Articles

Cyclobutane Thymine Dimers with a Disrupted Phosphodiester Bond Are Refractory to T4 Endonuclease V Digestion but Have Increased Sensitivity to UvrABC Nuclease†

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ABSTRACT: UV irradiation induces the dimerization of synthetic single-stranded, 80-mer oligonucleotides with self-complementary, alternating purine-pyrimidine sequences, and terminal 5′- and 3′-thymines; this process can be reversed by photoreactivation. The UV-induced 160-mers are sensitive to digestion by the restriction enzyme *Sna*BI, but monomers are insensitive to digestion, indicating that UV irradiation stabilizes the formation of double-stranded DNA. These results suggest that UV irradiation of these 80-mer oligonucleotide substrates induces the formation of a novel cyclobutane thymine dimer which lacks an intradimer phosphodiester bond (CPD*). This CPD*, linking the terminal thymines of two separate 80-mer molecules, is formed in a double-stranded DNA region created by self-annealing and intermolecular hybridization of the two 80-mer strands. We have found that these UV-induced CPD* in 160-mers are sensitive to cleavage by the nucleotide excision enzyme complex UvrABC nuclease, but resistant to cleavage by the cyclobutane pyrimidine dimer-specific enzyme T4 endonuclease V. However, pretreatment of the 160-mers with ligase reverses their sensitivity to these two enzymes, significantly reducing their susceptibility to cleavage by UvrABC nuclease but dramatically increasing their susceptibility to cleavage by T4 endonuclease. The biological significance of these findings is discussed.

Cyclobutane pyrimidine dimers (CPD)[1] represent the major photoproducts formed in the cellular DNA of cells irradiated with ultraviolet light (UV). Both in vitro and in vivo results have demonstrated that CPD may block transcription and DNA replication, or lead to base-mispairing/DNA replication errors during lesion bypass. UV-induced cytotoxicity and mutagenicity have been attributed to these two effects (*1*).

In both prokaryotic and eukaryotic cells, CPD formed in genomic DNA are removed mainly by the nucleotide excision repair pathway. In this pathway, multiple components are involved in recognizing the CPD and subsequently making dual incisions at both the 5′ and 3′ sides of the CPD. These components have been isolated from *E. coli* cells (UvrA, UvrB, and UvrC proteins) and from human cells (*1*−*3*). Although CPD can be efficiently removed in cells in vivo, paradoxically, purified nucleotide excision components, particularly those isolated from human cells, repair CPD quite poorly (*4*).

In organisms such as *M. luteus* and *E. coli* cells infected with T4 phage, CPD can be repaired by endonuclease V (endo V), a single peptide protein, as well as by nucleotide excision repair enzymes. In these cells, endo V functions as both a glycosylase and a phosphodiesterase, first hydrolyzing the 5′ side glycosylic bond and then the phosphodiester bond between two pyrimidines (*5*−*7*). In contrast to the purified multiple nucleotide excision repair components, endo V purified from either *M. luteus* or T4 phage infected cells incises CPD very efficiently. These two enzymes have been widely used for quantifying CPD formation and repair in a variety of different organisms (*1*).

Using a T4 endo V incision assay, van Zeeland et al. (*8*) found that while CPD in the genome of cultured human cells were repaired efficiently, CPD in cultured rodent cells were poorly repaired, even though both kinds of cells had comparable UV sensitivity. These findings initially led to the conclusion that rodent cells can survive without removing CPD from their genomes, and this phenomenon has been termed the "rodent cell repair paradox" (*9*). Although subsequent findings that both human and rodent cells are

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[1] Abbreviations: CPD, cyclobutane pyrimidine dimer(s); CPD*, cyclobutane pyrimidine dimer with a disrupted phosphodiester bond; T4 endo V, T4 endonuclease V; CHO, Chinese hamster ovary; UV, ultraviolet; bp, base pair(s); nt, nucleotide(s); EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol.

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capable of efficient removal of CPD from actively transcribed gene sequences have provided a partial explanation for this paradox (*9, 10*), critical questions such as how DNA replication resumes without removing CPD from the parental DNA in rodent cells remain unexplained.

It is known that both prokaryotic and eukaryotic cells are capable of preferentially repairing CPD in the transcribed strand of actively expressed genes, facilitating rapid removal of transcription-blocking damage from critical gene sequences (*1*). Although the precise mechanisms of transcription-facilitated CPD repair have yet to be resolved, critical genes for transcription-coupled repair have been identified in *E. coli* cells (*mfd)* (*11*), and in human (CS-B) and rodent (ERCC 6) cells (*12*−*14*). Mammalian cells may also be capable of modifying CPD in their genomic DNA in ways that may expedite recognition and repair, or facilitate lesion bypass by the DNA replication machinery. However, while recent findings have provided a much better understanding of the excision step of nucleotide excision repair (*1*), we still know very little about damage recognition or the specific modifications that may precede incision. Mitchell et al. (*15*) have reported that the antigenicity of CPD changes in UV-irradiated Chinese hamster ovary (CHO) cells. Patterson's laboratory has reported that 85% of excised CPD in human cells lack a phosphodiester bond (*16*, *17*). To test the possibility that such modifications might enhance damage recognition or facilitate repair, we have constructed substrates containing cyclobutane thymine dimers without a phosphodiester bond, and have characterized the susceptibility of these modified CPD photoproducts to cleavage by either the dimer-specific endonuclease T4 endo V or the *E. coli* nucleotide excision repair enzyme UvrABC nuclease.

