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Conformation of DNA Modified at a d(GG) or a d(AG) Site by the Antitumor Drug *cis*-Diamminedichloroplatinum(II)†

Annie Schwartz, Laurent Marrot, and Marc Leng*
Centre de Biophysique Moléculaire, 1A, Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France
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ABSTRACT: The purpose of this work was the comparison of the conformational changes induced in the double helix by the adducts formed at d(GG) and d(AG) sites in the reaction between the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) and DNA. Two duplexes (20-mer) containing either a single d(A*G*) or a single d(G*G*) adduct were studied by means of gel electrophoresis and artificial nuclease and chemical probes. It is shown that the d(G*G*) and the d(A*G*) adducts bend DNA similarly, but at the nucleotide level they distort differently the double helix. We suggest that the weaker interactions between platinated A residues and the other nucleotides, as compared to the interactions between platinated G residues and the other nucleotides, are largely responsible for the differences in the distortions induced in DNA by the d(A*G*) and d(G*G*) adducts. This suggestion is supported by the study of the distortions induced in duplexes by the d(G*G*) adducts, one of the platinated G residues being paired with a T residue.

cis-Diamminedichloroplatinum(II) (*cis*-DDP)[1] is a clinically important anticancer drug. Numerous studies suggest that both the cytotoxicity and the antitumor activities of *cis*-DDP are a consequence of its reaction with cellular DNA. In vivo and in vitro, *cis*-DDP reacts preferentially with adjacent purine residues. The two major adducts are *cis*-[$Pt(NH_3)_2${d(GpG)}](N7,N7) and *cis*-[$Pt(NH_3)_2${d(ApG)}](N7,N7), respectively, representing 65% and 25% of the bound platinum. Several results suggest that the antitumor activity of *cis*-DDP is related to the intrastrand adducts, but whether the two adducts contribute to the activity of *cis*-DDP is not yet known [for general reviews, see Zwelling (1986), Eastman (1987), Reedijk (1987), Sherman and Lippard (1987), and references cited therein]. In *Escherichia coli* and *Salmonella typhimurium*, *cis*-DDP is mutagenic (Brouwer et al., 1981; Beck & Brubaker, 1975; Andersen, 1979; Leopold et al., 1981). Recently, using a forward-mutation assay based on the inactivation of the tetracycline-resistant gene located on plasmid pBR322, Burnouf et al. (1987) determined the mutation spectrum induced in *E. coli* by *cis*-DDP. They found that the d(A*G*) adducts are at least five times more mutagenic than the d(G*G*) adducts and suggested that the local structure induced by *cis*-DDP upon the DNA helix differs when the binding occurs at a d(GG) site or at a d(AG) site.

Structural knowledge of the d(G*G*) adduct has been deduced from numerous studies [for general reviews, see Reedijk (1987), Sherman and Lippard (1987), and references cited therein]. Much less is known of the structure of the d(A*G*) adduct. NMR studies of platinated oligonucleotides (van Hemelryck et al., 1987; van der Veer et al., 1986) and competition experiments with monoclonal antibodies (Sundquist et al., 1987) favor a close similarity between the structures of d(A*G*) and d(G*G*) adducts. Both the d(G*G*) and d(A*G*) adducts bend DNA (Rice et al., 1988; Marrot & Leng, 1989).

Our purpose was a comparison of the distortions induced in DNA by the d(G*G*) and d(A*G*) adducts. We studied two platinated double-stranded oligodeoxynucleotides (20-mer) containing either a d(G*G*) adduct or a d(A*G*) adduct. In these duplexes, all the base pairs but one are the same (the 5′ G·C base pair adjacent to the central G·C base pair is replaced by an A·T base pair). To describe the distortions, we used several techniques giving either a global view of the

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[1] Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum; OsO_4, osmium tetraoxide; CAA, chloroacetaldehyde; OP–Cu, 1,10-phenanthroline–copper; bp, base pair. An asterisk denotes a base modified by *cis*-diamminedichloroplatinum(II).

