Alkali Reversal of Psoralen Cross-Link for the Targeted Delivery of Psoralen Monoadduct Lesion†

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ABSTRACT: Psoralen intercalates into double-stranded DNA and photoreacts mainly with thymines to form monoadducts and interstrand cross-links. We used an oligonucleotide model to demonstrate a novel mechanism: the reversal of psoralen cross-links by base-catalyzed rearrangement at 90 °C (BCR). The BCR reaction is more efficient than the photoreversal reaction. We show that the BCR occurs predominantly on the furan side of a psoralen cross-link. The cleavage does not result in the breaking of the DNA backbone, and the thymine base freed from the cross-link by the cleavage reaction appears to be unmodified. Similarly, BCR of the furan-side monoadduct of psoralen removed the psoralen molecule and regenerated the unaltered native oligonucleotide. The pyrone-side psoralen monoadduct is relatively resistant to BCR. One can use BCR to perform efficient oligonucleotide-directed, site-specific delivery of a psoralen monoadduct. As a demonstration of this approach, we have hybridized a 19 base long oligonucleotide vehicle containing a furan-side psoralen monoadduct to a 56 base long complementary oligonucleotide target strand and formed a specific cross-link at the target site with 365-nm UV. Subsequent BCR released the oligonucleotide vehicle and deposited the psoralen at the target site.

The cytotoxicity of psoralen plus long-wave UV light has been used for the treatment of skin ailments since ancient times (Scott et al., 1976). Psoralens are also useful probes of nucleic acid structures [for a review, see Cimino et al. (1985)]. The psoralen monoadducts and cross-links in cellular DNA are repaired by nucleotide excision repair systems, e.g., the UvrABC endonuclease system of *Escherichia coli* (Cole et al., 1976).

The 5'TpA sequences are preferred sites for the formation of 4,5',8-trimethylpsoralen (TMP)[1] monoadducts and cross-links (Yeung et al., 1987). The thymine–psoralen–thymine cross-links can be photoreversed (Shim & Kim, 1983; Thompson & Hearst, 1983; Kanne et al., 1982; Yeung et al., 1988a). Because the photoreversal of a psoralen cross-link occurs on both the furan side and the pyrone side, the yield of pyrone-side monoadduct by this method is not quantitative.

In the present study, we illustrate the ability of base-catalyzed rearrangement at 90 °C (BCR) to selectively and quantitatively cleave the psoralen cross-link at the furan side. We partially characterize some of the products of the cleavage reaction. We also demonstrate how BCR can be used to transfer a psoralen monoadduct from an oligonucleotide vehicle to a target site, via the formation and BCR cleavage of a cross-linked intermediate.

Experimental Procedures

Preparation of Oligonucleotides. Oligonucleotides were synthesized by an Applied Biosystem DNA synthesizer 380A and purified by fragment isolation on denaturing polyacrylamide gels. The DNA sequences of the oligonucleotides were verified by chemical DNA sequencing as described (Yeung et al., 1988b).

Electrophoresis of Oligonucleotides. All denaturing polyacrylamide gels used in this study were 0.5 mm × 30 cm × 40 cm, 10 or 20% acrylamide, 37.5:1 acrylamide to bis(acrylamide), 7 M urea, and 1X TBE (50 mM Tris–borate, 1 mM EDTA, pH 8.3). All gels were run between 43 and 49 °C at 2200 V for 3.5–4 h. The sample buffer contained 95% formamide and 0.025% bromophenol blue in 1X TBE. Samples were dissolved in 10 μL of sample buffer and heated for 30 s at 90 °C. The nondenaturing polyacrylamide gels used in this study were of the same dimension, 10% in acrylamide in 1X TBE, and were run at about 40 °C at about 2000 V. The nondenaturing gel sample buffer contained 1X TBE, 10% glycerol, and 0.025% bromophenol blue.

Preparation of ^{32}P-Labeled Oligonucleotides. Oligonucleotides were 5'-end-labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase as described (Maxam & Gilbert, 1980) except unlabeled ATP was used to chase the phosphorylation reaction to completion. Oligonucleotides were quantitated by UV absorption at 260 nm. One A260 of single-stranded oligonucleotide was taken as 30 μg. About 20 μg of oligonucleotide was used in a typical phosphorylation experiment and in subsequent gel purification. The use of this amount of DNA lessened the effect of acrylamide contamination on the quantitation of the labeled DNA by UV absorption. The labeled DNA was purified by electrohoresis and fragment isolation on a 10%–20% acrylamide 7 M urea DNA sequencing gel as described (Maxam & Gilbert, 1980). These gels were polymerized at least 18 h at room temperature to minimize the amount of nonpolymerized acrylamide that can be extracted with the DNA.

Photoreaction of Psoralen with the Oligonucleotides. The conditions for the formation of psoralen monoadducts and cross-links as well as the photoreversal reactions were previously described in detail (Yeung et al., 1988a). In general, single-stranded oligonucleotides do not react with TMP be-

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[1] Abbreviations: TMP, 4,5',8-trimethylpsoralen; HMT, 4'-(hydroxymethyl)-4,5',8-trimethylpsoralen; 8-MOP, 8-methoxypsoralen; oligo, oligonucleotide; BCR, base-catalyzed rearrangement at 90 °C; 1X TBE, 50 mM Tris–borate and 1 mM EDTA, pH 8.3; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; MA, monoadduct.

