An ultraviolet light-damaged DNA recognition protein absent in xeroderma pigmentosum group E cells binds selectively to pyrimidine (6-4) pyrimidone photoproducts

Daniel K.Treiber, Zhenghuan Chen and John M.Essigmann*

Division of Toxicology and Department of Chemistry, Whitaker College of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Received June 17, 1992; Revised and Accepted October 7, 1992

ABSTRACT

The binding specificity was defined of a human ultraviolet light-damaged DNA recognition protein (UV-DRP), the activity of which is absent in some xeroderma pigmentosum complementation group E cells. Our results suggest that cyclobutane pyrimidine dimers (CPDs) are not high affinity UV-DRP binding sites—a finding consistent with other reports on this protein (Hirschfeld et al., (1990) *Mol. Cell Biol.*, 10, 2041–2048). A major role for 6-4 photoproducts in UV-DRP binding was suggested in studies showing that irradiated oligonucleotides containing a T₄C UV box sequence, which efficiently forms a TC 6-4 photoproduct, was a superior substrate for the UV-DRP when compared to a similar irradiated oligonucleotide having a T₅ sequence. The latter sequence forms CPDs at a much higher frequency than 6-4 photoproducts. In a more direct approach, T₄C-containing oligonucleotides complexed with the UV-DRP were separated from the unbound oligonucleotide fraction and the frequencies of 6-4 photoproducts in the two DNA populations were compared. The UV-DRP-bound fraction was highly enriched for the 6-4 lesion over the unbound fraction supporting the conclusion that 6-4 photoproducts are the principal binding cues for the UV-DRP.

INTRODUCTION

Several studies have revealed the existence of proteins in mammalian cells that bind specifically to DNA that has been damaged by chemical or physical agents. DNA damage recognition proteins (DRPs) have been reported for a growing list of DNA modifications, including certain cisplatin crosslinks (1,2), 1,N⁶-ethenoadenine (3), G:T mismatches (4), apurinic/apyrimidinic (AP) sites (5), N-acetyl-2-aminofluorene-guanine adducts (6), and ultraviolet light (UV)-induced photoproducts (7–9). The cellular function of these DRPs is currently unknown, but it is reasonable to speculate that some

or all of these proteins may play roles in DNA repair (8,9), or in other biological activities related to the genotoxicity of the agent under investigation (1,2).

A body of indirect evidence suggests that a UV-damaged DNA recognition protein (UV-DRP) may be a component of a versatile mammalian excision repair complex. The UV-DRP is overexpressed in human cells that display resistance to the anticancer drug cisplatin; these cells also exhibit an enhanced capacity to reactivate cisplatin-damaged plasmids (10). Furthermore, the UV-DRP is induced 2–4 fold above constitutive levels in primate cells pretreated with UV (11). These cells also reactivate UV-damaged plasmids with increased efficiency. The most compelling result linking the UV-DRP to DNA repair is the apparent absence of this activity in cells from two related persons afflicted with the human DNA repair disorder xeroderma pigmentosum (XP) (8). More specifically, these individuals belonged to genetic complementation group E. The generality of this finding has recently been challenged because UV-DRP activity is detected in a number of XP-E cell lines derived from Japanese patients (12,13). It is possible, however, that these recently tested cell lines contain a form of the UV-DRP that is defective in a function other than DNA binding.

The possible role of the UV-DRP in DNA repair has not been established but, based on genetic evidence in yeast, the protein has been hypothesized to be the human homolog of photolyase (14), an enzyme that catalyzes the chemical reversal of cyclobutane pyrimidine dimers (CPDs) back to normal dipyrimidine sequences in DNA. Although the UV-DRP lacks the ability to photoreactivate DNA *in vitro*, it may help to recruit repair complexes to sites of DNA damage (14). In support of this model, the binding of *Escherichia coli* photolyase to CPDs has been shown to stimulate the excision of these lesions by the UVR-ABC excision repair complex *in vitro* (15). Another model suggests that the UV-DRP may perform a damage recognition function analogous to that of the *E.coli* UVR-A protein (9).

