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Physical properties of nucleoprotein cores from adenovirus type 5

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ABSTRACT

Analytical ultracentrifugation, thermal denaturation, and electron microscopy have been used to study nucleoprotein core particles, obtained from disrupted type 5 adenovirus and partially purified on glycerol density gradients. Electron microscopy at low salt concentrations has shown that the cores are homogeneous particles with characteristic structures, which vary with conditions of observation from a fairly loose network of fibers to a highly condensed, compact particle. Sedimentation measurements in the analytical ultracentrifuge, both by boundary and by band techniques, show that the cores are relatively homogeneous in solution and have sedimentation coefficients near 185 S at low salt concentrations, about 243 S in 1 or 2 M NaCl, and 376 S in 1 mM $MgCl_2$. Correlation of sedimentation data with electron microscopic observations suggests that the 185 S particle has a loose, fibrous structure, while the faster species are more highly condensed particles. The melting temperature of the cores in 5 mM Tris/HCl is 79°C, which is 10°C higher than the T_m for purified, viral DNA. This indicates that the protein enhances the stability of DNA in the nucleoprotein complex.

INTRODUCTION

Characteristics of the adenoviruses, DNA-containing mammalian viruses which have a small number of capsid and internal proteins, have been studied in detail (1, 2, 3). Many of the components have been obtained in pure form and their physical and antigenic properties determined. Human adenovirus type 5 consists of a single, linear DNA duplex, with a molecular weight of 23 x 10^6 (1, 4) and ten or more (3, 5) polypeptides. Three of these are the major capsid proteins, hexon, penton base and fiber, while two are internal proteins, closely associated with the DNA (2, 6, 7). Recent work has shown that controlled, partial degradation of type 5 adenovirus and velocity gradient centrifugation may be used to obtain nucleoprotein cores which are nearly free of capsid protein. One type of core complex, partially purified on glycerol gradients, consists of the DNA and two internal proteins (6). Electron microscopy of these cores has shown that they can be obtained as nearly homogeneous nucleoprotein particles, in which the associ-

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ated core proteins have markedly condensed the DNA (7).

In this paper additional properties of the nucleoprotein core complexes from type 5 adenovirus are described. Since the cores can be obtained as nearly homogeneous particles, their sedimentation coefficients have been measured under a variety of conditions. Electron microscopic observations and sedimentation results have been correlated in an attempt to interpret the effects of changes in ionic strength and of the presence or absence of divalent cations. Thermal denaturation measurements on the core complex and on adenovirus DNA have been carried out and compared.

MATERIALS AND METHODS

Chemicals and biochemicals. Except as noted here, reagent grade chemicals from commercial supply houses were used without further purification. Reagent grade phenol from Fisher Scientific Co. was redistilled and stored at -20°C. Prior to use it was saturated with SSC buffer (0.15 M NaCl, 0.015 M Na citrate, pH 7.0). Pronase, obtained from Sigma Chemical Co., was heat-treated and stored in 1.0 M NaCl at pH 7 as described by Kates and Beeson (8).

Virus and viral cores. Human adenovirus type 5 (strain Ad 75) was propagated and purified as described previously (6, 9). The purified virus was disrupted by heating 1 ml portions of the virus suspension in 5 mM Tris/HCl, pH 7.6, plus 0.5% (w/v) sodium deoxycholate for approximately 1 min at 56°C (6, 7). Nucleoprotein cores were separated from other viral components by centrifugation (55,000 x *g*; 1 hr; 4°C) in a linear, 10-40% (v/v) glycerol gradient (6, 7). Appropriate glycerol solutions were made by diluting the stock reagent with 5 mM Tris/HCl. When removal of the glycerol was necessary, fresh core preparations were dialyzed 48 hours at 4°C against at least four, large-volume changes of 5 mM Tris/HCl, pH 7.6.

