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Biochemistry

Association of the adenovirus DNA-binding protein with RNA both *in vitro* and *in vivo*

(protein–RNA UV-crosslinking *in vivo*/nucleic acid binding domain)

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ABSTRACT **The multifunctional DNA-binding protein (DBP) encoded by human adenovirus binds RNA. The association of purified DBP with RNA *in vitro* was demonstrated by using either a gel filtration or a filter binding assay. This association is sensitive to ionic strength and exhibits no apparent sequence specificity. DBP also interacts with RNA *in vivo*; it can be crosslinked to polyadenylylated RNA by UV-irradiation of intact cells during the late phase of adenovirus infections. The 46-kDa carboxyl-terminal domain of DBP binds RNA *in vitro* and was found to be associated with polyadenylylated RNA *in vivo*. This is the same domain that interacts with DNA. However, the differences in sensitivity of DBP to trypsin when bound to RNA versus DNA suggest that RNA and DNA either bind at different sites within this domain or induce different conformational changes within the protein.**

The early region 2A gene (*E2A*) of human adenovirus types 2 and 5 (Ad2 and Ad5) encodes a 72-kDa DNA-binding phosphoprotein (DBP) (1, 2), which is synthesized both early and late during an infectious cycle (3, 4). This protein has a number of purported functions in infected cells. Analysis of DBP mutants has implicated the protein in viral DNA replication, viral early and late gene expression, and virion assembly.

Studies of a DBP temperature-sensitive (ts) mutant, Ad5ts125, provides evidence of the protein's involvement in DNA replication. This mutant encodes a DBP that is thermolabile in its ability to bind to single-stranded (ss) DNA *in vitro* (5) and, at the nonpermissive temperature, is defective for viral DNA synthesis *in vivo* (6) and *in vitro* (7).

The role of DBP in the regulation of Ad early gene expression is more controversial. DBP has been implicated in stimulating expression of early region 1B (*E1B*) (8) and early region 4 (*E4*) (9), while negatively influencing expression of early genes at intermediate and late times (10, 11). The purported negative regulation of early genes mediated by wild-type (wt) DBP appears to operate at the transcription level for *E4* (12, 13) and at the level of mRNA turnover for regions *E1A*, *E1B*, and probably *E2A* (14). However, studies by Rice and Klessig (15) with an *E2A* deletion mutant Ad5d1802, which does not make DBP yet exhibits a pattern of early gene expression very similar to that of wt Ad5, casts doubt on the role of DBP as a regulator of early gene expression.

DBP role(s) in controlling late gene expression is based on studies with monkey cells; wt Ad fails to productively infect monkey kidney cells (abortive infections) because of a complex block to late viral gene expression. This block includes a reduction in the rate of transcription of late genes (16), alterations in the pattern of mRNA splicing for the fiber polypeptide (17, 18), and poor utilization of this mRNA *in vivo* (19). Host-range (hr) mutants of Ad, which overcome these blocks and thus are capable of productive growth in monkey cells, contain alterations in the DBP gene (20–22).

Two general classes of DBP mutants have been described: (*i*) ts mutants, which are defective for viral DNA replication and early gene expression, typified by Ad5ts125; and (*ii*) hr mutants, which overcome the block to late gene expression in monkey cells—e.g., Ad2hr400. Physical mapping of these two classes of mutations suggests that the DBP contains two functionally distinct domains (21–24). The ts, DNA replication-minus mutations are located in the 3′ two-thirds of the gene, while the hr mutations map in the 5′ portion of the gene.

These functional domains appear to correspond to physical domains because digestion of purified DBP with a variety of proteases generates a C-terminal fragment of ≈44–46 kDa and an N-terminal fragment of ≈24–26 kDa (2, 25). The C-terminal fragment binds ss DNA (2) and can substitute for intact DBP in an *in vitro* DNA replication system (26). The N-terminal region also appears to function independently because DBP encoded by the hr mutants is able to overcome the block to late gene expression in monkey cells, even when its DNA replication function is perturbed by a ts mutation in the C-terminus (27).

