Adenovirus Coded Deoxyribonucleic Acid Binding Protein. Isolation, Physical Properties, and Effects of Proteolytic Digestion†

Norman M. Schechter,‡ William Davies,§ and Carl W. Anderson*

ABSTRACT: A procedure has been developed for the purification of adenovirus type 2 DNA-binding protein (DBP) from nuclei of infected HeLa cells. This procedure routinely yields 0.2–0.6 mg of protein per 10^9 cells that is greater than 98% DBP. Binding protein so prepared does not precipitate at low ionic strength, interacts with both single- and double-stranded DNA, and complements Ad5 ts125 function in an in vitro DNA synthesizing system dependent upon exogenous DBP. An examination of the hydrodynamic properties of Ad2 DBP indicated that DBP undergoes a concentration-dependent self-association process. In high ionic strength solutions (1.0 M NaCl), self-association is a limited process observed at DBP concentrations above about 0.1 mg/mL; the product is a unit having a molecular weight of a trimer. At low ionic strengths (0.1 M NaCl), self-association is more extensive and is observed at lower protein concentrations. Our findings suggest that units other than the 72 000 molecular weight monomer may interact with DNA in the cell. Purified Ad2 DBP was digested with several proteolytic enzymes to determine if smaller DNA-binding products could be generated that resemble the 48 000 molecular weight species observed in extracts of infected cells. Digestion of purified DBP with Pronase or chymotrypsin produced relatively stable fragments with molecular weights of 45 000 and 53 000, respectively. Trypsin cleavage produced a 51 000 molecular weight fragment that upon continued incubation was further digested to produce a 35 000-M_r peptide. The production of the 35 000-M_r peptide by trypsin cleavage of the 51 000-M_r fragment was not observed if a sufficient amount of DNA was added to the DBP solution prior to trypsin digestion. This result indicates that bound DNA protects a trypsin-sensitive site(s) in the 51 000-M_r fragment, and it suggests that the 51 000-M_r fragment contains at least a part of the binding site for single-stranded DNA.

The ability to isolate the different components required for DNA replication in bacteria and the ability to recombine these components to form functional systems have contributed greatly to an understanding of the mechanisms of DNA replication (Wickner, 1978). We have been particularly interested in the replication of adenovirus DNA because it apparently replicates as linear molecules with replication intermediates that are never longer than the genome length. There are unique problems associated with the replication of such molecules which have been discussed by Cavalier-Smith (1974) and which may apply to the replication of chromosome ends in eucaryotic cells. In this paper we report an improved procedure for the isolation of one of the components required for adenovirus DNA replication, and we describe our preliminary studies of the physical properties of this component.

Adenovirus-infected cells contain within their nuclei a virus-coded protein having a polypeptide chain molecular weight (M_r) of 72 000 that has been shown to bind to single-stranded DNA (Levine et al., 1976; Linnē et al., 1977; Sugawara et al., 1977; van der Vliet & Levine, 1972). Genetic and biochemical studies have shown that the adenovirus DNA-binding protein (DBP)[1] has important roles in both the initiation and the elongation of DNA during virus replication (Arens et al., 1977; Ginsberg et al., 1977; Horwitz, 1978; van der Vliet et al., 1975, 1978). Large amounts of the DBP are found in replication complexes isolated from infected cells late after infection. Because the adenovirus DBP is produced in large amounts for an early virus protein, and because it interacts with single-stranded DNA in a non-sequence-specific manner, it has been speculated that this protein functions in a manner analogous to the gene 32 product of bacteriophage T4 (Alberts & Frey, 1970; Carroll et al., 1975). The fact that adenovirus replication proceeds by a strand-displacement mechanism (Lechner & Kelley, 1977), in which a high percentage of the replicating DNA is in the form of single strands, has been used as an argument to support this analogy.

Recent studies have indicated that the role of the DBP in virus replication may be more complicated than was originally suspected. Studies of the molecular properties of the adenovirus DBP show that although the protein saturates single-stranded DNA, it does not significantly alter the melting temperature of double-stranded DNA (Fowlkes et al., 1979; van der Vliet et al., 1978). In addition, we and others (Fowlkes et al., 1979; and this report) have shown that the adenovirus DBP possesses a binding activity for double-stranded DNA that was not recognized in earlier studies of this protein. Studies with temperature-sensitive mutants known to map in the DBP encoding region (61.2 to 68.5 map units; Chow et al., 1979) have also shown that the absence of functional DBP affects other virus-specific processes such as the amount of early mRNA synthesized (Carter & Blanton, 1978a,b) and the extent to which adenovirus DNA sequences are integrated in transformed cells (Mayer & Ginsberg, 1977). Very recently, mutants which map in the DBP cistron have been shown to allow adenovirus replication in normally nonpermissive monkey cells (Klessig & Grodzicker, 1979).

The purification method that we report here routinely yields milligram quantities of adenovirus DBP from 4×10^9 infected

† From the Biology Department, Brookhaven National Laboratory, Upton, New York 11973. *Received September 21, 1979.* This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Department of Energy. N.S. was supported by National Institutes of Health Training Grant DHEW-NIH CA-09121.

‡ Present address: Department of Dermatology, Duke University, Durham, NC.

§ Present address: Dartmouth Medical School, Hanover, NH.

[1] Abbreviations used: DBP, adenovirus 2 DNA-binding protein; MEM, minimal essential medium; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; Tos-PheCH_2Cl, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone; Tos-LysCH_2Cl, L-(1-chloro-3-tosylamido-7-amino-2-heptanone).

0006-2960/80/0419-2802$01.00/0

cells that is better than 98% pure. The protein is isolated from nuclei without the use of ionic detergents and is active in an in vitro DNA synthesizing system. This protein is now one of a very few proteins involved in DNA replication that can be obtained from eucaryotic cells in sufficient quantity and purity so that its physical and molecular properties may be thoroughly studied.

Materials and Methods

Cells and Virus. HeLa cells for suspension culture were obtained from J. Flint, Princeton University. They were grown in Joklik's modified minimal essential medium (MEM) with penicillin and streptomycin (Pacific Biologicals) supplemented with either 5% horse serum (Pacific Biological) or 5% calf serum (Pacific Biological). Adenovirus 2 was originally provided by Ulf Pettersson, Uppsala University, Sweden. Stocks with titers above 10^{10} plaque forming units (pfu) were prepared on HeLa cells in suspension after high multiplicity (greater than 10 pfu/cell) infection with non-plaque-purified seed stock essentially as described by Pettersson et al. (1967); these were stored at −70 °C. Adenovirus 2 was titered on HeLa cell monolayers (from J. Williams, Carnegie-Mellon University) essentially as described by Williams (1970) but employing 5% fetal calf serum in the overlay medium. Monolayer HeLa cells were routinely grown in Dulbecco's modification of Eagle medium (Pacific Biological) with high glucose (4500 mg/L), without antibiotics, and supplemented with 10% calf serum.

DNA-Binding Protein (DBP) Purification. HeLa cells were grown to a density of approximately 1×10^6 cells/mL; usually 4 L of cells at this density was used for a preparation. For infection, cells were concentrated to a density of about 8×10^6 cells/mL, and adenovirus was added to give a multiplicity of 20 pfu/cell. After a 1.5 to 2 h adsorption period at 37 °C, the infected culture was diluted to 1×10^6 cells/mL with fresh medium containing 5% serum. At 40–48 h after infection, the infected cells were harvested by centrifugation at 6000*g* (4 °C), and the cell pellets were washed once with phosphate-buffered saline (PBS). The washed pellets were usually processed immediately as described below, but they could be stored frozen at −70 °C.

