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Heavy and Light Particles of Adeno-Associated Virus

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KB cells coinfected with adenovirus and adeno-associated virus (AAV) yielded two kinds of infectious AAV particles that banded in CsCl at densities of 1.45 and 1.41 g/cm^2, respectively. The 1.45 band was found to be composed of a heterogeneous group of viral particles that could be subfractionated by velocity sedimentation. The main component from this band had a smaller S value (109) than the main component from the 1.41 band (111S), although both had the same DNA/protein ratio and the same density in metrizamide gradients. Continuous-label experiments showed that early after infection, both components (1.45 and 1.41) were generated in the same amounts, but this was followed by a relative increase in the proportion of the 1.41 component over the 1.45 particles. Pulse-chase analysis failed to demonstrate a precursor-product relationship between these two bands. The slower-sedimenting components from the 1.45 band were unstable in CsCl and were present in a greater proportion early after infection. These particles contained DNA that was enriched for the terminal sequences of the AAV genomes and was accessible to digestion with micrococcal nuclease.

Adeno-associated virus (AAV) grown in the presence of adenovirus as a helper can be fractionated by isopycnic centrifugation in CsCl into several predominant classes (7). The major band of infectious AAV, AAV (1.41), has a density of 1.40 to 1.42 g/cm^3, whereas a minor peak of infectious particles, AAV (1.45), is found at 1.45 to 1.47 g/cm^3 (7). Electron microscope studies revealed that the more dense, infectious particles were 10 to 20% smaller in diameter than the 1.41 particles (6). Immunoelectron microscopy studies indicated that AAV (1.41) and AAV (1.45) may exhibit some antigenic differences (15). In addition, the 1.45 particles were reported to be unstable in CsCl and to have a higher DNA/protein ratio than the 1.41 band (15). Furthermore, it was reported that the ratio of infectious units to physical particles was three orders of magnitude higher for the 1.41 particles than for the 1.45 particles (15). The particle bands at 1.35 and 1.32 g/cm^3 consist of a mixture of empty or partially empty AAV capsids and adenovirus components (18). The AAV particles from these regions include defective interfering (DI) particles since they can interfere with the replication of the standard virus, AAV (1.41), or AAV (1.45) and cannot replicate unless the standard virus is present (10). Restriction endonuclease analysis of these AAV DI genomes showed that they are DNA molecules which are deleted for internal region, but retain terminal regions of the AAV genome (4, 5).

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Most of these earlier studies on the AAV infectious particles used preparations that were purified only by banding in CsCl. In the present study, we used density centrifugation, velocity sedimentation, and restriction endonuclease analysis in order to further characterize the infectious 1.45 and 1.41 AAV constituents. Our results show that these particles purified only from CsCl gradients contain a heterogeneous mixture of particles with widely differing sedimentation rates. For both AAV (1.41) and (1.45), the major components had an identical DNA content and DNA/protein ratio but differed slightly in their sedimentation rates and differed significantly in their relative infectivity.

MATERIALS AND METHODS

Virus growth and purification. AAV was grown with adenovirus 2 (Ad2) as a helper in KB cells as previously described (4). The virus was purified with ultrasound, trypsin, and deoxycholate by the procedure described by Rose et al. (12).

Cesium chloride isopycnic centrifugation. The cell lysate was mixed with CsCl, adjusted to a density of 1.40 g/cm^3, and banded to equilibrium in the SW50 Spinco rotor at 35,000 rpm. Individual bands were collected into cellulose nitrate tubes (50 by 12 mm), and CsCl, of the same density as the virus band, was added to a total volume of 4.5 ml. The gradient was layered on top with mineral oil and spun at 45,000 rpm at 4°C for 4 h in the TV-865 vertical rotor, using the OTD-65 Sorval ultracentrifuge. The run was stopped by using the Reograd system, and the bands were collected from the bottom of the tube.

Density centrifugation in metrizamide. Labeled 1.45 and 1.41 AAV2 particles banded in CsCl and

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further purified by velocity sedimentation in sucrose gradients were layered on top of a 40% metrizamide solution (10 mM Tris-hydrochloride, pH 8.5–1 mM EDTA) and centrifuged for 40 h in the Sorvall TV-865 vertical rotor at 40,000 rpm. Fractions were collected from the bottom of the tube, and the densities were calculated by using the relationship: density (5°C) = 3.453 × refractive index (20°C) − 3.601 (19).

Velocity sedimentation. Virions were layered on a 12-ml 5 to 20% linear sucrose gradient (1 M NaCl–50 mM Tris-hydrochloride, pH 8.0–1 mM EDTA) and spun in the Beckman SW41 rotor at 35,000 rpm for 90 min at 4°C. Virus DNA was analyzed in 5 to 20% neutral or alkaline sucrose gradients as previously described (5).

