Purification and Characterization of a Human Protein That Binds to Damaged DNA†

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ABSTRACT: Xeroderma pigmentosum (XP) is an inherited disease characterized by defective repair of DNA damaged by ultraviolet (UV) radiation or agents that produce bulky DNA adducts. Human cells contain a factor that is deficient in a subset of patients from XP complementation group E and binds to DNA damaged by UV, cisplatin, or denaturation. This factor, XPE binding factor (XPE-BF), was purified to near homogeneity. The denatured protein migrated as a 125-kDa polypeptide on SDS–PAGE, and the native protein migrated primarily as a monomer on gel filtration and glycerol gradient sedimentation. Sedimentation revealed major peak in binding activity at 6.8 S, corresponding to the monomeric form, and a minor peak at 14.5 S, suggesting a homodimeric form. Binding activity was dependent on unmodified cysteine residues, stimulated by magnesium, and inhibited by zinc. Binding to UV-damaged nucleotides was 500 000-fold greater than for intact nucleotides, explaining how a molecule with an abundance of only 1–2 molecules per megabase can survey the genome for damaged DNA. Binding required a minimal DNA substrate of between 16 and 26 bp, as determined by a novel "shoe size" assay. Consistent with its previously noted versatility, XPE-BF bound to some cyclobutane pyrimidine dimers and at least one other UV-induced lesion. However, it may not bind to a subset of cyclobutane dimers, likely including the thymine dimer. These findings may explain the relatively mild phenotype of XP group E and suggest the existence of at least one other binding protein involved in the XP repair pathway.

The environment contains a multitude of agents that damage DNA. Some are indigenous to the biosphere, such as ultraviolet radiation from the sun or plant and fungal toxins. Others are man-made chemicals. How do living organisms recognize the broad spectrum of DNA lesions and target them for repair? In the bacterium *Escherichia coli*, several damage recognition proteins have been identified and, in some cases, purified and extensively characterized (Sancar & Sancar, 1988). For example, the uvrABC excision repair system is capable of recognizing and repairing a large variety of bulky DNA adducts. It is reasonable to suppose that some aspects of DNA repair in *E. coli* can be translated to humans. However, it is likely that the translation will be incomplete and that the recognition of damaged DNA will be a more complex process. Humans have a genome 10^3 times larger and a lifetime 10^6 times longer, presenting a larger target over longer periods of time to DNA-damaging agents. The requirements for damage recognition may prove to be significantly more stringent in humans than in bacteria.

The human counterpart of the *E. coli* uvrABC mutants may be the autosomal recessive disease xeroderma pigmentosum (XP), which is characterized by abnormal sensitivity to ultraviolet (UV) radiation, a marked predisposition toward skin cancer, and a defect in one of the early steps of DNA excision repair (Cleaver & Kraemer, 1989). Somatic cell fusion experiments have defined seven complementation groups, A–G, implying that XP involves a biochemical pathway containing multiple proteins. In addition to UV-induced lesions, XP cells are deficient in the repair of bulky adducts produced by a large number of agents, including the antitumor drug cisplatin (Chu & Berg, 1987). Thus, the XP repair pathway must be versatile enough to recognize many different lesions.

Human cell extracts contain a protein factor that binds to DNA damaged by UV radiation, cisplatin, or denaturation (Chu & Chang, 1988). The factor was present in nuclear and absent in cytoplasmic fractions. It was absent in two Dutch patients from XP complementation group E (Chu & Chang, 1988) and was designated XPE binding factor (XPE-BF). XPE-BF was present in 10 cell lines from the six remaining XP complementation groups (Chu & Chang, 1988) as well as 20 normal individuals (Y. Shen, M. Patterson, B. Hwang, and G. Chu, unpublished data). Subsequent studies have found binding activity to be present in eight of nine group E patients from Japan (Kataoka & Fujiwara, 1991; Keeney et al., 1992). It is possible that XPE individuals with normal binding activity have mutations in a domain of the protein involved in a function other than DNA binding, perhaps in the recruitment of other repair proteins needed for subsequent steps in DNA repair. The observation that XPE-BF was absent in a subset of XPE patients but present in every non-XPE individual was statistically significant, $p = 0.02$ by Fisher's exact test. Nevertheless, demonstration that the primary defect in XP group E involves XPE-BF must await cloning of the gene.

When human cell lines were grown in the presence of stepwise increasing doses of cisplatin, they became resistant to cisplatin cytotoxicity (Chu & Chang, 1990). Transfection of cisplatin-cross-linked plasmid DNA carrying a marker gene demonstrated that the resistant cells were capable of repairing damaged DNA more efficiently. Furthermore, the resistant cells expressed 3–5-fold higher levels of XPE-BF, suggesting that XPE-BF may be responsible for the development of some forms of drug resistance in human tumors.

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In summary, these studies support the conclusion that XPE-BF participates in a versatile DNA repair pathway: (1) it is deficient in XP group E, a disease of defective DNA repair; (2) it is increased in cells with increased DNA repair; and (3) it binds to damaged DNA. We propose that XPE-BF acts at the recognition step of the excision repair pathway defined by XP and that it then recruits additional repair proteins to the site of damage.

Although experiments with crude nuclear extracts have been fruitful, a number of important questions can be answered only by studying purified protein. Therefore, to better understand its role in recognizing damaged DNA, XPE-BF was purified from extracts of human cells. This report describes how purified XPE-BF recognizes and binds to damaged DNA.

MATERIALS AND METHODS

Buffers. The following buffers were used throughout these experiments: buffer A (10 mM potassium acetate, pH 4.5, 0.5 mM EDTA, 1 mM DTT, 20% glycerol); buffer B (10 mM Hepes, pH 7.9, 0.5 mM EDTA, 1 mM DTT, 20% glycerol); buffer C (12 mM Hepes, pH 7.9, 60 mM KCl, 5 mM $MgCl_2$, 4 mM Tris, 0.6 mM EDTA, 1 mM DTT, 12% glycerol).

Isolation of Cell Nuclei from Human Placenta. Human placentas from normal births were immediately placed on ice and within 8 h of delivery were washed clean of blood with buffer A. A single placenta yielded an amount of XPE-BF equivalent to 100 L of HeLa cells grown in spinner culture (E. Chang, B. Hwang, and G. Chu, unpublished data). The membranous sheath and as much connective tissue as possible were removed. The remaining tissue was minced into small pieces, washed with buffer A, and then strained on a cheesecloth to remove excess liquid. The damp tissue was weighed, mixed with fresh buffer A (5 mL/g of tissue), homogenized in a Waring blender with two pulses of 30 s each at 4 °C, and spun in a Sorvall GS-3 rotor at 10000*g* for 20 min at 4 °C. The pellet was resuspended in the same volume of buffer A and then homogenized and spun a second time. The pellet at this point was essentially a crude preparation of cell nuclei.

Preparation of Crude Nuclear Extract from Nuclei. The cell nuclei from 10 placentas were suspended in 1.2 L of buffer A, disrupted by three cycles of repeated freeze-thawing, and then spun at 10000*g* in a Sorvall GS-3 rotor for 20 min at 4 °C. The pellet was resuspended in 1.2 L of buffer A in 0.2 M KCl, held at 4 °C for 1 h with stirring, spun down, separated from the supernatant, resuspended in 1.2 L of buffer A in 0.5 M KCl, held at 4 °C for 6 h with stirring, and spun down again. The supernatant was kept and the pellet was extracted two more times with buffer A in 0.5 M KCl. The combined supernatants consisted of 3.5 L of crude nuclear extract containing a total of 14.6 g of protein (fraction I). Fractions I–VII are designated in Table I. This procedure of sequential extraction at pH 4.5, first in 0.2 M KCl and then in 0.5 M KCl, resulted in a 4-fold purification, since a large amount of protein but no binding activity was removed with the 0.2 M KCl extraction.

