amB23, 8.5% for petite particles, 14.0% for ptg19-80 normal-size particles, and 2.9% for giant particles.*

ably be prepared with sufficiently large pore size to permit electrophoretic migration of most viruses. Polyacrylamide gels have proven to be a useful medium for separating a wide variety of macromolecules; this is because they are mechanically stable even at low concentrations and can be prepared with a very wide range of pore sizes. For purposes of the present study, they possess an additional advantage, viz., they are very transparent to visible and UV light, in contrast to agarose or mixed agarose/polyacrylamide gels. This feature is important because it permits phage particles to be viewed directly as a visible light-scattering band during electrophoresis. The progress of an experiment can be readily followed as phage enter and migrate and different types separate. The data of Table 1 were obtained in this way. In the system as described, the rate of migration of T4 is relatively rapid, and separation of different types may be observed in as little as 60 min; a gel can be re-used over a period of a day, further increasing the number of samples that can be studied. Excellent reproducibility in the determination of *relative* electrophoretic mobility of different phage preparations was obtained by mixing them with wild-type particles and observing their rates of migration within the same gel (Table 1). The relative mobility was considered to be 1.0 when the mixture of phage particles migrated as a single band over a distance of 30 mm. When separation into two bands occurred, they were distinguished by varying the relative amounts of different phages and observing the resultant changes in light-scattering intensity of the bands. The identity of different bands has also been confirmed by slicing the gel and assaying for viable phage. Despite their soft consistency, unfrozen gels can be sliced with a device described earlier (2); however, frozen gels are easier to handle and viable phage can be recovered (60% in one experiment, data not shown).

The mobilities of the different phages that were examined fell into three classes (Table 1). Those with mobilities indistinguishable from that of wild type included a variety of mutants with defects in genes 7, 23, and 24. Stocks of two of these (*ptg19-80* and *E920g*) contained petite particles which also had the same mobility as wild-type particles. Failure to separate petite and normal-size particles is somewhat surprising considering that their head lengths differ by up to 20%. This suggests that the decreased frictional coefficient associated with the smaller particles is offset by a decreased net charge.

The only mutant particles that were found to migrate more slowly than wild-type particles were *amB23*. The mutation responsible for the altered mobility has been shown to be different from the *amB23* mutation, since am^+ revertants and recombinants retained their altered electrophoretic mobility. The site of this newly identified mutation (designated *eph1*) has not yet been identified. It is likely to be responsible for the introduction of an altered amino acid in a capsid protein, since its mobility is decreased to a greater extent than that of wild-type phage by decreasing the pH of the gel buffer from 8.3 to 5.0.

The electrophoretic properties of giant particles are particularly interesting. Their modal head length is about five times that of normal-size particles, yet they were found to migrate 8 to 35% *faster* than normal. This is in contrast to the case of M13, a filamentous coliphage, in which, as expected, double-length particles migrated much *slower* (about ¼ the rate) than single-length particles (1). We have no definitive explanation for this marked difference, but it appears that a gain in net negative charge (possibly due to the increased DNA content) offsets the expected increase in frictional coefficient due to their larger size. In fact, it is likely that giant particles are oriented with respect to the direction of their motion through gels, in which case their frictional coefficient might be much closer to that of normal-size particles than otherwise predicted.

Electrophoresis of T4 phage particles on polyacrylamide gels has led to the identification of a new mutant, *eph1*. This finding was fortuitous, but it suggests that the method can be used for isolating other such mutants. A stock grown from mutagenized phage could be subjected to electrophoresis, and the regions of the gel behind and ahead of normal phage could be isolated and tested for the presence of electrophoretic mutants. These mutant particles could be of normal size and shape with altered charge, or they could have an altered morphology, such as the giant particles we have tested. This approach may also be applicable for the study of other large viruses.

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