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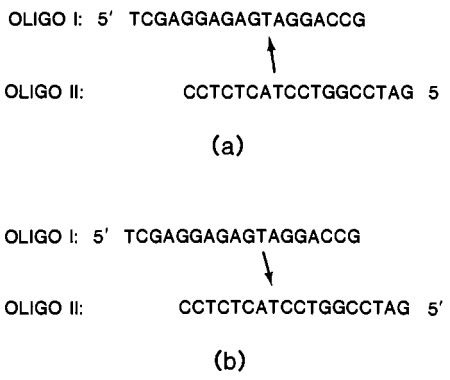

FIGURE 1: Sequences of the complementary oligonucleotides, oligo I and oligo II, used in this study. The arrows indicate the orientations of the psoralen molecule from the furan ring toward the pyrone ring. (a) and (b) are the two orientational isomers of psoralen cross-link at the 5' TpA sequence favoring the psoralen photoreaction: (a) upper cross-link; (b) lower cross-link.

cause of the absence of TMP intercalation. Thus, while we try to perform most reactions with the unlabeled oligonucleotide in slight excess of the labeled oligonucleotide, there is no need to keep their ratio close to one to one.

BCR Reversal of the Psoralen Cross-Links. The sequences of the 18 base long oligonucleotides used to demonstrate the properties of BCR are illustrated in Figure 1. A typical BCR reaction consisted of 150 μL of a mixture containing 0.2 μg of ^{32}P-labeled cross-linked oligonucleotide, 0.1 M in KOH, heated for 30 min at 90 °C. The mixture was neutralized with 33 μL of 3 M sodium acetate, pH 7.0. Absolute ethanol (0.7 mL) was added to precipitate the DNA. The DNA pellet from centrifugation was washed twice with 80% ethanol and dried in vacuo. The DNA was dissolved in 10 μL of sample buffer and heated for 30 s at 90 °C. Three microliters of this sample was loaded into a well of a DNA sequencing gel for analysis.

BCR Reversal of the Psoralen Monoadducts. The monoadducts tested were of three types. The first type was the furan-side TMP monoadduct from the forward photoreaction of TMP with an oligonucleotide duplex. The second type was the pyrone-side monoadducts generated in either DNA strand by the alkaline reactions of the TMP cross-linked DNA in experiments described above. The lactone ring of the psoralen molecule was expected to be opened by hydrolysis in these monoadducts. The third type of monoadducts was the furan-side monoadducts and pyrone-side monoadducts generated in either DNA strand by 254-nm UV photoreversal of isolated TMP cross-linked DNA at neutral pH. These monoadducts were gel purified. The BCR reactions for the monoadducts, sample preparation, and analyses were the same as for the analyses of BCR of the cross-links.

Experiment To Demonstrate the Targeted Delivery of Psoralen Monoadduct. The sequences of the DNA used for this experiment are shown in Figure 2, and the schematic presentation of the experiment is shown in Figure 3. The target to receive the psoralen monoadduct is a thymine at position 32 (numbered as in Figure 2) in a 5'TpA sequence in the middle of a 56 base long oligonucleotide TY19. TY12 is a 19 base long oligonucleotide vehicle that will hybridize to TY19. One A260 unit of TY12, labeled at the 5' termini with ^{32}P (Maxam & Gilbert, 1980), and TY15 hybridized to form a duplex. The duplex was reacted with TMP and 365-nm UV to form furan-side monoadducts on TY12 and TY15, as well as two cross-link isomers (see insert of Figure 3). The conditions for the reaction were previously described in detail (Yeung et al., 1988a). Briefly, the conditions were about 30 μg of each oligonucleotide, 5 μg/mL TMP, and irradiation at 23 400 J/m^2 by 365-nm UV light at 25 °C. The reacted DNA was extracted with chloroform twice to remove TMP, followed by ethanol precipitation and wash. The DNA was dried, dissolved in sample buffer, and applied to a denaturing polyacrylamide DNA sequencing gel to resolve the products of the photoreaction. TY12 containing a monoadduct migrates above the native TY12 as shown in the schematic drawing in the insert of Figure 3. The bands were visualized with autoradiogram and excised. The TY12-MA DNA (MA = monoadduct) was extracted as previously described (Yeung et al., 1988a). TY12-MA was mixed with 1 A260 unit of TY19 in 100 μL of 50 mM NaCl, 5 mM Tris-HCl, and 0.2 mM EDTA, pH 7.6. The mixture was heated to 90 °C, cooled to 70 °C for 10 min, and irradiated at 30 °C with 365-nm UV for 156 000 J/m^2 to convert the monoadduct on TY12 to a TY12::TY19 cross-linked duplex. The point of attachment of the cross-link to TY19 will be the thymine moiety at position 32 of TY19. The cross-linked TY12::TY19 duplex was isolated from a denaturing polyacrylamide gel containing 10% acrylamide run at 55 °C. The DNA was purified and subjected to BCR. The BCR reaction cleaved the cross-link on the furan side, producing a free TY12 and a TY19 containing a pyrone-side monoadduct. This TY19-MA oligonucleotide was gel purified from a denaturing polyacrylamide gel con-