EXPERIMENTAL PROCEDURES

Materials. The 80-mer oligonucleotide sequence shown in Figure 1 was synthesized in an Applied Biosystems DNA synthesizer, Model 381A. [γ-^{32}P]ATP (specific activity ~3000 Ci/mmol) was purchased from DuPont New England Nuclear. T4 polynucleotide kinase and T4 ligase were purchased from Boehringer-Mannheim. NACS PAC columns were purchased from Gibco-BRL. T4 endo V was a generous gift of Dr. S. Lloyd, University of Texas Health Science Center at Galveston, Galveston, TX. Calf thymus ligase II was a generous gift of Dr. A. Thompkin, University of Texas Health Science Center at San Antonio, San Antonio, TX. UvrA, UvrB, and UvrC were purified from cells carrying pD*R*3274 (*uvrC)*, pUNC211 (*uvrB*), or pUNC45 (*uvrA*) by previously described methods (*18*); these plasmids and photolyases were kindly provided by Dr. A. Sancar, University of North Carolina at Chapel Hill, Chapel Hill, NC. All other chemicals and electrophoretic materials were obtained either from Sigma or from Bio-Rad.

5′-End-^{32}P-Labeling and 80-mer Purification. The 80-mer oligonucleotide sequence shown in Figure 1 was synthesized using the 1 μM precusor Trityle-off program. After synthesis, the 80-mers were cleaved from the solid support by ammonium hydroxide hydrolysis, and purified by electrophoresis in a 8% denaturing polyacrylamide gel and then 5′-end-labeled with [γ-^{32}P]ATP according to the published procedure (*19*).

UV Irradiation and Isolation of the UV-Induced 160-mer. The purified, 5′-end ^{32}P-labeled 80-mers were dissolved in TE (Tris, 1 mM, pH 8.0, and EDTA, 0.1 mM) and irradiated with different fluences of UV light (germicidal lamp, major emission 254 nm). After UV irradiation, the DNAs were separated by electrophoresis in an 8% denaturing polyacrylamide gel. The 160-mer bands were cut out, eluted, and further purified by a NACS PAC column.

Photoreactivation. The purified 160-mers (5×10^{-5} nmol) were incubated with *E. coli* photolyase 1.5 μg (0.03 nmol), in photoreactivation buffer (5 mM Tris, pH 7.7, 1 mM EDTA, 100 mM NaC1, 10 mM DTT) and irradiated with black light (F15T8/BLB 15W Sylvania) for 90 min.

T4 Ligase and Calf Thymus Ligase II Reaction. The purified 160-mers (5×10^{-4} nmol) were reacted with either T4 ligase (10 units) or calf thymus ligase II in reaction buffer (66 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, and 1 mM ATP). The reaction mixtures were incubated at 18 °C for 14 h. The reactions were stopped by phenol and diethyl ether extractions, and followed by ethanol precipitation in the presence of aqueous ammonium acetate (2.5 M). The precipitated DNA was washed with 70% ethanol, and dried under vacuum.

UvrABC Nuclease and T4 Endonuclease V Reactions. The UvrABC nuclease reactions were carried out in a reaction mixture (25 μL) containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 10 mM $MgCl_2$, 1 mM ATP, 100 mM KCl, 1 mM DTT, 15 nM UvrA, 15 nM UvrB, 15 nM UvrC, and substrate DNA (2 nM). The T4 endo V reactions were carried out in a reaction mixture containing 100 mM NaCl, 1 mM EDTA, 5 mM Tris, pH 7.7, T4 endo V (6.5 nM), and substrate DNA (2 nM). Both reaction mixtures were incubated at 37 °C for 1 h, and the reactions were stopped by phenol and diethyl ether extractions, followed by ethanol precipitation in the presence of aqueous ammonium acetate (2.5 M). The precipitated DNA was washed with 70% ethanol, and dried under vacuum.

Gel Electrophoresis and Autoradiography. The ^{32}P-labeled 80-mers or 160-mers (either untreated or treated with UvrABC nuclease or T4 endo V) were suspended in sequencing tracking dye (80% v/v deionized formamide, 0.1% xylene cyanol, and 0.1% bromophenol blue), heated at 90 °C for 4 min, and quenched in an ice bath. The samples were applied to a 0.4 mm denaturing sequencing gel consisting of 8% polyacrylamide and 7 M urea in TBE buffer (89 mM Tris-HCl, 89 mM borate, and 2 mM EDTA, pH 8.3). The gels were dried in a Bio-Rad gel dryer, initially exposed to a phosphor screen and then to Kodak X-Omat RP film at −70 °C for various lengths of time. The intensity of the bands was determined by a PhosphorImager (Molecular Dynamics). For detection of the 12−13 base fragments generated from UvrABC digestion, the DNA samples were applied to a 1.5 mm 15% polyacrylamide gel containing 7 M urea in TBE buffer. The gels were directly exposed to film at −70 °C or a phosphor screen without further drying.

