UvrABC Incision of N-Methylmitomycin A-DNA Monoadducts and Cross-links*

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The Escherichia coli UvrABC endonuclease is a multisubunit enzyme that initiates the repair of a wide variety of DNA lesions in vivo by making dual incisions on a damaged strand at the eighth or ninth phosphodiester bond 3' to the modified base. It has been hypothesized that UvrABC is able to recognize a broad spectrum of lesions because it does not recognize the lesion per se but rather gross helical distortions that the lesion induces in the DNA. Several lesions have recently been studied which are thermal stabilizing and are not believed to distort the DNA grossly, including the CC-1065-N-3-adenine and anthramycin-N-2-guanine adducts. We have studied the activity of UvrABC in vitro on another thermal stabilizing and nondistortive adduct, N-methylmitomycin A (NMA), a bifunctional DNA-alkylating agent that reacts with guanine on the side facing the minor groove, yielding either monoadducts or interstrand cross-links. NMA adducts increase the thermal stability of DNA, and theoretical calculations indicate that NMA adducts do not grossly distort the DNA helix. Our results show that UvrABC makes incisions at the eighth phosphodiester bond 5' and the fifth phosphodiester bond 3' to an NMA monoadduct, consistent with the incision pattern observed for the majority of other lesions that are also recognized by UvrABC. DNA containing a site-specific NMA cross-link was also recognized and incised by UvrABC. The rate of incision of NMA cross-linked DNA was about 200-fold higher in supercoiled molecules than in relaxed molecules, whereas the rate of incision of DNA containing NMA monoadducts was stimulated approximately 2-fold by supercoiling. The signal for UvrABC recognition and incision of damaged DNA is discussed in relation to the ability of UvrABC to incise NMA adducts as well as other nondistortive lesions.

* This work was supported by National Institutes of Health Grants GM01389 and CA39238. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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(Received for publication, January 25, 1989)
possibly MC cross-links, are substrates for UvrABC. In this
study, we have used a closely related, biologically more active
derivative of MC, N-methylmitomycin A (NMA; Fig. 1b) (Teng
et al., 1989), to generate both a nonspecifically modified
dNA substrate containing a mixture of monoadducts and
cross-links, and a site-specifically modified DNA molecule
containing a single NMA cross-link. These DNA substrates
were used to investigate the mode of action of UvrABC on
both types of NMA adducts in vitro. The effects of DNA
topology on UvrABC incision of NMA-modified DNA were
also examined.

MATERIALS AND METHODS

DNAs, Enzymes, and Reagents—DNA oligonucleotides were syn-
thesized on an Applied Biosystems DNA synthesizer and purified by
electrophoresis through 12 or 20% denaturing polyacrylamide gels.
pBR322 was isolated from E. coli strain DH5α by alkaline lysis,
equilibrium density centrifugation in a gradient of CaCl2, followed by
exclusion chromatography on Sepharose CL-4B (Pharmacia LKB
Biotechnology Inc.).

Restriction enzymes and T4 DNA ligase were purchased from New
England Biolabs and Promega Biotec. T4 polynucleotide kinase was
from IBI, and calf intestinal alkaline phosphatase and sequencing
grade E. coli polymerase large fragment (Klenow enzyme) were from
Boehringer Mannheim. Protease K, topoisomerase I, and DNA
gyrase were from Bethesda Research Laboratories. Unless otherwise
indicated, reaction conditions were those recommended by the manu-
facturer. UvrA, UvrB (Yeung et al., 1986), and UvrC (Sancar and
Rupp, 1983) were purified as described previously.

NMA, a gift from S. J. Danishefsky and M. S. Egbertson (Depart-
ment of Chemistry, Yale University), was dissolved in Me2SO to a
final concentration of 75 mM and stored at −20 °C for up to a month.
Na2S2O4 (Fisher) was dissolved in degassed water and used imme-
diately.

Denaturing and native polyacrylamide gels were prepared and run as
described elsewhere (Maniatis et al., 1982). DNA was recovered from
polyacrylamide gels by soaking overnight in 10 mM Tris-HCl, pH 8.0,
followed by purification on Nensorb-20 columns (Du Pont-New England Nuclear).

