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DNA-binding properties of the major core protein of adenovirus 2

Bruce C.Black and Melvin S.Center

Division of Biology, Kansas State University, Manhattan, KS 66506, USA

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ABSTRACT

The major adenovirus core protein (P·VII) binds to various species of duplex and single-stranded DNA molecules as a linear function of P·VII concentration. P·VII progressively condenses 32S Ad2 DNA into rapidly sedimenting forms having an S value of around 2,280. P·VII does not coat DNA like cytochrome C, instead DNA-protein beads are visualized in the electron microscope at low protein concentration. These beads appear to interact forming larger structures and at high P·VII concentrations the DNA molecule becomes highly compacted. Analysis of DNA fragments formed after digestion of P·VII-DNA complexes and isolated cores with micrococcal nuclease suggest that the organization of the DNA in the two structures is essentially identical. The initial P·VII and DNA interaction is sensitive to both ionic and hydrophobic environments, whereas the in vitro DNA-P·VII complexes are extremely stable and are not disrupted in the presence of 3 M NaCl, 1% sarcosyl or 5% deoxycholate. Properties of these in vitro DNA-protein VII complexes share striking similarities to isolated viral core particles.

INTRODUCTION

The core particle of the adenovirus virion consists of duplex DNA complexed with 1,070 copies of protein VII and 180 copies of protein V.[1,2] Both of these proteins are coded for by the viral genome.[1,3] The major protein of the core particle, protein VII (P·VII), has a molecular weight of 18,500[1,2] and is highly basic due to an enrichment of arginine and lysine residues.[4,5,6] Despite extensive studies on the physical properties of P·VII, very little information is available concerning the interaction of this protein with DNA. This information is essential to understanding the functional role of the protein in virion structure. In the present study, we describe a detailed analysis of the DNA binding properties of P·VII and demonstrate some unusual features of the complex formed between this protein and DNA.

MATERIALS AND METHODS

Cells, virus, and DNA. KB cells were grown in Eagle's medium[7] supplemented with 7% calf serum. Human adenovirus type 2 (Ad2) was propagated in KB cells and was purified as described by Green.[8] The production of Ad2 radioactively labeled with [^{3}H]thymidine is described elsewhere.[9] Adenovirus DNA was extracted from CsCl-purified virions by methods reported previously.[8,9] Duplex T7 DNA labeled with [^{3}H]thymidine was prepared as described.[10] Circular, duplex DNA of bacteriophage PM2[11] labeled with [^{3}H]thymidine was prepared as previously reported.[10,12] ^{3}H-labeled DNA of polyoma virus was a generous gift from Robert Feighny. Single-stranded DNA molecules were prepared by boiling duplex DNA for five minutes, followed by rapid cooling in an ice-water bath.

Isolation of viral cores and protein VII. CsCl-purified preparations of adenovirus 2 (1 to 5 mg) were dialyzed into 20 mM Tris-HCl (pH 7.6) containing 1 mM EDTA. Viral cores were released after 16 freeze-thaw cycles.[4,13] Cores were subsequently pelleted three times by low speed centrifugation (1500 RPM for 15 min in Sorvall GLC-1) followed by resuspending cores in 2 ml of 20 mM Tris-HCl (pH 7.6) containing 1 mM EDTA. After the final wash cycle, core proteins were extracted during a 15 minute incubation at room temperature in one ml of saturated guanidine. DNA was precipitated by addition of 3 ml of absolute ethanol. After a 30 min incubation, the DNA was removed by low speed centrifugation and the supernate was dialyzed against 0.5 M NaCl-10 mM HCl. When the protein concentration exceeds 25 µg/ml, P·VII is found to precipitate during the dialysis procedure. The precipitate was collected by low speed centrifugation and thereafter washed three times with 10 mM Tris-HCl (pH 7.6) containing 1 mM EDTA. The washed precipitate was solubilized with saturated guanidine and thereafter dialyzed and stored at 0°C in 0.5 M NaCl-10 mM HCl.[14,15] Purified P·VII migrated as a single homogeneous protein in sodium dodecyl sulfate - 15% polyacrylamide gel electrophoresis.[16]

Assay for protein VII DNA binding activity. This assay measures the amount of either single-stranded or duplex DNA retained on a nitrocellulose membrane filter in the presence of appropriate amounts of protein.[10,17,18] The reaction mixture (0.1 ml) contained 0.1 to 0.5 µg of various ^{3}H-labeled DNA species, 20 mM Tris-HCl (pH 7.6), 25 mM NaCl and 5% dimethyl sulfoxide. Protein VII was added to the reaction mixture and, after incubating 15 minutes at 22°C, the reaction was terminated by the addition of 3.0 ml of 20 mM Tris-HCl (pH 7.6) containing 5% DMSO (buffer A). The solutions were

thereafter passed through nitrocellulose membrane filters (Scheicher and Schuell, B6). The reaction tubes were washed twice and filters were washed once with 3 ml aliquots of buffer A. The filters were dried, and radioactivity was determined with a scintillation spectrometer. In the absence of binding protein, about 1% of the input duplex DNA and 2% of the input single-stranded DNA was retained by the filter.

