

# Repair of 4,5',8-trimethylpsoralen monoadducts and cross-links by the *Escherichia coli* UvrABC endonuclease

(photoreaction/DNA cross-link/DNA repair/UV endonuclease/mutagenesis)

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Communicated by Richard B. Setlow, August 4, 1988 (received for review November 24, 1987)

**ABSTRACT** Using an oligonucleotide model substrate, we observed two unusual mechanisms of UvrABC endonuclease in the repair of 4,5',8-trimethylpsoralen monoadducts and cross-links. (i) UvrABC endonuclease usually incises a psoralen monoadduct only on the damaged strand. However, for one of the monoadducts we studied, incision on the complementary undamaged strand was also observed at a very low frequency, as though the adduct were on the thymine across from the damaged strand. Although the details of the erroneous incision are not yet known, such erroneous incision is potentially mutagenic. (ii) In cross-link repair, we observed that the UvrABC endonuclease incises the cross-linked DNA on either the furan side strand or the pyrene side strand. The incisions are not equally efficient. These data suggest that the structure of a psoralen cross-link, as seen by a repair enzyme, varies with the DNA sequence.

Psoralens are a family of photoreactive furocoumarins, planar three-ringed heterocyclic hydrocarbon compounds (1), which have been used to treat skin ailments such as psoriasis (2). 4,5',8-Trimethylpsoralen (TMP) is specific for 5'-TpA sequences and photoreacts with thymines in a 5'-TpA sequence in a DNA duplex to form first a furan side monoadduct and then an interstrand cross-link (3, 4). Both the monoadducts and the cross-links of psoralens are known to be repaired by nucleotide excision repair systems such as the UvrABC endonuclease system of *Escherichia coli* (5, 6).

With a psoralen monoadduct, UvrABC endonuclease incises at the eighth phosphodiester moiety 5' to the modified base and at the fifth phosphodiester moiety 3' to the modified base (3, 7). *In vitro*, at least, the incisions are not always bimodal and precise. Sometimes, only one of the two incisions is made (8). The reason for these single incisions is not known. The 5' incision at a psoralen monoadduct lesion can also be at the ninth instead of the eighth phosphodiester moiety (3). This finding suggests that the sequences around a lesion may exist in more than one conformation, some or all of which can be acted upon by the UvrABC endonuclease (3).

Depending on the orientation of a psoralen molecule in a given 5'-TpA site, two isomers of psoralen cross-links exhibiting different DNA helix stabilities are formed (4). Our present study will determine whether the UvrABC endonuclease would respond differently to the two cross-linked isomers. This paper also provides enzymatic evidence that the structure of a psoralen cross-link varies with the sequence of the DNA. To begin to examine how errors in DNA repair might be important to mutagenesis, we tested and observed that the UvrABC endonuclease can err and incise the undamaged DNA strand across from a psoralen monoadduct.