Restriction endonuclease digestion. DNA extracted from virus particles was digested with site-specific endodeoxyribonucleases *Hha*I and *Hae*III (New England Biolabs) and analyzed in acrylamide gels as described (4, 5).

Micrococcal nuclease digestion. Viral particles from the velocity sedimentation gradient were dialyzed against 50 mM Tris, pH 8.0, and digested with micrococcal nuclease (Worthington Biochemicals Corp.) at a concentration of 1,000 U/ml at 37°C for 1 h. The reactions were stopped by adding sodium dodecyl sulfate to 1% and EDTA to 10 mM and placing the reactions on wet ice. NaOH was added to a final concentration of 0.1 N, and the DNA was analyzed in alkaline sucrose gradients.

Infectivity assay. Infectivity of AAV components was determined on KB cell monolayers by using a fluorescent focus assay (10).

RESULTS

Cesium chloride equilibrium sedimentation. Extracts of AAV-infected cells were adjusted to a density of 1.40 g/cm^3, using cesium chloride, and banded to equilibrium in a swinging-bucket rotor. The second and third rebandings in CsCl were performed in the Sorvall vertical rotor (TV-865) at 45,000 rpm for 4 h at 4°C. This short centrifugation time was found to give a linear density gradient that did not change when the run was prolonged to 24 or 48 h. The 1.45, 1.41, and 1.35 peaks can easily be resolved in gradients in the swinging-bucket rotor. The 1.28 to 1.32 region yields a single broad band in the swinging-bucket rotor that can be resolved into three components of densities of 1.32, 1.31, and 1.28 g/cm^3 in the vertical rotor. Due to the shallow slope of the density gradient generated, this vertical rotor is extremely valuable for separating particles of similar density. An AAV2 preparation grown in the presence of ^{32}P was harvested at 40 h and purified; after the third banding in the vertical rotor, equal portions were collected, purified by velocity sedimentation, and then rebanded in CsCl in the same vertical rotor. The AAV (1.45) and AAV (1.41) banded as well-defined components (Fig. 1).

Centrifugation in metrizamide. To establish whether density differences between the 1.41 and 1.45 bands could be detected in a nonionic medium, purified 1.45 and 1.41 components were centrifuged in a metrizamide gradient (19). AAV2 or Ad2 duplex DNAs were used as density markers. Both AAV (1.45) and AAV (1.41) particles banded in metrizamide as a sharp peak at approximately 1.31 g/cm^3 (Fig. 2; average of

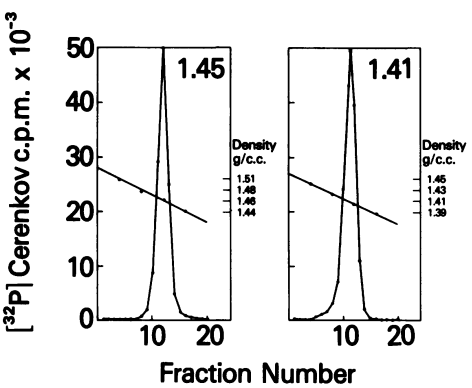

FIG. 1. *CsCl equilibrium density sedimentation of viral particles. ^{32}P-labeled virus extracted from KB cells coinfected with AAV2 and Ad2 were purified in CsCl, specific density bands were collected, and the particles were further fractionated by velocity sedimentation in sucrose gradients. The main component from each velocity sedimentation gradient was dialyzed and adjusted with CsCl to its specific density and spun in the Sorvall TV-865 vertical rotor at 45,000 rpm for 4 h at 4°C.*

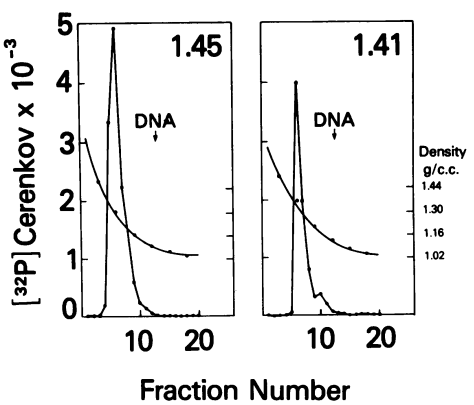

FIG. 2. *Centrifugation of the 1.45 and 1.41 AAV particles on metrizamide gradients. Velocity sedimentation-purified viral particles harvested 40 h after infection were layered on a 40% metrizamide solution and centrifuged at 40,000 rpm for 40 h at 4°C in the TV-865 vertical rotor. ^{3}H-labeled AAV DNA was used as internal density marker.*

three experiments with a standard deviation of ±0.02 g/cm^3), whereas the marker DNA banded at a density of 1.14 g/cm^3. To confirm this result, ^{32}P-labeled AAV (1.45) and [^{3}H]thymidine-labeled AAV (1.41) particles were mixed and centrifuged in a metrizamide solution under conditions similar to those used in the previous experiments. Again we found no differences between the densities of the two types of particles (data not shown).

