JOURNAL OF VIROLOGY, Dec. 1994, p. 8288–8295
0022-538X/94/$04.00+0

Vol. 68, No. 12

Stimulation of the Adenovirus Major Late Promoter In Vitro by Transcription Factor USF Is Enhanced by the Adenovirus DNA Binding Protein

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Received 13 June 1994/Accepted 21 September 1994

Previous studies have shown that the sequence-independent adenovirus DNA binding protein (DBP) increases transcription from several promoters, notably from the adenovirus major late promoter (MLP) and the adeno-associated virus P5 promoter, both of which contain a USF/MLTF binding site. In order to study this mechanism, we have investigated the effects of DBP on the binding of USF/MLTF to MLP and on transcription from MLP by a reconstituted in vitro system. As shown by gel retardation and DNase I footprinting, upon saturation of DNA, DBP enhances the binding affinity of USF^{43} to the promoter three- to fourfold without changing the footprint pattern. In contrast, the binding of the TATA box binding protein to the promoter is not influenced by DBP. No protein-protein interactions between DBP and USF^{43} could be observed in the absence of DNA, suggesting that enhanced binding is caused by a change in DNA structure induced by the DBP-DNA complex. Employing a transcription system reconstituted with purified general transcription factors, we show that USF^{43} enhances basal transcription and that USF^{43}-dependent transcription is further increased by DBP, while DBP alone does not have an effect on basal transcription. Our results suggest that transcription enhancement by DBP is based on a specific increase in the binding of a transcription factor to a promoter through subtle changes in DNA structure, similar to the mechanism by which DBP stimulates the initiation of DNA replication.

One of the major transcription factors involved in adenovirus late transcription is the cellular protein upstream stimulatory factor USF (MLTF) (4, 10, 25–27, 34–36, 52). It can bind to the adenovirus major late promoter (MLP) upstream sequence between −63 and −58, relative to the transcription start site, and activate transcription from MLP (10, 34, 35). In addition, USF is also involved in regulating the expression of several cellular genes, such as the human growth hormone gene (31), the mouse metallothionein I gene (5), and the rat γ-fibrinogen gene (9). Furthermore, a USF binding site can be found in a region encompassing the putative human origin of replication B48 (13, 15).

USF consists of two polypeptides with molecular masses of 43 and 44 kDa (36). Both polypeptides show independent DNA binding, and each binds either as a homo- or heterodimer to the palindromic CACGTG motif (34). The 43-kDa component (USF^{43}) is a member of the basic region helix-loop-helix leucine repeat (B-HLH-LR) class of transcription factors (16). Deletion mutagenesis identified two domains N terminal of the B-HLH-LR domain (amino acids 15 to 59 and 93 to 156) that contribute to transcriptional activation (20). The 44-kDa component is less well characterized. Recently, a full-length cDNA encoding USF^{44} was cloned, showing that USF^{44} and USF^{43} are members of the same protein family with highly conserved DNA binding and dimerization domains but quite divergent N-terminal amino acid sequences (37). A comparison of the protein sequence with previously published sequences showed that USF^{44} is identical to Fos interacting protein (1).

USF bound to its consensus recognition site upstream of the TATA box can stimulate transcription, but it can also bind to initiator elements encompassing the transcription start site and transactivate transcription (14, 24, 33). In in vitro transcription assays, crude preparations of USF can stimulate transcription up to 10-fold. As a result of purification, part of its activating potential is lost, and highly purified preparations can only activate transcription approximately threefold (34). Recombinant USF^{43} is also able to activate transcription in a manner indistinguishable from that of highly purified USF (32). USF interacts directly with the TFIID complex and binds DNA synergistically with this complex (34, 35). In the presence of TFIID, USF inhibits nucleosome assembly on promoter sequences and thereby facilitates the formation of preinitiation complexes during in vitro chromatin assembly (51). During adenovirus infection, the activation of transcription by USF requires DNA replication, which suggests that early in infection, adenovirus chromatin is not accessible to USF (45). Early in infection, adenovirus DNA is still complexed to protein VII, whereas late in infection, the situation is different and DNA may be complexed with the adenovirus DNA binding protein (DBP) (8).

DBP is a multifunctional protein of 529 amino acids that consists of two domains (22). The N-terminal domain (amino acids 1 to 173) is not well conserved among serotypes and harbors the nuclear localization signal (12, 28). The C-terminal domain is well conserved, contains DNA binding properties, and harbors most of the biological functions ascribed to DBP (21).

DBP binds RNA, single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA) in a sequence-independent man-

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ner. Binding to ssDNA is cooperative, and the monomeric binding site is 11 to 15 bases, varying slightly for different polynucleotides (23). Recently, the crystal structure of DBP was elucidated and DBP was shown to be a roughly globular protein with a striking 17-amino-acid C-terminal extension. This C-terminal hook can interact with a neighboring DBP molecule, which can lead to the formation of a protein chain. The deletion of this hook destroys cooperativity of ssDNA binding (46). Binding to dsDNA is not cooperative, and both association and dissociation are very rapid (39). Hydroxyl radical footprinting and electron microscopy show that DBP changes the structure of dsDNA. This DNA acquires a rigid structure; at the same time, DBP introduces changes in base-to-base positions and is able to remove higher-order structure from DNA. Cryoelectron microscopy suggests that under saturating conditions, DBP may form two interwound chains around the DNA helix (39).

