Recognition and Repair of 2-Aminofluorene– and 2-(Acetylamino)fluorene–DNA Adducts by UVRABC Nuclease†

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ABSTRACT: Recognition of damage induced by *N*-hydroxy-2-aminofluorene (*N*-OH-AF) and *N*-acetoxy-2-(acetylamino)fluorene (NAAAF) in both φX174 RFI supercoiled DNA and a linear DNA fragment by purified UVRA, UVRB, and UVRC proteins was investigated. We have previously demonstrated that *N*-OH-AF and NAAAF treatments produce *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF) and *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene (dG-C8-AAF), respectively, in DNA. Using a piperidine cleavage method and DNA sequence analysis, we have found that *all* guanine residues can be modified by *N*-OH-AF and NAAAF. These two kinds of adducts have different impacts on the DNA helix structure; while dG-C8-AF maintains the anti configuration, dG-C8-AAF is in the syn form. φX174 RF DNA–*Escherichia coli* transfection results indicate that while the *uvrA*, *uvrB*, and *uvrC* gene products are needed to repair dG-C8-AAF, the *uvrC*, but not the *uvrA* or *uvrB* gene products, is needed for repair of dG-C8-AF. However, we have found that in vitro the UVRA, UVRB, and UVRC proteins must work in concert to nick both dG-C8-AF and dG-C8-AAF. In general, the reactions of UVRABC nuclease toward dG-C8-AF are similar to those toward dG-C8-AAF; it incises seven to eight nucleotides from the 5′ side and three to four nucleotides from the 3′ side of the DNA adduct. Evidence is presented to suggest that hydrolysis on the 3′ and 5′ sides of the damaged base by UVRABC nuclease is not simultaneous and that at least occasionally hydrolysis occurs only on the 3′ side or on the 5′ side of the damage site. The possible mechanisms of UVRABC nuclease incision for AF-DNA are discussed.

2-(Acetylamino)fluorene (AAF) is a model carcinogen shown to cause liver and bladder carcinomas in both mice and rats. It is believed that the damage to DNA by this agent triggers carcinogenesis (Miller, 1978; Kriek & Westra, 1979). Three types of bulky adducts have been detected in DNA of animals fed AAF (Miller & Miller, 1976; Kriek, 1972; Kriek et al., 1967). The most prevalent adduct is the deacetylated form, *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF).[1] Two other adducts are 3-(deoxyguanosin-N^2-yl)-2-(acetylamino)fluorene (dG-N2-AAF) and *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene (dG-C8-AAF). It is possible in vitro by use of various derivatives of aminofluorene to modify DNA to contain the dG-C8-AF adduct or the dG-C8-AAF adduct. The synthetic chemical *N*-hydroxy-2-aminofluorene (*N*-OH-AF) reacts specifically at the C8 of guanine to form dG-C8-AF, while *N*-acetoxy-2-(acetylamino)fluorene (NAAAF) reacts with DNA in vitro to form mainly dG-C8-AAF with a minor percentage of dG-N2-AAF (5%) (Tang & Lieberman, 1983).

Although the two C8 adducts seem similar with an extra acetyl group as the only difference in their structure, these two adducts have quite distinct effects on DNA structure and replication. Molecular modeling results have shown that the acetylated form, dG-C8-AAF, assumes the syn conformation because of steric interference by the extra acetyl function (Fuchs & Daune, 1974; Hingerty & Broyde, 1982; Grunberger & Weinstein, 1978). This can cause the bulky (acetylamino)fluorene to swing into the base-pairing region of the helix, interrupting base pairing over a region of several base pairs. In contrast, dG-C8-AF is believed to have less effect on the secondary structure of the helix since the bulky adduct may lie outside the base-pairing region in the major groove as the guanine to which it is attached can assume the normal anti structure (Lipkowitz et al., 1982; Leng et al., 1980). Consistent with these molecular models are the results of S1 nuclease assays on *N*-OH-AF- or NAAAF-modified DNA with AF-adducted DNA being less sensitive to S1 than AAF-adducted DNA (Fuchs, 1975; Kriek & Spelt, 1979). These two kinds of DNA adducts tend to stall DNA replication at different positions; moreover, AAF adducts appear to stop DNA replication more efficiently than AF adducts (Moore et al., 1981; Michaels et al., 1987).

