Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5889–5893, August 1986
Biochemistry

Affinity purification of sequence-specific DNA binding proteins

(DNA-agarose affinity chromatography/competitor DNA/transcription factor Sp1/synthetic oligodeoxynucleotides)

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Communicated by Bruce M. Alberts, April 30, 1986

ABSTRACT **We describe a method for affinity purification of sequence-specific DNA binding proteins that is fast and effective. Complementary chemically synthesized oligodeoxynucleotides that contain a recognition site for a sequence-specific DNA binding protein are annealed and ligated to give oligomers. This DNA is then covalently coupled to Sepharose CL-2B with cyanogen bromide to yield the affinity resin. A partially purified protein fraction is combined with competitor DNA and subsequently passed through the DNA-Sepharose resin. The desired sequence-specific DNA binding protein is purified because it preferentially binds to the recognition sites in the affinity resin rather than to the nonspecific competitor DNA in solution. For example, a protein fraction that is enriched for transcription factor Sp1 can be further purified 500- to 1000-fold by two sequential affinity chromatography steps to give Sp1 of an estimated 90% homogeneity with 30% yield. In addition, the use of tandem affinity columns containing different protein binding sites allows the simultaneous purification of multiple DNA binding proteins from the same extract. This method provides a means for the purification of rare sequence-specific DNA binding proteins, such as Sp1 and CAAT-binding transcription factor.**

Many important cellular processes, such as transcription, replication, and recombination, involve the action of DNA binding proteins. For example, sequence-specific DNA binding proteins are directly involved in the regulation of mRNA transcription initiation in higher organisms (for reviews, see refs. 1–3). To study the biochemical properties of these transcription factors, it is necessary to purify the proteins to homogeneity. This would enable the factors to be characterized, facilitate the raising of antibodies, and ultimately provide a means for cloning the genes encoding these regulatory proteins. It has generally been very difficult, however, to obtain homogeneous preparations of these transcription factors because they typically constitute only 0.001% of the total cellular protein (M. R. Briggs, J.T.K., and R.T., unpublished data).

It has long been predicted that sequence-specific DNA binding proteins can be purified by chromatography through affinity resins that contain the proper DNA recognition sites attached to an immobile support (4–7). In the past, a number of DNA binding proteins, including various RNA and DNA polymerases, hormone receptors, and repressors, have been purified by nonspecific DNA-cellulose and DNA-agarose affinity chromatography (4, 5, 8). However, sequence-specific purification of DNA binding proteins has been performed only in a few cases (9, 10), and our attempts to purify eukaryotic promoter-specific transcription factors by the published methods have not been successful.

In the course of our studies on the regulation of mRNA synthesis by RNA polymerase II, we have found that conventional chromatography and HPLC of promoter-specific transcription factors resulted in preparations of only 1–2% purity. We were therefore prompted to develop an affinity chromatography method that could be successfully used for purification of low abundance sequence-specific DNA binding proteins, such as Sp1, CAAT-binding transcription factor (CTF; for reviews, see refs. 1 and 3), and activator protein 1 (AP-1; AP-1 is an RNA polymerase II transcription factor similar to Sp1 and CTF; W. Lee, P. Mitchell, and R.T., unpublished data). These proteins, which are typically derived from HeLa (human) cells, activate transcription by RNA polymerase II from a select group of promoters, such as the simian virus 40 (SV40) early and herpes simplex virus thymidine kinase promoters, that contain at least one properly positioned recognition site for Sp1, CTF, or AP-1. The complete purification and biochemical characterization of Sp1, CTF, and AP-1 will be described elsewhere (M. R. Briggs, J.T.K., and R.T., unpublished data; K. A. Jones, J.T.K., and R.T., unpublished data; W. Lee, P. Mitchell, and R.T., unpublished data). Here we report a simple and effective DNA affinity chromatography method that has allowed us to purify these transcription factors to homogeneity. This technique should be generally applicable for the purification of other sequence-specific DNA binding proteins.