Definition of the substrate specificity of a protein that interacts with other molecules is a key step toward elucidation of function. In the case of the UV-DRP, definition of the true physiological substrate is problematic because UV induces several forms of

* To whom correspondence should be addressed

DNA damage, including CPDs and pyrimidine (6-4) pyrimidone photoproducts (6-4 photoproducts). Each of these photoproducts causes distortions in DNA architecture (16–19) and is believed to be both cytotoxic and mutagenic (20–24). Interestingly, the relative repair rates of these lesions in mammalian cells (25–27) and cellular extracts (28) have been shown to be biased by a factor of 4–12 in favor of repair of the 6-4 photoproducts. The protein(s) responsible for this differential repair are currently unknown.

Previous studies examining the binding specificity of the UV-DRP have failed to identify definitively the nature of the UV lesion(s) recognized with highest affinity. It was shown, however, that a UV-DRP from human placenta had no measurable affinity for the major UV-induced photoproducts, CPDs (7). This same property was found to be true for the primate UV-DRP, although it was established that the presence of adjacent pyrimidines was an important prerequisite for high affinity binding (9). In the present report we have used a strategy involving oligonucleotide

synthesis, preparative electrophoretic mobility shift assays, and UV-photoproduct analysis to demonstrate that 6-4 photoproducts are high affinity binding sites for the UV-DRP. Furthermore, in agreement with the findings cited above, we have found no significant role for CPDs in the binding of this protein to UV-damaged DNA.

MATERIALS AND METHODS

Materials

Polynucleotide kinase was purchased from New England Biolabs and poly(dI-dC) · poly(dI-dC) from Pharmacia. T4 endonuclease V (T4 endo V) was kindly provided by R.S. Lloyd (Vanderbilt University) and HeLa cells were obtained from M. Chow (M.I.T.). Media and fetal calf serum were purchased from GIBCO.

Nuclear extract preparation

HeLa cells were grown in suspension in S-MEM supplemented with 5% fetal calf serum, and nuclear extracts were prepared according to a published procedure (29). Protein concentrations were determined by the Bradford assay (30).

Preparation of UV-treated synthetic oligonucleotide probes

Sixty nine-mer oligonucleotides T₅, T₄C, and Δ (*vide infra*) were synthesized on an Applied Biosystems 381-A DNA synthesizer. Oligomers were purified on 10% denaturing (7 M urea) polyacrylamide gels and electroeluted by using an Amicon centrifuge. Urea was removed from oligomers by several distilled water washes in Amicon Centricon 10 microconcentrators. T₅, T₄C, and Δ were end labeled with [γ -³²P]ATP (3000 Ci/mmol, New England Nuclear) by using polynucleotide kinase. Unincorporated label was removed by centrifugation through G-25 spun columns. Labeled strands were annealed to unlabeled complementary strands, and the resulting duplexes were purified by native polyacrylamide gel electrophoresis (PAGE) to remove contaminating single stranded DNA. Labeled probes were irradiated on a chilled surface with a 15-W germicidal lamp (maximum output at 254 nm) at a fluence of 5 J/m² until the desired dose was achieved (fluence measured with a UVX-25 sensor).

Analytical electrophoretic mobility shift assays

DNA-protein complexes were detected by using a published procedure (31). End labeled probes either unmodified or UV-treated (7×10^4 cpm) were incubated in the presence of crude nuclear extracts (10 μ g) and poly(dI-dC) · poly(dI-dC) (6 μ g) for 15 min at 37°C in binding buffer (2 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 10 mM NaCl, 50 mM ethylenediaminetetraacetic acid (EDTA), 1% glycerol, and 0.2 mM dithiothreitol) in a final volume of 15–25 μ l. Protein-DNA complexes were then resolved on 6% polyacrylamide gels [29:1 acrylamide/*N,N'*-methylenebis(acrylamide)]. Gels were electrophoresed in Tris-glycine buffer (50 mM Tris-HCl (pH 8.5), 380 mM glycine, 2 mM EDTA) for 4 hr at 30 mA. Gels were imaged and quantified by using a Molecular Dynamics Phosphorimager.

