Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3201–3205, June 1980
Biochemistry

Recognition of chemical carcinogen-modified DNA by a DNA-binding protein*

(*N*-acetoxy-2-acetylaminofluorene/methylnitrosourea/methylmethanesulfonate/ultraviolet radiation/DNA repair)

FRANCESCO MORANELLI AND MICHAEL W. LIEBERMAN

Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110

Communicated by Richard B. Setlow, February 29, 1980

ABSTRACT **Using a filter binding assay, we have detected and partially purified a protein from human placenta that has a high affinity for *N*-acetoxy-2-acetylaminofluorene-modified double-stranded DNA (AAF-[^{3}H]DNA) of bacteriophage T7. This protein has been partially purified from a 1 M NaCl extract of a crude nuclear fraction by a combination of ion-exchange and nucleic acid affinity chromatography. With AAF-[^{3}H]DNA as the substrate, the binding reaction reached equilibrium within 1 hr at 4°C, and the extent of binding was proportional to the amount of protein added. Complex formation was dependent on both pH and salt concentration and was unaffected by the presence of sulfhydryl-blocking agents. The purest protein fraction also recognizes DNA modified with methylmethanesulfonate or methylnitrosourea. It shows little or no recognition of single-stranded DNA, double-stranded DNA, supercoiled bacteriophage φX174 DNA, partially depurinated DNA, glucosylated bacteriophage T4 DNA, or UV-irradiated DNA. No endo- or exonuclease activity, DNA polymerase activity, or glycosylase activity for AAF-DNA was detectable in the preparation.**

Although the excision repair of chemical damage clearly shares many features with the repair of UV-induced damage (1), recent experiments have suggested that there may be differences in the ways in which lesions produced by these two types of agents are recognized and removed (2, 3). If differences do exist, a class of proteins may exist that selectively recognizes DNA modified by chemical carcinogens. This logic has led us to search for DNA-binding proteins that recognize *N*-acetoxy-2-acetylaminofluorene (NA-AAF)-modified DNA (AAF-DNA). To assay for such proteins, we have used filter binding techniques (4) similar to those used to demonstrate DNA-binding proteins that specifically recognize UV-damaged DNA (5) and partially depurinated DNA (6). In this paper we report the partial purification and characterization of a DNA-binding protein from human placenta that shows strong preferential binding to AAF-DNA and DNA modified with methyl methanesulfonate or methylnitrosourea (MMS-DNA or MNU-DNA, respectively) as compared to single- and double-stranded DNA, supercoiled bacteriophage φX174 DNA, partially depurinated DNA, glucosylated bacteriophage T4 DNA, or UV-irradiated DNA.

MATERIALS AND METHODS

Preparation of Binding Substrates. Bacteriophage T7 [^{3}H]DNA (1.21×10^5 cpm/μg, unless otherwise indicated) was prepared from phage grown and isolated as described in the literature (7, 8), and the DNA was purified from NaDoDSO$_4$-lysed, proteinase K-digested (Merck) phage (9).

AAF-[^{3}H]DNA was prepared as described (10). The modified DNA was then dialyzed against TNE buffer [10 mM Tris·HCl, pH 8.0 (25°C)/80 mM NaCl/1 mM EDTA], and the degree of modification was determined from the A_{305}/A_{260} absorbance ratio (11). The procedure resulted in 2–3 AAF molecules bound per 100 bases. DNA modified by methyl methanesulfonate (MMS, Aldrich) was prepared according to Paquette *et al.* (12), and DNA modified by methylnitrosourea (MNU, Ash Stevens, Detroit, MI) was prepared as follows: 20 μg of T7 [^{3}H]DNA [8.4×10^4 cpm/μg; in 500 μl of 250 mM Tris (pH 7.3 at 25°C) and 0.5 M NaCl] was treated with 20 μg of MNU for 6 hr at 37°C. Unbound carcinogen was then removed by extensive dialysis against TNE buffer. Depurinated single-stranded (ss) or double-stranded (ds) T7 [^{3}H]DNA (8.4×10^4 cpm/μg) was prepared according to published procedures (13, 14). Glucosylated T4 [^{3}H]DNA (2.34×10^5 cpm/μg) was isolated from bacteriophage (a gift from M. Bittner and C. F. Morris). To prepare UV-irradiated DNA, 114 μg of T7 [^{3}H]DNA in 10 ml of TNE buffer was exposed to 200 or 500 J/m^2 of UV light (predominantly 254 nm). ssDNA was prepared by heating T7 [^{3}H]DNA in a boiling water bath for 10 min followed by rapid cooling in ice water. Unlabeled supercoiled φX174 DNA was a gift from S. Dresler, and ^{3}H-labeled supercoiled φX174 DNA (11.9×10^4 cpm/μg) was obtained from Bethesda Research Laboratories (Rockville, MD). Deoxyguanosine monophosphate modified with [^{3}H]NA-AAF ([^{3}H]AAF-dGMP, 878 μCi/μmol; 1 Ci = 3.7×10^{10} becquerels) was a gift from Thea D. Tlsty.