MATERIALS AND METHODS

Materials. Sepharose CL-2B and T4 polynucleotide kinase were obtained from Pharmacia. T4 DNA ligase was from either New England Biolabs or Promega Biotec (Madison, WI). Econo-Columns were the products of Bio-Rad (no. 731-1550). Cyanogen bromide (CNBr; 97%) was purchased from Aldrich. Oligodeoxynucleotides were prepared with an Applied Biosystems 380A DNA synthesizer.

Preparation of DNA for Coupling to Sepharose. A scheme for the preparation of a sequence-specific DNA affinity resin is shown in Fig. 1. First, chemically synthesized complementary oligonucleotides, such as X and Y (see Fig. 2*A*), are annealed, 5′-phosphorylated, and ligated as follows. Gel-purified oligodeoxynucleotides (220 μg of each) are combined in 67 mM Tris·HCl buffer (pH 7.6) containing 13 mM $MgCl_2$, 6.7 mM dithiothreitol, 1.3 mM spermidine, and 1.3 mM EDTA in a total volume of 75 μl. This mixture is incubated at 88°C for 2 min, 65°C for 10 min, 37°C for 10 min, and room temperature for 5 min. ATP (20 mM) containing 5 μCi of [γ-^{32}P]ATP (pH 7; 15 μl; 1 Ci = 37 GBq) and T4 polynucleotide kinase (100 units; 10 μl) are then added to give a final volume of 100 μl, and the resulting solution is incubated at 37°C for 2 hr. This reaction is stopped by the addition of 5 M NH_4OAc, pH 5.5 (100 μl)/100 mM $MgCl_2$ (25 μl)/TE buffer (25 μl; TE is 10 mM Tris·HCl, pH 7.6/1 mM EDTA), and the mixture is heated at 65°C for 15 min to inactivate the kinase. The DNA is ethanol-precipitated; resuspended in TE buffer (200 μl), 3 M NaOAc (25 μl), and 100 mM $MgCl_2$ (25 μl);

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Abbreviations: CTF, CAAT-binding transcription factor; AP-1, activator protein 1; NFI, nuclear factor I; SV40, simian virus 40.

reprecipitated with ethanol; washed with 70% ethanol; and dried *in vacuo*. The DNA is then dissolved in 88 mM Tris·HCl, pH 7.5/13.3 mM $MgCl_2$/20 mM dithiothreitol/1.3 mM spermidine (75 μl), and the ligation reaction is initiated by the addition of 20 μl of 20 mM ATP (pH 7) and 5 μl of T4 DNA ligase (10–30 Weiss units) to give a final volume of 100 μl. This mixture is incubated at 16°C for 4 hr (depending on the sequence and the length of the oligodeoxynucleotides, the optimal temperature for ligation may vary from 4°C to 16°C), and the DNA is then phenol-extracted, precipitated with ethanol, dried *in vacuo*, and dissolved in water (100 μl). (Note: do not dissolve the DNA in TE buffer—it will interfere with the coupling reaction.) Analysis of the resulting DNA by agarose gel electrophoresis typically shows oligomers of the basic oligodeoxynucleotide unit ranging from 3-mers to 75-mers (Fig. 2*B*).

Coupling of DNA to Sepharose. The DNA oligomers are covalently attached to Sepharose CL-2B by slight modification of the method of Arndt-Jovin *et al.* (8). Sepharose CL-2B (settled volume, 10 ml) is extensively washed with 250 ml of water, suspended in water to give a 20-ml slurry, and then equilibrated to 15°C in a water bath. CNBr (1.1 g; 10 mmol) is dissolved in *N,N*-dimethylformamide (2 ml) and added dropwise over 1 min to the Sepharose, which is mixed by magnetic stirring. Then, 5 M NaOH (1.8 ml; 9 mmol) is slowly added dropwise to the resin over 10 min. The pH of the reaction, which generates HBr as a by-product, should not exceed pH 10. The reaction is stopped by the addition of ice-cold water (100 ml) followed by gentle suction filtration of the resin on a coarse sintered-glass funnel. It is very important that the activated Sepharose is not suction-filtered into a dry cake. The CNBr-derivatized resin is then extensively washed with ice-cold water (300 ml) and 10 mM potassium phosphate (pH 8.0; 100 ml).