DNA Fragment Isolation and Sequencing. The single 5′-end ^{32}P-labeled 176 bp *Eco*RI−*Hae*III fragments were isolated from 377 bp *Eco*RI−*Bam*HI fragments of PBR322 plasmids as described (*19*). Sequencing reactions were carried out by the Maxam and Gilbert method (*20*).

RESULTS

Dimerization of 80-mers by UV Irradiation. An 80-mer oligonucleotide sequence was designed in such a way that

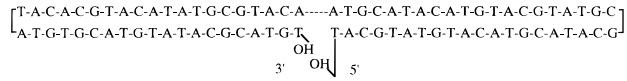

FIGURE 1: Nucleotide sequence of the synthetic 80-mer oligonucleotide. For clarity, the sequence is presented to illustrate its self-complementary nature and potential of forming a thymine−thymine cyclobutane dimer without a phosphodiester bond at the 5′ and 3′ ends of the sequence upon UV irradiation.

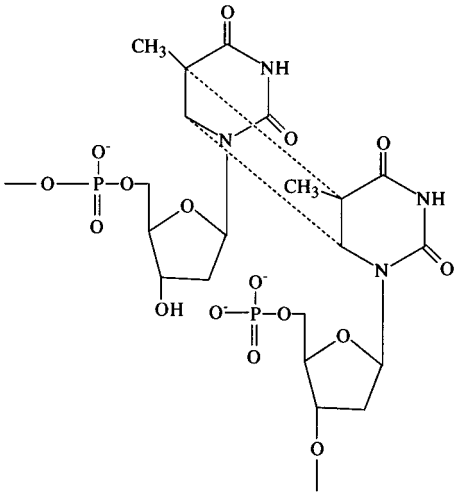

FIGURE 2: Chemical structure of cyclobutane thymine dimer with an interruption at the phosphodiester bond (CPD*).

folding-back and self-annealing of its palindromic 5′- and 3′-ends could form a double-stranded structure in which the 3′- and 5′-terminal thymine residues would be adjacent to one another (Figure 1). Upon UV-irradiation, these adjacent thymines should yield a novel cyclobutane thymine dimer photoproduct, a CPD with no phosphodiester bond between the two thymine residues (CPD*) (Figure 2). In an attempt to generate such photoproducts, gel-purified 5′-end ^{32}P-labeled 80-mers were irradiated with UV light (0−8000 J/m^2), and the DNA was then denatured and separated by electrophoresis in a denaturing 8% polyacrylamide gel. As shown in Figure 3A, UV irradiation of these 80-mer oligonucleotides produced a band with the mobility of a 160-mer; the intensity of this band appeared to be dependent upon UV fluence. These results suggest that UV irradiation induces dimerization of the 80-mer oligonucleotide sequence. The relationship between 160-mer formation and UV fluence is shown in Figure 3B; these results demonstrate that formation of 160-mers reaches a maximum at a UV fluence of 4000 J/m^2.

This UV-induced dimerization of 80-mers appears to be due to the formation of a cyclobutane thymine dimer between the terminal 3′- and 5′-thymines from two separate 80-mers. If this is indeed the case, then photoreactivation should be able to reverse this UV-induced dimerization, producing two 80-mers. To test this possibility, we purified UV-induced 160-mers and attempted to photoreactivate these DNAs using *E. coli* photolyase. Results in Figure 4 show that the photoreactivation treatment of UV-induced 160-mers does in fact produce 80-mers. These results demonstrate that most (approximately 88%) of the 160-mers produced by UV-induced dimerization can be reversed by photoreactivation. However, pretreatment of these UV-induced 160-mers with T4 ligase renders these 160-mers refractory to monomerization by photoreactivation (cf. lane 5 to lane 7). These

