Differences and Similarities in the Repair of Two Benzo[*a*]pyrene Diol Epoxide Isomers Induced DNA Adducts by *uvrA*, *uvrB*, and *uvrC* Gene Products†

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ABSTRACT: We have determined the role of the *uvrA*, *uvrB*, and *uvrC* genes in *Escherichia coli* cells in repairing DNA damage induced by three benzo[*a*]pyrene diol epoxide isomers. Using the *φX174* RF DNA–*E. coli* transfection system, we have found that BPDE-I or BPDE-II modified *φX174* RF DNA has much lower transfectivity in *uvrA*, *uvrB*, and *uvrC* mutant cells compared to wild type cells. In contrast, BPDE-III modification of *φX174* RF DNA causes much less difference in transfectivity between wild type and *uvr*⁻ mutant cells. Moreover, BPDE-I– and -II–DNA adducts are much more genotoxic than are BPDE-III–DNA adducts. Using purified UVRA, UVRB, and UVRC proteins, we have found that these three gene products, working together, incise both BPDE-I– and BPDE-III–DNA adducts quantitatively and, more importantly, at the same rate. In general, UVRABC nuclease incises on both the 5′ (six to seven nucleotides) and 3′ (four nucleotides) sides of BPDE–DNA adducts with similar efficiency with few exceptions. Quantitation of the UVRABC incision bands indicates that both of these BPDE isomers have different sequence selectivities in DNA binding. These results suggest that although UVR proteins can efficiently repair both BPDE-I– and BPDE-III–DNA adducts, in vivo the *uvr* system is the major excision mechanism for repairing BPDE-I–DNA adducts but may play a lesser role in repairing BPDE-III–DNA adducts. It is possible the low lethality of BPDE-III–DNA adducts is due to less complete blockage of DNA replication. Alternatively, *E. coli* cells may have an alternative mechanism for repairing BPDE-III adducts that is not active in the repair of BPDE-I– or BPDE-II–DNA adducts.

Benzo[*a*]pyrene (BP)[1] is a potent environmental mutagen and carcinogen (Weinstein, 1981). Metabolically activated diol epoxide forms of BP are reactive with DNA, and the DNA adducts have been ascribed as the initial lesions that trigger mutagenesis and carcinogenesis (Newbold & Brookes, 1976; Buening et al., 1978; Slaga et al., 1977; Weinstein, 1981). Macromolecular adducts formed by several isomeric diol epoxides of BP have been found in vivo, including BPDE-I, BPDE-II, and BPDE-III (Jeffrey et al., 1977; Pruess-Schwartz & Baird, 1986; Ivanovic et al., 1978; MacLeod et al., 1980). These three diol epoxide forms of BP covalently bond to the exocyclic amino group of guanine and adenine bases in DNA through the C10 position; however, the stereostructural differences of these isomers apparently greatly affect the conformation of the adducted DNA helix (Hogan et al., 1981). Results from several laboratories (Geacintov et al., 1978; MacLeod et al., 1982; Harvey & Geacintov, 1988) suggest that while covalently bound BPDE-I adducts tend to be external to the DNA helix, the major BPDE-III adduct is intercalated in the helix structure. In contrast, the BPDE-II–DNA adducts exist in both external and intercalating structures (Undemann et al., 1983). It is conceivable that the stereostructural differences of the BPDE isomers and their different effects on DNA helix structure will affect their repairability, and these effects may in turn contribute to their various potencies in mutagenesis and carcinogenesis. Indeed, BPDE-II–DNA adducts are removed faster in mammalian cells than are BPDE-I adducts (Pruess-Schwartz et al., 1988; MacLeod et al., 1991a).

In mammalian and *Escherichia coli* cells mutants that are deficient in excision repair of UV-induced DNA damage are also sensitive to BPDE-I-induced DNA damage; therefore, the repair of BPDE-I–DNA damage seems to occur via the same pathways as cyclobutane dipyrimidine (Py⟨⟩Py) photoproducts (Seeberg et al., 1983). Recent results indicate that repair-deficient mammalian cells are also hypersensitive to the cytotoxic and mutagenic affects of BPDE-II and BPDE-III (MacLeod et al., 1991a; MacLeod et al., 1991b). In *E. coli* cells the three gene products *uvrA*, *uvrB*, and *uvrC* are involved in the recognition and initial incision of Py⟨⟩Py and a variety of bulky chemical induced DNA damages (for review, see Sancar & Sancar, 1988; Grossman & Yeung, 1990; Van Houten, 1990). We have found that purified UVRA, UVRB, and UVRC proteins work together to incise a variety of kinds of DNA damage such as Py⟨⟩Py, dG-C8-AAF, and dG-G8-AF; however, in vivo *uvr* mutants respond differently to certain kinds of DNA adducts such as dG-C8-AF and anthramycin-N2-guanine DNA adducts (Tang et al., 1982; Pierce et al., 1989; Tang et al., 1991). These findings raise the possibility that in vivo the *uvr* gene products may have more versatile functions than observed in vitro and that in vivo in the presence of other cellular repair factors the function of *uvr* gene products may be much more dependent on DNA adduct structure.

In light of the stereostructural differences between the DNA adducts formed by these BPDE isomers, we determined the in vivo *uvr* gene function in repair of DNA adducts formed by these BPDE isomers. We also characterized the mode and the rate of repair of BPDE-I– and BPDE-III–DNA adducts in vitro. We found that purified UVRA, UVRB, and UVRC proteins working together incise both BPDE-I– and -III–DNA adducts at a similar rate and efficiency and that the incisions occur both 5′ (six to seven bases away) and 3′ (four bases

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[1] Abbreviations: BP, benzo[*a*]pyrene; BPDE-I, (±)-*r*-7,*t*-8-dihydroxy-9,*t*-10-epoxy-7,8,9,10-tetrahydro-BP; BPDE-II, (±)-*r*-7,*t*-8-dihydroxy-9,*c*-10-epoxy-7,8,9,10-tetrahydro-BP; BPDE-III, (±)-*r*-9,*t*-10-dihydroxy-7,*c*-8-epoxy-7,8,9,10-tetrahydro-BP; dG-C8-AAF, *N*-(deoxyguanosine-8-*C*-yl)-2-acetylaminofluorene; dG-C8-AF, *N*-(deoxyguanosin-8-*C*-yl)-2-aminofluorene.

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away) from the BPDE-I– and -III–DNA adducts. Our transfection results suggest that in vivo *uvr* gene products are the major excision repair pathway for BPDE-I– and BPDE-II–DNA adducts. In contrast, there may be other repair pathways besides the *uvr* system that play an important role in repairing BPDE-III–DNA adducts.