DBP is intimately involved in the viral life cycle. It functions in both the initiation and elongation phases of DNA replication (for reviews, see references 18, 38, and 47), in virus assembly, in the stability of mRNA, and in the replication of adeno-associated viruses (for reviews, see references 7, 18, 19, and 48). DBP is also involved in transcriptional regulation. DBP is able to enhance its own synthesis. Mutant analysis suggests that DBP enhances its own expression only when it is present in a highly phosphorylated form (29). By in vitro transcription runoff assays and transfection assays, DBP was found to specifically repress transcription from the adenovirus E4 promoter (6, 17). Furthermore, transfection experiments with DBP expression constructs showed that DBP enhances the expression of reporter genes controlled by several different adenovirus or adeno-associated virus promoters, with the strongest effect on genes controlled by the adenovirus MLP and the adeno-associated virus P5 promoter, both of which contain a USF binding site (6). The mechanism by which DBP modulates transcription is unknown.

We and others reported previously that DBP is able to enhance the binding of the cellular transcription factor nuclear factor I (NFI) to its recognition site in the adenovirus auxiliary origin of replication, which leads to enhanced initiation of DNA replication in the presence of DBP (11, 40). In this work, we investigated the influence of DBP on transcription from the adenovirus MLP. We find that DBP enhances the binding of USF^{43} to its recognition site upstream of the adenovirus MLP. Furthermore, we show that DBP does not interact directly with USF^{43}, suggesting that the stimulation of USF binding is the result of structural alterations of the binding site induced by DBP. Finally, we show that DBP enhances the stimulation of transcription by USF^{43} in a reconstituted in vitro transcription assay.

MATERIALS AND METHODS

Purification of proteins. The coding region of USF^{43} was cloned 3′ of the glutathione *S*-transferase region in the bacterial expression vector pGEX2T (13a). The resulting expression plasmid pGST-USF43 was transformed into the SF8 strain of *Escherichia coli*. Transformed bacteria (500 ml) were grown at 37°C until they reached an optical density at 600 nm of 0.6. Next, protein expression was induced by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) (final concentration, 1 mM). After 4 h, the culture was centrifuged at 5,000 × *g* for 5 min at 4°C, the bacterial pellet was resuspended in 12.5 ml of 50 mM Tris-HCl (pH 8.0)–1 mM EDTA–0.4 mM $Na_2S_2O_5$–4 mM dithiothreitol (buffer A), and 10% (wt/vol) sucrose was added. The solution was frozen and thawed once, lysozyme was added to 0.25 mg/ml, and the solution was frozen and thawed twice more before being sonicated three times for 30 s each. NaCl was added to 500 mM, and the solution was centrifuged at 35,000 rpm in a Sorvall SW41 rotor for 90 min at 4°C. The supernatant was diluted to 150 mM NaCl with buffer A, and 2 ml of 50% glutathione-agarose (GA) beads, equilibrated in buffer A and containing 150 mM NaCl, was added. Binding was for 60 min at 4°C on a rotating wheel. Subsequently, the slurry was packed in a column, washed, and eluted with 20 mM glutathione in buffer A. DBP was purified as described previously (53). Both proteins were more than 95% pure as judged by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Coomassie staining.

The TATA box binding protein (TBP) preparation used in gel retardation assays contained recombinant, histidine-tagged TBP, which was purified by Ni^{2+}-NTA chromatography (44) and was 30 to 50% pure as judged by Coomassie staining.

The coding region of the DNA binding domain of NFI (NFI-BD) was cloned in the bacterial expression vector pET-15b. Histidine-tagged NFI-BD (His-NFI-BD) was purified by Ni^{2+}-NTA chromatography and was homogeneous.

