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Biochemistry

Enzymatic properties of purified *Escherichia coli* uvrABC proteins

(DNA repair/UV endonuclease/*uvrA*/*uvrB*/*uvrC*)

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ABSTRACT **The cloned *uvrA* and *uvrB* genes of *Escherichia coli* K-12 were amplified by linkage to the P_L promoter of plasmid pKC30. The *uvrC* gene was amplified in the high-copy-number plasmid pRLM 24. The three gene products (purified in each case to greater than 95% purity) and ATP are required to effectively incise UV-damaged DNAs. The uvrABC proteins bind tightly to damaged sites in DNA, requiring the initial attachment of the uvrA protein in the presence of ATP before productive binding of the uvrB and uvrC proteins. Using a cloned tandem double insert of the *lac p-o* region as a damaged DNA substrate for the uvrABC complex and analyzing the incision both 5′ and 3′ to each pyrimidine dimer, we found that one break occurs 7 nucleotides 5′ to a pyrimidine dimer and a second break is made 3–4 nucleotides 3′ from the same pair of pyrimidines in the dimer. No such breaks are found in the strand complementary to the dimer. The size of the incised fragment in the DNA suggests that incision may be coordinated with excision reactions in repair processes.**

There are at least two mechanistic responses to UV-induced pyrimidine cyclobutane dimers in DNA, depending on the biological systems examined. The least complicated mechanism occurs in those UV-resistant organisms such as *Micrococcus luteus* or T4 phage-infected *Escherichia coli*. In these cell systems the 5′-pyrimidine *N*-glycosylic linkage of the pyrimidine dimer is acted on by a unique dimer DNA glycosylase, generating an apyrimidinic site juxtaposed to a thymine-thymidylate dimer (1–5). This unusual site is recognized by a 3′-apyrimidinic site endonuclease specifically generating a hydroxylated 3′-apyrimidinic site and a 5′-phosphorylated thymine-thymidylate dimer site (1). The dimer-specific endonucleases from both *M. luteus* and T4-infected *E. coli* are bifunctional proteins possessing both the DNA glycosylase and the 3′-apyrimidinic site endonuclease activities (1, 6, 7).

The *uvr*-encoded system necessary for incision in *E. coli* is composed of the *uvrA*, *uvrB*, and *uvrC* gene products and incises not only UV-irradiated DNA but also DNAs affected by a variety of agents that form "bulky" adducts. Incision by the *uvrA*, *uvrB*, and *uvrC* gene products *in vitro* was demonstrated by Seeberg (8, 9) through the complementation of various *uvr*$^-$ mutant extracts with partially purified uvr proteins. Further elaboration of these findings was achieved through the cloning, expression, and amplification of the three *uvr* genes, and the site of their catalytic action was subsequently assessed on UV-irradiated DNA fragments of defined sequence (10, 11). It was from these studies that Rupp and his colleagues suggested that the uvrABC endonuclease complex generates two breaks on either side of a dimer site. It is the purpose of this communication to show that the highly purified *uvrA*, *uvrB*, and *uvrC* gene products act bifunctionally and that the active complex also hydrolyzes single phosphodiester bonds.

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METHODS

Purification of the *uvrA*, *uvrB*, and *uvrC* Proteins. Having cloned the *uvrA*, *uvrC* (12), and *uvrB* (unpublished data) genes from the chromosome of *E. coli* strain AB1157 we made use of the strong P_L promoter of phage λ to amplify the uvrA protein to 7% of the total protein of *E. coli* (13) and the uvrB protein in a similar manner to 5% of total *E. coli* protein (unpublished data). The uvrC protein was amplified about 300-fold by increasing the *uvrC* gene dosage through its cloning in high-copy-number plasmid pRLM24 (source: Roger L. McMacken, The Johns Hopkins University). The uvrA protein has been purified to >95% purity by using three chromatographic steps: (*i*) Affi-Gel blue (Bio-Rad), (*ii*) phosphocellulose P-11 (Whatman), and (*iii*) single-stranded DNA-agarose affinity chromatography (Fig. 1). The uvrB protein was purified to >95% purity in four chromatographic steps: (*i*) Affi-Gel blue, (*ii*) DEAE-Sephacel (Pharmacia), (*iii*) phenylagarose, and (*iv*) Sephadex G-150 (Pharmacia) (Fig. 1). The uvrC protein was purified to >95% purity by three chromatographic steps: (*i*) Affi-Gel blue, (*ii*) phosphocellulose P-11, and (*iii*) single-stranded DNA-agarose affinity chromatography (Fig. 1).