The excision repair mechanisms for AF– or AAF–DNA adducts apparently share to a certain extent the same pathway with the excision repair pathway of ultraviolet light (UV)–DNA damage; both eukaryotes and prokaryotes that are sensitive to UV irradiation are also sensitive to AF or AAF damage (Maher et al., 1975; Thompson et al., 1983; Tang et al., 1982). In *Escherichia coli*, three genes—*uvrA*, *uvrB*, and *uvrC*—control the initial step of repairing the DNA damage induced by bulky chemicals like AAF, *cis*-platinum, and psoralen, and by UV radiation. It has been shown that in vitro these three gene products—UVRA, UVRB, and UVRC—act in concert to excise 12–13 nucleotides of the damaged region of DNA (Sancar & Sancar, 1988). However, using the φX174 RF DNA–*E. coli* transfection assay we have found that only *uvrC* mutants display heightened sensitivity to dG-C8-AF,

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[1] Abbreviations: UV, ultraviolet light; NAAAF, *N*-acetoxy-2-(acetylamino)fluorene; *N*-OH-AF, *N*-hydroxy-2-aminofluorene; dG-C8-AAF, *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene; dG-C8-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; dG-N2-AAF, 3-(deoxyguanosin-N^2-yl)-2-(acetylamino)fluorene; RFI, replicative form I; bp, base pairs, DTT, dithiothreitol; ATP, adenosine triphosphate; HPLC, high-performance liquid chromatography; dTTP, thymidine triphosphate; TFA, trifluoroacetic acid; SDS, sodium dodecyl sulfate.

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while *uvrA*, *uvrB*, and *uvrC* mutants are all markedly more sensitive than wild-type cells to dG-C8-AAF. Moreover, we have shown that dG-C8-AF is about 10 times less lethal than dG-C8-AAF (Tang et al., 1982). These results suggest that in vivo the *uvr* system is more critical for the repair of dG-C8-AAF than for dG-C8-AF. This also implies that repair of dG-C8-AAF requires the products of the *uvrA*, *uvrB*, and *uvrC* genes while repair of dG-C8-AF adducts can occur in the absence of fully functional *uvrA* and *uvrB* gene products.

In this paper we present a biochemical study of the role of the *uvr* gene products in repairing these two kinds of adducts. We have found that the incision of supercoiled ϕX174 RFI DNA containing either dG-C8-AAF or dG-C8-AF requires purified UVRA, UVRB, and UVRC proteins. Using ^{32}P-end-labeled defined DNA fragments modified with *N*-OH-AF or NAAAF as substrate, we have found that the modes of incisions for both adducts by UVRABC nuclease are the same; it occurs mainly at the eighth or ninth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to the damaged base. The possible mechanisms of how these UVR proteins recognize and repair dG-C8-AF and dG-C8-AAF in vitro and in vivo are discussed.

Materials and Methods

Materials. *N*-Hydroxy-2-aminofluorene (*N*-OH-AF) and *N*-acetoxy-2-(acetylamino)fluorene (NAAAF) were obtained from the National Cancer Institute Carcinogen Repository and a gift from Dr. Fredric A. Beland, National Toxicological Center, Little Rock, AR. Restriction enzymes *Hae*III, *Bam*HI, and *Eco*RI, T4 polynucleotide kinase, acrylamide, bis(acrylamide), agarose, and NACS Prepacs convertible columns (NACS PACS) were obtained from Bethesda Research Laboratories. Restriction enzyme *Bst*NI was obtained from New England Biolabs. Bacterial alkaline phosphatase and yeast tRNA were obtained from Sigma Chemical Co. All ^{32}P-labeled nucleotides were obtained from Amersham Co.

DNA Fragment Isolation. Plasmid pBR322 was isolated and purified by cesium chloride density gradient centrifugation. The 174-bp *Eco*RI–*Hae*III 5′-end-labeled fragment was prepared by digesting pBR322 with *Eco*RI and *Bam*HI and resolving the resulting 375-bp fragment on a 1% agarose gel. Care was taken during this procedure not to expose the fragment to UV light to prevent any unwanted damage to the DNA. The DNA was then bound and eluted from a NACS PAC by the procedures recommended by the manufacturer to remove any contaminants introduced by the agarose. The eluate was ethanol precipitated, redissolved in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA (TE), and reprecipitated with 2.5 M ammonium acetate and ethanol. The DNA pellet was dried and redissolved in 50 mM Tris-HCl, pH 8.0, and 50 mM NaCl. Bacterial alkaline phosphatase was added, and the solution was incubated for 1 h at 60 °C. The reaction was stopped by phenol extraction followed by diethyl ether extraction. The DNA was precipitated with ethanol in the presence of sodium acetate (0.33 M), washed in 75% ethanol, and dried under vacuum. The dried pellet was redissolved in 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, and 5 mM dithiothreitol (DTT) and reacted with T4 polynucleotide kinase at 37 °C for 30 min in the presence of 50 μCi [γ-^{32}P]ATP. The reaction was terminated by ethanol precipitation in the presence of ammonium acetate. The labeled DNA was then digested with *Hae*III for 2 h at 37 °C and applied to a 5% acrylamide gel, where the 174-bp fragment was resolved from the free ATP and the two other resulting fragments. The fragment was electroeluted, cleaned on a NACS PAC, ethanol precipitated, and dried.