Assay for protection of DNA-protein VII complexes against digestion with micrococcal nuclease. As a convenient means for comparing complexes formed upon incubation of DNA with varying amounts of protein VII, we determined the extent to which the complexes were digested with micrococcal nuclease. The reaction mixture (0.2 ml) contained 0.1 to 0.5 µg of various ^{3}H-labeled DNA species, 20 mM Tris-HCl (pH 7.6), and 25 mM NaCl. Protein VII was added to one tube whereas a second, which did not contain P·VII, was processed in an identical manner. After incubating 15 minutes at 22°C, the reaction mixtures were adjusted to contain 10 mM Tris-HCl (pH 7.6), 1 mM $CaCl_2$, 20 mM NaCl, and 1 µg/ml micrococcal nuclease (Sigma, grade IV). Incubation was continued for 30 min at 37°C and the reaction was terminated by the addition of 10 mM EDTA and 20 µg of salmon sperm DNA. Trichloroacetic acid was added to 5% and after centrifugation for 10 min at 10,000 RPM in the SE-12 Sorvall rotor, acid soluble radioactivity was determined.

Electron microscopy. The electron microscopy techniques used in this work have been described in detail.[19,20] Varying amounts of protein VII were incubated with viral DNA (1-1.6 µg/ml) for 15 minutes at room temperature in 20 mM Tris-HCl (pH 7.6) containing 25 mM NaCl. Samples were placed on parafilm for one minute and were thereafter adsorbed to carbon supporting films on 400 mesh copper grids treated with a 10,000 volt discharge for about 1 minute at 200 mm Hg pressure prior to use. The grid was touched to a drop of 0.25 M ammonium acetate, and subsequently stained and dehydrated in 0.05 M uranyl acetate in 95% ethanol. The grids were shadowed at 7° with 80% platinum - 20% paladium and the specimens were examined in a Phillips 201 electron microscope. The magnification was calibrated with a carbon grating replica having 2,160 lines/mm.

Other methods and materials. Protein was determined by the procedure of Lowry et al.[21] using bovine serum albumin and calf thymus histones as standards. All radioactively labeled compounds were purchased from New England Nuclear.

RESULTS

DNA binding properties of protein VII. The DNA-binding activity of protein VII was measured using the nitrocellulose filter assay as described in Methods. As shown in Figure 1, protein VII binds to both Ad2 double- and single-stranded DNA. The binding activity is linear with respect to protein concentration. Additional studies have shown that at a given concentration of protein VII, the molar equivalent of T7, PM2, and polyoma DNA retained on the filter is essentially identical to that obtained with Ad2 DNA.

We have also examined the effect of increasing concentrations of various salts on the binding activity of protein VII and have compared this activity to that obtained with a mixture of the four calf thymus histones, H2a, H2b, H3, and H4 (Figure 2). Increasing salt concentrations were found to interfere with the binding activity of protein VII, and at 0.75 M NaCl the reaction is only 1% of the control. Protein VII does, however, bind to DNA in the presence of lower salt concentrations and this binding is considerably more pronounced than exhibited by calf thymus histones.

The binding activity of protein VII has a broad pH optimum from pH 5.5 to pH 8.5. Above pH 9, the binding activity of protein VII declines rapidly

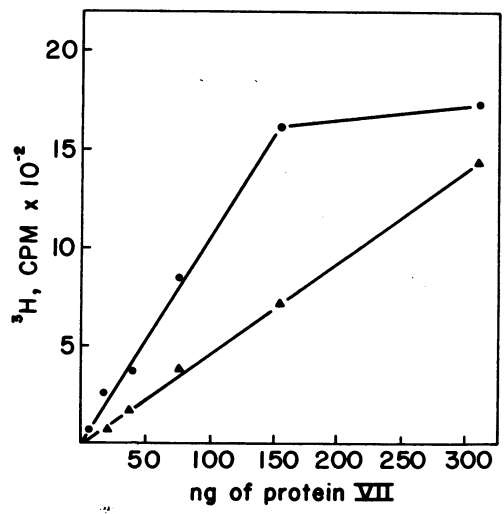

Figure 1. DNA binding activity of isolated protein VII. DNA binding activity using the nitrocellulose membrane filter assay was as described in Methods. Ad2 DNA was held constant at 0.35 µg/assay. DNA retention typically reached a plateau 80% of the total input. Symbols: ●---●, double-stranded Ad2 DNA; ▲---▲, single-stranded Ad2 DNA.