MATERIALS AND METHODS

Materials. BPDE-I, -II, and -III isomers were obtained from the National Cancer Institute's Chemical Carcinogen Repository. Restriction enzymes *Eco*RI, *Bam*HI, and *Ava*I, T4 polynucleotide kinase, bacterial alkaline phosphatase, acrylamide, bis(acrylamide), and agarose were obtained from Bethesda Research Laboratories. Restriction enzyme *Bst*NI was obtained from New England Biolabs. All ^{32}P-labeled nucleotides were obtained from New England Nuclear, Du Pont.

Bacterial Strains and φX174 RF DNA Transfection. The details of strain construction and the genotype of the wild type *E. coli* (MST1) and the *uvr* mutants *uvrA6*(MST13), *uvrB5*(MST3), and *uvrC34*(MST8) were described previously (Tang et al., 1982). Methods for preparing competent cells, DNA transfection, and calculating relative transfection frequency were the same as previously described (Tang et al., 1982).

Purification of UVRA, UVRB, and UVRC Proteins. UVRA, UVRB, and UVRC proteins were isolated from *E. coli* K12 strain CH296 carrying plasmids pUNC45 (*uvrA*), pUNC211 (*uvrB*), or pDR3274 (*uvrC*) (Thomas et al., 1985). These plasmid-carrying strains were kindly provided by Dr. A. Sancar, University of North Carolina, Chapel Hill, NC. UVRA and UVRC proteins were purified through (1) phosphocellulose and (2) single-stranded DNA–cellulose columns, and UVRB proteins were purified through (1) DEAE-Bio-Gel-A, (2) phenylagarose, and (3) Affigel-Blue columns. The detailed purification procedures are essentially the same as Sancar and Rupp's method (1983), except that the steps of protein precipitation by polyethylenimine and ammonium sulfate were eliminated and a continuous salt gradient replaced the step gradient to elute the proteins. The purified UVRA, UVRB, and UVRC proteins were free of nonspecific nuclease contamination as detected by the standard UVRABC nuclease reaction conditions and showed only single band in PAGE gel stained with Coomasie Blue (Tang et al., 1991).

DNA Isolation and ^{32}P-End Labeling. Supercoiled *φX174* RFI DNA was prepared from *E. coli* C *supE* cells infected with *φX174* $_{am}3_{cs}70$ phages by clear lysate method and cesium chloride density gradient centrifugation. All preparations used contained greater than 85% supercoiled molecules as determined by agarose gel electrophoresis. pBR322 DNA and its 174-bp *Eco*RI-*Hae*III and 129-bp *Eco*RI-*Bst*NI fragments were prepared and 5′-end and 3′-end labeled with ^{32}P, respectively, the same way as previously described (Pierce et al., 1989).

BPDE Modifications. Purified *φX174*-RF DNA and defined DNA fragments were modified with BPDE-I or BPDE-II as previously described (MacLeod & Tang, 1985). Since BPDE-III is less reactive than BPDE-I, BPDE-III modification reactions were carried out for longer periods of time. For transfection experiments, modification proceeded for 40 h. To prepare BPDE-III modified substrates for biochemical studies, reaction times of 5 or 16 h were used. In most experiments, the level of adduction with BPDE-III was determined by a fluorescence method. Salmon DNA was modified in parallel to *φX174* DNA, and the level of adduction in the salmon DNA was determined by absorbance

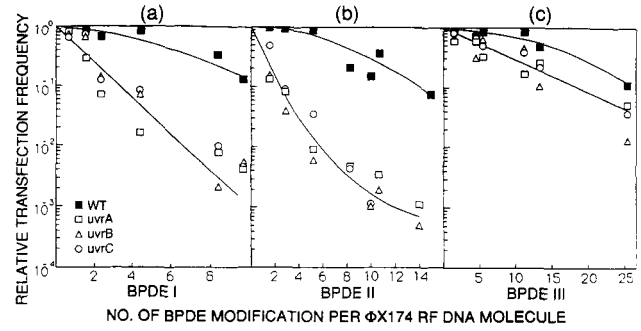

FIGURE 1: Relative transfection frequency of (a) BPDE-I, (b) BPDE-II, and (c) BPDE-III modified *φX174* RF DNA in *E. coli* wild type (■), *uvrA6* (□), *uvrB5* (△), and *uvrC34* (○) mutant cells. The preparation of BPDE-modified *φX174* RF DNA, DNA–*E. coli* cell transfection and the calculation of relative transfection frequency are described in Materials and Methods. Each point is an average of two to four experiments.

spectroscopy using E_m^{352} = 29 500 (Hogan et al., 1981). This DNA was then diluted and used to construct a standard curve by measuring fluorescence excitation spectra in an SLM/Aminco 500 spectrofluorimeter. The emission signal at 400 nm was integrated for excitation wavelengths from 320 to 359 nm. In control experiments where large quantities of *φX174*-DNA were used, allowing absorption measurements of adduct levels to be made directly, the absorbance and fluorescence methods gave essentially identical values for the levels of adduction. Aliquots of BPDE-modified *φX174* RF DNA were linearized by digestion with *Ava*I and 3′-end ^{32}P labeled as described previously (Tang et al., 1991).

Reaction of UVR Proteins with DNA. Standard UVRABC reactions were carried out in 25–50 μL containing 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1.0 mM DTT, 10 mM $MgCl_2$, 1 mM ATP, 100 mM KCl, 15 nM UVRA, 12 nM UVRB, 15 nM UVRC, and substrate DNA (0.2 μg). The mixtures were incubated for different periods of time at 37 °C. For *φX174* RF DNA the reactions were stopped by adding 0.1% SDS and heating at 65 °C for 5 min, and the DNAs were electrophoresed in a 1% agarose gel in TAE buffer (40 mM Tris–acetate, pH 8.0, 1 mM EDTA) at 1 V/cm for 14 h. The gel was stained with 0.5 μg/mL ethidium bromide to visualize supercoiled and relaxed forms of DNA. For radioactively labeled DNA fragments and linearized *φX174* RF DNA, the reactions were stopped by phenol extractions and followed by diethyl ether extractions and precipitation with 75% ethanol; 50 μg/mL tRNA was added as carrier. The precipitated DNA was recovered by centrifugation and washed with 80% ethanol. The DNA was denatured by neutral formamide treatment. A 90-μL aliquot of fresh deionized formamide solution was added to 10 μL of DNA solution, and the mixture was incubated at 37 °C for 30 min. Immediately after incubation the linearized *φX147* RF DNAs were electrophoresed at 5 V/cm for 3 h in a preformed 1% agarose gel in TBE buffer (50 mM Tris-HCl, pH 7.9, 50 mM sodium borate, 5 mM EDTA) with 0.5 μg/mL ethidium bromide. After electrophoresis the gel was dried and exposed to Kodak X-Omat RP film at −70 °C for various times.