Velocity sedimentation of viral particles. ^{32}P-labeled AAV was fractionated in a CsCl gradient, and each individual density species was purified in three successive CsCl gradients in the vertical rotor. Virus particles were then analyzed by velocity sedimentation on a neutral sucrose gradient (Fig. 3). The AAV (1.45) particles yielded a main peak of 109S (see below) and a heterogeneous group of slower-sedimenting components (12S to 70S). The AAV (1.41) band, on the other hand, consisted almost exclusively of the 111S peak with a small trailing shoulder.

To demonstrate that the sedimentation rates of AAV (1.41) and AAV (1.45) particles are different, the peak fractions from gradients similar to those in Fig. 3 were resedimented, together with ^{3}H-labeled minute virus of mice (MVM) particles, in a neutral sucrose gradient (Fig. 4). A sedimentation value of 110S has recently been obtained for a pooled sample of the 1.45 and 1.40 bands of MVM (D. C. Ward, personal communication).

In relation to the ^{3}H-MVM marker, AAV (1.41) sedimented faster, whereas AAV (1.45) sedimented slower (Fig. 4). To confirm these results, AAV2 preparations were labeled with [^{3}H]thymidine or with ^{32}P$_i$, and the 1.45 and 1.41 particles from two different labeled preparations were mixed and analyzed. In all cases, the 1.41 particles migrated with a higher sedimentation

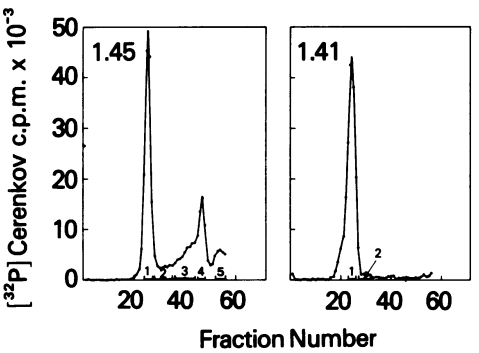

FIG. 3. *Velocity sedimentation analysis of the CsCl virus bands. Specific density viral components from the CsCl density gradients were dialyzed against 10 mM Tris (pH 8.0)-10 mM NaCl-1 mM EDTA and layered on top of a 5 to 20% neutral sucrose gradient. The particles were spun in the SW41 rotor at 35,000 rpm for 90 min at 4°C. Portions were collected from the bottom of the tube and counted. (Sedimentation is from right to left in all figures.)*

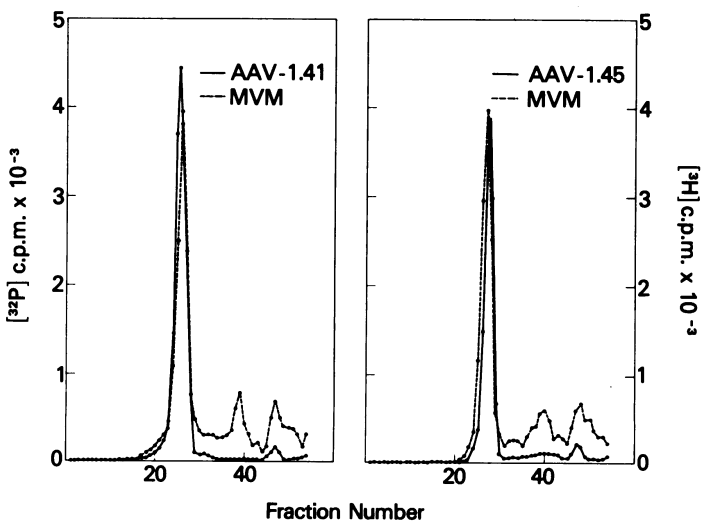

FIG. 4. *Sedimentation value of AAV (1.45) and (1.41) particles. The peak fraction of the main component from the 1.45 and 1.41 regions shown in Fig. 3 was dialyzed against 1× SSC (SSC = 0.15 M NaCl + 0.015 M sodium citrate), pooled with [^{3}H]thymidine-labeled MVM particles (a pool of 1.45 and 1.41; gift of D. Ward), and layered on top of a 5 to 20% linear sucrose gradient. After centrifugation (35,000 rpm, 90 min at 4°C) in the SW41 rotor, fractions were collected from the bottom and counted.*