The uvrABC UV-Endonuclease Assay. The uvrABC endonuclease assay employed in these experiments exploits the ability of nitrocellulose filters to discriminate between replicative form (RF)-I DNA species and the single-stranded components of the enzyme-generated RF-II DNA (15). The assay conditions were essentially those described by Seeberg (8, 9), and included in a 140-μl reaction volume are 85 mM KCl, 40 mM potassium morpholinopropanesulfonate (Mops) at pH 7.6, 1 mM NaEDTA, 1 mM dithiothreitol, 15 mM $MgSO_4$, 2 mM ATP, 39 fmol (0.16 μg) of ^{3}H-labeled phage fd DNA (5×10^4 cpm/μg), 490 fmol of uvrA protein, 535 fmol of uvrB protein, and 516 fmol of uvrC protein. The mixture is incubated at 37°C. The number of pyrimidine dimers in each fd DNA molecule averaged between 1 and 6 according to the needs of different experiments.

Assembly of the uvrABC Complex. The mechanism of uvrABC protein–DNA complex formation will be the subject of a forthcoming publication. The amount of fd DNA that has uvrABC proteins associated as a protein–DNA complex was measured by the nitrocellulose filter binding assay (15). The assembly reaction conditions were identical to those employed for studying incision assays.

Preparation of Labeled DNA Fragments. All uvrABC incision reactions monitored by DNA sequence analysis employed four separate substrates derived from the double *lac*

Abbreviations: bp, base pair(s); DNG, dimer *N*-glycosylase; RF, replicative form. "Dimer" indicates a cyclobutane dimer of T-T, C-C, C-T, or T-C combinations; T◇T, C◇C, C◇T, and T◇C are the symbols for the same photoproducts.

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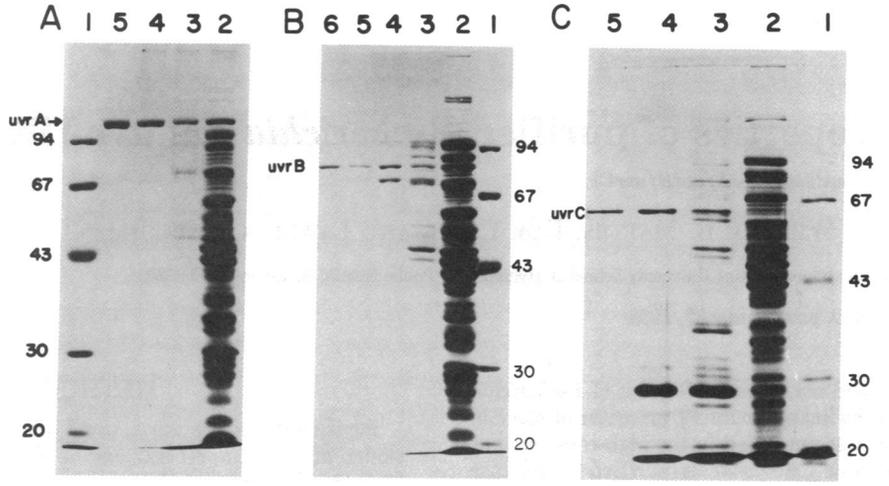

FIG. 1. Purification of the uvrA, uvrB, and uvrC proteins. Purification procedures for the uvrA, uvrB, and uvrC proteins were monitored by sodium dodecyl sulfate/9% polyacrylamide gel electrophoresis according to the method of Laemmli (14). (*A*) Purification of the uvrA protein. Lane 1, molecular weight standards, labeled $\times 10^{-3}$; lane 2, crude extract of 42°C-induced GHY8533 in which *uvrA* was expressed from the cloned P_L promoter of λ; lanes 3, 4, and 5, pooled uvrA protein peak fractions from the Affi-Gel blue column, phosphocellulose P-11 column, and single-stranded DNA-agarose columns, respectively. (*B*) Purification of the uvrB protein. Lane 1, molecular weight standards; lane 2, 42°C-induced BM1731 cells in which the *uvrB* gene is expressed from the cloned P_L promoter of λ; lanes 3, 4, 5, and 6, pooled uvrB protein peak fractions from the Affi-Gel blue column, DEAE-Sephacel column, phenyl-agarose column, and Sephadex G-150 column, respectively. (*C*) Purification of the uvrC protein. Lane 1, molecular weight standards; lane 2, crude extract of EOH-013 in which *uvrC* was amplified by its cloning in pRLM 24; lanes 3, 4, and 5, pooled uvrC protein peak fractions from the Affi-Gel blue column, phosphocellulose P-11 column, and single-stranded DNA-agarose column, respectively.