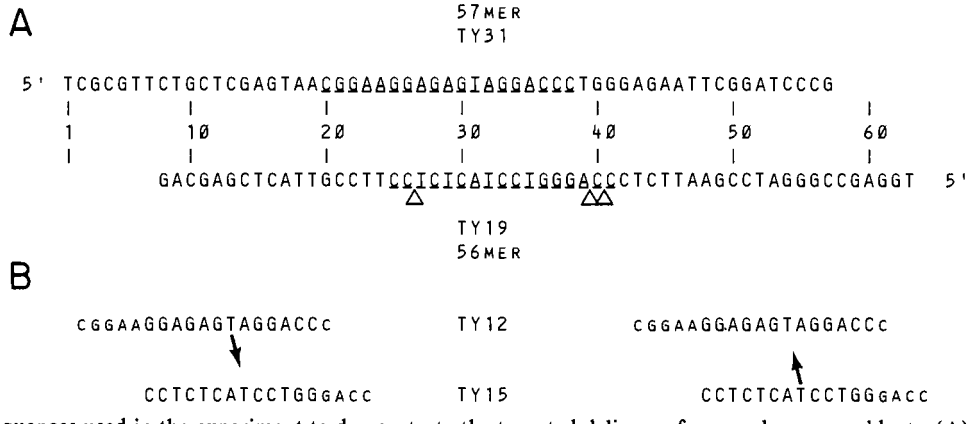

FIGURE 2: DNA sequences used in the experiment to demonstrate the targeted delivery of a psoralen monoadduct: (A) TY19 is the target strand. TY31 is complementary to TY19. The numbering system is with respect to TY31. The thymine residue at position 32 of TY19 is the intended site to receive the pyrone-side psoralen monoadduct in the BCR transfer reaction. The thymine residue at position 18 of TY19 is the secondary thymine residue, where mishybridization of a 3'CTCAT5' sequence can cause a misdelivery of the psoralen monoadduct. (B) TY12 and TY15 are of the same sequence as the underlined regions in TY31 and TY19, respectively. The parts of TY12 and TY15 that are the same as oligo I and oligo II, respectively, are shown in larger print. The arrows between TY12 and TY15 indicate the two possible directions a psoralen molecule can point in a given 5'TpA site. The three open triangles indicate the UvrABC endonuclease incision sites observed.

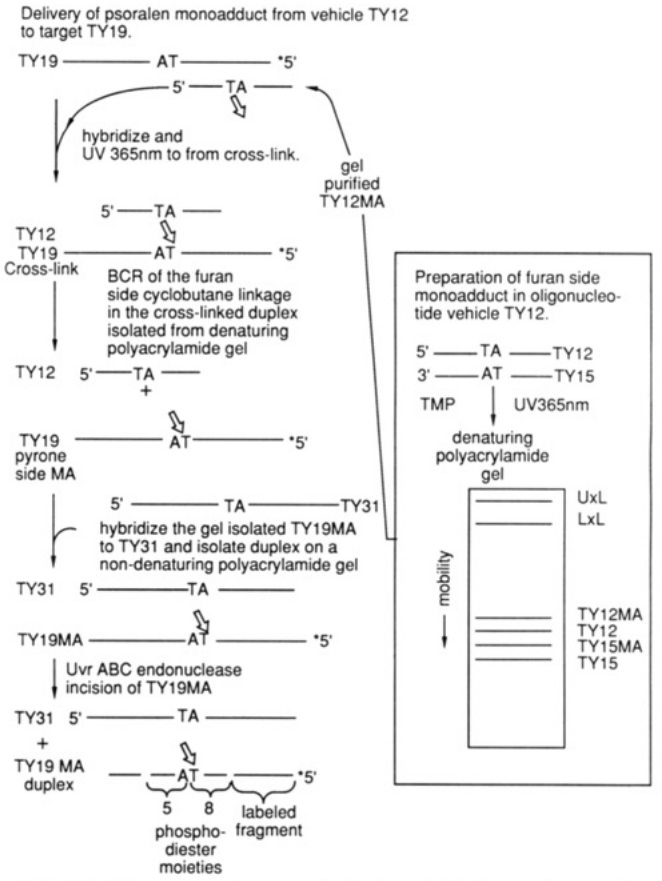

FIGURE 3: Schematic to demonstrate the targeted delivery of a psoralen monoadduct. The insert illustrates the resolution of the products of the photoreaction of TY12::TY15 with TMP and 365-nm UV by a denaturing polyacrylamide gel. The oligonucleotides will be labeled with ^{32}P and will be visualized with autoradiography. TY12-MA will be isolated from this gel, purified, and reacted with TY19 (labeled at 5′ termini with ^{32}P) to form a cross-link at the target thymine site on TY19. Subsequent BCR will deposit the pyrone-side monoadduct on TY19. UvrABC endonuclease will recognize this monoadduct as a lesion and will incise the TY19 to produce a labeled band that can be visualized on a denaturing DNA sequencing gel. The hollow arrow denotes a psoralen molecule pointing from the furan side toward the pyrone side.

taining 10% acrylamide and then hybridized to a complementary strand TY31 (a 57-mer). The paired duplexes were separated from the excess single-stranded oligonucleotide by isolation of the DNA duplex with a 10% nondenaturing polyacrylamide gel run at 40 °C. The single-stranded DNA migrated faster than the double-stranded DNA in this system. The TY19-MA::TY31 duplex was treated with the UvrABC endonuclease as described (Yeung et al., 1987). Briefly, the reaction consisted of about 40 fmol of TY19MA::TY31 duplex incubated with 500 fmol each of purified UvrA, UvrB, and UvrC proteins (Yeung et al., 1986a) at 37 °C in a 140-μL reaction that contained 10 mM MgCl, 2 mM ATP, 85 mM KCl, 40 mM 3-(*N*-morpholino)propanesulfonate (MOPS) buffer, and 1 mM dithiothreitol, pH 7.6. After 1 h of incubation, EDTA at pH 7.0 was added to the mixture to 50 mM, and the mixture was incubated for 10 more min at 37 °C to dissociate the UvrABC endonuclease postincision nucleoprotein complex (Yeung et al., 1986b). The DNA in the mixture was then ethanol precipitated by adjusting to 0.35 M in sodium acetate, followed by the addition of 2 volumes of ethanol and centrifugation for 40 min in an Eppendorf centrifuge at 4 °C without freezing. The DNA pellet was washed with 80% ethanol twice and dried. The DNA was dissolved in 10 μL of sample buffer, heated for 30 s at 90 °C, and 3 μL was