DNA preparation. Adenovirus DNA was prepared from cores by a modification of the procedure of Kates and Beeson (8). Cores were dialyzed into 5 mM Tris/HCl, pH 7.5, and incubated with heat-treated pronase (1 mg/ml) for 30 min at 37°C. Sarcosyl was added to a final concentration of 0.5% (w/v) and the incubation was continued for an additional 30 min. All subsequent steps were done at 4°C. The solution was made 0.5 M in sodium perchlorate and extracted with an equal volume of chloroform:isoamyl alcohol, 24:1 (v/v). DNA was precipitated from the aqueous phase with two volumes of absolute ethanol. The DNA was redissolved in SSC, then extracted three times with SSC-saturated phenol. The phenol was removed by dialysis of the aqueous phase for 48 hours against five large-volume changes of 5 mM Tris/HCl.

T2 bacteriophage DNA, prepared by phenol extraction (10), was a gift from Dr. K. E. Rich.

Polyacrylamide gel electrophoresis. Electrophoresis was accomplished in the presence of sodium dodecyl sulfate and urea, as described by Russell et al. (6).

Electron microscopy. Procedures for routine examination of core preparations by electron microscopy were identical to those reported previously (7).

Analytical ultracentrifugation. Sedimentation velocities of various core preparations were measured at 18-24°C in Beckman Model E ultracentrifuges, one equipped with ultraviolet absorption optics and the other with a photoelectric scanner. Data were recorded as a function of time on Ilford N4 E.50 film, converted to a graph on a Joyce, Loebl MK III recording microdensitometer, and results were obtained from the graphs or scanner tracings by standard procedures. Sedimentation coefficients, corrected to standard conditions (11, 12), were calculated from the least-squares slope of a $\ln x$ vs time function, where x is the distance from the center of rotation. In cases where standard deviations of the data from the least-squares line were too large, obviously erroneous points appearing on a graph of $\ln x$ vs time, or the entire run, were discarded.

Boundary sedimentation runs on core preparations were made by standard techniques (11) at 10,000 to 15,000 rpm in either 12 mm, Kel-F, 2°, or 30 mm, aluminum, 4°, single-sector centerpieces. A^{260} of the samples, measured in 1 cm cuvettes, varied from 0.2 to 0.9. All boundary runs were in 5 mM Tris/HCl, pH 7.6.

Band sedimentation experiments (12, 13) were done at 12,000 rpm in 12 mm, Type II, Kel-F, 4°, single-sector centerpieces or in 12 mm, Type I, charcoal-filled Epon, double-sector centerpieces. Samples were solutions of cores in 5 mM Tris/HCl, pH 7.5-7.6, with A^{260} from 1 to 2. Sample volumes were 0.03 ml in the single-sector and 0.015 ml in the double-sector cell. The single-sector cell chamber was filled with 0.52 ml and the double-sector chambers with 0.34 ml of the solvents in Table II. The procedures described by Studier (12) were followed as closely as possible.

Thermal denaturation. Melting experiments were performed in a Gilford model 2000 spectrophotometer equipped with a thermostated cell compartment. Temperature was regulated by a Haake model FS circulating water bath and measured with a platinum resistance thermometer cemented into a cuvette, which was located between the blank and the sample. All samples were dialyzed at 4°C for at least 48 hours against five large volumes of 5 mM

Tris/HCl, pH 7.5; the last dialysate was used as the blank. Before denaturation all solutions, with the initial A^{260} between 0.3 and 0.5, were bubbled with helium (14) and placed in Teflon-stoppered cuvettes with 1 cm path lengths. The temperature of the water bath was raised manually. At least 30 min were allowed for the achievement of thermal equilibrium at each temperature. Throughout the transition, steady-state absorbances were measured within 1 hr after setting the temperature increment. Spectra for some samples were measured between 230 and 400 nm at various temperatures. Absorbance measurements were corrected for thermal expansion of the solvent, which was assumed to be the same as that for H_2O. No corrections for light scattering were made (15). The observed melting temperature, T_m, was taken as the temperature at the midpoint of the change in absorbance at a specified wavelength.

In low ionic strength buffers without chelating agents, trace contamination by multivalent cations can have a large effect on the T_m (14, 16). To minimize this problem, all solutions, glassware, and dialysis tubing (17) were handled with appropriate precautions. In order to detect contamination, an aliquot of T2 DNA was dialyzed along with each sample of adenovirus cores or DNA, and the T_m of T2 DNA in 5 mM Tris/HCl, pH 7.5, was determined. When this was done, the T_m's of the T2 DNA were within 0.6°C of the expected value of 57.2°C (18, 19).