The activated Sepharose is immediately used for coupling to DNA as follows. The resin is transferred to a 15-ml polypropylene screw-cap tube, and 10 mM potassium phosphate (pH 8.0, 4 ml) is added to give a thick slurry. The ligated DNA (100 μl in water) is then added to this mixture, and the coupling reaction is carried out at room temperature for 16 hr on a rotary shaker. The resin is collected on a sintered-glass funnel, washed with water (200 ml) and 1 M ethanolamine·HCl (pH 8.0; 100 ml), and suspended in 1 M ethanolamine·HCl (pH 8) to give a final volume of 14 ml. This inactivation of unreacted CNBr-derivatized Sepharose by ethanolamine is carried out at room temperature for 4–6 hr on a rotary shaker. The resin is collected on a sintered-glass funnel and washed with 10 mM potassium phosphate (pH 8.0; 100 ml), 1 M potassium phosphate (pH 8.0, 100 ml), 1 M KCl (100 ml), water (100 ml), and 10 mM Tris·HCl (pH 7.6) containing 0.3 M NaCl, 1 mM EDTA, and 0.02% (wt/vol) NaN_3 (100 ml). The resin is stored at 4°C in 10 mM Tris·HCl (pH 7.6) containing 0.3 M NaCl, 1 mM EDTA, and 0.02% (wt/vol) NaN_3.

Because the DNA is labeled with ^{32}P, the efficiency of DNA attachment to the Sepharose can be crudely estimated by comparing the amount of radioactivity that is retained on the resin with the amount of radioactivity that remains in solution after the coupling reaction. The efficiency of DNA incorporation into the resin is usually 40–70%. Thus, the concentration of covalently bound DNA in the affinity resin is 20–30 μg of DNA per ml of resin.

DNA Affinity Chromatography. This procedure, which is described here for the purification of Sp1, should work, with only minor modification, for any high-affinity sequence-specific DNA binding protein. In one affinity chromatography step, the recovery of Sp1, as measured by a DNase I footprint assay, is typically 50–60%. All operations are performed at 4°C. Sp1 DNA affinity resin (1 ml) is equilibrated in a Bio-Rad Econo-Column with buffer Z containing 0.1 M KCl [20 ml; buffer Z is 25 mM Hepes (K^+), pH 7.8/12.5 mM $MgCl_2$/1 mM dithiothreitol/20% (vol/vol) glycerol/0.1% (vol/vol) Nonidet P-40]. A crude fraction of Sp1 (total protein, 5–10 mg, derived from 50 g of HeLa cells; ≈0.1% Sp1; 25 ml) in buffer Z containing 0.1 M KCl is combined with sonicated calf thymus DNA (440 μg; 200 μl of a 2.2 mg/ml solution in TE) and allowed to stand for 10 min. The protein–DNA mixture is passed through the affinity resin by gravity flow (≈15 ml/hr), and the resin is washed four times with 2 ml of buffer Z containing 0.1 M KCl. The passage of buffer through the column is stopped, buffer Z containing 1.0 M KCl (1.2 ml) is added to the column, and the resin is thoroughly mixed with the buffer by using a narrow siliconized glass rod. The resin is allowed to stand for 10 min, and the protein is eluted to give the major Sp1 eluate (≈1.2 ml). The column is then washed with buffer Z containing 0.5 M KCl (1.2 ml) to give the minor Sp1 eluate (≈1.2 ml). Alternatively, Sp1 could be recovered by using a 0.1 M to 1.0 M KCl gradient, where the protein typically elutes from the resin from 0.4 M to 0.6 M KCl. Also, if further purification is desired, the Sp1 fractions can be diluted to 0.1 M KCl with buffer Z without KCl, mixed with competitor DNA, and reapplied to the affinity resin.

The affinity columns are regenerated by washing with 5 mM Tris·HCl, pH 7.6/2.5 M NaCl/0.5 mM EDTA (25 ml), and 10 mM Tris·HCl, pH 7.6/0.3 M NaCl/1 mM EDTA/0.02% (wt/vol) NaN_3 (10 ml). The columns are then stored at 4°C in 10 mM Tris·HCl, pH 7.6/0.3 M NaCl/1 mM EDTA/0.02% (wt/vol) NaN_3.