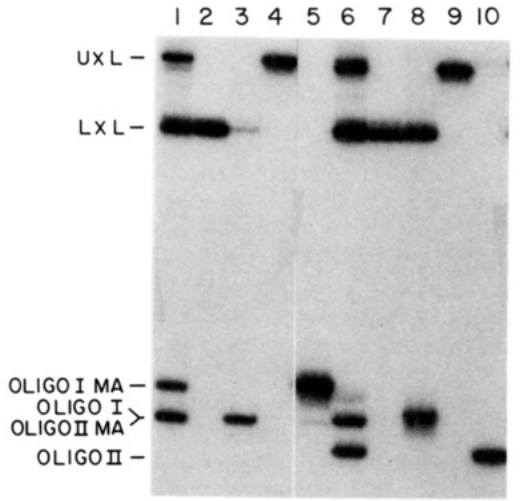

FIGURE 4: BCR of TMP cross-links. The upper cross-link (UXL) and the lower cross-link (LXL) from the photoreaction of TMP with oligo I and oligo II were purified as described under Experimental Procedures. Oligo I and oligo II were 5′-labeled with ^{32}P in lanes 1–5 and lanes 6–10, respectively, to visualize the reactions on the two DNA strands independently: (Lane 1) TMP reaction standards. (Lane 2) Lower cross-link before BCR. (Lane 3) Lower cross-link after BCR, producing oligo I free of TMP. (Lane 4) Upper cross-link before BCR. (Lane 5) Upper cross-link after BCR, producing oligo I–TMP monoadduct (MA = monoadduct). (Lane 6) Oligo II labeled TMP reaction standards (the upper cross-link showed band splitting because the unlabeled oligo II used to form this cross-link was not completely phosphorylated). (Lane 7) Lower cross-link before BCR. (Lane 8) Lower cross-link after BCR, producing oligo II–TMP monoadduct. The BCR reaction was not completed in this lane. (Lane 9) Upper cross-link before BCR. (Lane 10) Upper cross-link after BCR, producing oligo II free of TMP. The monoadduct bands in the reaction standard lanes were furan-side monoadducts. Furan-side monoadduct and pyrone-side monoadduct show similar, although nonidentical, mobilities in our electrophoresis experiments (Yeung et al., 1988a).

loaded into a well in a DNA sequencing gel. We found that phenol extraction of the proteins was unnecessary if the incubation in EDTA was included.

Results and Discussion

BCR Reversal of TMP Cross-Links in the Oligonucleotides. Figure 4 shows the results of the BCR of the gel-isolated upper cross-link (UXL) and lower cross-link (LXL). By labeling only one of the two strands of DNA in each experiment, the reactions on the two DNA strands can be separately visualized. Oligo I was labeled in lanes 1–5. Oligo II was labeled in lanes 6–10. The presence of a molecule of TMP on an oligonucleotide causes the oligonucleotide to migrate about one base spacing slower in a DNA sequencing gel (Yeung et al., 1988a). Figure 4 (lane 5) shows that BCR of upper cross-link produced a band migrating about one base spacing above the labled oligo I, indicating that a TMP monoadduct was attached to the oligo I molecule. This result suggested that cleavage of the cross-link occurred on the furan side of TMP because the pyrone ring of TMP was attached to the labeled oligo I in the upper cross-link (Yeung et al., 1988a). Lane 10 shows that BCR of the upper cross-link produced a band with the same mobility as oligo II, indicating that the labeled oligo II produced had no TMP attached. This result is consistent with the above conclusion that cleavage of the cross-link occurred on the furan side of TMP because the furan ring of TMP was attached to the labeled oligo II in upper cross-link. The lower cross-link was tested in the same manner (lanes 3 and 8). In the lower cross-link, the furan ring of TMP was attached to oligo I (Yeung et al., 1988a). BCR of the lower cross-link produced

labeled oligo I without TMP attached (lane 3) and labeled oligo II with a TMP monoadduct attached (lane 8). Alkali condition was required for the BCR reaction. The cross-links were stable at 90 °C in 10 mM Tris and 0.4 mM EDTA, pH 7.6 (data not shown). It can be seen in Figure 4 that the alkali-generated monoadduct–oligonucleotide band was slower in migration than the purified furan-side monoadduct markers. The difference in mobility may be due to the differences between a furan-side monoadduct and a pyrone-side monoadduct or due to alkali opening of the lactone ring. This experiment also suggested that pyrone-side monoadducts generated by BCR of a cross-link are resistant to further BCR. The lower cross-link is more resistant to BCR than the upper cross-link. This observation is consistent with our previous finding that the lower cross-link in this duplex is more resistant to denaturation than the upper cross-link (Yeung et al., 1988a).

Characterization of the Products of the BCR Reaction. When the 3,4 double bond of the pyrone ring reacted with the 5,6 double bond of a thymine residue to form a cyclobutane ring, the resultant pyrone-side monoadduct of psoralen no longer absorbed 365-nm UV. As a result, the pyrone-side monoadduct is incapable of further photoreaction to form a DNA cross-link (Parsons, 1980; Kanne et al., 1982). When the gel-purified labeled oligo I-MA, formed by BCR of upper cross-link (expected to be the pyrone-side monoadduct), was hybridized with unreacted oligo II and irradiated with 365-nm UV, no cross-linking reaction was observed (Figure 5, lane 3). Experiments with labeled oligo II-MA, formed by BCR of lower cross-link, gave the same result (Figure 5, lane 8). These observations were expected if BCR did not not produce a new pyrone-side derivative that is photoreactive. We next examined the thymine residue that was connected to the furan ring of psoralen in the cross-link before the BCR reaction. No cleavage of the DNA backbone by the BCR reaction, which should produce a truncated oligonucleotide, had been observed in any of the experiments presented in this study.

The 5,6 double bond of the thymine base was probably fully regenerated by BCR as shown by the experiment in Figure 5. When the labeled oligo I released by BCR from the lower cross-link was photoreacted in the presence of a slight excess of unlabeled oligo II and fresh TMP, upper cross-link and lower cross-link with correct gel mobilites were formed. A similar result was obtained when this experiment was repeated with labeled oligo II, regenerated from BCR of upper cross-link (Figure 5, lane 10). These photo-cross-linking reactions with TMP should not be possible if the 5,6 double bond of the thymine was absent in the 5′TpA sites of the oligonucleotides generated by BCR. The two cross-link bands should not have migrated normally in the gel if the pyrimidine ring in the thymine were opened in the BCR reaction.