RESULTS

Polyacrylamide gel electrophoresis. The reproducibility and polypeptide composition of adenovirus and core preparations were followed routinely by gel electrophoresis, as well as by observations of band position and appearance in glycerol gradients. All core samples contained the two core polypeptides and some viral hexon, as reported by Russell *et al*. (6). Because many of the experiments for the present study required dialyzed core preparations or ones which had been stored at 4°C for relatively long times, effects of these procedures were examined. Dialysis of fresh samples did not change the gel patterns of cores (6). However, dialyzed samples, stored at 4°C for extended times, showed breakdown products which were similar to those observed by Pereira and Skehel (20) and were presumably due to trace amounts of endogenous proteases.

Electron microscopy. Fresh core samples, both before and after dialysis against dilute Tris/HCl, pH 7.6, appeared as shown in Figures 5-8 of Nermut *et al*. (7). The structure of cores either in glycerol or in dilute Tris/HCl was unchanged by storage at 4°C up to a month, but older samples showed a

gradual loosening and disappearance of the condensed centers, usually observed by the adsorption technique (7). Because of these changes, experiments in following sections were performed on cores within one month after their preparation. Electron microscopic results obtained on the samples used for this investigation have been reported previously (7).

Analytical ultracentrifugation. One observation made on core preparations with the electron microscope is that the particles are relatively uniform in appearance and apparently not aggregated (7). In order to test whether the nucleoprotein is homogeneous in solution, boundary sedimentation experiments were run on cores in 5 mM Tris/HCl, pH 7.6. Figure 1 summarizes the boundary results, which give a straight line over a four-fold range of concentration. Average deviation from the line is ± 5%, which is the approximate range of experimental uncertainty. Since the buffer was so dilute, the solvent was assumed to be equivalent to H_2O in making corrections to standard conditions. The value of $s^0_{20,w}$ is 213 ± 10 S. Traces of the sedimenting boundaries, even at the lowest concentrations, were nearly symmetrical, except for a suggestion in some runs of a somewhat faster component or components. The major difficulty encountered, which made it necessary to discard about one-third of the runs, was instability of boundaries, especially at low concentrations. Apparently this was due to convection (21), which was eliminated with the careful manual temperature control described by Studier (12). The symmetrical and stable boundary indicated that the nucleoprotein cores were as homogeneous in solution as they appeared to be in the electron microscope.

Results from the boundary experiments indicated that the core preparations were homogeneous enough, so that effects of ionic strength, pH, and divalent cations on hydrodynamic properties could be determined. It is advantageous to use the band centrifugation technique mentioned in Methods, because of the small amount of material needed and the

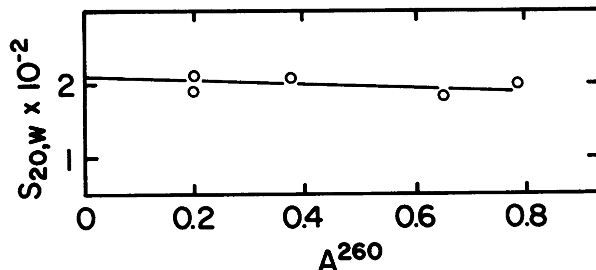

Figure 1: Plot of $s_{20,w}$ vs A^{260} for dialyzed cores in 5 mM Tris/HCl, pH 7.6.

ease of changing solvent conditions. However, changes in the solvent necessitate making large corrections to standard conditions which require auxiliary data (11, 12). For purposes of this paper, the necessary data have been estimated from various sources in the literature. If the composition of the cores is taken as 2.5 g protein per g DNA (6, 9), their partial specific volume can be estimated to be 0.69 from the assumption of additivity of the partial specific volumes for DNA (12) and for proteins

Table I

Calculated Densities, ρ, and Relative Viscosities, η_r, of Solvents at 20°C

Solvent	ρ (g/cm^3)[a]	η_r[b]
1.0 M NaCl	1.03849_1	1.086_8
2.0 M NaCl	1.07697_1	1.195_8
$46._9$% D_2O[c]	1.0482_8	1.114_5
$47._9$% D_2O	1.0493_7	1.116_9
$48._6$% D_2O	1.0501_1	1.118_5
$49._4$% D_2O	1.0510_1	1.120_4
$49._8$% D_2O	1.0514_4	1.121_4
$47._0$% D_2O - 0.01 M NaCl	1.0487_1	1.115_8
$46._9$% D_2O - 0.01 M KCl	1.0487_6	1.114_3

[a]Densities were calculated from specific gravities and the density of water, 0.998203 g/cm^3 (25).