Ion Exchange and DNA Affinity Columns. DE-52 DEAE-cellulose and P-11 phosphocellulose (Whatman) were precycled as described by Bollum (15) prior to use. DNA-celluloses (1.2 mg/ml of slurry) were prepared as described by Fox and Pardee (16), using Munktell 410 cellulose (Bio-Rad) and heat-denatured or NA-AAF-modified calf thymus DNA (Worthington).

Filter Binding Assay. DNA binding proteins were assayed in a total volume of 200 μl containing 20 mM Tris at pH 8.0 (4°C), 24 mM NaCl, approximately 66 ng of AAF-[^{3}H]DNA, ss[^{3}H]DNA, or ds[^{3}H]DNA (1.21×10^5 cpm/μg unless otherwise indicated), and an appropriate aliquot of DNA-binding protein preparation. The mixture was then incubated at 4°C for 90 min (unless otherwise indicated), and the reaction was terminated by the addition of 2 ml of cold twice-concentrated standard saline citrate (NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate for the standard concentration). The samples were then filtered through Millipore HAWP filters (0.45 μm pore size) that had been previously washed with 0.3 M NaOH (17) for 10 min and with deionized water until the pH reached

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Abbreviations: AAF-DNA, MMS-DNA, and MNU-DNA, DNA modified with *N*-acetoxy-2-acetylaminofluorene (NA-AAF), methyl methanesulfonate (MMS), and methylnitrosourea (MNU), respectively; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; AAF-dGMP, deoxyguanosine monophosphate modified with NA-AAF; PhMeSO$_2$F, phenylmethylsulfonyl fluoride; NaCl/Cit, standard saline citrate.

* A preliminary report of this work has been presented: Moranelli, F. & Lieberman, M. W. (1979) in *XIth International Congress of Biochemistry: Abstracts*, Toronto, Canada, p. 34 (abstr.).

neutrality, and then stored in 0.5× NaCl/Cit. Once filtered, the samples were washed with 15 ml of 0.5× NaCl/Cit, and the filters were dried and their radioactivities were measured. Except for assays on column fractions, all assays were carried out in duplicate; the duplicates usually agreed to within ±5%. Appropriate control experiments were also carried out in the absence of the binding activity to determine the background (2.5–3.7% of input AAF-[^{3}H]DNA, and less than 1% of ss- and ds[^{3}H]DNA), and these values were subtracted from those obtained in the presence of binding protein.

Enzyme Assays. Exonuclease activity was assessed under conditions similar to those for DNA-binding activity except that 0.5 mg of bovine serum albumin (Sigma) and 5 mM $MgCl_2$ were included. The reaction took place at 37°C for 1 hr and the amount of trichloroacetic acid-soluble radioactivity released was determined. Endonuclease activity was assayed by determining changes in the electrophoretic mobility (18) of unmodified and AAF-modified supercoiled ϕX174 DNA. To assay for glycosylase activity, we have made use of the observation that guanine-AAF adducts are acid insoluble and ethanol soluble (19). [^{14}C]AAF-[^{3}H]DNA (from KB cells, 21,200 ^{3}H and 1100 ^{14}C cpm/μg DNA) was used as the substrate. DNA polymerase activity was assayed with poly(dA)·$(dT)_{10}$ (Miles) as template-primer and [^{3}H]dTTP (46 Ci/mmol, Amersham) as the substrate, as described (20).