Cellular lysis, isolation of nuclei, and solubilization of nuclear material were similar to a procedure described by Bonner et al. (1968) for the isolation of chromatin. Cell pellets (ca. 4×10^9 cells) were lysed by suspension in 80 mL of 0.01 M $NaHCO_3$ (pH 8.0), 0.15 M NaCl, and 0.5% NP-40 (Nonidet P-40, Shell Chemical). Phenylmethanesulfonyl fluoride (PMSF) was added to the lysis solution (final concentration 10 μg/mL) and to the first nuclear wash to minimize proteolysis. Nuclei were collected by sedimentation at 600*g* for 3 min (4 °C) and then washed by centrifugation with 80 mL of each of the following solutions: (1) once with the above lysis solution without NP-40, (2) twice with 25 mM NaCl and 8 mM NaEDTA (pH 8.0), and (3) twice with 0.01 M Tris-HCl (pH 8.0). During the final two washes, nuclear structure is destroyed, and centrifugation was increased to 1600*g* for 5 min in order to sediment the disrupted preparation. Analysis of the supernatant and nuclear fractions by $NaDodSO_4$–polyacrylamide gel electrophoresis indicated that little material was released from nuclei after the first wash.

Solubilization of the nuclear material was accomplished by sonication. After resuspension in 80 mL of 0.01 M Tris-HCl (pH 8.0), the nuclear material was sonicated for 10 × 30 s using a Branson Model W185 sonifier set at full power and equipped with a 0.5-in. diameter probe. Between sonication intervals, the nuclear suspension was cooled to 2 °C with a dry ice–acetone bath. The sonicate was brought to 0.15 M NaCl and was clarified by sedimentation at 50000*g* for 30 min at 4 °C using a Beckman Model L-50 ultracentrifuge and a type 35 rotor. Virtually all of the DBP was contained in the supernatant as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis.

After clarification, solid ammonium sulfate was added to the sonicate to give 25% saturation (144 g/L), and the precipitate formed at 4 °C was removed by centrifugation at 35000*g* for 30 min (4 °C). Solid ammonium sulfate was then added to the supernatant to achieve 60% saturation (290 g/L), and the precipitate, which contained most of the DBP as determined by $NaDodSO_4$–polyacrylamide gel analysis, was removed by centrifugation at 35000*g* (4 °C). The remaining unprecipitated protein consisted primarily of histones. The material precipitating between 25 and 60% saturation was gently solubilized in 0.1 (i.e., 4 mL) the original volume of 0.01 M Tris-HCl (pH 8.0) and 0.05 M NaCl, and it was then dialyzed overnight against 1 L of this same solution to remove residual ammonium sulfate. After dialysis, insoluble material was removed by centrifugation at 100000*g* for 1 h (4 °C); the resulting supernatant contained virtually all of the DBP as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis.

The solubilized ammonium sulfate fraction containing the DNA-binding protein was next purified by phosphocellulose chromatography. Phosphocellulose (Whatman P-10) was prepared as described by Peterson (1970), and a 5 × 2.0 cm column was poured and equilibrated with 0.01 M Tris-HCl (pH 8.0) and 0.05 M NaCl at 4 °C. The DBP was found to elute from the phosphocellulose column between 0.30 and 0.45 M NaCl when a 0.05 to 0.90 M linear NaCl gradient was applied to the column. A second major peak eluted from the phosphocellulose column between 0.1 and 0.3 M NaCl and contained adenovirus 100K protein as its major protein component (data not shown); in contrast to some other investigators (Hodge et al., 1977; Russell & Blair, 1977; Sundquist et al., 1977), we have consistently found the bulk of the 100K protein in the nuclear fraction. To quicken the procedure, the DBP-containing ammonium sulfate fraction was routinely dialyzed against 0.25 M NaCl and 0.01 M Tris-HCl (pH 8.0) and loaded onto a column equilibrated with the same solution. After extensive washing of the column, the DBP was eluted with 0.5 M NaCl and 0.01 M Tris-HCl (pH 8.0), and fractions of 3.0 mL were collected. Peak fractions, which contained 2–3 mg/mL protein, were pooled and prepared for DNA-cellulose chromatography or immediately frozen at −70 °C.

DNA-cellulose was prepared as described by Alberts & Herrick (1971) except that commercial salmon sperm DNA (Sigma Chemical Co.) was used. Before use, the DNA-cellulose was extensively washed with 2.0 M NaCl and 0.01 M Tris-HCl (pH 8.0) and with 0.2 M NaCl and 0.01 M Tris-HCl (pH 8.0). Columns (2.0 × 1.5 cm diameter) were prepared from ca. 0.5 g of dry resin, and they were equilibrated with 0.2 M NaCl and 0.1 M Tris-HCl (pH 8.0) until the eluate had an A_{260} of less than 0.03. DNA binding protein fractions from the phosphocellulose column were diluted with 0.01 M Tris-HCl (pH 8.0) to reduce the NaCl concentration to 0.2 M and to have a protein concentration of less than 1 mg/mL. The column was loaded at approximately 2 mL/h, and the DBP was eluted stepwise with 0.5 M NaCl and 0.01 M Tris-HCl (pH 8.0) and subsequently with 2.0 M NaCl and 0.01 M Tris-HCl (pH 8.0); 1.0-mL fractions were collected.

The DNA-binding protein eluting at 2.0 M NaCl was greater than 98% pure as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis. Samples were normally stored in

2.0 M NaCl at 4 °C and dialyzed into an appropriate buffer immediately prior to use; however, storage at −70 °C in 2.0 M NaCl had no obvious adverse effects. Rigorous studies to determine the effects of long-term storage under different conditions have not been performed.

Preparation of Radioactive Adenovirus DNA. Adenovirus 2 DNA labeled with radioactive [^{3}H]thymidine was prepared from infected HeLa cells grown in suspension essentially as described by Pettersson & Sambrook (1973), except that [^{3}H]thymidine (New England Nuclear) was added to 1.25 μCi/mL (specific activity 2.5 Ci/mmol) 12 h after infection. Purified virions were digested with Pronase at 1.0 mg/mL in 0.5% $NaDodSO_4$ for 30 min at 37 °C to remove the adenovirus terminal protein. Purified DNA was stored in 10 mM Tris-HCl (pH 7.9) and 1 mM EDTA at 4 °C with 1 drop of $CHCl_3$. Preparations typically yielded 1 mg of DNA per 5 × 10^8 infected cells at a specific activity of 16 000 dpm/μg of DNA. The DNA was judged to be essentially intact by neutral and alkaline agarose gel electrophoresis.

Nitrocellulose Filter Binding Assay. The interaction of adenovirus DNA-binding protein with DNA was measured by using the nitrocellulose binding assay first described by Oey & Knippers (1972). Appropriate amounts of DNA and DNA-binding protein were allowed to react for 15 to 30 min at 4 °C. Reactions were performed in 0.3 mL of NaCl (0.1 to 0.25 M) and 0.01 M Tris-HCl (pH 8.0). The reaction mixture was filtered through a moist 13-mm diameter Millipore nitrocellulose filter (1.2 μm pore size) under vacuum adjusted to give a flow rate of about 3 mL/h. Filters were washed twice with 0.5 mL of reaction solution before being removed and dried for scintillation counting with 4 mL of Econofluor (NEN). One hundred percent bound values were determined by drying an appropriate aliquot of DNA solution directly on a filter and counting as described above.

Adenovirus DNA was denatured to single strands by heating sonicated adenovirus DNA at 100 °C for 10 min and immediately plunging the solution into liquid nitrogen. The sonicated double-stranded fragments had an average size of about 1000 base pairs.