[b]Relative viscosity is the viscosity of the indicated solvent relative to water at 20°C.

[c]% D_2O is volume % (v/v).

(19). Values of densities and relative viscosities of most solvents used in this study were obtained by interpolating available data on salt solutions (22), D_2O mixtures (23, 24), and on H_2O (25). Effects of added salts were estimated with an appropriate linear combination of data for H_2O-salt and H_2O-D_2O mixtures, or were neglected for salt concentrations below 0.01 M. The results, summarized in Table I, were used to make corrections of band sedimentation coefficients to standard conditions (12). Corrections due to deuterium exchange were neglected.

Band sedimentation coefficients of the cores were measured as described in Methods and corrected to standard conditions as noted above. Although at least half of the measurements attempted gave unstable bands, probably due to thermal convection or to insufficient stabilizing gradients (26), satisfactory results were obtained in a number of solvents. The data for all acceptable runs (see Methods) are summarized in Table II, in which

the indicated uncertainty is the average deviation of two or more determinations. Results with no indicated error are from single runs. Sedimentation patterns of the cores in D_2O-5 mM Tris/HCl are illustrated in Figure 2. The band in this figure is somewhat broad, due to the large volume of sample used, but is relatively symmetrical, except for downfield skewing expected as a result of concentration effects (26, 27). In general such a stable and symmetrical band indicates the sample is homogeneous. Clearly, there

Table II

Band Sedimentation Coefficients of Adenovirus Cores in Various Solvents

Solvent[a]	$s_{20,w}$ (S)
1.0 M NaCl - 4 mM Tris/HCl	239 ± 10 (373 ± 9)[b]
2.0 M NaCl - 3 mM Tris/HCl	247 ± 8 (371 ± 6)[b]
49.4% D_2O - 5 mM Tris/HCl	182 ± 10
49.8% D_2O - 5 mM Tris/HCl - 1 mM $MgCl_2$	376 ± 34
49.8% D_2O - 5 mM Tris/HCl - 1 mM EDTA	183 ± 7
47.9% D_2O - 5 mM Tris/HCl, pH 9	176
47.0% D_2O - 5 mM Tris/HCl - 0.01 M NaCl	195
46.9% D_2O - 5 mM Tris/HCl - 0.01 M KCl	201 ± 1

[a]Solvent is the bulk mixture in which sedimentation occurs. All solvents had a measured pH between 7.2 and 7.7, except where noted.

[b]The sedimentation value in parentheses is that for a resolvable, faster component which appeared in the experiment.

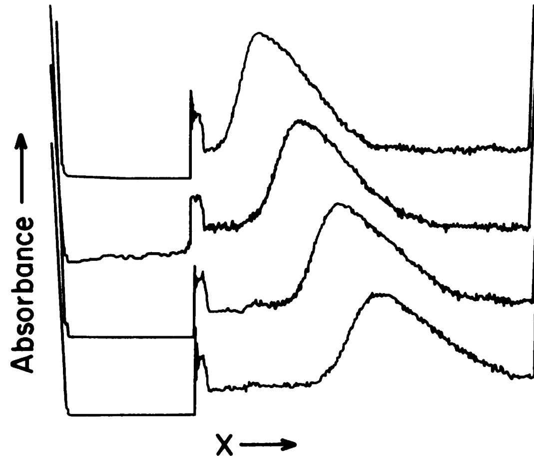

Figure 2: Band sedimentation velocity patterns for cores at 12,000 rpm in 49.4% D_2O - 5 mM Tris/HCl. Initial A^{260} of cores in 5 mM Tris was 1.9. Sedimentation was from left to right. The exposure at the top was taken 16 min after reaching speed; subsequent ones shown were at 12 min intervals. Run temperature was 20.9°C. Other experimental details are described in the text.

is no unusual distribution of material resulting from nonspecific aggregation. The band results confirm the conclusions from boundary sedimentation and electron microscopic observations that the core preparations are homogeneous enough for reliable hydrodynamic studies.