How the DBP accomplishes this varied set of purported functions is still poorly understood. No enzymatic functions have been attributed to it, and only its binding to DNA has been biochemically defined. Some of the phenotypic characteristics of both ts and hr DBP mutants suggest that this polypeptide might affect mRNA synthesis, processing, and/or utilization. These effects on mRNA could be mediated through association of DBP with RNA. This paper provides evidence both *in vitro* and *in vivo* of such a direct interaction between DBP and RNA.

MATERIALS AND METHODS

Nucleic Acids. Phage MS2 [^{3}H]RNA was a gift from P. Hine. Phage M13 ss [^{32}P]DNA was prepared *in vivo* by the procedure of Rivera *et al.* (28) for M13 RNA, with the exception that labeling was allowed to proceed for 4 hr. Both labeled and unlabeled ss M13 DNA were isolated as described (22).

Purification of DBP. DBP was isolated by a modification of the procedure of Schechter *et al.* (25). HeLa cells (12 liters) at 36–40 hr after infection with 20 plaque-forming units per cell of Ad2 were harvested by centrifugation at 600 × g (4°C) followed by two washes with phosphate-buffered saline

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Abbreviations: DBP, virus-encoded DNA-binding protein; Ad, adenovirus; Ad2 and Ad5, Ad types 2 and 5; ts, temperature sensitive; hr, host range; wt, wild type; ss, single stranded; *E*, early region gene.

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(PBS; ref. 20). Cells were resuspended and allowed to swell 15 min (0°C) in 200 ml of buffer D (10 mM sodium phosphate, pH 7.0/10 mM NaCl/1.5 mM $MgCl_2$/0.5 mM dithiothreitol/30 μg of phenylmethanesulfonyl fluoride per ml) before homogenizing with 20 strokes of a Bellco pestle type B (0°C). Nuclei were collected by sedimentation at 500 × *g* for 8 min (4°C) and washed with buffer D. DBP was extracted from nuclei by resuspension in 100 ml of buffer E (10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.5 mM dithiothreitol) containing 0.6 M LiCl and 30 μg of phenylmethylsulfonyl fluoride per ml, followed by rocking vigorously for 30 min (4°C). Nuclei were removed by sedimentation at 17,000 × *g* for 10 min (4°C), and the extraction was repeated. DBP from the pooled supernatants was precipitated by using a 32–55% ammonium sulfate cut. The precipitate was dissolved in buffer E containing 2 M NaCl and 7% (vol/vol) glycerol, dialyzed against the same solution with 0.15 M NaCl, clarified by centrifugation, and chromatographed on a 1.5 × 13 cm phosphocellulose column (Whatman P-11). Peak-DBP-containing fractions (0.25–0.3 M NaCl) eluted with a 0.15–1 M linear NaCl gradient were pooled, dialyzed against buffer A (25 mM Tris·HCl, pH 7.4/1 mM EDTA/0.5 mM dithiothreitol/20% glycerol) containing 0.1 M NaCl and 2 μg of aprotinin per ml, and applied to a 1 × 13 cm ss DNA-cellulose column. ss DNA-cellulose was prepared from Bio-Rad Cellex 410 and calf-thymus DNA (Sigma) as described by Alberts and Herrick (29). DBP, which was eluted at 0.45–0.5 M NaCl in a 0.1–1 M linear NaCl gradient, was stored at −70°C.

Standard *in Vitro* Binding Reaction. Purified Ad2 DBP, either intact or proteolytically cleaved with chymotrypsin, was incubated with RNA or DNA for 20 min (0°C) in TE buffer (10 mM Tris, pH 7.4/1 mM EDTA) containing variable concentrations of NaCl.

Nitrocellulose Filter Binding Assays. Nitrocellulose membranes (6-mm diameter; BA85, Schleicher & Schuell) were prepared for use by boiling in water for 10 min and then soaking overnight at 4°C in TE buffer of the appropriate ionic strength. Binding reactions were filtered through the nitrocellulose membranes by gravitation at 4°C, and the membranes were then washed 10 times with the same binding buffer and air-dried. The amount of labeled nucleic acid retained on the membrane was determined by assay in Econofluor (New England Nuclear).