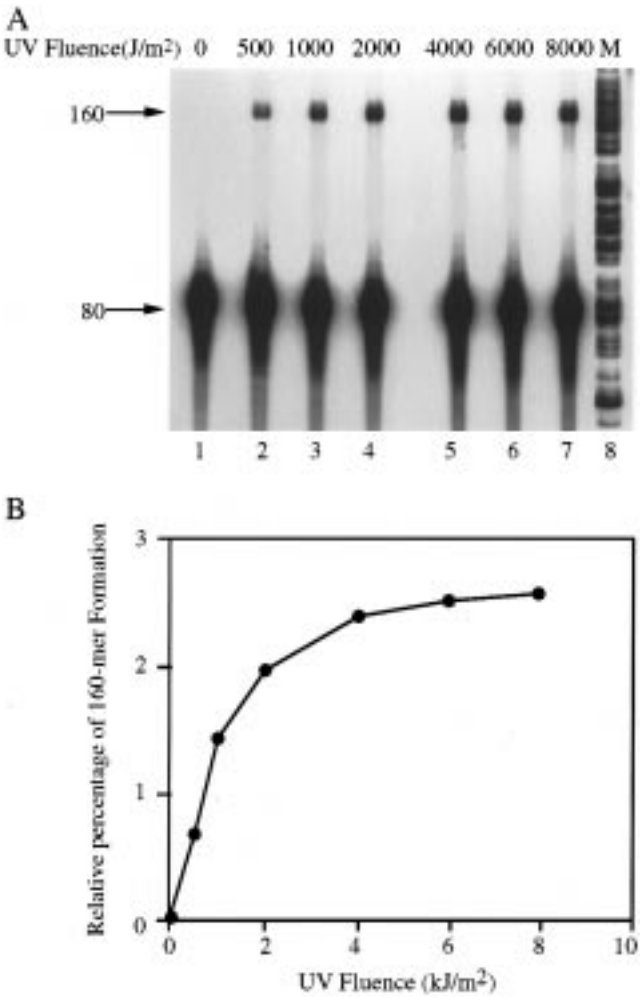

FIGURE 3: (A) UV fluence-dependent dimerization of 80-mers. 5′-end ^{32}P-labeled 80-mers were irradiated with different fluences of UV light (0, 500, 1000, 2000, 4000, 6000, and 8000 J/m^2 for lanes 1−7) and then were separated by electrophoresis in an 8% denaturing polyacrylamide gel. In lane 8 are DNA size markers. The positions of 80 and 160 nt are indicated by arrows. (B) Relationship between UV fluence and the degree of dimerization of 80-mers. Band intensities of the 80-mer and the 160-mer were scanned in a PhosphorImage densitometer, and the percentages of the 160-mers formed by UV irradiation were calculated.

results indicate that the UV-induced 160-mers contain novel CPD* and ligation treatment may convert these CPD* to CPD.

The sequence of the 80-mer oligonucleotide was intentionally designed to contain two -TACGTA- sites, allowing us to detect any double-strandedness for these regions produced by either self-annealing of sequences within an 80-mer or intermolecular annealing between two 80-mers, which would render these sites susceptible to cutting by the restriction enzyme *Sna*BI. Figure 5 shows that the 80-mers are resistant to *Sna*BI digestion. It appears that no *Sna*BI-sensitive double-strand DNA structures are formed in unirradiated 80-mers or, alternatively, that 80-mers may form unknown unusual structures which are insensitive to *Sna*BI digestion. The majority of the UV irradiation-induced 160-mers are sensitive to *Sna*BI digestion. These results suggest that UV-induced cyclobutane thymine dimer formation may stabilize both intermolecular annealing between 80-mers and self-annealing within the 160-mer. The multiple bands produced by *Sna*BI digestion of UV irradiation-induced 160-mers may

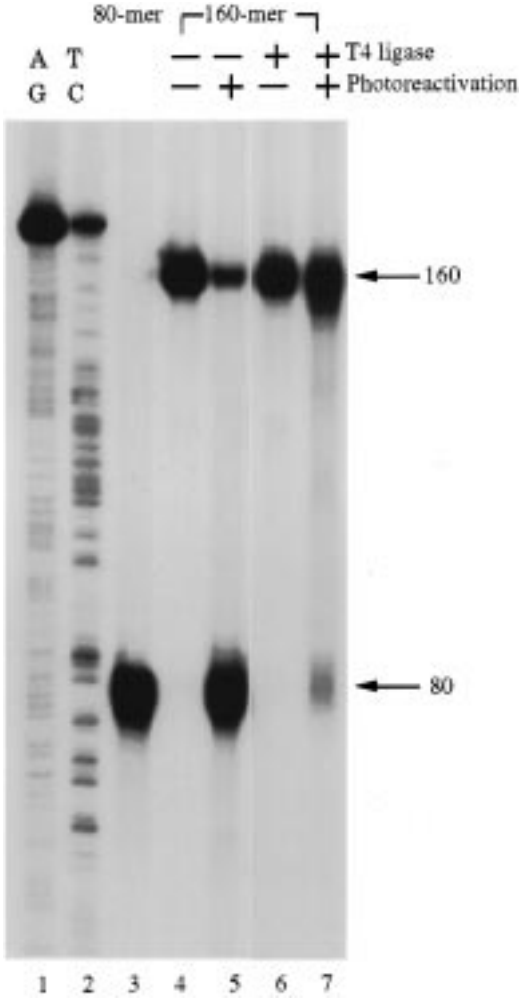

FIGURE 4: Monomerization of UV-induced dimerized 80-mers by photoreactivation. UV-induced 160-mers with and without pretreatment of T4 ligase were reacted with photolyase and black light, and the resultant DNAs were separated in an 8% polyacrylamide gel as described in the text. Lanes 1 and 2 are DNA size markers using DNA fragments resulting from Maxam and Gilbert AG and TC reactions of 5′ ^{32}P-labeled *Eco*RI−*Hae*III 176 bp of pBR322 plasmid DNA. DNAs in lane 3 are 80-mers. The positions of 80 and 160 nt are indicated by arrows.

