Wavelength Dependence for the Photoreactions of DNA-Psoralen Monoadducts.

2. Photo-Cross-Linking of Monoadducts†

Yun-bo Shi and John E. Hearst*

Department of Chemistry, University of California, Berkeley, Berkeley, California 94720

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ABSTRACT: The photoreactions of HMT [4'-(hydroxymethyl)-4,5',8-trimethylpsoralen] monoadducts in double-stranded DNA have been studied with complementary oligonucleotides. The HMT was first attached to the thymidine residue in the oligonucleotide 5'-GAAGCTACGAGC-3' as either a furan-side monoadduct or a pyrone-side monoadduct. The HMT-monoadducted oligonucleotide was then hybridized to the complementary oligonucleotide 5'-GCTCGTAGCTTC-3' and irradiated with monochromatic light. In the case of the pyrone-side monoadducted oligonucleotide, photoreversal was the predominant reaction, and very little cross-link was formed at all wavelengths. The course of the photoreaction of the double-stranded furan-side monoadducted oligonucleotide was dependent on the irradiation wavelength. At wavelengths below 313 nm, both photoreversal and photo-cross-linking occurred. At wavelengths above 313 nm, photoreversal of the monoadduct could not be detected, and photo-cross-linking occurred efficiently with a quantum yield of 2.4 × 10⁻².

†This work was supported by NIH Grant GM1:1180.
*Author to whom correspondence should be addressed.

The use of psoralens (furocoumarins) in the medical and biological fields is highly dependent upon the ability of these compounds to cross-link double-stranded nucleic acids through photoreactions with adjacent pyrimidine bases on opposite strands. The cross-link formation is well understood and occurs in three steps (Song & Tapley, 1979; Parson, 1980; Parson, 1980; Cimino et al., 1985). First, psoralens intercalate between base pairs of a double-stranded nucleic acid. Second, the intercalated psoralens photoreact with pyrimidine bases to form psoralen–pyrimidine monoadducts under UV (320–400 nm) irradiation. Finally, the monoadducts photoreact with adjacent pyrimidine bases by absorbing a second photon to form diadducts that cross-link the two strands of the nucleic acid together. It has been well established that the furan-side monoadduct can be easily driven to a diadduct in a double-

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stranded nucleic acid. Gasparro et al. (1984) have reported the action spectrum for the AMT \[4',4'-(aminomethyl)-4,5',8\text{-trimethylpsoralen}\] cross-linking of pBR322 DNA in the wavelength region from 300 to 380 nm. They found that the wavelength dependence for cross-link formation correlates with the absorption spectrum of the furan-side monoadduct. Tessman et al. (1985) have reported the photo-cross-linking of another furan-side monoadduct, thymidine-8-methoxypsoralen furan-side monoadduct, in calf thymus DNA. They observed that the monoadduct could be converted to the diadduct at 341.5 nm with a quantum yield of \(2.8 \times 10^{-2}\). The pyrone-side monoadducts do not absorb light above 320 nm, so they cannot be driven to diadducts with 320–380-nm light.

In the preceding paper (Shi & Hearst, 1987), we reported the wavelength dependence for the photoreversals of DNA–HMT monoadducts \(\text{HMT}: 4',4'-(\text{hydroxymethyl})-4,5',8\text{-trimethylpsoralen}\) and isolated T-HMT monoadducts. We found that both the DNA–HMT and the T–HMT pyrone-side monoadducts are photoreversible at wavelengths below 334 nm, which is consistent with the absorption spectrum of the T–HMT pyrone-side monoadduct. The DNA–HMT and T–HMT furan-side monoadducts can be photoreversed at wavelengths up to 365 nm, which also correlates with the absorption spectrum of the T–HMT furan-side monoadduct. The quantum yield of the photoreversion of the furan-side monoadduct is, however, only \(7 \times 10^{-4}\) at wavelengths above 285 nm, which is much smaller than the photo-cross-linking quantum yield of the thymidine–8-methoxypsoralen furan-side monoadduct at 341.5 nm as reported by Tessman et al. (1985).

Here we report the complete action spectra for the photoreactions of DNA–HMT monoadducts in double-stranded DNA. We observed that the DNA–HMT pyrone-side monoadduct yielded very little cross-link and was predominantly photoreversed upon UV irradiation in its absorption bands. In the case of the DNA–HMT furan-side monoadduct, UV irradiation at wavelengths \(\geq 313.2\) nm yields only cross-link with a quantum yield similar to that observed by Tessman et al. (1985), whereas UV irradiation at wavelengths \(\leq 302.2\) nm yields both photo-cross-linking and photoreversal products.