value than the 1.45 particles. We have assigned a value of 111S to the 1.41 particles and a value of 109S to the 1.45 component. This difference in sedimentation was maintained when the viral particles were extracted from the cells with deoxycholate-Freon (7) or with butanol (M. Myers and B. Carter, unpublished data), when the sucrose concentration in the gradient was 5 to 20% or 15 to 30%, when the temperature was varied from 4 to 25°C, or when minor variations in concentration of EDTA (0.5 to 10 mM) were introduced. Equal portions from the main components of the 1.45 and 1.41 regions and from the slower-sedimenting particles of the 1.45 band were dialyzed and digested with micrococcal nuclease. After digestion, the reaction products were analyzed by sedimentation through alkaline sucrose gradients. Over 95% of the DNA molecules from the 111S AAV (1.41) particles and over 85% of the 109S component from the AAV (1.45) particles were resistant to nuclease digestion. On the other hand, over 95% of the DNA molecules from the slower-sedimenting particles of the 1.45 band (i.e., fractions 36 through 55 of Fig. 3) were accessible to complete digestion by the nuclease.

Spectrophotometric analysis of the 1.45 and 1.41 particles. It was recently reported that after CsCl purification, the 1.45 particles have a higher DNA/protein ratio than the 1.41 band (15). To investigate whether that difference could be accounted for by the presence of the slow-sedimenting particles found in the 1.45 band, spectrophotometric analysis of the virions after velocity sedimentation was performed. In three independent experiments, we obtained identical UV spectra for the 111S AAV (1.41) and 109S AAV (1.45) particles; each had an A_{280}/A_{260} (absorbancy at 280 nm/absorbancy at 260 nm) ratio of 0.72 + 0.02. In confirmation of this, the ratio of [^{35}S]methionine to [^{3}H]thymidine counts in a double-labeled preparation of AAV particles was found to be identical for the 109S (1.45) and 111S (1.41) particles (Myers and Carter, unpublished data). In addition, the UV spectra of the slower-sedimenting particles from the 1.45 region were examined. In this case, the A_{280}/A_{260} ratio progressively decreased in parallel with a decrease in the S value of the particles. For example, AAV (1.45) pool 3 (Fig. 3) had an average ratio of 0.70, and pool 4 had a value of 0.61. These observations suggest that there is an increased DNA/protein ratio for the slow-sedimenting components.

Kinetics of appearance of the heavy and light AAV particles. For some nondefective parvoviruses such as MVM and H-1, there is evidence indicating a precursor-product relationship between the 1.45 and 1.41 density particles (11, 16, 17). To analyze this possibility for AAV, continuous-label and pulse-chase experiments were performed. Continuously labeled virus was obtained by infecting KB cells with AAV2 and Ad2 and adding [^{3}H]thymidine 16 h after infection. Two hours after the addition of the label (18 h postinfection), very few counts were incorporated into viral particles, and the ratio of the 1.45 to 1.41 component was approximately 1:1 (Fig. 5). At later times, there was a progressive incorporation of labeled DNA into viral particles. More interestingly, there was also a progressive increase in the amount of ^{3}H label in 1.41 particles relative to 1.45 particles at the time of harvest after infection increased. By 22 or 24 h the ratio of 1.41 to 1.45 was about 3:1. This result might indicate either a precursor-product relationship between the 1.45 and 1.41 virions or independent synthesis and turnover of the two types of particles. To analyze these possibilities further, infected cells were pulsed-labeled with ^{32}P for 1 h beginning at 16 h after infection and then chased with unlabeled medium. Portions of the culture were then lysed and banded to equilibrium in CsCl gradients. The 1.45 and 1.41 components were already labeled 4 h after the pulse (Fig. 6). Labeling of a dense component that pelleted onto the fluorinet cushion (fractions 1 through 3; Fig. 6), and which was free DNA, progressively decreased during the chase. There was no apparent chase of the counts from 1.45 to the 1.41 component.

The stability and purity of each component in Fig. 6 was further analyzed by CsCl isopycnic centrifugation and by velocity sedimentation (data not shown). The 1.45 and 1.41 peaks collected at 21 h after infection behaved as a heterogeneous population when rebanded in CsCl. At subsequent times, the 1.41 band remained as a stable component upon rebanding, whereas the 1.45 peak again lost its homogeneous density in the last harvest. This finding may explain the reported observation that the 1.45 particles are less stable in CsCl than the 1.41 particles (15). Portions of each CsCl band (Fig. 6) were dialyzed and sedimented in neutral sucrose gradients (data not shown). The 1.45 band contained a main 109S component and several slower-sedimenting components similar to those shown in Fig. 3. During the chase there was a progressive increase in the ratio of full AAV particles to slower-sedimenting components, and about 50% of the small particles disappeared by 49 h after infection. On the contrary, the 1.41 particles obtained throughout the chase period sedimented as a homogeneous 111S peak.