## METHODS

**Preparation of Homogeneous DNA Substrates Containing TMP Monoadducts and Cross-Links.** To avoid UV damage, all oligonucleotides and substrates were purified from polyacrylamide gels by isolation of the bands located by autoradiography. All purification gels were run with the lightly labeled oligonucleotides ( $^{32}\text{P}$ ,  $\approx 100,000$  dpm/ $\mu\text{g}$ ) migrating at least 35 cm in 10% or 20% polyacrylamide gels such that the full-length oligonucleotides were well resolved from the  $n - 1$  length side products of DNA synthesis and from the non-fully deblocked oligonucleotides. A TMP monoadduct of a 57-mer strand can be resolved from an unreacted strand. Fig. 1 shows the sequences of the oligonucleotides used in this study. The TMP monoadduct-containing TY12 and TY15 were prepared as described (4) and as follows: TY12 and TY15 were lightly labeled by phosphorylation with T4 polynucleotide kinase to completion and used in TMP photoreactions to produce the furan side monoadducts. These monoadducts were gel purified. For those oligonucleotides in which the presence of a 5'  $^{32}\text{P}$  will interfere with interpretation of the results of later experiments, the label was removed by calf intestinal alkaline phosphatase and replaced with unlabeled phosphate. The 57-mer containing a monoadduct was prepared as follows: the monoadduct containing TY12 was ligated to TY20 and TY13, using the full-length TY19 as template, to obtain maximum hybridization and ligation. Five micrograms of each of the three short oligonucleotides and 15  $\mu\text{g}$  of TY19 were incubated with 125 units of T4 DNA ligase for 45 min at 22°C. Products of incomplete ligation were removed by strand purification of the 57-mer on denaturing polyacrylamide gels. The monoadduct-containing 56-mer was made in the same manner. Labeling of the 57-mer duplex at one of the four termini was done after the gel isolation, using methods previously described (9). The gel purified monoadduct-containing full-length oligonucleotide was hybridized to a full-length complementary strand, and precise pairing was assured by purification of the duplex on a 10% polyacrylamide nondenaturing gel. The cross-linked substrates were prepared as follows: the paired full-length monoadduct-containing substrate was further irradiated by 365-nm UV light at 156 kJ/m<sup>2</sup> to form the cross-links and was then gel purified on 10% polyacrylamide/7 M urea denaturing gel at 49°C as described (4). When cross-link incisions and monoadduct incisions are compared, both substrates have identical specific activities because they were prepared from the same lot of labeled 57-mer DNA and were used simultaneously. The results described in this paper have been repeated in at least three independent experiments.

**The UvrABC Endonuclease Incision Assay.** Purified UvrABC endonuclease (10) incision conditions were the same as in our previous studies with other forms of substrates (3, 8, 11).

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Abbreviation: TMP, 4,5',8-trimethylpsoralen.

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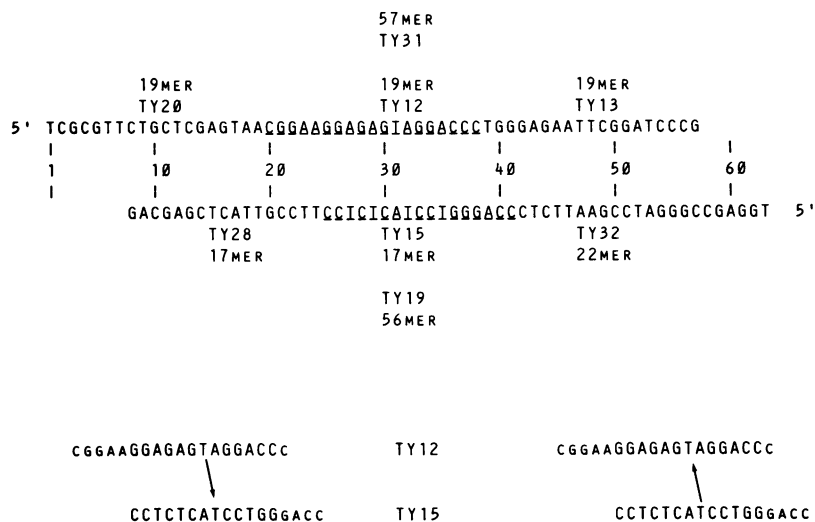


FIG. 1. Oligonucleotides used in this study. TY31 is a 57-mer which can also be assembled from TY20, TY12 (underlined), and TY13. When labeled at the 3' end by the Klenow fragment and dGTP, it becomes 58 bases long. TY19 is a 56-mer, which can also be assembled from TY28, TY15 (underlined), and TY32. When labeled in the 3' end by the Klenow fragment and dATP and dCTP, it becomes 59 bases long. The TY12 and TY15 duplex contains a single 5'-TpA site capable of forming TMP monoadducts in two orientations. (Lower Left) Furan ring of the TMP bonding to TY12. The direction of the arrow indicates the TMP molecule pointing from the furan side to the pyrone side. (Lower Right) Furan ring bonding to TY15. The bases in larger capital letters represent the core sequences of this duplex conserved from the oligonucleotides I and II used in a previous study (4), which demonstrated that the two TMP cross-links in this DNA sequence have different helix stabilities.