Other Methods. The partial purification of Sp1 has been described (11, 12). The complete purification of Sp1 to homogeneity as well as biochemical identification and characterization of the protein will be described elsewhere (M. R. Briggs, J.T.K., and R.T., unpublished data). Oligodeoxynucleotides were synthesized, separated from contaminants by electrophoresis on a denaturing 20% polyacrylamide gel, and then recovered by ethanol precipitation. The A_{260nm} and A_{280nm} of each oligodeoxynucleotide was measured, and the concentration of each sample was estimated by assuming that 1 A_{260nm} absorbance unit corresponds to 40 μg of DNA per ml. DNase I footprinting was carried out as described by Galas and Schmitz (13) and Dynan and Tjian (12). Reconstituted *in vivo* transcription reactions in the presence or absence of Sp1 were performed as described (11). DNA techniques were carried out as recommended by Maniatis *et al.* (14). Polyacrylamide gel electrophoresis of proteins in the presence of $NaDodSO_4$ was done according to the method of Laemmli (15).

RESULTS

Preparation of the DNA Affinity Resin. A scheme for the preparation of an affinity resin for the purification of sequence-specific DNA binding proteins is shown in Fig. 1. The DNA affinity resin was designed to have the following properties. First, to maximize the recovery of the desired protein and to minimize contamination by other proteins, the DNA that is attached to the resin consists only of tandem repeats of a strong binding site for the desired protein. We have found that it is best to survey different recognition sequences to determine a high-affinity binding site. Second, to ensure that the DNA is accessible to protein and stably bound to the resin, each DNA molecule is attached to an agarose bead by an average of one covalent bond. These aims were realized by the use of chemically synthesized oligodeoxynucleotides, which can be quickly and easily prepared in micromole quantities. Complementary oligodeoxynucleotides [such as X and Y (Fig. 2*A*), which, when annealed, possess complementary 5′-protruding ends] are annealed, 5′-phosphorylated, and ligated to give oligomers of the basic