Analysis of the DNA by velocity sedimentation. The DNA molecules obtained from AAV particles that were purified by three bandings in

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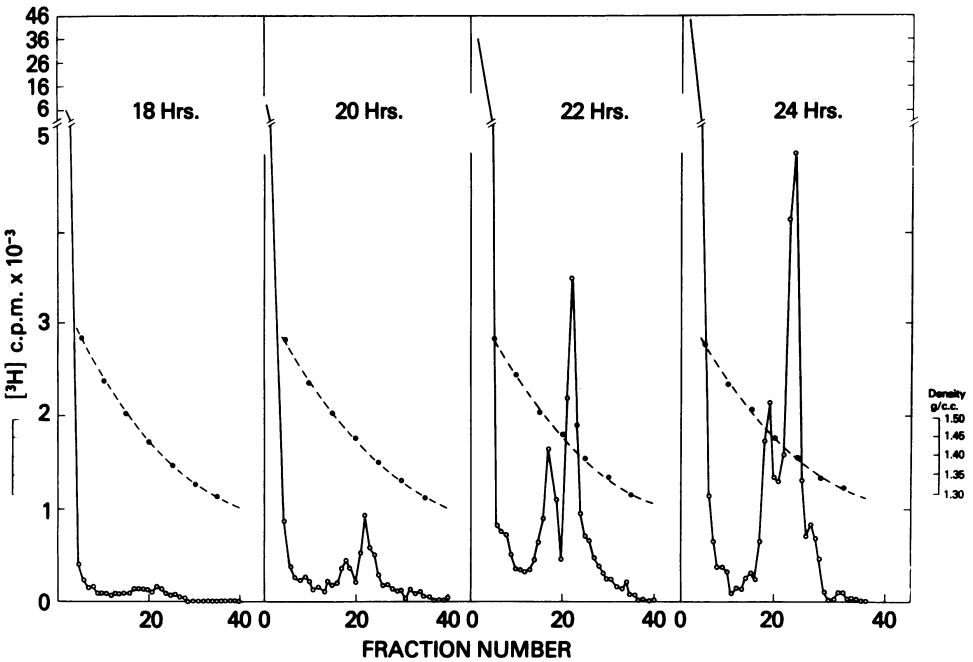

FIG. 5. *CsCl gradients of continuously labeled viral particles. KB cells coinfected with Ad2 and AAV2 were labeled starting 16 h after infection with [^{3}H]thymidine. Portions were taken at 18, 20, 22, and 24 h, extracted with trypsin-deoxycholate, and centrifuged to equilibrium on a CsCl gradient. Fractions were collected from the bottom of the gradient, the refractive index was measured, and portions were counted on the Beckman LS-250 scintillation system.*

CsCl followed by the velocity sedimentation (see Fig. 3) were analyzed by sedimentation through alkaline and neutral sucrose. Figure 7 shows the neutral and alkaline sucrose gradients of the 1.45 and 1.41 particles. The 109S (1.45-1) and 111S (1.41-1) peaks contained mostly full-length AAV DNA. A similar pattern was observed for the 1.45-2 and 1.41-2 components, although a trailing shoulder was present in both the neutral and alkaline gradients. Virions from the 1.45-4 peak contained both full-length DNA molecules or shorter DNA corresponding to approximately 50% of the genome size. These shorter molecules were similar to those previously described for DNA from AAV DI particles (5) as shown below. Thus, the pool 4 particles appeared to contain full-length or DI genomes. Most of the DNA from the 1.45-5 peak was small in size in both alkaline and neutral conditions.

Restriction endonuclease digestion. DNA extracted from pools of viral particles after velocity sedimentation (Fig. 3) was reassociated, precipitated with alcohol, and analyzed by cleavage with restriction enzymes. *Hha*I or *Hae*III was added to obtain a complete digestion, and the reaction products were analyzed by polyacrylamide gel electrophoresis (Fig. 8). All the normal digestion products were present in the DNA from the 1.45-1,2,3 and 1.45-4 viral particles. However, densitometric scanning of the cleavage products suggested that the terminal fragments were present in a higher molar ratio in the slow-sedimenting particles (1.45-3 and 1.45-4) than in the main component (1.45-1). To verify this observation, the DNA from the 1.45-4 particles was denatured and fractionated on an alkaline sucrose gradient (Fig. 8). In addition to a main component (fraction 22; Fig. 8) sedimenting with the same S value as the marker, linear AAV DNA, a second component (fraction 27; Fig. 8) with an S value equivalent to that of half-size molecules was also obtained. The DNA from these two components was reassociated and digested with *Hha*I. The slower-sedimenting DNA contained almost exclusively the terminal 20 to 30% region of the AAV ends (Fig. 8, right panel). Another clear indication of the enrichment of the slower-sedimenting particles for the AAV terminal regions is the presence of the *Hha*I 1.2 and *Hae*III 1.6 bands. These frag-