DNA Sequencing Gel Electrophoresis. The ^{32}P-labeled 174-bp or 129-bp DNA fragments with or without various UVR protein treatments were precipitated, dried, and denatured in neutral formamide (90%). Samples were electrophoresed in 8% (w/v) polyacrylamide sequencing gels with 50% urea in TBE buffer in parallel with Maxam and Gilbert (1980) sequencing reactions at 40 V/cm. After electrophoresis the gels were dried on a Bio-Rad slab gel dryer and exposed to Kodak X-Omat RP film at −70 °C for various periods of time.

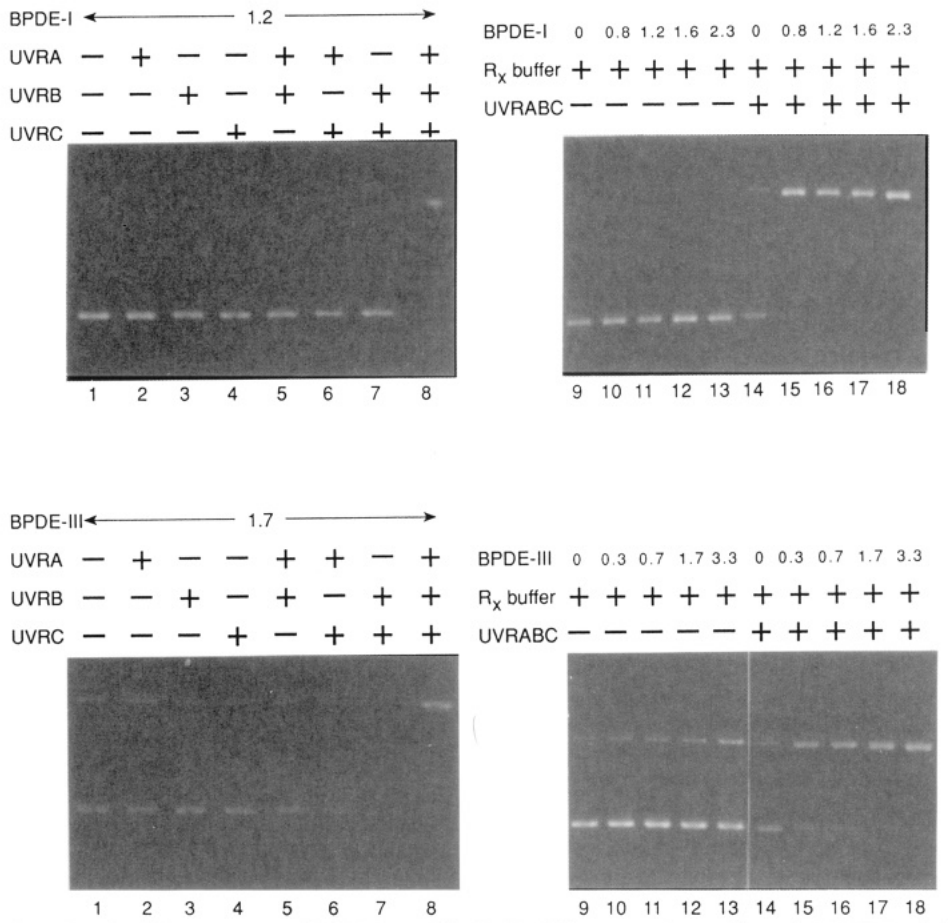

FIGURE 2: UVRABC nuclease incisions on supercoiled DNA modified with different numbers of (a, top) BPDE-I and (b, bottom) BPDE-III adducts. The number of BPDE modifications indicated on the top of each panel was calculated on the basis of the radioactive specific activity for BPDE-I and the fluorescence measurements for BPDE-III. The BPDE-modified supercoiled *φX174* RF DNA was reacted with UVRABC nuclease for 60 min at 37 °C to achieve maximum incisions, and then the DNAs were electrophoresed in 1% agarose. The + and – signs represent whether the enzyme and the reaction buffer (Rx buffer) were added or not.

The intensities of UVRABC nuclease incision bands were determined with a BioImage Visage 100 System, consisting of a high-resolution digitizing camera and whole band analysis software.

RESULTS

Effects of uvr Mutations on the Transfectivity of BPDE-I, BPDE-II, and BPDE-III Modified φX174 RF DNA. Metabolically activated or chemically synthesized BPDE isomers react strongly with nucleophilic components in cells such as RNA and proteins besides DNA (Ivanovic et al., 1978; MacLeod et al., 1980); BPDE-induced damage in these components may affect cellular processes such as DNA repair and replication and also may affect macromolecular syntheses. In order to establish the direct causal relationship between biological effects and BPDE–DNA adducts per se, we have used a phage–DNA–*E. coli* cell transfection system to assess the genotoxicities of BPDE–DNA adducts and the host-cell genes that are involved in repair of these adducts. Purified *φX174* RFI DNA was modified with different concentrations of BPDE-I, BPDE-II, or BPDE-III to achieve different levels of DNA modification. The number of BPDE–DNA adducts per *φX174* RF DNA molecule was calculated on the basis of the radioactivity for BPDE-I and BPDE-II modification. However, due to lack of radioactivity labeled BPDE-III, the DNA adducts formed by this isomer were determined by the fluorescence emission of BPDE-III–DNA adducts after excitation at 343 nm.

The BPDE-I, BPDE-II, and BPDE-III modified *φX174* RFI DNAs were subsequently transfected into wild type *E. coli* cells or into isogenic *uvrA*, *uvrB*, or *uvrC* mutant cells, and the relative transfection frequencies were determined using a plaque assay. Figure 1a,b shows that BPDE-I and BPDE-II modified *φX174* RF DNAs have significantly lower tranfectivity in *uvrA*, *uvrB*, and *uvrC* mutant cells than in wild type cells. While an average of 1.4 and 1.0 modification of BPDE-I and BPDE-II per DNA molecule reduces the transfectivity of the DNA to 37% (D_{37}) of control levels in these *uvr*⁻ mutant cells, the D_{37} for wild type cells are 6.5 and 8 for BPDE-I and BPDE-II, respectively. These results suggest that the repair of BPDE-I and BPDE-II induced DNA adducts requires *uvrA*, *uvrB*, and *uvrC* gene products, and this *uvr* gene controlled excision mechanism is the major excision repair pathway for these two kinds of DNA adducts.