DNA-damaging Treatments—Photodimers were introduced into
dNA by irradiating samples with 254 nm UV light from a GE G15T8 germicidal lamp. The number of dimers formed was calculated using
7 J m−2 dimer−1 kilobase (Sancar and Rupp, 1983).

NMA lesions were introduced by treating DNA with NMA in the
presence of Na2S2O4. Tris-free DNA samples contained 20 mM potas-
sium phosphate, pH 7.5, 10% Me2SO, and 0.12-15 mM NMA. Before
adding Na2S2O4, the otherwise complete reaction mixture was flushed
with nitrogen gas for at least 1 min. A 4-fold excess of Na2S2O4
was then added. The bubbling of nitrogen gas was stopped 1 min
after the addition of Na2S2O4, and the reaction was allowed to
continue for another hour in tightly capped tubes. The entire reaction
was performed on ice. Unreacted NMA was removed by precipitating
the DNA in ethanol and washing the pellet thoroughly with 95%
ethanol.

Preparation of Nonspecifically Modified Plasmid DNA Substrates—
To introduce NMA lesions, a 98-μl sample containing 43 μg of
pBR322 was treated with 0.1 mM NMA and 4.6 mM Na2S2O4. Control
DNA (Na2S2O4-treated DNA) was prepared similarly, except that
Me2SO was added to the reaction in place of NMA. DNA containing
3 UV dimers/pBR322 molecule was prepared as described above.
After treatment, the plasmids were relaxed by incubating with calf
thymus topoisomerase I (Bethesda Research Laboratories) using the
reaction conditions described by the manufacturer. Aliquots were
subjected to electrophoresis through a 0.8% agarose gel to verify the
completeness of the reaction. Reaction mixtures were deproteinized

Fig. 1. Molecular structures of mitomycin C, N-methylmitomycin
A, and adducts with guanosine. a, mitomycin C; R1 = –NH2; R2 = –H; b, N-
methylmitomycin A; R1 = –CH2O; R2 = –CH3. c, MC monoadduct. d, MC
cross-link.
by incubation with proteinase K followed by phenol/chloroform extraction and precipitation in ethanol.

Preparation of Nonspecifically Modified Linear DNA Substrates—The 187-bp EcoRI/EcoRV fragment of pBR322 was labeled at the 3' end of the EcoRI site by incubating with [α-32P]dATP in the presence of dCTP, dGTP, and dTTP, and Klenow enzyme. To obtain the same 3' end-labeled 187-bp fragment of pBR322, pBR322 was digested with EcoRI, treated with calf intestinal alkaline phosphatase, and labeled with [γ-32P]ATP and T4 polynucleotide kinase. After precipitation in ethanol, the DNA was digested with EcoRV. Both the 5' and 3′ end-labeled 187-bp EcoRI/EcoRV fragments were purified by electrophoresis through an 8% native polyacrylamide gel. A 10-mg sample of each purified fragment was treated with 15 μM NMA in Me2SO. Control DNA was treated with 6 mM Na2S2O4 and Me2SO.

Positive control DNA containing photodimers was prepared by UV-irradiating 19 μg of each fragment with 110 J m⁻². The complementary oligonucleotides, oligo-1 and oligo-2 (see Fig. 5a), were phosphorylated at the 5' end with [α-32P]dATP and T4 polynucleotide kinase. After labeling, 19 nmol of oligo-1 was annealed to 19 nmol of oligo-2 by heating to 65°C and slowly cooling to 4°C. Reaction with NMA was conducted as described above in a final volume of 200 μl in the presence of 15 mM NMA and 60 mM Na2S2O4.