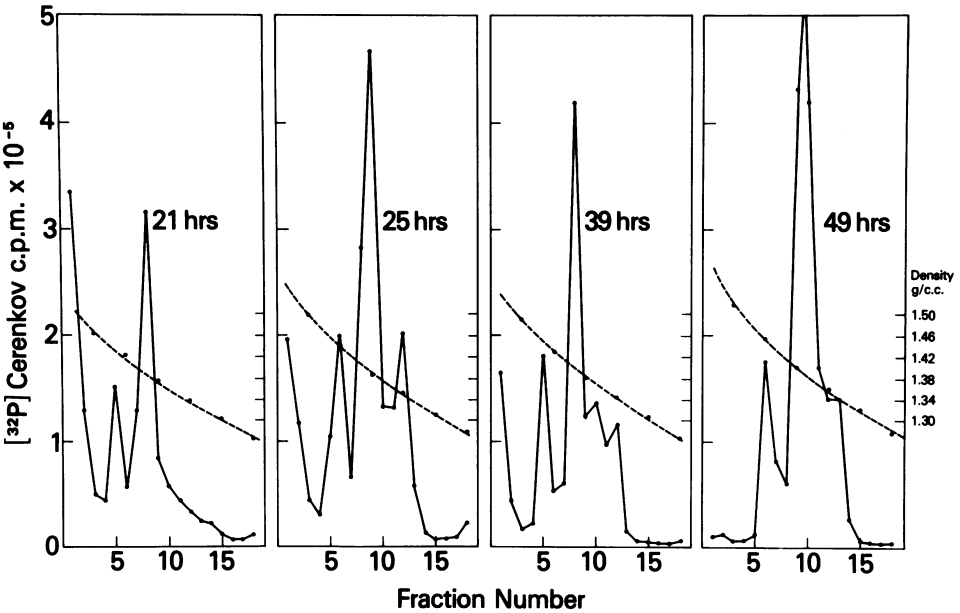

FIG. 6. *Analysis of the pulse-chase experiment by CsCl centrifugation. AAV2 was pulsed with ^{32}P from 16 to 17 h after infection, and the label was chased with cold medium. Portions of the viral preparation were collected at 21, 15, 39, and 49 h postinfection and centrifuged to equilibrium on CsCl.*

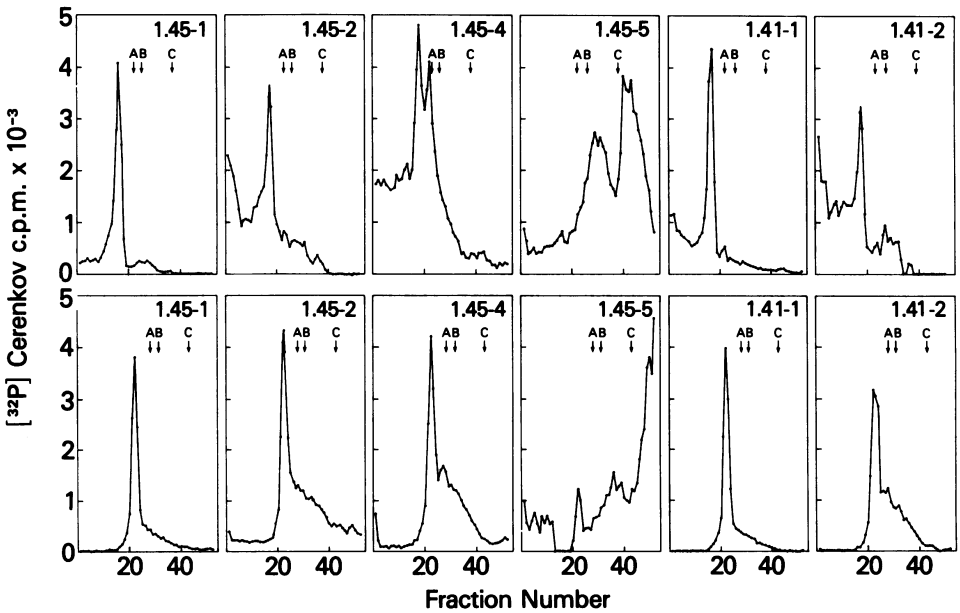

FIG. 7. *Neutral (top) and alkaline (bottom) sucrose gradients of the DNA from velocity sedimentation purified viral particles. Regions of the velocity sedimentation gradients were pooled as indicated in Fig. 3. ^{3}H-labeled linear AAV DNA cleaved with EcoRI was used as internal marker (4). The marker peaks A, B, and C correspond to 57.3, 38.1, and 4.6% of the AAV genome, respectively.*