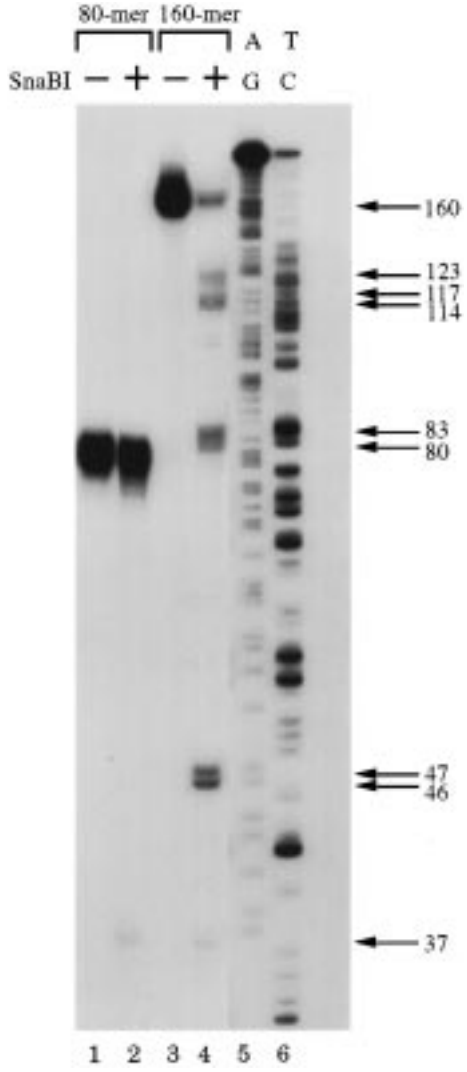

FIGURE 5: Restriction enzyme digestion results of 160-mer formed by UV-induced dimerization of 80-mer. ^{32}P-Labeled 80-mers and the 160-mers formed by UV-irradiation of the 80-mers were digested with restriction enzyme *Sna*BI (10 units/μg of DNA) and then separated by electrophoresis in an 8% denaturing polyacrylamide gel. The sizes of the DNA fragments in the band are indicated. Lanes 5 and 6 are DNA size markers the same as described in Figure 4.

reflect either (1) incomplete digestion by *Sna*BI due to the formation of cyclobutane thymine dimers at *Sna*BI sequences or (2) a mixed population of isomeric 160-mers with either one or two cyclobutane dimers. Based on the multiple bands (160, 123, 117, 114, 83, 80, 47, 46, and 37 nt) observed following *Sna*BI digestion, we propose that at least five different forms of 160-mers may be induced by UV irradiation of 80-mers; Figure 6 is a schematic representation of how UV irradiation might produce various 160-mer forms, involving the induction of either one or two cyclobutane thymine dimers and the size of the DNA fragments that these 160-mer forms might generate after *Sna*BI digestion. We will refer to the three isomeric 160-mers containing two cyclobutane thymine dimers as forms I, II, and III, and to the two isomeric 160-mers containing only one cyclobutane thymine dimer as forms IV and V. Except for the 3-mers, all the bands that appear in the gel can be accounted for by complete and partial *Sna*BI digestion of form I to form V of 160-mers. We believe that the 3-mers produced by *Sna*BI digestion were run off the gel. Although no 47-mers were expected to be formed by *Sna*BI digestion, we believe that the band corresponding to the size of 47-mers may result from 46-mers which contain different stereoisomers (cis-syn, trans-syn, cis-anti, and trans-anti) of cyclobutane thymine dimers. It should be noted that the cyclobutane thymine dimers in these 160-mers contain no phosphodiester bond. We term this kind of photodimer a CPD* (Figure 2).

T4 Endo V and UvrABC Nuclease Sensitivities of UV-Induced 160-mer. If UV-induced 160-mers are formed by linking two 80-mers by a CPD*, then it is reasonable to assume that such UV-induced 160-mers will be sensitive to cleavage by the cyclobutane pyrimidine dimer specific enzyme, T4 endo V, as well as by the nucleotide excision repair enzyme, UvrABC nuclease (for a review, see *1*, *2*). To test this possibility, we have treated the UV-induced 160-mers with either T4 endo V (Figure 7) or UvrABC nuclease (Figure 8). Since T4 endo V should break both the glycosylic bond at the 5′-end of a cyclobutane thymine dimer and the phosphodiester bond between the two thymines, we would expect T4 endo V to generate two 80 nt fragments