Construction of Site-specific NMA Cross-linked DNA Substrate—The cross-linked oligonucleotides, oligo-1 and oligo-2 (see Fig. 5a), were phosphorylated at the 5' end with [α-32P]dATP and T4 polynucleotide kinase. After labeling, 19 nmol of oligo-1 was annealed to 19 nmol of oligo-2 by heating to 65°C and slowly cooling to 4°C. Reaction with NMA was conducted as described above in a final volume of 200 μl in the presence of 15 mM NMA and 60 mM Na2S2O4. The cross-linked product was purified by electrophoresis through a 10% denaturing polyacrylamide gel (see Fig. 5b).

A 1497-bp circle containing a single site-specific NMA cross-link (pBR-XL) was constructed by ligation of the cross-linked oligonucleotides (pBR/AuNI fragment of pBR322 (see Fig. 5c)) into 30 pmol of the EcoRI/AuNI fragment was incubated with 30 pmol of cross-linked oligonucleotides in 1.5 ml of IHI ligase buffer containing 90 units of T4 DNA ligase. The covalently closed circular (CCC) product was isolated by equilibrium density centrifugation in a CsCl gradient. Fractions were collected from the gradient, and the peak of CCC DNA was located by running 5% of each fraction on a 1.5% agarose gel containing 1 μg/ml EtBr. The CCC peak was well separated from the open circular and linear DNA peak. Appropriate fractions were pooled and desalted by precipitation in ethanol. Undamaged circular control DNA, pBR-C, was constructed identically, except that 30 pmol of annealed untreated oligo-1 and oligo-2 were substituted for the cross-linked oligonucleotides.

UvrABC Digestion of DNA—Unless otherwise noted, UvrABC digestion were performed by incubating 5.3 pmol of UvrA, 4.5 pmol of UvrB, 2.1 pmol of UvrC, and DNA in 40 μl of buffer containing 150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 2 mM ATP, and 2 mM dithiothreitol at 37°C. For time course determinations, the mixture minus UvrC was prewarmed to 37°C for 1 min before UvrC was added to start the reaction. Aliquots were withdrawn at the appropriate time points and mixed with a 2-fold M excess of EDTA to stop the reaction (Sancar and Rupp, 1983).

RESULTS

UvrABC Nicking of NMA-modified Plasmids—To determine if DNA containing mitomycin adducts is a substrate for UvrABC in vitro, we compared the rate of cleavage of pBR322 modified by NMA with that of UV-irradiated or control samples, which consisted of unirradiated DNA or DNA treated with Na2S2O4 in the absence of NMA (Fig. 2). The Na2S2O4-treated control pBR322 was nicked by UvrABC at the same low level as the undamaged DNA; approximately 1 and 1.5% of the DNA was nicked/min for relaxed and supercoiled substrates, respectively (Fig. 2, A and B). In contrast, the NMA-modified pBR322 DNA was rapidly nicked by UvrABC, with 20% of the relaxed and 50% of the supercoiled DNA substrates becoming nicked in the 1st min of the reaction (Fig. 2A). These results are similar to those achieved with the UV-damaged DNA (Fig. 2B). Thus, DNA modified with NMA in the presence of Na2S2O4 is a substrate for the UvrABC endonuclease.

The rate of UvrABC nicking is independent of the number of lesions above a lesion density of approximately four DNA circle (Yeung et al., 1987). Although the exact number of NMA lesions/DNA circle is not known for the experiment shown in Fig. 2A, DNA damaged with a 20-fold higher concentration of NMA was incised at approximately the same rate (data not shown). Similarly, increasing the duration of UV irradiation 5-fold over that used in Fig. 2B did not change the rate of incision (data not shown). Thus, in each of the two experiments shown in Fig. 2, the concentration of lesions is sufficiently high that it does not affect the rate of UvrABC incision. UvrABC activity in vitro has been observed to be affected by supercoiling of the DNA substrate. The rate of incision of psoralen cross-links is highly dependent on supercoiling, whereas the rate of incision of anthramycin-modified DNA has been suggested to be inhibited by supercoiling (Walter et al., 1988). We investigated the dependence of UvrABC cleavage of NMA adducts on DNA topology by comparing the rate of cleavage of supercoiled and relaxed NMA-treated plasmids (Fig. 2). We found that the initial rate of cleavage of supercoiled plasmids was 2-fold higher than that of relaxed plasmids for NMA-treated DNA. A similar effect was observed for UV-irradiated DNA.