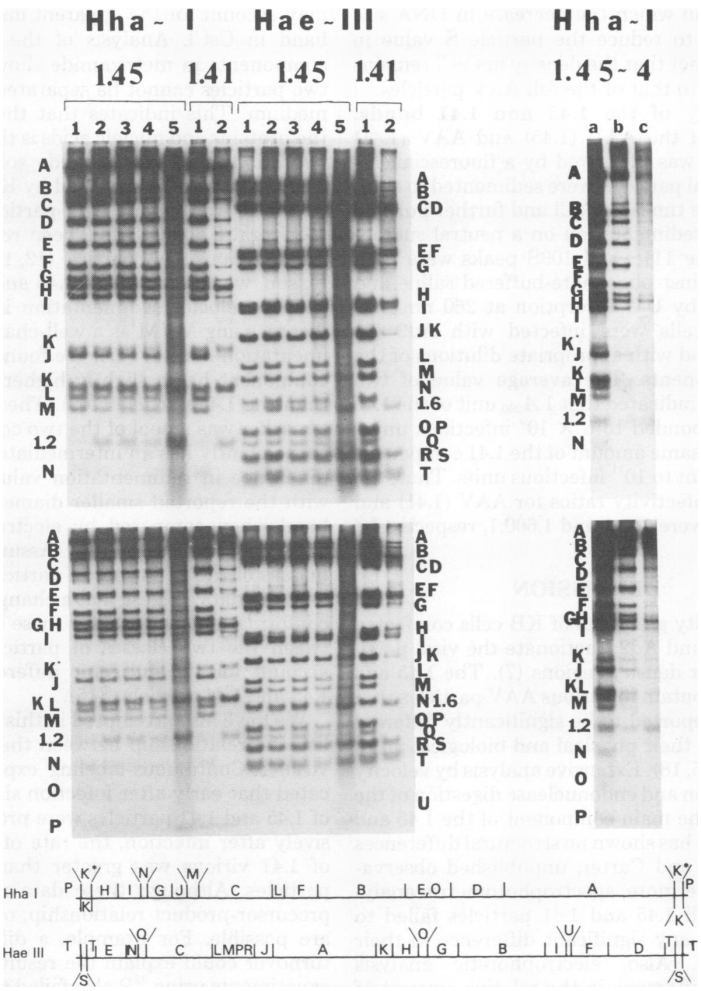

FIG. 8. *Polyacrylamide gel electrophoresis of the DNA extracted from pools of AAV particles from the 1.45 and 1.41 CsCl bands. Pools of particles (as indicated in Fig. 3) were collected; then the DNA was extracted, reassociated, and digested with HhaI or HaeIII, and the products were analyzed on a 3 to 10% (top) or 5 to 16% (bottom) acrylamide slab gel. The digestion products of the 1.45-4 pool were further analyzed (right). In this case, the DNA from the 1.45-4 pool was fractionated in alkaline sucrose gradients; the fast (b)- and slow (c)-sedimenting DNAs were pooled separately, reassociated, and digested with HhaI. The (a) track is a digestion of the 1.45-4 pool before fractionation in alkaline gradient.*

ments correspond to the replicating form hairpin structure of the DI DNA molecules which map at the termini of AAV DNA (5; L. de la Maza and B. Carter, J. Biol. Chem., in press). It can be seen in the 3 to 10% gel of the *Hae*III products (Fig. 8) that the molar proportion of the N fragment in relation to the 1.6 fragment varied greatly from the full particles to the slower-sedimenting components. Digestion of the DNA from the 1.41-1 pool gave the usual products expected from linear AAV DNA, whereas the 1.41-2 component was clearly enriched for the terminal regions of the AAV genome. Similarly to the slow-sedimenting 1.45 particles, the 1.41-2 pool contained DNA molecules with a significant amount of the *Hha*I 1.2 and *Hae*III 1.6 fragments. The cleavage patterns of the 1.41-2 particle DNA are similar to those previously described for AAV DI DNA (5). This shows that it is possible to partially separate AAV particles containing the largest DI genomes from standard AAV particles by velocity sedimentation in

those cases in which the decrease in DNA size is sufficient to reduce the particle S value in spite of the fact that the density in CsCl remains very similar to that of the full AAV particles.