Gel retardation. Binding reactions were carried out on ice or at room temperature for 60 min in a total volume of 20 μl containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.5), 60 mM NaCl, 2 mM $MgCl_2$, 10% glycerol, 50 μg of bovine serum albumin (BSA) per ml, 10,000 cpm of TATA or UBS DNA, and indicated amounts of proteins. UBS DNA consisted of the 140-bp *Pvu*II-*Xba*I fragment from pMLTF (3), which was Klenow end labelled with [α-^{32}P]dCTP. TATA DNA consisted of the 133-bp *Eco*RI-*Hin*dIII fragment from pBS-MLP, which was Klenow end labelled with [α-^{32}P]dATP. pBS-MLP contains the MLP sequence from −51 to +33 cloned in the *Sma*I site of pBS$^-$. After 60 min, 2.5 μl of loading buffer containing 0.09% Nonidet P-40 was added. When USF^{43} binding was assayed, 400 ng of poly(dI-dC) · poly(dI-dC) was added to the loading buffer added to the sample at the moment of loading the sample onto the gel. Since DBP dissociates rapidly from dsDNA (39), this procedure prevents the occurrence of shifted bands because of DBP binding without affecting USF^{43} binding. When TBP binding was assayed, no poly(dI-dC) · poly(dI-dC) was added, since this addition may also affect TBP binding. The binding conditions for both USF^{43} and TBP were identical up to the moment of loading, and therefore, the results can be compared directly. When DBP binding was assayed, UBS DNA was denatured by boiling for 2 min. Free DNA and protein-DNA complexes were resolved on a native 5% polyacrylamide gel which was run in 0.5× Tris-borate-EDTA buffer–0.01% Nonidet P-40 for 2 h at 8 V/cm. Gels were subsequently dried and autoradiographed. Results were quantified with an LKB Ultrascan XL gel scanner.

DNase I footprinting. Binding reactions were carried out at room temperature for 60 min in the same buffer used for gel retardation, containing 10,000 cpm of UBS DNA and indicated amounts of proteins. After 60 min, 0.1 U of DNase I together with 250 ng of poly(dI-dC) · poly(dI-dC) was added, and digestion was allowed for 90 sec at 30°C. Reactions were terminated by the addition of 3 μl of 0.2 M EDTA–10% SDS. Samples were extracted once with phenol-chloroform, precipitated with ethanol, and analyzed on a 10% denaturing polyacrylamide gel.

Affinity chromatography. Three micrograms of USF^{43} in 100 μl of buffer A containing 60 mM NaCl (buffer B) was incubated with 100 μl of GA beads, which were equilibrated in the same buffer, for 1 h at 4°C. Subsequently, the beads were packed in a column. Ten micrograms of DBP in 1 ml of buffer