Preparation of AAF-DNA-Binding Protein Extract. Fresh human placentas from normal deliveries were obtained from a local hospital; the tissues were immersed in cold isotonic KCl to remove any excess blood, and all subsequent operations were carried out in the cold. After the umbilical cord and membraneous sheath were excised, the damp tissue was strained of excess liquid, weighed, cut into small pieces, and homogenized with a Waring blender in 3 vol of buffer A [20 mM Tris·HCl, pH 8.0 (25°C)/1 mM EDTA/40 mM KCl/1 mM 2-mercaptoethanol/5% (vol/vol) glycerol], adjusted to 1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$, Sigma). The homogenate was then centrifuged at 6500 × g for 20 min. The supernatant was discarded and the pellet was resuspended in 1.5 vol (based on original tissue weight) of the same buffer. The suspension was then adjusted to 0.25% Triton X-100 (Sigma), stirred for 30 min, and recentrifuged as above; the supernatant was discarded. The pellet was then resuspended in 1.5 vol of buffer B (buffer A containing 0.35 M KCl) plus 1 mM $PhMeSO_2F$, stirred for 20 min, and recentrifuged. Again the supernatant was discarded, and the pellet was resuspended in another 1.5 vol of buffer B plus 1 mM $PhMeSO_2F$. The suspension was then sonicated 6 times with a Branson sonifier at a setting of 5 (50-W output) for 1 min each time. After recentrifugation, the pellet was resuspended in 1.5 vol of a 1 M NaCl solution plus 1 mM $PhMeSO_2F$ and stirred for 4 hr. The suspension was recentrifuged and the supernatant was retained. The pellet was reextracted with 0.5 vol of 1 M NaCl containing 1 mM $PhMeSO_2F$. This step was followed by centrifugation, and the supernatant was combined with that of the first 1 M NaCl extraction (fraction I) and used for subsequent purification of the AAF-DNA-binding activity.

RESULTS

Purification of AAF-DNA-Specific Binding Protein. Fraction I was dialyzed overnight against 25 vol of cold deionized water and centrifuged at 6500 × g for 30 min to remove debris (21). The supernatant (fraction II) was chromatographed on DEAE-cellulose, and the fractions were assayed for DNA-binding activities (Fig. 1A). The binding activity with the highest specificity towards AAF-[^{3}H]DNA eluted with the 0.1 M salt wash. The activity in this region of the column showed a preference for AAF-[^{3}H]DNA relative to ss[^{3}H]DNA and did not bind to ds[^{3}H]DNA. Other peaks showed less specificity and were therefore discarded. The active fractions from the 0.1 M salt wash were pooled (fraction III, Table 1) and applied to a phosphocellulose column equilibrated with buffer