Preparative electrophoretic mobility shift assays

Purification of UV-DRP-bound T₄C probes was achieved by scaling up the protein-DNA binding reaction by one order of magnitude. The UV-DRP-specific band and the non-protein

Figure 1. Characterization of UV box oligonucleotides. The relevant portions of the UV box oligonucleotide sequences are shown to the right. Sixty nine-mer oligonucleotides T₄C, T₅, and Δ were designed such that adjacent pyrimidines are absent in both strands except in the 'UV boxes' shown in large type near the center of each sequence. Δ contains no adjacent pyrimidines as the UV box was replaced with a purine/pyrimidine stretch. Ten bp GC clamps are present on the ends of each oligonucleotide, and the intervening DNA sequences between the GC clamps and the UV box were ATATGCGTACATGTGCG for the region upstream of (5' to) the UV box and ACGCACGCACGTACATGTGCG-ACGTGTAT for the region downstream. The autoradiogram to the left shows the photoproduct distribution in irradiated UV box oligonucleotides. Control and irradiated (10^4 J/m²) UV box oligonucleotides were treated with either T4 endo V (Endo V) for 1 hr at 37°C or with 1M piperidine (PIP) for 0.5 hr at 90°C to determine the distribution of CPDs and 6-4 photoproducts, respectively. The resulting cleavage products were resolved on a DNA sequencing gel adjacent to the appropriate chemical sequencing markers. Lanes 1–6, Δ, lanes 7–12, T₅, and lanes 13–18, T₄C. T4 endo V analysis of irradiated oligonucleotides is shown in lanes 1 (Δ), 7 (T₅), and 13 (T₄C) and a similar analysis of unirradiated oligonucleotides is shown in lanes 2 (Δ), 8 (T₅), and 14 (T₄C). Analysis of piperidine treated irradiated oligonucleotides is shown in lanes 3 (Δ), 9 (T₅), and 15 (T₄C) and in lanes 4 (Δ), 10 (T₅), and 16 (T₄C) a similar analysis of unirradiated oligonucleotides is shown. C+T chemical sequencing reactions are shown in lanes 5 (Δ), 11 (T₅), and 17 (T₄C), and A+G reactions are shown in lanes 6 (Δ), 12 (T₅), and 18 (T₄C).

bound band were excised from the gel and electroeluted. Gel-purified bound and free probes were deproteinized by a phenol:chloroform:isoamyl alcohol (29:29:1) extraction. Deproteinized material was extracted with chloroform, ethanol precipitated, and resuspended in buffer containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (TE).

6-4 photoproduct and cyclobutane pyrimidine dimer assays

6-4 photoproducts were detected as alkali-labile sites in irradiated DNA (26,32). Piperidine (Fisher) was diluted freshly in distilled water before use and added to end labeled DNA samples to a final concentration of 1 M. Samples were heated at 90°C for 0.5 hr. In some cases it was necessary to extend the incubation time to several hr. Piperidine was removed by vacuum centrifugation and samples were washed by resuspension in distilled water followed by vacuum centrifugation. Washed samples were resuspended in TE buffer. Piperidine treated samples were subjected to denaturing PAGE to quantify and to map 6-4 photoproducts. Chemical sequencing reactions (33) were used as calibration standards. CPDs were detected as T4 endo V-sensitive sites in DNA. End labeled DNA fragments were treated with T4 endo V (1 μ l of a 0.02 mg/ml solution of enzyme in TE and 100 μ g/ml BSA) in the presence of 10 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 50 mM NaCl, and 1 mg/ml BSA at 37°C for 1 hr. Digestion products were analyzed by denaturing PAGE. The T4 endo V reaction was shown to proceed to completion by 1 hr.

RESULTS

Analysis of UV-DRP binding specificity using synthetic oligonucleotides

A protein was present in human nuclear extracts that retarded the electrophoretic mobility of UV-damaged DNA fragments. This protein, the UV-DRP, has been observed by others (7–9), and has been shown to be lacking in two cell lines from XP patients belonging to complementation group E (8); the UV-DRP activity detected in our experiments was likewise absent in these same XP-E cell lines (data not shown). It has become clear that CPDs, the most abundant photoproducts, are not high affinity binding sites for the UV-DRP. In support of this conclusion is