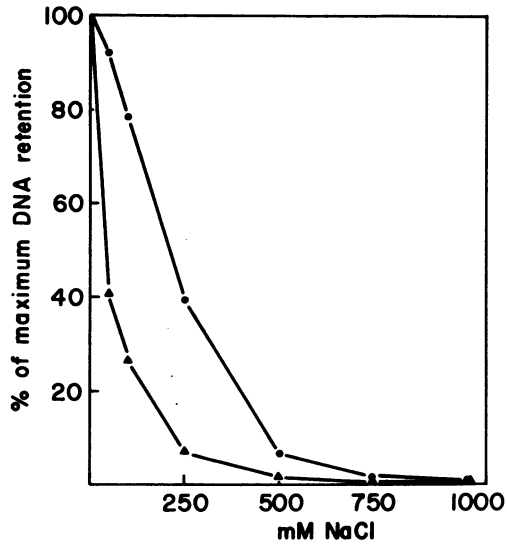

Figure 2. Effect of ionic strength on the formation of DNA-P·VII and DNA-histone complexes. DNA-binding activity was carried out using the nitrocellulose membrane filter assay as described in Methods. Standard reaction mixtures were incubated in the absence or presence of varying concentrations of NaCl. Concentrations of P·VII and calf thymus histones were adjusted to retain 50% of the input DNA under optimal conditions. Data is presented as percent of the maximum DNA retention on nitrocellulose filters. Symbols: ●---●, protein VII; ▲---▲, calf thymus histones.

and at pH 9.6, there remains only 24% of the optimal binding activity.

Sedimentation analysis of Ad2 DNA-protein VII complexes. Incubation of protein VII with Ad2 DNA results in a considerable increase in the sedimentation rate of the DNA (Figure 3). The DNA-protein VII complex sediments at a rate faster than either naked Ad2 DNA (Figure 3a) or adenovirus (Figure 3b). Examination of the fast sedimenting complexes reveal that those contained in fractions 10-15 (Figure 3a) are partially condensed structures similar to those shown in Figure 6, d-i. The complexes in fractions 2-4 (Figure 3a) are more highly compacted and are similar to those structures shown in Figure 7. Partially condensed complexes from 40% to 60% micrococcal nuclease protected are typically heterogeneous during sedimentation but become increasingly homogeneous as complexes are increasingly condensed. The S value for the highly condensed complexes compared with Ad2 virions as a marker is about 2,280. In addition to Ad2 DNA, incubation of protein VII with other DNAs (T7, PM2) results in their conversion to a rapidly sedimenting form. Evidence obtained

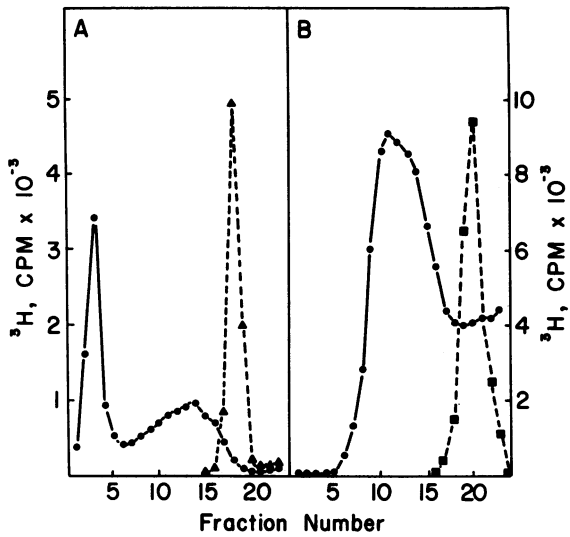

Figure 3. Zone sedimentation analysis of DNA-protein VII complexes. DNA-protein VII complexes were prepared by reacting 0.3 µg (panel A) or 0.8 µg (panel B) of P·VII with 0.4 µg [^{3}H]-labeled Ad2 DNA in 0.2 ml of 20 mM Tris-HCl (pH 7.6) for 15 min at 22°C. At the end of the incubation period complexes were centrifuged in either sucrose or glycerol gradients. Panel A. Samples were sedimented through a linear 5 to 20% sucrose gradient in 20 mM Tris-HCl (pH 8.0) containing 0.2 mM EDTA on top of a 0.5 ml saturated CsCl cushion at 40,000 RPM for 90 minutes at 4°C in a Spinco SW50.1 rotor. Panel B. Samples were sedimented through a linear 30 to 70% glycerol gradient in 20 mM Tris-HCl (pH 8.0) containing 0.2 mM EDTA at 20,000 RPM for 45 minutes at 4°C in a Spinco SW50.1 rotor. Fractions (0.2 ml) were collected from the bottom of the tube. Data shown as acid precipitable counts. Symbols: ●---●, Ad2 DNA; markers sedimented in parallel gradients of either; ▲---▲, [^{3}H]Ad2 DNA; or ■---■, [^{3}H]Ad2 virions.

with electron microscopy (Figure 6) suggest that the fast sedimentation rate of the complex is due to the formation of a collapsed DNA structure and not to aggregation effects.