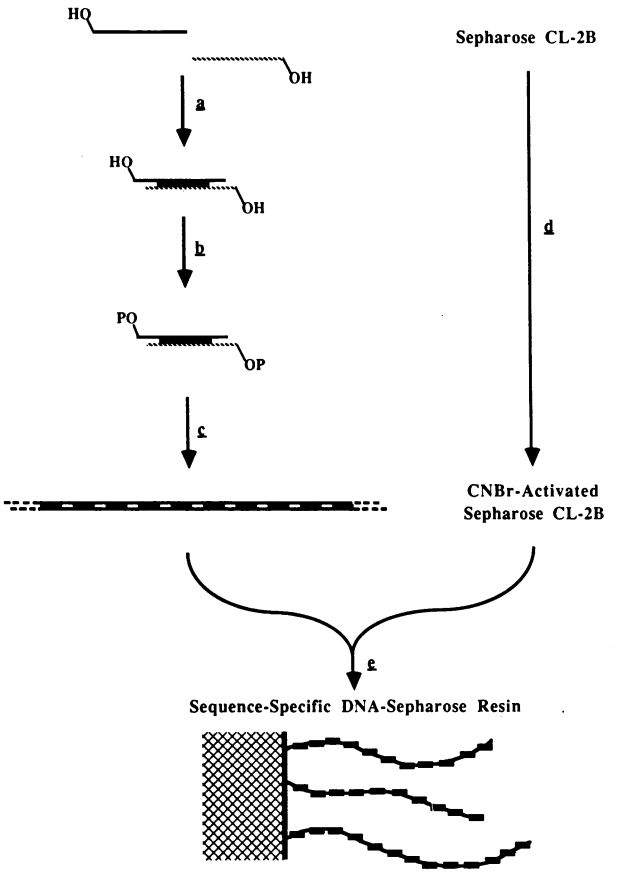

FIG. 1. Preparation of a sequence-specific DNA-Sepharose resin. Solid black rectangles represent the recognition site of a sequence-specific DNA binding protein. (*a*) Annealing of complementary oligodeoxynucleotides; (*b*) phosphorylation of 5′ protruding ends with T4 polynucleotide kinase and ATP; (*c*) polymerization of the complementary oligodeoxynucleotides with T4 DNA ligase and ATP; (*d*) activation of Sepharose CL-2B with CNBr; (*e*) coupling of the ligated DNA to the activated Sepharose to give the affinity resin. It is assumed that the DNA is covalently bound to the resin by the primary amino groups of the residues in the 5′ protruding ends; however, the exact position(s) where the DNA is attached to the resin is not known.

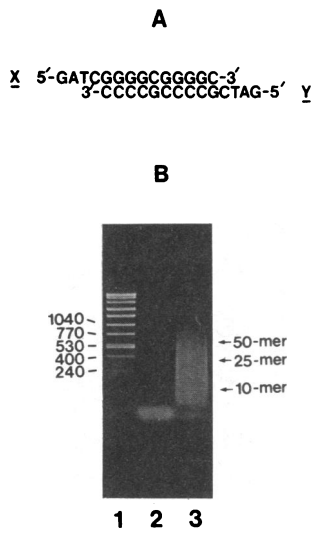

FIG. 2. Polymerization of complementary 5′-phosphorylated oligodeoxynucleotides. (*A*) DNA sequence of complementary oligodeoxynucleotides, X and Y. When annealed, oligodeoxynucleotides X and Y create the high-affinity Sp1 binding site, 5′-GGGGCGGGGC-3′ (1, 3). (*B*) Analysis of the ligated DNA by 2% agarose gel electrophoresis. The DNA was visualized by ethidium bromide staining and ultraviolet light fluorescence. Lanes: 1, linear double-stranded DNA molecular size markers (sizes of selected fragments are given in base pairs); 2, oligodeoxynucleotides X and Y before 5′-phosphorylation and ligation (1 μg); 3, oligodeoxynucleotides X and Y after 5′-phosphorylation and ligation (2 μg). The estimated migration of multimers is indicated by arrows.

oligodeoxynucleotide unit that range from 3-mers to 75-mers (Fig. 2*B*). The ligated DNA is then covalently coupled to Sepharose CL-2B with CNBr. The efficiency of coupling of the DNA to the Sepharose is usually between 40% and 70%, and most of the DNA molecules are probably attached to the resin by a single covalent bond (8). We have successfully used oligodeoxynucleotides that range in length from 14 to 50 nucleotides. The concentration of DNA bound to the Sepharose is typically 20–30 μg/ml, which corresponds to a theoretical protein binding capacity of 2–3 nmol/ml if there is one recognition site per 15 base pairs. We have also observed that the resin is stable for at least 1 year and can be reused >30 times without any detectable loss of protein binding capacity.

DNA Affinity Chromatography. We have purified transcription factor Sp1 to an estimated 90% homogeneity by using any of three affinity resins that contain different Sp1 binding sites as well as different flanking oligodeoxynucleotide sequences. Similarly, we have purified CTF (16) and AP-1 to near homogeneity (K. A. Jones, J.T.K., and R.T., unpublished data; W. Lee, P. Mitchell, and R.T., unpublished data).

The results of a typical affinity chromatography experiment with Sp1 as a model protein are presented in Fig. 3. A partially purified preparation of Sp1 (total protein, 5–10 mg, derived from 50 g of HeLa cells; ≈0.1% purity; see *Materials and Methods*) is combined with sonicated calf thymus competitor DNA, allowed to stand for 10 min, and then applied to an Sp1 affinity resin. The protein–DNA mixture is passed through the resin by gravity flow, the column is washed with buffer, and the Sp1 is eluted with 0.5 M KCl. The Sp1 DNA binding activity was monitored by DNase I footprinting of the 21-base-pair repeat sequences in SV40 (12). As displayed in Fig. 3*A*, <20% of the initial Sp1 activity is in the flowthrough fraction, and the recovery of Sp1 is 50–60%. Fig. 3*B* is a silver-stained $NaDodSO_4$/polyacrylamide gel that shows Sp1 before affinity purification and after one and two affinity chromatography steps, and Fig. 3*C* shows activation of transcription from the SV40 early promoter by purified Sp1 in a reconstituted *in vitro* transcription assay. Sp1 is purified 500- to 1000-fold with 30% yield to an estimated 90% homogeneity by two sequential affinity chromatography steps.