The 129-bp 3′ end fragment was isolated from a 1857-bp *Bst*NI fragment of pBR322 purified on a 1% agarose gel. The 1857-bp fragment was then reacted for 20 min at 25 °C with 3 units of Klenow fragment of DNA polymerase in the presence of 10 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 7.5 mM DTT, and approximately 50 μCi of [α-^{32}P]dTTP. The labeled DNA was then phenol extracted, diethyl ether extracted, and ethanol precipitated. The DNA was then digested with *Eco*RI for 2 h and applied to a 5% acrylamide gel from which the resulting 129-bp singly 3′-end-labeled fragment was isolated as was done for the 174-bp 5′ end fragment. The 174-bp 5′-end-labeled fragment and the 129-bp 3′-end-labeled fragment contain the same sequence of DNA from the unique *Eco*RI site to the *Bst*NI site at base 129. Thus, by use of these two labeled fragments, hydrolysis on either the 3′ or 5′ side of a damaged base could be estimated.

N-OH-AF and NAAAF Modification of DNA. *N*-OH-AF was dissolved in argon-purged absolute ethanol to a concentration of 1 mg/mL. DNA to be modified was dissolved in 100 μL of TE to which 100 μL of 0.02 M sodium acetate, pH 5.5, and 20 μL of the *N*-OH-AF solution was added, and the resulting solution was incubated at 25 °C for 3 h. Unreacted *N*-OH-AF was removed by extensive diethyl ether extractions. The modified DNA was then ethanol precipitated, washed with 75% ethanol, and dried under vacuum. This modification condition produced 0.1–0.3 AF adduct per 174-bp fragment.

NAAAF was dissolved in dimethyl sulfoxide to a concentration of 1 mg/mL and stored at −20 °C. DNA to be modified was dissolved in 100 μL of TE to which 100 μL of 0.02 M sodiuum acetate, pH 5.5, and 270 μL of the NAAAF solution were added, and the resultant solution was incubated for 3 h at 25 °C. Unreacted NAAAF was removed by extracting extensively with diethyl ether. The modified DNA was then ethanol precipitated, washed with 75% ethanol, and dried under vaccum. This modification condition produced about ≥1 AAF adduct per 174-bp fragment. For UV irradiation, the end-labeled DNA fragments in TE were irradiated with 254-nm UV at a fluence rate of 10 J M^{-2} s^{-1} to produce about 0.1–1 cyclobutane dipyrimidine per DNA molecule.

Piperidine Hydrolysis and High-Performance Liquid Chromatography (HPLC) Analysis of N-OH-AF- and NAAAF-Modified DNA. DNA was dissolved in 100 μL of 1 M piperidine and incubated for 30 min at 90 °C in 1.5-mL screw-capped microfuge tubes. After incubation, the DNA was transferred to a new tube, ethanol precipitated in the presence of 100 μg of yeast tRNA and ammonium acetate, and dried extensively under vacuum. Twice 10 μL of H_2O was added to each tube and dried again under vacuum.

HPLC analysis was performed according to the method of Tang and Lieberman (1983). Modified DNA was solvolyzed in trifluoroacetic acid (TFA) and analyzed on a C18 column with a gradient of 21–70% methanol. Fractions were counted in a scintillation counter to identify fractions with ^{3}H-labeled adduct.

UVRABC Nuclease Reactions. UVRA, UVRB, and UVRC proteins were purified from *E. coli* K12 strain CH296 carrying plasmids pUNC45 (*uvrA*), pUNC211 (*uvrB*), or pDR3274 (*uvrC*) (Tang et al., 1988). These plasmids and strain CH296 were kindly provided by Dr. A. Sancar, University of North Carolina, Chapel Hill, NC.

Activity of the UVRABC proteins was measured as single strand nicking in ϕX174 RFI DNA damaged with various agents. An aliquot of 0.2 μg of DNA was reacted with 0.2 pmol of UVRA, 2.2 pmol of UVRB, and 0.22 pmol of UVRC for 90 min at 37 °C in 50 mM Tris-HCl, pH 7.5, 10 mM

$MgCl_2$, 100 mM KCl, 1 mM ATP, and 1 mM DTT. The reaction was stopped with 0.1% SDS and heating at 65 °C for 5 min. The DNA was then electrophoresed in TAE (40 mM Tris–acetate, pH 8.0, 1 mM EDTA) buffer on 1% agarose at 1 V/cm for 16 h and visualized with UV light by staining with ethidium bromide (0.5 μg/mL). Damage-specific nicking was detected by the difference in mobility of supercoiled φX174 RFI DNA and the slower migrating relaxed or linear forms of the DNA. The same reaction conditions were used for ^{32}P-labeled DNA, but the reaction was terminated by phenol extractions. The labeled DNA was then ethanol precipitated, dried, and dissolved in freshly deionized formamide and incubated at 37 °C for 30 min before it was applied to a sequencing gel and run in parallel with sequencing reactions. This treatment was sufficient to denature the DNA while no detectable hydrolysis was observed in the DNA at adduct sites.