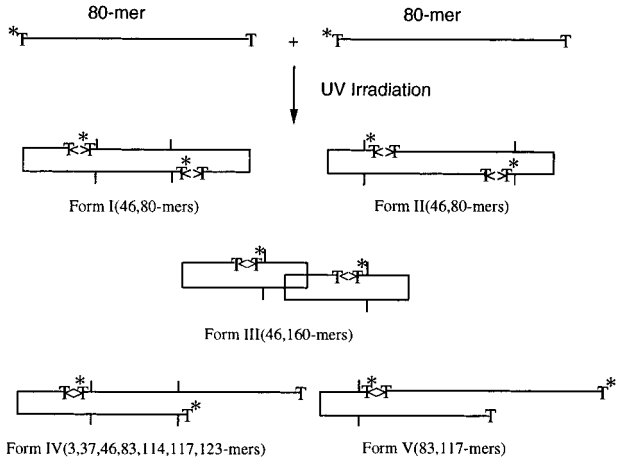

FIGURE 6: Schematic presentation of the UV induced dimerization of 80-mer via the formation of cyclobutane pyrimidine dimer between the two thymine residues at the 5′- and 3′-ends of two 80-mers. The thymines at the 5′-end labeled with ^{32}P are indicated by asterisks. The lines represent strands with complementary sequences and the antiparallel structure. The *Sna*BI sites are indicated by perpendicular lines, and the sizes of the DNA fragments generated by *Sna*BI digestion are depicted in parentheses.

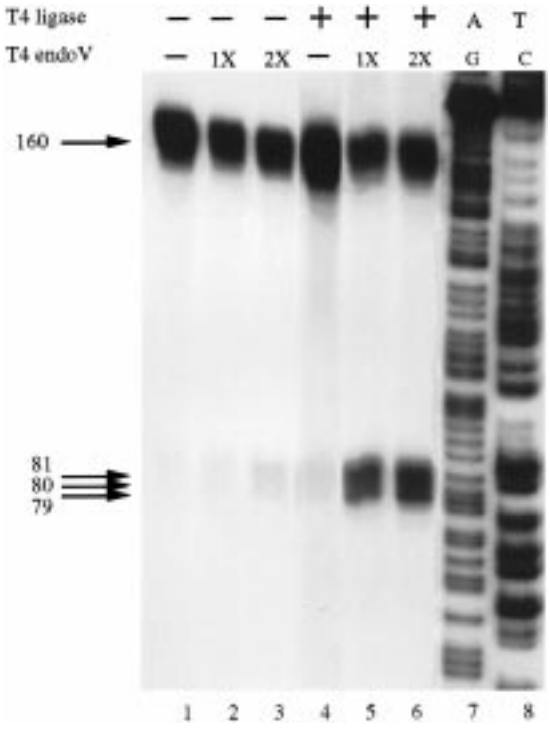

FIGURE 7: Effect of ligase treatment on the T4 endo V sensitivity of the 160-mer formed by UV-induced dimerization of 80-mer. UV-induced 160-mers purified by gel electrophoresis were treated with T4 ligases or calf thymus ligase II and then treated with T4 endo V as described in the text. The resultant DNAs were then separated by electrophoresis in an 8% denaturing polyacrylamide gel. T4 endo V cut generates DNA fragments with sizes of 79, 80, and 81 nucleotides. 1X and 2X represent 1- and 2-fold T4 endo V treatment.