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double helix (electrophoresis) or a local view (chemical probes and artificial nuclease).

Materials and Methods

The oligonucleotides synthesized on an Applied Biosystems solid-phase synthesizer were purified by ion-exchange FPLC. Klenow polymerase, T4 polynucleotide kinase, T4 DNA ligase, P1 nuclease, and alkaline phosphatase were purchased from Boehringer-Mannheim and from Bethesda Research Laboratories. Electrophoresis-grade acrylamide, bis(acrylamide), and sodium cyanide were from Merck.

Reaction of Platination. The reactions between *cis*-DDP (kindly provided by Dr. J. L. Butour, Toulouse, France) and single-stranded oligonucleotides were performed as previously described (Marrot & Leng, 1989). The platinated oligonucleotides were purified by ion-exchange FPLC. The sites of platination were verified by reverse-phase HPLC analysis of the digests after incubation of the platinated oligonucleotides with P1 nuclease (Fichtinger et al., 1985) and then with alkaline phosphatase (Eastman, 1986).

Chemical Modifications. These were performed as previously described (Marrot & Leng, 1989). The duplexes were labeled at one 3′ end with Klenow polymerase and [α-^{32}P]-deoxynucleotide triphosphate (from Amersham).

Phenanthroline–Copper Digestion. Digestions of the uniquely 3′ end-labeled duplexes were performed according to the procedure described (Yoon et al., 1988) with the following minor modifications: the digestions were carried out for 5 min at 20 °C and the solutions then passed through prespun Sephadex G-25 columns. After evaporation to dryness, the products were dissolved in 0.2 M NaCN. They were incubated for 12 h at 45 °C and then passed through prespun Sephadex G-25 columns.

Ligations and Electrophoresis. These were performed as described by Koo et al. (1986).

Material. A Camag microdensitometer was used to collect the data from sequence gels.

Results and Discussion

The sequences of the duplexes used in this work are

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5′ C T T C T C T T C T G G T C T T C T C T
     A A G A G A A G A C C A G A A G A G A G  5′

5′ C T T C T C T T C T A G T C T T C T C T
     A A G A G A A G A T C A G A A G A G A G  5′
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After reaction with the single-stranded oligonucleotides (upper strands), *cis*-DDP was bound either to the adjacent G residues or to the A residue adjacent to the G residue. The double-stranded oligonucleotides were obtained by mixing the platinated or the unplatinated 20-mer with the complementary strands (lower strands), adjusted so that the resulting duplexes have cohesive ends (19 bp were formed). We will name the duplexes by their two central base pairs.

Electrophoretic Mobility. Recently, it has been proposed from gel electrophoresis experiments that the d(G*G*) and the d(A*G*) adducts bend DNA (Rice et al., 1988; Marrot & Leng, 1989). A value of 35–45° has been estimated for the d(G*G*) adduct induced bend angle (Rice et al., 1988). The present work compares the electrophoretic mobility, in polyacrylamide gels, of the multimers of the ligated d(G*G*/CC) and d(A*G*/TC) duplexes. The same retardation of gel mobility occurs with DNA fragments containing d(G*G*) or d(A*G*) adducts (Figure 1, lanes 5 and 6).