Buoyant density of Ad2 DNA-protein VII complexes. The buoyant density of DNA-protein VII complexes was examined after equilibrium centrifugation in CsCl gradients. DNA-protein VII complexes which are 50% protected from micrococcal nuclease have a prominent peak at a density of 1.65 g/cc. These complexes are, however, quite heterogeneous having densities which range from 1.65 g/cc to 1.51 g/cc (Figure 4a). Fully condensed DNA-protein VII complexes have a homogeneous density in CsCl at 1.51 g/cc (Figure 4b). Under

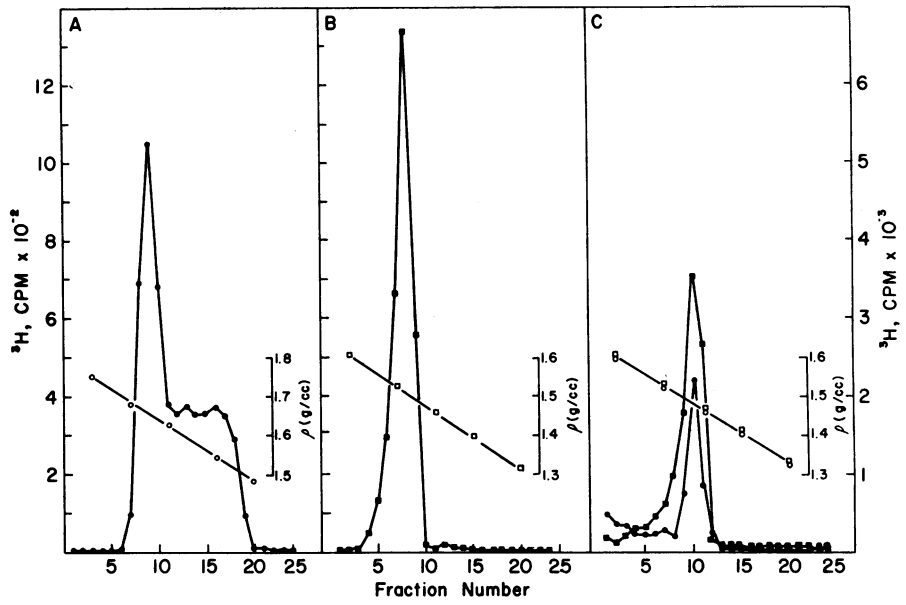

Figure 4. Buoyant density of DNA-protein VII complexes. DNA-protein VII complexes were prepared as described in the legend to Figure 3. After the incubation, samples to be nuclease treated were incubated with micrococcal nuclease (1 µg/ml) in the presence of 1 mM $CaCl_2$ for 45 min at 37°C. The reaction was terminated by the addition of EDTA to a final concentration of 10 mM. DNA-protein VII complexes were overlaid on preformed CsCl gradients prepared in 20 mM Tris-HCl (pH 7.6) - 1 mM EDTA. Centrifugation was carried out in the Spinco 50.1 rotor for 15 hrs at 40,000 RPM and 16°C. Acid insoluble radioactivity in fractions collected from the bottom of the tube was determined. Density of CsCl gradients was determined by refractive index. Panel A. Complexes formed by reacting 0.3 µg of protein VII with 0.4 µg [^{3}H]-labeled Ad2 DNA. Panel B. Complexes formed by reacting 0.8 µg of protein VII with 0.4 µg [^{3}H]-labeled Ad2 DNA. Panel C. Micrococcal nuclease treated complexes prepared by reacting 0.3 µg (●---●) or 0.8 µg (▲---▲) of protein VII with 0.4 µg [^{3}H]-labeled Ad2 DNA.

identical centrifugation conditions, core particles and Ad2 DNA have respective densities of 1.38 g/cc and 1.71 g/cc. Essentially identical results are obtained for complexes with and without formaldehyde fixation of protein VII to DNA. As will be described in the next section, the complex of protein VII with DNA is extremely stable.

The mass ratios of DNA-protein complexes from CsCl gradients were determined[22,23] and the number of protein molecules bound per DNA molecule was estimated by the following equation:

Number of molecules of protein VII = $[(\%\ \text{protein} \div \%\ \text{DNA})MW_{DNA}] \div MW_{VII}$

DNA-protein VII complexes having a density of 1.51 g/cc are calculated to have 895 molecules of protein VII bound per DNA molecule. Partially and fully condensed complexes after a micrococcal nuclease digestion both have an identical density in CsCl of 1.485 g/cc (Figure 4c). Thus the binding by protein VII to DNA must occur as a constant protein to DNA ratio to account for the shift to a common density of the various complexes after nuclease treatment.