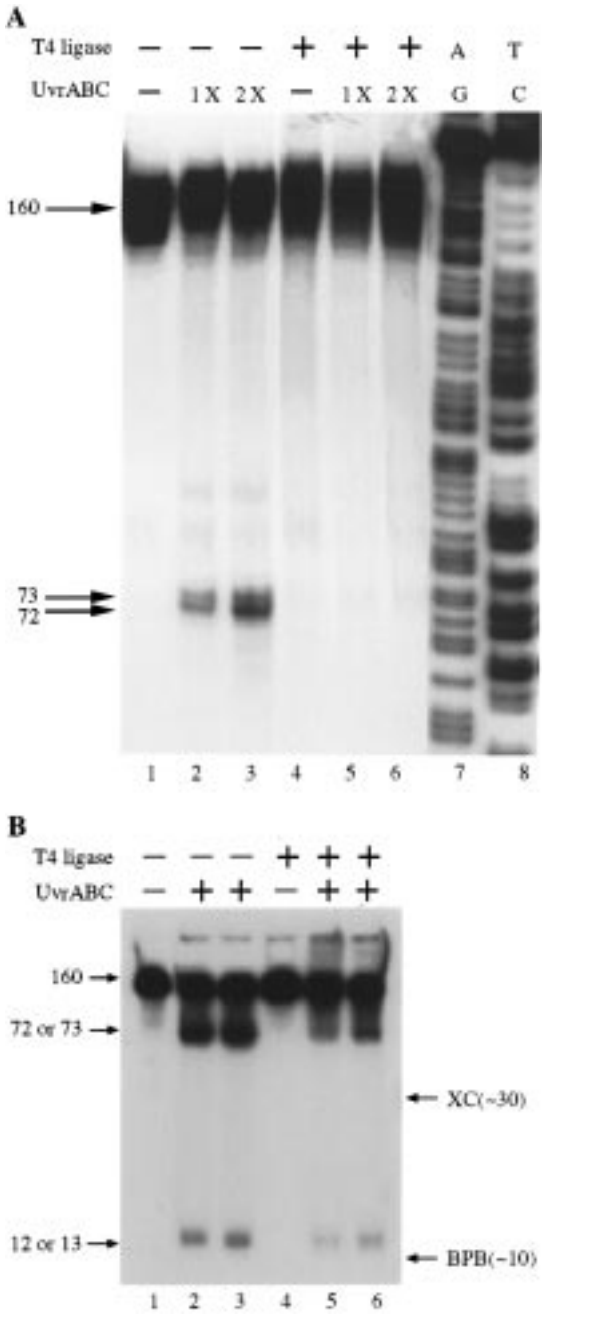

FIGURE 8: Effect of ligase treatment on the UvrABC nuclease sensitivity of the 160-mer formed by UV-induced dimerization of 80-mer. UV-induced 160-mers purified by gel electrophoresis were treated with T4 ligases or ligase II purified from calf thymus and then treated with UvrABC nucleases as described in the text. The resultant DNAs were then separated by electrophoresis in an 8% denaturing polyacrylamide gel (A) or in a 15% denaturing polyacrylamide gel (B). If we assume that UvrABC nuclease makes dual incisions 7−8 bp 5′ and 3−4 bp 3′ to a CPD or a CPD*, then this cut should generate a band with a size corresponding to 12−13 nt and a band with a size corresponding to a 72−73 nt. Lanes 7 and 8 in (A) are DNA size markers the same as described in Figure 4. In (B), the positions of xylene cyanol (XC) and bromophenol blue (BPB) are used as size markers.

from a form I or form II 160-mer, and 81 and 79 nt fragments (both are ^{32}P-labeled) from a form IV or form V 160-mer. We instead found that UV-induced 160-mers are refractory to cleavage by T4 endo V (Figure 7, lanes 2 and 3), suggesting that either (1) CPD* photodimers are not cleaved by T4 endo V, (2) 160-mers are formed by some unknown photochemical reactions other than by formation of one or two CPD* between two 80-mers, or (3) 160-mers are single-stranded DNA, as represented by Form III. To investigate the first possibility, we have treated these UV-induced 160-mers with T4 ligase. If it is indeed a CPD* which bridges two 80-mers to form the 160-mer, then T4 ligase treatment should convert this CPD* into a conventional CPD by sealing

Table 1: Effect of Ligase Treatment on the T4 Endo V and UvrABC Nuclease Sensitivity of the 160-mer Formed by UV-Induced Dimerization of 80-mer

	% cut	
T4 ligase treatment	−	+
control	0	0
T4 endo V	3.9	38.3
UvrABC	9.3	0.6

the phophodiester bond interruption, thereby rendering the UV-induced 160-mer sensitive to T4 endo V cleavage. As shown in Figure 7, a significant fraction of UV-induced 160-mers become sensitive to T4 endo V cleavage following T4 ligase treatment; this treatment produces three bands (81, 80, and 79 nt). These results lead us to conclude that a significant portion of UV-induced 160-mers are indeed formed by linking two 80-mers by one or two CPD* as forms I, II, IV, and V as depicted in Figure 6. Repeated treatments with T4 ligase or calf thymus ligase II (data not shown) do not increase the fraction of 160-mers which are sensitive to T4 endo V digestion; these results indicate that the conversion of CPD* to CPD by ligase may be inefficient and/or that a significant portion of UV-induced 160-mers may be form III single-stranded DNA.

Figure 8 shows that some of these UV-induced 160-mers are sensitive to UvrABC nuclease digestion. This enzyme treatment produces two bands corresponding in size to 72 and 73 nt (Figure 8A), as well as a band corresponding in size to 12−13 nt (Figure 8B). It is well established that UvrABC nuclease makes dual incisions 7−8 bp 5′ to and 3−5 bp 3′ to damaged bases (*2*, *3*). If we assume that UvrABC nuclease cuts both 5′ and 3′ of a CPD*, then we would expect that only 12−13 nt ^{32}P-labeled fragments should be generated from form I or II 160-mers, while both 12−13 and 72−73 nt ^{32}P-labeled fragments might be generated from forms IV and V 160-mers. The single-stranded form III should be refractory to UvrABC digestion. Since both 12−13 and 72−73 nt fragments were observed and many 160-mers were not cleaved at all, these results suggest all five forms of 160-mers may be induced by UV irradiation. Intriguingly, ligase treatment greatly decreased the sensitivity of these 160-mers to UvrABC nuclease digestion (compare lanes 2 and 3 to lanes 5 and 6 in Figure 8A,B).