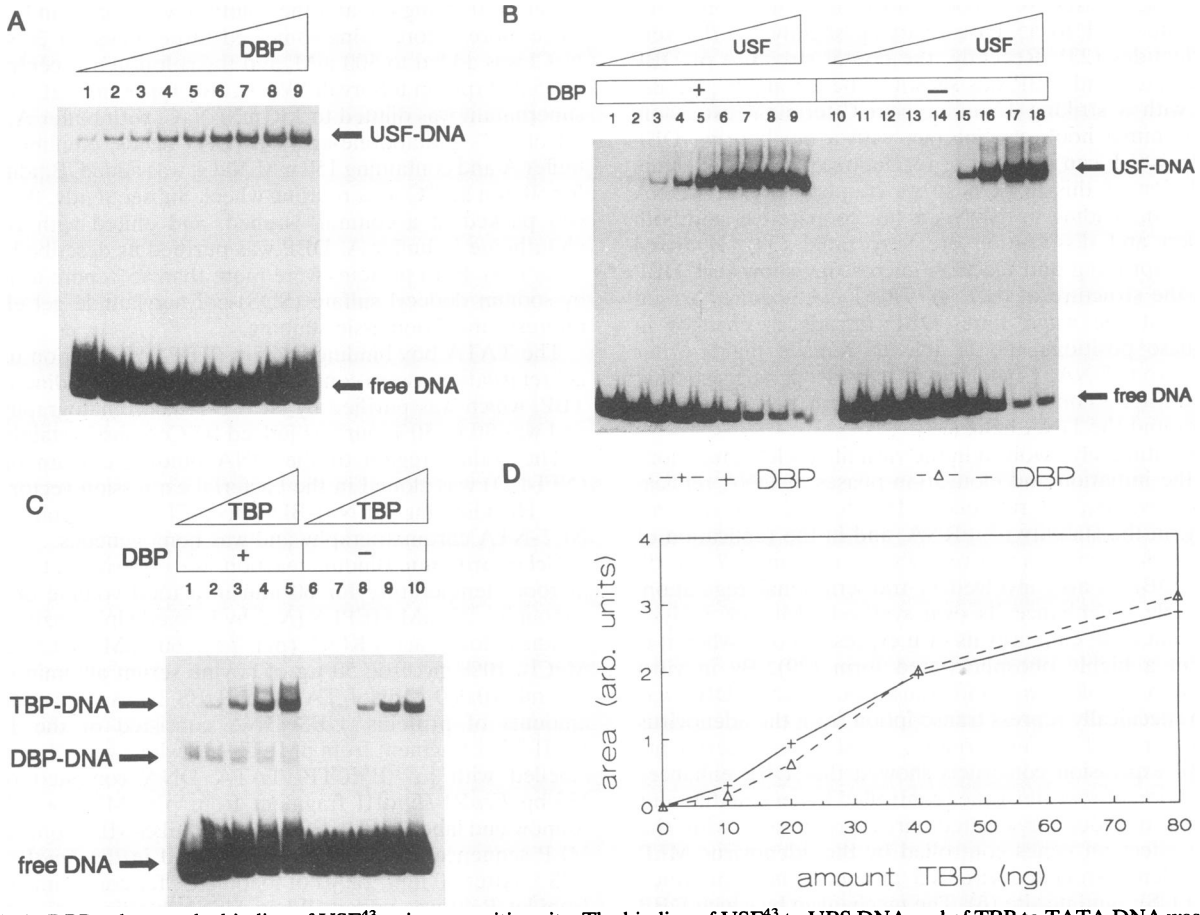

FIG. 1. DBP enhances the binding of USF[43] to its recognition site. The binding of USF[43] to UBS DNA and of TBP to TATA DNA was assayed by gel retardation. (A) Autoradiograph of a 5% nondenaturing gel; 10,000 cpm of UBS DNA was incubated with increasing amounts of DBP in the presence of 0.8 ng of USF[43]. Lanes 1 to 9, 0, 2, 5, 10, 20, 40, 80, 160, and 320 ng of DBP added, respectively. (B) Autoradiograph of a 5% nondenaturing gel; 10,000 cpm of UBS DNA was incubated with increasing amounts of USF[43] in the presence (lanes 1 to 9) or absence (lanes 10 to 18) of 150 ng of DBP. The amounts of USF[43] (in nanograms) added were 0 (lanes 1 and 10), 0.5 (lanes 2 and 11), 1 (lanes 3 and 12), 2 (lanes 4 and 13), 4 (lanes 5 and 14), 8 (lanes 6 and 15), 16 (lanes 7 and 16), 32 (lanes 8 and 17), and 64 (lanes 9 and 18). (C) Autoradiograph of a 5% nondenaturing gel; 10,000 cpm of TATA DNA was incubated with increasing amounts of TBP in the presence (lanes 1 to 5) or absence (lanes 6 to 10) of 300 ng of DBP. The amounts of TBP (in nanograms) added were 0 (lanes 1 and 6), 10 (lanes 2 and 7), 20 (lanes 3 and 8), 40 (lanes 4 and 9), and 80 (lanes 5 and 10). (D) Graphic representation of the results of panel C. The amounts of shifted probe as determined by scanning the autoradiograph (arbitrary units) are plotted against the amount of TBP added in the absence or presence of DBP.

B was passed over this column four times. The column was washed with 1 ml of buffer B and eluted with 20 mM glutathione in buffer B. One-hundred-microliter fractions were collected. USF[43] and DBP contents were assayed by gel retardation, with UBS DNA and heat-denatured UBS DNA as the probe, respectively.

Reconstituted transcription. Reactions were carried out, and samples were processed as described previously (43, 44). Briefly, reaction mixtures were assembled on ice and contained 12 mM HEPES-KOH (pH 7.9), 60 mM KCl, 12% glycerol, 0.6 mM EDTA, 0.3 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, 5 mM $MgCl_2$, 100 μg of BSA per ml, and 10 U of RNAguard (Promega). The transcription factors present were TFIID, TFIIA, and TFIIF, which were partially purified fractions from a HeLa cell extract; RNA polymerase II, purified from an Ama1 CHO cell extract and more than 90% pure as judged by silver staining; and TFIIB and TFIIE, both of which were bacterially expressed recombinant proteins purified to homogeneity as judged by Coomassie staining. The transcription templates were pΔML(C_2AT)200, which contains a 210-nucleotide (nt) G-less cassette preceded by the sequence −53 to +10 from the adenovirus MLP, and pML112(C_2AT), which contains a 380-nt G-less cassette preceded by the sequence −112 to +10 from the adenovirus MLP. Incubation was for 60 min at 30°C. Products were resolved on a denaturing 5% polyacrylamide gel.

RESULTS

DBP enhances the binding of USF[43] to its recognition site. We tested the effect of DBP on the binding of subsaturating amounts of USF[43] to its recognition site in a gel retardation assay. Figure 1A shows that the binding of USF[43] is markedly increased when DBP is present. This effect is dependent on the DBP concentration and reaches a plateau at approximately 80 to 160 ng of DBP (Fig. 1A, lanes 7 and 8). In these experiments, there was an approximately 100-fold molar excess of DBP over USF[43]. The reason for this is that there is only one USF binding site on this DNA, whereas the saturation of this DNA by DBP requires approximately 10 to 15 molecules of

DBP. Furthermore, DBP binds unstably to dsDNA and high concentrations are required for saturated binding. Interestingly, and stressing the relevance of the conditions chosen, high concentrations of DBP (approximately 2×10^7 molecules per cell) accumulate within the infected cell, resulting in an approximately 1,000-fold molar excess of DBP over USF (49). In a reciprocal experiment, when the USF^{43} concentration was varied in the absence or presence of 150 ng of DBP, the concentration of USF^{43} at which half of the DNA was bound was lowered threefold when DBP was present (Fig. 1B). USF^{43} was stable for at least 3 h at 4°C and for 1 h at 37°C, regardless of the presence of DBP, indicating that the effect of DBP on the binding affinity of USF^{43} was not due to stabilization (data not shown). To determine if this enhancement of binding was specific for USF^{43}, we tested to see if the binding of TBP was similarly affected by DBP. Figure 1C shows that TBP binding is not increased when DBP is present, even at 300 ng. In this particular experiment, the binding of DBP to the double-stranded probe is also observed, in contrast to the results in Fig. 1A, lane 9, when the same amount of DBP was added. The reason for this is that we did not add poly(dI-dC) · poly(dI-dC) to the sample at the moment of loading, since it also competes for TBP binding. The enhancement of TBP binding by DBP was not observed when the DBP concentration was varied and the concentration of TBP was fixed (Fig. 1D) or when binding was assayed by DNase I footprinting (data not shown).