Gel Filtration Chromatography. Approximately 20 μg of purified DBP, either intact or proteolytically cleaved with chymotrypsin, was incubated with 20 μg of MS2 [^{3}H]RNA or ss M13 [^{32}P]DNA in a standard binding reaction (0.16-ml volume) and then chromatographed on a preequilibrated Sepharose CL-6B (Pharmacia) column (5 × 200 mm bed volume) at 4°C or 23°C. The TE buffer having ionic strengths corresponding to those in the binding reaction was used for the preequilibration of and elution from columns. Elution behavior of DBP and nucleic acids was monitored by collecting successive 0.5-ml fractions, which were then subjected to (*i*) radioactive quantitations by assay in Aquasol (New England Nuclear), (*ii*) protein quantitation with the Bio-Rad protein assay, or (*iii*) protein separation on $NaDodSO_4$/15% polyacrylamide gels with Coomassie blue staining. Samples were prepared for polyacrylamide gels by acetone precipitation using 4 μg of soybean trypsin inhibitor as carrier.

Chymotrypsin Proteolysis of DBP. Approximately 20 μg of purified Ad2 DBP (300 μg/ml) was incubated with chymotrypsin (3 μg/ml; Sigma) at 37°C for 5 min in TE buffer containing 100 mM NaCl. Proteolysis was stopped by the addition of tosylamide-2-phenylethyl chloromethyl ketone to 50 μg.

UV-Crosslinking of DBP to RNA. Mock- or Ad5-infected HeLa cell monolayers (100-mm plates) 24 hr after infection were irradiated and solubilized as described by Dreyfuss *et al.* (30, 31) with the following modifications. After UV-irradiation, cells were scraped from plates, pelleted, and resuspended for 5–10 min (0°C) in high-salt RSB (10 mM Tris·HCl, pH 7.4/1 mM EDTA/1.5 mM $MgCl_2$/1 M NaCl) supplemented with 10 μg of aprotinin, 1 μg of leupeptin, and 1 μg of pepstatin A per ml. Cells were solubilized by addition of Empigen BB (Albright and Wilson, Old Tappan, NJ) and Nonidet P-40 to final concentrations of 1% each for 5–10 min (0°C). Viscosity was reduced by addition of DNase I [DPFF grade, Worthington, further treated with iodoacetate (32)] to 50 μg/ml, $MgCl_2$ to 10 mM, and vanadyl ribonucleoside complex (Bethesda Research Laboratories) to 10 mM with subsequent incubation at 37°C for 20 min. Finally, the extracts were passed through a 25-gauge needle four times.

Polyadenylylated RNA was selected as described by Dreyfuss *et al.* for their nuclear fraction (31), with the exception that LiCl was added to 750 mM and EDTA to 2 mM before oligo(dT) chromatography. RNA-associated proteins, after release with RNase, were concentrated by precipitation with acetone before being subjected to immunoblot analysis on 10% polyacrylamide gels as described (33), with the modification that nonfat dried milk was used in place of bovine serum albumin (34).

RESULTS

Association of DBP with RNA *in Vitro*. The association of DBP with RNA *in vitro* was demonstrated by using both a nitrocellulose filter binding assay and a gel filtration chromatography assay. The filter binding assay has been used previously to study the binding of DBP to DNA (25, 35). We used it to demonstrate directly the interaction of DBP with a variety of RNAs in addition to DNA. Fig. 1 shows the results of treating approximately equal amounts of phage MS2 RNA or phage M13 ss DNA with increasing concentrations of purified DBP.