Stability of the DNA-protein VII complex. Extensive studies have been performed to examine the conditions required for dissociating the DNA-protein VII complex. Assays for detecting DNA-protein VII association and dissociation were carried out by measuring the formation of rapidly sedimenting collapsed DNA-protein complexes as described in the legend of Table 1. The stability of the DNA-protein VII complexes was compared to that of isolated adenovirus cores (Table 1). The results given in Table 1 demonstrate that the initial interaction of P·VII with DNA is sensitive to ionic and hydrophobic environments but not to environments that strongly interfere with hydrogen bonding. After binding of protein VII to DNA, the complex is extremely stable. The complex is completely stable in 3 M NaCl or 6 M urea. However, in the presence of a mixture of 6 M urea and 1 M salt, disruption of the complex is readily evident. The DNA-protein complex is also quite stable in 1% sarcosyl and 5% deoxycholate although 1% sodium dodecyl sulfate dissociates the complex. It can also be seen from Table 1 that in many instances, conditions preventing association of protein VII with DNA have essentially no effect on the dissociation of the complex. Of particular interest is the finding that the conditions required for dissociation of the DNA-protein VII complex and isolated cores are essentially identical. Thus, the in vitro binding of protein VII to DNA appears to be quite similar to the structural stability imparted to the core particle generated in vivo.

The in vitro DNA-protein VII complex and virion cores can also be distinguished from DNA-histone interactions since in the latter case, the complex is labile to salt concentrations exceeding 1 M NaCl.[24,25]

Specificity of protein VII-DNA interaction. In these experiments, in vitro DNA-protein VII complexes were digested with micrococcal nuclease and the deproteinized DNA fragments were analyzed by velocity sedimentation and polyacrylamide gel electrophoresis. In sucrose gradients, the DNA fragments were observed to have a sedimentation coefficient of 5S. This value corresponds to 135,000 daltons or approximately 200 base pairs of DNA. DNA frag-

Effect of various reagents on the association and dissociation of P·VII and core complexes

Standard Reaction Buffer Containing:	Association DNA-VII % Control	Dissociation DNA-VII % Control	Dissociation Core % Control
Control	100.0	100.0	100.0
Pronase	0.0	0.0	1.3
1 M NaCl	2.0	89.0	100.0
2 M NaCl	-	100.0	96.0
3 M NaCl	-	91.0	100.0
6 M Urea	96.8	86.0	82.0
6 M Urea, 0.25 M NaCl	-	61.0	-
6 M Urea, 1.0 M NaCl	-	11.0	13.0
1% SDS	0.1	0.3	0.2
1% Sarcosyl	0.1	100.0	98.0
5% Sarcosyl	-	2.0	5.0
5% DOC	0.5	100.0	100.0
5% Pyridine	87.0	95.0	98.0
6 M Guanidine	1.0	1.0	2.0

The effect of various reagents on the association of P·VII with DNA was determined by reacting 0.5 µg of ^{3}H-labeled T7 DNA with 0.9 µg of P·VII in 0.2 ml of 20 mM Tris-HCl (pH 7.6) containing the various reagents indicated in the table. After a 15 min incubation period, the solutions were adjusted to 1 ml with reaction mixture. The effect of various reagents on the dissociation of P·VII-DNA complexes or viral cores was determined by reacting 1.9 µg of cores containing ^{3}H-labeled DNA or 0.5 µg of ^{3}H-labeled T7 DNA with 0.9 µg of protein VII in 0.2 ml of 20 mM Tris-HCl (pH 7.6). After a 15 min incubation period, the reaction mixture was adjusted to 1.0 ml with 20 mM Tris-HCl (pH 7.6) containing the various agents indicated in the table. These samples were centrifuged at 10,000 RPM for 20 min and 4°C in the Sorvall SE-12 rotor. Under these conditions, P·VII-DNA complexes or core associated DNA will pellet to the bottom of the tube. Supernate was discarded and pelleted material was solubilized in 0.05 ml of 0.6% SDS, 10 mM EDTA, and 10 mM Tris-HCl (pH 7.6). The radioactivity in the entire solubilized sample was determined. Controls of DNA without protein in various reaction mixtures were about 1% of the total input radioactivity. For certain experiments, complexes were treated with pronase (1 mg/ml) for 15 min at 37°C prior to centrifugation.

ments derived from micrococcal nuclease treatment of in vitro DNA-protein VII complexes and analyzed in 6% polyacrylamide gels (Figure 5a) reveal a similar size as those DNA fragments obtained by an identical micrococcal nuclease