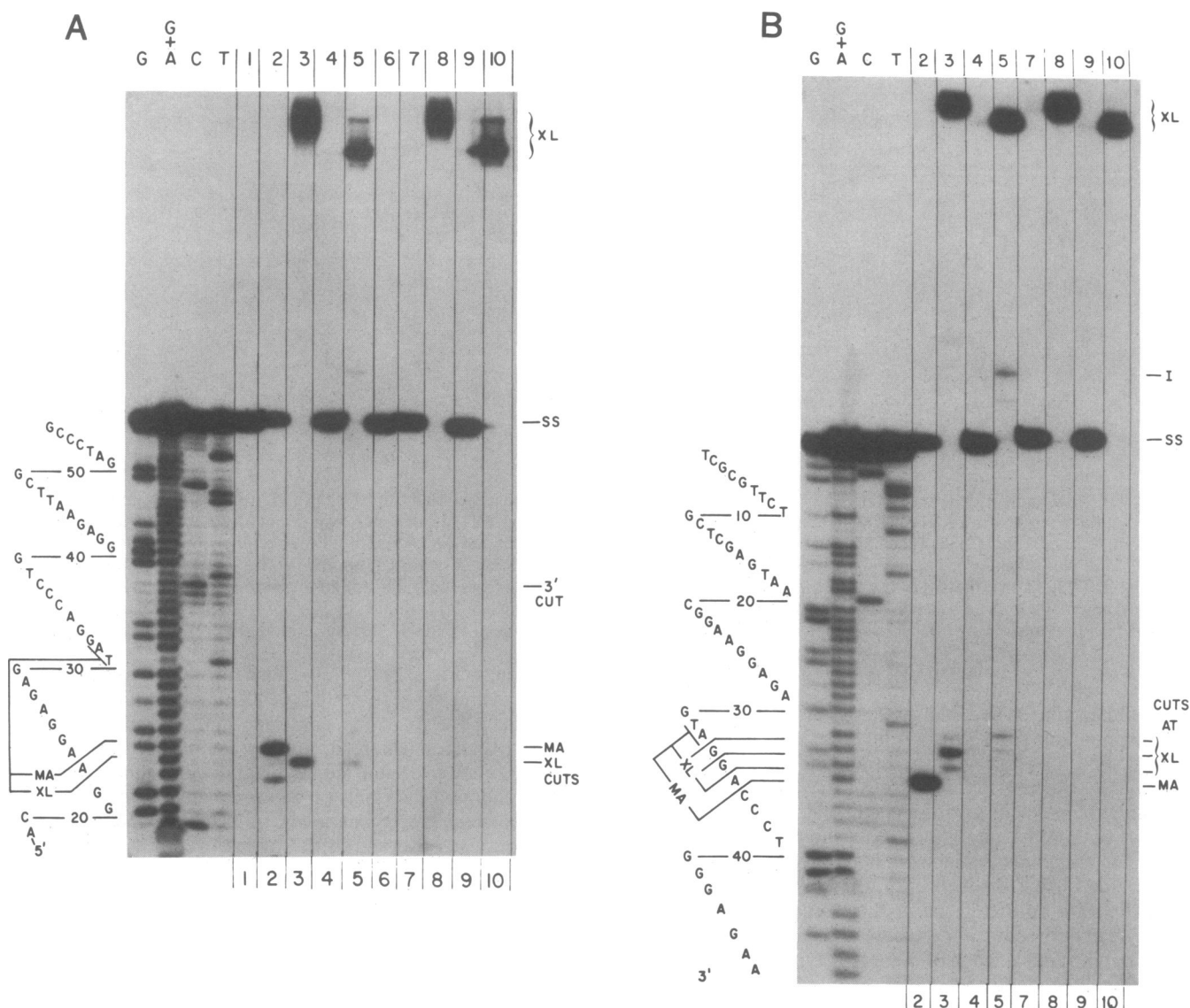
**RESULTS**

We studied four types of psoralen substrates: (i) the furan side monoadduct of TY19, (ii) the furan side monoadduct of TY31, (iii) cross-link with the furan side on TY31, and (iv) cross-link with the furan side on TY19. It is necessary to examine the mechanisms of incision of UvrABC endonuclease on both DNA strands in each substrate. Because the UvrABC endonuclease incision is usually bimodal, once on either side of the modified base, the DNA strand has to be labeled either on the 5' end or the 3' end in each strand in separate experiments to score both of the incisions. Thus, the two DNA strands of each of these four types of adducts were labeled independently on one of the two 5' termini or one of the two 3' termini, producing 16 different substrates. All these substrates were prepared simultaneously. Together, the possibilities of incision by UvrABC endonuclease on both the 5' and 3' sides of each lesion, on either DNA strand, were examined. Each of the four panels in Figs. 2 and 3 contains DNA labeled at only one of the four termini of the TY31/TY19 DNA duplex. Using the four chemical DNA sequencing lanes as markers in each panel, we compared the incisions of the four types of TMP adducts. Details of the relationship of positions of the UvrABC endonuclease incision bands to the DNA sequencing ladder have been described (8). The results of the incision analyses are summarized in Fig. 4 for each of the four types of TMP adducts.