Sites of UvrABC Incision of a 187-Mer Nonspecifically Modified by NMA—The positions of the incisions made by UvrABC 3' and 5' to an NMA lesion were determined by incubating 3' or 5' end-labeled NMA-treated DNA samples with UvrABC, followed by separation of the products on high resolution denaturing polyacrylamide gels alongside the Maxam-Gilbert chemical cleavage products of the same DNA molecules (Maxam and Gilbert, 1980) (Fig. 3). We compared...
FIG. 3. Sites of UvrABC incision on the 187-bp EcoRI/EcoRV fragment of pBR322. The 187-bp EcoRI/EcoRV fragment of pBR322 was labeled at the 3' or the 5' end of the EcoRI site. The end-labeled fragment was treated with Na$_2$S$_2$O$_4$, alone or with Na$_2$S$_2$O$_4$ and 15 mM NMA or with UV (110 J m$^{-2}$). 200 ng of each DNA sample was then incubated with UvrABC for 15 min at 37°C. The reaction products were precipitated in ethanol and analyzed on an 8% denaturing polyacrylamide gel. Lengths of DNA fragments produced by UvrABC incision were determined by comparison with Maxam-Gilbert sequencing lanes of the same end-labeled DNA. Numbering of the top strand is 5' to 3' starting from the first T in the EcoRI recognition site of pBR322. Numbering of the bottom strand starts from the first A in the EcoRI recognition site of pBR322. The numbers for the bottom strand are primed to indicate that numbering is 3' to 5': a, UvrABC digestion of 5' end-labeled DNA. b, UvrABC digestion of 3' end-labeled DNA.

The mobilities of the UvrABC digestion products of NMA-treated or UV-irradiated DNA with the marker lanes as described previously (Sancar and Rupp, 1983; Royer-Pokora et al., 1981). The incision pattern observed for the UV-irradiated controls was consistent with previous results (Sancar and Rupp, 1983). All but one of the major products of UvrABC digestion of 3' end-labeled DNA containing NMA lesions migrated at a position corresponding to a fragment 4 nucleotides shorter than a guanine-terminating fragment in the sequencing lane of the same 3' end-labeled DNA fragment. Also, with just one exception, all of the major products of UvrABC digestion of the 5' end-labeled DNA containing NMA lesions migrated at a position corresponding to a fragment 6.5 nucleotides shorter than a guanine-terminating fragment in the corresponding DNA-sequencing lanes. None of the bands in the lanes containing NMA-modified DNA was observed in the control lanes containing untreated or Na$_2$S$_2$O$_4$-treated DNA. We conclude that the UvrABC digestion pattern for the NMA-treated EcoRI/EcoRV fragments is due to specific nicks made at the fifth phosphodiester bond 3' and the eighth phosphodiester bond 5' to NMA-guanine adducts, consistent with incision patterns observed with other adducts. The incision pattern for the NMA-modified DNA fragment is illustrated in Fig. 4.

Mitomycin treatment of random sequence DNA yields 10–20 times more monoadducts than cross-links (Iyer and Szybalski, 1964), and presumably the pattern of modification by NMA is similar. Guanine is the essential base for mitomycin-DNA interaction (Tomasz et al., 1974, 1986, 1987). Furthermore, NMA interstrand cross-linking has been shown to be sequence specific, occurring only at 5'-CpG sequences (Teng et al., 1989). We therefore expected that all guanines could potentially form NMA monoadducts and that some guanines in CpG sequences could also form cross-links. Thus, in the case of guanine residues not in CpG sequences, the lesion recognized by UvrABC must be a monoadduct. In the case of guanine residues within CpG sequences, it is not clear whether cross-linked DNA was incised by UvrABC because as shown below, the NMA cross-links in relaxed DNA are poorly incised by UvrABC.