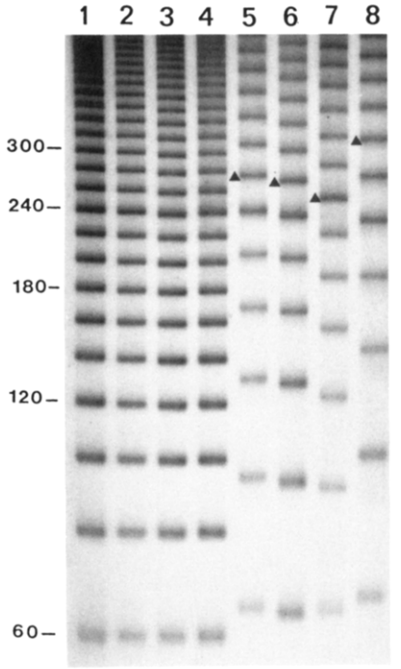

FIGURE 1: Comparison of the migration of platinated to unplatinated multimers on a nondenaturing 8% polyacrylamide gel at room temperature. Lanes 1–4, respectively, are relative to the d(GG/CT), d(GG/TC), d(GG/CC), and d(AG/TC) oligomers ligated to multimers; lanes 5–8, respectively, are relative to the d(A*G*/TC), d(G*G*/CC), d(G*G*/TC), and d(G*G*/CT) oligomers ligated to multimers. The triangles (▲) indicate the platinated 180-bp DNA fragments.

Similar results were obtained at 4 and 37 °C. The multimers of the ligated duplexes d(GG/CC) (lane 3) and d(AG/CC) (lane 4) and 10-bp *Bam*HI linkers (not shown) have normal mobility. Thus, judging from the electrophoretic mobility, the d(G*G*) and d(A*G*) adducts induce a similar distortion in DNA.

For reasons given later, we studied the migration of DNA fragments containing a base-pair mismatch (G·T). The G·T mismatch destabilizes the double helix but introduces a minimal distortion in the global conformation [for general reviews, see Kennard (1987), Patel et al. (1987), and references cited therein]. The electrophoretic mobilities of the d(GG/CT), d(GG/TC), and d(GG/CC) multimers are approximately the same (Figure 1, lanes 1–3), which confirms that the mismatches do not distort the global conformation. On the other hand, the corresponding platinated multimers are all retarded, but the retardation is the largest for the d(G*G*/CT) multimers (Figure 1, lanes 6–8). For example, the *K* factor (apparent length to sequence length ratio) is equal to 1.55, 1.47, and 1.83 for the 180-bp d(G*G*/CC), d(G*G*/TC), and d(G*G*/CT) fragments, respectively.

Phenanthroline–Copper Digestion. The nucleolytic agent 1,10-phenanthroline–copper (OP–Cu) attacks DNA from a binding site within the minor groove and cleaves DNA in a sequence-dependent manner but not in a base-specific manner (Yoon et al., 1988; Sigman, 1986; Sigman et al., 1985). The cleavage patterns of the unplatinated duplexes d(GG/CC) and d(AG/TC) by OP–Cu are approximately similar (Figures 2 and 3). The comparison between the cleavage patterns relative to the platinated and unplatinated duplexes reveals variations in the helices' geometry. Within d(G*G*/CC), the central sequence TG*G*T becomes less reactive. The C and T nucleotides adjacent to this sequence on the 5′ side are slightly hyperreactive, whereas the complementary G and A nucleotides are strongly hyperreactive. Within d(A*G*/TC),