**UvrABC Endonuclease Incision at the Monoadduct Lesions.** The simplest incision pattern was seen for the furan side monoadduct of TY19 where UvrABC endonuclease incised the psoralen-containing DNA strand once at the eighth phosphodiester moiety on the 5' side of the adduct (Fig. 3A, lane 2) and once at the fifth phosphodiester moiety on the 3' side of the adduct (Fig. 3B, lane 2), with no apparent incision on the undamaged strand (Fig. 2A and B, lanes 4). For the furan side monoadduct of TY31, the incisions were mainly in the psoralen-containing DNA strand at the eighth phosphodiester moiety on the 5' side of the adduct (Fig. 2A, lane 2) and at the fifth phosphodiester moiety on the 3' side of the adduct (Fig. 2B, lane 2). Two other types of incisions that differ from this incision pattern were also observed. One type consisted of minor incisions 10 phosphodiester moieties on the 5' side of the modified base on the damaged strand (Fig.

2A, lane 2; Fig. 3A, lane 2). These unusual incisions support our previous suggestion that the lesion-containing DNA may assume more than one conformation recognizable by the UvrABC endonuclease (3). In Figs. 2A and 3A (lanes 2), three other minor psoralen monoadduct-specific aberrant UvrABC endonuclease incision bands can also be observed, but the exact cause is unknown. A second type of unusual incision was observed in the undamaged strand, the TY19 from a full-length synthesis, which had never participated in any psoralen reaction or UV irradiation (Fig. 3B, lane 4). The incision was produced at a low frequency on the undamaged complementary strand as though the adduct was on a 5'-TpA site across from the damaged strand, corresponding to the fifth phosphodiester moiety 3' from that thymine base. The band that corresponds to the 5' incision at this phantom lesion position tends to be very weak and difficult to demonstrate (data not shown). Whether double-stranded breaks can result from these incisions has not been examined.