Subsequently, we tested if DBP changes the binding of USF^{43} qualitatively by DNase I footprint analysis. No differences in the protection pattern of the USF binding site could be detected when DBP was present during incubation (Fig. 2C, compare lanes 5 and 9), although the lower border of the footprint is not clearly visible in this figure. Bands below the lowest band of the footprint were faint, but upon overexposure, it was obvious that the lowest band shown represents the lower border of the footprint in the absence or presence of DBP. Again, USF^{43} was found to bind more efficiently in the presence of DBP. Figure 2B shows that with a fixed amount of USF^{43}, the protection of the USF binding site is increased from 25 to 80% by the addition of DBP, and Fig. 2D shows that the binding affinity of USF^{43} is increased two- to sixfold in the presence of DBP. DBP alone showed no protection of the USF binding site.

DBP does not interact directly with USF^{43}. The stimulation of USF^{43} binding by DBP can in principle be mediated through different mechanisms. DBP might interact directly with USF^{43} and tether it to DNA, or DBP might change the structure of dsDNA, leading to more efficient binding by USF^{43}.

The construction of a USF^{43} fusion protein containing a GST tag enabled us to immobilize USF^{43} on GA beads and test whether DBP can bind to the bead-protein complex.

Figure 3 shows that USF^{43} can be immobilized on GA beads and eluted with 20 mM glutathione (lanes 6 to 8). Using conditions under which DBP stimulated USF^{43} binding to DNA, we tested whether a direct interaction between USF^{43} and DBP could be observed. In this experiment, approximately 1 μg of USF^{43} was immobilized on a GA column. Whereas DBP could be detected in the flowthrough and first wash fraction (Fig. 3, lanes 14 to 16), no DBP was present in the eluate containing USF^{43}. Since the detection limit for DBP in this assay was approximately 0.3 ng, we can conclude that less than 0.3 ng of DBP per 100 ng of USF^{43} was complexed.

DBP is also known to stimulate the binding of another transcription factor, NFI, to its recognition site (11, 40). The binding of NFI-BD is stimulated by DBP in a manner indistinguishable from that of the intact NFI protein (2). In a way similar to that used for USF^{43} but employing Ni^{2+}-NTA