As shown in Fig. 4, the addition of competitor DNA to the crude protein fraction can be critical for successful affinity chromatography. In a typical experiment with a 1-ml affinity column that contains ≈20 μg of covalently bound DNA, 440 μg of sonicated calf thymus DNA, which is greater than a 20-fold excess over the synthetic DNA bound to the resin, is added to the crude protein fraction before chromatography. Thus, each passage through the affinity resin can give up to a 20-fold enrichment of Sp1 relative to other DNA binding proteins as well as separation from proteins that do not bind to DNA. In the absence of sufficient competitor DNA, nonspecific proteins will bind to the resin and subsequently contaminate the Sp1. On the other hand, a large excess of competitor DNA can result in a low recovery of Sp1, presumably due to weak binding of the protein to the competitor DNA (data not shown). Interestingly, the use of competitor DNA is important for affinity chromatography of Sp1 from crude extracts of ≈0.1% purity (Fig. 4*A*) but not for isolation of Sp1 from protein fractions of ≈1% purity (Fig. 4*B*). In the purification of a new protein, the composition and

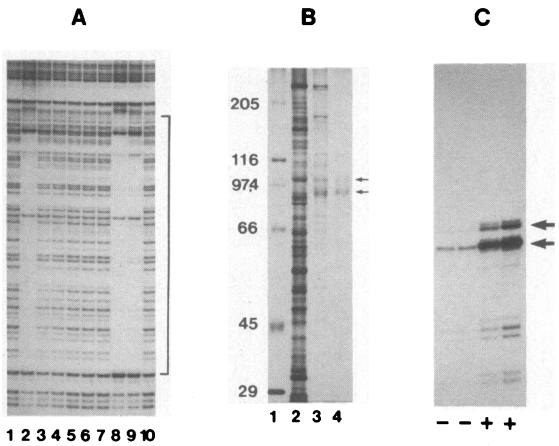

FIG. 3. Sequence-specific DNA affinity chromatography of transcription factor Sp1. (*A*) DNase I footprint assay of Sp1 binding to the SV40 21-base-pair repeat sequences. The degenerate 21-base-pair repeats in the SV40 genome contain six tandem Sp1 binding sites (12, 17, 18). Lanes 1 and 10, control DNase I digestion in the absence of protein. Lane 2, crude protein fraction before chromatography (25 μl of 6-ml fraction). Lane 3, affinity resin flowthrough (25 μl of 6-ml fraction). Lane 4, first column wash (25 μl of 2-ml fraction). Lane 5, second column wash (25 μl of 2-ml fraction). Lane 6, third column wash (25 μl of 2-ml fraction). Lane 7, fourth column wash (25 μl of 2-ml fraction). Lane 8, major Sp1 eluate (10 μl of 1-ml fraction). Lane 9, minor Sp1 eluate (10 μl of 0.6-ml fraction). The region of DNA protected from DNase I digestion by Sp1 is indicated by a bracket. (*B*) $NaDodSO_4$/polyacrylamide gel electrophoresis of affinity purified Sp1. Protein samples were precipitated with trichloroacetic acid, subjected to electrophoresis on a $NaDodSO_4$/7% polyacrylamide gel, and stained with silver. Lanes: 1, protein molecular size markers; 2, crude Sp1 fraction (15 μg total protein); 3, Sp1 fraction after one affinity chromatography step (1.5 μg total protein); 4, Sp1 after two sequential affinity chromatography steps (0.3 μg total protein). The protein samples applied to lanes 3 and 4 contained roughly 10 times the Sp1 DNA binding activity as the protein sample applied to lane 2. The two polypeptides of 95 and 105 kDa, which are indicated by arrows, have been shown to be Sp1 (M. R. Briggs, J.T.K., and R.T., unpublished data). The size of each of the protein markers is also given in kDa. (*C*) Activation of *in vitro* transcription from the SV40 early promoter by affinity-purified Sp1. RNA synthesis was measured by a primer extension assay, and the arrows indicate the cDNA strands that derive from transcripts generated *in vitro*. The symbols + and − indicate the presence or absence of affinity-purified Sp1.