Two anomalous incisions were observed: one between Gua$^{115}$ and Gua$^{116}$, 6 phosphodiester bonds 3' from a guanine residue, and the other between Gua$^{72}$ and Thy$^{73}$, either 5 or 10 phosphodiester bonds 5' from guanine residues. These products were dependent upon NMA treatment of the DNA, as they were not observed in either the untreated or UV-irradiated DNA. Since there is strong evidence that nearly all mitomycin DNA adducts occur at guanine residues (Tomasz et al., 1974), it is unlikely that UvrABC is responding in standard fashion to NMA modification of a base other than guanine. Although we cannot absolutely rule out the presence of minor adducts at bases other than guanine, these data indicate that UvrABC incises some NMA-guanine monoadducts in an atypical manner. Such anomalies are not unique to NMA adducts (Jones and Yeung, 1988; Myles et al., 1987).

We also found that not all guanine residues in the sequence had a corresponding UvrABC incision. This may be due to DNA sequence-dependent differences in the rate of incision by UvrABC or to differences in the reactivity of the drug to different sequences. Myles et al. (1987) have observed that
UV photoproducts at some sites were completely removed by UvrABC, whereas at other sites, only 10–15% of the photoproducts present were removed; similarly, in one study, only one of five CC-1065 adducts was incised by UvrABC, perhaps due to effects of local sequence on UvrABC recognition (Tang et al., 1988). Sequence-dependent reactivity has been demonstrated for many DNA-modifying agents such as UV (Brash and Haseltine, 1982), psoralens (Kanne et al., 1982), and N-acetoxy-2-acetylaminofluorene (Fuchs and Seeberg, 1984). Thus, we cannot distinguish these two possibilities with our current data.

Construction of a Circular DNA Substrate Containing a Single Site-specific NMA Cross-link—To show that UvrABC is able to initiate repair of NMA cross-links, we prepared a circular DNA substrate containing a single site-specific NMA cross-link. The two complementary oligonucleotides shown in Fig. 5a were annealed and treated with NMA. The cross-linked product (oligo-XL) was purified from unmodified and monoadducted oligonucleotides by electrophoresis through a denaturing polyacrylamide gel (Fig. 5b). Since each oligomer contained a single CpG sequence, the cross-linked oligomers were known to be modified at that site; since the only guanine in each oligomer was contained within the CpG sequence, the cross-linked oligomers were also known to be free of monoadducts. The cross-linked product consists of two enantiomers corresponding to the two possible orientations of the NMA molecule. These co-purified during this preparation. No bands corresponding to oligonucleotides containing monoadducts were observed even after extended electrophoresis through a 20% denaturing polyacrylamide gel (not shown).

The efficiency of UvrABC incision has been observed to decrease sharply near DNA termini (Van Houten et al., 1986a, 1987). For this reason and to enable us to study the effects of
supercoiling on UvrABC incision of the mitomycin cross-link, the ends of oligo-XL were extended by ligating them to a 1477-bp EcoRI/AlwNI fragment of pBR322 to form a CCC DNA substrate, pBR-XL (Fig. 6c). In a typical preparation of pBR-XL, 30 pmol of oligo-XL was ligated to 30 pmol of the 1477-bp EcoRI/AlwNI fragment of pBR322. To prepare equivalent unmodified circular DNA, we ligated 30 pmol of the undamaged 5'-phosphorylated oligo-1-oligo-2 duplex to 30 pmol of the EcoRI/AlwNI fragment from the same preparation to make pBR-C. After purification on CsCl-EtBr gradients, approximately 1.5 pmol (1.5 μg) of covalently closed circular DNA was recovered in each case. To verify that the recovered DNA was the desired product and to determine the fraction of molecules that were cross-linked, we digested each DNA with Ddel, labeled the 3' ends of the restriction fragments, and ran the reaction products on an 8% denaturing polyacrylamide gel (Fig. 5d). The restriction pattern was diagnostic of ligation at both the EcoRI and AlwNI ends. Furthermore, the reduced mobility of the 365-nucleotide fragment of pBR-XL in the denaturing gel is consistent with the presence of the cross-link in this fragment. Overexposure of the gel (not shown) revealed a minor band at the same position as the 365-nucleotide fragment of pBR-C. Using densitometric measurements to compare the intensity of the retarded versus unretarded bands, we estimate that greater than 96% of the molecules contained a cross-link within the 365-bp fragment, presumably at the CpG site at position 84 of pBR-XL.