Sequencing Reactions. Chemical sequencing was carried out as described by Maxam and Gilbert (1980) with the following modifications. The G+A reaction was performed by adding 25 μL of 88% formic acid to 20 μL of DNA dissolved in H_2O and incubating the reaction for 5–8 min. All modification reactions were ethanol precipitated in the presence of 2.5 M ammonium acetate and yeast tRNA (100 μg) and washed in 75% ethanol. After piperidine hydrolysis, all reactions were again ethanol precipitated, dried under vacuum, resuspended in 10 μL of H_2O, and dried again to remove the last traces of piperidine.

Results

Incision Assay on AF- or AAF-Modified φX174 RFI DNA. As described before, our previous viral–DNA-*E. coli* transfection results suggested that in vivo the *uvrA*, *uvrB*, and *uvrC* products are required for repair of dG-C8-AAF; however, the *uvrC*, but not the *uvrA* or *uvrB* gene product is involved in repair of dG-C8-AF (Tang et al., 1982). To investigate the role of the individual UVR proteins in repair of these two kinds of DNA adducts in supercoiled φX174 RFI DNA, we have reacted purified UVRA, UVRB, and UVRC proteins with modified DNA in the presence of Mg^{2+} and ATP. The results in Figure 1 demonstrate that the UVR proteins singly or in pairwise combinations cannot incise AF- or AAF-modified DNA or UV-irradiated DNA; these three proteins must work in concert to incise the modified φX174 RFI DNA. These results are consistent with the results of Fuchs and Seeberg (1984).

Sequence Effect on AF and AAF Adduct Formation in Linear DNA Fragments. Since dG-C8-AF and dG-C8-AAF have very different impacts on the DNA helix structure, it is possible that the DNA sequence may play an important role in the efficiency of adduct formation and repair. To address these two questions, we have modified restriction fragments from pBR322 DNA with *N*-OH-AF or NAAAF to produce dG-C8-AF or dG-C8-AAF, respectively. These modified fragments were then subjected to either piperidine hydrolysis or UVRABC nuclease treatment and subsequently analyzed in DNA sequencing gels to identify any sequence specificity in either adduct formation or repair. The types of adducts formed in modified DNA analyzed by HPLC were consistent with published results (Tang & Lieberman, 1983) that *N*-OH-AF modification produces solely dG-C8-AF while NAAAF modification produces 95% dG-C8-AAF and 5% dG-N2-AAF in DNA (Figure 2).

It has been shown that treatment of AF adducts with piperidine and heat results in strand scission at the 3′ phosphodiester bond of each modified deoxyguanosine, and when ^{32}P-end-labeled DNA with this treatment is analyzed in sequencing gels, bands corresponding to each modified deoxyguanosine are formed (Bichara & Fuchs, 1985; Bases et al., 1983). The intensity of each band should, therefore, be proportional to the degree of modification by the chemical. Whether or not piperidine treatment results in strand scission for AAF adducts is in dispute in the literature. Bichara and Fuchs (1985) failed to demonstrate strand scission in this manner and suggested that the acetylated adduct cannot undergo β elimination of the guanine base as the deacetylated adduct can. However, Bases et al. (1983), using the same piperidine treatment, demonstrated that strand scission does occur for AAF-adducted DNA. It is known that under basic conditions the AAF adduct can be deacetylated, yielding the AF adduct (Kriek & Westra, 1980). It is therefore reasonable to assume that deacetylation can occur in piperidine solutions because of the alkalinity of these solutions. Figure 2 shows the DNA adduct analysis profile of NAAAF-modified DNA after piperidine treatment; the results demonstrate that a significant fraction of the AAF adducts are converted to the deacetylated form. Thus, AAF adducts containing DNA must be susceptible to piperidine cleavage of the phosphodiester

