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Filter-binding assay for covalent DNA–protein complexes: Adenovirus DNA–terminal protein complex

(glass fiber filter/salt-dependent binding/restriction endonuclease)

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ABSTRACT **A rapid, simple, and quantitative filter-binding assay using glass fiber filters has been developed to detect the covalent adenovirus DNA–terminal protein complex. The assay is unusually sensitive because binding of protein-free DNA generally is less than 0.1%. Binding of the adenovirus complex to filters is mediated by terminal protein. We have found that: (*i*) the adenovirus complex binds maximally to filters in NaCl at concentrations higher than 0.2 M; (*ii*) noncovalent complexes between protein-free DNA and adenovirus proteins bind to filters in salt at concentrations lower than 0.4 M but not in concentrations higher than 0.7 M; and (*iii*) protein-free DNA alone binds to filters in guanidine·hydrochloride at concentrations higher than 0.8 M. By varying the ionic conditions, "all or none" modulation of these interactions can be achieved.**

There are now clear examples of proteins covalently joined to nucleic acids. Covalent nucleic acid–protein complexes occur as structural elements of the chromosomes of DNA viruses (1–10) and RNA viruses (11–14). A number of methods have been used to detect DNA–protein complexes. In the case of adenovirus, a 55,000-dalton protein (7), called "terminal protein," is covalently linked to each 5′ end of the linear, double-stranded DNA molecule (15). The adenovirus complex has been visualized directly in the electron microscope as protease-sensitive circles and other more complicated structures (2, 3, 7, 8). These molecular forms presumably arise from end-to-end interactions mediated by terminal protein because only linear DNA molecules can be detected after protease treatment. Terminal protein prevents adenovirus DNA or terminal restriction endonuclease fragments of viral DNA from entering agarose gels during electrophoresis (16, 17). Recently, several laboratories have discovered that the adenovirus DNA–terminal protein complex binds tightly to benzoylated-naphthoylated-DEAE-cellulose under conditions such that protein-free DNA does not (B. W. Stillman and A. D. J. Bellett, personal communication; L. M. Kaplan and M. S. Horwitz, personal communication).

Each of these assays, however, suffers with respect to quantitation or ease of use or both. Our laboratory has now developed a rapid, simple, and quantitative filter-binding assay for the adenovirus DNA–terminal protein complex. In this paper we show that the adenovirus complex or terminal restriction endonuclease fragments of the complex are quantitatively retained (>98%) on glass fiber filters under conditions such that protein-free DNA is not (<0.1%). The adenovirus complex binds maximally to filters at NaCl concentrations greater than 200 mM. This means that the adenovirus complex can be assayed even in the presence of other proteins that interact ionically with DNA. It is likely that the filter-binding assay will prove useful for detecting and quantitating covalent nucleic acid–protein complexes from a wide range of organisms.

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MATERIALS AND METHODS

Adenovirus. A plaque-purified stock of type 2 adenovirus was kindly supplied by J. Weber. The growth of virus in HeLa cells, the purification of virus, and the extraction of viral DNA from virus particles have been described (18, 19). The specific activity of virus labeled with [^{32}P]orthophosphate (New England Nuclear) or [*methyl*-^{3}H]thymidine (New England Nuclear) was 5×10^5 or 5×10^4 cpm/μg of DNA, respectively.

Buffers. TE buffer is 10 mM Tris·HCl/1 mM EDTA, pH 7.5. TEN buffer is TE buffer containing 300 mM NaCl. Sodium phosphate buffer contains equal molar concentrations of the mono- and dibasic salts at pH 6.8.