Purification of XPE-BF from Crude Nuclear Extract. Protein concentration during purification was monitored by absorbance at 280 nm or by the method of Bradford (Bradford, 1976). Binding activity was precipitated from the crude extract by the addition of solid ammonium sulfate to 75% saturation followed by centrifugation at 37 000 rpm in a Beckman Ti45 rotor for 90 min at 4 °C. The pellet was resuspended in buffer B and dialyzed against buffer B with 0.2 M KCl. Insoluble material was removed from the dialyzed extract by centrifugation at 37 000 rpm in a Beckman Ti45 rotor for 90 min at 4 °C. The cleared extract (fraction II) was loaded onto a 500-mL Affi-Gel blue column (Bio-Rad) equilibrated with buffer B containing 0.2 M KCl. After the column was washed with the equilibrium buffer, protein was eluted with a linear salt gradient of 0.2 M to 1.0 M KCl in buffer B. Binding activity eluted between 0.3 and 0.4 M KCl. The fractions containing binding activity were pooled (fraction III), and solid KCl was added to bring the concentration of KCl up to 2 M, and the fractions were loaded onto a 30-mL phenyl-Sepharose column (Pharmacia) equilibrated with buffer B containing 2 M KCl. Binding activity was then step-eluted with buffer B (fraction IV). This method of removing salt was used instead of dialysis because at this stage of purification significant amounts of XPE-BF stuck to dialysis membrane.

The active fractions were pooled and loaded onto a 2-mL double-stranded DNA–cellulose column (Sigma Type 50) equilibrated with buffer B containing 0.1 M KCl. Binding activity was eluted with a linear salt gradient of 0.1 M to 2.0 M KCl in buffer B. Binding activity eluted between 0.3 and 0.4 M KCl. Active fractions were pooled (fraction V), diluted with buffer B to bring the salt concentration down to 0.2 M KCl, supplemented with $MgCl_2$ to a concentration of 5 mM, and loaded onto a 0.3-mL UV-DNA–cellulose affinity column equilibrated with buffer B containing 0.2 M KCl and 5 mM $MgCl_2$ and then eluted with step salt gradients from 0.25 M to 1.0 M KCl in buffer B plus 5 mM $MgCl_2$ followed by 1.0 M KCl in buffer B without $MgCl_2$.

The active fractions from the first UV-DNA–cellulose column were pooled (fraction VI), and a small portion (0.1%) was incubated with 200 μg of supercoiled pUC18 DNA for 2 h at 4 °C and loaded onto a 20-μL UV-DNA–cellulose column, which was packed in a micropipet tip and equilibrated with buffer C containing 0.2 mg/mL pUC18 DNA. The undamaged pUC18 DNA was used as a competitor to remove nonspecific DNA-binding proteins that would otherwise bind to the column, which contained about 100 μg of UV-irradiated salmon sperm DNA. The column was washed with buffer C containing 0.2 M KCl and step-eluted with buffer C containing 0.4 M KCl and then 1.0 M KCl (fraction VII). The eluate was stored in aliquots at −80 °C.

Preparation of UV-Damaged DNA–Cellulose. To prepare the UV-damaged DNA–cellulose affinity column, a total of 60 mg of salmon sperm DNA was diluted to a concentration of 50 μg/mL, placed in an open glass vessel (20 × 30 cm), exposed to a UV germicidal lamp to a dose of 6200 J/m^2 with shaking, and then concentrated by ethanol precipitation.

The UV-irradiated DNA was adsorbed to 4 g of acid-washed cellulose (Sigma Type 50) by the method of Alberts and Herrick (Alberts & Herrick, 1971). Following adsorption, the DNA was cross-linked to the cellulose as previously described (Litman, 1968) by suspending the DNA–cellulose resin in ethanol and exposing the mixture to further UV irradiation to a dose of 10 000 J/m^2 with continuous slow stirring. The coupling efficiency was about 50%, corresponding to 5 mg of DNA/mL of column volume. By comparison, the more recent procedure of covalent coupling of DNA to cyanogen bromide-activated Sepharose (Kadonaga & Tjian, 1986) yielded only 30–50 μg of DNA/mL of column volume (data not shown) and was therefore more than 100-fold less efficient. This was a crucial difference, since the carbohydrate matrix bound contaminating proteins nonspecifically and XPE-BF irreversibly. The reduction in column volume improved

both separation from contaminating proteins and yield of XPE-BF.

SDS–PAGE. Proteins were resolved under denaturing conditions by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) with 7.5% polyacrylamide by the method of Laemmli (Laemmli, 1970). After electrophoresis, the proteins were silver-stained as described previously (Merril, et al., 1980), except that the gels were fixed in 40% methanol/10% trichloroacetic acid, followed by 10% ethanol/5% acetic acid, and finally deionized water.

Preparation of UV-Damaged DNA Probe f148. The DNA fragment f148 consisted of 148 bp from the bacterial chloramphenicol acetyltransferase gene. The fragment was labeled by two methods. In the first method, f148 was end-labeled with the large fragment of DNA polymerase I in the presence of [α-^{32}P]dCTP to fill in the 5′ overhang generated by *Hin*dIII (Patterson & Chu, 1989). In the second method, f148 was incubated with exonuclease III to generate extended 5′ overhangs and then incubated with the large fragment of DNA polymerase I in the presence of [α-^{32}P]dCTP for 20 min and then in the presence of 0.2 mM unlabeled dCTP for an additional 20 min to fill in the 5′ overhangs. The exonuclease III labeling method incorporated about 10-fold more radioactivity than the first method. The reaction volume was heat inactivated at 65 °C for 10 min, and unincorporated nucleotides were removed by spun-column chromatography (Sambrook et al., 1989). The labeled f148 was UV irradiated at a DNA concentration of 0.2 μg/mL with a germicidal lamp (Sylvania G15T8) at a flux of 10.4 J/m^2 s^{-1} for a total dose of 5000 J/m^2, unless specified otherwise.

Gel Mobility Shift Assay. Binding activity to the UV-irradiated DNA probe f148 was measured by a gel mobility shift assay essentially as described previously (Chu & Chang, 1988). A reaction mixture of 10 μL contained 0.2 ng of DNA probe in buffer C together with protein extract, which was always added last. Unlabeled competitor DNA consisting of a 1:2 mixture of sonicated salmon sperm DNA and the alternating copolymer poly(dI-dC)·poly(dI-dC) was added to the reaction mixture in different amounts depending on the purification stage to eliminate nonspecific DNA binding by contaminating proteins. After the addition of protein extract, the reaction mixture was incubated at room temperature for 30 min and then resolved by nondenaturing gel electrophoresis through 5% polyacrylamide in TGE (50 mM Tris, pH 8.5, 380 mM glycine, 2 mM EDTA). The gel was dried onto Whatman 3MM paper and exposed to X-ray film.

Removal of Cyclobutane Pyrimidine Dimers from the DNA Probe. Cyclobutane pyrimidine dimers in UV-irradiated f148 DNA were repaired enzymatically by purified photolyase from the cyanobacterium *Anacystis nidulans* (a kind gift of Andries Eker) leaving a DNA probe containing the remaining UV-induced photoproducts (Eker et al., 1990). The UV-irradiated DNA probe was incubated with photolyase in buffer containing 20 mM potassium phosphate, pH 7.0, 100 mM NaCl, 5 mM 2-mercaptoethanol for 20 min at room temperature in the dark, followed by photoreactivation for 20 min under a blue fluorescent lamp (Phillips TLADK 30W/03, λ_{max} = 425 nm). The DNA was then extracted with phenol/chloroform, extracted with ether, precipitated with ethanol, and resuspended in 10 mM Tris, pH 8.0, 1 mM EDTA.