p-o DNA fragment: the 5′-labeled 117-base-pair (bp) fragment, the 3′-labeled 117-bp fragment, the 5′-labeled 168-bp fragment, and the 3′-labeled 168-bp fragment (Fig. 2). Because the DNA sequence in the original 285-bp fragment is a tandem head-to-tail repeat, the use of these four labeled fragments allows analyses of breaks on the strand complementary to the position of a UV-induced pyrimidine dimer as well as breaks on the strand containing the dimer.

Defined DNA fragments were prepared and labeled according to procedures described by D'Andrea and Haseltine (16) with modification. To prepare large amounts of this DNA fragment for study, the 285-bp *Eco*RI fragment containing the *lac p-o* was excised from pLJ3 (17) and inserted into the unique *Eco*RI site of the replication control-defective plasmid pRLM 24 to produce pPYC3. The *E. coli* C-600 strain containing the plasmid was grown in Luria broth to stationary phase in the presence of kanamycin at 20 μg/ml and the plasmids were isolated as described by Tanaka and Weisblum (18). To avoid the use of UV light required for visualizing fragments during the isolation, 125 μg of *Eco*RI-digested plasmid was 5′-end labeled by treatment with bacterial alkaline phosphatase (Bethesda Research Laboratories) for 2 hr at 60°C followed by polynucleotide kinase and [γ-^{32}P]ATP (19). The labeled reaction mixture was mixed with 1 mg of *Eco*RI-hydrolyzed pPYC3 and then separated on a 6% nondenaturing polyacrylamide gel. After visualization by autoradiography, the 285-bp fragment was isolated from the gels as described by Maxam and Gilbert (20). To increase the specific activity of the fragment, 1-μg aliquots of 285-bp fragment were dephosphorylated to completion and 5′-end labeled with polynucleotide kinase and [γ-^{32}P]ATP (>3,000 Ci/mmol; 1 Ci = 3.7×10^{10} Bq). The fragment was precipitated with ethanol and digested with an 8-fold excess of *Hae* III (Bethesda Research Laboratories). The resulting 117- and 168-bp fragments were separated on 8% polyacrylamide gels and purified (20). The 3′-end-labeled fragments were isolated in a similar manner with the exception that the 3′-end label was prepared by repair synthesis catalyzed by 2 units of the *E. coli* DNA polymerase I Klenow fragment with [α-^{32}P]dATP (>3,000 Ci/mmol) and dTTP (19). These synthetic repair reactions were quenched by two extractions with phenol followed by four extractions with ether and precipitation with ethanol. No carrier tRNA was used during the fragment isolation.

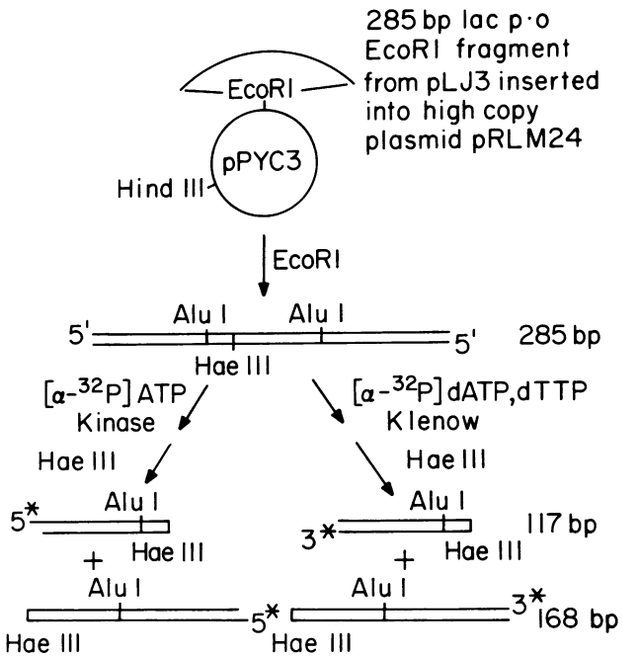

FIG. 2. Strategy for preparation and end-labeling of the 117- and 168-bp fragments that contain the *lac p-o* fragment. ^{32}P in DNA is indicated by *.