Infectivity of the 1.45 and 1.41 bands. Infectivity of the AAV (1.45) and AAV (1.41) components was measured by a fluorescent assay (10). Viral particles were sedimented to equilibrium three times in CsCl and further purified by velocity sedimentation on a neutral sucrose gradient. The 111S and 109S peaks were then dialyzed against phosphate-buffered saline and quantitated by UV absorption at 260 nm. KB monolayer cells were infected with Ad2 (10 PFU/cell) and with appropriate dilutions of the AAV components. The average value of two experiments indicated that 1 A_{260} unit of the 1.45 band corresponded to 6×10^9 infectious units, whereas the same amount of the 1.41 component was equivalent to 10^{11} infectious units. Thus, the particle-to-infectivity ratios for AAV (1.41) and AAV (1.45) were 100:1 and 1,600:1, respectively.

DISCUSSION

CsCl density gradients of KB cells coinfected with AAV2 and Ad2 fractionate the viral particles into four density regions (7). The 1.45 and 1.41 bands contain infectious AAV particles that have been reported to be significantly different in respect to their physical and biological properties (6, 7, 15, 18). Extensive analysis by velocity sedimentation and endonuclease digestion of the DNA from the main component of the 1.45 and 1.41 particles has shown no structural differences (de la Maza and Carter, unpublished observations). Furthermore, spectrophotometric analysis of the full 1.45 and 1.41 particles failed to demonstrate any significant difference in their UV spectra. Also, electrophoretic analysis showed no difference in the relative amount of proteins in either particle (L. M. de la Maza, F. T. Jay, M. W. Myers, and B. I. Carter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S22, p. 243). These findings contradict earlier reports of differences in the protein/DNA ratio between the heavy and light AAV particles after CsCl gradients (6, 8, 15). We find a 16-fold difference in infectivity between 1.41 and 1.45 particles. This is less than the 300-fold difference reported by Roy et al. (15). We think that these differences between our work and that of others may be due to the presence of a heterogeneous population of viral particles in the 1.45 region. In addition to the full-size particles, there are several classes of slow-sedimenting particles having a low protein/DNA ratio that probably account for the reported data (6, 8, 15). This slow-sedimenting component constitutes a large proportion of the 1.45 band early after infection and may account for the apparent instability of this band in CsCl. Analysis of the 1.45 and 1.41 components in metrizamide showed that these two particles cannot be separated in a nonionic medium. This indicates that the hydration of the proteins and nucleic acids is the same for the two bands in a metrizamide solution. Similar findings have been reported by Kongsvick et al. (9) for H-1 heavy and light particles.

Several S values have been reported for the full infectious AAV particle (12, 13, 14). For that reason, we compared the 1.45 and 1.41 full virions by velocity sedimentation in sucrose gradients, using MVM as a well-characterized sedimentation marker (17). We found that the 1.41 component has a slightly higher S value (111) than the 1.45 particle (109). The MVM used as a marker was a pool of the two components and consequently has an intermediate S value. This difference in sedimentation value corresponds with the reported smaller diameter of the 1.45 band when compared by electron microscope with the 1.41 region (6). Assuming that the masses of the two types of particles are equivalent, the data suggest that a change in structural conformation accounts for these differences between the two classes of particles. This may account for the antigenic differences between 1.45 and 1.41 particles (15).

We have also attempted in this work to establish the relationship between the 1.45 and 1.41 virions. Continuous-labeling experiments indicated that early after infection similar amounts of 1.45 and 1.41 particles were present. Progressively after infection, the rate of accumulation of 1.41 virions was greater than that of 1.45 particles. Although these data may suggest a precursor-product relationship, other pathways are possible. For example, a different rate of turnover could explain the results. Pulse-chase experiments using ^{32}P also failed to provide conclusive evidence on a precursor-product relationship between the two components. Other pulse-chase experiments using [^{3}H]thymidine or [^{35}S]methionine and a butanol extraction procedure for virus purification also failed to clearly demonstrate a precursor-product relationship between these particles (Myers and Carter, unpublished data). These results with AAV are in contrast to work with MVM in which it has been reported that the MVM (1.45) particle is a precursor of the MVM (1.41) particle (11, 16, 17).

Cleavage with restriction enzymes of the DNA from viral particles after velocity sedimentation indicated that full 1.41 particles can be at least partially separated from virions of the same CsCl density containing incomplete genomes. This finding will facilitate the study of AAV DI particles. In addition, digestion with restriction enzymes showed that the slow-sedimenting parti-

cles from the 1.45 band contained preferentially the terminal regions of the genome. This observation may be significant for the maturation process of AAV. AAV assembly appears to occur by interaction of the DNA strand with a preformed empty capsid (Myers and Carter, submitted for publication). Assuming that the terminal region of the AAV genome is the first one to interact with the capsid proteins, it is possible that nicking of the single strand by an endonuclease will result in the production of the apparently empty 1.32 virions or in the production of the slow-sedimenting 1.45 components, both of which contain preferentially the terminal repetition. We note, however, the presence of a full range of particles of different buoyant densities containing incomplete genomes of varying size (1, 10).

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