Successful removal of the cyclobutane pyrimidine dimers was monitored by purified phage T4 endonuclease V (a kind gift of Ann Ganesan), which nicks DNA specifically near the site of the dimer (Gordon & Haseltine, 1980). DNA was incubated with T4 endonuclease V in 10 mM Tris, pH 8.0, 10 mM EDTA, 100 mM NaCl at 37 °C for 20 min, added to an equal volume of denaturing buffer (98% formamide, 10 mM EDTA, pH 8.0, 0.025% xylene cyanol, 0.025% bromophenol blue) and heated to 95 °C for 5 min., and then resolved by denaturing gel electrophoresis in 8% polyacrylamide, 7 M urea, TBE (89 mM Tris, pH 8.0, 89 mM borate, 2 mM EDTA). After electrophoresis, the gel was dried onto Whatman 3MM paper and exposed to X-ray film (Kodak XAR-5).

Preparation of DNA Probes for Measurement of the Shoe Size. The shoe size experiment included a mixture of f148 and DNA fragments, 16, 26, 34, 44, 68, 89, 110, and 174 bp in size. The 16-bp fragment was in either one of two forms:

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5' GATCCGTCAACCTGCA 3'
3' CTAGGCAGTTGGACGT 5'

5' --------G------- 3'
3' --------C------- 5'
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The first form contained one site for a potential TT dimer, and both forms contained multiple sites for other pyrimidine dimers. Both forms gave identical results (data not shown). The remaining fragments were generated by *Ava*II and *Hpa*II cleavage of pUC18 plasmid DNA followed by purification from a 2% agarose gel to isolate the fragments from 26 to 174 bp.

The 16-bp fragment was end-labeled by incubation with [γ-^{32}P]ATP and T4 DNA kinase for 20 min at 37 °C. The pUC18 fragments were end-labeled with the large fragment of DNA polymerase I as described previously. The labeled DNA mixture was UV-irradiated with a germicidal lamp at a flux of 20.8 J/m^2 s^{-1} to doses of 12, 24, and 48 kJ/m^2.

Calculation of Molecular Mass of Native Protein. The molecular mass of native XPE-BF may be calculated from the formula (Siegel & Monty, 1966): $M = 6\pi\eta Nas/(1-v\rho)$, where η is the viscosity of water (0.01 poise), N is Avogadro's number (6.02×10^{23}), a is the Stokes radius in centimeters, s is the sedimentation coefficient in water in seconds, v is the partial specific volume, and ρ is the density of water (1 g/mL). The partial excluded volume may be calculated from the amino acid content of the protein. However, in the absence of such information, an average value v = 0.725 mL/g may be used. Most proteins have a value for v between 0.700 and 0.750 mL/g (Lee & Timasheff, 1974). Protein shape asymmetry is measured by the frictional ratio f/f_0, which may be calculated from the equation: $f/f_0 = a/(3vM/4\pi N)^{1/3}$ (Siegel & Monty, 1966).

Gel Filtration. XPE-BF (fraction VI) was applied to a 1 × 30 cm Superose 12 gel filtration FPLC (Pharmacia) column, equilibrated with buffer B containing 0.2 M KCl. Fractions of 0.3 mL were collected at a rate of 0.3 mL/min. Marker proteins were detected by absorbance at 280 nm. Molecular masses and Stokes radii of the marker proteins were ferritin (440 kDa, 61 Å); catalase (232 kDa, 52.2 Å); aldolase (158 kDa, 48.1 Å); bovine serum albumin (66 kDa, 35.5 Å); ovalbumin (43 kDa, 30.5 Å); and cytochrome *c* (12.4 kDa, 17 Å).

Glycerol Gradient Sedimentation. Purified XPE-BF (fraction VII) was loaded onto 1.3 mL of a 20%–45% (v/v) glycerol gradient in 10 mM Hepes, pH 7.9, 1 mM DTT, 200 mM KCl, 4 mM $MgCl_2$. The gradient was centrifuged for 19 h at 50 000 rpm (166000*g*) at 4 °C in a tabletop ultracentrifuge (Beckman TL100, rotor TLS-55). Fractions of 40 μL were collected from the top of each tube. XPE-BF activity was assayed by the gel mobility shift assay and the binding activity was quantitated by densitometry.

Table I: Purification[a] of XPE Binding Factor

fraction	total protein (mg)	volume (mL)	total binding (units × 10^6)	specific binding ((units × 10^2)/mg)	net yield (%)	cumulative purification
I sequential salt extraction	14600	3500	35.0	24	100	4
II $AmSO_4$ precipitation	5700	690	31.0	54	88	9
III Affi-Gel blue agarose	90	500	8.0	890	23	150
IV phenyl-Sepharose	60	100	7.0	1200	20	200
V DNA–cellulose	6	5	4.0	6700	11	1100
VI first UV DNA–cellulose	0.5	5	2.8	56000	8	9300
VII second UV-DNA–cellulose[b]	5×10^{-5}	0.2	6×10^{-4}	120000		20000

[a] Purification was performed on extracts from 10 pooled human placentas as described in Materials and Methods. One unit of binding activity was defined as 33% binding to 0.2 ng of UV-damaged f148 DNA after a 30-min incubation at room temperature. Thus, one unit represents binding to 0.68 fmol of the DNA probe. [b] 0.1% of the output of first UV-DNA affinity column was applied onto the second UV-DNA affinity column.

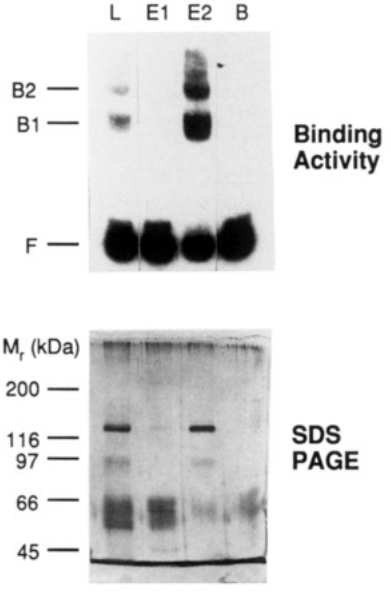

FIGURE 1: Purification of XPE-BF. Purification of XPE-BF was assayed by SDS–PAGE followed by silver staining and by the gel mobility shift binding assay. Fractions with binding activity from the first UV-DNA–cellulose column were pooled (fraction VI) and loaded onto a second UV-DNA–cellulose column (L), eluted at 0.4 M KCl (E1), and eluted at 1.0 M KCl (E2). A lane with no protein shows the silver-staining background (B). The purified binding activity in E2 (fraction VII) contained a major band at 125 kDa and a minor band at 93 kDa. Molecular mass markers were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa).

RESULTS

Purification of XPE-BF to Near Homogeneity. Table I summarizes the protocol, in which the final steps were dependent on affinity columns made from undamaged double-stranded DNA cellulose and from UV-irradiated DNA cellulose. When protein was eluted from the first UV-DNA cellulose column, binding activity was recovered in step elutions of 0.3 M, 0.35 M, 0.4 M, 0.6 M, and 1.0 M KCl (data not shown). The elution of XPE-BF over such a broad range of salt concentrations suggests that the UV-DNA–cellulose contains a heterogeneous mixture of binding sites with a broad range of affinities for XPE-BF. The UV dose of 10 000 J/m^2 which was used to make the column is known to produce a number of different lesions, including cyclobutane dimers, (6–4) photoproducts, thymine glycols, and interstrand cross-links (Spivak et al., 1988).

Figure 1 shows that the pooled eluate from the UV-DNA–cellulose column (fraction VI, lane L) consisted of approximately 50% of a 125-kDa polypeptide. This sample was loaded onto a second UV-DNA–cellulose column. The 0.4 M KCl eluate (E1) contained almost all the contaminating low molecular weight proteins while the 1.0 M KCl eluate (E2) contained predominantly the 125-kDa band with a minor band at 93 kDa. Binding activity was nearly absent in E1 but present at high levels in E2, paralleling the presence of the 125-kDa band.