DBP binding to RNA showed both similarities and differences to its association with DNA. Approximately equal amounts of each nucleic acid could be retained on the filter at low ionic strengths. Moreover, as with DNA, DBP

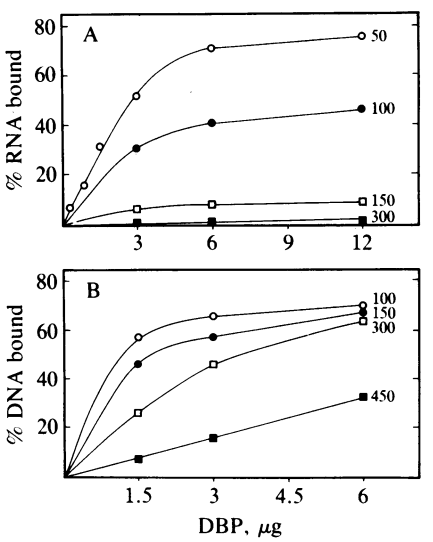

FIG. 1. Interaction of Ad2 DBP with ss RNA or DNA at different ionic strengths. A constant amount of labeled nucleic acid (0.5 μg) was incubated with different amounts of purified Ad2 DBP in a standard binding reaction. The resultant DBP–nucleic acid complexes were collected on nitrocellulose filters and quantitated by liquid scintillation analysis. Values are expressed as the percentage of total labeled nucleic acid retained on filters. The different binding curves generated with various millimolar concentrations of NaCl indicated by each curve are shown for MS2 [^{3}H]RNA (*A*) or for M13 [^{3}P]DNA (*B*). Note the difference in DBP concentration scales.

appeared to associate with RNA in a nonsequence-specific manner, since total cytoplasmic RNA isolated from mock- or Ad2-infected cells either during the early or during late phases of infection gave binding profiles similar to that observed for MS2 RNA (data not shown). However, in general a higher concentration of DBP was required to obtain maximum binding with RNA than with DNA. Furthermore, the association of DBP with RNA was much more influenced by ionic strength than is its binding of DNA. For example, in 100 or 150 mM NaCl, RNA binding was reduced to ≈60% and ≈15% of that seen in 50 mM NaCl and effectively ceased in 300 mM NaCl. In contrast, in 150, 300, and 450 mM NaCl, the amount of DNA associated with DBP was approximately 95%, 90%, and 75%, respectively, of that observed in 100 mM NaCl.

The association of DBP with RNA also was demonstrated by using gel filtration chromatography. In this assay, the nucleic acids (MS2 [^{3}H]RNA or M13 [^{32}P]DNA) and any associated protein were excluded from the Sepharose CL-6B matrix, while free protein was included (Fig. 2*B*). In low-ionic-strength buffer (50 mM NaCl), the majority of the DBP coeluted in the void volume with MS2 RNA (Fig. 2*A*) or M13 ss DNA (data not shown), indicating that most of the purified DBP is capable of binding either of the nucleic acids. To ensure the specificity of the assay, bovine serum albumin was substituted for DBP. It did not coelute with RNA or DNA but was found in the included volume (data not shown).

Again the binding of RNA, by this assay, was more salt-dependent than was DNA binding. The greater salt sensitivity of RNA binding probably reflects a higher dissociation rate rather than a lower association rate because, when the DBP was treated with RNA in 150 mM NaCl at 0°C and then subjected to gel filtration at different temperatures, little, if any, DBP remained associated with the RNA at 23°C, whereas at 4°C the majority of DBP comigrated with the RNA.

UV-Crosslinking of DBP to RNA *in Vivo*. Since RNA closely resembles ss DNA, the association of this ss DBP with RNA *in vitro* could be artifactual. Thus, it was essential to demonstrate that such an interaction occurs *in vivo*. This was achieved by crosslinking DBP to RNA in intact cells with UV-irradiation. Mock- or Ad5-infected HeLa cell monolayers were UV-irradiated at hour 24 postinfection. Cells were then solubilized and treated with DNase I to reduce viscosity. Polyadenylylated RNA was selected by two rounds of oligo(dT) chromatography in the presence of the denaturant $NaDodSO_4$. Protein tightly (covalently) bound to the polyadenylylated RNA was released by RNase A and micrococcal nuclease treatment and subjected to immunoblot analysis with anti-DBP serum. A small portion of the total DBP was found covalently attached to polyadenylylated RNA after UV-irradiation (Fig. 3, lanes 4–7). This crosslinking was specific to RNA because treatment with RNase, but not with DNase, prior to oligo(dT) chromatography eliminated association of DBP with the oligo(dT)-selected material (Fig. 3, lane 3).