Filter-Binding Assay. The standard filter-binding assay used 2.4-cm Whatman GF/C glass fiber filters held in a commercially available stainless steel filter holder. Filters were wetted with 1–2 ml of the sample buffer to establish a hydrostatic head, and the samples (up to 1 ml) were applied freely. The filter was rinsed with an additional 2 ml of the appropriate buffer and sucked dry. In some cases (for example, 1 M sodium phosphate buffer) the filter was rinsed last with 1 ml of TEN buffer prior to suction drainage. Both the filter and the filtrate were assayed for radioactivity to determine the percentage DNA bound to the filter. Miniature filters (3.5 mm diameter), called "microdot filters," were cut from stock GF/C filters. Microdot filters were mounted in a special holder constructed from a 1-ml polystyrene pipet (detailed instructions for constructing the holder are available upon request). The microdot filter facilitates handling of volumes less than 0.1 ml. Bound DNA–protein complexes could be eluted from the microdot filter in 10–25 μl of solutions containing detergents or proteolytic enzymes. During elution, the liquid was carefully passed down and up through the filter several times by use of suction and pressure. The solution was removed from above the filter with a micropipet.

Enzymes. *Eco*RI endonuclease was isolated according to the method of Greene *et al.* (20). *Hae* III endonuclease was prepared as described by Middleton *et al.* (21). *Bam*HI endonuclease was purified by the method of Wilson and Young (22). *Sma* I endonuclease was purified as described by McParland *et al.* (23, 24). (Some restriction enzyme preparations contain nonionic detergents—for example, Triton X-100—that may artifactually reduce binding of DNA–protein complexes to filters.) Pronase (grade B, Calbiochem) and Proteinase K (EM Biochemicals) were incubated at 37°C for 2 hr at a concentration of 10 mg/ml before use.

Radioactivity Determinations. Dried filters were assayed by liquid scintillation spectrometry using a toluene/2,5-diphenyloxazole/1,4 bis[2-(5-phenyloxazolyl)]benzene mixture (18). ^{32}P radioactivity was determined by Cerenkov radiation. Isotope overlap corrections were computed on a Hewlett-Packard 9821A calculator.

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Gdn·HCl, guanidine·hydrochloride; bp, base pairs.

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RESULTS

Purification of the Adenovirus DNA-Terminal Protein Complex. The purification of the adenovirus DNA-terminal protein complex is shown in Fig. 1*A*. ^{32}P-Labeled virus particles were disrupted with 4 M guanidine·hydrochloride (Gdn·HCl) (2, 3, 7). The DNA-protein complex containing only the 55,000-dalton terminal protein (7) was separated from the other dissociated viral proteins by chromatography on a Sepharose 2B-CL column in the presence of 4 M Gdn·HCl. ^{32}P-Labeled complex emerged quantitatively from the column in the excluded volume. The A_{260}/A_{280} ratio of the complex was greater than 2.0 and was indistinguishable from the ratio of protein-free DNA. No radioactivity was associated with material eluting from the column in the included volume. This latter material had an A_{260}/A_{280} ratio less than 0.9 and contained all of the virion proteins when analyzed by gel electrophoresis (not shown). We have also isolated, by Sepharose chromatography in 0.005% sodium dodecyl sulfate ($NaDodSO_4$), the adenovirus complex from virus particles disrupted at 80°C with 0.1% $NaDodSO_4$ (not shown). Complex prepared by either procedure had identical properties. Chinnadurai *et al.* (25) have also independently purified the adenovirus complex by Sepharose chromatography.