XPE-BF Consists of a 125-kDa Polypeptide. These results suggested that XPE-BF contains one or more subunits of a 125-kDa polypeptide. To rule out the possibility that XPE-BF might include a minor contaminant such as the 93-kDa polypeptide, the stoichiometry of binding was measured. Therefore, the pooled eluate from the first UV-DNA–cellulose column (fraction VI) was resolved by SDS–PAGE (Figure 1, lane L), electroblotted to PVDF membrane, and stained with Coomassie Blue to identify the protein bands, which were then subjected to amino acid analysis. The concentration of the 125-kDa protein was 10-fold greater than for the 93-kDa protein. The purified sample (E2, fraction VII) contained an even greater preponderance of the 125-kDa band, approximately 20-fold greater than the 93-kDa band.

Next, fraction VI was tested for binding activity as a function of the amount of protein. As demonstrated in Figure 2, binding activity was a linear function of the amount of protein added to the reaction. Note that the binding activity in band B2 represents two independent binding events (see below). Thus, the addition of fraction VI containing 60 pg of the 125-kDa polypeptide and 6 pg of the 93-kDa polypeptide resulted in binding to 10% of 0.2 ng of UV-f148 probe, or 0.20 fmol of protein–DNA complex.

This degree of binding could not be accounted for by the 93-kDa protein, since 6 pg of the 93-kDa polypeptide corresponds to only 0.065 fmol. On the other hand, 60 pg of the 125-kDa polypeptide corresponds to 0.48 fmol. Thus, the stoichiometry is consistent with binding by either a homodimer of the 125 kDa protein if almost all the protein in the reaction is bound to DNA or a monomer if 50% of the protein remains unbound. Therefore, XPE-BF appears to consist of a 125-kDa polypeptide that binds to damaged DNA either as a monomer or as a homodimer.

XPE-BF Exists Primarily as a Monomer in Solution. The native molecular mass of a protein may be estimated from its Stokes radius and sedimentation coefficient (Siegel & Monty, 1966). Gel filtration of XPE-BF led to an estimate for the Stokes radius of 47 Å (Figure 3). Sedimentation led to an estimate for the sedimentation coefficient of $S_{20,w} = 6.8 \times 10^{-13}$ s (Figure 4). There was a small but reproducible shoulder in the binding activity centered at $S_{20,w} = (14.5 \pm 1.0) \times 10^{-13}$ s. Thus, the predominant native species of XPE-BF in solution has a molecular mass of M = 134 kDa ± 20 kDa, where the error includes uncertainty in the partial specific volume. This agrees with the molecular mass estimate from SDS–PAGE of 125 kDa. Therefore, XPE-BF exists primarily as a monomer in solution. The frictional ratio for XPE-BF is 1.39. For an

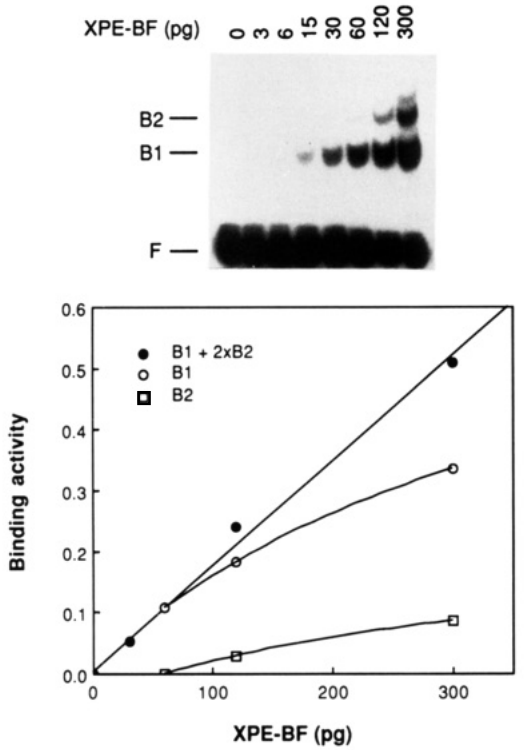

FIGURE 2: Titration of binding activity with purified XPE-BF. (A, top) Mobility shift gel: Fraction VI was analyzed for binding activity as a function of protein concentration. Each 10-μL binding reaction contained 0.2 ng of UV-f148 probe DNA and from 0 to 300 pg of the 125-kDa polypeptide. (B, bottom) Binding activity: Quantitation was done by scanning densitometry of the autoradiograph. The open circles represent the fraction of probe shifted to band B1. The open squares represent the fraction of probe shifted to band B2. Band B2 corresponds to two independent binding events on the same probe. Therefore, the closed circles represent total binding activity, B1 plus twice B2.

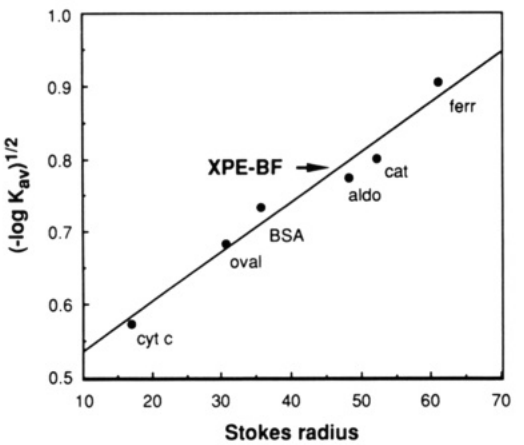

FIGURE 3: Gel filtration of XPE-BF. Fraction VI was separated by gel filtration with fractions assayed for binding activity. The parameter K_{av} is defined in terms of the elution volume of the sample V_e, the void volume V_0 (measured with blue dextran), and the gel bed volume V_t of the column, $K_{av} = (V_e - V_0)/(V_t - V_o)$. Theoretical calculations predict that the Stokes radius is a linear function of $(-\log K_{av})^{1/2}$ (Siegel & Monty, 1966). Linear interpolation yielded a Stokes radius of 47 Å for XPE-BF.

idealized ellipsoid of revolution, this corresponds to an axial ratio of 7, implying that XPE-BF has a significant degree of asymmetry.

Metal Ions Affect Binding to Damaged DNA. Figure 5 shows that XPE-BF binding activity increased markedly in the presence of magnesium ion, provided that the protein concentration in the reaction was low enough. At higher protein concentrations, the stimulation by $MgCl_2$ disappeared.

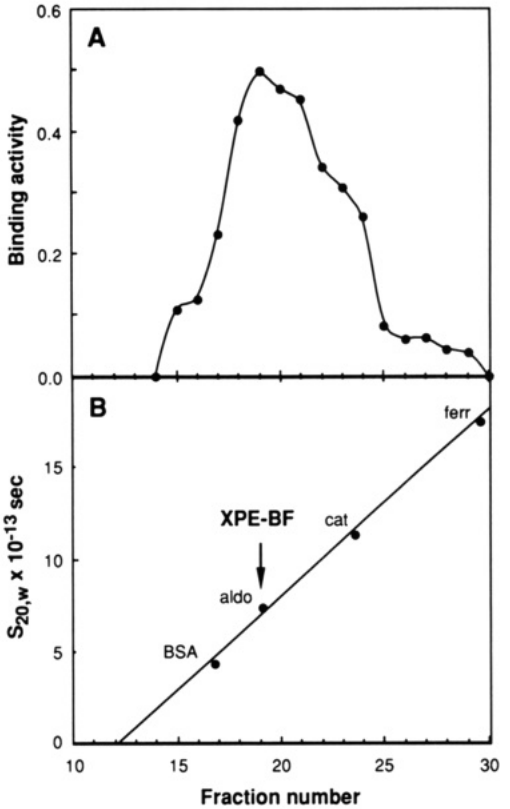

FIGURE 4: Glycerol gradient sedimentation of XPE-BF. (A) Gel mobility shift assay of glycerol gradient fractions: XPE-BF activity was assayed by the gel mobility shift assay and the binding activity was quantitated by densitometry. (B) Sedimentation coefficient of XPE-BF: The sedimentation coefficient was determined by the position of marker proteins, which were loaded onto the same glycerol gradient with XPE-BF. The marker proteins were detected by SDS–PAGE followed by staining with Coomassie blue. Sedimentation coefficients at 20 °C in water ($\times 10^{-13}$ s) of the marker proteins were ferritin (ferr, 17.5); catalase (cat, 11.3); aldolase (aldo, 7.4); and bovine serum albumin (BSA, 4.3).