BCR of the TMP Monoadduct-Containing Oligonucleotides. Since the monoadducts generated by the BCR of cross-link may be different from the monoadducts formed without BCR, we have tested whether the specific cleavage of the furan side of psoralen also occurs on psoralen monoadducts generated without the use of BCR. The forward reaction of TMP photoreaction produces monoadducts mainly of the furan side (Yeung et al., 1988a). Both furan-side monoadduct and pyrone-side monoadduct were prepared by photoreversal of isolated cross-links as previously described (Yeung et al., 1988a). These oligonucleotides containing monoadducts were gel purified and subjected to BCR. As seen in Figure 6 (lanes 2 and 6), the furan-side monoadducts from the forward reaction of TMP with oligo I and oligo II are cleaved by BCR to form the native oligonucleotides. The loss

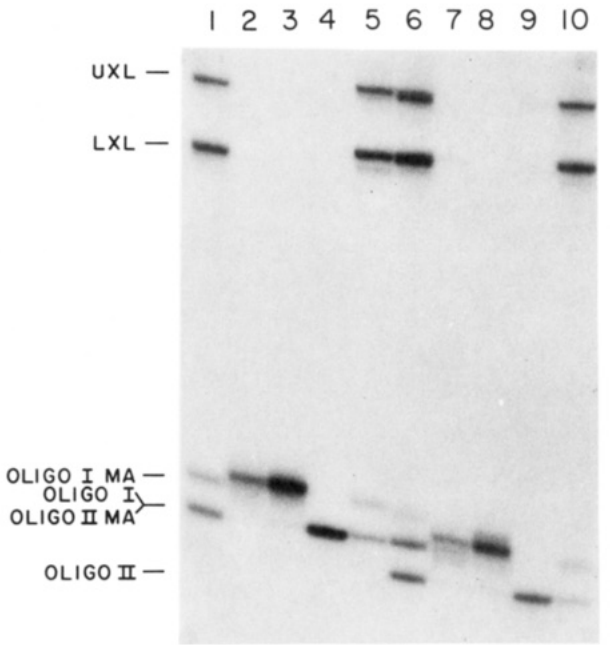

FIGURE 5: Photoreactivity of the two DNA strands separated by BCR from a TMP cross-linked duplex. By performing the experiments in Figure 4 in preparative scale, the oligonucleotide–TMP-MA strands and the TMP-free oligonucleotides were isolated and tested for their ability to participate in further TMP photoreaction in the presence of the complementary strand. Oligo I and oligo II were 5′-labeled with ^{32}P in lanes 1–5 and lanes 6–10, respectively, to visualize the reactions on the two DNA strands independently: (Lane 1) TMP reaction standards. (Lane 2) Gel-isolated oligo I–TMP monoadduct from BCR of purified upper cross-link. (Lane 3) Oligo I–TMP monoadduct from BCR of purified upper cross-link, hybridized to a slight excess of oligo II and irradiated with 365-nm UV for 20 min. No cross-link formation occurred. (Lane 4) Gel-isolated oligo I, TMP-free strand, from BCR of purified lower cross-link. (Lane 5) Oligo I, TMP-free strand, from BCR of purified lower cross-link, hybridized to a slight excess of oligo II and irradiated with 365-nm UV for 20 min in the presence of fresh TMP. Cross-link formation occurred at the normal rate, with both cross-links showing normal mobilities as if the oligo I was totally unmodified. (Lane 6) Oligo II labeled TMP reaction standards (the upper cross-link showed band splitting because the unlabeled oligo I used to form this cross-link was not completely phosphorylated). (Lane 7) Gel isolated oligo II–TMP monoadduct from BCR of purified lower cross-link. (Lane 8) Oligo II–TMP monoadduct from BCR of purified lower cross-link, hybridized to a slight excess of oligo I and irradiated with 365-nm UV for 20 min. No cross-link formation occurred. (Lane 9) Gel-isolated oligo II, TMP-free strand, from BCR of purified upper cross-link. (Lane 10) Oligo II, TMP-free strand, from BCR of purified upper cross-link, hybridized to a slight excess of oligo I and irradiated with 365-nm UV for 20 min in the presence of fresh TMP. Cross-link formation occurred at the normal rate, with both cross-links showing normal mobilities as if the oligo II was totally unmodified.

of TMP rendered these oligonucleotides incapable of forming cross-links when they were irradiated by 365-nm UV after hybridization to the complementary strands. Lanes 3 and 7 show that the oligonucleotides containing the furan-side monoadducts, formed from the photoreversal of TMP cross-links, lost the TMP molecules after BCR, resulting in gel mobilities equal to those of the native oligonucleotides. Lanes 4 and 8 showed that the oligonucleotides containing the pyrone-side monoadducts from photoreversal are resistant to the removal of the psoralen molecule by BCR, with only minor loss of the TMP molecule visible in this overexposed autoradiogram. Similar to the pyrone-side monoadducts generated by BCR of TMP cross-links, these oligonucleotides containing pyrone-side monoadducts also showed slightly slower gel mobilities than the oligonucleotides containing the furan-side monoadduct.