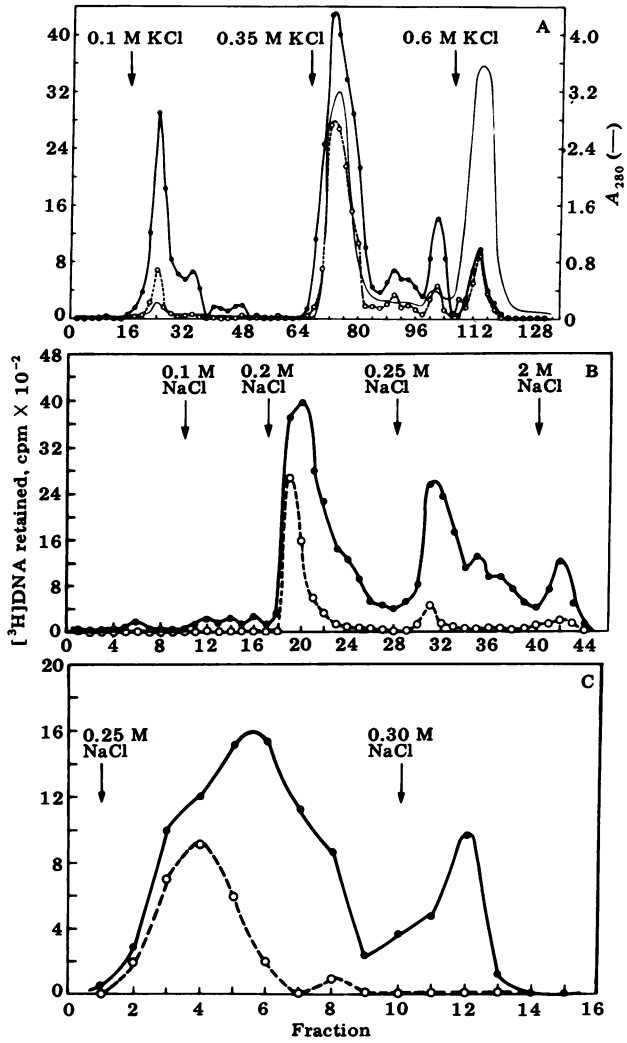

FIG. 1. (A) Elution profile of DNA-binding activities from DEAE-cellulose. Fraction II was adjusted to 20 mM Tris, pH 8, and applied to a DE52 column (5 × 20 cm) equilibrated with buffer A. The column was then washed with one column vol of Buffer A, followed by 1.75 column vol of buffer A containing 0.1 M KCl and 1.5 column vol of buffer A containing 0.35 or 0.6 M KCl. Fractions (13.5 ml) were collected, starting with the wash with buffer A containing 0.1 M KCl. Samples (20 μl) of alternate fractions were then diluted 1:10 with deionized water, and 10-μl aliquots were assayed for DNA-binding activities with either AAF-[^{3}H]DNA (●—●) or ss[^{3}H]DNA (○---○) as the binding substrates. (B) ssDNA-cellulose elution profile of DNA-binding activities. Fraction IV was diluted with 3 vol of buffer D [10 mM Tris, pH 7.5 (25°C)/1 mM EDTA/1 mM 2-mercaptoethanol/10% (vol/vol) glycerol] containing 0.1 mg of bovine serum albumin per ml and applied to a ssDNA-cellulose column (1.5 × 7 cm) equilibrated with buffer D. The column was then washed with 20 ml each of buffer D and buffer D containing 0.1 M NaCl, followed by 30-ml portions of buffer D containing 0.2 and 0.25 M NaCl and finally with 20 ml of buffer D containing 2 M NaCl. Approximately 2.6-ml fractions were collected, and 10-μl aliquots of each were assayed for binding activities. (C) AAF-DNA-cellulose elution profile of DNA-binding activities. A 10-ml sample of fraction V was applied to an AAF-DNA-cellulose column (1.5 × 4 cm) equilibrated with buffer D, and the DNA-binding activities were eluted with 20-ml washes of buffer D containing 0.25 M and 0.3 M NaCl. Approximately 3.4-ml fractions were collected, and 10-μl aliquots of each were assayed for binding activities under standard conditions except that the length of incubation was 30 min.

C [20 mM potassium phosphate, pH 7.4/1 mM EDTA/1 mM 2-mercaptoethanol/40 mM KCl/10% (vol/vol) glycerol]. The sample was eluted with a stepwise salt gradient of 0.2 and 0.3 M KCl in buffer C (data not shown). The material eluting with the 0.3 M KCl wash (fraction IV) showed the highest specificity towards AAF-[^{3}H]DNA. Fraction IV was then chromatographed on a ssDNA-cellulose affinity column (Fig. 1*B*). The fraction eluting with the 0.25 M NaCl wash (fraction V) showed the highest specificity towards AAF-[^{3}H]DNA and bound little ss[^{3}H]DNA (Fig. 1*B* and Table 1). Fraction V was then further purified on the AAF-DNA-cellulose column (Fig. 1*C*). The 0.3 M NaCl wash (fraction VI) showed the greatest specificity for AAF-[^{3}H]DNA relative to ss[^{3}H]DNA (15:1) and did not bind ds[^{3}H]DNA. This fraction was used for all subsequent experiments.