Analysis of the Site of Incision of the uvrABC UV-Endonuclease. About 0.05 μg of the ^{32}P-labeled 117-bp fragment and 0.07 μg of the 168-bp fragment were used in each uvrABC incision reaction, in which 10% of each reaction mixture was eventually loaded onto each sequencing lane. About 1 μg of lyophilized ^{32}P-labeled fragment was dissolved in 100 μl of 50 mM Tris·HCl/2 mM EDTA, pH 7.8; 75 μl of the DNA solution was irradiated to 2,000 J/m^2. Each incision reaction employed 5 μl of the irradiated DNA. The conditions used for the uvrABC incision reaction were those described above except for a 1-hr

incubation time and a 5-fold increase in the concentrations of all three proteins. At the end of the uvrABC incision reaction, 6.5 μl of 0.5 M NaEDTA, pH 8.0, was added to bring the reaction mixture to 25 mM in EDTA, after which 4 μg of tRNA carrier was added. After 10 min at 37°C the uvrABC complex dissociated from the DNA and 300 μl of phenol was added to extract the proteins. The aqueous phase was removed and reaction products were resolved from nucleotides and inorganic phosphate by desalting on a 900-μl column of Sephadex G-25 (21) or a 100-μl column of DEAE-Sephacel. The fragments were then precipitated in 2.5 M sodium acetate with 2 vol of isopropyl alcohol at −20°C overnight. The DNA was pelleted the next day, washed with 95% (vol/vol) ethanol, and vacuum dried. The pellet was dissolved in 10 μl of tracking dye buffer [0.1% xylene cyanol/0.1% bromophenol blue/30% (vol/vol) glycerol], and 1 μl was used for each sequencing gel lane. The *M. luteus* UV-endonuclease reaction was carried out in 100-μl reaction volumes in 50 mM Hepes/1 mM EDTA/5 mM $MgCl_2$, pH 7.6, as described by Grafstrom *et al.* (1). Approximately 5 units of the Sephadex G-75 fraction was used in each reaction. The incubation was carried out for 1 hr at 37°C.

The DNA sequencing reactions that were employed were those described by Maxam and Gilbert (20); the thymine>adenine tracts were generated by treatment with neocarzinostatin (Kayaku Antibiotics, Tokyo) (16). The enzyme and chemical reaction products were analyzed on 8% polyacrylamide/7 M urea sequencing gels (0.03 × 35 × 100 cm), in which electrophoresis was carried out for 4 hr at 2,500 V.

RESULTS

Purification of the uvrA, uvrB, and uvrC Proteins. Although the same purification steps were used for the uvrA and uvrC proteins, the two proteins were well resolved because they can be eluted from the column at significantly different salt concentrations. All three purified enzymes are isolated in an undegraded form and their activities are stable to storage.

The uvrA, uvrB, uvrC Protein Complementation Incision Assay. A complementation assay for measuring the uvrABC protein/MgATP-dependent incision of UV-irradiated DNA was demonstrated by Seeberg, using crude extracts of mutants of the *uvrA*, *uvrB*, and *uvrC* genes (8). The purified uvrA, uvrB, and uvrC proteins described in this communication are similarly capable of incising UV-irradiated DNA efficiently when these three proteins are combined in the presence of MgATP (Fig. 3). Omission of the uvrA, uvrB, or uvrC proteins, or Mg^{2+}, or ATP results in loss of the UV-dependent DNA incision. While analyzing the amount of the uvrA, uvrB, and uvrC proteins required to effect maximal UV-dependent incision activity, we found that the optimal ratio of uvrA to uvrB to uvrC in the reaction was 1:1:1. This stoichiometry is the same as that required for the optimal formation of the uvrABC incision complex on UV-irradiated DNA (data not shown).