The relative transfectivity of BPDE-III modified *φX174* RF DNA in *E. coli* cells is very different from that of BPDE-I and BPDE-II modified DNA (cf. Figure 1c with Figure 1a,b). The D_{37} of BPDE-III modification is 16 for wild type cells and 8.5 for *uvr*⁻ mutant cells. These results indicate that the BPDE-III–DNA adducts are less genotoxic for *E. coli* cells and also indicate that *uvr* gene products play a lesser role in repairing BPDE-III–DNA adducts than in repairing BPDE-I or BPDE-II adducts in vivo. Levels of BPDE-I or BPDE-II modification that reduce the relative transfection to 10^{-1} in wild type cells reduce the relative transfection in *uvr* cells to 5×10^{-3} to 5×10^{-4}; in contrast, BPDE-III modification that gives 10^{-1} relative transfection frequency in wild type cells produces a relative tranfection frequency at $(1–5) \times 10^{-2}$ in *uvr* cells (Figure 1c).

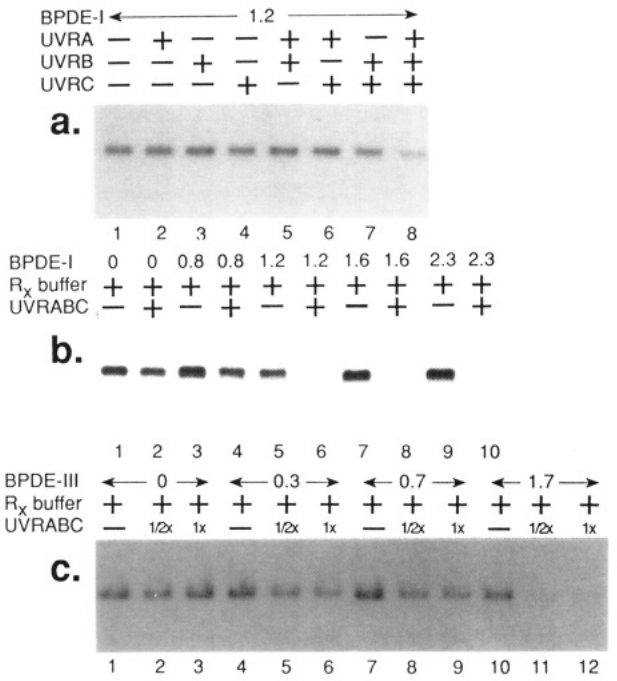

FIGURE 3: UVRABC nuclease incisions on linear DNA modified with different numbers of (a and b) BPDE-I and (c) BPDE-III adducts. The BPDE-modified supercoiled $\phi X174$ RF DNAs described in Figure 2 were linearized, 3′-end ^{32}P labeled, and then reacted with UVRABC nuclease for 60 min at 37 °C. The resultant DNAs were denatured and separated by electrophoresis in a 1% agarose gel. The methods for denaturation of the DNA and electrophoresis were described in Materials and Methods. The signs are the same as described in Figure 2, and 1× and 1/2× represent that the whole and the half standard amounts of UVR proteins were used, respectively.

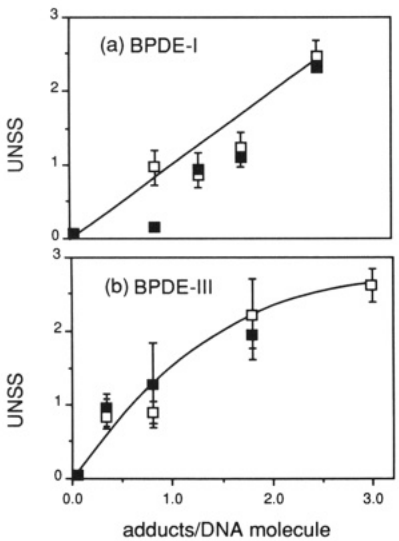

FIGURE 4: Quantitative correlation of the numbers of (a) BPDE-I and (b) BPDE-III modifications and the numbers of UVRABC nuclease incisions. The numbers of UVRABC nuclease incisions were calculated on the basis of the Poisson distribution equation and the fraction of DNA in CCC form in Figure 2 or the fraction of DNA that was full length in Figure 3. Symbols: (□) supercoiled DNA, (■) linear DNA, and (UNSS) UVRABC nuclease sensitive site. Each point is an average of two experiments, and the error bars of the measurement are presented.

The major role of *uvr* gene products in excision repair is making specific incisions on both the 5′ and 3′ sides of the damaged bases, facilitating their removal and subsequently allowing the repair synthesis mechanism to proceed (for review, see Sancar & Sancar, 1988; Grossman & Yeung, 1990; Van Houten, 1990). One possible explanation for the results shown in Figure 1 is that the *uvr* gene products may repair BPDE-I– and BPDE-III–DNA adducts with quantitative (rate and/or efficiency) and/or qualitative differences (such as positions of incisions). The following experiments were designed to determine the efficiency, rate, and mode of UVRABC nuclease in incising BPDE-I– and BPDE-III–DNA adducts.

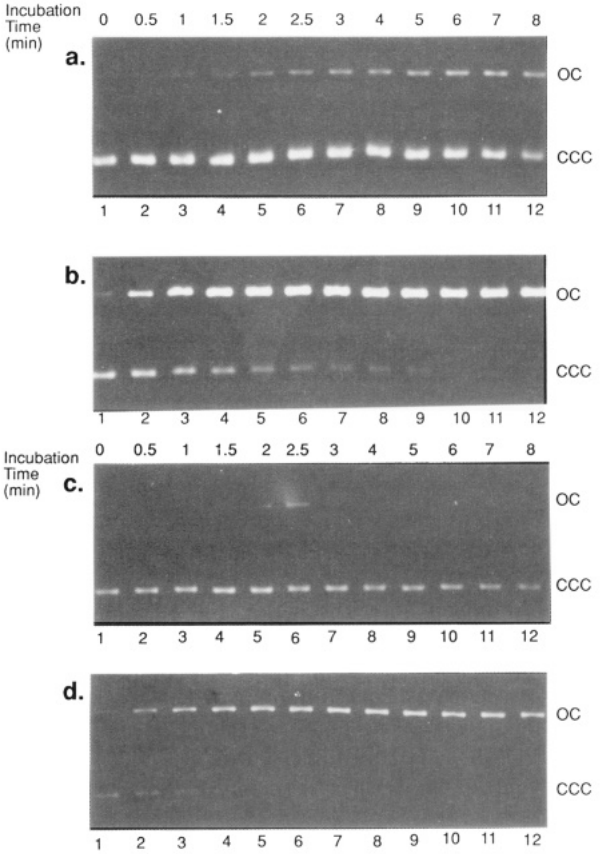

FIGURE 5: Time course of UVRABC nuclease incision on (a) and (c) mock, (b) BPDE-I modified and (d) BPDE-III modified supercoiled $\phi X174$ RF DNA. The supercoiled DNAs modified with 1.2 BPDE-I adducts or 1.0 BPDE-III adducts per DNA molecule, or unmodified DNAs were treated with UVRABC nuclease at 37 °C for 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 min (lanes 1–12). DNAs in (a) were BPDE-I mock treated and DNAs in (c) were BPDE-III mock treated. After removal of the UVR proteins by phenol extractions, the DNAs were electrophoresed in a 1% agarose in TAE buffer. OC, open circular DNA; CCC, covalently closed circular DNA.