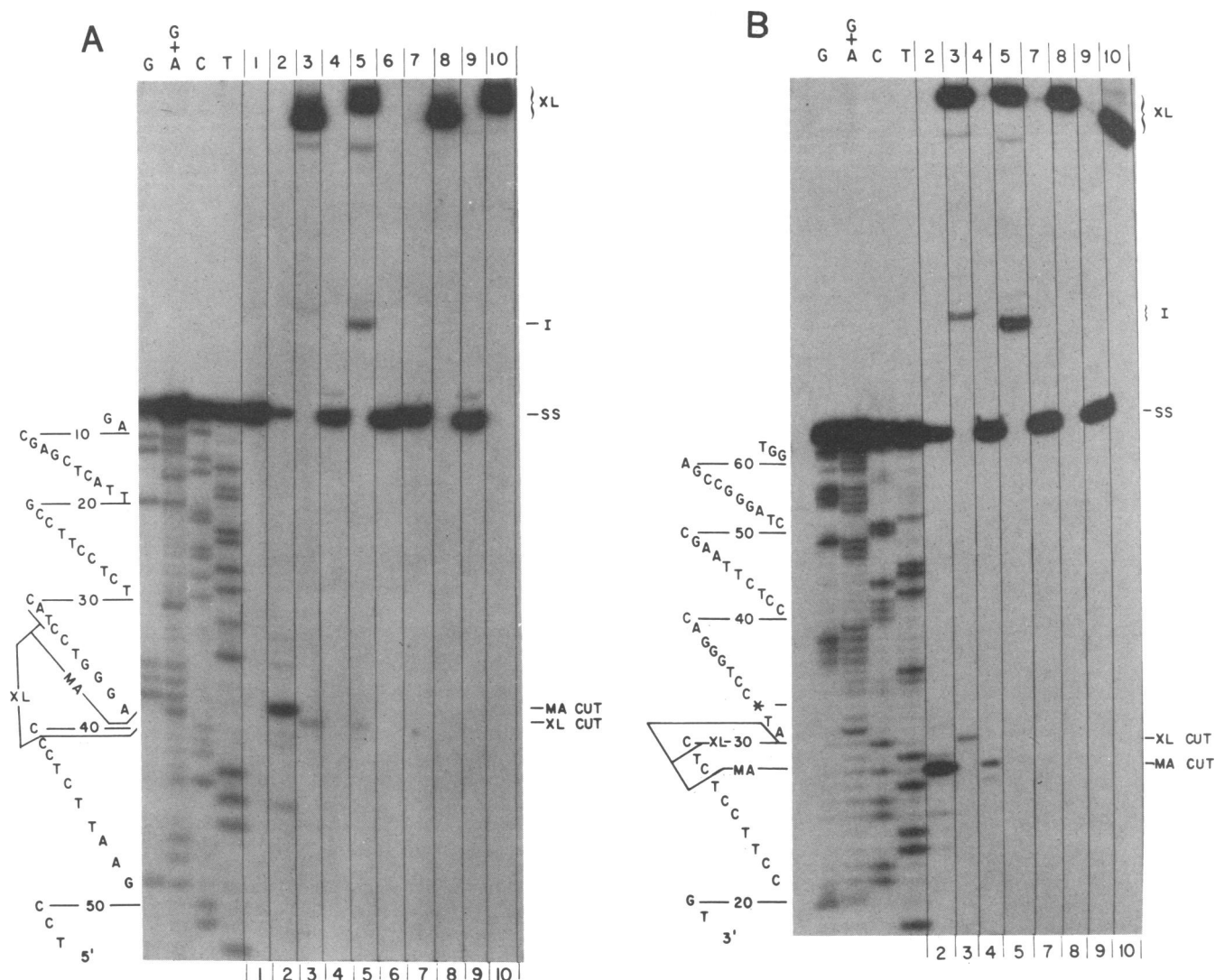
**UvrABC Endonuclease Incision of the TMP Interstrand Cross-Link.** The furan ring of TMP was attached to TY31 in Fig. 4C and to TY19 in Fig. 4D. All incisions for the cross-links were slow relative to the monoadduct incisions. Depending on the incision position in the cross-linked helix, the rate varies from ≈6% to 20% of the incision rate at the monoadduct lesions (these preliminary data were obtained by cutting out the bands on the gel, guided by an autoradiogram, eluting the DNA, and quantitating by scintillation counting; data not shown). The incisions may occur on either strand for a given orientation of the psoralen molecule between the two strands. The 5' incision in each case is at the ninth phosphodiester moiety from the modified thymine (Figs. 2A and 3A, lanes 3 and 5). The 3' incision is at the second, third, or fourth phosphodiester moiety from the modified thymine, with the major incision at the third phosphodiester moiety (Figs. 2B and 3B, lanes 3 and 5). The most efficient 5' and 3' incisions are those in TY31 with the furan ring on TY31 (Fig. 4C and Fig. 2, lanes 3). For the same cross-link, the incisions in TY19 (Fig. 3, lanes 5) are at least 3 times less efficient, but approximately equal to the incisions for the cross-link of the other psoralen orientation (Fig. 4D), on both TY31 (Fig. 2, lanes 5) and TY19 (Fig. 3, lanes 3), when the furan ring is on TY19.