Assembly of the uvrA–uvrB–uvrC–Protein Complex. Kacinski and Rupp demonstrated that uvrB protein binds to unirradiated single-stranded DNA in the presence of uvrA protein (22). Nitrocellulose filters are capable of trapping protein-bound DNA molecules (23). Making use of this property, Seeberg showed that the uvrA protein has an ATP-dependent affinity for UV-irradiated duplex DNA (24). Extending these findings, we show that the uvrB protein, which has no affinity for damaged DNA by itself, enhances the ability of the uvrA protein to bind to UV-irradiated duplex DNA. It can be seen in Fig. 4 that the maximal enhancement of uvrA binding to only UV-irradiated DNA was obtained at equimolar ratios of uvrB to uvrA protein. Further, this enhanced binding was completely ATP dependent. The uvrA protein will bind significantly to both irradiated and unirradiated double-stranded DNA in the presence of MgATP (Fig. 4). The structure of the uvrAB–irradiated DNA complex can be distinguished from that of the uvrA–irradiated DNA complex by the latter's unique sensitivity to chelators and ionic strength during washing procedures. Under conditions in which the uvrAB–irradiated DNA complex is stable to chelators and high salt ($t_{1/2}$ for dissociation = 55 min) the uvrA–irradiated DNA complex has a half-life of less than 5 sec.

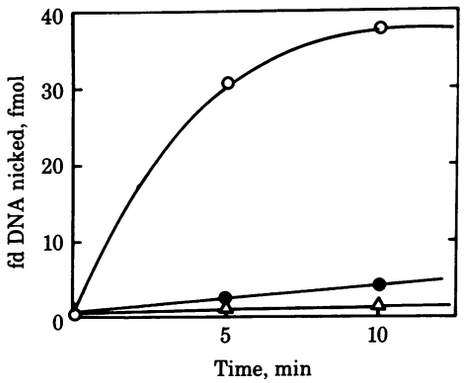

FIG. 3. Incision of UV-irradiated DNA by the purified uvrA, uvrB, and uvrC proteins. The incision of UV-irradiated fd RF-I DNA (containing 6 dimers per fd DNA molecule) by the uvrABC endonuclease at 37°C was followed by the conversion of RF-I DNA to the RF-II form (15). ○, uvrA + uvrB + uvrC + UV + Mg^{2+} + ATP; ●, uvrA + uvrB + uvrC − UV + Mg^{2+} + ATP; △, any of the following experiments: uvrA + uvrB + uvrC + UV + Mg^{2+} − ATP, uvrA + uvrB + UV + Mg^{2+} + ATP, uvrB + uvrC + UV + Mg^{2+} + ATP, uvrA + uvrC + UV + Mg^{2+} + ATP, uvrA + UV + Mg^{2+} + ATP, uvrB + UV + Mg^{2+} + ATP, uvrC + UV + Mg^{2+} + ATP. The assay contained 39 fmol of ^{3}H-labeled fd DNA (8,000 cpm), 490 fmol of uvrA protein, 535 fmol of uvrB protein, and 516 fmol of uvrC protein.

Site of Incision of the uvrABC UV-Endonuclease. It is feasible for a site-specific endonuclease to hydrolyze phosphodiester bonds at the site of damage, opposite to such a recognition site, or both. Moreover, more than one phosphodiester bond may be hydrolyzed on each side of the recognition site. The use of the defined sequences of the *lac p-o* region and its cloning as a tandem duplicate in the plasmid pLJ3 (17) permit

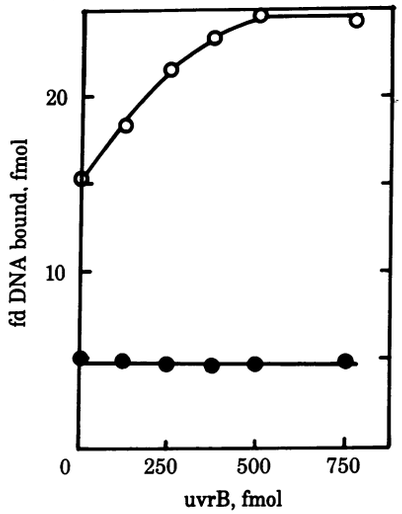

FIG. 4. Formation of uvrA–uvrB–irradiated DNA complex. uvrA protein (490 fmol) was incubated with 38.7 fmol of ^{3}H-labeled fd RF-I DNA under conditions identical to those used for the nicking assay as described for Fig. 3. Different amounts of uvrB protein, 0–750 fmol, were also included in the assembly reaction mixture. After 20 min at 37°C, the reaction mixture was diluted with 5 ml of 0°C assay buffer containing no ATP, then passed slowly through a nitrocellulose filter to collect the protein–DNA complex. ○, + UV (6 dimers per fd DNA molecule); ●, − UV.