Figure 2. UV dose-response analysis of UV-DRP binding to UV box oligonucleotides. UV box oligonucleotides T₄C, T₅ and Δ were irradiated to doses of 0.1–20 kJ/m² and incubated with 10 μ g of HeLa nuclear extract. The mixtures were then analyzed by native PAGE. The amount of probe incorporated into the protein/DNA complex was quantified and is reported in arbitrary phosphorimager units (PIU).

the observation that UV-treated DNA rendered devoid of CPDs by enzymatic photoreactivation was recognized with undiminished affinity by the UV-DRP (9, and our unpublished observations). These studies have definitively ruled out CPDs as the high affinity targets of the UV-DRP, and they suggested as one logical possibility that the 6-4 lesion might be the true physiological target, an hypothesis buttressed by the observation that the presence of adjacent pyrimidines was a critical feature of high affinity UV-DRP probes (9). In a formal biochemical sense, however, the importance of 6-4 photoproducts in UV-DRP binding has yet to be addressed in a direct and definitive manner.

Our primary goal in this work was to assess the importance of 6-4 photoproducts in UV-DRP binding. In the absence of an enzyme that repairs 6-4 photoproducts specifically, we deemed it useful to employ three nearly identical 69-mer oligonucleotides, T₅, T₄C, and Δ (Figure 1). Sequences were chosen such that adjacent pyrimidines are found exclusively in five bp 'UV boxes' that reside near the center of the oligonucleotides. GC clamps on both ends of the oligomers increased the annealing efficiency but did not alter the outcome of experiments performed with these sequences (unpublished observation). These oligonucleotides permitted an evaluation of the relative importance of CPDs, 6-4 photoproducts, and non-dimer damage in UV-DRP binding. The oligonucleotide Δ contains no adjacent pyrimidines and therefore cannot form pyrimidine dimers. The T₅ probe has a run of Ts, making it an excellent target site for CPD formation; 6-4 photoproducts form rarely at TT sequences (26,34) and, hence, irradiation of this probe was expected to yield a product that contains mainly CPDs. The T₄C sequence differs from T₅ by a single T to C transition, which provides a TC sequence following a run of pyrimidines. Such sequences are known hotspots for the formation of 6-4 photoproducts (24,34). As a result, T₄C should have a much higher frequency of 6-4 photoproducts than either Δ or T₅.

Photoproduct analysis of irradiated T₅, T₄C and Δ revealed that these sequences formed the predicted distribution of photoproducts (Figure 1). T4 endo V analysis of T₅ showed that this sequence has a high frequency of CPDs mapping primarily to the 3' TT sites in the UV box (lane 7). Conversely, 6-4 photoproducts were not detected in the T₅ oligomer as UV specific piperidine cleavage products were not observed (compare lanes 9 and 10). The T₄C sequence formed both CPDs and 6-4 photoproducts (lanes 13 and 15, respectively). CPDs were most abundant in the central TT sites, but were also evident in the flanking TT and TC dipyrimidines. The exclusive hotspot for the formation of 6-4 photoproducts was the TC site as evidenced by a UV specific piperidine cleavage product (lane 15) migrating 1 nucleotide above the corresponding C in the chemical sequencing lane (lane 17). This banding pattern was not surprising because piperidine cleavage of TC 6-4 photoproducts present on a 5' end-labeled fragment has been shown to yield a product that has a reduced mobility relative to sequencing standards (35). As expected, the Δ oligomer contained neither CPDs nor 6-4 photoproducts (lanes 1–4).

A UV dose-response gel mobility shift assay was performed to compare binding of the UV-DRP to T₅, T₄C, and Δ. Results from this experiment are in Figure 2 and illustrate that T₄C was a superior substrate for the UV-DRP by a five to seven fold margin at all doses tested. In agreement with the aforementioned photoreactivation studies, T₅ and Δ were equally poor substrates. On the basis of these findings it was speculated that the major UV-DRP binding site is the 6-4 photoproduct while