Binding of the Adenovirus DNA-Terminal Protein Complex to Glass Fiber Filters. Portions of each fraction from the Sepharose column depicted in Fig. 1*A* were mixed with protein-free adenovirus [^{3}H]DNA, diluted 1:10 into TE buffer or 1 M sodium phosphate buffer (all samples had a final concentration of 0.4 M Gdn·HCl after dilution), and passed through glass fiber filters. Fig. 1*B* shows that the profile of ^{32}P radioactivity bound to filters agreed exactly with the profile of ^{32}P-labeled complex eluting from the Sepharose column. An average of 98.5% of ^{32}P-labeled complex diluted in either buffer bound to filters. Less than 0.1% of [^{3}H]DNA added to fractions containing complex (fractions 5–8) bound to filters in either buffer, demonstrating that the complex by itself did not interact with protein-free DNA. On the other hand, more than 95% of [^{3}H]DNA added to fractions containing viral proteins (fractions 17–22) bound to filters after dilution in TE buffer, but less than 0.2% bound after dilution in 1 M sodium phosphate buffer. Because we could qualitatively demonstrate by gel electrophoresis that all virion proteins were bound to filters after dilution in either buffer (not shown), 1 M sodium phosphate buffer presumably disrupted ionic bonds in noncovalent complexes between viral proteins and protein-free DNA. Similar results have been reported for other noncovalent nucleic acid–protein complexes on cellulose nitrate filters (26).

The binding of purified adenovirus DNA-terminal protein complex was salt-dependent. Fig. 2 shows that, after exhaustive dialysis of purified complex against TE buffer, less than 2% of the complex bound to filters. As the solution containing complex was adjusted to higher ionic strengths and filtered, an increasing percentage of the complex was retained. More than 99% of the ^{32}P-labeled complex bound to filters when the concentration of NaCl exceeded 0.2 M. Complex was quantitatively retained by filters at all concentrations up to 4 M NaCl (not shown). The binding curve with KCl was virtually identical to the curve for NaCl (not shown). Fig. 2 also shows that an average of 0.4% (range, 0.1–0.7%) of protein-free [^{3}H]DNA bound to filters in concentrations of salt (NaCl or KCl) ranging up to 1 M although marked binding of [^{3}H]DNA occurred between 4 and 5 M (not shown). Concentrations of Gdn·HCl as low as 65 mM elicited maximal binding of the complex (Fig. 2). [^{3}H]DNA did not bind to filters in concentrations of Gdn·HCl lower than 0.4 M. However, whether mixed with complex or not, protein-free DNA bound quantitatively (>99%) to filters at concentrations of Gdn·HCl greater than 0.8 M. Most buffers (TE, TEN, etc.) released bound DNA from filters, but 1 M sodium phosphate buffer was the most efficient (95% released). We have used this unusual property to concentrate DNA from dilute solutions (27). Preliminary experiments indicate that the volume of solution and the size and concentration of DNA do not affect the binding of protein-free DNA in 1 M Gdn·HCl. The capacity of microdot filters (3.5 mm diameter) is about 5 μg of DNA.