The purification of the AAF-DNA-specific binding activity from 500 g of human placenta is summarized in Table 1. The activity detected with AAF-[^{3}H]DNA as the binding substrate has been purified at least 165-fold at the phosphocellulose step (fraction IV). This estimate probably represents only the minimal degree of purification actually achieved, because earlier fractions were contaminated with other DNA-binding proteins (see Table 1) that might be detectable with the assay. The purification after the final step (fraction VI) is probably much greater but cannot be calculated due to the low protein concentration. An enrichment of AAF-DNA-specific binding activity during purification is clearly indicated by the increased preference for AAF-[^{3}H]DNA relative to ss[^{3}H]DNA and ds[^{3}H]DNA with fractionation (Table 1, columns 5 and 6). The apparent low recovery of AAF-DNA-binding activity may result from an overestimate of its abundance in the initial fraction, which contains a great many different types of DNA-binding proteins. In addition, the relative instability of the activity (see below) and the sacrifice of yield for purity have reduced recovery.

Characterization of AAF-DNA-Binding Activity. Several lines of evidence indicate that the material binding to AAF-DNA is a protein. The activity is abolished by heating to 98°C for 2 min, by the addition of 0.1% NaDodSO$_4$ to the reaction mixture, and by treatment with proteinase K or trypsin.

The binding is linear as a function of protein concentration (Fig. 2*A*) and shows great specificity for AAF-modified T7 DNA compared to ssDNA (Fig. 2*B*). When the binding is measured with albumin (0.25–1.2 mg/ml) in place of fraction VI, no retention of AAF-[^{3}H]DNA, ss[^{3}H]DNA, or ds[^{3}H]DNA on the filter is observed. At 4°C, the usual reaction temperature, the reaction is about 50% complete in 10 min and reaches equilibrium in about 1 hr (Fig. 2*B*).

Binding is increased approximately 3-fold when NaCl or KCl at 20 and 40 mM is added to the reaction mixture. Higher concentrations of salt result in a decrease in activity (Fig. 3) relative to that observed under optimal salt conditions. $MgCl_2$ and $MnCl_2$ are inhibitory at concentrations above 0.5 mM and produce 50% inhibition at about 4 and 1.5 mM, respectively. The pH optimum for the binding activity is in the range of 7.4–8.3 (Fig. 4).

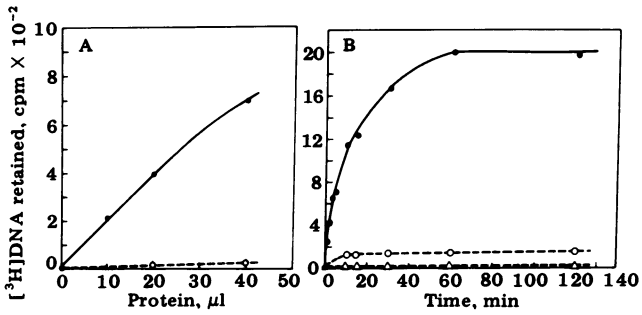

FIG. 2. (*A*) DNA-binding activity as a function of protein concentration. DNA-binding activity was assessed with AAF-[^{3}H]DNA (●) or ss[^{3}H]DNA (○) as the binding substrates under standard binding conditions except that the NaCl concentration was 60 mM and various concentrations of protein (denoted as μl of sample per assay) were used. (*B*) DNA-binding activity as a function of time. DNA-binding activities were assayed under standard conditions for various incubation times with AAF-[^{3}H]DNA (●), ss[^{3}H]DNA (○), or ds[^{3}H]DNA (△) as the binding substrates.

The substrate specificity of fraction VI is shown in Table 2. As indicated, the most efficient substrates in the binding reaction are AAF-[^{3}H]DNA, MMS-[^{3}H]DNA, and MNU-[^{3}H]DNA. We have also observed that when the latter two substrates are stored in the cold (see MMS-DNA, Table 2) or heated at 54°C (up to 2 hr, data not shown), they become much more effective in the binding reaction. These data suggest that the binding protein recognizes not only the initial modification(s) produced on the DNA by these alkylating agents but also some secondary byproduct(s) (see *Discussion*) produced slowly after the initial modifications. This enhanced binding of substrate upon "aging" or heating was not observed with AAF-[^{3}H]DNA. Heat-denatured AAF-[^{3}H]DNA (ssAAF-[^{3}H]DNA) is also recognized by the binding protein, although to a much lesser extent than nondenatured AAF-[^{3}H]DNA. These data, along with the low recognition for ss[^{3}H]DNA, suggest that specificity is conferred by both the AAF moiety and the secondary structure of the DNA. The binding protein showed no recognition of ds[^{3}H]DNA, supercoiled φX174 [^{3}H]DNA, or glucosylated T4 [^{3}H]DNA.