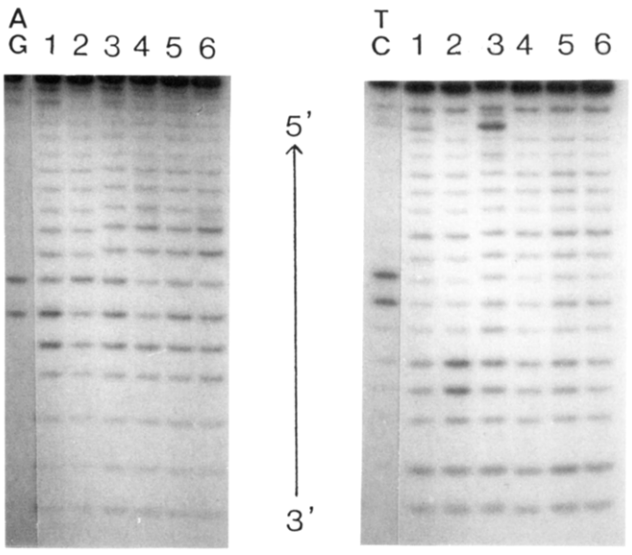

FIGURE 2: Autoradiogram of a denaturing 24% polyacrylamide gel of a OP–Cu scission reaction product. (Left, upper strands) Lanes 1, 3, and 5 are relative to the duplexes d(AG/TC), d(GG/CC), and d(GG/TC), respectively. Lanes 2, 4, and 6 are relative to the duplexes d(A*G*/TC), d(G*G*/CC), and d(G*G*/TC), respectively. (Right, lower strands) Lanes 1, 3, and 5 are relative to the duplexes d(GG/CC), d(AG/TC), and d(GG/TC), respectively. Lanes 2, 4, and 6 are relative to the duplexes d(G*G*/CC), d(A*G*/TC), and d(G*G*/TC), respectively. GA (left) and TC (right) are Maxam–Gilbert specific reactions for the unplatinated duplexes.

the A* nucleotide is more reactive and the next G*, T, and C nucleotides are less reactive. In the lower strand, the C residue complementary to the G* nucleotides and the next 3′ A nucleotides are less reactive. Our conclusion is that (i) the geometry of the double helix is altered over several base pairs by the d(A*G*) and the d(G*G*) adducts and (ii) the conformational changes induced by the two adducts are different. NMR studies (van Hemelryck et al., 1984, 1986; den Hartog et al., 1984, 1985) of duplexes platinated at a d(GG) site have shown that the distortion spreads over 4 bp (the two base pairs of the adduct and the 5′ and the 3′ base pairs adjacent to the adduct).

With regard to the G·T mismatch, the cleavage pattern of the d(GG/TC) duplex resembles that of the d(GG/CC) duplex (Figures 2 and 3). This suggests small conformational changes in the minor groove. After platination, the cleavage pattern is modified. The two nucleotides C and T adjacent to the adduct on the 5′ side are more reactive, the two G* nucleotides are less reactive, and the next two 3′ nucleotides are more reactive. Minor changes occur in the lower strand. The cleavage pattern does not resemble that of the d(G*G*/CC) or d(A*G*/TC) duplexes.

Osmium Tetraoxide and Chloroacetaldehyde. The rate of reactivity of several reagents with individual base residues is strongly dependent upon the structure of DNA. Osmium tetraoxide (OsO_4) is hyperreactive with T residues in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (Lilley & Palecek, 1984; Johnston & Rich, 1985; Palecek et al., 1987). Chloroacetaldehyde (CAA) is hyperreactive with A and C residues in single-stranded nucleic acids and with A residues in Z-DNA as compared to B-DNA (Lilley, 1983; Kohwi-Shigematsu et al., 1987; McLean et al., 1987; Vogt et al., 1988). These two probes were previously used to characterize, at the nucleotide level, the conformational changes induced by the d(A*G*) adduct within the d(A*G*/CT) duplexes (Marrot & Leng, 1989). A similar study is now carried out with the duplexes d(G*G*/CC), d(G*G*/TC), and d(G*G*/CT).