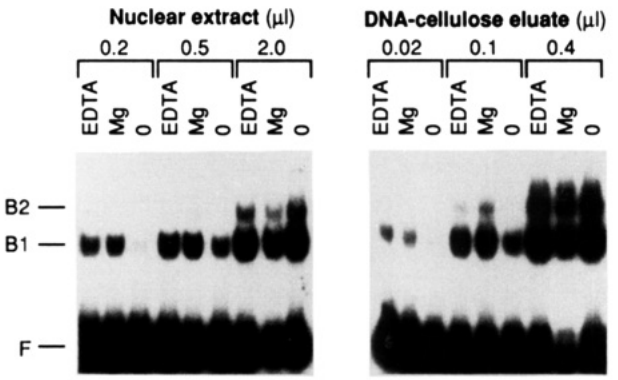

FIGURE 5: Metal ions affect the binding of XPE-BF to damaged DNA. The dependence of binding activity was assayed at different protein concentrations in the absence (0) or presence of either 5 mM $MgCl_2$ (Mg) or 5 mM EDTA. Both fraction I (nuclear extract, 4.17 mg/mL) and fraction V (DNA–cellulose eluate, 1.2 mg/mL) were used to demonstrate that the effects remained after extensive purification of XPE-BF.

Furthermore, the effect was present throughout the purification of XPE-BF through the DNA–cellulose eluate (fraction V). Representative data from fractions I and V are shown. More highly purified fractions contained $MgCl_2$ and so could not be tested.

Figure 5 also shows that EDTA stimulates XPE-BF binding activity, even in the absence of $MgCl_2$. This suggests that a chelatable metal ion other than magnesium was present in the extracts and that it inhibits binding. For example, 2 mM

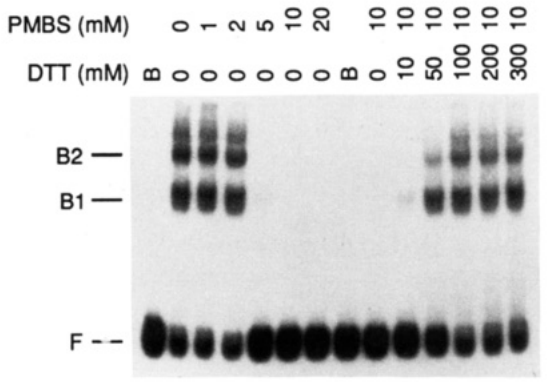

FIGURE 6: Binding activity depends on free sulfhydryl groups. To determine whether free sulfhydryl groups are necessary for XPE-BF binding activity, the reversible sulfhydryl reagent *p*-(hydroxymercuri)-benzylsulfonate (PMBS) was added to the binding reaction containing purified XPE-BF (fraction VII). To demonstrate that the PMBS effect was specific for sulfhydryl groups, DTT and PMBS were added together to the binding reaction. Lanes marked B demonstrate the background when no protein was added.

$ZnCl_2$ strongly inhibited binding and the inhibition could be reversed by EDTA (data not shown). The decrease in total radioactivity in the Mg lanes at the highest protein concentrations was due to a contaminating magnesium-dependent nuclease activity. Magnesium did not affect the sedimentation profile of XPE-BF: the relative sizes of the major peak at 6.8 S and the minor peak at 14.5 S were unchanged (data not shown), so that the stimulation in binding activity by magnesium was not due to a shift in the sedimentation characteristics of XPE-BF.

By contrast, sedimentation in the presence of zinc ions resulted in the disappearance of the 6.8S and 14.5S species and the formation of species with a broad peak in binding activity that reached a maximum at 18 S (data not shown). These results suggest that zinc inhibits binding by inducing the formation of inactive aggregates of XPE-BF. Aggregation must be reversible since the glycerol gradient fractions displayed binding activity in the gel mobility shift assay after the samples were diluted in the binding reaction mixture in the presence of EDTA.

Binding Activity Depends on Unmodified Cysteine Residues. The reversible sulfhydryl reagent *p*-(hydroxymercuri)-benzylsulfonate (PMBS) binds to free sulfhydryl groups on cysteine residues. Figure 6 shows that PMBS will inhibit XPE-BF binding activity at concentrations as low as 5 mM. The effect was dependent on the reactivity of PMBS with sulfhydryl groups, since the simultaneous addition of DTT blocked the inhibition. Furthermore, binding activity was unaffected by the addition of as much as 300 mM DTT, indicating that XPE-BF binding activity does not depend on cystine disulfide cross-links.

XPE-BF Has a Strong Preference for Binding to UV-Damaged DNA. To quantify the specificity of XPE-BF for damaged DNA, binding activity was measured in the presence of cold competitor DNA that was intact or damaged by either UV irradiation or heat denaturation. As shown in Figure 7, there was no competition with 300 ng of intact double-stranded DNA. In fact, only a small amount of competition could be detected with the addition of 3000 ng of intact DNA (data not shown). By contrast, as little as 0.3 ng of UV-irradiated competitor DNA, either single-stranded (ss) or double-stranded (ds), produced more than a 50% decrease in binding to the probe. Thus, XPE-BF has at least a 10 000-fold specificity for UV-damaged (5 kJ/m^2) ss or ds DNA over intact ds DNA.

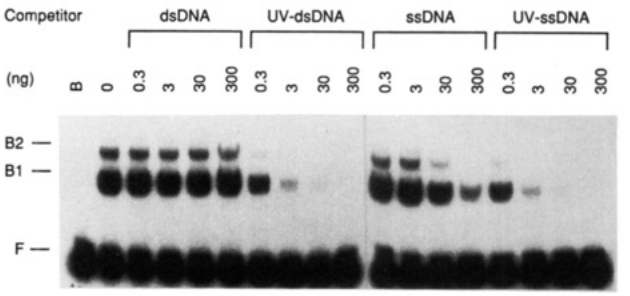

FIGURE 7: Specificity of XPE-BF for damaged DNA. To demonstrate its specificity for damaged DNA, 150 pg of purified XPE-BF (fraction VII) was incubated with UV-f148 DNA probe (0.2 ng) in the presence of different amounts of unlabeled competitor, pUC18 DNA linearized by digestion at a single site by *Hin*dIII. The competition was carried out with intact double-stranded DNA (dsDNA), UV-irradiated double-stranded DNA (UV-dsDNA), single-stranded DNA (ssDNA), or UV-irradiated single-stranded DNA (UV-ssDNA). Single-stranded DNA was prepared by heating at 95 °C for 5 min followed by rapid cooling. The lanes marked B and 0 corresponded to a control reaction with XPE-BF omitted and a reaction containing XPE-BF but no competitor DNA, respectively.

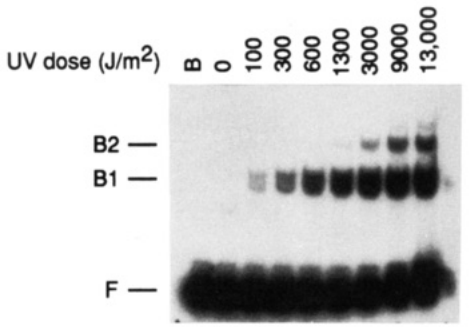

FIGURE 8: Binding of XPE-BF to UV-irradiated DNA as a function of UV dose. The f148 probe DNA was exposed to different doses of UV irradiation ranging from 0 to 13 000 J/m^2, incubated with 150 pg of purified XPE-BF (fraction VII), and then analyzed in the gel mobility shift assay. The lane marked B contained no XPE-BF.

Single-stranded DNA (made by heat denaturation) competed for binding activity starting at around 30 ng, confirming similar results in crude extracts using M13 ss DNA (Patterson & Chu, 1989). Thus, XPE-BF has an approximately 100-fold specificity for ss DNA over intact ds DNA.