UV-irradiated [^{3}H]DNA and partially depurinated [^{3}H]DNA

Table 1. Purification of AAF-DNA-binding activity from human placenta

Fraction	Volume, ml	Protein, mg	Units* of binding activity observed with various DNA substrates: AAF-[^{3}H]DNA	2 × ss[^{3}H]DNA†	ds[^{3}H]DNA‡
I. 1 M NaCl extract	955	2,388	169,725	74,382 (32.3)	13,237 (12.8)
II. Dialysate	920	497	77,274	25,502 (3)	1,822 (42.4)
III. DEAE-cellulose	246	17.2	8,251	2,030 (4.1)	0 (∞)
IV. Phosphocellulose	43	<0.043	502	78 (6.4)	—
V. ssDNA-cellulose	24	—	43	3.8 (11.3)	—
VI. AAF-DNA-cellulose	13	—	3	0.2 (15)	—

Five hundred grams of tissue from two human placentas was processed. Protein concentration for fractions I and II was determined by the method of Lowry *et al.* (22), and that for fractions III and IV by the method of Warburg and Christian (23). No material absorbing at 280 nm could be detected in fractions V and VI.

* A unit of binding activity is defined as the number of μl of undiluted fraction required to cause the retention of 1 μg of T7 [^{3}H]DNA on the filter. The ssDNA values have been multiplied by 2 to compensate for the reduction in the amount of radioactivity per molecule.

† Numbers in parentheses are AAF-[^{3}H]DNA retained/2 × ss[^{3}H]DNA retained.

‡ Numbers in parentheses are AAF-[^{3}H]DNA retained/ds[^{3}H]DNA retained.

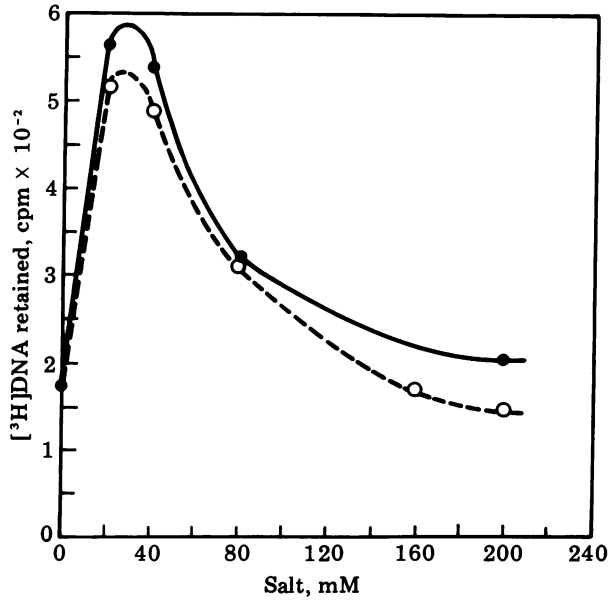

FIG. 3. Effect of salt on AAF-DNA-binding activity. A 2-ml sample of fraction VI was dialyzed overnight against buffer D to remove the NaCl. A fixed amount of this dialysate was then assayed with AAF-[3H]DNA as the binding substrate in the presence of various concentrations of either NaCl (●) or KCl (○).

Table 2. Binding substrate specificity of AAF-DNA-binding protein

Substrate	Activity, %
T7 AAF-[3H]DNA	100
Heat-denatured T7 AAF-[3H]DNA	19
T7 MMS-[3H]DNA*	
Day 1	103
Day 8	190
Day 17	227
T7 MNU-[3H]DNA	138
Partially depurinated T7 [3H]DNA	4.6
UV-irradiated T7 [3H]DNA	1.6
T7 ss[3H]DNA	3.2
Glucosylated T4 [3H]DNA	0
Supercoiled φX174 [3H]DNA	0
T7 ds[3H]DNA	0

Binding activity was assayed under standard conditions with the DNA substrates indicated. The amount of substrate present in the assay ranged from 42 ng in the case of glucosylated T4 [3H]DNA to 119 ng in the case of MMS-[3H]DNA, MNU-[3H]DNA, and partially depurinated [3H]DNA. Other substrates were present at a concentration of 82–84 ng per assay. Specific activities were as indicated in *Materials and Methods*. Activity of 100% is equal to 7–19 ng of AAF-[3H]DNA retained.