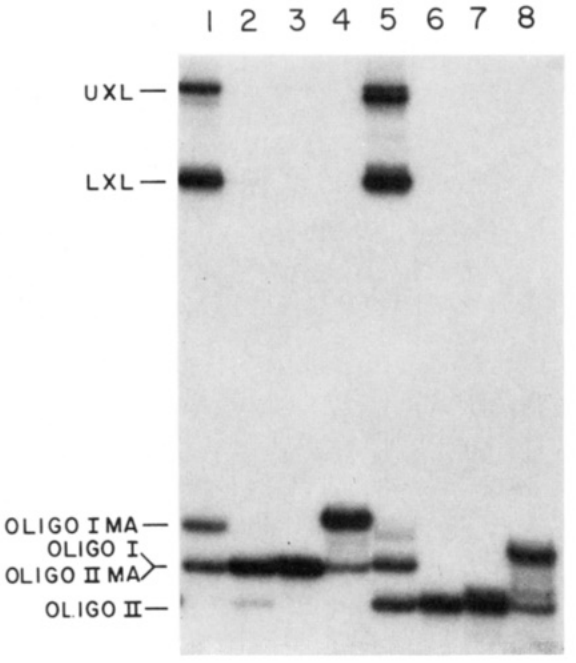

FIGURE 6: BCR of the furan-side TMP monoadduct from the forward photoreaction and of the furan-side and the pyrone-side TMP monoadducts from photoreversal reactions. Oligo I and oligo II were 5′-labeled with ^{32}P in lanes 1–4 and lanes 5–8, respectively: (Lane 1) TMP reaction standards. (Lane 2) The furan-side monoadduct of oligo I from the forward photoreaction was subjected to BCR, purified, hybridized to oligo II, and irradiated again with 365-nm UV. No cross-links were formed. (Lanes 3 and 4) The purified furan-side and the pyrone-side monoadducts of oligo I, respectively, from 254-nm UV photoreversal of the lower cross-link and the upper cross-link, were tested for stability to BCR. (Lane 5) TMP reaction standards (the upper cross-link showed band splitting because the unlabeled oligo I used to form this cross-link was not completely phosphorylated). (Lane 6) The furan-side monoadduct of oligo II from the forward photoreaction was subjected to BCR, purified, hybridized to oligo I, and irradiated again with 365-nm UV. No cross-links were formed. (Lanes 7 and 8) The purified furan-side and the pyrone-side monoadducts, respectively, from 254-nm UV photoreversal of the upper cross-link and the lower cross-link, were tested for stability to BCR.

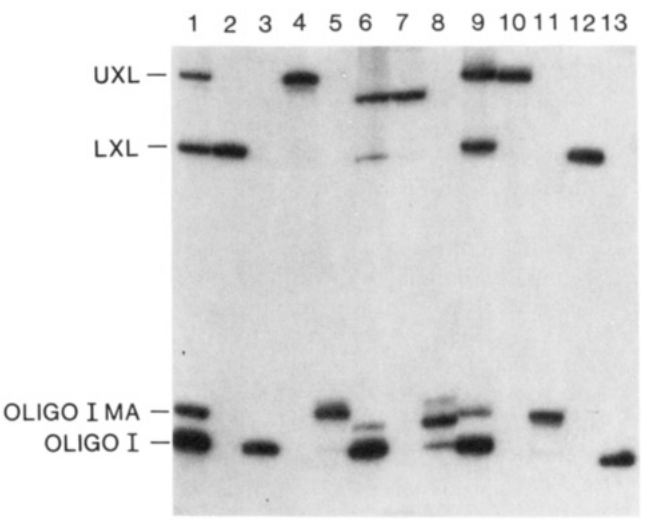

FIGURE 7: BCR of cross-links of HMT and 8-MOP. Isolated cross-link isomers of HMT and 8-MOP were subjected to BCR as described under Experimental Procedures. The results were analyzed by a denaturing polyacrylamide gel as described for Figure 4. Oligo I was labeled with ^{32}P at the 5′ terminus in each lane: (Lane 1) Standards of TMP reaction with oligo I and oligo II duplex. (Lane 2) Purified TMP lower cross-link. (Lane 3) TMP lower cross-link + BCR. (Lane 4) Purified TMP upper cross-link. (Lane 5) TMP upper cross-link + BCR. (Lane 6) 8-MOP reaction with oligo I and oligo II duplex. (Lane 7) 8-MOP upper cross-link. (Lane 8) 8-MOP upper cross-link + BCR. (Lane 9) HMT reaction with oligo I and oligo II duplex. (Lane 10) Purified HMT upper cross-link. (Lane 11) HMT upper cross-link + BCR. (Lane 12) Purified HMT lower cross-link. (Lane 13) HMT lower cross-link + BCR.

BCR of the Cross-Links of 4′-(Hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) and 8-Methoxypsoralen (8-MOP). The results of BCR of the cross-links of 8-MOP and HMT are shown in Figure 7. Oligo I was labeled in this experiment, and BCR of TMP was included as a control. For 8-MOP, since the upper cross-link is formed preferentially in this DNA sequence (Yeung et al., 1988a), the lower cross-link was not examined in this experiment. The results are the same for all three psoralens. The pyrone side of psoralen is attached to the oligo I in the upper cross-link (Yeung et al., 1988a), and BCR of upper cross-link, at the furan side, produced oligo I with pyrone-side monoadduct attached (lanes 5, 8, and 11). The furan side of psoralen is attached to oligo I in the lower cross-link, and BCR of lower cross-link produced psoralen-free oligo I (lanes 3 and 13). The cross-links of 8-MOP appeared to be less stable in the BCR reaction than the cross-links of TMP and HMT. Some cleavage at the pyrone side may have formed the small amount of oligo I free of 8-MOP (lane 8). Damage of the 8-MOP rings in a monoadduct may have formed the minor band above the 8-MOP monoadduct band in the same lane. This experiment was repeated with oligo II labeled instead of oligo I. Again, BCR of all three psoralens occurred preferentially on the furan side, independent of the orientation of the psoralen molecule between the two DNA strands (data not shown).

Possible Mechanism of BCR of the Furan–Thymine Cyclobutane Ring. It is known that [2 + 2]cycloreversions can occur in thermal reactions [for a review, see Schaumann and Ketcham (1982)]. However, the BCR of psoralen cross-link is unlikely to be just a thermal rearrangement because most of the thermal cycloconversions occur at temperatures in the range of hundreds of degrees centigrade. Alternatively, the mechanism of BCR may be a simple base-catalyzed rearrangement that is more rapid at higher temperatures. A third possible mechanism may be hydrolysis of the furan ring by BCR since the thymine residue seems unaltered in these experiments. The lower reactivity of the pyrone-side cyclobutane ring to BCR may be due to the opening of the lactone ring in alkali. Obviously, the elucidation of the mechanism of BCR on the two sides of a psoralen cross-link will require further investigation.