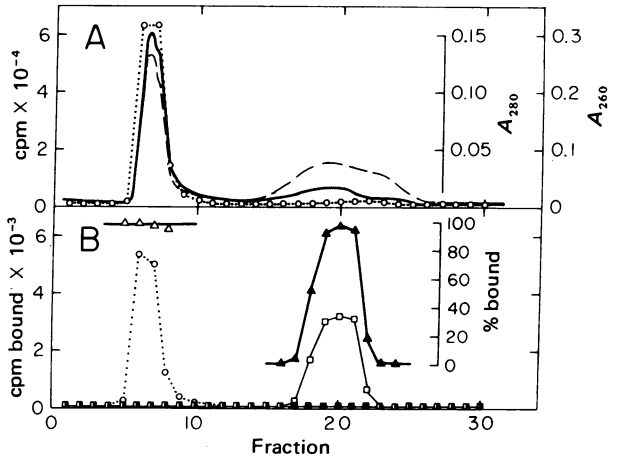

FIG. 1. Purification of the adenovirus DNA-terminal protein complex by Sepharose chromatography. (*A*) Column profile. ^{32}P-Labeled adenovirus particles were disrupted by the addition of an equal volume of 8 M guanidine·HCl (Gdn·HCl)/2 mM 2-mercaptoethanol. A 0.3-ml sample was layered on a 1.5 × 13 cm column of Sepharose 2B-CL (Pharmacia). The column was eluted with TE buffer containing 4 M Gdn·HCl/0.1% Sarkosyl/1 mM 2-mercaptoethanol. The flow rate was 0.2 ml/min, and 0.7-ml fractions were collected; the absorbances at 260 and 280 nm were recorded continuously by using an Altex UV monitor. ○, ^{32}P radioactivity; solid line, A_{260}; dashed line, A_{280}. Fractions containing ^{32}P-labeled complex were pooled and stored at −20°C or dialyzed exhaustively against TE buffer and stored at 4°C. (*B*) Filter-binding profiles. Samples (100 μl) of each fraction from the column were mixed with [^{3}H]DNA, diluted 1:10 into TE buffer or 1 M sodium phosphate buffer (the final concentration of Gdn·HCl in all samples was 0.4 M after dilution), and passed through glass fiber filters. ○, ^{32}P-Labeled complex bound after dilution in TE or phosphate buffers; △, % ^{32}P-labeled complex bound after dilution in TE or phosphate buffers; □, [^{3}H]DNA bound after dilution in TE buffer; ■, [^{3}H]DNA bound after dilution in phosphate buffer; ▲, % [^{3}H]DNA bound after dilution in TE buffer.

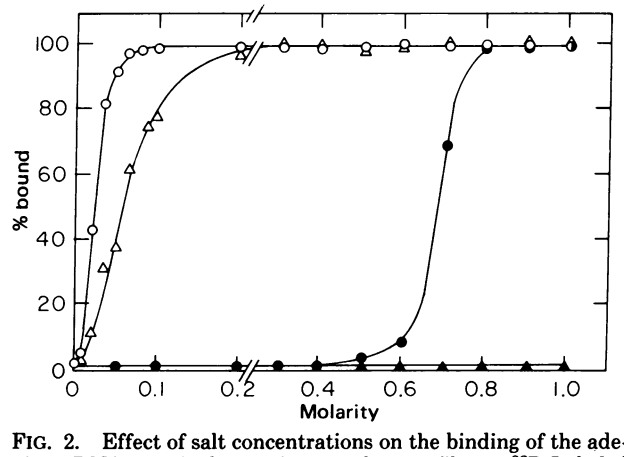

FIG. 2. Effect of salt concentrations on the binding of the adenovirus DNA-terminal protein complex to filters. ^{32}P-Labeled complex was purified as described in Fig. 1 and dialyzed exhaustively against TE buffer. Samples of ^{32}P-labeled complex were mixed with [^{3}H]DNA, adjusted to the indicated concentration of NaCl or Gdn·HCl, and passed through glass fiber filters equilibrated with the same salt solution. ○, ^{32}P-Labeled complex in Gdn·HCl; ●, [^{3}H]DNA in Gdn·HCl; △, ^{32}P-labeled complex in NaCl; ▲, [^{3}H]DNA in NaCl.

Other experiments (not shown) demonstrated that binding of complex was independent of concentration over a 50-fold range (0.37–20 μg of DNA/ml or 0.03–1.7 pM terminal protein, assuming two protein molecules per DNA molecule) and independent of time of incubation in Gdn·HCl from 2 sec to 30 min.