The C-Terminal 46-kDa Fragment of DBP Interacts with RNA. To determine which domain(s) of DBP interacts with RNA, purified DBP was digested with chymotrypsin to cleave it into a 46-kDa C-terminal fragment and a 24-kDa N-terminal fragment. Only the 46-kDa C-terminal fragment of DBP was associated with RNA and, thus, was eluted in the void volume of the Sepharose CL-6B column (Fig. 4). This is the same domain that has been shown to bind DNA (2). The binding of the 46-kDa fragment to RNA shows the same salt dependence as does intact DBP (data not shown). Moreover, the cleaved DBP exhibited RNA binding activity similar to that of the intact polypeptide in a filter binding assay (data not shown).

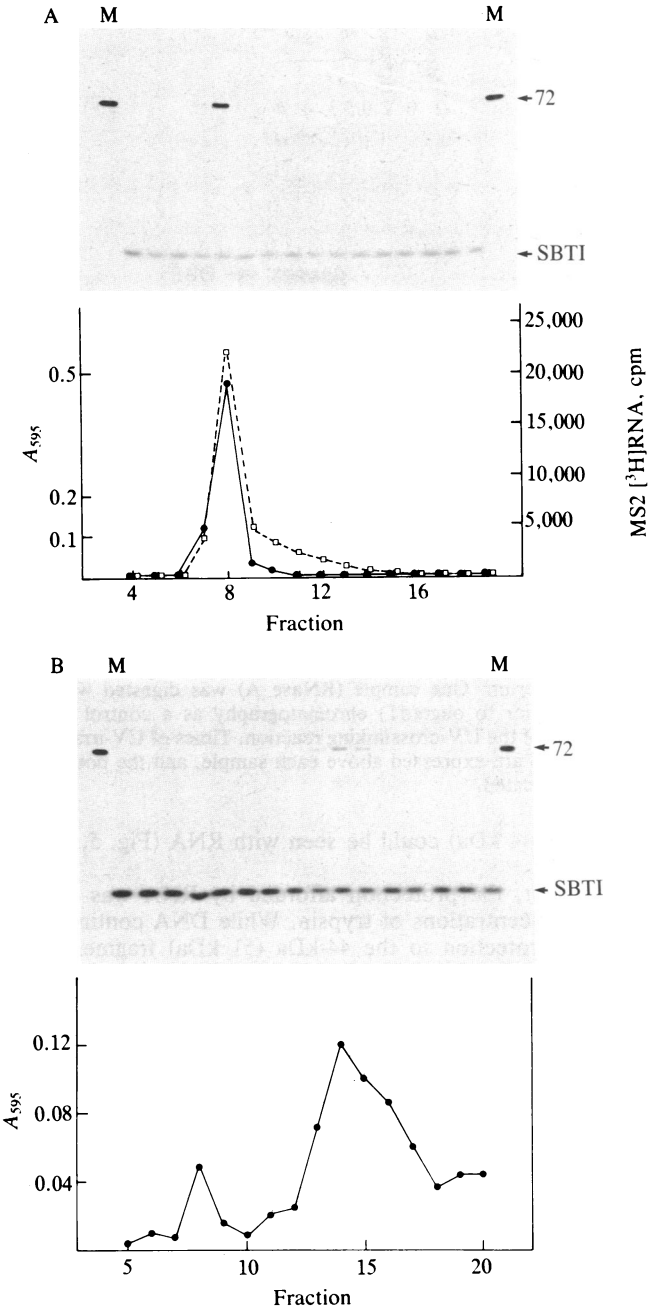

FIG. 2. Gel filtration analysis of DBP–RNA complexes. (*A*) Phage MS2 [^{3}H]RNA (20 μg) was incubated with purified Ad2 DBP (20 μg) in a standard binding reaction in the presence of 50 mM NaCl. The reaction was then subjected to gel filtration column chromatography at room temperature as described. (*A Upper*) Coomassie brilliant blue-stained $NaDodSO_4$/polyacrylamide gel of aliquots of each column fraction. The positions of the 72-kDa DBP and carrier soybean trypsin inhibitor (SBTI) are indicated to the right of the gel. (*A Lower*) Elution profiles of MS2 [^{3}H]RNA (□) and protein (●). (*B*) Purified Ad2 DBP (20 μg) was treated as in *A* except that no RNA was added to the reaction (note the difference in adsorbance scales).