Results obtained from the band sedimentation experiments gave some indication of the effects of ionic strength on the properties of the cores. Efforts to determine the effects of 0.2 M and 0.5 M NaCl in band experiments were unsuccessful. No bands formed and all ultraviolet-absorbing material disappeared. Early time photographs at very slow rotor speeds (2500 rpm) showed that, at these salt concentrations, the cores quickly sedimented to the bottom of the cell. Since sedimentation was so rapid at low speed and left no ultraviolet-absorbing material in the liquid column, it was concluded that the cores had precipitated, rather than being dispersed in an unstable gradient (26, 27). With 1.0 M NaCl as the sedimentation medium, a band with $s_{20,w}$ = 239 (Table II) formed and persisted, although repeated runs gave band patterns with considerable variation in faster components. In 2.0 M NaCl, a good band formed and then split into two or three well-resolved components, as indicated in Table II. In addition about half the sample sedimented as a broad, unsymmetrical band, ahead of the slowest components. This fast-sedimenting material is likely to be aggregates of cores. In 0.01 M NaCl or KCl plus D_2O and 5 mM Tris/HCl, the sedimentation coefficient is about 200 (Table II). There is no significant difference in $s_{20,w}$ in the presence of dilute K^+ or Na^+. Because of experimental uncertainties it is not possible to judge if the difference between $s_{20,w}$ = 180 in 50% D_2O/Tris and 200 in 0.01 M salt is significant (Table II).

Preliminary tests of the effects of pH and divalent cations were made in band sedimentation experiments. In 50% D_2O at an approximate pH 5, precipitation occurred immediately. Experimental observations of this effect were similar to those described above for 0.2 and 0.5 M NaCl. At pH 9, the sedimentation coefficient ($s_{20,w}$ = 176) was quite similar to that obtained in the same medium at pH 7.6. There was no gross change in sedimentation properties of the cores. In 1 mM EDTA (ethylenediaminetetraacetic acid, disodium salt), also, there was no marked change in $s_{20,w}$ (Table II). The largest change occurred when 1 mM $MgCl_2$ was added to the sedimentation medium. In this case a higher $s_{20,w}$ of 376 was obtained for the major component (Table II). A second, faster component consisting of less than 25% of the sample was also observed. The high sedimentation coefficient of 376 S may indicate that the sedimenting particles in 1 mM

$MgCl_2$ have a tightened core structure. Further comments are in the Discussion.

Thermal denaturation. The melting behavior of viral cores, monitored at 260 nm, is illustrated in Figure 3. One major transition with a T_m of 79.0 ± 0.5°C was observed. The breadth of this transition, defined as the temperature interval between 25% and 75% of the absorbance change, was 6.2 ± 0.8°C. In addition, a variable absorbance change amounting to less than 10% of the total hyperchromicity was often detected at 60-65°C. Measurements on the cores were complicated by variable contributions from light scattering, which was monitored at wavelengths from 320 to 400 nm. Although experimental uncertainty was high, absorption changes with temperature in this part of the spectrum appeared to parallel the transitions observed at 260 nm.

Initial experiments with viral DNA gave variable results with T_m values equal to or above those for the cores. When precautions against contamination during dialysis of samples were taken as described in Methods, the melting profile shown in Figure 3 was obtained. The T_m for DNA was 69.5 ± 1.5°C and the breadth of the transition (25-75%, as described above) was 9.1 ± 0.2°C, which is significantly larger than the value for cores. Absorbances between 320 and 400 nm for DNA samples were low and showed little or no temperature dependence. Therefore, light scattering effects for these preparations were considered to be negligible.