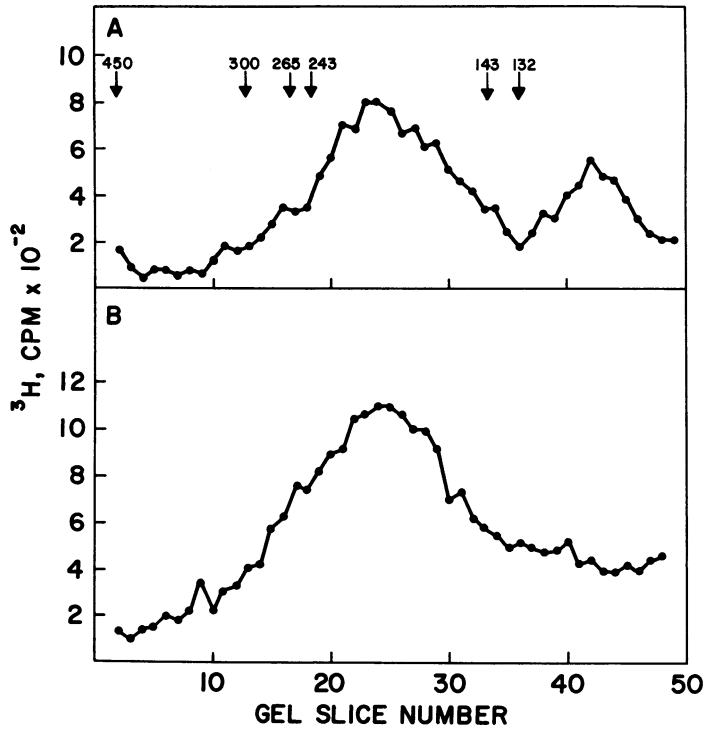

Figure 5. Polyacrylamide gel electrophoresis of DNA fragments formed after treatment of DNA-protein VII complexes and Ad2 core particles with micrococcal nuclease. Core particles and complexes were incubated in the standard reaction mixture containing micrococcal nuclease (1 µg/ml) for 30 min at 37°C. Longer incubation with micrococcal nuclease does not change the pattern of DNA fragments observed. The reaction mixture was thereafter adjusted to contain self-digested pronase (0.5 mg/ml), 2.5% SDS, 0.01 M EDTA and 5% glycerol and incubation was allowed to continue for 1 hr at 37°C. Portions of the reaction mixture were applied to 6% polyacrylamide tube gels (10 x 0.6 cm) and electrophoresis was carried out in the Loening buffer[26] for 4 hrs at 6 mA/gel. Gels were sectioned and radioactivity determined. Panel A. DNA fragments from complexes formed by reacting 1.5 µg of P·VII with 2 µg [^{3}H]-labeled Ad2 DNA. Panel B. Fragments formed from digestion of isolated cores containing 2.8 µg of ^{3}H-labeled DNA. Polyacrylamide gels were calibrated using PM2 DNA that was cleaved by Hae III restriction nuclease.[27] Arrows indicate the numbers of base pairs in the PM2 fragments.

treatment of core particles (Figure 5b). The size of the major fraction of DNA fragments corresponds to 170-225 base pairs for both in vitro complexes and adenovirus core particles. The peak fractions correspond to about 205 base pairs. An additional peak in figure 5a of about 100 base pairs may

represent a population of DNA fragments that are not protected from micrococcal nuclease by a specific complex of protein VII. Corden et al. have reported similar size DNA fragments following micrococcal nuclease treatment of viral core particles.[30] In studies carried out thus far, a distinct population of DNA fragments of about 170-225 base pairs has been obtained following micrococcal nuclease treatment of complexes, irregardless of the extent of protection conferred by protein VII.

Electron microscope examination of DNA-protein VII complexes. DNA-protein VII complexes visualized in the electron microscope are shown in Figures 6 and 7. Complexes prepared using either Ad2 or T7 DNAs were indistinguishable by electron microscopy. Incubation of T7 DNA with protein VII results in a condensation of the DNA molecule. The compactness of the DNA increases with increasing protein VII concentration. As our micrococcal nuclease data would predict, the complexes do not exhibit a thickening of the DNA molecules as is observed with cytochrome C coating of DNA. Instead, small protein beads are located apparently at random on DNA molecules (Figure 6b, c, i) at low protein VII concentrations. These beads have an average diameter of 190 $\pm$ 18 angstroms and must represent at least several molecules of protein VII. Samples of DNA-protein VII complexes treated with pronase lost all of their beaded and/or condensed structure and were converted to molecules similar to naked (native) DNA (Figure 6a). Large numbers of these small protein beads do not accumulate after binding to DNA. Instead, these protein beads apparently interact with each other forming larger protein structures (Figure 6d-i). As shown in Figures 6d-i and 7, the integrity of individual beads was lost in these complexes. Additional structural features reveal the presence of loops of naked DNA which extend from these complexes. These DNA loops are not associated with individual protein beads. In larger complexes, many loops of naked DNA are associated with a central, dense structure and appear as rosettes (Figures 6f and 7b). Brown et al.[28] and Mirza and Weber[29] have shown that gradually disrupted virion cores also contain similar loops of free DNA. As the protein concentration is increased to a ratio of P·VII to DNA of 1:1, the length of free DNA extending from these complexes decreases and the DNA molecules are progressively condensed appearing more compact (Figure 7). Increasing the protein concentration to a ratio of P·VII to DNA of 2:1 produces structures essentially identical to those shown in Figure 7.