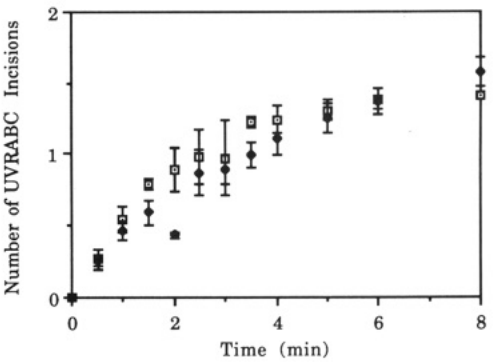

FIGURE 6: The rate of UVRABC nuclease incision on BPDE-I (□) and BPDE-III (♦) modified supercoiled form DNA. The intensity of the DNA in CCC and OC bands in Figure 5 was measured by densitometric scanning, and the calculations of the number of UVRABC nuclease incisions was the same as described in Figure 4. The results are averaged from three experiments, and the bars represent the standard errors.

Efficiency and Rate of UVRABC Nuclease Incision on BPDE-I or BPDE-III Modified DNA. In all the UVRABC incision experiments the UVRABC nuclease/DNA adduct ratio was maintained larger than 1. This is critical for

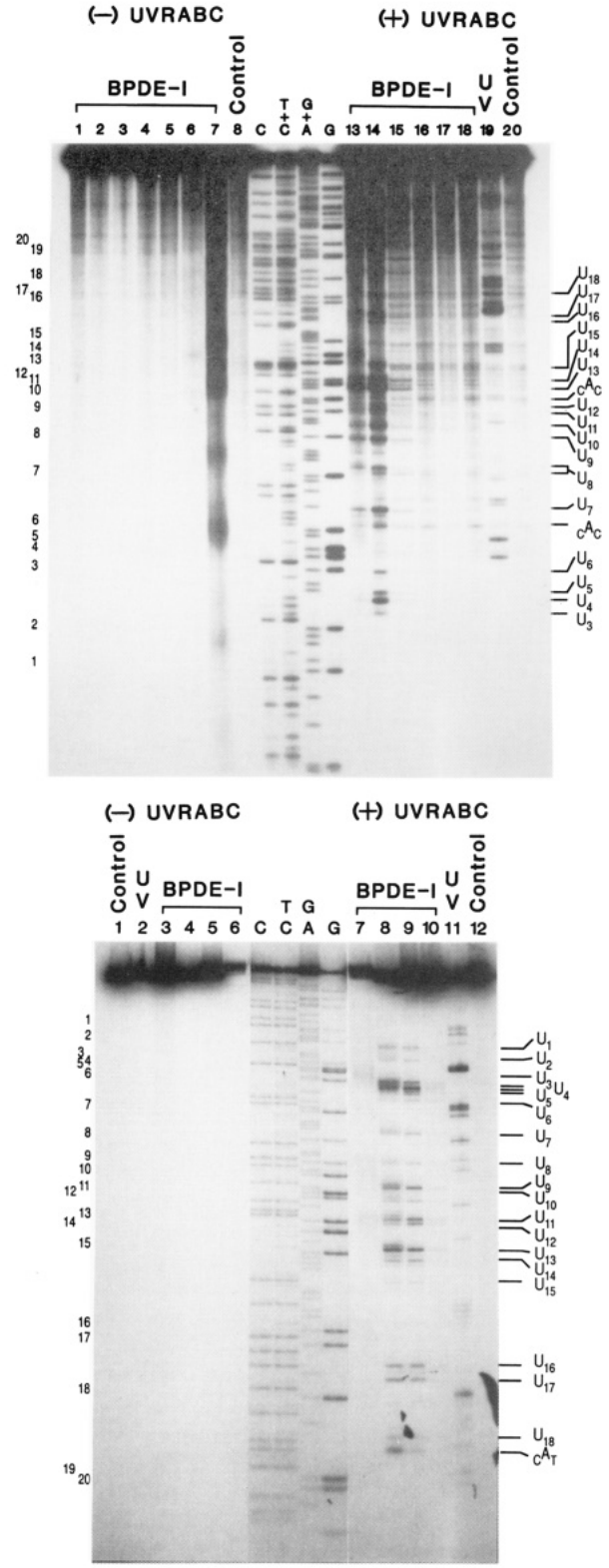

FIGURE 7: Identification of UVRABC nuclease incision sites on BPDE-I modified (a, top) 5′-end-labeled 174-bp *Eco*RI-*Hae*III and (b, bottom) 3′-end-labeled *Eco*RI-*Bst*NI 129-bp DNA fragments of pBR322. In (a) the DNAs in lanes 1 and 18, 2 and 17, 3 and 16, 4 and 15, 5 and 14, 6 and 13, 7, and 8 and 20 were modified with 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 0 mg/mL of BPDE-I, respectively. In (b) the DNAs in lanes 6 and 7, 5 and 8, 4 and 9, 3 and 10, and 1 and 12 were modified with 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 0 mg/mL BPDE-I, respectively. DNAs in lanes 19 in (a) and lanes 2 and 11 in (b) were irradiated with UV. (+) and (−) UVRABC represent that DNA was treated with or without UVRABC nuclease. The BPDE–DNA adduct induced UVRABC incision bands depicted as U_1 to U_{18} in the right side of the panel correspond to the guanine residue G_1 to G_{18}, respectively. The UVRABC incision induced by BPDE–adenine adducts were depicted by -$_C$A$_T$- and -cAc- signs which include the neighbor bases of modified adenine residues.