As shown in Fig. 2, 0.4 M Gdn·HCl elicited maximal binding of complex to filters but was not sufficient to prevent nonspecific binding of protein-free DNA mediated by viral proteins other than the terminal protein (Fig. 1*B*). ^{32}P-Labeled virus particles, mixed with adenovirus [^{3}H]DNA, were disrupted with 4 M Gdn·HCl, diluted 1:10 into TE buffer (0.4 M Gdn·HCl after dilution), and filtered. Fig. 3 shows that, as expected, both ^{32}P-labeled complex and [^{3}H]DNA were bound (98.8%). However, when the solution of disrupted virus particles and free DNA was diluted 1:10 into salt solutions of increasing ionic strength, nonspecific binding of DNA was progressively reduced although complex was still retained maximally. Less than 0.5% of [^{3}H]DNA was bound at any concentration of sodium phosphate or NaCl greater than 0.3 M (each solution, of course, still contained 0.4 M Gdn·HCl). Potassium phosphate and KCl gave similar results (not shown). In a separate experiment (not shown), the mixture of disrupted virus and protein-free DNA was adjusted to give solutions ranging from 0.4 to 1.4 M Gdn·HCl. Complex was bound quantitatively at all concentrations of Gdn·HCl. However, nonspecific binding of free DNA was progressively reduced to a minimum of 12% as the concentration of Gdn·HCl increased from 0.4 to 0.7 M. Then, as the concentration exceeded 0.7 M, binding of DNA increased and became maximal at 0.9 M or higher (compare with Fig. 2). It is clear that the *total* salt concentration must be at least 0.7 M (i.e., 0.4 M Gdn·HCl plus 0.3 M NaCl, or 0.7 M Gdn·HCl alone) to prevent nonspecific binding of DNA by virion proteins (see *Discussion*).

Binding of the Adenovirus DNA–Terminal Protein Complex Is Mediated by Protein. ^{32}P-Labeled complex in solution was incubated for 30 min at 37°C with various concentrations of Pronase or Proteinase K and then filtered. Fig. 4 shows that progressively less complex bound to filters as the concentration of either enzyme increased. Less than 0.2% of ^{32}P-labeled complex was retained by filters when the concentration of either protease was greater than 50 μg/ml, and digested complex

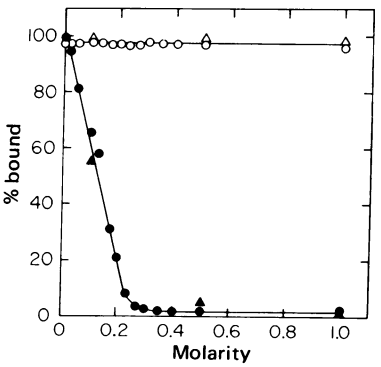

FIG. 3. Binding of the adenovirus DNA–terminal protein complex from virus particles disrupted with 4 M Gdn·HCl. A solution of ^{32}P-labeled adenovirus particles and Pronase-treated adenovirus [^{3}H]DNA (molar ratio, 3:1) was mixed with an equal volume of 8 M Gdn·HCl to disrupt the virus particles. The total DNA concentration in 4 M Gdn·HCl was 30 μg/ml. Samples were further diluted 1:10 into buffer designed to give the indicated concentration of NaCl or sodium phosphate (the final concentration of Gdn·HCl was 0.4 M in all samples) and filtered. ○, ^{32}P-Labeled complex bound after dilution in phosphate buffer; ●, [^{3}H]DNA bound after dilution in phosphate buffer; △, ^{32}P-labeled complex bound after dilution in NaCl; ▲, [^{3}H]DNA bound after dilution in NaCl.

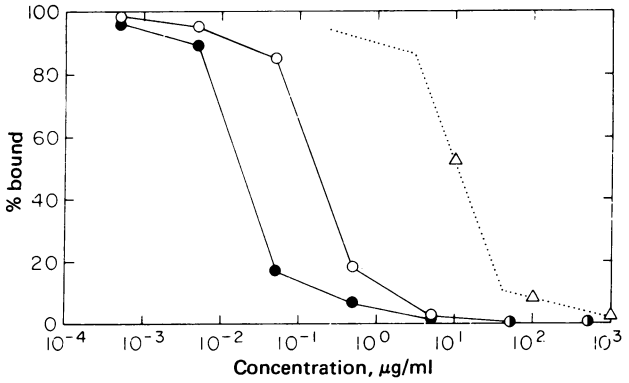

FIG. 4. Proteolytic digestion of the adenovirus DNA–terminal protein complex. ^{32}P-Labeled complex was incubated with the indicated concentration of Pronase (○) or Proteinase K (●) for 30 min at 37°C in TEN buffer. The reaction mixtures were diluted 1:10 into TEN buffer and filtered. In another experiment, microdot filters containing bound ^{32}P-labeled complex were covered with 25 μl of Pronase solution at the indicated concentrations in TEN buffer, incubated for 30 min at 37°C, and rinsed with 300 μl of TEN buffer (△).