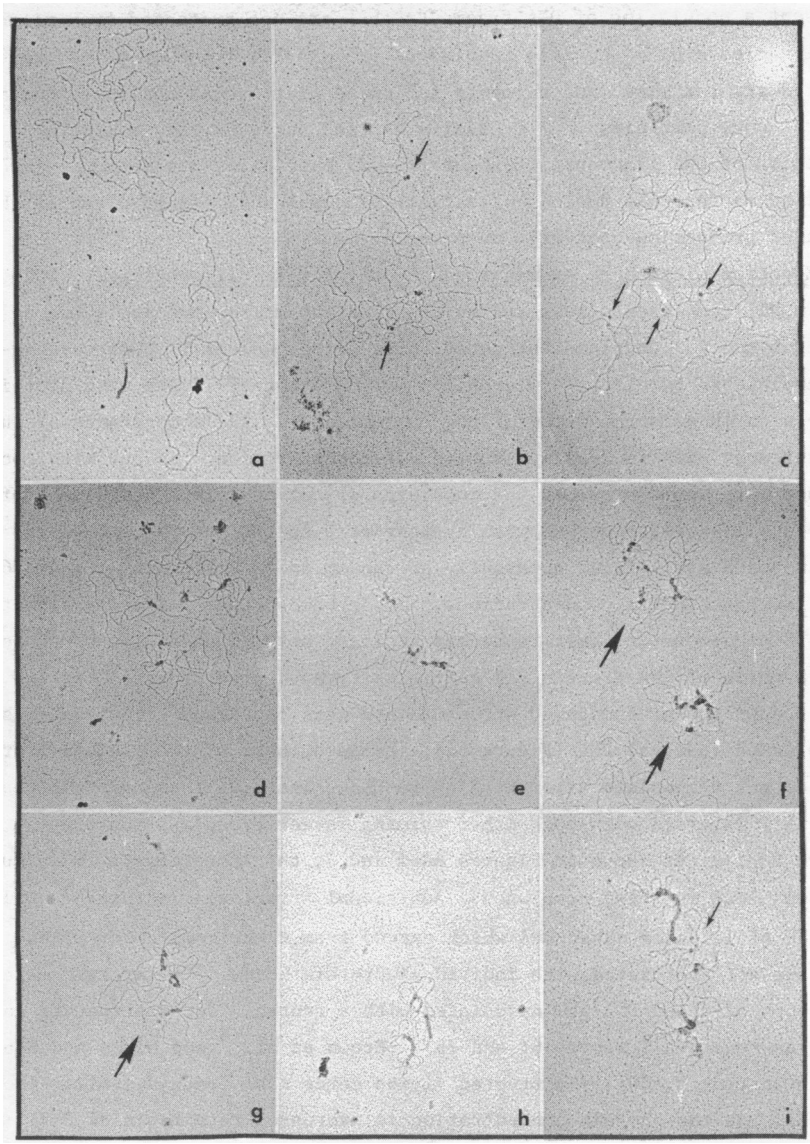

Figure 6. Electron micrographs of DNA-protein VII complexes. Procedures used for electron microscopy are described in Methods. a, T7 DNA incubated without protein. b-c, complexes visualized after incubating T7 DNA (1.6 μg/ml) with protein VII (0.35 μg/ml). d-i, complexes visualized after incubating T7 DNA (1.6 μg/ml) with 0.7 μg/ml of protein VII. Magnification: a-h, 34,500X; i, 46,750X. Small arrows indicate the presence of protein beads attached to T7 DNA. Large arrows show rosettes of DNA emanating from large protein structures. The percent solubilization of the DNA by micrococcal nuclease was 85% (b-c) and 60% (d-i).