Experiment To Demonstrate the Targeted Transfer of a Psoralen Monoadduct. The results of the targeted delivery of psoralen monoadduct are shown in Figure 8. The position of the pyrone-side monoadduct formed on TY19 was diagnosed with incision of the DNA by the UvrABC endonuclease. The UvrABC endonuclease incises the psoralen-modified DNA strand in a DNA duplex twice, once at eight phosphodiester moieties 5′ to the modified thymine and once at five phosphodiester moieties 3′ to the modified thymine (Yeung et al., 1986b). When the DNA is labeled at the 5′ position as in this experiment, the 5′ incision site resulted in a labeled strand that is eight phosphodiester bonds shorter than the position the modified thymine would indicate on a DNA sequencing gel. The chemical sequencing ladders are used as reference in this figure. The rationale for comparing the position of a UvrABC endonuclease incision band with a chemical DNA sequencing ladder was previously described (Yeung et al., 1983). In lane 4 of Figure 8, a major UvrABC endonuclease incision band is seen at this position, together with a minor band one base shorter. The minor band is known to occur as an alternative UvrABC endonuclease incision at nine phosphodiester moieties from the modified thymine (Yeung et al., 1987). These two

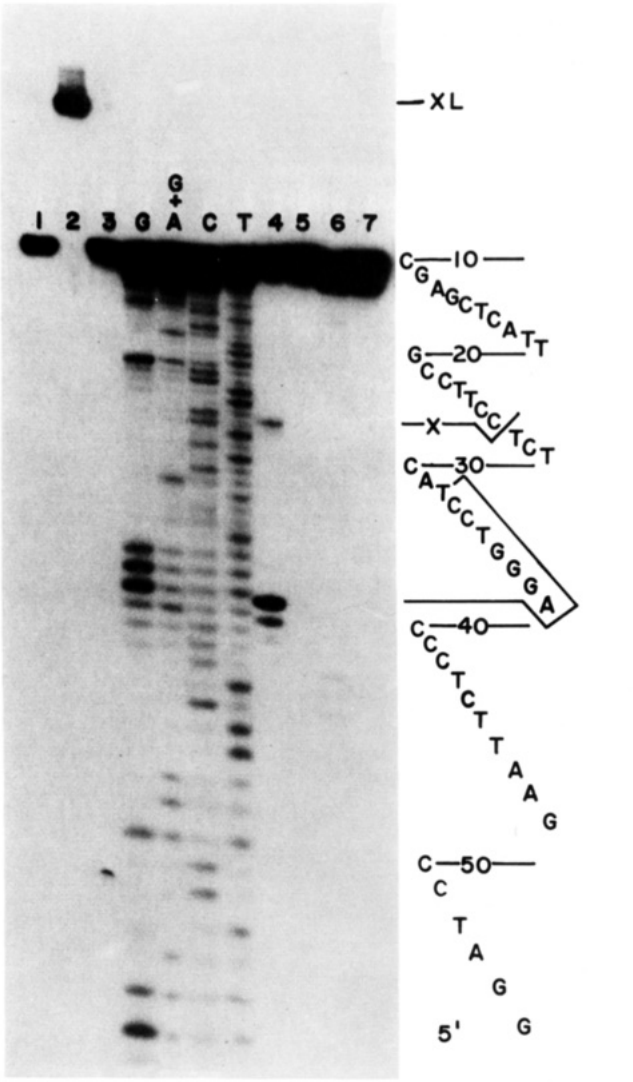

FIGURE 8: Analysis of the product of the targeted delivery of the psoralen monoadduct. The picture is an autoradiogram of a polyacrylamide DNA sequencing gel. TY19 was labeled at the 5′ termini with ^{32}P in this experiment. The chemical DNA sequencing lanes are indicated in the figure. (Lane 1) Untreated TY19. (Lane 2) Cross-link of TY12::TY19 by psoralen; the band position is indicated by XL on the side of the figure. (Lane 3) TY19-MA (pyrone-side monoadduct formed by BCR of the cross-link seen in lane 2). (Lanes 4 and 5) Incubation of the TY19-MA::TY31 duplex with or without UvrABC endonuclease, respectively. A line is traced from the UvrABC endonuclease incision band through the phosphodiester bond broken by the enzyme to the thymine base with which the incision site correlates. (Lanes 5 and 6) Incubations of unmodified TY19::TY31 duplex, with or without UvrABC endonuclease, respectively. A very low level of UvrABC endonuclease incision may be visible in lane 6. These positions correlate with G bases that are modified at a low frequency in oligonucleotides synthesized by the methoxyphosphoramidite chemistry (Yeung et al., 1988b).