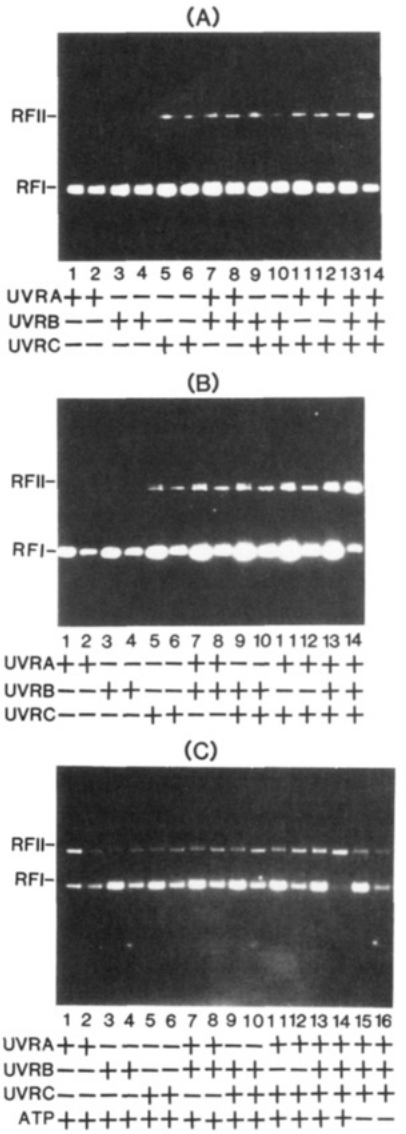

FIGURE 1: Reaction of UVRA, UVRB, and UVRC proteins with (A) UV-, (B) *N*-OH-AF-, and (C) NAAAF-modified φX174 RFI DNA. The proteins were added singly or in combinations as indicated by the + and − signs. Reaction conditions are described in the text. Odd-numbered lanes are control (unmodified) DNA, and even-numbered lanes have DNA modified as described.

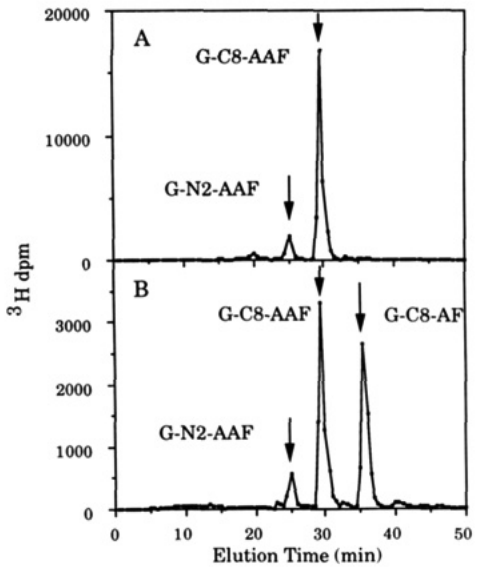

FIGURE 2: HPLC profiles of NAAAF-modified DNA digested in anhydrous TFA. Plasmid pBR322 DNA was modified with [^{3}H]-NAAAF, solvolyzed by TFA, and chromatographed as described in the text. The arrows indicate the positions of elution of the internal standards G-C8-AAF and G-C8-AF. The position of G-N2-AAF was calculated on the basis of its R_f relative to that of G-C8-AAF and G-C8-AF (Tang & Lieberman, 1983). (A) NAAAF-modified DNA; (B) NAAAF-modified DNA treated with 1 M piperidine for 30 min at 90 °C.

bond, and the bands observed on a sequencing gel resulting from piperidine cleavage of AAF-adducted DNA should be proportional to the degree of modification at the corresponding deoxyguanosine in the sequence. Figures 3 and 4 (lanes 10 and 13) show the results of sequence analysis of piperidine-treated *N*-OH-AF and NAAAF-modified DNA fragments. Since piperidine treatment produces bands corresponding to *every* deoxyguanosine position in the sequence of *N*-OH-AF and NAAAF-modified but not in nonmodified DNA, we conclude that every deoxyguanosine in the sequence is modified by either agent. No significant difference was observed in the intensities of the piperidine cleavage bands for AAF-modified DNA. In contrast, G82 and G84 show a 2-fold higher level of modification by AF than the other guanines in the sequence. These results suggest that in the sequence the "hot spots" for modification by these two agents are different.

Incision of dG-C8-AF and dG-C8-AAF in Linear DNA by UVRABC Nuclease. Since all guanines in the fragment appear to be modified by both *N*-OH-AF and NAAAF, we then investigated (1) whether the mode of incision on AF– and AAF–DNA adducts by UVRABC nuclease is the same and (2) whether there are sequence preferences for the recognition of AF– and AAF–DNA adducts by the UVRABC nuclease. The (*Eco*RI–*Hae*III) 174-bp fragment and the (*Eco*RI–*Bst*NI) 129-bp fragment from pBR322 with a 129-bp common sequence were 5′ and 3′ end labeled, respectively, modified with *N*-OH-AF or NAAAF, and then reacted with UVRABC. The resultant DNA from these reactions was analyzed on sequencing gels. The results of these experiments are shown in Figures 3 and 4. As shown in Figure 3 the UVRABC nuclease produced strand breaks in 5′-end-labeled NAAAF- or *N*-OH-AF-modified DNA at seven or eight bases 5′ to all guanine residues (Figure 3, lanes 9 and 12). Two "extra" bands (positions 87 and 89) that do not correspond to the expected bands resulting from cleavage on the 5′ side of damaged bases are readily observed in the UVRABC-treated AF–DNA. These extra bands can be accounted for by assuming that UVRABC nicked only on the 3′ side of AF-damaged guanines