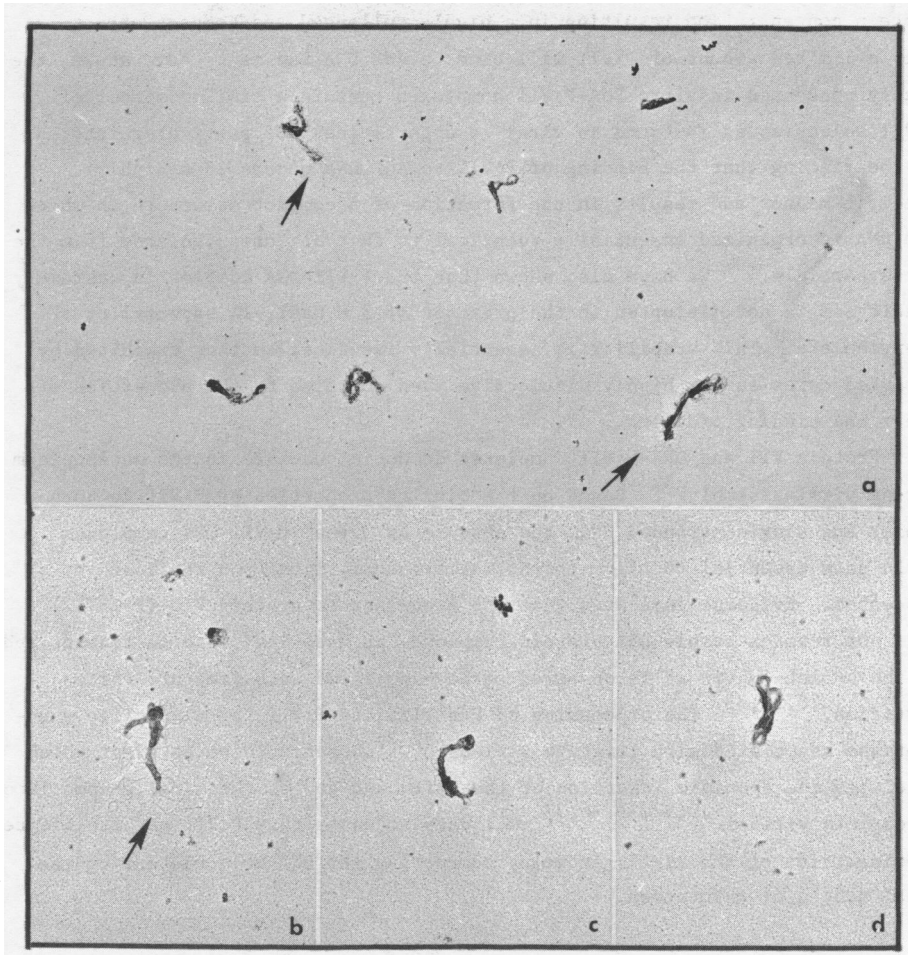

Figure 7. Electron micrographs of DNA-protein VII complexes. Procedures used for electron microscopy are described in Methods. a-d, complexes visualized after incubating T7 DNA (1.6 μg/ml) with protein VII (1.4 μg/ml). Magnification: a, 26,700X; b-d, 59,550X. Arrows indicate the presence of rosettes of DNA emanating from large protein structures. Twenty percent of the DNA in the complexes was solubilized after treatment with micrococcal nuclease.

DISCUSSION

In the present study, we have carried out a detailed analysis of the DNA binding properties of the major adenovirus core protein (P·VII). The results of our study show that P·VII binds Ad2 DNA as a linear function of increasing

protein concentration resulting in a highly collapsed and compact structure. Only a limited amount of P·VII will bind to Ad2 DNA and as we have shown, the highly condensed in vitro DNA-P·VII complexes contain a similar number of P·VII molecules as is found in viral core particles. Of particular interest is the finding that the binding of P·VII to Ad2 DNA occurs in a highly specific manner and results in the formation of a compact structure in which the DNA is organized essentially identical to that of cores isolated from the virus particle.[30] We have also shown that the P·VII-DNA complex is extremely stable and is not disrupted in the presence of 3 M NaCl, 1% sarocsyl or 5% deoxycholate. This stability is essentially identical to that exhibited by isolated cores and is highly distinctive when compared to the properties of other DNA binding proteins.[24,25]

Protein VII and DNA-P·VII complexes do not accumulate in the nucleoplasm during viral assembly.[31] Based on the binding properties of P·VII to both duplex and single-stranded DNA, the absence of "free" P·VII-DNA complexes would seem essential to allow for normal transcriptional and replicative processes. Evidence indicates that the precursor to protein VII (Pre VII) does not form as stable DNA-protein complexes as does P·VII and is transported to the nucleus where it is observed to be associated with immature virion particles.[28,32,33] The processing of Pre VII into P·VII, by a specific endo-protease associated with immature virions,[32-36] appears to be an event which regulates the specific insertion of the left-hand end of the viral genome into incomplete virions.[28,32,34,36,37] Our data suggests that P·VII may facilitate the insertion of the viral genome by compacting the DNA molecule during the final stages of maturation.

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