now behaved in all respects like protein-free DNA. Proteinase K was 10 times more efficient on a weight basis, compared to Pronase, at removing protein from the complex: 200 ng of Pronase per ml was required to reduce binding to 50% compared to 20 ng of Proteinase K per ml. This result is most likely due to differences in the specific activities of the enzyme preparations. Because the link between the complex and the filter is protein, proteolytic digestion should release bound complex in the form of free DNA. Fig. 4 also shows that more than 98% of the ^{32}P-labeled complex was released from filters after digestion with Pronase at a concentration of 1 mg/ml. Pronase digestion on the filter was 1/50th as efficient as digestion in solution: 50% of the ^{32}P-labeled complex was removed at 10 μg of Pronase per ml.

The adenovirus DNA–terminal protein complex was efficiently eluted with $NaDodSO_4$ from filters in the form of complex. Fig. 5 shows that progressively more complex was eluted from filters as the concentration of $NaDodSO_4$ increased. More than 99% of bound ^{32}P-labeled complex was recovered with 1% $NaDodSO_4$ in TE buffer. $NaDodSO_4$ in TE buffer containing 0.1 M NaCl (or 0.3 or 0.5 M NaCl) was slightly more efficient (3-fold) at eluting complex compared to $NaDodSO_4$

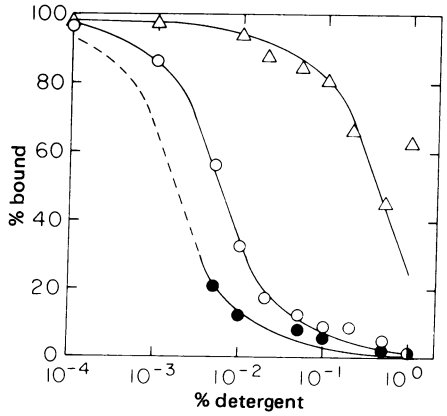

FIG. 5. Elution of the adenovirus DNA–terminal protein complex from glass fiber filters with detergents. ^{32}P-Labeled complex was bound to standard filters. Filters were rinsed with 5 ml of buffer containing the indicated concentrations of detergent. ○, $NaDodSO_4$ in TE buffer; ●, $NaDodSO_4$ in TE buffer containing 0.1 M NaCl; △, Sarkosyl in TE buffer.

in TE buffer alone. However, Sarkosyl was 1/70th as efficient as $NaDodSO_4$: 50% of ^{32}P-labeled complex was eluted with 0.4% Sarkosyl compared to 0.006% $NaDodSO_4$. To restore the affinity of $NaDodSO_4$-eluted complex for filters, solutions were diluted or dialyzed to give a $NaDodSO_4$ concentration lower than 0.005%. $NaDodSO_4$ was removed by chromatography on a Sepharose 2B-CL column in 4 M Gdn·HCl; 98.5% of the $NaDodSO_4$-free complex was retained by filters. Alternatively, solutions of complex in 0.005% $NaDodSO_4$ were adjusted to 0.5 M KCl, chilled to 0°C for 10 min, and centrifuged at 5000 rev/min for 5 min at 0°C to remove precipitated $NaDodSO_4$. The filters retained 96.5% of the complex in the supernatant fraction; the pellet contained only 1.9% of the complex. However, 93% of the ^{32}P-labeled complex was precipitated along with the $NaDodSO_4$ when 0.5 M KCl was added to a solution containing 1% $NaDodSO_4$.

Complex was also eluted from filters with 8 M urea. Four successive washes removed approximately 80% of the bound complex. Although urea released complex less efficiently than $NaDodSO_4$, urea may not interfere with the infectivity of the complex (17, 25).