Proteolytic Digestion of the DNA Binding Protein. Tos-PheCH_2Cl-trypsin, Tos-LysCH_2Cl-chymotrypsin, and Pronase were obtained from Worthington. Stock solutions of trypsin and chymotrypsin were prepared in 0.001 N HCl at 1 mg/mL and stored frozen at −20 °C; Pronase was prepared at 1 mg/mL in distilled water. Digestions were performed at room temperature in 25 μL of solution containing 0.15 to 0.25 M NaCl and 0.01 M Tris-HCl (pH 8.0). Reactions were stopped by adding 5 to 10 μL of 10% $NaDodSO_4$ and 2% 2-mercaptoethanol solution and heating to 70 °C for 10 min. Denatured samples were stored at −20 °C until analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Coomassie brilliant blue stained gels were photographed with Polaroid Type 55P/N film, and densitometer tracings of the negative were made with a Joyce-Lobel microdensitometer.

Ultracentrifugation. Sedimentation velocity and sedimentation equilibrium experiments were performed by using a Beckman Model E analytical ultracentrifuge equipped with UV optics, a Beckman An-D rotor, and double-sector cells having a 1.2-cm path length. The protein concentration (C) at different radial positions (r) was determined from the absorbance at 280 nm. Sedimentation velocity studies were performed at 48 000 rpm with centrifuge cells having sapphire windows. The radial position of the protein–solvent boundary was measured at 4-min intervals, and experiments lasted between 40 and 60 min. Although it is clear that concentration-dependent aggregation was occurring during these studies, plots of ln r vs. time did not deviate significantly from linearity. Sedimentation coefficients were calculated, converted to Svedbergs (S), and corrected to 20 °C and the viscosity of water ($s_{20,w}$) as described by Chervenka (1969). All experiments were performed at 20 °C, and $\bar{\nu}$ for the DBP was taken to be 0.73 cm^3/g as calculated by van der Vliet et al. (1978) from the reported amino acid composition (Linnê et al., 1977). For experiments performed in 1.0 M NaCl, values of 1.04 for density and 1.09 for viscosity were used (Weast, 1978).

Sedimentation equilibrium studies were performed at 20 °C in 1.0 M NaCl and 0.01 M Tris-HCl (pH 8.0). The experiment shown (Figure 7) had a protein sample at 0.2 mg/mL. Weight-average molecular weights were determined from the slope of ln C vs. r^2 plots (Chervenka, 1969); linear regression analysis was used to determine slopes. After all ultracentrifugation experiments, the samples were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis to verify that protein degradation had not occurred.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. $NaDodSO_4$–polyacrylamide slab gels were prepared and run as previously described by Anderson et al. (1973). The running gel contained 17.5% acrylamide and 0.8% bis(acrylamide) unless otherwise noted. Gels were stained with 0.75% Coomassie brilliant blue R in 50% methanol and 7.5% acetic acid and were destained in 14% methanol and 7.5% acetic acid. Protein molecular weight standards were obtained from Worthington or from Sigma.

Protein Concentration. Protein concentration was determined by the sulfobromophthalein assay described by McGuire et al. (1977) using bovine serum albumin (Sigma, fraction V) as a standard. For determinations made on DNA-containing samples, DNA was first removed by acid hydrolysis. Samples were heated to 100 °C for 10 min in 10% perchloric acid, collected by centrifugation, and then washed twice in 10% perchloric acid at room temperature. Determinations made on purified DBP were related to absorbance measurements at 280 nm in order to determine a working extinction coefficient. A DBP solution of 1 mg/mL by the above assay was found to have an absorbance at 280 nm of 0.86 for a 1-cm path length.

Results

Our purification procedure for the 72K adenovirus DNA-binding protein differs in several important respects from previously published procedures. The major differences are that the DBP isolated by our procedure is obtained from the nucleus rather than from the cytoplasmic fraction or from whole cell extracts and that ionic detergents are not required to solubilize purified DBP. Analysis of cytoplasmic and nuclear fractions by $NaDodSO_4$–polyacrylamide gel electrophoresis (data not shown) indicated that virtually all of the 72K DBP was found in the nucleus 44 h after infection, whereas nearly all of the soluble virion components (e.g., hexon, penton base, fiber, etc.) appeared in the cytoplasmic fraction after detergent lysis. Removal of the soluble virion components and the cellular cytoplasmic components makes nuclear isolation a particularly effective first step. The washed nuclear fraction contained only about a dozen major protein species including, in addition to the DBP, adenovirus components 100K, V, VII, and the cellular histones. Subsequent fractionation of the DBP by ammonium sulfate precipitation and by chromatography on phosphocellulose yielded a product approximately 90% pure with respect to other proteins (Figures 1 and 4E), but contaminated with 10 to 25 residues of nucleic acid per 72 000-M_r polypeptide (A_{280}/A_{260} = 0.76–1.0).

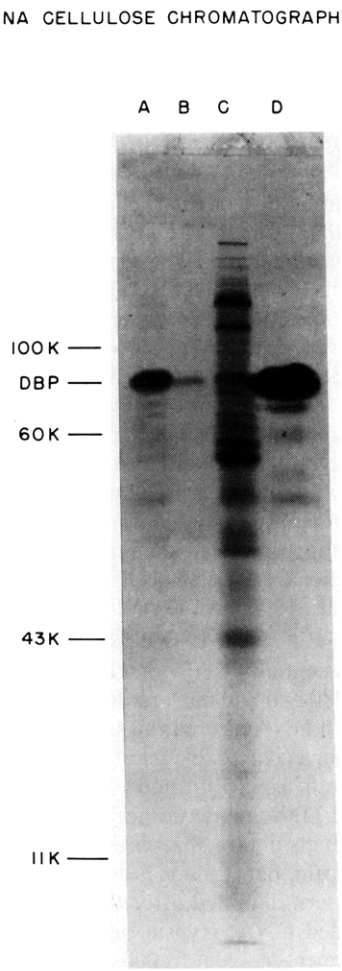

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of fractions obtained from DNA-cellulose chromatography of phosphocellulose-purified adenovirus 2 DNA-binding protein. Column A depicts the Coomassie blue stained polypeptides in a DBP preparation purified through the phosphocellulose chromatography step. Column B presents the material eluting from DNA-cellulose in 0.2 M NaCl; column C presents the material eluting in 0.5 M NaCl; column D presents the material eluting in 2.0 M NaCl. The numbers to the left side of the figure indicate the position of molecular weight standards: phosphorylase *a* (100 000), catalase (60 000), ovalbumin (43 000), α-chymotrypsinogen (27 000), and lysozyme (11 000). Approximately 10 μg of protein was analyzed in each gel column.

Sedimentation velocity studies (data not shown) indicated that the phosphocellulose purified material contained large aggregates of widely varying molecular weights, and analysis of the nucleic acid by agarose gel electrophoresis indicated that it contained significant amounts of DNA, about 1% the size of the adenovirus genome (ca. 400 base pairs).

Various methods were tried in order to remove the nucleic acid remaining after phosphocellulose chromatography. Incubation of the nuclear material with DNase I had little or no effect on the amount of copurifying nucleic acid. Chromatography on DEAE-Sephadex was also unsuccessful. Chromatography of the phosphocellulose purified fraction on DNA-cellulose resulted, however, in the apparent displacement of the bound nucleic acid from the DBP.

The final DNA-cellulose-purified DBP preparation was greater than 98% binding protein by $NaDodSO_4$–polyacrylamide gel analysis (Figure 1) and had a 280 nm/260 nm absorbance ratio of about 1.47. Between 0.8 and 2.4 mg of DBP is obtained from 4×10^9 infected cells. DBP eluting from DNA-cellulose at 2.0 M NaCl is highly soluble when diluted or dialyzed to physiological ionic strength and pH. The purified DBP was found to be active (data not shown) in the in vitro DNA synthesizing system developed by Horwitz (1978). This assay measures the ability of exogenously added protein to complement the temperature-sensitive DBP produced by the ts125 mutant for the elongation of in vivo initiated adenovirus DNA strands. The behavior of the DBP during purification, its apparent polypeptide chain molecular weight of 72 000, and the fact that the purified product binds to single-stranded DNA demonstrate that the DBP isolated here is the virus-encoded DNA-binding protein that has been described by others (Levine et al., 1976; Linnê et al., 1977; Sugawara et al., 1977; van der Vliet & Levine, 1972). The protein eluted from DNA-cellulose columns with 2.0 M NaCl was used for all experiments reported below.