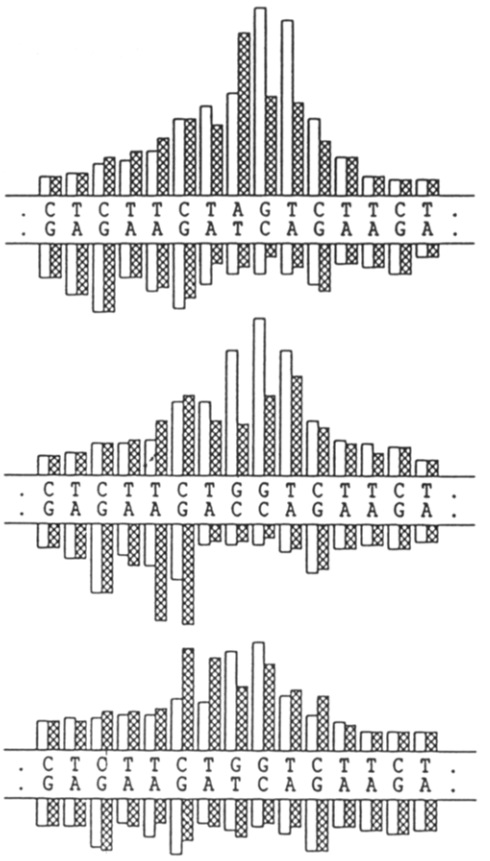

FIGURE 3: Quantitation of the OP–Cu digestion. The autoradiogram was quantitated by microdensitometry. The unshaded columns represent digestion of the unplatinated duplexes, and the shaded columns represent digestion of the platinated duplexes. The surfaces of the columns are proportional to the band intensity on the autoradiogram.

CAA does not react with any of the C residues complementary to the d(G*G*) adducts (not shown). Thus, in our experimental conditions, the d(G*G*) adducts do not locally denature the double helix, in agreement with other results (van Hemelryck et al., 1984, 1986; den Hartog, 1984, 1985; Kozelka et al., 1986; Marrot & Leng, 1989).

Lanes 1 and 2 (left) in Figure 4 are relative to the reactivity of OsO_4 with the upper strands of the duplexes d(GG/CC) and d(G*G*/CC). The T residues adjacent to the adduct are not (or only very weakly) reactive. In our previous study (Marrot & Leng, 1989), we found that within the d(A*G*/TC) duplex, only the three T residues located on the 5′ side of the adduct were reactive with OsO_4. Thus, as judged by OsO_4, the distortions induced by the d(G*G*) adducts and by the d(A*G*) adducts are different.

Two main factors are involved in these distortions: (i) the structure of the adducts; (ii) the interactions between adducts, complementary nucleotides, and adjacent nucleotide residues. The importance of the latter factor was studied by comparing the reactivity of the T residues within the two duplexes d(G*G*/TC) and d(G*G*/CT). In both the duplexes, the 5′ and 3′ T residues adjacent to the d(G*G*) adduct are OsO_4-susceptible, but their susceptibility depends upon the mismatch (Figure 4, left, lanes 4 and 6). Within the duplex d(G*G*/TC), the 5′ T residue reacts more strongly than the 3′ T residue, while within the duplex d(G*G*/CT), the 3′ T residue reacts more strongly than the 5′ T residue (the ratio of the band intensities is equal to 4 in the former duplex and to 2 in the latter). Thus, the chemical probe OsO_4 reveals that the distortions induced by the d(G*G*) adduct within re-