Figure 3. 6-4 photoproduct analysis of UV-DRP-bound T₄C. T₄C, either non-protein bound (Free, lanes 1–5) or associated with the UV-DRP (Bound, lanes 6–10), was purified from a preparative electrophoretic mobility shift assay and subjected to 1 M piperidine at 90°C for 0.5–6.0 hr. Equal amounts of each reaction mixture were analyzed by denaturing PAGE. The two major degradation products map to the UV box as determined by comparison to standards (not shown, refer to Figure 1). The percentage of T₄C containing a 6-4 photoproduct in the UV box was determined by quantifying the radioactivity of the two major cleavage products after 6 hr and dividing by the amount of full length DNA (69 nucleotides; 69N) in the 0 hr lane. Nonspecific degradation of the full length probes by piperidine necessitated this mode of data analysis as it was not feasible to measure the photoproduct frequency accurately by monitoring the disappearance of full length probes.

a cryptic non-pyrimidine dimer photoproduct(s) represents a rare or low affinity class of binding sites. Once again, there was no evidence to suggest that CPDs are recognized.

T₄C bound to the UV-DRP is enriched for 6-4 photoproducts

If 6-4 photoproducts represent the major class of UV-DRP binding sites, then it follows that protein-bound DNA should contain these lesions. Our aim was to determine whether 6-4 photoproducts were disproportionately represented in the material retarded in gel shift assays (UV-DRP-bound T₄C). Since a small fraction (2–5%) of the total T₄C used in a gel shift assay becomes incorporated into a protein-DNA complex, it was necessary to scale up the assays by an order of magnitude in order to generate sufficient bound DNA for analysis. The UV-DRP-bound DNA was purified and subjected to T4 endo V or piperidine treatment. A similar protocol was followed for unbound DNA. A prolonged exposure of these DNAs to piperidine revealed that UV-DRP-associated material was enriched for 6-4 photoproducts mapping to the UV box sequence of T₄C (Figure 3). The enrichment was substantial in that 90% of bound T₄C contained a 6-4 photoproduct (detected as an alkali-labile site) at this position relative to 20% for the unbound material (the mode of data analysis is explained in the Figure legend). The major site of 6-4 photoproduct formation was at the TC sequence as evidenced by the electrophoretic mobility of the piperidine cleavage product relative to standards (not shown, refer to Figure 1). A minor cleavage product, ~1 nucleotide smaller than the more abundant fragment, was also enriched in the bound population. This fragment is possibly the consequence of a rare 6-4 photoproduct occurring between the third and fourth Ts. These findings demonstrate clearly that the UV-DRP can distinguish between DNA possessing or lacking 6-4 photoproducts. Photoproduct frequencies in bound T₅ and Δ oligonucleotides were not determined because these sequences are recognized by the UV-DRP so weakly that it was not possible to obtain sufficient material for analysis.

Figure 4. CPD analysis of UV-DRP-bound T₄C. Bound and Free T₄C were purified as in Figure 3. These samples were digested by T4 endo V and subjected to denaturing PAGE (lanes 5 and 6). Equal amounts of DNA were loaded in each lane. The four major cleavage products mapped to the UV box as determined by comparison to standards (not shown, refer to Figure 1). The percentage of T₄C containing a CPD in the UV box was determined by quantifying the amount of full length DNA (69N) remaining after digestion and dividing by the amount of full length DNA in the T4 endo V-treated, unirradiated control (lane 4). In lanes 1–3, the DNAs were not digested with T4 endo V to show that the cleavage products in lanes 5 and 6 were caused by neither UV light nor UV-DRP binding.

T4 endo V analysis of T₄C complexed, and uncomplexed, with the UV-DRP showed that free and, to a lesser extent, bound T₄C contained CPDs mapping to the UV box region (Figure 4). As predicted from Figure 1, the internal TT sequences in the UV box were hotspots for CPD formation, and CPDs formed to a lesser extent at the flanking TT and TC sites. Interestingly, bound T₄C was impoverished by two fold for the CPD photoproducts relative to free T₄C (as evidenced by 11% degradation of T₄C by T4 endo V in the bound fraction vs. 22% degradation in the free fraction). In light of the concomitant enrichment for 6-4 photoproducts, this result may be attributable to two non-mutually exclusive phenomena. First, the UV-DRP may not bind with high affinity to a UV box containing both a CPD and a 6-4 photoproduct. Second, the presence of a 6-4 photoproduct in the UV box may significantly lower the propensity of upstream Ts to form CPDs.