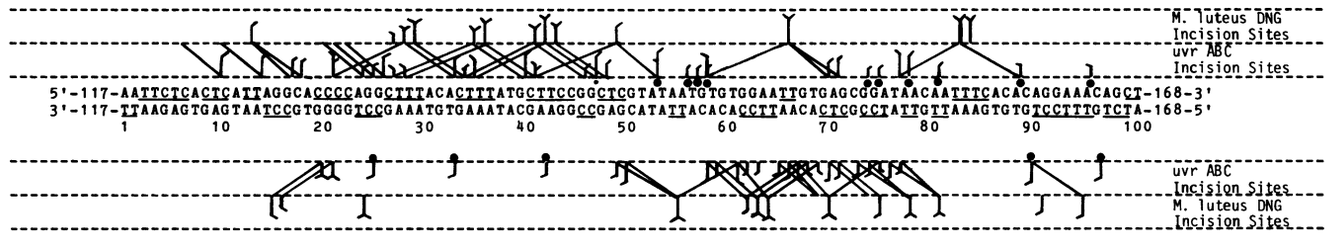

FIG. 5. Summary of the sites of incision by the uvrABC complex and the *M. luteus* UV endonuclease on the UV-irradiated *lac p-o* fragments. The 117- and 168-bp *lac p-o* fragments labeled at either the 5′ or the 3′ termini were used as substrates in the analysis of incision sites by the uvrABC complex and the *M. luteus* UV endonuclease. The results are diagrammatically presented in this figure. In the upper sequence: ⌉, incision sites on the 5′-labeled 117-bp fragment; ⌈, incision as on the 3′-labeled 168-bp fragment. In the lower sequence: ⌋, incision on the 3′-labeled 117-bp fragment; ⌊, incision on the 5′-labeled 168-bp fragment. Dimerizable pyrimidines are underlined. The incision site created by the *M. luteus* UV endonuclease (dimer *N*-glycosylase, DNG), symbolized by Y|, is the same whether a 5′- or 3′-labeled fragment was used and coincided at the sites of incision. The height of the bars reflect an approximate intensity of the band on the sequencing gel. Under the conditions used in this experiment, the uvrABC complex appeared to produce two incision sites for each of the potential sites for formation of T◇T, C◇C, T◇C, and C◇T dimers. Single incision sites are designated by ● at the base of the bar for that site.

direct analysis of the sites of incision by the uvrABC endonuclease, whether hydrolysis occurs on the DNA strand with the resident pyrimidine dimer or on the DNA strand opposite to such dimers. More importantly, the fact that two breaks are introduced by uvrABC proteins at each pyrimidine dimer is documented here by analysis of the incision events at each dimer with the same DNA sequence, first with the DNA sequence labeled at the 5′ terminus, then with the sequence labeled at the 3′ terminus. Fig. 5 summarizes the sequencing data of the incision sites on the UV-irradiated *lac p-o* fragment by the uvrABC UV endonuclease and the *M. luteus* UV endonuclease, whose site of action is also diagnostic for the location of pyrimidine dimers and is used as a control for incision. The *M. luteus* UV endonuclease hydrolyzes the phosphodiester bond between the sugar moieties of the pyrimidine dimers in UV-irradiated DNA, generating a 3′-apyrimidinic site with a 3′-OH terminus, and a 5′-PO_4 at the other terminus generated by incision (1, 5). Thus, the sites of incision at each pyrimidine dimer by this enzyme are coincident whether the incision reaction is carried out on a 5′-labeled fragment or a 3′-labeled fragment. In contrast, the incision sites whose formation is catalyzed by the uvrABC complex are distal to the potential di-