XPE-BF Binds to at Least Two Different UV-Induced Photoproducts on DNA. To understand the effect of UV dosage on the binding of XPE-BF to DNA, the f148 probe DNA was treated with doses ranging from 0 to 13 000 J/m^2. Because the binding reactions were carried out with purified XPE-BF, no cold competitor DNA was added. Therefore, the assay was not compromised by the presence of competitor DNA, which can mask some binding activity. With undamaged DNA probe, no binding was observed (Figure 8, lane 0). However, with a UV dose as low as 100 J/m^2, binding activity was detected and increased successively with each dose up to the maximum dose used of 13 000 J/m^2. At low doses, the predominant lesions are cyclobutane pyrimidine dimers. The number of cyclobutane dimers increases linearly up to about 1000 J/m^2 but levels off at higher doses, reaching a plateau for doses above 4000 J/m^2 (Spivak et al., 1988). However, at high doses, there is a continued accumulation of other lesions, including (6–4) photoproducts (Lippke et al., 1981), thymine glycols, and interstrand cross-links (Spivak et al., 1988). Therefore, the progressive increase in binding at 9000 and 13 000 J/m^2 raised the possibility that XPE-BF might also bind to other lesions as well.

To determine if XPE-BF recognizes cyclobutane pyrimidine dimers, we prepared f148 probe DNA containing either the

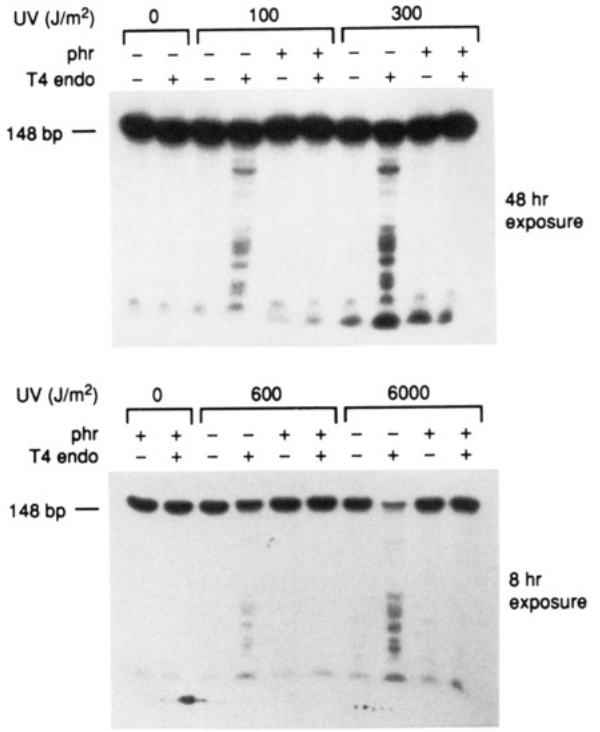

FIGURE 9: Preparation of photoreactivated DNA probe. The DNA fragment f148 was labeled by the exonuclease III method and exposed to different doses of UV irradiation (0, 100, 300, 600, and 6000 J/m²). At each UV dose, the probe was either untreated (–) or treated (+) with the photoreactivating (phr) enzyme photolyase, which repairs cyclobutane dimers. T4 endonuclease V (T4 endo) nicks DNA near the site of cyclobutane dimers. Nicking activity was measured by denaturing the DNA at 95 °C for 5 min, resolving the products by denaturing gel electrophoresis, and preparing an autoradiograph of the gel. At low doses of UV (100 and 300 J/m²), the film was exposed for 48 h, and at high doses (600 and 6000 J/m²) the film was exposed for 8 h.

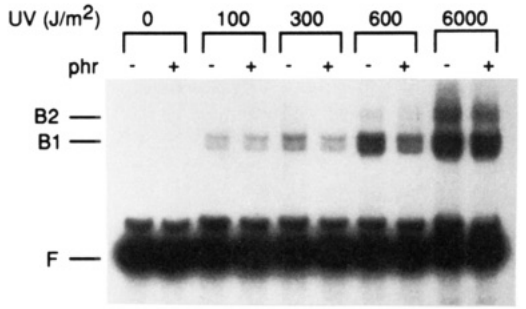

FIGURE 10: Dependence of XPE-BF binding activity on cyclobutane dimers in the DNA probe. Binding activity of 300 pg of purified XPE-BF (fraction VII) was measured by the gel mobility shift assay with DNA probe damaged at different UV doses. At each UV dose, the probe was either untreated (–) or treated (+) with photolyase so that the cyclobutane dimers were completely removed. Note the presence of two closely spaced bands of shifted mobility at the position designated as band B1. We have not yet identified the source of this phenomenon, but both bands are specific for UV-damaged DNA.

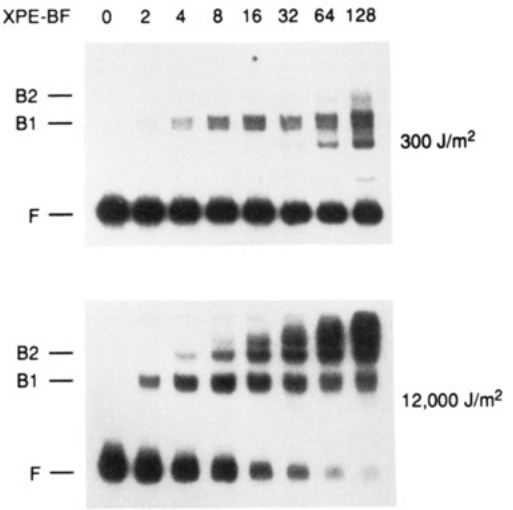

FIGURE 11: Higher order mobility shifts are due to multiple independent binding events. The f148 probe DNA was irradiated with either a low (300 J/m²) or high (12 000 J/m²) UV dose and incubated with increasing amounts of partially purified XPE-BF (fraction VI). The amount of extract is noted in nanoliters above each lane in the gel. The 300 J/m² probe produced a band just below B1 when incubated with 64 and 128 nL of extract. This band was not damage specific and arose from contaminants in the partially purified sample.

full spectrum of UV photoproducts or only the subspectrum that remains after treatment with the photoreactivating enzyme photolyase, which specifically removes cyclobutane pyrimidine dimers (Eker et al., 1990). Cyclobutane dimers in the DNA were quantitated by introducing a nick near the site of each dimer with the highly specific enzyme T4 endonuclease V (Gordon & Haseltine, 1980) and then resolving the products by denaturing gel electrophoresis. As shown in Figure 9, this procedure detected the presence of cyclobutane dimers even at doses as low as 100 J/m². It also demonstrated the complete removal of the dimers by photolyase at all doses from 100 to 6000 J/m².

Figure 10 shows that the removal of cyclobutane dimers leads to a substantial decrease in binding at 300, 600, and 6000 J/m². Therefore, XPE-BF appears to bind to cyclobutane dimers. Moreover, after complete removal of the cyclobutane dimers significant binding activity is still present. Therefore, XPE-BF must also bind to at least one other UV-induced photoproduct. The (6–4) photoproduct is a likely candidate because it is the next most abundant lesion particularly at doses less than 1000 J/m².

It is noteworthy that there is no decrease in binding activity with photoreactivation of the probe DNA at the dose of 100 J/m². This result was reproducible and occurred despite the easily observed presence of cyclobutane pyrimidine dimers at that dose (see Figure 9).

Binding to UV-Irradiated DNA Produces Multiple Mobility Shifts Due to Multiple Independent Binding Events. The gel mobility shift assay for XPE-BF often produces multiple bands of shifted mobility. The two most abundant bands are designated B1 and B2, but even higher order bands may be seen. The multiple bands may be explained in two possible ways. On one hand, they may arise from the formation of multimeric protein complexes at the site of a given lesion. Alternatively, they may be simply due to independent binding events at different DNA lesions on the same DNA molecule.