**FIG. 2.** Autoradiogram of UvrABC endonuclease incision in TY31, of TMP monoadducts and cross-links, analyzed by a denaturing polyacrylamide gel. (A) The 5' side of TY31 was labeled by T4 polynucleotide kinase and [ $^{32}$ P]ATP. (B) The 3' side of TY31 was labeled by Klenow fragment with [ $^{32}$ P]dGTP. About 100–200 fmol of end-labeled DNA was incised by 300 fmol of UvrA, 300 fmol of UvrB, and 760 fmol of UvrC in a vol of 140  $\mu$ l, containing 85 mM KCl, 40 mM potassium morpholinopropanesulfonate (pH 7.6), 1 mM dithiothreitol, 10 mM MgCl<sub>2</sub>, and 2 mM ATP. The reactions were terminated with the addition of EDTA to 50 mM, followed by phenol extraction, ether extractions, ethanol precipitation, and washes. The final DNA pellet was dissolved in tracking dye (90% formamide/50 mM Tris borate/1 mM EDTA, pH 8.3, plus bromophenol blue and xylene cyanol) and analyzed by autoradiography after resolution on a 10% polyacrylamide/7 M urea denaturing gel as described (3). The chemical DNA sequencing lanes for reference are indicated in the figures. The same substrates are used in corresponding numbered lanes in the two panels. Lanes 1 and 6 are missing in B. (A) Lanes 1–5 and 6–10, plus or minus UvrABC endonuclease in the incubations, respectively. (B) Only lanes 2–5 were treated with UvrABC endonuclease. Half as many counts of radioactivity was loaded in lanes 2 as in the other lanes. Lanes: 1 and 6, undamaged DNA; 2 and 7, furan side monoadduct on TY31; 3 and 8, TMP cross-link with the furan ring on TY31; 4 and 9, furan side monoadduct in TY19; 5 and 10, TMP cross-link with the furan ring of TMP on TY19. A line was traced from each UvrABC endonuclease incision band, through the phosphodiester moiety that the incision corresponded to, to the thymine base modified by TMP. Substrates in lanes 2–5 correspond to the substrates in lanes 4, 5, 2, and 3 in Fig. 3, respectively. XL, TY31/TY19 cross-linked duplex; SS, single-stranded TY31; I, intermediate of cross-link repair, with the 11 bases between the two incisions on TY19 cross-linked to the full-length TY31. The cuts on TY31 at the cross-link (XL) and monoadduct (MA) are indicated.

That the UvrABC endonuclease incisions can occur on both the furan side strand and the pyrone side strand of a cross-link can be determined in an alternative manner. Consider the lanes of UvrABC endonuclease incision of the cross-links in Figs. 2 and 3: Besides the incision bands 21–25 bases long, there are bands between the single-stranded length of 57 bases and the double-stranded cross-links of 114 bases at apparently three different locations in those lanes. The appearance of these bands are UvrABC endonuclease dependent. Importantly, our thorough protocol of substrate preparation had eliminated all bands that might have resulted from incomplete ligation. In addition, the conditions of our gel analyses had eliminated

bands of alternative mobilities for each cross-linked DNA. The simultaneous appearance of UvrABC endonuclease incision bands 21–25 bases long and bands longer than 57 bases requires that UvrABC endonuclease nick either the labeled strand or the unlabeled strand in different cross-linked duplexes. For example, consider the autoradiogram in Fig. 3B (lane 3) illustrated by the drawing in Fig. 4D. In this experiment, TY19 was 3'-labeled and the incision on TY19 at the 3' side of the modified thymine produced the incision bands 25 and 24 bases long (labeled "XL cuts"). In separate duplexes, other incisions, probably at both the 5' and the 3' side of the modified base in TY31 without incisions in TY19, produced