Possible Conformation Differences in DBP Bound to RNA Versus DNA. A further comparison of the binding of DBP to RNA and DNA was performed by using mild trypsin digestion of DBP in the presence or absence of associated DNA or RNA. Schechter *et al.* (25) showed that, when intact 72-kDa DBP is bound to DNA, mild digestion with trypsin generates a 51-kDa fragment that is more resistant to further degradation to a 35-kDa fragment than in the absence of bound DNA. Similar protection of the 51-kDa fragment (which in our gel

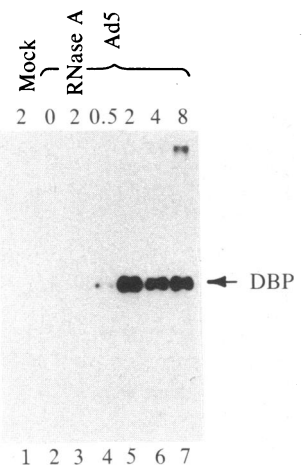

FIG. 3. UV-crosslinking of DBP to polyadenylylated RNA *in vivo*. Mock- or Ad5-infected HeLa cell monolayers were subjected to UV-irradiation at hour 24 postinfection. The cell contents were solubilized with detergent and high salt, treated with DNase to reduce viscosity, and chromatographed twice on oligo(dT)-cellulose in the presence of the denaturant $NaDodSO_4$. Polyadenylylated RNA and covalently associated material were eluted from the column, digested with RNases, and subjected to immunoblot analysis with anti-DBP serum. One sample (RNase A) was digested with this nuclease prior to oligo(dT) chromatography as a control for the specificity of the UV-crosslinking reaction. Times of UV-irradiation (in minutes) are expressed above each sample, and the position of DBP is indicated.

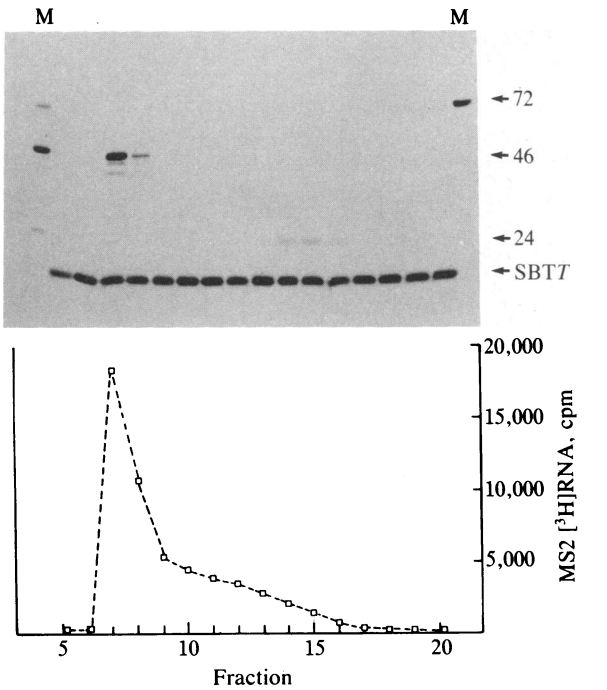

FIG. 4. Complex formation between the C-terminal fragment of DBP and RNA. Purified Ad2 DBP (20 μg) was proteolytically cleaved with chymotrypsin to separate the protein into a 46-kDa C-terminal fragment, and a 24-kDa N-terminal fragment. The mixture of DBP fragments were then incubated in the presence of phage MS2 [^{3}H]RNA (20 μg) in a standard binding reaction in the presence of 50 mM NaCl. Protein fragments bound to RNA were separated from unbound protein by gel filtration column chromatography. (*Upper*) Coomassie brilliant blue-stained $NaDodSO_4$/polyacrylamide gel of aliquots from each column fraction. Intact DBP and chymotrypsin-generated fragments were run in lanes M as standards and are indicated in kDa. Soybean trypsin inhibitor (SBTI) was used as carrier. (*Lower*) Elution profile of phage MS2 [^{3}H]RNA. In the absence of RNA, the 46-kDa fragment and the 26-kDa fragment were included in the column (data not shown).