To distinguish between these two possibilities, we prepared DNA probes containing different numbers of DNA lesions. At a dose of 300 J/m², only a small minority of DNA probe molecules contained pyrimidine dimers, since T4 endonuclease V left most of the DNA intact (Figure 9). Therefore, since most UV-induced lesions are pyrimidine dimers at that dose, an insignificant number of DNA molecules contained 2 or more lesions. Figure 11 shows that DNA probe treated with 300 J/m² produced only band B1 and no evidence of band B2, even when incubated with large amounts of XPE-BF. At a dose of 12 000 J/m², the DNA probe contained multiple lesions, since T4 endonuclease V degraded most of the DNA even at 6000 J/m² (Figure 9). DNA probe treated with 12 000 J/m² produced both bands B1 and B2 as well as at least two additional higher order bands as larger amounts of XPE-BF

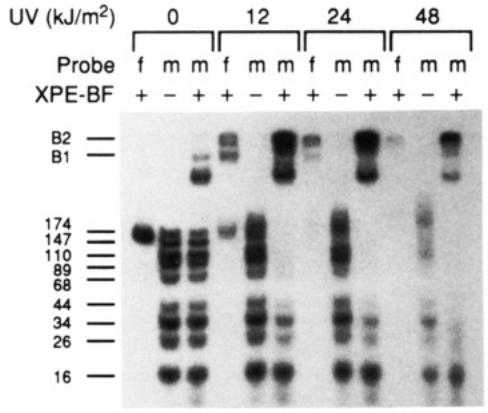

FIGURE 12: XPE-BF shoe size. DNA probes were either f148 (f) or a mixture (m) of DNA fragments. The DNA was UV-irradiated with 0, 12, 24, or 48 kJ/m², incubated with binding buffer either in the presence (+) or in the absence (−) of excess partially purified XPE-BF (fraction VI), and then resolved on a 7% native polyacrylamide gel. As the UV dose increased, all DNA fragments larger than 26 bp were shifted to lower mobilities while the 16-bp fragment remained unshifted. Two bands of shifted mobility coincident with and just below B1 arose from contaminants in the partially purified sample and were not damage specific since they were present with unirradiated probe.

were added to the reaction. Thus, multiple independent binding events at different DNA lesions produce the multiple bands of shifted mobility.

Measurement of DNA Shoe Size. From the experiment in Figure 11, the DNA probe f148 is capable of supporting at least four independent binding events. This implies that the binding site for XPE-BF must be smaller than one-fourth the size of f148, and hence smaller than 37 bp.

A more accurate measurement of the DNA binding site can be made by a DNA footprinting experiment. However, it is not easy to synthesize a DNA fragment with a single UV lesion at a well-defined location. We therefore employed a novel procedure to determine what we refer to as the DNA "shoe size" for XPE-BF. A mixture of DNA fragments was labeled and irradiated with a range of UV doses, sufficiently high to introduce lesions into even the smallest fragment. Incubation with an excess of XPE-BF then resulted in a decrease in intensity of bands for unbound DNA fragments provided that the fragments were large enough to allow the formation of a DNA–protein complex with XPE-BF. Thus, this procedure allows the analysis of protein binding to DNA fragments damaged at random locations. Figure 12 demonstrates the application of this method. Addition of XPE-BF caused a decrease in intensity of DNA fragments 26 bp or larger but had no effect on the 16-bp fragment. Thus, XPE-BF can bind to DNA fragments 26 bp or larger but not to a DNA fragment of 16 bp. Therefore, the DNA shoe size for XPE-BF is between 16 bp and 26 bp.

DISCUSSION

XPE-BF Is Primarily a Monomeric 125-kDa Polypeptide. We have described the purification of XPE binding factor, a protein that appears to be involved in DNA repair in human cells. Purification through two UV-DNA–cellulose column steps yielded a preparation containing detectable amounts of only two polypeptides of molecular masses of 125 kDa and 93 kDa, forming about 95% and 5% of the preparation, respectively. The stoichiometry of binding demonstrated that there was insufficient 93-kDa polypeptide to account for the binding activity observed with the purified preparation. Therefore, XPE-BF must contain the 125-kDa polypeptide. Furthermore, each mole of XPE-BF contains either 1 or at most 2 mol of the 125-kDa polypeptide. It is conceivable that XPE-BF also contains one or more additional polypeptides of molecular weight low enough to remain unresolved by SDS–PAGE or escape detection by silver staining. However, in the absence of any direct evidence for such polypeptides, we currently believe that XPE-BF consists solely of the 125-kDa polypeptide that binds to damaged DNA as either a monomer or homodimer. The native molecular mass of the predominant species of XPE-BF was 134 kDa ± 20 kDa, in agreement with the molecular mass of the denatured protein on SDS–PAGE. Therefore, XPE-BF is predominantly a monomer in solution.

Although magnesium ions stimulated XPE-BF activity, the effect disappeared at high XPE-BF concentrations. Why did this occur? One possible explanation is that the XPE-BF–DNA complex is stabilized by $MgCl_2$ but that the effect is no longer observable when the concentration of XPE-BF is so high that the potential DNA binding sites are saturated. A second possible explanation is that binding requires the assembly of a homodimeric complex of XPE-BF at the site of the DNA lesion and that the assembly is favored either by the presence of $MgCl_2$ or by high concentrations of XPE-BF.

XPE-BF Has an Affinity for UV-Damaged DNA High Enough To Scan the Genome. The purification of XPE-BF permitted an accurate determination of its relative affinity for UV-damaged DNA. XPE-BF bound to UV-damaged DNA (exposed to 5000 J/m²) with at least a 10 000-fold specificity over undamaged double-stranded DNA. The actual preference may be expressed as a preference for damaged nucleotides over undamaged nucleotides. UV irradiation at 254 nm produces a broad range of lesions, including thymine glycols and interstrand cross-links. However, the great majority of lesions consists of covalent linkages between adjacent pyrimidines: cyclobutane dimers and (6–4) photoproducts, which are introduced into DNA at a ratio of about 3 to 1 (Mitchell, 1988). A dose of 5000 J/m² produces about 30 cyclobutane dimers (Spivak et al., 1988) and thus about 10 (6–4) photoproducts per 1000 bp. The total number of lesions is therefore approximately 40 per 1000 bp, or 1 UV lesion for every 25 bp. Thus, XPE-BF binds to damaged nucleotides with at least a 250 000-fold preference (25 times 10 000) over undamaged nucleotides. Since XPE-BF does not appear to bind to a major subset of cyclobutane dimers including TT dimers (see below), the true preference may be at least twice as high, or 500 000-fold. Thus, XPE-BF should be able to find a single UV-induced lesion within a minimum of 500 kb. By contrast, the 40/42-kDa protein that complements XP group A cells has a relative affinity for UV-damaged over undamaged nucleotides of only 1000 (Robins, et al., 1991).

The strong preference of XPE-BF for damaged nucleotides is consistent with its relatively low abundance, roughly 10 000 molecules of XPE-BF per cell (Chu & Chang, 1988). Since the diploid genome contains about 6000 megabase pairs, this corresponds to only 1 molecule of XPE-BF for every 600 kb of DNA. (The 600-kb estimate may be somewhat high, since the abundance of XPE-BF was derived from assays on crude extract using a large excess of competitor DNA.) Thus, XPE-BF has a relative affinity for damaged DNA that is consistent with its abundance in the nucleus.

XPE-BF Recognizes at Least Some Cyclobutane Dimers and at Least One Other UV-Induced Lesion. Which UV-induced lesions are recognized by XPE-BF? Purified XPE-BF continued to bind to a UV-irradiated DNA probe even after it was treated with photolyase to remove all cyclobutane dimers. Therefore, XPE-BF must be able to recognize one

or more photoproducts that are not cyclobutane dimers. Since this effect was easily observed at all doses from 6000 J/m^2 to as low as 100 J/m^2, and since the most abundant of such lesions is the (6–4) photoproduct, it is likely that XPE-BF recognizes (6–4) photoproducts. Proof of this awaits more careful studies with better defined DNA probes.