incisions showed that a psoralen monoadduct was located on the thymine residue at position 32 of TY19. Not all the TY19-MA was incised by the UvrABC endonuclease in this reaction. This result does not indicate that some TY19-MA molecules do not contain a psoralen monoadduct, because it is known that UvrABC endonuclease cannot incise all the substrate molecules at a given DNA lesion. We attributed this phenomenon to our observation that a lesion in a DNA sequence may exhibit several DNA conformations at the same time, not all of which can be incised by the UvrABC endonuclease (Yeung et al., 1986b) and some of which forms nonproductive dead-end Uvr–protein–lesion complexes (unpublished data). Since the TY19-MA was produced by BCR of the gel-isolated cross-linked DNA, we conclude from the UvrABC endonuclease incision data that we can produce a population of TY19 molecules in which most molecules contain a pyrone-side monoadduct each at the thymine residue at position 32. A third UvrABC endonuclease incision band, marked X in Figure 8, was also observed. This band did not arise from the TT pair at position 18–19 of TY19, as a pyrimidine dimer, because the TY19 DNA had not been exposed to short-wave UV light. There are two possible explanations for this band: (1) A second site in TY19 at a thymine residue at position 18 may have received a psoralen monoadduct, at a lower frequency than the thymine at position 32. The DNA sequence 3′ to this position has some homology with the sequence around position 32 (both 3′CTCAT5′). Since the intercalation of a psoralen monoadduct increases the DNA helix stability (Cimino et al., 1985), the low stringency we used in our hybridization and cross-linking reaction had probably allowed this mishybridization to occur. Higher stringency in the hybridization should be able to cure this problem. The drawback of this explanation is that the incision at X is nine phosphodiester moieties from the putative thymine modification at position 18, instead of the usual distance of eight phosphodiester moieties. (2) A second explanation is that sometimes one incision can occur without the other (Yeung et al., 1983). The absence of a 5′ incision when there is a 3′ incision results in a DNA fragment 12 phosphodiester moieties (12 bases) longer than the 5′ incision band would indicate on the DNA sequencing gel. The band labeled X is 13 bases longer than the 5′ incision band for the thymine at position 32 of TY19. Thus, X may be produced by a single 3′ incision that is six phosphodiester moieties from the thymine residue at position 32 of TY19, instead of the usual distance of five phosphodiester moieties. Further experiments need to be performed to ascertain whether the UvrABC endonuclease incises the monoadducts produced by BCR in a manner slightly different from the incisions expected of unmodified pyrone-side psoralen monoadducts.

In summary, we have demonstrated that BCR of a 4,5′,8-trimethylpsoralen cross-link does not involve the breaking of the DNA backbone. The cross-link is specifically cleaved on the furan side of the TMP molecule. The cleavage of TMP–thymine linkage is also observed for the furan-side TMP–thymine monoadduct but not for the pyrone-side TMP–thymine monoadduct. The thymine moiety released by the BCR reaction appeared unmodified. These results suggested that the cyclobutane ring can be cleaved by BCR under appropriate conditions, such as the environment at the furan side of a TMP–thymine adduct. The BCR of the cross-links of 8-MOP and HMT was similar to the BCR of TMP cross-links. We have demonstrated in this study that BCR at the furan side of psoralen can quantitatively transfer a psoralen monoadduct from vehicle to a target site. This approach to the site-specific covalent transfer of a psoralen monoadduct may be useful to experiments of DNA replication, repair, recombination, and gene expression where it may be desirable to place a "bulky" adduct at a specific site in the DNA to produce a blockage of enzymatic processes, and it may also be useful in studies of nucleic acid structure. It may be possible to design psoralen derivatives that are very sensitive to furan-side cross-link cleavage. Such psoralen derivatives may allow the targeted delivery of a psoralen monoadduct under physiological conditions.

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Photochemical Cleavage of DNA by Nitrobenzamides Linked to 9-Aminoacridine†

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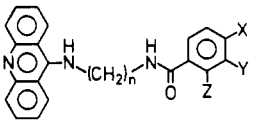
ABSTRACT: Nitrobenzamido ligands linked to the DNA intercalator 9-aminoacridine via poly(methylene) chains induce single-strand nicks in DNA upon irradiation with long-wavelength ultraviolet light ($\lambda \geq 300$ nm). Optimal photocleavage activity was found for the reagent 9-[[6-(4-nitrobenzamido)hexyl]amino]-acridine. Removal of the acridinyl ligand or changing the position of the nitro group from the 4- to the 2-position caused a 10-fold decrease in photocleavage efficiency, whereas a change to the 3-position caused a 30-fold reduction. The DNA cleavage was 5-fold enhanced by subsequent piperidine treatment and showed some sequence dependency with predominant cleavage at G and T residues. Furthermore, significant differences in cleavage preference were observed when the poly(methylene) linker length was changed.

Photochemical single-strand cleavage of DNA has been observed with several DNA binding reagents. The DNA photocleavage by methylene blue (Friedman & Brown, 1978; OhUigin et al., 1987), acridine orange (Freifelder et al., 1961; Bowler et al., 1984), or porphyrins (Praseuth et al., 1986) proceeds by an oxidative mechanism via singlet oxygen or via electron transfer, whereas the photocleavage by transition metal complexes of bleomycin or triphenanthrolines occurs via either singlet oxygen or a radical mechanism depending on the metal ion (Chang & Meares, 1984; Subramanian & Meares, 1985, 1986; Barton, 1986; Fleischer et al., 1986). Furthermore, a chemical DNA cleaving reagent has been developed by linking EDTA to the DNA intercalator methidium (Dervan, 1986), and this reagent has been used to footprint the binding of proteins to DNA in vitro (Van Dyke & Dervan, 1983) and in situ (Cartwright & Elgin, 1984). Such synthetic "nucleases", could be very useful tools in molecular biology, especially for studying protein–DNA interactions in vivo.

Table I



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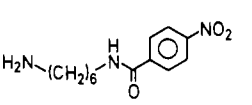

2

reagent	X	Y	Z	*n*	photocleavage act.[a]
1a	NO_2	H	H	4	90
1b	NO_2	H	H	6	100
1c	NO_2	H	H	8	80
1d	H	NO_2	H	6	3
1e	H	H	NO_2	6	10
1f	NO_2	NO_2	H	6	10
1g	NO_2	H	NO_2	6	75
1h	H	H	H	6	<1
2					10

[a] The relative photocleavage activity was determined from the reagent concentration that under otherwise identical conditions, as specified in the legend to Figure 1, gave 50% relaxation of the pUC 19 DNA.

We are presently designing novel "photonucleases" based on previous work with polyfunctional DNA-intercalating 9-aminoacridine derivatives (Buchardt et al., 1984, 1987; Nielsen, 1982, 1985; Nielsen et al., 1983, 1984; Jeppesen et

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