* Number of days after the DNA had been modified and stored in NaCl/Cit at 4°C.

were only minimally recognized. The failure to recognize UV-irradiated DNA is probably not a result of different extents of damage to the DNA. Indeed, even when T7 DNA was damaged with 500 J/m^2, there was no appreciable binding of it by the binding protein. Likewise, when the depurination reaction of T7 DNA was carried out for as long as 17 hr at 54°C its effectiveness as a substrate was not improved. We emphasize that the percent activities shown in Table 2 are only approximate, because the phage DNAs have different molecular weights, and the numbers of modifications per molecule differ.

We also investigated the ability of the binding protein to recognize low molecular weight derivatives of NA-AAF. We found that neither [3H]AAF-dGMP nor a mixture of [3H]-NA-AAF hydrolysis products was recognized by the binding protein, as determined by the filter assay.

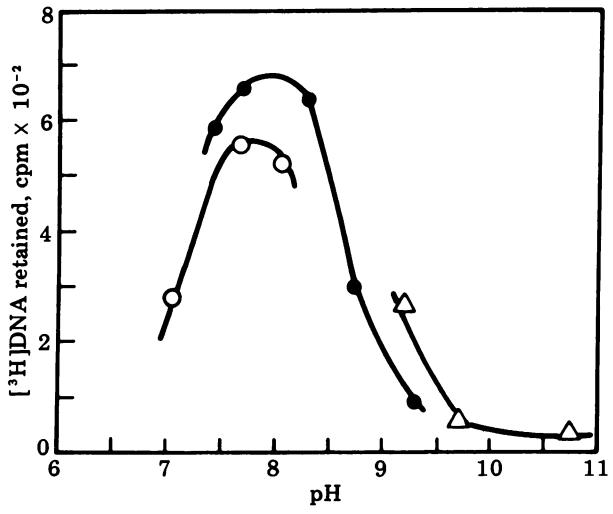

FIG. 4. Effect of pH on AAF-DNA-binding activity. A 1-ml sample of fraction VI was dialyzed overnight against buffer D containing 0.3 M NaCl and lacking 10 mM Tris, pH 7.5. The sample was then assayed at a fixed concentration under standard conditions except that the NaCl concentration was 30 mM and 20 mM phosphate (○), Tris (●), or sodium carbonate (△) buffer, at the indicated, pH values, was used.

Preliminary evidence suggests that the amount of binding activity observed is dependent on the degree to which the DNA has been modified with NA-AAF or MMS. Treatment of a fixed amount of T7 [3H]DNA with increasing amounts of either NA-AAF or MMS resulted in increased levels of retention of the DNAs in the presence of a constant amount of protein (data not shown).

We have also studied the effects of various compounds on the binding reaction. *N*-Ethylmaleimide (10 mM) and iodoacetamide (10 mM) had no effect on binding, suggesting that the DNA-binding material is not a sulfhydryl-containing protein or at least that any sulfhydryl groups that may be present are not essential for binding. EDTA (8 mM) had little effect on the reaction, and this datum together with the lack of stimulation by Mg^{2+} or Mn^{2+} discussed above indicates that divalent cations are not involved in the binding reaction. Caffeine, which is known to bind to single-stranded regions of DNA (24), had no effect on the binding activity at concentrations up to 2.5 mM.

Although the binding activity (fraction VI) is stable for at least one month at 4°C in buffer D containing 0.3 M NaCl, activity is rapidly lost under conditions of low salt or elevated temperature. This instability and the low protein concentration in the final fraction have made it difficult to estimate its molecular weight. Usual techniques such as electrophoresis, gel filtration, and velocity sedimentation have been unsuccessful. Data from concentrating procedures indicate that the protein is retained by a Millipore immersible concentrator with an exclusion limit of 10,000 daltons.