Together, the results shown in Figures 7 and 8 reveal the opposite effects that T4 ligase treatment has on T4 endo V and UvrABC nuclease cleavage of UV-induced 160-mers. These effects are better demonstrated in Table 1; while T4 ligase treatment increases the sensitivity of UV-induced 160-mers to T4 endo V cleavage ∼10-fold, the same treatment greatly reduces the sensitivity of this substrate to cleavage by UvrABC nuclease.

DISCUSSION

We have found that UV irradiation of synthetic 80-mer oligonucleotides containing 5′- and 3′-terminal thymidine residues induces the formation of 160-mers containing one or two site-specific-CPD*. This conclusion is supported by our finding that these UV-induced 160-mers are reversible to 80-mers by photoreactivation. These substrates allowed us to examine how the nucleotide excision enzyme UvrABC nuclease and the cyclobutane pyrimidine dimer specific glycosylase/phosphodiesterase T4 endo V react with these modified CPD* in comparison with their reactions with CPD. The low efficiency of T4 endo V cleavage at these CPD* sites is consistent with the results of Lewis and Hanawalt (*21*), who reported that UV-induced dimers formed between thymidines at the ends of two oligonucleotides are refractory to T4 endo V digestion. In contrast, our finding that UvrABC cuts CPD* much more efficiently than CPD is much more surprising. It has been long recognized that, in comparison to many bulky chemical−DNA adducts such as 2-acetylaminofluorene− or benzo[*a*]pyrene diol epoxide−DNA adducts, CPD are poor substrates for UvrABC nuclease (*22*). Nevertheless, CPD are repaired rapidly and efficiently in *E. coli* cells (*23*). It has been shown that in a Uvr$^+$ genetic background, Phr$^-$ cells are more sensitive to UV irradiation than Phr$^+$ cells; however, this *phr* gene effect was not observed in Uvr$^-$ cells (*24*, *25*). These results suggest that other CPD-binding proteins besides Uvr proteins may facilitate CPD excision repair. Indeed, Sancar et al. (*26*) have shown that CPD bound by photolyase are more efficiently repaired by the UvrABC system. Since the number of photolyase molecules per *E. coli* cell is in the range of 10−20 and the CPD−photolyase binding is rather stable (*27*, *28*), it is unlikely the rapid CPD removal in irradiated cells is solely facilitated by photolyase binding. However, it is possible that in vivo, CPD may be subject to certain kinds of modifications that render them more readily recognizable by Uvr enzymes.

The *uvr* system recognizes and repairs a wide range of substrates and types of DNA damage. It is conceivable that the versatility of this system may come at the expense of efficiency; some types of DNA damage are certainly repaired more efficiently than others. On the other hand, CPD represent the major UV-induced photoproducts in DNA. If CPD are poor substrates for UvrABC nuclease per se, it is inconceivable that, over millions of years of evolution, cells would not have developed accessory mechanisms for enhancing recognition of CPD by the Uvr system. Enhancement of UvrABC incision of CPD by photolyase binding is just one example. Our results suggest that cleavage of the phosphodiester bond within a CPD may be another way to enhance Uvr recognition. It is notable that Patterson et al. (*17*) have reported that 85% of excised cyclobutane pyrimidine dimers in human cells contain no phosphodiester bond between the two pyrimidines. The conversion of CPD → CPD* may be an important preincision step for enhancing recognition of pyrimidine dimers by the nucleotide excision enzymes. Whether this CPD processing occurs in vivo in *E. coli* cells remains to be seen. However, our finding that a CPD* is a better in vitro substrate for UvrABC nucleases than a CPD suggests that processing CPD to CPD* as a preincision step might be a distinct possibility.

We do not know why UvrABC and T4 endo V appear to cut only a portion of the UV irradiation-induced 160-mers. Formation of the single-stranded form III 160-mers may account for some of this resistance to enzyme digestion. Since a relatively high UV fluence (4000 J/m^2) was used for generating these 160-mers, it is possible that some 160-mers may contain more than one CPD* and/or other photoproducts. It is known that CPD and other photodamaged bases may alter DNA structure significantly (*29*);

perhaps the dramatic changes in DNA structure produced by multiple CPD* or other photoproducts within such a small (160-mer) region may hinder recognition by UvrABC, T4 endo V, or even restriction enzymes. We have generally found that short DNA fragments modified with CC-1065, mitomycin C, anthramycin, tomaymycin, BPDE, and 7,12-dimethylbenz[*a*]anthracene diol epoxide (particularly at high levels of modification) are poor substrates for UvrABC nucleases (*18*, *30*).

The question of whether an interruption of the phosphodiester bond in cyclobutane pyrimidine dimers other than thymine dimers will change their susceptibility toward T4 endo V and UvrABC nuclease is currently under investigation. Since thymine dimers represent the major UV-induced CPD, our findings that CPD* can be photoreactivated and that ligase treatment can dramatically change the T4 endo V and UvrABC nuclease sensitivities of CPD* may provide useful tools for studying the processing of CPD in vivo, and determining whether the conversion of CPD → CPD* may be an important preincision step for enhancing recognition of pyrimidine dimers by the nucleotide excision repair enzymes.

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