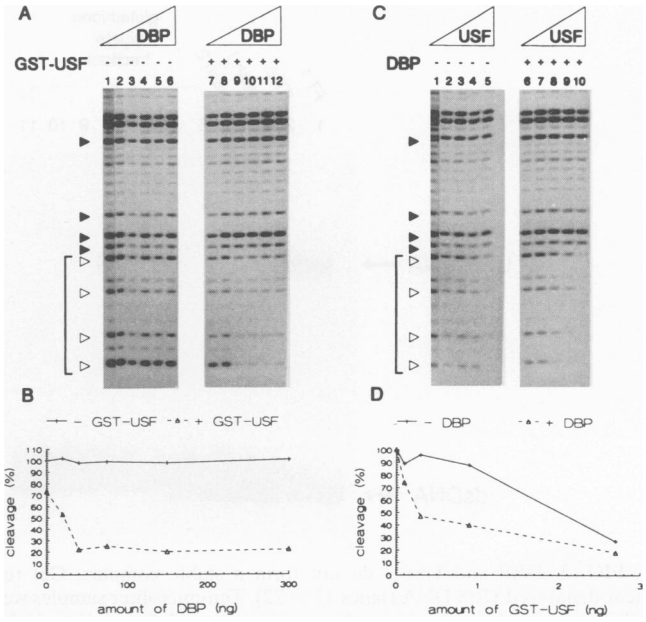

FIG. 2. DBP increases the binding affinity but does not change the footprint pattern of USF^{43}. (A and C) DNase I footprint analyses of USF^{43} binding in the presence or absence of DBP. The protected binding site is shown in brackets. (A) UBS DNA (10,000 cpm) was incubated with increasing amounts of DBP in the absence (lanes 1 to 6) or presence (lanes 7 to 12) of 12 ng of USF^{43}. The amounts of DBP (in nanograms) added were 0 (lanes 1 and 7), 20 (lanes 2 and 8), 40 (lanes 3 and 9), 80 (lanes 4 and 10), 150 (lanes 5 and 11), and 300 (lanes 6 and 12). (B) Graphic representation of the results in panel A. Average cleavage at the positions of the open triangles in panel A (corresponding to the USF binding site) relative to cleavage at positions of the closed triangles (which is a measure of the degree of occupancy of the USF binding site) is plotted as a function of the amount of DBP added. Relative cleavage in lane 1 is set up as 100%. (C) UBS DNA (10,000 cpm) was incubated with increasing amounts of USF^{43} in the absence (lanes 1 to 5) or presence (lanes 6 to 10) of 150 ng of DBP. The amounts of USF^{43} (in nanograms) added were 0 (lanes 1 and 6), 1 (lanes 2 and 7), 3 (lanes 3 and 8), 9 (lanes 4 and 9), and 27 (lanes 5 and 10). (D) Graphic representation of the results in panel C. Relative cleavage is plotted as a function of the amount of USF^{43} added in the presence or absence of DBP.

affinity chromatography, we tested for possible direct interaction between His-NFI-BD and DBP. We immobilized His-NFI-BD on a Ni^{2+}-NTA column and found that DBP was not retained on such a column under conditions at which DBP stimulates the binding of NFI-BD (data not shown).

Since we were unable to show direct interaction between DBP and either USF^{43} or His-NFI-BD, NFI-BD and USF^{43} are unrelated proteins, and DBP is known to induce extensive structural alterations in dsDNA, we favor the explanation that DBP enhances the binding of transcription factors by affecting the general structure of DNA.

DBP enhances transcription stimulation by USF^{43}. Transcription in vivo from the adenovirus MLP is stimulated by the binding of USF^{43} to its recognition site upstream of the promoter. We checked if transcription in a reconstituted in vitro system could also be stimulated by USF^{43}. The in vitro system employed contained homogeneous preparations of recombinant basal transcription factors TFIIB and TFIIE and purified preparations of TFIIA, TFIID, TFIIF, and RNA polymerase II. In in vitro transcription reactions, we used the following two templates: pML112(C_2AT), in which a 380-nt

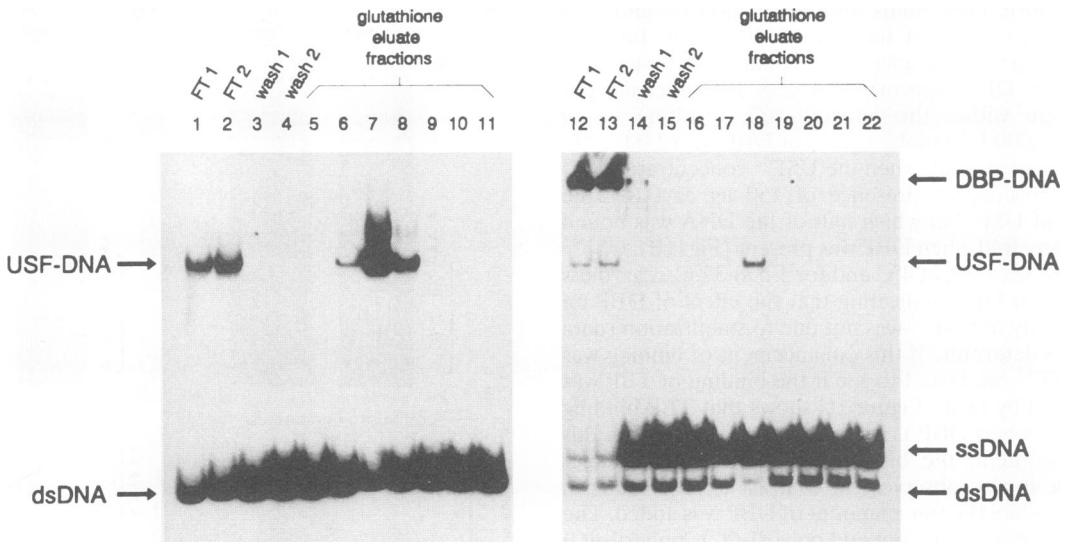

FIG. 3. DBP and USF^{43} do not form a stable complex. Gel retardation assay of column fractions with UBS DNA (lanes 1 to 11) or heat-denatured UBS DNA (lanes 12 to 22). Ten-microliter samples were assayed. FT 1, sample taken after DBP was passed over the column once; FT 2, sample taken after four column passages; wash 1, first 100 μl of the wash; wash 2, the remaining 900 μl. The positions of free ds- and ssUBS DNA and ssUBS DNA-DBP and dsUBS DNA-USF^{43} complexes are indicated.