We have analyzed fraction VI for a series of enzymatic activities. The protein preparation was devoid of exonuclease, endonuclease, glycosylase, and DNA polymerase activities. Some preparations contained RNase activity; however, this activity appears to be a contaminant because other preparations were completely devoid of it.

DISCUSSION

DNA modified with the chemical carcinogens NA-AAF or MNU or the alkylating agent MMS seems to be required for recognition by the binding protein preparation. Other DNAs are poorly recognized, if at all (Table 2). NA-AAF, MNU, and MMS modify DNA at many sites, but it is of interest that the major site of damage for each of these agents is the imidazole ring of guanine. NA-AAF attacks the C8 position (25), whereas MMS and MNU attack the N7 position (26). Furthermore, damage by these agents at the C8 and N7 positions predisposes to imidazole ring opening (27, 28). Thus, one determinant of recognition may be damage to the imidazole ring of guanine, and the enhanced binding of DNA damaged with MMS or MNU with "aging" or heating may result from a secondary reaction such as ring opening. Whether or not the protein can recognize other types of damage produced by these agents such as adenine damage, O^6-guanine alkylation, phosphotriester formation, or aryl addition at the guanido group (25, 26) is unknown. The possibility that recognition has one variable constraint (damage) and one fixed constraint (DNA) should make further studies with this protein interesting. At present it is not certain that a single protein species is responsible for the binding to various chemically modified DNAs. Although for purposes of discussion we have assumed that this is the case, additional experiments are necessary to explore this problem.

At present the function of the protein is unknown. The preparation lacks DNA endo- and exonuclease activities, glycosylase activity against AAF-damaged DNA, and DNA polymerase activity when assayed under conditions of maximal binding activity or in the presence of 5 mM $MgCl_2$. It would be of interest to know if the protein functions in recognizing chemical lesions in DNA during repair. At present we have not evaluated this possibility. Alternatively, the protein may have some function entirely unrelated to the recognition of DNA damage and repair and may be involved in some as yet unidentified process that requires recognition of chemically modified DNA.

The isolated protein appears to differ from other proteins reported to bind carcinogen alone or damaged DNA. Proteins such as ligandin (glutathione *S*-transferase B) (29), H-protein (30), and cortisol-binding proteins (31) that bind carcinogens have no requirement for DNA in the binding step and appear to be primarily involved in the transport and metabolism of hydrophobic compounds. Because the protein does not recognize UV-irradiated DNA and has no absolute requirement for monovalent or divalent cations, the AAF-DNA-binding protein is probably different from the protein isolated by Feldberg and Grossman (5). In addition, its inability to bind partially depurinated DNA, its lack of sensitivity to caffeine and EDTA, and its less specific salt requirement indicate that it is different from the protein isolated by Deutsch and Linn (6).

We believe that the detection and isolation of proteins that are highly selective for different types of DNA damage is a useful approach to analyzing the molecular events involved in DNA repair. Once isolated, such proteins may be assayed not only for repair functions (e.g., nuclease activity, polymerase activity, etc.) but also for their ability to stimulate or redirect the function of known repair proteins. In addition, the effectiveness of these proteins in augmenting or complementing the repair of specific types of damage in permeable normal or repair-deficient human cells (e.g., ref. 32) may be determined. Thus, by focusing on a single event presumed to occur during repair, the binding of proteins to damaged DNA, one may be able to identify repair functions that have previously gone unrecognized.

We are indebted to Drs. M. Bittner and C. F. Morris for gifts of phage and their many thoughtful comments. We also thank S. M. Cohn and Dr. M. J. Smerdon for their valuable suggestions in the preparation of this manuscript. This work was supported by National Institutes of Health Grant CA 20513. F.M. is a Monsanto Fund Fellow in Toxicology. Additional support was provided by the following companies: Brown & Williamson Tobacco Corp.; Larus & Brother Co., Inc.; Liggett & Myers, Inc.; Lorillard, a Division of Loews Theatres, Inc.; Philip Morris, Inc.; R. J. Reynolds Tobacco Co.; United States Tobacco Co.; and Tobacco Associates, Inc.

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