UvrABC nicking of site-specifically cross-linked circles—To determine whether NMA cross-links are substrates for UvrABC, we compared the rate of nicking by UvrABC of unmodified pBR-C with that of pBR-XL (Fig. 6). Prior to treatment with DNA gyrase, pBR-XL, which was circularized in a relaxed state, was nicked at only a slightly faster rate than the unmodified pBR-C DNA. Introduction of three photodimers in pBR-XL resulted in complete nicking of the DNA. Prior to the gel (not shown) revealed a minor band at the same position.

No band corresponding to the position of linear DNA was observed in the agarose gel after as much as 20 min of incubation of supercoiled pBR-XL with UvrABC (not shown). We estimate that double strand breaks in 5% of the substrate molecules would have been detected, indicating that UvrABC does not make incisions in both strands at the site of an NMA cross-link. These experiments do not allow us to determine whether UvrABC cuts preferentially on one side of the NMA cross-link. In previous studies with psoralen interstrand cross-links, double strand breaks were not observed (Van Houten et al., 1986b).

Based on the data presented in Fig. 6, we estimate the rate of UvrABC incision of the NMA cross-link to be at least 200-fold higher in the supercoiled versus the relaxed substrate. This value was determined as follows. Lesion-specific UvrABC nicking was measured by subtracting the level of nonspecific nicking, determined from the pBR-C substrate, from the value obtained for the cross-linked substrate. Thus, 55% of the supercoiled pBR-XL DNA and 15% of the supercoiled pBR-C DNA were nicked in the first 2 min of incubation; i.e. 40% of the pBR-XL was nicked in a lesion-specific way. By a similar analysis, we estimate that 1% of the relaxed substrate is nicked in a lesion-specific manner in the first 10 min. Taking into account the 5-fold difference in incubation times, we conclude that the supercoiled substrate is incised at least 200-fold faster than the relaxed substrate. The actual value may be higher because the rate of lesion-specific incision of the relaxed substrate is near the detection limits of the assay.

**Discussion**

We have shown that mitomycin adducts should be included among the minor groove-modifying nondistortive class of lesions that can be recognized and incised by UvrABC. Unlike CC-1065 adducts, which are inefficiently incised by UvrABC, NMA-treated plasmids are incised by UvrABC about as efficiently as UV-irradiated plasmids. NMA-modified DNA is cut at the eighth phosphodiester bond 5' and the fifth phosphodiester bond 3' to NMA monoaducts. Because significant incision of NMA cross-linked DNA was not observed in relaxed DNA, it is unlikely that cross-links contributed to the UvrABC digestion pattern for NMA-modified linear DNAs. The sites of incision at NMA adducts are the same as the sites for CC-1065 (Selby and Sancar, 1988) and the majority of distortive major groove adducts. Interestingly, incision occurs at the sixth or seventh phosphodiester bond 5' and the third or fourth phosphodiester bond 3' to anthramycin adducts (Walter et al., 1988). Anthramycin and mitomycin both modify DNA at the N-2 position of guanine, and the structural similarities of molecular models of the drug-DNA complexes have been noted (Rao et al., 1986b). Despite the predicted structural similarities, the UvrABC incision pattern is different in the two cases. This difference may be due to dissimilar conformations of the UvrABC-drug-DNA complexes.