The UV-DRP did not appear to promote the formation of strand breaks nor AP sites in bound DNA. The UV-DRP-bound DNA exhibited the same mobility in denaturing gels as full length control DNA. The lack of AP sites in this DNA is evidenced by its resistance to the AP endonuclease activity of T4 endo V.

DISCUSSION

The goal of this work was to determine the nature of the UV-induced photoproduct that is recognized with high affinity by a human UV-DRP. We have found that this DRP binds almost exclusively to 6-4 photoproducts and, in agreement with previous studies, has no apparent affinity for CPDs. Experiments with UV box oligonucleotides showed that T₄C sites, but not T₅ sequences, formed high affinity UV-DRP binding sites upon irradiation (Figures 1 and 2). Subsequent photoproduct analysis revealed that the UV-DRP-bound T₄C oligonucleotides were enriched by 4–5 fold for 6-4 photoproducts as compared to the unbound fraction (Figure 3). Conversely, the level of CPDs in bound DNA was reduced relative to that in the free fraction (Figure 4).

Both CPDs and 6-4 photoproducts are believed to distort the DNA helix and hence the UV-DRP does not simply seem to be recognizing general DNA distortion. It is unclear at present how the UV-DRP differentiates between these lesions, but chemical and structural data suggest that the two types of photoproducts induce dissimilar helix irregularities. Hydrogen bonding moieties critical to base pairing are chemically disrupted in 6-4 photoproducts but not in CPDs (17), and NMR studies with dinucleotides suggest that 6-4 photoproducts, in which the pyrimidine and pyrimidine rings occupy perpendicular planes, cause a perturbation in DNA structure more severe than that of CPDs (17–19). Whether the grossly distorted structure observed for the 6-4 photoproduct dinucleotide is also manifested as a similar alteration in double stranded DNA is unknown. Studies with duplex DNA containing CPDs showed that these lesions bend ($\sim 30^\circ$) and probably unwind ($14\text{--}20^\circ$) the helix (16). It is possible that subtle differences in the degree of helix bending, unwinding, and melting induced by the two classes of photoproducts mediate differential recognition by the UV-DRP. Such differences have been hypothesized to explain the differential recognition of various cisplatin-DNA crosslinks by a human cisplatin-DRP (1) and the chromosomal protein HMG-1 (2).

The UV-DRP also has detectable affinity for non-pyrimidine dimer UV damage as evidenced by the observation that irradiated oligonucleotides lacking adjacent pyrimidines showed a weak, but significant, level of binding to the UV-DRP (Figure 2). The modest binding of the UV-DRP to these probes suggests that the non-dimer photoproduct(s) is either rare or recognized with low affinity. The chemical nature of the photoproduct(s) responsible for this activity is unknown, but interstrand crosslinks are not likely candidates because these lesions were not detected by denaturing PAGE. Pyrimidine glycols and pyrimidine hydrates may be UV-DRP binding sites, but we have yet to address this issue. The relative contribution of this unidentified photoproduct(s) to overall UV-DRP binding in random DNA sequences is likely to be considerably lower than in the case of the UV box oligonucleotides in which sequences that permit 6-4 photoproduct formation are underrepresented.

The binding specificity of the UV-DRP is of possible importance to the mechanism by which mammalian DNA repair complexes remove 6-4 photoproducts more efficiently than CPDs. The UV-DRP could play an active role in the physical delivery of repair complexes to sites in DNA containing 6-4 photoproducts. Acting in this manner the UV-DRP would function analogously to the *E. coli* UVR-A protein as the lesion recognition subunit (36). Alternatively, the UV-DRP may, as hypothesized previously (14), increase the efficiency of excision repair by the mode proposed for the *E. coli* photolyase, which enhances the rate of the UVR-ABC catalyzed excision of CPDs *in vitro* (15). By this model, the UV-DRP would occupy DNA regions containing 6-4 photoproducts and act as an antenna to recruit repair complexes. The antenna function of the UV-DRP could be mediated by protein-protein interactions, or by inducing structural changes in the DNA (e.g., bending and/or unwinding) that enhance the binding of repair proteins.