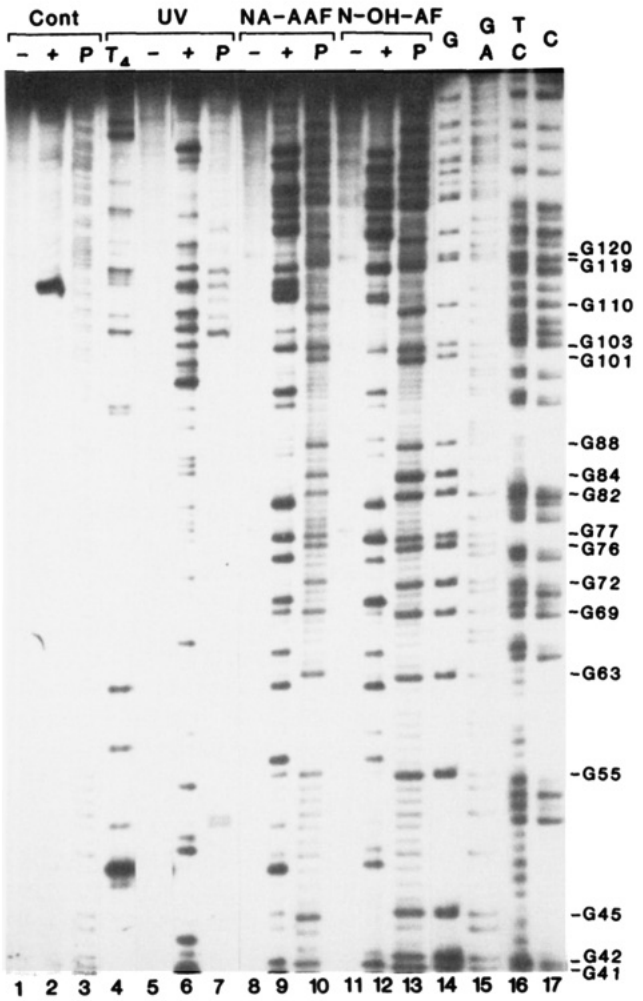

FIGURE 3: Electrophoresis of UVRABC nuclease and piperidine-treated *N*-OH-AF, NAAAF, and UV-treated 5′-end-labeled (*Eco*RI–*Hae*III) 174-bp fragment. The DNA fragments with or without modifications were incubated with UVRABC nuclease or piperidine as described in the text and then electrophoresed in an 8% polyacrylamide–urea sequencing gel. (Lanes 1–3) Mock modified DNA; (lanes 4–7) UV-irradiated DNA; (lanes 8–10) NAAAF-modified DNA; (lanes 11–13) *N*-OH-AF-modified DNA; (lanes 14–17) Maxam and Gilbert sequencing reactions; (lanes 1, 5, 8, and 11) no enzymes; (lanes 2, 6, 9, and 12) UVRABC treated; (lanes 3, 7, 10, and 13) piperidine treated; (lane 4) DNA treated with T4 endonuclease V.

at G82 and G84. These extra bands were also observed in UVRABC-treated AAF–DNA (lane 9), but they are less distinguishable compared to those observed in AF–DNA (lane 12). Other less intense bands (positions 49, 59, and 68) were also observed corresponding to apparent nicking on the 3′ side only of three guanines (G45, G55, and G72) in the sequence. These results indicate that, for a certain subpopulation of the DNA molecules, UVRABC may nick on only one side of the AF- and AAF-damaged guanines at certain positions. It is worth noting that these irregular UVRABC nuclease incisions on AAF–DNA were not observed by Sancar et al. (1985).

Figure 4 shows the results of 3′-end-labeled DNA with the aforementioned treatment; UVRABC nuclease produced breaks at three or four bases 3′ to all guanine residues (lane 9) in NAAAF-modified DNA. Similar results were observed in *N*-OH-AF-modified DNA with UVRABC nuclease (lane 12), producing breaks at three or four bases 3′ to all guanine residues with an exception. That is, there is no UVRABC incision band corresponding to guanine position 101. Nonetheless, an expected band that corresponds to a 5′ incision seven nucleotides from guanine 101 was observed on the 5′-end-la-