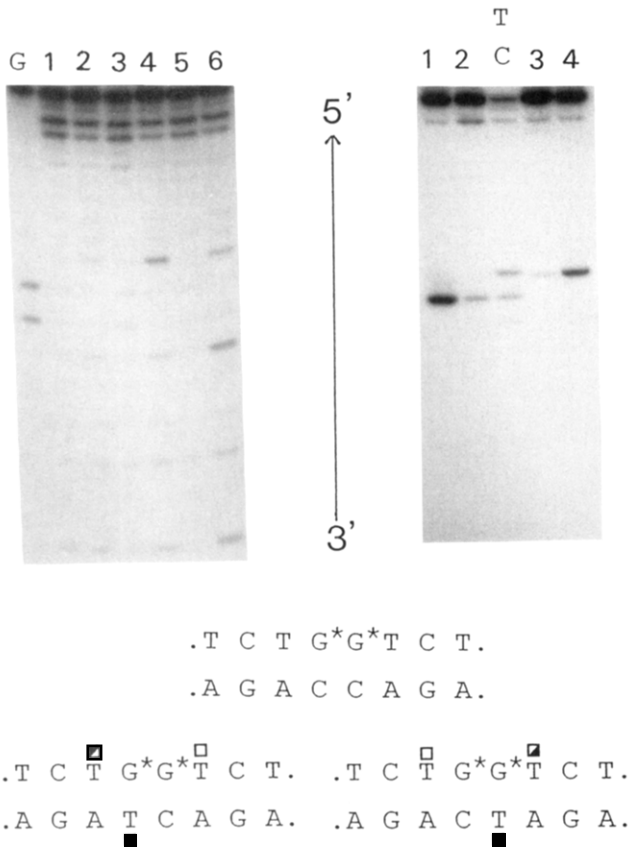

FIGURE 4: Piperidine-induced specific strand cleavage at OsO_4-modified bases in platinated and unplatinated duplexes. (Left, upper strands) Lanes 1, 3, and 5 are relative to the duplexes d(GG/CC), d(GG/TC), and d(GG/CT), respectively. Lanes 2, 4, and 6 are relative to the duplexes d(G*G*/CC), d(G*G*/TC), and d(G*G*/CT), respectively. (Right, lower strands) Lanes 2 and 3 are relative to the duplexes d(GG/TC) and d(GG/CT), respectively. Lanes 1 and 4 are relative to the duplexes d(G*G*/TC) and d(G*G*/CT), respectively. G (left) and TC (right) are Maxam–Gilbert specific reactions for the unplatinated duplexes. A summary of the changes in chemical reactivity is given at the bottom of the figure. Filled, half-filled, and open symbols indicate strong, intermediate, and low hyperreactivity, respectively.

spectively the three duplexes d(G*G*/CC), d(G*G*/TC), and d(G*G*/CT) are different whereas the electrophoretic mobility (Figure 1) shows mainly a difference between the d(G*G*/CC) and d(G*G*/CT) multimers. By analogy with these results, we assume that even if the d(A*G*) and d(G*G*) adducts have the same structure, as suggested by NMR data (van Hemelryck et al., 1987; van der Veer et al., 1986), they distort the double helix differently because the interactions between the A* residues and the other nucleotides are weaker than those between the G* residues and the other nucleotides.

Within the duplexes d(GG/TC) and d(GG/CT), the T residues, when mismatched with the central G residues, are reactive with OsO_4 (Figure 4, right, lanes 2 and 3). The accessibility of these T residues to the chemical probe confirms X-ray and NMR studies (Kennard, 1987; Patel et al., 1987) showing that the mismatched T residues move into the major groove. The advantage of OsO_4 for the detection of mismatches in DNA, as recently reported (Cotton et al., 1988), is worth noting. Within the duplexes d(G*G*/TC) and d(G*G*/CT), the mismatched T residues are strongly reactive with OsO_4 (Figure 4, right, lanes 1 and 4). Within the duplex d(A*G*/TC), the T residue complementary to the A* residue is also OsO_4-sensitive (Marrot & Leng, 1989).

To summarize, the substitution of a G*·C base pair within the duplex d(G*G*/CC) by a less stable A*·T or G*·T base pair transforms an apparently symmetrical distortion into an asymmetrical distortion larger on the side of the substituted base pair. If we examine the mutation spectrum in *E. coli* (Burnouf et al., 1987), among the 24 base-pair-substitution mutants, 14 occur at d(AG) sites and 9 at d(GG) sites. It is striking that all the 14 mutations occurring at the d(AG) sites affect the adenine residues. In agreement with Burnouf et al. (1987), we assume that the mutagenic event is related to the larger distortion of the double helix on the 5′ side of the d(A*G*) adducts rather than on the 3′ side. With regard to the mutations at the d(GG) sites, some affect the 5′ G residues and some the 3′ G residues. We suggest that the stability of the base pair adjacent to the d(G*G*) adducts plays a major role in inducing an asymmetrical distortion and, consequently, that the mutation occurs at the G* residue adjacent to the less stable sequence. This assumption is supported by three mutations occurring at d(GG) sites [Table 1 in Burnouf et al. (1987)]. One mutation affecting the 3′ G residue is in the sequence GAGGAT, and two mutations affecting the 5′ G residues are in the sequence TCGGGC. We did not take into account the data relative to the sequence GGCCGGC, which contains three *cis*-DDP potential binding sites. If two adjacent sites are platinated, the geometry of the distortion differs largely from that induced by only one platinated site (Marrot & Leng, 1989). Work is in progress to collect more mutation data.