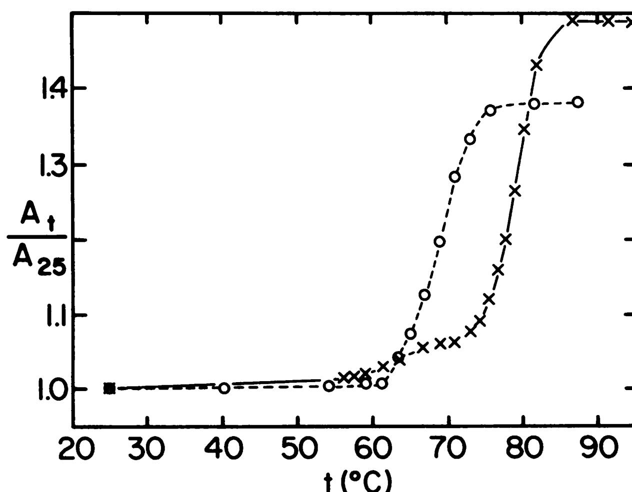

Figure 3: Thermal melting profiles at 260 nm for adenovirus DNA (O) and cores (x) in 5 mM Tris/HCl, pH 7.5. A_t/A_{25} is the ratio of absorbance at the temperature, t, to that at 25°C.

Because of effects of trace contaminants on the T_m of viral DNA, melting profiles were redetermined on viral cores in which contamination during dialysis was eliminated, as described in Methods. The T_m of cores prepared under these conditions was unchanged from the value previously determined. Similar observations have been reported by Olins et al. (16) who found that in dilute buffer trace contamination by multivalent cations raised the T_m of free DNA by 10-12°C, while the T_m of DNA-polypeptide complexes was unaffected.

DISCUSSION

Sedimentation coefficients for adenovirus cores were obtained from both boundary and band experiments. The value of $s^0_{20,w}$ = 213 ± 10, obtained from boundary data in Figure 1, is consistently higher than the results from band experiments (Table II), in which $s_{20,w}$ varies between 176-201. Much of the variation in the band results may be due to band instability from thermal convection or from unstable gradients (27). The fact that the band results are lower than $s^0_{20,w}$ may be due to the relatively high concentrations of cores and thick lamellae which had to be used with available equipment. The tendency for $s_{20,w}$ to decrease with increasing concentration is illustrated in Figure 1. Furthermore, some of the discrepancies can be accounted for by the approximations used in making corrections to standard conditions (see Results). In view of these considerations, it is likely that the cores which sediment in band experiments with values of $s_{20,w}$ = 176-201 are the same as those which give $s^0_{20,w}$ = 213 in the boundary experiments. Components with sedimentation coefficients outside these values probably are different species.

The cores with $s_{20,w}$ around 190 S are quite different from either purified virus or viral DNA. In similar solvents, intact type 5 adenovirus should have a sedimentation coefficient of 795 S (28) and the purified viral DNA has $s^0_{20,w}$ = 31 S (4). Clearly, the sedimentation of cores is consistent with that of a heavier and more compact particle than the DNA, yet lighter and more loose than the intact virus. This is consistent with the structure of cores (7) and whole virions (3, 29) observed in the electron microscope.

Observations from the sedimentation studies showed that core particles were soluble and homogeneous at low ionic strengths, precipitated at intermediate salt concentrations (0.2 and 0.5 M), and were soluble again at salt concentrations above 1.0 M (Table II). Qualitatively, this behavior was consistent with that reported for nucleohistone (21, 30, 31). It was also observed (Table II) that in 1.0 and 2.0 M NaCl the core samples sedimented at a faster rate than in low salt. This increased sedimentation coefficient of the

cores in high salt is opposite to the effects observed by Lewis et al. (21) for nucleohistone.