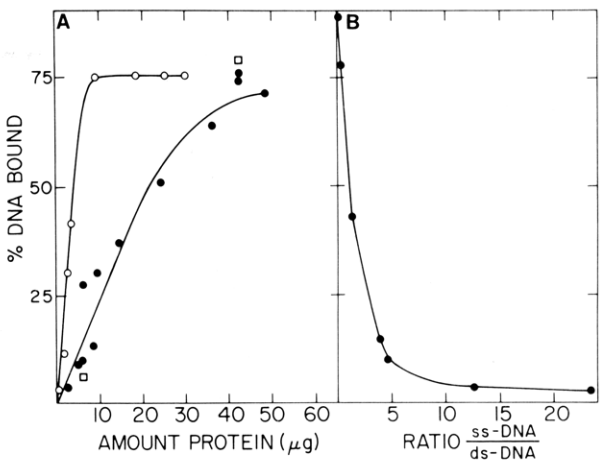

FIGURE 2: Interaction of adenovirus 2 DBP with single-stranded and double-stranded adenovirus. (A) Different amounts of adenovirus 2 DBP were reacted at 4 °C with ^3H-labeled single-stranded adenovirus 2 DNA or ^3H-labeled full-length, double-stranded adenovirus DNA, and the DBP–DNA complex was collected on nitrocellulose filters. Closed circles represent experiments performed using 8 μg of single-stranded DNA per assay (26.7 μg/mL), open circles represent experiments performed with 4 μg of single-stranded DNA per assay (13.3 μg/mL), and the open squares represent values obtained with 8 μg (26.7 μg/mL) of double-stranded DNA. (B) Competition between double-stranded adenovirus DNA (8 μg) and different amounts of single-stranded adenovirus DNA for DBP. Each reaction contained 9 μg of Ad2 DBP. Reaction conditions and the quantitation of bound protein were as described under Materials and Methods.

Interaction of the Ad2 DNA-Binding Protein with Double-Stranded and Single-Stranded DNA. The interaction of DNA-cellulose purified DBP with single-stranded and double-stranded radioactive Ad2 DNA was studied by using a nitrocellulose filter disk assay. Figure 2A demonstrates that at saturation about 75% of the single-stranded Ad2 DNA fragments could be specifically bound to nitrocellulose filters by the addition of DBP to the reaction solution. This result indicates that the Ad2 DBP can interact with many different regions of the adenovirus genome, and thus the interaction probably involves little if any sequence specificity. The fact that only 75% of the radioactivity was found bound to the filters probably resulted from errors in determining the 100% value; the apparent lack of complete binding is not considered significant.

When the binding of Ad2 DBP to native, full-length, double-stranded Ad2 DNA was examined by the filter disk assay, it was found that 75 to 80% of the double-stranded DNA could be retained on the filter disk by addition of sufficient DBP (Figure 2A). This result was unexpected, as previous investigators had failed to find the binding of double-stranded DNA by either Ad5 (van der Vliet et al., 1978) or Ad2 (Sugawara et al., 1977) DBP. The binding observed with double-stranded

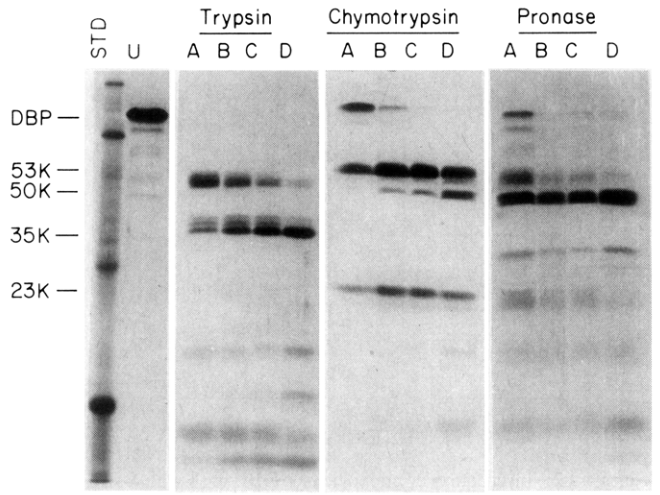

FIGURE 3: Proteolytic degradation of the adenovirus 2 DNA-binding protein. Approximately 5 μg of DBP was incubated with trypsin, chymotrypsin, or Pronase, and the products of digestion were analyzed by NaDodSO$_4$–polyacrylamide gel electrophoresis. Samples labeled A and B were incubated with a weight ratio of DBP to protease of 500:1; samples labeled C and D were incubated with a DBP to protease weight ratio of 1:200. Samples A and C were incubated at room temperature (20 °C) for 15 min, whereas samples B and D were incubated for 30 min. The undigested DBP is shown in the sample labeled U; the sample marked STD displays the molecular weight standards (phosphorylase *a*, catalase, α-chymotrypsinogen, and lysozyme). The molecular weights of the major proteolytic products of DBP digestion are indicated at the left of the figure.

DNA is not thought to result from nicks in the DNA, as the DNA used for these experiments was found to be essentially intact by alkaline agarose gel electrophoretic analysis. The viral DNA preparation had also been treated with Pronase; thus, binding to double-stranded DNA could not have been due to an interaction between the DBP and the terminal protein that is covalently linked to the 5′ end of each DNA strand. The addition of DBP to mixtures containing radioactive double-stranded DNA and increasing amounts of unlabeled single-stranded Ad2 DNA decreased the amount of double-stranded DNA retained by the filter (Figure 2B). This result indicates that the entity in solution that caused the retention by the nitrocellulose filter of double-stranded DNA also binds to single-stranded DNA. The binding of adenovirus DBP to double-stranded DNA has also been observed independently by Fowlkes et al. (1979), and they have shown that the binding of Ad5 ts125 DBP to double-stranded DNA is temperature sensitive. Together our results show that the DBP itself possesses binding activities for both single-stranded and double-stranded DNA. The competition by single-stranded DNA with double-stranded DNA binding thus suggests that the binding site for double-stranded DNA overlaps that for single-stranded DNA, although the possibility of inhibition due to a conformational change in the DBP after binding single-stranded DNA cannot be ruled out.

Proteolytic Digestion of Native Ad2 DNA-Binding Protein. The native DNA-cellulose purified 72K DNA-binding protein was digested with trypsin, chymotrypsin, or Pronase, and the products obtained after different digestion times were analyzed by NaDodSO$_4$–polyacrylamide gel electrophoresis (Figure 3). Under the conditions employed, each of the proteases gave a distinctive series of proteolytic products; however, a common feature of all digestions was the appearance of relatively stable peptide fragments of about 50 000 M_r (a similar fragment has been found after protease V8 digestion: E. Buzash-Pollert, unpublished experiments). That such large peptide fragments were obtained after Pronase digestion was surprising, since Pronase is a very nonspecific peptidase. This result suggests that the DBP contains a compact rigid domain that includes about two-thirds of the polypeptide chain. The sizes of these proteolytic cores are similar to that of the naturally produced 48 000-M_r fragment(s) described by van der Vliet et al. (1975) and others (Levine et al., 1976; Rosenwirth et al., 1976).