Conclusion

The distortions induced in DNA by the d(A*G*) and the d(G*G*) adducts are similar as judged by electrophoretic mobility. On the other hand, at the nucleotide level, the distortions are not similar as shown by the reactivity of the platinated oligonucleotides with OsO_4 and OP–Cu. It seems unlikely that the DNA replication machinery is less sensitive to subtle conformational changes in the DNA double helix than chemical probes or artificial nuclease, and thus one expects different biochemical consequences whether *cis*-DDP binds to a d(AG) or a d(GG) site.

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A New Mechanism for Repairing Oxidative Damage to DNA: (A)BC Excinuclease Removes AP Sites and Thymine Glycols from DNA†

Jing-Jer Lin and Aziz Sancar*
Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599
Received July 18, 1989; Revised Manuscript Received August 3, 1989

ABSTRACT: *Escherichia coli* (A)BC excinuclease is the major enzyme responsible for removing bulky adducts, such as pyrimidine dimers and 6–4 photoproducts, from DNA. Mutants deficient in this enzyme are extremely sensitive to UV and UV-mimetic agents, but not to oxidizing agents, or ionizing radiation which damages DNA in part by generating active oxygen species. DNA glycosylases and AP[1] endonucleases play major roles in repairing oxidative DNA damage, and thus it has been assumed that nucleotide excision repair has no role in cellular defense against damage by ionizing radiation and oxidative damage. In this study we show that the *E. coli* nucleotide excision repair enzyme (A)BC excinuclease removes from DNA the two major products of oxidative damage, thymine glycol and the baseless sugar (AP site). We conclude that nucleotide excision repair is an important cellular defense mechanism against oxidizing agents.

Active oxygen species (the superoxide radical, $O_2^{\bullet-}$, hydroxyl radical, $OH^{\bullet}$, and hydrogen peroxide, H_2O_2) generated by incomplete reduction of oxygen during aerobic metabolism are serious threats to cellular integrity. All aerobic organisms appear to have molecular mechanisms to protect themselves against and to repair the damage caused by these agents. The protective enzymes include superoxide dismutase, which converts $O_2^{\bullet-}$ into H_2O_2, and catalase, which converts H_2O_2 into molecular oxygen and H_2O, as well as specific peroxidases [see Fridovich (1989)]. In addition to these protective measures there are several enzymes that are involved in repairing the oxidative damage to DNA. In *Escherichia coli* it has been shown that exonuclease III (Demple et al., 1983), endonucleases III and IV (Cunningham et al., 1986), and RecA protein (Imlay & Linn, 1986) play important roles in repairing DNA damaged by oxidative damage, as well as by ionizing radiation which damages DNA mainly through generation of $OH^{\bullet}$ radicals [see Von Sonntag (1987) for a review]. Enzymes that repair oxidative DNA damage (redoxyendonuclease) have also been found in yeast (Gossett et al., 1988) and in bovine and human cells (Doetsch et al., 1987).

The repair of damage caused by either ionizing radiation or active oxygen species is accomplished by removal of the saturated (e.g., thymine glycol) or fragmented (urea, methyltartronylurea) base by so-called redoxyendonucleases

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* Address correspondence to this author.

[1] Abbreviations: AP, apurinic/apyrimidinic; TG, thymine glycol; AP-DNA, DNA with apurinic/apyrimidinic sites; TG-DNA, DNA containing thymine glycols; Nth, *E. coli* endonuclease III; Nfo, *E. coli* endonuclease IV.

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