determining the efficiency of UVRABC in incising DNA adducts since it has been found that in the absence of repair synthesis and the subsequent DNA ligation steps the UVRABC nuclease has negligble turnover rate or does not turnover at all (Husain et al., 1985; Caron et al., 1985). To quantify the efficiency of the *uvr* gene products in incising BPDE-I– and BPDE-III–DNA adducts in supercoiled DNA, *ϕX174* RFI DNA modified with BPDE-I or BPDE-III was reacted with optimal amounts of UVRA, UVRB, and UVRC proteins at 37 °C for 60 min. Under these conditions the UVRABC nuclease incision reaches plateau levels. The resultant DNAs were then separated by neutral agarose gel electrophoresis, and the results in Figure 2 show that UVR proteins, singly or pairwise, cannot incise the BPDE-modified *ϕX174* RF DNA, but that all three proteins are needed to incise either BPDE-I– or BPDE-III–DNA adducts in supercoiled DNA. For both isomers, the degree of UVRABC incision is dependent on the extent of BPDE modification. It is worth noting that the BPDE-III modification process produces a small amount of nicking in DNA, and the extent of the nicking is proportional to the concentration of BPDE-III (Figure 2b, lanes 9–13). Although no detectable DNA nicking was observed in BPDE-I modification, the process of BPDE-I modification, however, produces site that are sensitive to UVRABC nuclease incision (Figure 2a, lane 14).

To quantify the efficiency of UVRABC nuclease in incising BPDE-I– and BPDE-III–DNA adducts from linear DNA, the BPDE-modified *ϕX174* RFI DNAs were restricted at the unique *Ava*I site, subsequently 3′-end ^{32}P labeled, and reacted with UVR proteins under the same conditions as those for supercoiled DNA. The results in Figure 3 show that the incision of BPDE–DNA adducts in linear DNA also requires UVRA, UVRB, and UVRC to work together, and the degree of UVRABC incision for both BPDE-I– and BPDE-III–DNA adducts is dependent on the extent of modification.

The quantitative relations between BPDE-I and BPDE-III modification and UVRABC nuclease incision on both linear and supercoiled form DNA are shown in Figure 4. These data clearly demonstrate that UVRABC nuclease incises both BPDE-I– and BPDE-III–DNA adducts quantitatively regardless of whether the adducts are in linear or supercoiled DNA.

To compare the rates of UVRABC nuclease incision at BPDE-I– and BPDE-III–DNA adducts, *ϕX174* RFI DNAs modified to similar levels with BPDE-I or BPDE-III were reacted with enzymes for different periods of time. The electrophoretic separation of the resultant DNAs is shown in Figure 5, and the quantitation of the rate of incision is shown in Figure 6. These two figures demonstrate that the initial rates of UVRABC nuclease incision on BPDE-I and BPDE-III adducted DNA were very similar: 0.52 per min for the former and 0.45 per min for the latter.

Mode of Incision of BPDE-I– or BPDE-III–DNA Adducts in Linear DNA by UVRABC Nuclease. Since UVRABC nuclease incised both BPDE-I– and BPDE-III–DNA adducts quantitatively, we then investigated (1) whether the mode of incision on BPDE-I and BPDE-III modified DNA by UVRABC nuclease was the same and (2) whether there were any differences in sequence preferences for the BPDE-I– and BPDE-III–DNA binding. The *Eco*RI-*Hae*III 174-bp fragment and the *Eco*RI-*Bst*NI 129-bp fragment from pBR322 containing a 129-bp common sequence were 5′- and 3′-end-labeled, respectively, modified with different concentrations of BPDE-I or BPDE-III, and then reacted with UVRABC. The use of both 5′-end-labeled and 3′-end-labeled DNAs allowed us to determine the precise positions of UVRABC

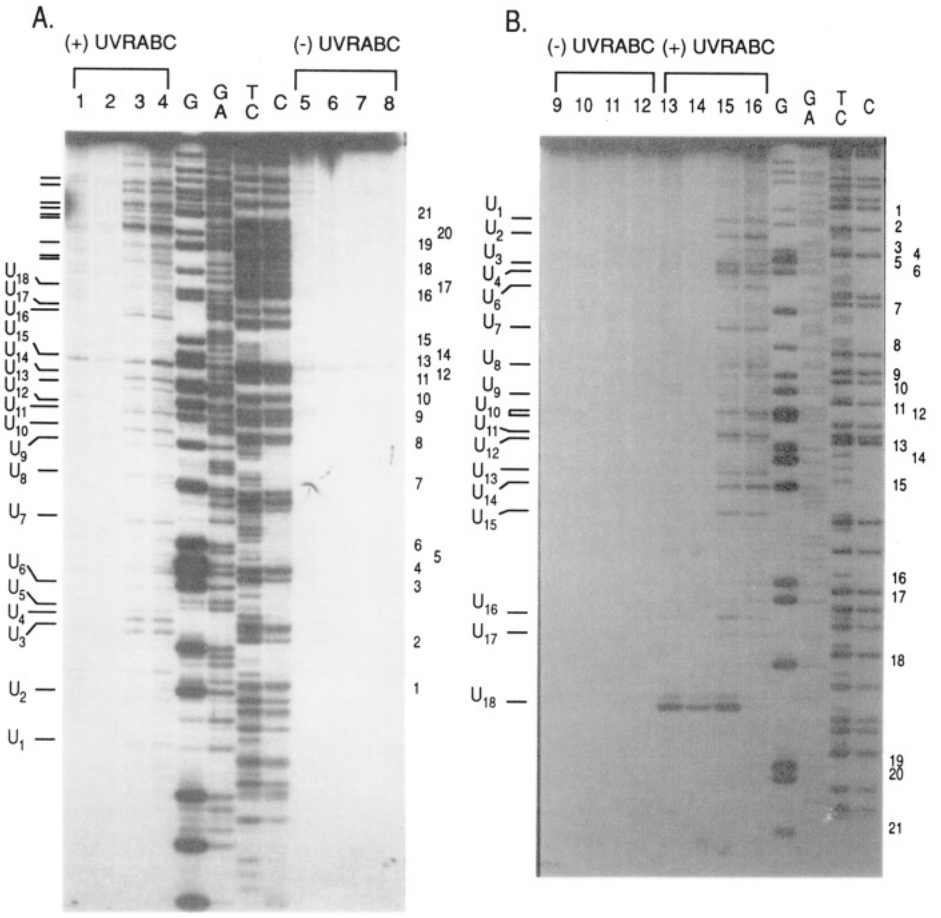

FIGURE 8: Identification of UVRABC nuclease incision sites on BPDE-III modified (A) 5′-end-labeled 174-bp *Eco*RI-*Hae*III and (B) 3′-end-labeled *Eco*RI-*Bst*NI 129-bp DNA fragments of pBR322. DNAs in lanes 1, 8, 9, and 13; 2, 7, 10 and 14; 3, 6, 11 and 15; and 4, 5, 12 and 16 were modified with 0, 0.2, 2, and 20 μM of BPDE-III, respectively. (+) and (–) UVRABC represent that DNA was treated with or without UVRABC nuclease. The BPDE–DNA adduct induced UVRABC incision bands depicted as U_1 to U_{18} in the left side of each panel correspond to the guanine residue G_1 to G_{18}, respectively.