A closer examination of the products of chymotrypsin digestion shows that after short digestion times only two major cleavage products are found. The combined molecular weights of these products, 53 000 and 23 000 M_r, account for the size of the intact molecule. Densitometry of the photographic negative from the Coomassie blue stained gel indicates that the two initial peptide products have masses in the ratio of 2:1, as expected for fragments representing $^2/_3$ and $^1/_3$ of the initial species. The stability of the smaller molecular weight fragment to chymotrypsin may indicate that it and the larger fragment fold independently into separate domains. Alternatively, the 23 000-M_r fragment may not contain aromatic amino acids. A molecule composed of two domains might be expected to have a high frictional coefficient as has been found for the DBP (Sugawara et al., 1977; van der Vliet et al., 1978).

The DNA-binding protein used for the digestions shown in Figure 3 was essentially free of nucleic acid as judged by its absorbance at 280 and 260 nm. To determine if the interaction of the DBP with DNA altered the sensitivity of DBP to proteolytic digestion, a comparable set of digestions was performed in the presence of single-stranded DNA. The presence of single-stranded DNA made no noticeable difference in the pattern of products obtained after digestion with Pronase or with chymotrypsin, but it made a dramatic difference in the proteolytic pattern obtained after trypsin digestion. In the absence of added DNA, trypsin digestion first produced a proteolytic fragment of about 51 000 M_r that was subsequently digested to yield a 35 000-M_r peptide. In the presence of added DNA, the stability of the 51 000-M_r peptide to further trypsin cleavage was markedly increased (Figure 4A–D). The above experiment implies that the 51 000-M_r DBP fragment contains at least part of the binding site for single-stranded DNA and that bound DNA may sterically block the access of trypsin to the cleavage site. To demonstrate that cleavage inhibition is not due to the proximity of adjacent molecules, the digestion of DBP in the presence of different amounts of single-stranded DNA was examined (Figure 4, C and D). No difference was observed in the digestion patterns performed at DNA to protein ratios between 1:20, where the DNA should be just saturated, and 1:2, where DBP monomers should be spaced an average of 90 nucleotides apart. It should also be noted that the proteolytic digestions were done at sufficiently high DBP concentrations so that much of it would have been aggregated even in the absence of DNA.

The fact that the binding of single-stranded DNA by adenovirus DBP prevents further cleavage of the 51 000-M_r peptide fragment provides an independent method to determine the size of the binding site at saturating protein levels. If a fixed amount of single-stranded DBP is allowed to react for a suitable time with increasing amounts of DNA, and the products of this reaction are then digested with trypsin, the point at which 35 000-M_r peptide markedly disappears or reaches a constant background value may be taken as a saturation of the protein sites by DNA. The results of two such experiments (data not shown) indicate that the saturation point is between 3 and 9 nucleotides per 72 000-M_r subunit. For technical reasons there was some variation between the two experiments; however, these low values are in good agreement with the relatively low saturation level of 7 nucleotides per

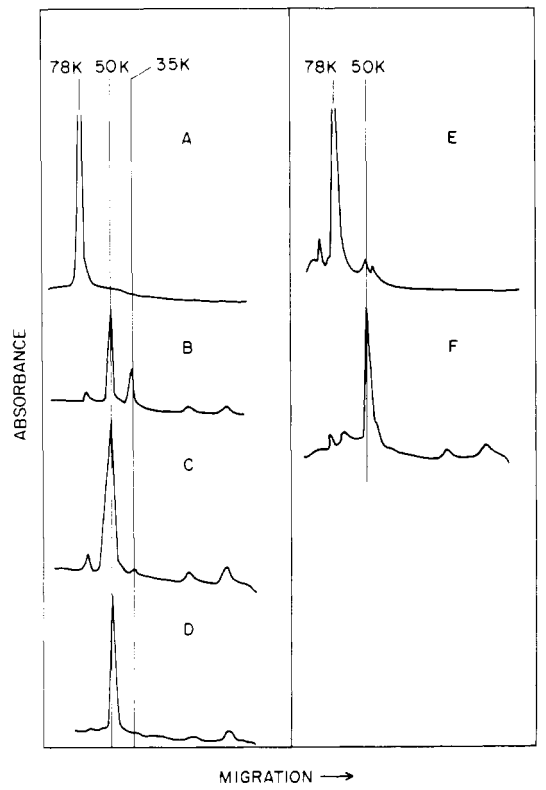

FIGURE 4: Effect of bound DNA on the digestion of adenovirus 2 DNA-binding protein by trypsin. Approximately 4 μg of DBP was incubated with single-stranded adenovirus 2 DNA under the conditions used to measure binding. Trypsin (1:500 weight ratio) was then added to the reaction mixture, and digestion was allowed to proceed for 20 min at room temperature. Digestion was stopped by the addition of $NaDodSO_4$ and mercaptoethanol, and the digestion products were analyzed by $NaDodSO_4$-polyacrylamide slab gel electrophoresis. Molecular weights of the starting material and the major tryptic products are indicated at the top of the figure. Tracings are denoted by letters and represent analyses of the following: (A) DNA-cellulose purified, undigested DBP; (B) the products obtained by trypsin digestion of the DBP in the absence of added DNA; (C) the trypsin digestion products obtained when DBP had first been incubated with a 1:20 weight ratio of single-stranded adenovirus DNA to DBP (at this weight ratio the DNA should just be saturated with DBP assuming that one 72 000 molecular weight DBP binds to 10 nucleotides and that all of the protein is active); (D) the trypsin digestion products obtained at a DNA to DBP weight ratio of 1:2 (DNA excess); (E) DBP preparation obtained after phosphocellulose chromatography (this material had a A_{280}/A_{260} ratio of ca. 0.8, which indicates that it contains nucleic acid); (F) the products obtained after trypsin digestion of the phosphocellulose-purified material shown in E.

72 000-M_r subunit that has been reported by van der Vliet et al. (1978). This technique has two advantages over alternative ways of obtaining saturation values. One is that none of the components need be radioactively labeled. The second is that an inactive protein fraction potentially may be detected by the generation of novel peptides or by the appearance of the 35 000-M_r fragment at DNA to DBP ratios below the saturation point.

The adenovirus DBP obtained after phosphocellulose chromatography is relatively pure with respect to other proteins, but it is heavily contaminated with a substance presumed from the absorbance profile to be nucleic acid. This profile suggests that the DBP as isolated from infected cell nuclei is bound to about 10 nucleotides per 72 000-M_r subunit (i.e., probably to replicating adenovirus DNA). If the DBP is bound to DNA in the same manner as happens in vitro, then digestion of the phosphocellulose material with trypsin should yield the 53 000-M_r peptide rather than the 35 000-M_r peptide. Parts A and B of Figure 4 show that this is indeed the case. Thus,

Table I: Effect of Ionic Strength and Protein Concentration on the Sedimentation Coefficient of the Adenovirus Type 2 DNA-Binding Protein[a]

protein concn (mg/mL)	ionic strength (M)	$s_{20,w}$
0.14	1.0	4.5
0.20	1.0	5.5, 6.0
0.80	1.0	7.0
0.12	0.1	6.0
0.20	0.1	5.6
0.40	0.1	8.0

[a] Sedimentation coefficients were determined with an analytical ultracentrifuge, and the results were corrected to $s_{20,w}$ as described under Materials and Methods. The ionic strength given indicates the concentration of NaCl (determined from the refractive index) at which the data were obtained. Solutions were buffered at pH 8.0 with 0.01 M Tris-HCl. After each experiment the sample was analyzed by $NaDodSO_4$-polyacrylamide gel electrophoresis for proteolytic degradation; none was observed.