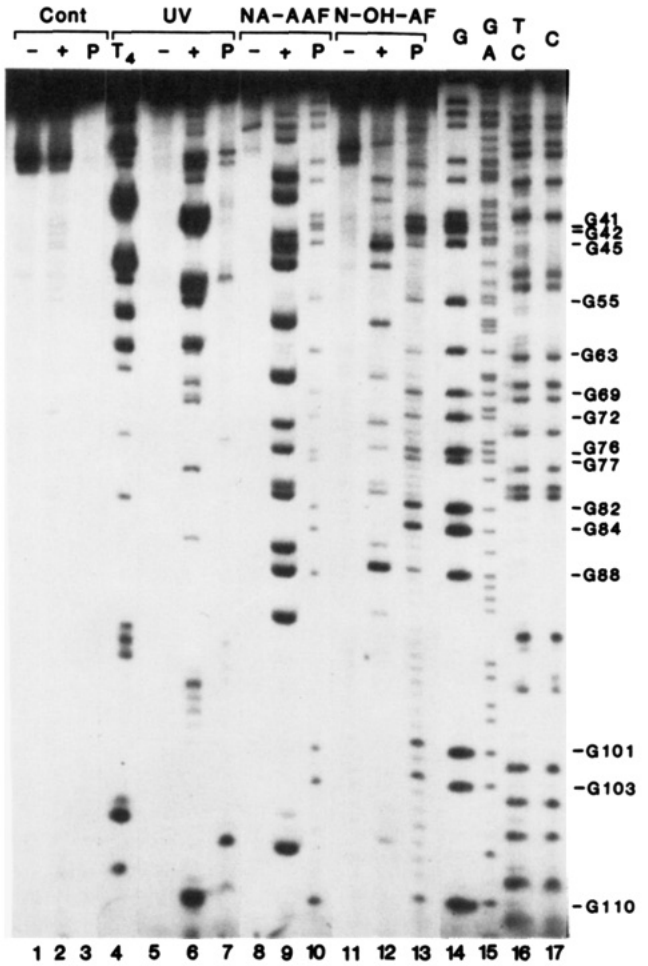

FIGURE 4: Electrophoresis of UVRABC nuclease and piperidine-treated *N*-OH-AF, NAAAF, and UV-treated 3′-end-labeled (*Eco*RI–*Bst*NI) 129-bp fragment. The DNA fragments with or without modification were incubated with UVRABC nuclease or piperidine as described in the text and then electrophoresed in an 8% polyacrylamide–urea sequencing gel. (Lanes 1–3) Mock unmodified DNA; (lanes 4–7) UV-irradiated DNA; (lanes 8–10) NAAAF-modified DNA; (lanes 11–13) *N*-OH-AF-modified DNA; (lanes 14–17) Maxam and Gilbert sequencing reactions; (lanes 1, 5, 8, and 11) no enzymes; (lanes 2, 6, 9, and 12) UVRABC treated; (lanes 3, 7, 10, and 13) piperidine treated; (lane 4) DNA treated with T4 endonuclease V.

beled DNA (Figure 3, lane 12, position 94). We conclude that UVRABC only cuts on one side (5′) of the AF damage at guanine position 101. Taken together, these data suggest that, in general, the mode of incision toward dG-C8-AF and dG-C8-AAF by UVRABC is the same; i.e., incisions occur at seven or eight nucleotides from the 5′ side and three or four nucleotides from the 3′ side of damaged bases. However, UVRABC may occasionally nick on only one side of some damaged bases. This occurs more often in AF–DNA than in AAF–DNA. Moreover, in the 5′-labeled *N*-OH-AF- and NAAAF-modified DNA we observed bands corresponding to 3′ cuts in addition to bands corresponding to 5′ cuts for some damaged guanines; we did not observe any extra bands in the 3′-labeled *N*-OH-AF- or NAAAF-modified DNA. One plausible explanation for these results is that the 5′ and the 3′ nicks are not simultaneous for dG-C8-AF or dG-C8-AAF at some sequences; incision at the 3′ side may occur before it occurs at the 5′ side, and it may occur with or without subsequent incision at the 5′ side of the adduct.

Figures 3 and 4 also show that UVRABC nuclease incises seven or eight bases 5′ and four or five bases 3′ to pyrimidine–pyrimidine sequences of UV-irradiated DNA fragments. This mode of UVRABC nuclease incision for UV-irradiated DNA is identical with Sancar's et al. result (1985). These results indicate that irregular UVRABC nuclease incisions on AF- or AAF–DNA we observed are unlikely due to any possible differences in our preparation of the UVR proteins.