Binding of the Adenovirus DNA–Terminal Protein Complex is Mediated by Terminal Protein. Table 1 demonstrates that, after digestion with *Bam*HI, *Eco*RI, *Sma*I, or *Hae* III restriction endonuclease, the percentage binding of restricted ^{32}P-labeled complex agreed very well with the expected values based on the sums of the sizes of terminal DNA fragments. Fig. 6 confirms that glass fiber filters selectively retained terminal restriction endonuclease fragments of the complex. ^{32}P-Labeled complex was cleaved separately with *Bam*HI (Fig. 6*A*) or *Sma* I (Fig. 6*B*) endonucleases and filtered. Fragments retained by the filters were removed with Pronase and analyzed by gel electrophoresis. Fragments in the filtrate were similarly analyzed. In each case, the terminal fragments were retained by filters while internal fragments passed through. Identical results were obtained after cleavage with *Eco*RI and *Hae* III endonucleases (not shown). Fragments bound to filters from the left end of the adenovirus genome ranged from 275 base pairs (bp) (*Hae* III) up to 20,300 bp (*Eco*RI), and fragments from the right end ranged from 320 bp (*Hae* III) up to 14,200 bp (*Bam*HI). We conclude from these experiments that terminal protein mediates binding of terminal restriction endonuclease fragments (and, by extension, intact complex) and that terminal protein is sufficient to anchor any length of DNA (at least up to 20,300 bp) to the filter.

DISCUSSION

A filter-binding assay using glass fiber filters has been developed to detect the adenovirus DNA–terminal protein complex. The assay is unusually sensitive because glass fiber filters generally bind less than 0.1% of protein-free DNA molecules. Binding of the adenovirus DNA–terminal protein complex to filters is mediated by terminal protein (Figs. 4 and 6; Table 1). Because the 55,000-dalton terminal protein (7) is covalently linked to both 5′ ends of linear adenovirus DNA (15), prior treatment of the complex with phenol, 8 M urea, 6 M Gdn·HCl, or 0.1% $NaDodSO_4$ at 80°C does not affect binding. The adenovirus complex binds maximally to filters in concentrations of salt greater than 65 mM Gdn·HCl or 200 mM NaCl (Fig. 2). By contrast, high concentrations of salt interfere with filter-binding assays for noncovalent nucleic acid–protein complexes containing proteins such as RNA polymerase (28) and the *lac* repressor (29) as well as with other types of filter-binding assays (26). Hence, the adenovirus complex can be assayed under conditions that disrupt noncovalent complexes. We have found that ionic complexes between protein-free DNA and virion proteins are bound to filters at concentrations of salt below 0.4 M but not at concentrations above 0.7 M (Figs. 1 and 3). Finally, protein-free DNA alone binds to filters in Gdn·HCl at higher than 0.8 M (Fig. 2). By varying the ionic conditions, we have achieved "all or none" modulation of these interactions.

Because all adenovirus proteins bind, at least qualitatively, to filters (not shown), it is likely that many other proteins may bind as well (26, 30). Thus, the filter-binding assay may prove useful to detect both naturally occurring as well as artificially induced covalent DNA–protein complexes. Such complexes include the chromosomes of some *Bacillus subtilis* bacteriophages (1, 4–6, 9, 10), plasmid relaxation complexes (31), the ϕX174 DNA-*cis*A protein complex (32, 33), and the complexes between DNA and nicking-closing enzyme (34) or DNA gyrase (35, 36). In this regard, we have preliminary evidence, obtained with our filter-binding assay, that proteins are very tightly, if not covalently, linked to cellular DNA in HeLa cells (27). These proteins, of as yet unknown function, are distributed at long range along cellular DNA molecules with an average spacing of 50,000 ± 5,000 bp.