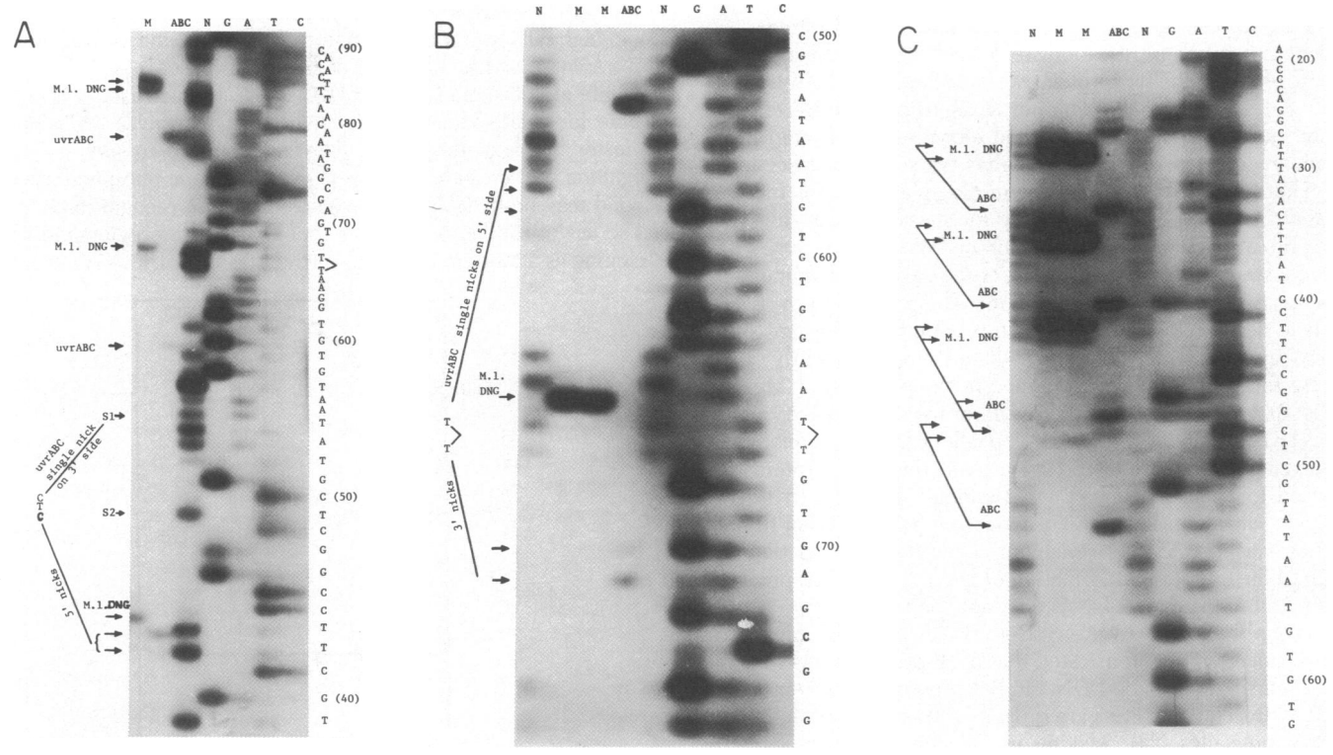

FIG. 6. Analysis of the uvrABC incision sites by DNA sequencing gels. Lanes: M, incision of UV-irradiated fragments by the *M. luteus* dimer-specific endonuclease (Sephadex G-75 fraction); ABC, incision of UV-irradiated fragments by the uvrABC complex; N, A>T reaction of the fragments treated with neocarzinostatin; G, G sequencing reaction; A, A+G sequencing reaction; T, T+C sequencing reaction; C, C sequencing reaction. (*A*) The 5′-labeled fragments; (*B* and *C*) the 3′-labeled 117- and 168-bp fragments, respectively. Analysis of a T◇T dimer at position 66-67 is shown in *A*, in which incision occurs on the 5′ side of this dimer at position 58-59. *B* depicts incisions on the 3′ side of the same dimer at position 70-71. In the case of this dimer site the uvrABC complex incised on both sides of the dimer; 7 nucleotides 5′ and 3–4 nucleotides 3′ to this dimer. In addition to incision on both sides of this dimer, some DNA fragments were singly incised on the 5′ side of the dimer site as shown by the three arrows in *B* at positions 56-57, 57-58, and 58-59. Similarly, single incisions that resulted in breaks on the 3′ side of the dimer were observed in *A*: (*i*) a faint band marked by the symbol S1 at position 53-54 acting at the C-T-C dimerizable site (position 48-50); and (*ii*) another faint single incision site symbolized by S2 at position 47-48 near dimerizable sites 41-45. *C* depicts a more obvious example of the uvrABC incision in which 3–4 nucleotides are offset from the site hydrolyzed by the *M. luteus* UV endonuclease (M.l. DNG) when acting on the 3′-labeled 168-bp fragment.