amount of competitor DNA must be carefully determined because the affinity of a test protein for different DNAs will vary. We have successfully used both sonicated calf thymus DNA and polydeoxyinosinic/polydeoxycytidylic acid as competitor DNAs.

In addition to variation of the competitor DNAs, the composition of the buffer is critical for successful DNA affinity chromatography. The most obvious constituents to vary are the concentration of mono- and divalent metal cations, such as sodium, potassium, and magnesium ions, as well as the buffer itself and the pH. If nucleases are present in the crude protein fraction, then divalent metal cations should be omitted and EDTA should be added to protect the resin. Also, specific ligands that affect the DNA binding properties of a protein could be used in some situations.

Finally, the affinity resins can assist in the identification of sequence-specific DNA binding proteins. For example, any protein that is enriched by chromatography through an affinity resin that contains Sp1 recognition sites is likely to be either Sp1 or a protein that is closely associated with Sp1. To eliminate the possibility that such proteins are not species that fortuitously bind either to DNA sequences other than the Sp1 binding sites or to the Sepharose CL-2B support, fractions containing Sp1 can be passed through DNA-

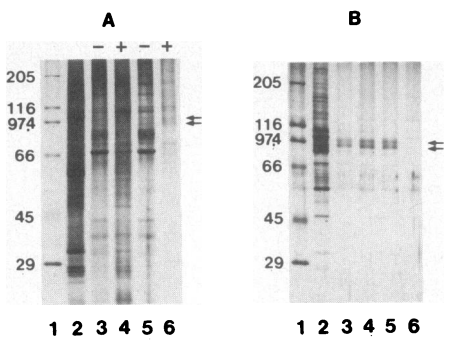

FIG. 4. Effects of competitor DNA and binding sites on the affinity purification of Sp1. Protein samples were precipitated with trichloroacetic acid, subjected to electrophoresis on a $NaDodSO_4$/8% polyacrylamide gel, and stained with silver. Sp1 is indicated by arrows, and the size of each of the protein markers is given in kDa. (*A*) Affinity chromatography of crude protein fraction containing 0.05–0.1% Sp1. Lanes: 1, protein molecular size markers; 2, crude protein fraction before chromatography; 3 and 4, Sp1 fractions after one affinity chromatography step; 5 and 6, Sp1 fractions after two affinity chromatography steps. The symbols + and − indicate the presence or absence of calf thymus competitor DNA in the protein fractions before chromatography. (*B*) Affinity chromatography of protein fraction containing ≈1% Sp1. Lanes: 1, protein molecular size markers; 2, protein fraction before chromatography; 3–5, eluate fractions from one passage through Sp1 affinity resins; 6, eluate fraction from one passage through an affinity resin that does not contain Sp1 binding sites. The relative amounts of calf thymus competitor DNA used in the affinity chromatography experiments shown in lanes 3, 4, 5, and 6 are 0, 0.2, 1, and 1, respectively. The two bands that migrate between the 45- and 66-kDa size markers derive from 2-mercaptoethanol in the electrophoresis sample buffer.

Sepharose resins that do or do not possess Sp1 recognition sequences. As shown in Fig. 4*B*, two polypeptides of 105 and 95 kDa, which have subsequently been shown to be Sp1 (M. R. Briggs, J.T.K., and R.T., unpublished data), are purified by affinity chromatography through a resin that contains Sp1 recognition sequences but are not enriched by passage through a similar resin that does not possess Sp1 binding sites.