nuclease incision 5′ and 3′ relative to a modified base, respectively. Figure 7a shows the UVRABC nuclease incision bands in the 5′-end-labeled BPDE-I modified DNA; there are 17 bands that can be attributed to UVRABC nuclease incision six or seven bases 5′ to a guanine residue and two bands that can be attributed to enzyme incision six to seven bases 5′ to an adenine position.

Results in Figure 7b show the UVRABC nuclease incision bands in the 3′-end-labeled BPDE-I modified DNA; again, there are 19 bands that can be attributed to UVRABC nuclease incision four to five bases 3′ to a guanine position. However, no band can be attributed to UVRABC enzyme incision three to five bases 3′ to any adenine position. Figure 7 shows that all guanine residues (17) but only 3 out of 22 adenine residues in the readable region of the gel have corresponding UVRABC nuclease incision bands although the intensity of these bands varies significantly.

These results suggest that all the guanines but not all the adenines are potentially modifiable by BPDE-I and there are sequence preferences of BPDE-I modification. The UVRABC nuclease incises at six or seven bases 5′ to and four or five (mainly four) bases 3′ to a BPDE-I modified guanine. However, for three BPDE-I modified adenine residues, only 5′ incisions by UVRABC nuclease were detected.

Results in Figure 8A show the UVRABC nuclease incision bands in the 5′-end-labeled BPDE-III modified DNA; we identified 18 bands that can be attributed to UVRABC nuclease incision at six or seven bases 5′ from a guanine residue. The intensity of the UVRABC incision bands also varies significantly at different sequences.

Results in Figure 8B show the UVRABC nuclease incision bands in the 3′-end-labeled BPDE-III modified DNA; we identified 18 bands that can be attributed to UVRABC nuclease incision at four or five bases 3′ from a guanine residues. Similar to the results obtained from 5′-end-labeled DNA, the intensity of the UVRABC incision bands also varies at different sequences. No significant enzyme incision bands are found to correspond to guanine at positions 5 and 9.

Relative Binding Affinity of BPDE-I and BPDE-III at Different Sequences Detected by the UVRABC Nuclease Incision Method. Since the results from Figures 3 and 4 demonstrate that UVRABC nuclease incises both BPDE-I– and BPDE-III–DNA adducts quantitatively, it is reasonable to conclude that the intensity of the UVRABC nuclease incision bands reflects primarily the level of BPDE modification at different sequences rather than the efficiency of enzymatic cutting at different adduct-containing sequences. Hence, the relative preference of BPDE–DNA binding at different sequences can be determined by the UVRABC incision methods. The intensity of UVRABC incision bands in BPDE-I and BPDE-III modified 129-bp DNA fragments shown in Figures 7 and 8 were scanned, and the relative intensities of each band are shown in Figure 9. The DNA binding of both BPDE isomers shows significant sequence selectivity, and BPDE-I shows more selectivity than BPDE-III.

For BPDE-I adducted DNA, in general, the guanine positions that render a strong 5′ UVRABC incision band also render a strong 3′ incision band. However, there are a few exceptions: at G15, BPDE-I modification induces a strong