merizable sites. When 5′-labeled DNA fragments are employed as substrates, the site of incision occurs about 7 nucleotides 5′ to the pyrimidine dimer. Furthermore, when 3′-labeled DNA fragments are used as substrates, the site of incision appears 3 or 4 bases 3′ to the pyrimidine dimer. Analysis of the strand complementary to the pyrimidine dimer did not reveal any breaks in those regions under conditions in which the uvrABC complex generates two incision sites for each dimer. In addition to sites 5′ and 3′ to the dimer, some incision events do occur at the 3′ side of the dimer without an accompanying incision at the 5′ side, and vice versa, even under conditions of >20-fold overdigestion. These single incision sites are observed on the sequencing gel as bands that migrate at the 3′-incision site on a 5′-labeled fragment, and at the 5′ incision position on a 3′-labeled fragment. That the uvrABC complex hydrolyzes DNA as a double incision event on the same strand as the pyrimidine dimer is best exemplified by the analysis of incision of the T◇T dimer at position 66-67 in the top strand of the DNA in Fig. 5. The pyrimidine dimer site is singly incised by the *M. luteus* UV endonuclease at the dimer position. However, the uvrABC complex-catalyzed incision occurs at a position 7 nucleotides 5′ from this T◇T dimer on this particular fragment (Fig. 6*A*). When the 168-bp 3′-labeled fragment was used as a substrate the *M. luteus* UV endonuclease incised at the same position within the T◇T dimer, whereas the uvrABC-catalyzed incision occurred 3 or 4 nucleotides 3′ to the T◇T dimer (Fig. 6*B*). In addition, a minor band was observed at the position of 5′ incision relative to the dimer when this 3′-labeled fragment was used as a substrate, indicating that potential 3′ incision sites on some molecules were not acted upon (Fig. 6*B*). In Fig. 6*C*, analysis of a 3′-labeled 168-bp fragment shows that the uvrABC complex incises at the 3′ side of several dimerizable sites that are 3–4 nucleotides 3′ to the site incised by the *M. luteus* UV endonuclease. It should be emphasized that, like the incision reactions with fd RF-I DNA, witholding any one of the uvr proteins, ATP, or Mg^{2+} in the reaction prevents incision (data not shown).

DISCUSSION

The uvrABC endonuclease complex incises UV-damaged double-stranded DNA such that most of the time two breaks are made in the vicinity of sites of potential pyrimidine cyclobutane dimers. These breaks are found only on the strand of DNA containing the dimer site and occur on both sides of the site, suggesting that the uvrABC endonuclease may release a 12- to 13-base oligonucleotide containing the pyrimidine dimer. However, the possibility that other UV-induced damages in DNA can be incised by the uvrABC endonuclease in the same manner cannot be examined by the experiments presented in this paper.

If the *M. luteus* dimer-specific endonuclease is used as a model for a damage-specific endonuclease it might have been expected that the *E. coli* uvr-mediated repair system should have acted similarly. However, the *M. luteus* dimer-specific endonuclease is limited to pyrimidine dimers and is unable to act upon the broad spectrum of seemingly unrelated damaged sites that the *E. coli* uvrABC complex can accommodate. This complex must recognize, therefore, a distortional site somewhat common to a range of damage that can include, in addition to pyrimidine dimers, benzo[*a*]pyrene-guanine adducts, acetylaminofluorene-C^8-guanine adducts, thymine-psoralen-thymine crosslinks, psoralen-thymine monoadducts, and O^6-alkylguanine adducts. It seems appropriate that such a complex recognizes and acts at the extremes of the structural distortion imposed by these disparate chemical and photochemical structures. An additional compelling property of this system of incision is its potential for a coordinated incision–excision reaction in which an oligomer 12 nucleotides in length is potentially excisable.

We have presented evidence that a stable complex of uvrA and uvrB proteins with UV-irradiated double-stranded DNA can be demonstrated under conditions conducive for the incision reaction. In experiments to be presented in another publication, we have found that addition of purified uvrC to the preformed uvrAB protein–irradiated DNA complex results in rapid, specific incision of the irradiated DNA. After the incision event, a uvr protein–DNA complex seems to persist. Although the nature of the postincision complex is not yet elucidated, the stability of the incised DNA–uvrABC complex *in vitro* raises the possibility that the uvr system may not turn over. It would appear that for productive excision the described uvrABC system may be limiting with respect to other gene products. The uvrABC-incised DNA complex is amenable to an examination of other proteins that may facilitate the excision reaction. For example, proteins such as the *uvrD* gene product, which can, because of its helicase activity, possibly displace single-stranded regions containing a dimer and single-stranded DNA binding proteins, which can facilitate strand displacement or DNA polymerase I action, which because of its preference for nicked sites may be able to displace the uvrABC protein complex in a coordinated excision-reinsertion reaction at the short gaps occupied by the excisable damaged fragment.

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