G-less cassette is preceded by the sequence −112 to +10 from the adenovirus MLP and which contains one USF binding site; and pΔML(C_2AT)200, which served as an internal reference, contains a 210-nt G-less cassette preceded by the sequence −53 to +10 from the adenovirus MLP, and lacks this USF binding site. When increasing amounts of USF^{43} were added, transcription of the 380-nt cassette was increased relative to transcription of the 210-nt cassette in a concentration-dependent fashion (Fig. 4A, lanes 1 to 5). Maximal stimulation was about 2.5-fold in the absence of DBP (lane 4). (An additional band is always seen just below the 380-nt product; this band presumably arises from reinitiation and premature termination rather than internal initiation, since this band is also increased in the presence of USF^{43} and its intensity compared with that of the 380-nt band is always approximately 10%, regardless of added proteins.) When DBP is added (lanes 6 to 10), the level of transcription of the 380-nt cassette, relative to transcription of the 210-nt cassette, is consistently higher than in the absence of DBP. DBP alone does not influence transcription at the concentrations used here (Fig. 4A, lanes 11 to 15). The enhancement of USF^{43}-stimulated transcription at a suboptimal USF^{43} concentration increases with an increasing DBP concentration and reaches a plateau at 1,000 to 2,000 ng of DBP (Fig. 4A, lanes 16 to 20, and C). This concentration is 16 to 32 times higher than that required for maximal stimulation of USF^{43} binding. The most likely explanation for this is that in the transcription reaction, 200 ng of DNA is present as opposed to 0.1 to 0.2 ng of DNA in binding reactions. This excess DNA effectively lowers the concentration of unbound DBP, and therefore, more DBP is necessary in the transcription assay to reach the same level of stimulation.

DISCUSSION

In this paper, we have shown that DBP enhances the binding of USF^{43} to its recognition site. We have also shown that this increase in binding affinity in the presence of DBP results in an enhanced stimulation of transcription in vitro by USF^{43}.

Previously, DBP had been shown to increase the binding affinity of NFI-BD (2, 11, 40). Although the structure of NFI-BD is not yet known, on the basis of sequence comparison, it does not belong to the class of B-HLH-LR transcription factors and most likely its structure is unrelated to USF^{43} (30). The increase in the binding affinity of both transcription factors does not appear to be the result of stable interaction with DBP but is mediated through structural changes imposed on DNA upon DBP binding. DBP does not enhance the binding of all transcription factors to their recognition sites. Previous work showed that the binding of the POU domain of NFIII/Oct-1 is unaffected by DBP (40), and we observed the same results for the binding of TBP to the TATA box.

At present, we do not know what determines whether the binding of a transcription factor can be enhanced by DBP. The saturation of dsDNA with DBP does not change the length of the DNA, which excludes the possibility that the DNA is extensively wrapped around the protein, unlike the situation for the ssDNA-DBP complex. Circular dichroism measurements indicate the introduction of base-to-base distortions in complexed DNA, consistent with altered helical pitch or distance between base pairs. Furthermore, hydroxyl radical footprinting shows a disappearance of positions of hypersensitivity present in naked DNA. Since circular dichroism measurements indicate the introductions of base-to-base distortions, the regular structure, as suggested by hydroxyl radical footprinting, is interpreted to be the result of a large number of short-lived DBP-mediated distortions. Because interactions are brief, the net result is a regular dsDNA structure in the presence of DBP, brought about as a consequence of an increase in the dynamic flexibility of DNA. Such a conformation, devoid of stable secondary structure, may be preferentially bound by some transcription factors.

The stimulation of USF binding to DNA by DBP is strongest at saturating DBP concentrations. We know that within infected cells, DBP accumulates to high levels (approximately 2×10^7 molecules per cell), presumably reaching very high concentrations within discrete clusters (41). Therefore, it is considered very likely that adenovirus dsDNA is coated with