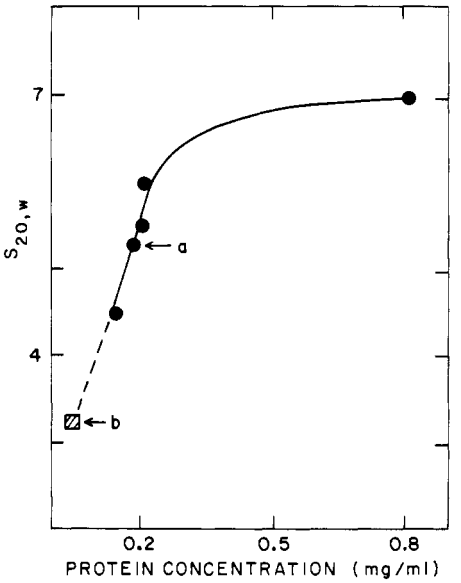

FIGURE 5: Sedimentation coefficient of adenovirus 2 DBP as a function of protein concentration in 1.0 M NaCl. Sedimentation coefficients represented as solid circles are those reported in Table I. They were obtained by analytical centrifugation of DNA-cellulose purified DBP as described in the legend to Figure 6. The value marked a was obtained by using material purified through the phosphocellulose chromatography step. This material contained nucleic acid that is presumed to have dissociated in the 1.0 M NaCl against which the preparation was dialyzed prior to the experiment. The sedimentation value marked b (square) was taken from Sugawara et al. (1977) and van der Vliet et al. (1978).

even at 40 h after infection, virtually all of the adenovirus DBP appears to be bound to single-stranded DNA.

Hydrodynamic Properties of the Adenovirus 2 DNA-Binding Protein. The sedimentation coefficient of the Ad2 DBP was obtained as a function of protein concentration in 0.1 and 1.0 M NaCl (pH 8.0) by centrifugation in the Model E ultracentrifuge (Table I, Figure 5). In 1.0 M NaCl, the sedimentation coefficient was found to increase with increasing protein concentration. In a homogeneous system, this type of behavior is characteristic of a self-association process. The lowest sedimentation value found in our studies was 4.5, a value that is significantly greater than the values of 3.1 to 3.4 reported by others (Sugawara et al., 1977; van der Vliet et al., 1978). Our s value was obtained at a protein concentration of 0.14 mg/mL, whereas we estimate that the lower sedimentation values reported by others were obtained at protein concentrations below 50 μg/mL. When a sedimentation

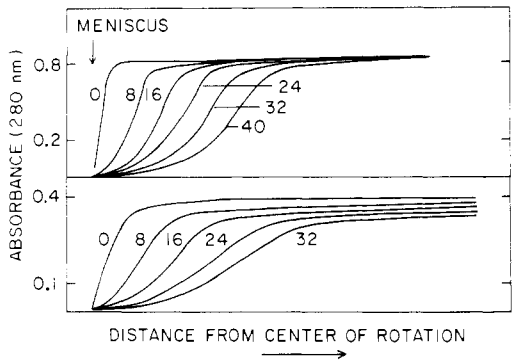

FIGURE 6: Sedimentation velocity of adenovirus 2 DNA-binding protein in 1.0 and 0.1 M NaCl. The experiments were performed with DNA-cellulose purified DBP as described under Materials and Methods. Shown are composites of absorbance scans taken at 8-min intervals; individual scan times are given in the figure. In the experiment depicted in the upper figure, sedimentation was in 1.0 M NaCl and 0.01 M Tris-HCl (pH 8.0), and the initial protein concentration was 0.8 mg/mL. The lower figure depicts an experiment performed in 0.1 M NaCl and 0.01 M Tris-HCl (pH 8.0); the initial protein concentration was 0.4 mg/mL.

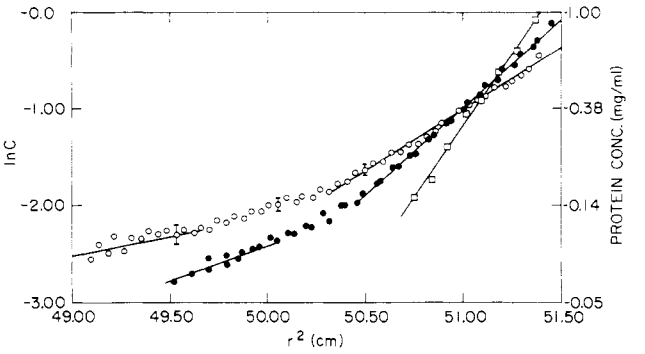

FIGURE 7: Sedimentation equilibrium analysis of the adenovirus 2 DNA-binding protein in 1.0 M NaCl. The data shown were obtained with DNA-cellulose purified DBP. The sample was analyzed at three different speeds. The centrifuge was first adjusted to 16000 rpm (□), and data shown were obtained after 13 h of centrifugation. The centrifuge was then adjusted to 13000 rpm (●), and data were obtained after 36 and 48 h of additional centrifugation. Finally, the centrifuge was adjusted to 10000 rpm (○), and data were obtained after 28 h of additional centrifugation. The error bars indicate an uncertainty in the absorbance measurement. The slopes obtained for the indicated data points using a linear regression model were as follows: 1.237 ± 0.017 (10000 rpm, high concentration); 1.799 ± 0.022 (13000 rpm, high concentration); 2.538 ± 0.129 (16000 rpm); 0.522 ± 0.116 (10000 rpm, low concentration); 0.811 ± 0.055 (13000 rpm, low concentration). The errors reported for the calculated molecular weights (see text) reflect only the standard deviation found for these slopes and thus do not reflect additional factors (e.g., errors associated with the absolute value of the protein concentration, the specific volume, etc.) which affect the absolute accuracy of the reported values.

coefficient of ca. 3.4 and a Stokes radius of 4.9 nm (obtained from gel filtration studies) were used to calculate a molecular weight, that molecular weight (van der Vliet et al., 1978) is consistent with the value of 72000 M_r for the peptide chain obtained by $NaDodSO_4$ gel electrophoresis. The sedimentation coefficient of 3.1–3.4 therefore represents that of the most dissociated form of DBP.

At protein concentrations between 0.14 and 0.2 mg/mL the sedimentation constant for the DBP increases rapidly (Figure 5), but, between 0.2 and 0.8 mg/mL, the sedimentation value increased by only about 1 S. The lack of a significant increase in s above 0.2 mg/mL suggests that the self-association process is not endless but stops after the association of a specific number of subunits to produce a 7S aggregate. The concept that the self-association process is limited is supported by the homogeneous appearance of the sedimentation profile for the 0.8 mg/mL sample which is displayed in Figure 6 (top). Throughout the experiment, the protein boundary remained sharp, and the plateau region, the value of which is sensitive to small quantities of high molecular weight aggregates, did not decrease more than is expected from radial dilution of the sample as it sediments. This profile should be compared with that of a highly aggregated sample (Figure 6, bottom) that gave an apparent sedimentation coefficient of 8 S. Here, the loss of the high molecular weight aggregates from the plateau region can readily be observed. In the upper sedimentation profile (Figure 6), the lower portion of the protein boundary did not clear as sharply from the meniscus as would be expected for a homogeneous protein species. This behavior indicates the presence of slower sedimenting components, and it is expected if the self-association process is spontaneously reversible.

The aggregation state of the DBP was further examined by sedimentation equilibrium analysis in the Model E ultracentrifuge. In sedimentation equilibrium, the weight-average molecular weights (M_w) of the components in the sample are related to the slope of the concentration gradient by a logarithmic function (Chervenka, 1969). For a homogeneous protein that does not undergo concentration-dependent aggregation, a plot of ln C vs. r^2, where r is the distance from the center of rotation and C is the protein concentration at any point in the sample, gives a straight line; from this slope, M_w may be calculated. If, however, the protein undergoes a concentration-dependent self-association process, a plot of ln C vs. r^2 will give a curved line, but the slope of the tangent to the curve will still give M_w for the mixture of components present at that point in the cell.