DISCUSSION

In this paper, we describe a method for affinity purification of sequence-specific DNA binding proteins that is fast and effective. This technique provides a means for the purification of low abundance mammalian transcription factors, such as Sp1, CTF, and AP-1. In addition, multiple sequence-specific DNA binding factors can be simultaneously purified from a single protein fraction with the use of tandem affinity columns that contain the appropriate binding sites.

A general strategy for the purification of sequence-specific DNA binding proteins can now be outlined. (*i*) First, it is important that the protein is partially purified by conventional chromatography to remove nucleases and other contaminating activities that might adversely affect the affinity column. (*ii*) Next, the recognition sequence should be identified by various methods, such as DNase I footprinting (13), methidiumpropyl-EDTA·Fe(II) footprinting (19), and dimethyl sulfate methylation protection (20). (*iii*) If more than one binding site is known, the highest affinity site for the protein should be determined because low-affinity recognition sequences may not be useful for the purification of proteins (unpublished data). (*iv*) To assist in the identification of the desired protein, two or more DNA-Sepharose resins could be prepared that contain the highest affinity recognition site with different flanking oligodeoxynucleotide sequences in addition to a control resin that does not possess any recognition sites. If a protein is purified by passage through two different

affinity resins, then contamination by proteins that bind fortuitously to flanking oligodeoxynucleotide sequences is minimized. The control resin is used to identify proteins that bind nonspecifically to DNA-Sepharose. (*v*) Last, the conditions for successful affinity chromatography should be established by variation of such factors as competitor DNAs, pH, metal ion concentrations, ligands, recognition sequences, and the purity and quantity of the crude protein fraction.

Recently, affinity purification of nuclear factor I (NFI), a sequence-specific DNA binding protein from HeLa cells that stimulates adenovirus DNA replication, has been described (9). The NFI affinity resin consisted of plasmid DNA containing 88 copies of the NFI binding site adsorbed onto cellulose by the method of Alberts and Herrick (5). Highly purified NFI was obtained from a crude protein fraction by chromatography on a nonspecific *Escherichia coli* DNA-cellulose column followed by two passages through the sequence-specific DNA-cellulose resin in the absence of any competitor DNA.

Sequence-specific DNA affinity chromatography has also been reported for SV40 large tumor antigen (T antigen), polyoma virus large T antigen, and *E. coli lac* repressor. SV40 large T antigen was purified in a similar manner as NFI by chromatography with plasmid DNA-cellulose (10), while a variation of the DNA-cellulose method was used to enrich for polyoma virus large T antigen (21). Last, the feasibility of sequence-specific affinity chromatography of *E. coli lac* repressor was investigated by comparison of the salt concentrations that were required to elute the protein from sequence-specific or nonspecific plasmid DNA resins (7).

Our previous attempts to purify Sp1 by sequence-specific DNA-cellulose chromatography with plasmid DNA containing multiple copies of a binding site failed to enrich for Sp1 relative to other DNA binding proteins (unpublished data). There are some significant differences between the previously described affinity chromatography procedures and the method we describe here that may account for the success of the synthetic DNA-Sepharose resins. First, the most important distinction is probably the addition of competitor DNA directly to the crude protein fraction before passage through the DNA-Sepharose resin. The presence of competitor DNA in solution minimizes retention of nonspecific DNA binding proteins on the affinity resin. Second, the use of synthetic oligodeoxynucleotides maximizes the specificity of the DNA-Sepharose resins. The oligodeoxynucleotides are not only more selective than plasmids containing multiple binding sites, but they can also be quickly and easily prepared. Finally, affinity chromatography has been previously performed with DNA-cellulose resins consisting of plasmid DNA adsorbed to cellulose, whereas, in this study, we have used DNA-Sepharose resins that were prepared by covalent coupling of synthetic DNA to an agarose support.

We are grateful to Kathy Jones, Mike Briggs, Bill Dynan, and Mark Biggin for their helpful suggestions. We also thank Bruce Malcolm for synthesizing the oligodeoxynucleotides that were used in this study. J.T.K. is a Fellow of the Miller Institute for Basic Research in Science. This work was funded by grants from the National Institutes of Health and by partial support from a National Institute for Environmental Health Sciences grant to R.T.

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