The effects of 1 mM Mg^{2+} and of EDTA on the sedimentation behavior of the cores have been described in Results. In the electron microscope it was observed that addition of 1 mM $MgCl_2$ to cores (prepared for microscopy by adsorption) had little or no effect on the "normal" core structure consisting of arms radiating from condensed centers (Figures 5 and 7, ref. 7). In the presence of 1 mM EDTA, cores prepared by the same technique had a more loose, fibrous structure (similar to Figures 6 and 8, ref. 7). This behavior appears to be consistent with the band sedimentation data in Table II, in which the more relaxed core material in a solvent with EDTA has a lower sedimentation coefficient than in the presence of Mg^{2+}. However, this does not explain why cores in the presence and in the absence of EDTA have the same sedimentation coefficient, but have markedly different structures in the electron microscope (7). The most probable explanation of this discrepancy is that, during the preparation of cores for electron microscopy in the absence of EDTA, contamination with divalent cations altered the core structure. Several observations indicate that such effects may have occurred. (a) Core structures seen in the electron microscope are variable and can be altered with changing conditions (7). (b) Addition of EDTA changed the structure observed by electron microscopy, while addition of Mg^{2+} had no effect (7). (c) Contamination of some solutions did occur, as indicated by changes in the T_m of DNA (see Results). (d) When precautions against contamination during dialysis (see Methods) were extended to the preparation of samples in 5 mM Tris/HCl for electron microscopy by adsorption, loose, fibrous structures similar to those seen in the presence of EDTA were observed for cores (unpublished observations). A second explanation of the discrepancy between the sedimentation and electron microscopic results might be that aggregation of the cores had occurred, thus increasing the sedimentation coefficient of cores in 1 mM $MgCl_2$. This seems unlikely on the basis of available results. In 1 mM $MgCl_2$ the cores showed no evidence of aggregation in the electron microscope (7). Under similar conditions in the ultracentrifuge, 75% of the sample sedimented with $s_{20,w}$ = 376 (Table II). Although a second, faster component, which might have been a dimer, was observed, there was no evidence of further aggregation or precipitation. On the basis of these considerations it appears that the cores with sedimentation coefficients between 176 and 201 S (Table II) have in solution the relaxed structures corresponding to those in Figures 6 and 8 of Nermut et al. (7), and the rapidly sedimenting

particles (376 S) in 1 mM Mg^{2+} have more highly condensed structures similar to those illustrated in Figures 5 and 7 of Nermut et al. (7).

Interpretation of the results in 1.0 and 2.0 M NaCl is more difficult. The 243 S specie (Table II) may be a monomeric particle with a structure intermediate to those in 5 mM Tris/HCl and in 1 mM $MgCl_2$. In this case the 372 S component may be a dimer. However, an alternative hypothesis is that the 372 S component in high salt is similar to the 376 S specie in 1 mM $MgCl_2$, and that the slower particle (243 S) has a more relaxed structure resulting from removal of core protein by NaCl (32). These and other possibilities are currently under investigation.

After elimination of the problems with cation contamination, as described in Methods and in Results, it was found that the T_m of viral DNA was 69.5 ± 1.5°C. This result was somewhat higher than the value of 67°C, estimated for the base composition of the DNA (18, 19) and the solvent used. The relatively high uncertainty in the T_m for adenovirus DNA could be due to residual protein in the samples. Much of the difference between the calculated and experimental values could also be due to residual protein or to errors associated with the extrapolation (18) used for estimating the T_m. Clearly, however, the proteins in the cores raised the T_m of viral DNA by at least 10°C. This indicated that the associated proteins had a stabilizing effect on the secondary structure of the viral DNA, in addition to the effects on tertiary structure observed in electron microscopic (7) and sedimentation studies. The denaturation results showed further that the cores melted with a sharper transition, and therefore more cooperatively, than did the viral DNA. This is similar to effects observed for synthetic polypeptide-DNA (15) and protamine-DNA (16) complexes. Unfortunately, variability in the contributions of light scattering to the spectra of different core preparations made it impossible to obtain more specific information about the DNA-protein complex from dispersion measurements of hyperchromicity (14, 15, 33). A more detailed study of the effects of divalent cations on the cores may help minimize the scattering problems and determine whether or not EDTA and Mg^{2+} have effects on thermal denaturation, which relate to those observed by electron microscopy (7) and ultracentrifugation.

The results presented in this paper, along with earlier reports in the literature, make it possible to draw several conclusions about the structure and properties of the core particles from adenovirus. The data are consistent with other observations (6, 9, 34, 35) which show that the cores are complexes of DNA and identifiable viral proteins. The sedimentation re-

sults confirm the conclusion from earlier studies (7, 34), that the core polypeptides markedly condense the viral DNA. Thermal denaturation results show further that the internal polypeptides stabilize the secondary structure of DNA. Separate effects of the two core polypeptides on structure, on stability of the DNA, and on biological functions of the virus remain to be defined.

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