The results of a sedimentation equilibrium experiment in 1.0 M NaCl with DBP at an initial protein concentration of 0.2 mg/mL are shown in Figure 7. The protein sample was brought to equilibrium at three different speeds, 10000, 13000, and 16000 rpm, and a plot of ln C vs. r^2 is shown for each speed. As expected, the plots were significantly curved for the two lowest speeds, indicating that the system did indeed contain multiple molecular weight components. At protein concentrations above 0.14 mg/mL (ln C of −2.00), however, the data points formed relatively good straight lines. The molecular weights calculated from these slopes were 228000 ±3000 at 10000 rpm, 196000 ± 2500 at 13000 rpm, and 196000 ± 3000 at 16000 rpm. These molecular weights are similar and consistent with that expected for a trimer composed of three 72000 M_r monomers. The lack of noticeable slope curvature over a large concentration range (ca. 0.14–1.0 mg/mL) suggests that aggregates larger than trimers were not present.

Accurate estimates of M_w at low protein concentrations are more difficult due to difficulties inherent in obtaining accurate protein concentrations. Nevertheless, the values obtained in the experiment shown in Figure 7 for concentrations below 0.10 mg/mL also form a reasonably straight line when plotted as ln C vs. r^2. The least-squares values for M_w calculated from these values are 96000 ± 21000 at 10000 rpm and 88000 ± 6000 at 13000 rpm. These values are close to that of the monomer as determined by Sugawara et al. (1977) and van der Vliet et al. (1978).

To exclude the possibility that a large proportion of the sample analyzed in the above experiments had sedimented to the bottom of the cell, the method of Schechter et al. (1976) was used to determine if obtained data were representative of the total amount of starting material. Assuming that the majority of the protein within the cell was in the form of a

216 000-M_r trimer, 65 to 100% of the total protein sample could be accounted for. The recovery must be closer to the higher value for, if as much as 45% of the sample had been highly aggregated, the rapid loss of material from the plateau region would have been noticed in the sedimentation velocity study shown in Figure 6 (top).

The sedimentation coefficients obtained for DBP at low ionic strength (0.1 M NaCl; Table I, bottom) also increased as a function of protein concentration; however, the increase appeared to be less consistent that that observed in 1.0 M NaCl. This inconsistency may be related to the fact that the material for the 0.2 mg/mL sample was from a different preparation than the material used for the 0.12 and 0.40 mg/mL samples. At all concentrations examined, the sedimentation coefficients at 0.1 M NaCl were higher than for the same concentration at 1.0 M NaCl. That aggregates larger than trimer are formed in 0.1 M NaCl is suggested by the fact that a sedimentation coefficient of 8 was obtained at 0.4 mg/mL DBP. The presence of larger aggregates is clearly demonstrated by the sedimentation profile for this sample shown in Figure 6 (bottom). The sample did not contain a significant amount of precipitated material; if it had, a considerable loss of material from the plateau region would have occurred prior to the first scan. Soluble preparations at protein concentrations up to 0.5 mg/mL were achieved; the maximum limit to solubility in 0.1 M NaCl, pH 8.0, was not determined.

The sedimentation coefficient obtained at 0.12 mg/mL in 0.1 M NaCl was 6.0, a value similar to that found for the trimer aggregate at 1.0 M NaCl. Whether the DBP actually exists predominantly as a trimer at this protein concentration in 0.1 M NaCl has not been determined. The data obtained from sedimentation equilibrium experiments at 0.1 M NaCl were not good enough to determine accurate molecular weights. Most of the protein appeared to be highly aggregated, and much of it had sedimentated to the bottom of the cell.

Discussion

Adenovirus 2 DBP was isolated from infected cell nuclei late (ca. 44 h) after infection. Purification of the DBP was not complicated by large amounts of late adenovirus proteins, since most of these were found to diffuse readily from nuclei after lysis of the cytoplasmic membrane. DBP was solubilized from disrupted nuclei by sonication, but it appeared to remain bound to nucleic acid as judged by analysis of the phosphocellulose-purified material. The binding of purified DBP to single-stranded DNA results in an altered susceptibility of the protein to trypsin; further degradation of the 51K trypsin fragment was prevented by bound single-stranded DNA. Inhibition of this cleavage was also observed for the phosphocellulose-purified DBP (in the absence of added single-stranded DNA) but not for the DNA-cellulose-purified (DNA-free) protein incubated with double-stranded adenovirus DNA at DNA to protein ratios in excess of those needed for cleavage inhibition with single-stranded DNA (E. Buzash-Pollert, unpublished experiments). This result suggests that DBP as solubilized from nuclei is bound to single-stranded DNA, but it does not preclude an interaction with double-stranded DNA as well. That bound single-stranded DNA affects the trypsin cleavage pattern is a fact not previously described, and it may prove useful for the analysis of DBP subunit structure and function.

Several cellular proteins that bind tightly to DNA-cellulose have been purified from calf thymus (Herrick & Alberts, 1976). The best characterized of these, UP1, is a helix-unwinding protein with a polypeptide chain molecular weight of 24 000, a value considerably smaller than that of the adenovirus DBP. Proteins similar to UP1 have been isolated from a variety of cultured mammalian cells (Herrick & Alberts, 1976), but no protein of this size was observed to copurify with adenovirus DBP. We estimate that contamination of the adenovirus DBP by a UP1-like protein at a level of 1% would readily have been detected by polyacrylamide gel electrophoretic analysis.

Nuclear adenovirus DBP is similar in many respects to DBP isolated from cytoplasmic (Sugawara et al., 1977) or whole cell extracts (Linné et al., 1977; van der Vliet et al., 1978). It stimulates the in vitro elongation of virus DNA, and it saturates single-stranded DNA in a non-sequence-specific manner. A binding site for single-stranded DNA of between three and nine nucleotides determined by the trypsin cleavage assay is similar to the value of seven nucleotides reported by van der Vliet et al. (1978) for the adenovirus 5 DBP. The adenovirus 2 nuclear DBP was also shown to bind to double-stranded DNA, but single-stranded DNA could inhibit double-stranded DNA binding. This result suggests that both binding sites overlap. A double-stranded binding activity had not previously been found by others (Sugawara et al., 1977; van der Vliet et al., 1978), but recently Fowlkes et al. (1979) found that both adenovirus 2 and 5 DBPs possess double-stranded binding activity.

The hydrodynamic studies presented show that adenovirus 2 DBP self-associates at both high and low ionic strengths. The self-association process is extensive in 0.1 M NaCl at protein concentrations around 0.4 mg/mL, but DBP did not precipitate in 0.1 M NaCl at this concentration. Although the solubility properties of the nuclear DBP have not been extensively studied, the pH-dependent precipitation observed by van der Vliet et al. (1978) for adenovirus 5 DBP was not observed. Also, it was not necessary to use detergents as described by Sugawara et al. (1977) to keep the protein in solution. Poor solubility of the nuclear adenovirus 2 DBP was noted if DEAE-Sephadex was used in place of DNA-cellulose as the last step in purification. The reason for this observation is unknown.

In 1.0 M NaCl, self-association of the adenovirus DBP was a limited process that resulted in formation of a trimer. Trimer was readily evident from the slope of ln C vs. r^2 plots from equilibrium sedimentation studies, at protein concentrations above 0.14 mg/mL (10^{-6} M). Late after infection, infected cells contain about 50×10^6 molecules (0.6 mg/10^9 cells); thus the average nuclear concentration should be about 1 mg/mL (assuming 5×10^{-10} mL/nucleus). This concentration is sufficiently high for the intranuclear formation of trimer, but, as the strength of DBP interactions with double- and single-strand DNA under physiological conditions is not accurately known, the concentration of free DBP cannot be accurately estimated. However, even if there is not sufficient free DBP to form trimers, the formation of trimer in vitro may be indicative of interactions between bound adjacent proteins on the DNA chain. At low ionic strength, DBP at 0.12 mg/mL had a sedimentation coefficient similar to trimer (6 S vs. 7 S). Thus, at physiological ionic strength, trimer may be stable at free protein concentrations below those required for trimer formation in 1.0 M NaCl.