Discussion

The *uvrA*, *uvrB*, and *uvrC* controlled excision repair system in *E. coli* is a versatile system; it repairs both helix destabilizing (Sancar & Sancar, 1988) and stabilizing DNA damage (Tang et al., 1988; Walter et al., 1988). Although the biochemical details of how these gene products recognize such a variety of DNA damage are not clear, it has been shown that in vitro these three gene products work together to "excise" a 12–13 nucleotide fragment including the damaged base(s) (Sancar & Rupp, 1983; Sancar et al., 1985). Previously, using a ϕX174 RFI DNA–*E. coli* transfection assay, we have found that the *uvr* genes function differently in repair of dG-C8-AF and dG-C8-AAF (Tang et al., 1982). While the repair of dG-C8-AAF or cyclobutane dipyrimidine requires functional *uvrA*, *uvrB*, and *uvrC* gene products, in contrast, the repair of dG-C8-AF requires only a functional *uvrC* gene. In this paper we have shown that the purified UVRC protein singly cannot incise dG-C8-AAF or dG-C8-AF; however, in combination with the UVRA and UVRB protein it can incise both kinds of adducts. Similar results have been reported by Fuchs and Seeberg (1984). These results raise a perplexing question: how does the *uvrC* gene product act in repairing dG-C8-AF? Although in vitro the UVR proteins work in concert in repairing different kinds of DNA damage in an apparently similar manner, in vivo UVRC still may be able to complex with factors other than UVRA and UVRB proteins to repair some types of DNA damage. There are at least two possibilities to reconcile these paradoxical in vitro and in vivo observations: (1) In vivo UVRC may complex with mutated UVRA or UVRB to form a functional nuclease for repair of dG-C8-AF but not for cyclobutane dipyrimidine and dG-C8-AAF damage. (2) In vivo UVRC may complex with a protein other than UVRA and UVRB for repair of dG-C8-AF.

Bichara and Fuchs (1987) have recently shown that *uvrAuvrC* double mutants have the same phenotype as *uvrA* rather than *uvrC* mutants in regard to repair of dG-C8-AF. Therefore, they have proposed that the UVRC protein can only complex with UVRA and UVRB in repair of dG-C8-AF. They also proposed that the defective UVRC protein can complex with UVRA and UVRB proteins and bind to dG-C8-AF but cannot repair the damage. Because of the defective function of the mutated UVRC protein, the UVRABC complex cannot be released from the DNA by the normal repair replication mechanism, which has been demonstrated to require helicase II and DNA polymerase I, and, therefore, acts as a block to DNA replication and, consequently, is a lethal event for phage DNA survival or plasmid DNA transformation. If Bichara and Fuch's hypothesis is correct, then we would expect that the dG-C8-AF adduct would be highly lethal in a *uvrC* cell. However, in comparison with dG-C8-AAF or cyclobutane dipyrimidine, dG-C8-AF is 10 times less lethal for ϕX174 RFI DNA transfectivity in *uvrC* mutants and in wild-type cells. It is possible that a defective UVRABC complex might have a lower affinity or bind more weakly to AF–DNA adducts than to AAF–DNA adducts. One plausible alternative explanation for their finding that *uvrAuvrC* double mutants have the same phenotype as *uvrA*, but not *uvrC*, mutants for repair of dG-C8-AF is that the defective UVRA and UVRC proteins can form a complex with the UVRB protein and repair dG-C8-AF, but not cyclobutane di-

pyrimidine or dG-C8-AAF adducts.

Although in general the UVRABC nuclease incises both dG-C8-AF and dG-C8-AAF in the same fashion, seven or eight nucleotides 5′ from and three to four nucleotides 3′ from the adducts, irregular incisions occur more often for AF–DNA than for AAF–DNA (Figure 3). These results indicate that the dG-C8-AF adducts in different sequences may have different effects on the helix structure and consequently may affect the mode of cutting by UVRABC nuclease. Three different models have been proposed for dG-C8-AF conformation: (1) The AF moiety is placed in the major groove of DNA helix, and the deoxyguanosine remains in the anti conformation (Evans et al., 1980). (2) AF is stacked with the neighboring base with the deoxyguanosine still in the anti conformation (Broyde & Hingerty, 1983). (3) AF is inserted into the DNA helix in the same conformation as dG-C8-AAF, thereby introducing significant denaturation as the AF adduct interacts strongly with the surrounding DNA bases (Spodheim-Maurizot et al., 1979). On the basis of spectroscopic results, van Houte et al. (1987) have concluded that "insertion" is the most abundant conformation of dG-C8-AF in double-stranded DNA. If this is the case in our *N*-OH-AF-modified linear DNA, then this may be the reason that in general the mode of incision by UVRABC nuclease for dG-C8-AF and dG-C8-AAF is the same. It is also possible that the mode of UVRABC nuclease incision may be different when the dG-C8-AF adducts are in a base stacking or outside-binding mode. This may explain the extra bands we observed in 5′-labeled *N*-OH-AF-modified DNA.

It is worth noting that by comparing the relative extent of UVRABC incision induced by AAF- or AF–DNA adducts at different sequences, we have found that at 6 of 11 sites the enzyme shows a 1.5–2.5-fold difference in recognition of these two kinds of adducts at the same sequence (Figures 3 and 4). The efficiency of enzyme recognition between the acetylated and the deacetylated aminofluorene–DNA adducts apparently is governed by the sequence surrounding the DNA adduct. These results are consistent with the notion that the DNA adducts at different sequences may vary in their effects on the helical structure. Further molecular modeling using various sequences adjacent to the DNA adduct may help to visualize this DNA sequence effect on the adduct conformation.

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