Of the two models considered above, the putative photolyase-like mechanism is more consistent with the XP-E phenotype than the model involving UVR-A. XP-E cells exhibit a reduced, but still significant, level of DNA repair and UV resistance when compared to normal cells (37,38), suggesting that a non-vital component of the repair machinery is defective in this complementation group. Other XP groups have a more severe

repair deficiency and are likely to be lacking, or have defects in, proteins as essential to DNA repair in mammalian cells as UVR-A is to the *E. coli* excision nuclease. If the model based on the photolyase analogy presented above is valid, then it also follows that XP-E cells should lack the repair bias in favor of 6-4 photoproducts that exists in normal cells. The literature contains data showing that the repair of 6-4 photoproducts is deficient in XP-E cells (39). A deficient repair of CPDs is also evident in these cells, but the extent of the deficiency is unclear as conflicting data have been reported (39,40). Further repair studies are clearly required in order to test the proposed model conclusively.

The data cited above suggest that XP-E cells may be somewhat deficient in CPD repair. The UV-DRP may be involved indirectly in CPD repair by facilitating the release of repair complexes from 6-4 photoproducts subsequent to the DNA incision event. This process would effectively increase the turnover number for the repair complex and lead to the enhanced repair of all photoproducts. A similar model was proposed to explain the stimulation of UVR-ABC activity by photolyase (15). This model is not necessarily consistent with the UV-DRP having a role in the biased repair of 6-4 photoproducts and may necessitate the existence of other protein(s) that mediate this phenomenon.

In summary, we have presented results showing that a human UV-DRP has 6-4 photoproducts as a primary high affinity binding site in irradiated DNA. We were unable to demonstrate any binding of this protein to CPDs, although an as yet unknown form of non-pyrimidine dimer damage seems to be a rare or low affinity binding cue. It is possible that this protein mediates the efficient repair of 6-4 photoproducts in UV-damaged DNA. Alternatively, the UV-DRP could be of more general importance in the repair of UV damage by increasing the rate at which repair complexes dissociate from 6-4 photoproducts subsequent to DNA incision. The methodologies we have employed could be of broad use for determining the binding specificities of other DRPs.

ACKNOWLEDGEMENTS

Financial support was provided by Grants R35-CA52127 and T32-ES07020 from the National Institutes of Health. We acknowledge T. Connors, and B. Demple for helpful discussions, P. Songsanand for technical assistance, and D. Harrington for assistance with the figures. We also thank B. A. Donahue, S. J. Lippard, and K. J. Yarema for critically reading the manuscript.

REFERENCES

1. Donahue, B. A., Augot, M., Bellon, S. F., Treiber, D. K., Toney, J. H., Lippard, S. J. and Essigmann, J. M. (1990) *Biochemistry*, **29**, 5872–5880.
2. Pil, P. M. and Lippard, S. J. (1992) *Science*, **256**, 234–237.
3. Rydberg, B., Dosanjh, M. K. and Singer, B. (1991) *Proc. Natl. Acad. Sci. U. S. A.*, **88**, 6839–6842.
4. Jiricny, J., Hughes, M., Corman, N. and Rudkin, B. B. (1988) *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 8860–8864.
5. Lenz, J., Okenquist, S. A., LoSardo, J. E., Hamilton, K. K. and Doetsch, P. W. (1990) *Proc. Natl. Acad. Sci. U. S. A.*, **87**, 3396–3400.
6. Moranelli, F. and Lieberman, M. W. (1980) *Proc. Natl. Acad. Sci. U. S. A.*, **77**, 3201–3205.
7. Feldberg, R. S. and Grossman, L. (1976) *Biochemistry*, **15**, 2402–2408.
8. Chu, G. and Chang, E. (1988) *Science*, **242**, 564–567.
9. Hirschfeld, S., Levine, A. S., Ozato, K. and Protic, M. (1990) *Mol. Cell Biol.*, **10**, 2041–2048.
10. Chu, G. and Chang, E. (1990) *Proc. Natl. Acad. Sci. U. S. A.*, **87**, 3324–3328.