That aggregates greater than trimer were not observed in 1.0 M NaCl indicates that the trimer is a defined structural unit formed by the triangular arrangement of three protein subunits. The alternative, a linear arrangement, would imply an indefinite type of association that would have resulted in the formation of larger aggregates, that in turn would have produced a continued curvature of plots of ln C vs. r^2 at protein

concentrations above 0.14 mg/mL. The frictional coefficient for the trimer can be calculated from its sedimentation coefficient and its molecular weight if one assumes a partial specific volume (taken to be 0.73 cm^3/g) and hydration (taken to be 0.2 g/g of protein). Assuming the trimer to be a 7S aggregate of 204 000 M_r, f/f_0 would be 1.64 (f/f_{min} = 1.78). This result corresponds to the frictional coefficient expected for a prolate ellipsoid with axial dimensions of 12:1 or an oblate ellipsoid with axial dimensions of 15:1 (Tanford, 1961). Thus, like the monomer, the trimer is highly asymmetric. Dimensions calculated for a hydrated protein of mass 204 000 are 4.4 × 54.0 nm if it were a prolate ellipsoid ($2a/2b$ = 12) and 1.7 × 25.2 nm if it were an oblate ellipsoid ($2a/2b$ = 15); determination of the actual shape and size of the trimer will require additional study. One can imagine that trimers might associate at physiological ionic strength to form filaments to which DNA can bind. It is interesting to note that "thick" filaments have been observed in nuclear spreads of adenovirus-infected cells (Miller & Hodge, 1975; Kedinger et al., 1978); however, these filaments are somewhat thinner than would be expected for a DBP trimer–DNA complex.

Structural studies using proteolytic enzymes suggest that DBP monomer may have a complex internal structure consisting of separately folded domains of specialized function. Digestion by different proteolytic enzymes indicated that about two-thirds of the DBP peptide chain is folded into a rigid core that contains at least part of the binding site for single-stranded DNA. These observations may help to explain the origin of the several ca. 48 000-M_r DBP fragments found in many DBP preparations (Levine et al., 1976; Rosenwirth et al., 1976). Cleavage of DBP by chymotrypsin produced two stable fragments of 50 000 and 23 000 M_r that are representative of the whole molecule. We hope to use this finding to further define the domain structure of the DBP monomer and to relate this structure to possible functions.

Acknowledgments

We thank Bill Bauer (State University of New York, Stony Brook) for the use of his Model E ultracentrifuge, Betsy Sutherland and Bill Farland (Brookhaven National Laboratory) for advice on the filter-disk binding assay, and Marshall Horwitz for testing the activity of purified DBP for its ability to stimulate adenovirus DNA replication in vitro. We also thank Dana Fowlkes for making available to us his manuscript prior to publication and Greg Ballantyne and Sydell Lamb for providing valuable technical assistance.

References

Alberts, B. M., & Frey, L. (1970) *Nature (London) 227*, 1313–1317.

Alberts, B., & Herrick, G. (1971) *Methods Enzymol. 21D*, 198–217.

Anderson, C. W., Baum, P. R., & Gesteland, R. F. (1973) *J. Virol. 12*, 241–252.

Arens, M., Yamashita, T., Padmanabhan, R., Tsurvo, T., & Green, M. (1977) *J. Biol. Chem. 252*, 7947–7954.

Bonner, J., Chalkley, G. R., Dahmus, M., Fambrough, D., Fujimura, F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B., & Windholm, J. (1968) *Methods Enzymol. 12B*, 3–65.

Carroll, R. B., Neet, K., & Goldthwait, D. A. (1975) *J. Mol. Biol. 91*, 275–291.

Carter, T. H., & Blanton, R. A. (1978a) *J. Virol. 25*, 664–674.

Carter, T. H., & Blanton, R. A. (1978b) *J. Virol. 28*, 450–456.

Cavalier-Smith, T. (1974) *Nature (London) 250*, 467–470.

Chervenka, C. H. (1969) in *A Manual of Methods for the Analytical Ultracentrifuge*, pp 23–67, Beckman Instruments, Inc., Palo Alto, CA.

Chow, L. T., Broker, T. R., & Lewis, J. B. (1979) *J. Mol. Biol. 134*, 265–304.

Fowlkes, D. M., Lord, S. T., Linné, T., Pettersson, U., & Philipson, L. (1979) *J. Mol. Biol. 132*, 163–180.

Ginsberg, H. S., Lundholm, U., & Linné, T. (1977) *J. Virol. 23*, 142–151.

Herrick, G., & Alberts, B. (1976) *J. Biol. Chem. 251*, 2124–2132.

Hodge, L. D., Mancini, P., Davis, F. M., & Heywood, P. (1977) *J. Cell Biol. 72*, 194–208.

Horwitz, M. S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4291–4295.

Kedinger, C., Brison, O., Perrin, F., & Wilhelm, J. (1978) *J. Virol. 26*, 364–379.

Klessig, D. & Grodzicker, T. (1979) *Cell 17*, 957–966.

Lechner, R. L., & Kelley, T. J. (1977) *Cell 12*, 1007–1020.

Levine, A. J., van der Vliet, P. C., Rosenwirth, B., Anderson, C. W., Rubek, J., Levinson, A., & Anderson, S. (1976) *J. Mol. Cell. Biochem. 11*, 79–95.

Linné, T., Jörnvall, H., & Philipson, L. (1977) *Eur. J. Biochem. 76*, 481–490.

Mayer, A. J., & Ginsberg, H. S. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 785–788.

McGuire, J., Taylor, P., & Green, L. A. (1977) *Anal. Biochem. 83*, 75–81.

Miller, O. L., & Hodge, L. D. (1975) *J. Cell Biol. 67*, 284 (Abstr.).

Oey, J. L., & Knippers, R. (1972) *J. Mol. Biol. 68*, 125–138.

Peterson, E. A. (1970) in *Laboratory Techniques in Biochemistry and Molecular Biology* (Work, T. S., & Work, E., Eds.) Vol. 2, pp 223–400, American Elsevier, New York.

Pettersson, U., & Sambrook, J. (1973) *J. Mol. Biol. 73*, 125–130.

Pettersson, U., Philipson, L., & Högland, S. (1967) *Virology 33*, 575–590.

Rosenwirth, B., Anderson, C. W., & Levine, A. J. (1976) *Virology 69*, 617–625.

Russell, W. C., & Blair, G. E. (1977) *J. Gen. Virol. 34*, 19–35.

Schechter, N. M., Sharp, M., Reynolds, J. A., & Tanford, C. (1976) *Biochemistry 15*, 1897–1904.

Sugawara, K., Gilead, Z., & Green, M. (1977) *J. Virol. 21*, 338–346.

Sundquist, B., Persson, T., & Lindberg, U. (1977) *Nucleic Acids Res. 4*, 899–915.

Tanford, C. (1961) *Physical Chemistry of Macromolecules*, p 327, Wiley, New York.

van der Vliet, P. C., & Levine, A. J. (1972) *Nature (London) New Biol. 246*, 170–174.

van der Vliet, P. C., Levine, A. J., Ensinger, M. J., & Ginsberg, H. S. (1975) *J. Virol. 15*, 348–354.

van der Vliet, P. C., Keegstra, W., & Jansz, H. S. (1978) *Eur. J. Biochem. 86*, 389–398.

Weast, R. C., Ed. (1978) *CRC Handbook of Chemistry and Physics*, pp D299–D300, Chemical Rubber Publishing Co., West Palm Beach, FL.

Wickner, S. H. (1978) *Annu. Rev. Biochem. 47*, 1163–1191.

Williams, J. F. (1970) *J. Gen. Virol. 9*, 251–255.