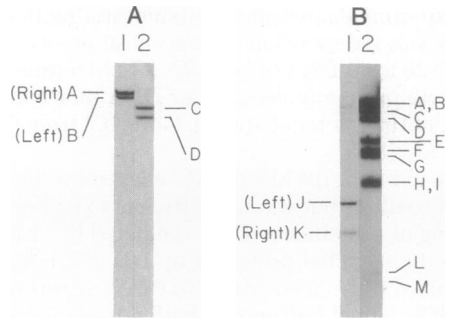

FIG. 6. Identification of terminal restriction endonuclease fragments. ^{32}P-Labeled adenovirus DNA–terminal protein complex in 100 μl of TE buffer containing 100 mM KCl/10 mM $MgCl_2$, pH 7.2, was incubated at 37°C with 10 μl of the indicated restriction endonuclease for 80 min. The reaction was continued for another 80 min after a second addition of 10 μl of enzyme. The reaction mixtures were adjusted to 0.3 M NaCl and filtered through microdot filters. DNA in the filtrate was precipitated with ethanol, suspended in 50 μl of TE buffer, and analyzed by gel electrophoresis. DNA fragments were recovered from the filter for electrophoresis by incubation for 30 min at 37°C in 25 μl of Pronase solution (1 mg/ml in TE buffer). Electrophoresis was for 4 hr at 50 V on a 0.3 × 10 cm 1% agarose-ethidium bromide slab gel. The gel was dried and autoradiated with Kodak XR-5 x-ray film using an intensifier screen. (*A*) *Bam*HI endonuclease. Lanes: 1, DNA bound to the filter; 2, DNA in the filtrate. (*B*) *Sma* I endonuclease. Lanes: 1, DNA bound to the filter; 2, DNA in the filtrate. When the sizes of large internal fragments exceeded by a factor greater than 10 the size of the smallest terminal fragment, we occasionally detected traces of large internal fragments. For example, 0.5% nonspecific binding of the *Sma* I A fragment would constitute 1/17 by mass (but 1/200 by number) of the *Sma* I K fragment.

Table 1. Binding of terminal restriction fragments

Enzyme	% bound	
	Expected	Observed
Control	(100)	97.8
*Bam*HI	70.1	66.3
*Eco*RI	68.8	64.6
Sma I	4.6	4.8
Hae III	1.7	2.0

^{32}P-Labeled complex was digested with the indicated restriction endonuclease as described in Fig. 6. Reaction mixtures were diluted 1:10 into 1 M sodium phosphate buffer and filtered. The percentage radioactivity bound was calculated. The expected value for each enzyme was calculated from the sum of the sizes of the terminal fragments (23, 24).

While this work was in progress we learned that a binding assay using cellulose nitrate filters had also been developed for the adenovirus complex (37). In our experience, the conditions for binding the adenovirus complex to cellulose nitrate filters were essentially identical to those used with glass fiber filters. However, the complex was attached to cellulose nitrate filters so tightly that it was difficult to remove it with detergent. We estimated that elution of complex with $NaDodSO_4$ was 1/50th as efficient compared to glass fiber filters. Protein-free DNA also bound to cellulose nitrate filters in Gdn·HCl at concentrations higher than 1 M, but the binding varied erratically (10–80%), even with filters from the same lot. We emphasize this result because the assay on cellulose nitrate filters uses 4 M Gdn·HCl (37).

We are indebted to Dr. A. J. Robinson for suggestions and help during the development of the filter-binding assay. H. Mark Engelking and Marda Brown provided excellent technical help. Dr. R. H. McParland purified the restriction endonucleases. Dr. R. J. Roberts suggested the use of the Sepharose column to purify the adenovirus complex. We thank Drs. B. W. Stillman and A. J. D. Bellett for a copy of their manuscript prior to publication. This research was supported by Grants NP-67D and NP-67E from the American Cancer Society and U.S. Public Health Service Grant CA 17699 from the National Cancer Institute. D.H.C. was supported in part by U.S. Public Health Service Postdoctoral Fellowship 5 F32 CA 05242.

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