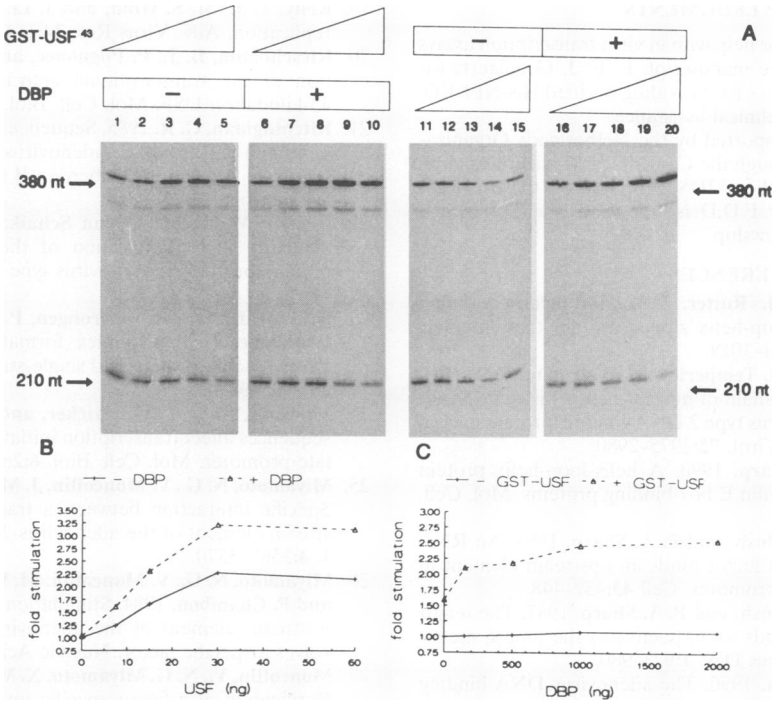

FIG. 4. Stimulation of reconstituted in vitro transcription by USF[43] is enhanced by DBP. (A) Reconstituted in vitro transcription was performed with increasing amounts of USF[43] in the presence (lanes 1 to 5) or absence (lanes 6 to 10) of 1,000 ng of DBP and with purified proteins and increasing amounts of DBP in the presence (lanes 11 to 15) or absence (lanes 16 to 20) of 15 ng of USF[43]. The amounts of USF[43] (in nanograms) were 0 (lanes 1 and 6), 7.5 (lanes 2 and 7), 15 (lanes 3 and 8), 30 (lanes 4 and 9), and 60 (lanes 5 and 10). The amounts of DBP (in nanograms) were 0 (lanes 11 and 16), 150 (lanes 12 and 17), 500 (lanes 13 and 18), 1,000 (lanes 14 and 19), and 2,000 (lanes 15 and 20). Graphic representation of the results from lanes 1 to 10 (B) and 11 to 20 (C) of panel A. The level of transcription of the 380-nt cassette relative to that of the 210-nt cassette is plotted as a function of the amount of USF[43] added in the presence or absence of DBP, with the relative level in lane 1 set up as 1, (B) or as a function of the amount of DBP added in the presence or absence of USF[43], with the relative level in lane 11 set up as 1 (C).

DBP late in infection (39), which underlines the relevance of these data for the in vivo situation.

Although we observe only a two- to fourfold increase in the binding affinity of USF in the presence of DBP, this may result in a much larger increase in the occupancy of USF binding sites in vivo and hence in a much larger increase in USF-activated transcription. This should be the case when the intracellular concentration of USF, estimated at approximately 10^4 molecules per cell, is limiting. Such a situation may arise late in infection when 10^4 to 10^5 progeny DNA molecules per infected cell have accumulated. Furthermore, USF may be recruited specifically to viral DNA since in vivo footprinting shows that USF, although present at the same concentration throughout the infection cycle, can bind only to replicated viral DNA. Upon entering the nucleus shortly after infection, adenovirus DNA is still complexed to viral protein VII (8). The DNA structure is changed after replication, and DNA may be complexed to DBP at this stage, since no protein VII, which is made late in infection as a precursor and which is cleaved at a late step in virus assembly, is present to bind newly synthesized DNA. This implies that USF binding to DNA is inhibited when DNA is complexed with protein VII but that complex formation of DNA with DBP allows and stimulates USF binding (45). Interestingly, the stimulation of transcription by DBP in vivo is strongest for genes controlled by the adenovirus MLP and the adeno-associated virus P5 promoter, both of which contain a functional USF binding site (6).

We showed that DBP does not stimulate basal transcription in vitro. This means that at least in vitro, the unwinding activity of DBP, which we demonstrated recently (54), does not lead to an increase in transcription.

Several sequence-independent DBPs have been shown to alter the binding of regulatory transcription factors. However, a relation between modulations of binding and transcription has not been documented in most instances. For example, the high mobility group (HMG) proteins can influence the binding of sequence-specific DBPs. HMG 1 and 2 increase and slightly alter the binding of purified USF/MLTF, as has been shown by gel retardation and DNase I footprinting analysis, but whether HMG 1 and 2 also stimulate USF-activated transcription has not been determined (50). HMG I(Y) increases the binding of NF-κB to the PRDII site in the beta interferon promoter, while it leaves the binding of NF-κB to IGκB and H-2 sites unaffected (42). Since both NF-κB and HMG I(Y) bend DNA, by introducing a bend at the PRDII site, HMG I(Y) might lower the free energy required for NF-κB binding to PRDII (42). Since there is no indication that DNA is severely bent when complexed to DBP, a similar mechanism for the enhancement of transcription factor binding by DBP is unlikely.

The enhancement of transcription factor binding to DNA may be a general mechanism for achieving specificity of gene activation. In the case of DBP, viral transcription may be specifically enhanced. Adenovirus uses cellular transcription factors to promote its own transcription as well as DNA replication, and in order to avoid competition with cellular binding sites, the viral binding sites are converted into high-affinity sites upon complex formation with DBP.

ACKNOWLEDGMENTS

We thank F. C. P. Holstege for help with in vitro transcription assays and for a critical reading of the manuscript, F. E. J. Coenjaerts for providing purified DBP, J. Dekker for providing purified His-NFI-BD, and W. van Driel for expert technical assistance.

This work was financially supported by the Netherlands Organization for Scientific Research through the Council for Medical Research and by grant 8203-48 from the AIDS program of the Instituto Superiore di Sanita, Rome, Italy. F.D.D. is supported by a SISSA/ISAS (Trieste, Italy) predoctoral fellowship.

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