system is 44 kDa) could be seen with RNA (Fig. 5, traces A–C).

However, the protection afforded by RNA was lost at higher concentrations of trypsin. While DNA continued to provide protection to the 44-kDa (51 kDa) fragment, the bound RNA did not and actually decreased the stability of the 35-kDa fragment under these more rigorous digestion conditions (Fig. 5, traces D–F). These results were consistently found with a variety of ss DNAs (i.e., M13, pUC119, denatured calf thymus, and denatured Ad2 DNAs) and RNAs (i.e., MS2 RNA, globin mRNA, and cytoplasmic RNA from Ad-infected cells).

DISCUSSION

Previous studies with DBP mutants suggest that this protein might carry out some of its functions via direct binding of RNA. This association has been demonstrated both *in vitro* and *in vivo*. Two independent *in vitro* binding assays (filter binding and gel filtration) document the affinity of DBP for RNA. More importantly, DBP–RNA complexes form *in vivo* as shown by UV-crosslinking studies. While only a small percentage (≈0.1%) of DBP was crosslinked to polyadenylylated RNA, this number probably reflects the low efficiency of crosslinking of DBP to RNA via UV-irradiation rather than the actual amount of DBP associated with RNA. For example, it was difficult to demonstrate crosslinking of purified DBP to RNA *in vitro*, despite the fact that the majority of the protein was associated with RNA as shown by gel filtration. The crosslinking of a small amount of DBP to RNA has also been observed by other investigators (ref. 40; J. Nevins, personal communication).

The association of DBP *in vitro* with RNA or with DNA is similar but not identical. Both nucleic acids are bound with no apparent sequence specificity. Moreover, the 46-kDa C-terminal fragment of DBP, known to bind DNA (2), is also responsible for RNA association. This same domain seems to associate also with RNA *in vivo*, since the 46-kDa DBP fragment was found to be crosslinked to polyadenylylated RNA via UV-irradiation (data not shown). However, it is not known if this fragment normally binds to RNA in a manner independent of the N terminus, as has been demonstrated *in vitro*, or whether proteolysis of the intact protein occurs after RNA binding, perhaps during the fractionation procedures. Since the 35-kDa fragment, generated by continued trypsin digestion of the 44-kDa polypeptide, is much more sensitive to complete cleavage with trypsin in the presence than in the absence of RNA, the 35-kDa fragment must also interact with RNA.

DBP showed several differences in its binding to RNA versus DNA. The ability to discriminate between these two nucleic acids may provide a mechanism by which DBP performs its varied functions, as has been demonstrated for the gene *32* protein of bacteriophage T4 (36). DBP showed different sensitivity to cleavage by trypsin when bound to RNA compared to DNA. This suggests either that the RNA and DNA binding sites are different or that their binding induces different conformational changes within the protein. The association of DBP with RNA *in vitro* was also much more sensitive to ionic strength than was its binding to DNA. Similar salt dependencies for protein–RNA interactions have been observed by others (37). Perhaps noteworthy is the observation that binding of DNA by ts DBP is more sensitive to ionic strength at the nonpermissive temperature than is DNA binding by wt DBP (38).