**FIG. 3.** Autoradiogram of UvrABC endonuclease incision in TY19, of TMP monoadducts and cross-links, analyzed by a denaturing polyacrylamide gel. (A) The 5' side of TY19 was labeled. (B) The 3' side of TY19 was labeled with dATP and [ $^{32}$ P]dCTP. The chemical DNA sequencing lanes for reference are indicated in the figures. The same substrates are used in corresponding numbered lanes in the two panels. Lanes 1 and 6 are missing in B. (A) Lanes 1–5 and 6–10, plus or minus UvrABC endonuclease in the incubation, respectively. (B) Lanes 2–5, treated with UvrABC endonuclease. Half as many counts of radioactivity was loaded in lanes 2 as in the other lanes. Lanes: 1 and 6, undamaged DNA; 2 and 7, furan side monoadduct on TY19; 3 and 8, TMP cross-link with the furan ring on TY19; 4 and 9, furan side monoadduct in TY31; 5 and 10, TMP cross-link with the furan ring of TMP on TY31. XL, TY31/TY19 cross-linked duplex; SS, single-stranded TY19; I, intermediate of cross-link repair, with the 11 bases between the two incisions on TY31 cross-linked to the full-length TY19. The cuts on TY19 at the cross-link (XL) and monoadduct (MA) are indicated. Note that the monoadduct-modified TY19 was purposely used in the DNA sequencing ladder in B. The presence of the psoralen caused (i) the modified T not to react with  $\text{KMnO}_4$  in the T-specific reaction, (ii) all the bands 5' of the modified T to shift one band spacing upwards, thus (iii) creating a phantom T\* band position above the T position. This method shows that  $\approx 100\%$  of our substrate contained the psoralen modification at the location we intended.

the band  $\approx 70$  bases long in lane 3 (labeled "I"). This band may represent the full-length 3'-labeled TY19 cross-linked to an 11-base-long oligonucleotide produced by the double incisions in TY31 by UvrABC endonuclease. At a lower frequency, putative single incisions on either the 5' or the 3' side of the modified base, but not both sides, may be observed as bands of even slower mobility. Similar reasoning can be applied to all the UvrABC endonuclease incision lanes of cross-linked substrates in this study to indicate that either strand of a given cross-link may be incised by UvrABC endonuclease. Preliminary results have indicated that we cannot detect double-stranded cuts in our substrate, although it might occur at a very low frequency.

We do not think photoisomerization of cross-links (12) occurred in our TMP studies to contribute to the pyrone side incisions: first, the two TMP cross-link isomers are easily

separated in the denaturing gel even if both were present (data not shown); second, additional 365-nm UV irradiation of the isolated cross-links did not produce increased pyrone side UvrABC endonuclease incision (data not shown); third, the pyrone side monoadduct did not accumulate, nor was there any UvrABC endonuclease incision band that corresponded to incisions at monoadducts (Figs. 2 and 3) in any of these cross-link incision lanes.

## DISCUSSION

**UvrABC Endonuclease Repair of Monoadduct Lesions.** UvrABC endonuclease repairs a spectrum of apparently dissimilar lesions (6, 13) that share the characteristics of "bulkiness" and covalent bonding. This flexibility suggests that the UvrABC endonuclease can accommodate a variety of DNA conformations. One may predict that such a plastic