Purified XPE-BF also binds to cyclobutane dimers, since its binding activity to a UV-irradiated DNA probe clearly decreased when the DNA was pretreated with photolyase. The percentage change in binding activity was pronounced at UV doses of 300 J/m^2 and 600 J/m^2. It decreased somewhat at 6000 J/m^2, as would be expected since photoproducts other than cyclobutane dimers assume relatively greater importance at such high doses. There is also functional evidence that XP group E cells are defective in the repair of at least some cyclobutane dimers. Tanaka et al. introduced purified T4 endonuclease V into group E cells (XP2RO) (Tanaka et al., 1977), which have been shown to be lacking binding activity (Chu & Chang, 1988). Both UV-induced unscheduled DNA synthesis and UV-survival increased significantly (Tanaka, et al., 1977). Thus, specific incision of DNA at the site of cyclobutane dimers by T4 endonuclease V was able to rescue XP group E cells after UV radiation.

Comparison to Other Mammalian UV-Damaged DNA Binding Proteins. Others have reported the purification of proteins that bind to UV-damaged DNA. For example, Feldberg et al. (Feldberg, 1980; Feldberg & Grossman, 1976; Feldberg et al., 1982) purified a UV-damaged-DNA specific protein from human placenta using a filter binding assay. Binding activity was not tested for XP group E, and molecular weight was not estimated by SDS–PAGE. Furthermore, they reported that their protein did not bind to cyclobutane pyrimidine dimers (Feldberg & Grossman, 1976), in contrast to XPE-BF. Therefore their protein may be distinct from XPE-BF. Robins et al. (Robins et al., 1991) recently purified a protein from calf thymus that complements XP group A cell extracts in a cell-free DNA repair assay. Their protein also has affinity for UV-damaged DNA but migrates on SDS–PAGE as a doublet of 40 and 42 kDa, so that it is clearly distinct from XPE-BF.

Recently, a damaged-DNA binding (DDB) protein was identified and purified from monkey CV1 cells (Abramic et al., 1991; Hirschfeld et al., 1990). The molecular mass of DDB protein on SDS–PAGE was 126 kDa, very similar to that of XPE-BF. However, a number of results were different from ours. Zinc ions inhibited DDB activity (similar to XPE-BF) but magnesium ions had no effect (unlike XPE-BF). DDB protein was reported to be a homodimer in solution, but the estimate of native molecular mass was based on the Stokes radius and did not include the sedimentation coefficient (Siegel & Monty, 1966). DDB protein had a modest affinity for UV-damaged DNA (6.5 kJ/m^2) over undamaged DNA of 5.5–17-fold. By contrast, XPE-BF had a greater than 10 000-fold affinity for UV-damaged DNA (5 kJ/m^2). Interestingly, DDB protein did not bind to a probe containing a single TT cyclobutane dimer. Thus, the monkey DDB protein and the human XPE-BF may be different proteins, despite their similarity in molecular mass. Alternatively, the different results may be due to differences in the probes or assay conditions.

Size of DNA Binding Site. In the absence of a completely well-defined DNA template for DNA footprinting, we measured the minimal size DNA fragment required for binding to XPE-BF, the DNA "shoe size". The result was a shoe size of between 16 and 26 bp. By comparison, the DNA footprint for *E. coli* mutS, which recognizes base pair mismatches, is 20 bp (Su et al., 1988). The footprint for *E. coli* UvrA around a psoralen–thymine monoadduct is somewhat larger: 33 bp. Formation of the $(UvrA)_2/(UvrB)_1$ complex at the site of damage leads to an expansion of the footprint to 45 bp; but subsequent dissociation of UvrA results in a 19-bp UvrB footprint (Bertrand-Burggraf et al., 1991). Finally, we have previously shown that XPE-BF has similar binding properties to *Saccharomyces cerevisiae* photolyase (Patterson & Chu, 1989), which has a strong footprint of 14 bp and a weaker footprint extending to 25 bp (Baer & Sancar, 1989). The *E. coli* photolyase has a slightly different footprint both in position relative to the TT dimer and in size, with a strong footprint of 14 bp extending to a weak footprint of 20 bp (Baer & Sancar, 1989). Each of these proteins may recognize distortions in the DNA helix rather than specific nucleotide changes, since they bind to lesions located at different sequences. It is noteworthy that many of the binding sites are of approximately equivalent size, despite significant variation in molecular mass: MutS (97 kDa) (Lahue, et al., 1989), *E. coli* and *S. cerevisiae* photolyases (54 and 66 kDa) (Sancar, 1990), UvrA and UvrB (104 kDa and 76 kDa) (Sancar & Sancar, 1988), and XPE-BF (125 kDa).

The size of the DNA binding site for XPE-BF is consistent with the physical dimensions of the protein. A binding site of 16–26 bp extends between 54 Å and 88 Å. This is somewhat larger than the XPE-BF Stokes radius of 47 Å but consistent with an asymmetric protein with a moderately high frictional ratio of 1.39 corresponding to an axial ratio of 7 in an equivalent ellipsoid of revolution.

XPE-BF May Not Recognize All Cyclobutane Dimers. Surprisingly, at the low UV dose of 100 J/m^2, XPE-BF binding activity did not decrease with photoreactivation of the probe DNA. This observation was reproducible and occurred despite the formation of an easily detectable number of cyclobutane dimers at that dose. TT cyclobutane dimers are the predominant cyclobutane dimer at low UV doses. For example, in *E. coli* DNA irradiated with 264-nm UV light at a dose of 200 J/m^2, 59% of the cyclobutane dimers are TT while 34% are TC or CT and 7% are CC (Setlow & Carrier, 1966). The ratio should be even more biased in favor of TT dimers in the f148 probe, which has a higher thymine content (30%) compared to *E. coli* (25%). This suggests that XPE-BF might not bind to TT cyclobutane dimers but only binds to a less abundant subset of the remaining possible cyclobutane dimers: CT, TC, or CC.

Specificity for only a subset of the possible cyclobutane dimers seems at first to be surprising. The structures of the different possible dimers are quite similar, and XPE-BF clearly shows significant versatility in its ability to recognize at least one other UV photoproduct, as well as single-stranded DNA and cisplatin-cross-linked DNA. Nevertheless, such a combination of versatility and specificity is not unprecedented. For example, in the *E. coli* mismatch repair system, the mutS protein recognizes all eight possible mismatches, but with widely differing dissociation constants ranging from 39 nM for G-T mismatches to 480 nM for C-C mismatches (Su et al., 1988). Further investigation regarding the binding of XPE-BF to different cyclobutane dimers awaits the construction of more precise DNA templates.

If XPE-BF fails to bind with high affinity to TT cyclobutane dimers, that may explain why XP complementation group E is characterized by only mild to moderate sensitivity to UV irradiation. The loss of XPE-BF activity might disturb the recognition of only a subset of UV lesions, and the repair of

the remaining lesions (including, for example, the abundant TT cyclobutane dimers) would remain intact.

Since XPE-BF may not recognize every UV photoproduct, it is possible that the normal cell contains a second factor that recognizes a different subset of lesions, perhaps including the TT cyclobutane dimer. We predict that this factor will be defective in a different XP complementation group. The presence of this second factor was not observed in XP group E extracts, although it may have escaped detection either because the factor was not easily extractable from nuclei or because the corresponding protein–DNA complex was not stable enough to survive gel electrophoresis. In conclusion, future studies of proteins that bind specifically to damaged DNA promise to reveal insights into human DNA repair.

ADDED IN PROOF

Recent experiments with crude HeLa extracts give direct evidence that XPE-BF binds to (6–4) photoproducts (Treiber et al., 1992). These same authors also searched for binding of XPE-BF to cyclobutane dimers. However, their experiments were sensitive enough to detect binding primarily to TT cyclobutane dimers and not to other dimers. Their results are consistent with our conclusion that XPE-BF does not bind to TT cyclobutane dimers (D. Treiber, private communication).

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