Potential differences between RNA and DNA binding were suggested by earlier studies of abortive infections of monkey cells using the mutant Ad2ts400, which contains both a hr

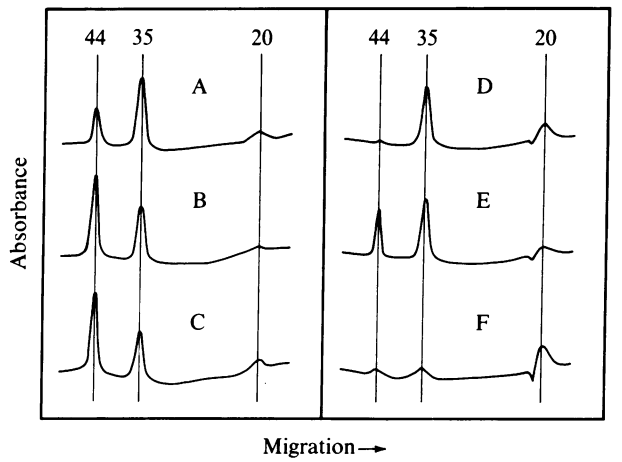

FIG. 5. Effects of bound RNA or DNA on the cleavage of Ad2 DBP by trypsin. Approximately 4 μg of purified Ad2 DBP was incubated with 4 μg of either RNA or DNA in a standard binding reaction in the presence of 50 mM NaCl (30-μl total volume). After binding, different concentrations of trypsin were added to the reactions, and proteolysis was allowed to proceed for 15 min at room temperature. The proteolytic digestion was stopped by adding 10% $NaDodSO_4$/2% 2-mercaptoethanol and heating the reaction mixtures at 70°C for 10 min. Products were analyzed by $NaDodSO_4$/15% PAGE. Gels were stained with Coomassie brilliant blue and photographed. The tracings shown above were made with a Joyce–Loebl Chromoscan 3 densitometer from photographic negatives. The molecular weights (kDa) of the major tryptic fragments obtained by using a 1:100 weight ratio of trypsin to DBP (traces A–C) or a 1:50 weight ratio of trypsin to digest DBP (traces D–F) in the absence of nucleic acid (traces A and D), in the presence of M13 ss DNA (traces B and E), or in the presence of globin mRNA (traces C and F) are indicated at the top of the figure.

mutation and a ts DNA-replication-negative mutation in DBP. After entry into the late phase of Ad2ts400 infection of monkey cells at the permissive temperature, the DNA binding and replication function of the ts DBP located in the C terminus could be perturbed by shifting to the nonpermissive temperature. However, the hr mutation, located in the other domain (N-terminal) of the protein, continued to relieve the block to viral late gene expression (27). If DBP must associate with RNA to carry out its role in late gene expression, then these results suggest that the binding of RNA is not significantly altered by the ts mutation.

The significance of DBP's association with RNA is unclear. Studies with DBP hr mutants, which efficiently multiply in monkey cells, indicate that changes in this protein can directly or indirectly alter the patterns of splicing of late viral transcripts—in particular, those processed into fiber mRNA (17, 18). Moreover, the translatability of the fiber mRNA *in vivo* is affected by these hr mutations, which may or may not be a consequence of the altered structure of the message. The hr mutations, however, reside in the N-terminal 24-kDa domain, whereas RNA binding is localized to the C-terminal, 46-kDa domain. Thus, while in some assays these two domains function independently (7, 27), this independence is not complete.

That the two domains may interact to carry out some of the roles of this multifunctional polypeptide is suggested by several other observations. First, ts mutations located in the C-terminal domain affect phosphorylation of the N-terminal region (39). Second, the level of phosphorylation of the N-terminal region alters the DNA binding activity of the protein, which is located in the C-terminal domain (2). Third, hr mutations in the N terminus depress the elevated efficiency of morphological transformation of rodent cells exhibited by mutants with a ts lesion in the C-terminal domain (S. A. Rice, D.F.K., and J. Williams, unpublished results). Fourth, a deletion mutant (Ad5d1804) that encodes a truncated DBP-related protein that contains the entire N-terminal domain derived from an hr DBP mutant fails to relieve the block to late viral gene expression in monkey cells coinfected with wt Ad (15). In this coinfection the other essential DBP functions should be provided by the wt DBP. Thus, if DBP's association with RNA is essential for viral late gene expression, then it appears that both domains are required to carry out its role in RNA processing and/or utilization.

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