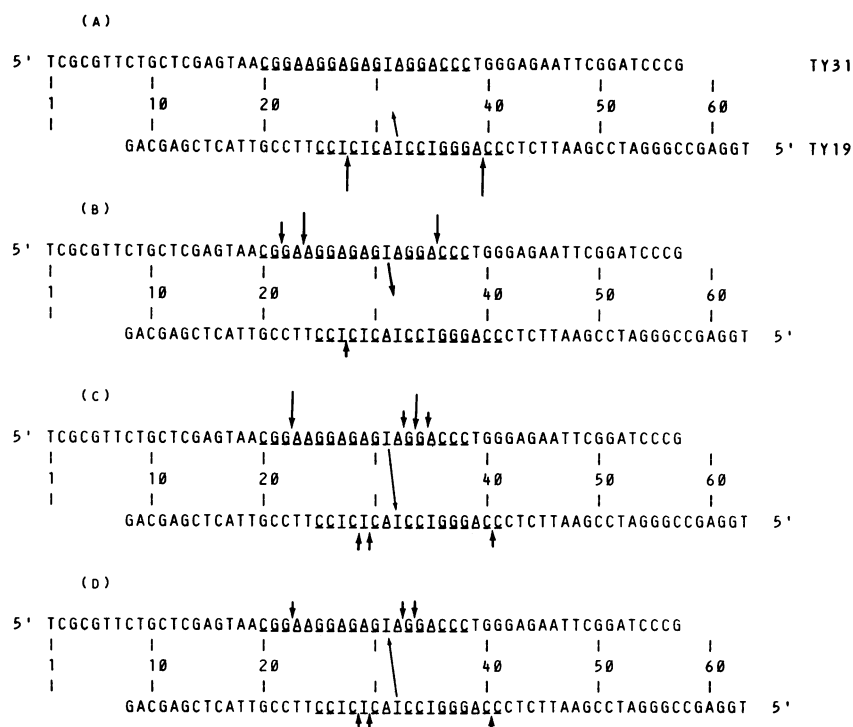


FIG. 4. Patterns of incision of UvrABC endonuclease at the four kinds of psoralen adducts at the same 5'-TpA site. (A) Furan side monoadduct in TY19, (B) furan side monoadduct on TY31, (C) cross-link with the furan side of psoralen on TY31, (D) cross-link with the furan side of psoralen on TY19. Data were obtained from Figs. 2 and 3. The positions of the psoralen molecules are indicated by arrows between the two DNA strands as in Fig. 1 (Lower). The directions of the arrows indicate for the furan ring to the pyrone ring. The phosphodiester moieties broken are indicated by vertical arrows, the lengths of which approximate the relative efficiencies of the observed incisions.

enzyme system may, at certain DNA sequences and frequency, incise the undamaged DNA strand across from a DNA lesion. We have now observed this phenomenon in the incision of the undamaged TY19 due to the presence of a TMP monoadduct on the complementary strand TY31. It is possible that this observation is unique for some psoralen monoadducts because a monoadduct of TMP is essentially equivalent to an intercalator of infinite affinity. Such high-affinity intercalation may impose a distortion on the opposite strand of the DNA to make it a substrate for UvrABC endonuclease. Because this incision across from a monoadduct was not observed when the monoadduct was on TY19, such incisions may be sequence dependent. It will be meaningful to examine other nonintercalator substrates of UvrABC endonuclease to see if similar incisions across from a lesion can be observed. Erroneous cleavage at only a very small fraction of the UvrABC endonuclease repairable lesions in a cell would be numerically significant if they lead to mutations at high probability. The nick in the DNA may not be readily ligated if a protein-DNA complex persists at the incision site. Alternatively, gap filling by DNA polymerase I using a lesion-containing template may be error prone.

**UvrABC Endonuclease Repair of Cross-Link Lesions.** In this study, we have observed that the UvrABC endonuclease incision can occur on either strand of a cross-linked helix. Since the rates of incisions were about the same for monoadducts with the TMP molecule located on either strand, the cross-links must have imposed new conformations on the DNA. That Van Houten *et al.* (14) observed only furan side incisions and Seeberg and co-workers (15) reported no incisions at psoralen cross-links can be rationalized as follows: We propose that psoralen cross-links, as seen by UvrABC endonuclease, exhibit at least four major conformations in a DNA sequence-dependent manner. A given cross-link isomer will exhibit one major conformation, which causes UvrABC endonuclease incision to be (i) mainly on the furan side, (ii) mainly on the pyrone side, (iii) same efficiency

on either side, or (iv) no incisions on either side. The results of this paper are consistent with this hypothesis.

These studies were supported, in part, by grants from the National Science Foundation (DMB84-17419 and DMB88-02091) to A.T.Y., institutional grants from the National Institutes of Health to the Fox Chase Cancer Center (CA06927 and RRO5539), an appropriation from the Commonwealth of Pennsylvania, and a grant from the Glenmede Trust to the Fox Chase Cancer Center.

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