Supercoiling enhances the rate of UvrABC incision of NMA adducts. The rate of incision of supercoiled plasmids containing either thymine dimers or mitomycin adducts is about 2-fold higher than the rate of incision when the same plasmids are relaxed. Using a substrate containing a site-specific cross-link, we estimated that supercoiling increases the rate of damage-specific incision about 200-fold. These data imply that the observed cutting of relaxed NMA-treated plasmids is due to cutting at monoaducts and that the rate of cutting of
NMA monoadducts is, at most, only 2-fold dependent on supercoiling. These results are consistent with our observation that the effect of supercoiling depends strongly upon the type of lesion. 2-Acetylaminofluorene monoadducts, psoralen monoadducts, and thymine intrastrand cross-links are incised rapidly by UvrABC in either relaxed or supercoiled DNA, whereas psoralen cross-links, like NMA cross-links, are nicked rapidly only when the DNA is supercoiled. In contrast, Walter et al. (1988) have suggested from indirect evidence that the structurally similar anthramycin-N-2-quanine adduct is recognized better by UvrABC in linear DNA than in supercoiled DNA. It will be interesting to determine at which stage incision of relaxed NMA cross-links is inhibited. Footprinting studies should allow us to distinguish impaired recognition of the lesion from normal recognition but impaired incision.

Our finding that NMA lesions are recognized by UvrABC and incised at the same sites as many adducts that induce significantly different DNA structures adds to the growing list of UvrABC substrates. The structural and chemical diversity of these lesions makes defining characteristics common to all increasingly difficult. The spectrum of recognized damage spans (i) bulky nucleotide-modifying adducts and UV-induced fused bases that are chemically only slightly different from the undamaged precursors; (ii) monoadducts and intra- or interstrand cross-links; (iii) adducts that extend or intercalate into the major groove and adducts that lie in the minor groove; (iv) adducts that either stabilize or destabilize the DNA helix, and (v) lesions that cause kinking or bending of the DNA and those that cause little detectable distortion. What, then, does UvrABC recognize?

One possibility is that UvrABC recognizes lesion-imposed restrictions of the normal range of conformations assumable by DNA. The lesion-recognizing component of the UvrABC complex, UvrA, or the UvrAB subcomplex, may actively “check” DNA for normal helical parameters by inducing limited conformational changes in the DNA structure on binding to it. A normal binding interaction would be followed by translocation or dissociation of the UvrAB(A) complex. A lesion prevents a localized region of DNA from occupying a subset of the conformations available to undamaged DNA; lesion recognition would occur because the UvrAB(A) complex cannot fully flex this damaged DNA. This “jamming” of the normal DNA-binding mechanism would cause the formation of a UvrAB(A) complex that is trapped in a high affinity conformation to which UvrC subsequently binds. By this mechanism, the UvrABC endonuclease can recognize lesions that do not necessarily alter the static structure of the DNA but rather affect the dynamic nature of the DNA helix. This model therefore suggests that we should not expect a priori that the various lesions recognized by UvrABC should have a common structural motif, a conclusion that is supported by the diversity of the predicted three-dimensional structures adopted by DNA containing lesions known to be recognized by UvrABC.

The most important feature of this model is the dynamic nature of the interaction between UvrAB(A) and normal DNA. UvrA has DNA-unwinding activity (Oh and Grossman, 1986), and the UvrAB subcomplex exhibits both DNA-unwinding activity and limited DNA helicase activity on undamaged DNA (Oh and Grossman, 1986, 1987). These activities may be a reflection of the active distortion of the helix by UvrA or UvrAB, which we propose is central to the recognition process.

If distortion of the helix involving DNA unwinding is crucial to the ability of UvrAB(A) to distinguish damaged DNA, then the effect of negative supercoiling on making DNA unwinding energetically more favorable may enhance lesion recognition and hence be responsible for the dramatic increase in incision rate which we have observed when DNA containing NMA cross-links is negatively supercoiled. Alternatively, the more favorable unwinding energy of supercoiled DNA may increase the rate at which the UvrAB-damaged DNA complex is converted to an incision-competent complex in a process requiring ATP hydrolysis. This transition is characterized by helical unwinding and the appearance of DNase I-sensitive bands in the UvrAB-damaged DNA footprint (Oh and Grossman, 1989; Van Houten et al., 1988).

Acknowledgments—We thank Dr. Cinzia Cera for her generous assistance in the preparation of NMA-modified substrates and Dr. Donald M. Crothers for his insightful discussions.

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