11. Protic,M., Hirschfeld,S., Tsang,A.P., Wagner,M., Dixon,K. and Levine,A.S. (1989) *Mol. Toxicol.*, **2**, 255–270.
12. Kataoka,H. and Fujiwara,Y. (1991) *Biochem. Biophys. Res. Commun.*, **175**, 1139–1143.
13. Keeney,S., Wein,H. and Linn,S. (1992) *Mutat. Res.*, **273**, 49–56.
14. Patterson,M. and Chu,G. (1989) *Mol. Cell Biol.*, **9**, 5105–5112.
15. Sancar,A., Franklin,K.A. and Sancar,G.B. (1984) *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 7397–7401.
16. Husain,I., Griffith,J. and Sancar,A. (1988) *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 2558–2562.
17. Franklin,W.A., Doetsch,P.W. and Haseltine,W.A. (1985) *Nucleic Acids Res.*, **13**, 5317–5325.
18. Rycyna,R.E. and Alderfer,J.L. (1985) *Nucleic Acids Res.*, **13**, 5949–5963.
19. Kemmink,J., Boelens,R. and Kaptein,R. (1987) *Eur. Biophys. J.*, **14**, 293–299.
20. LeClerc,J.E., Borden,A. and Lawrence,C.W. (1991) *Proc. Natl. Acad. Sci. U. S. A.*, **88**, 9685–9689.
21. Protic-Sabljic,M., Tuteja,N., Munson,P.J., Hauser,J., Kraemer,K.H. and Dixon,K. (1986) *Mol. Cell Biol.*, **6**, 3349–3356.
22. Brash,D.E., Seetharam,S., Kraemer,K.H., Seidman,M.M. and Bredberg,A. (1987) *Proc. Natl. Acad. Sci. U. S. A.*, **84**, 3782–3786.
23. Glickman,B.W., Schaaper,R.M., Haseltine,W.A., Dunn,R.L. and Brash,D.E. (1986) *Proc. Natl. Acad. Sci. U. S. A.*, **83**, 6945–6949.
24. Brash,D.E. and Haseltine,W.A. (1982) *Nature*, **298**, 189–192.
25. Mitchell,D.L., Haipke,C.A. and Clarkson,J.M. (1985) *Mutat. Res.*, **143**, 109–112.
26. Mitchell,D.L., Brash,D.E. and Nairn,R.S. (1990) *Nucleic Acids Res.*, **18**, 963–971.
27. Thomas,D.C., Okumoto,D.S., Sancar,A. and Bohr,V.A. (1989) *J. Biol. Chem.*, **264**, 18005–18010.
28. Wood,R.D. (1989) *Biochemistry*, **28**, 8287–8292.
29. Dignam,J.D., Lebovitz,R.M. and Roeder,R.G. (1983) *Nucleic Acids Res.*, **11**, 1475–1489.
30. Bradford,M.M. (1976) *Anal. Biochem.*, **72**, 248–254.
31. Carthew,R.W., Chodosh,L.A. and Sharp,P.A. (1985) *Cell*, **43**, 439–448.
32. Franklin,W.A., Lo,K.M. and Haseltine,W.A. (1982) *J. Biol. Chem.*, **257**, 13535–13543.
33. Banaszuk,A.M., Deugau,K.V., Sherwood,J., Michalak,M. and Glick,B.R. (1983) *Anal. Biochem.*, **128**, 281–286.
34. Lippke,J.A., Gordon,L.K., Brash,D.E. and Haseltine,W.A. (1981) *Proc. Natl. Acad. Sci. U. S. A.*, **78**, 3388–3392.
35. Brash,D.E., Franklin,W.A., Sancar,G.B., Sancar,A. and Haseltine,W.A. (1985) *J. Biol. Chem.*, **260**, 11438–11441.
36. Orren,D.K. and Sancar,A. (1989) *Proc. Natl. Acad. Sci. U. S. A.*, **86**, 5237–5241.
37. Lehmann,A.R., Kirk-Bell,S., Arlett,C.F., Harcourt,S.A., de Weerd-Kastelein,E.A., Keijzer,W. and Hall-Smith,P. (1977) *Cancer Res.*, **37**, 904–910.
38. Andrews,A.D., Barrett,S.F. and Robbins,J.H. (1978) *Proc. Natl. Acad. Sci. U. S. A.*, **75**, 1984–1988.
39. Mitchell,D.L. (1988) *Photochem. Photobiol.*, **48**, 51–57.
40. Zelle,B. and Lohman,P.H. (1979) *Mutat. Res.*, **62**, 363–368.