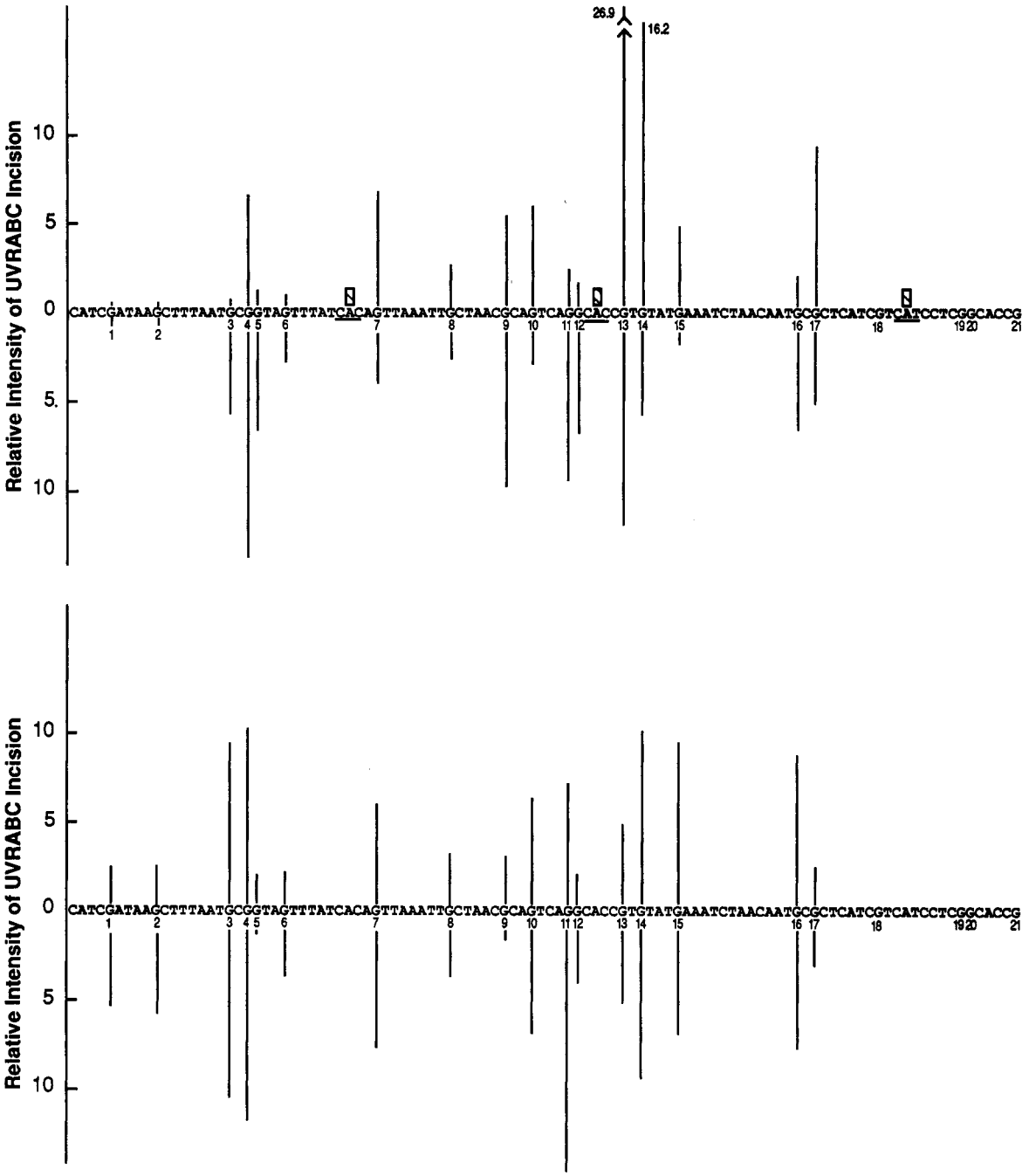

FIGURE 9: Relative intensity of UVRABC nuclease incision on the 5′ (upper) and 3′ (lower) sides of (a, top) BPDE-I– and (b, bottom) BPDE-III–DNA adducts in *Eco*RI-*Bst*NI 129-bp sequence of pBR322 DNA. The intensity of UVRABC nuclease incision bands in Figures 8 and 9 was measured by desitometric scanning. The quantitation was described in Materials and Methods.

UVRABC cut on the 5′ side but only a weak cut on the 3′ side; on the other hand, at G3, G5, and G12, BPDE-I modifications induce strong UVRABC 3′ cuts but weak 5′ cuts. For BPDE-III adducted DNA the intensities of the 5′ and 3′ cuts flanking each G residue were also similar except for G5 and G9 residue.

DISCUSSION

We have presented evidence that purified UVRABC nuclease incises DNA damage produced by two isomeric diol epoxides, BPDE-I and BPDE-III, not only quantitatively but also at the same rate and with the same mode of cutting. Paradoxically these two kinds of DNA adducts have very different effects on the transfectivity of *ϕX174* RF DNA: first, the BPDE-III–DNA adducts are much less genotoxic than BPDE-I–DNA adducts, and second, the BPDE-I modifications cause a much larger difference in transfectivity between wild type and *uvr*⁻ mutant cells than do BPDE-III–DNA adducts.

Our previous studies (MacLeod & Tang, 1985) indicated that most BPDE-I–DNA adducts derive from deoxyguanosine in both linear salmon DNA and supercoiled *ϕX174* RF DNA; a minor fraction (5%) of the total adducts is derived from deoxyadenosine. HPLC analysis of BPDE-III–deoxyribonucleoside adducts is hampered by our lack of radioactivity-labeled BPDE-III. However, in preliminary studies using fluorescence detection we have found that the major BPDE-III adduct is apparently a deoxyguanosine derivative, with lower levels of deoxyadenosine adducts presented. Therefore, it is unlikely that the different lethality of BPDE-I and BPDE-III resulted from different modified bases but rather is likely due to the stereostructural differences of the DNA adducts induced by these two isomers. Evidence from fluorescence quenching (MacLeod et al., 1982) and linear dichroism experiments (N.E. Geacintov & M. C. MacLeod, unpublished results) demonstrates that the DNA adducts formed by these two isomers have distinctly different impacts on helix structure,

and this difference may consequently result in various effects on DNA replication and RNA transcription. Furthermore, the DNA adducts may have different efficiencies of being recognized and repaired.

We propose two possibilities to account for the relatively low lethality of BPDE-III–DNA adducts in mutants of *E. coli* that lack the *uvr* excision repair system. One possibility is that in addition to the *uvr* system there exists another efficient repair pathway for these DNA adducts in *E. coli* cells. The high lethality of BPDE-I- and BPDE-II–DNA adducts in the *uvr*⁻ cells suggests that these adducts would not be substrates for the postulated alternative repair pathway. The second possibility is that BPDE-III–DNA adducts are less efficient than BPDE-I–DNA adducts in blocking DNA replication and RNA transcription and are therefore intrinsically less lethal to $\phi X174$. BPDE-I–DNA adducts have been shown to block DNA replication efficiently (Moore et al., 1981; Brown & Romano, 1991), but the effects of BPDE-III–DNA adducts are not known. Determination of the effects of BPDE-III–DNA adducts on the effects of DNA replication is important because if both kinds of DNA adducts block DNA synthesis equally, then the possibility that there is a new pathway for repairing bulky chemical–DNA adducts that intercalate in the DNA helix becomes prominent.

Although in general the BPDE modification induces relatively equal UVRABC incisions at both the 5′ and 3′ sides of guanines base, there are exceptions at several positions, such as G3, G5, G10, G12, and G15 in BPDE-I modified DNA and G5 and G9 in BPDE-IIII modified DNA. No visible bands are found that can be attributed to UVRABC incision at the 3′ side of a adenine position, although there are three bands which correspond to enzyme incisions at the 5′ side of a adenine. These results suggest that although in most sequences UVRABC nuclease incises at both the 5′ and 3′ sides of a BPDE-modified base, at some sequences the enzyme only incises at one side of the damaged base. It is likely that the conformation of BPDE–DNA adducts at different sequences may vary and consequently affect the protein binding or the processing of the damaged DNA region by the UVR proteins.

The majority of the UVRABC nuclease incision bands can be attributed to the chemical modification at guanine residues. However, there are three bands in BPDE-I modified DNA that correspond to enzyme cutting at two -CAC- and one -CAT- sites. These results are consistent with our HPLC adduct analysis which shows that the bases modified by BPDE-I are mainly guanines (95%) and adenines (5%). It is worth noting that the two BPDE-I–adenine bonding sequences we identified are -CAC- sites, and this sequence has been found to be a hot spot for T→A transversion mutations induced by BPDE-I in the *lac I* gene in *E. coli* genes (Bernelot-Moens et al., 1990).

Although the results we obtained from the analysis of 129-bp fragments do not provide sufficient data to deduce the effect of DNA sequence on the BPDE-DNA binding, nonetheless it clearly demonstrates significant variation of BPDE binding at different sequences. It is likely that DNA with different conformations and DNA in vivo with or without proteins and other factors associated may have even more profound changes in BPDE binding. Since our results indicate that UVRABC nuclease incises BPDE-modified bases quantitatively, this enzyme can be used for unraveling the sequence selectivity of BPDE–DNA binding. This approach could be applied to the study of DNA damage and repair in cells or tissues of BP-treated animals.

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