**Materials and Methods**

**Materials.** All materials were obtained as described in the preceding paper (Shi & Hearst, 1987).

**Preparation of 5'-End Labeled DNA Oligonucleotides.** The DNA oligonucleotides used in this study are 5'-GAAGCT-ACCGAGC-3', 5'-GCTCGTAGCTTC-3', and 5'-TCGTAAGCT-3', respectively called oligonucleotide A, B, and C. The furan-side DNA–HMT monoadduct, 5'-GAAGCT[HMT][F]-ACCGAGC-3' (MF-A), and the pyrone-side DNA–HMT monoadduct, 5'-GAAGCT[HMT][T]-ACCGAGC-3' (MT-A), were prepared as described in the preceding paper (Shi & Hearst, 1987). All unmodified and HMT-monoadducted oligonucleotides were 5'-phosphorylated with \(\gamma\text{-32P}\text{ATP}\) and T4 polynucleotide kinase and subsequently chased with cold ATP (Maniatis et al., 1982; Shi & Hearst, 1986). The kinase exchange reaction was used to label oligonucleotides with \(5'\text{-phosphate}\) (Maniatis et al., 1982; Shi & Hearst, 1987).

The concentrations of the unmodified and HMT-monoadducted DNA oligonucleotides were determined by absorption measurements at 260 nm. The extinction coefficients for oligonucleotides A–C and \(M_F\)-A and \(M_T\)-A are \(1.2 \times 10^3\), \(1.1 \times 10^3\), \(7.4 \times 10^4\), \(1.3 \times 10^5\), and \(1.3 \times 10^5\), respectively (Shi & Hearst, 1986).

**Photo-Cross-Linking of the DNA–HMT Monoadducts.** Monochromatic irradiations were done with the apparatus as described by Cimino et al. (1986). The bandwidth was maintained constant at 5.0 nm. The light intensity was monitored continuously with an in-line photoioder, which was calibrated by actinometry with \(K_3\text{Fe(C$_2$O$_4$)$_3$}\). The photo-cross-linking of the DNA–HMT monoadducts, both \(M_F\)-A and \(M_T\)-A, was done in the presence of the cold 5'-phosphorylated complementary oligonucleotide B or C. The solutions were irradiated with monochromatic light in a stirred cuvette, which was covered with Parafilm, at 4°C in 100 mM NaOAc, 10 mM MgCl\(_2\), and 0.1 mM EDTA, pH 6.0, or as otherwise indicated. A total of 16 μg of carrier tRNA was added to each sample after irradiation, and the sample was then concentrated in a Speedvac concentrator (Savant Instrument Inc.) to 400 μL (irradiation volume was \(2.5 \times 10^{-5}\) μL) and EiOH precipitated overnight (2.5 v/v EiOH) at -20°C. The sample pellets were then dissolved in 8 M urea, 0.02% bromophenol blue, and 0.02% xylene cyanol FF loading buffer and electrophoresed on a 20% polyacrylamide–7 M urea gel (40 cm × 40 cm × 0.05 cm, 35 W for 3–4 h). Band positions of the photoproducts and the reagents were located by autoradiography. The bands were then excised and quantified by 32P Cerenkov counting with a Beckman LS-230 liquid scintillation counter.

**Kinetics of the Photoreactions.** The photoreactions of DNA–HMT monoadducts in double-stranded DNA are relatively complicated. Figure 1 shows the possible reactions of a double-stranded DNA formed between oligonucleotides A and B, with an HMT monoadduct attached to A. The initial reactions are the photoreversal and the photo-cross-linking of the monoadduct. Photo-cross-linking yields a cross-link that links A and B together. Upon absorbing a second photon, the cross-link can be photoreversed to yield a DNA–HMT monoadduct with the HMT attached to either B or A. Photo-reversal of the monoadduct gives a complex with the HMT molecule intercalated in the double-stranded helix A:B. This complex can undergo either a photoreaction to yield a DNA–HMT monoadduct or a reversible dark dissociation to yield a free HMT molecule, which can be subsequently photodamaged.

Instead of relying upon the complicated kinetic equations, we used initial rate analysis to obtain the rate constants for the photoreversal and photo-cross-linking of the monoadducts.

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1 Abbreviations: HMT, 4',4'-(hydroxymethyl)-4,5',8-trimethylpsoralen; EiOH, ethanol; ATP, adenosine 5'-triphosphate; EDTA, ethylenediaminetetraacetic acid. Ttris (tris(hydroxymethyl)aminomethane; M_F, furan-side monoadduct; M_T, pyrone-side monoadduct; 5'-T[HMT][A]-3', thymidine–HMT furan-side monoadduct with HMT on the 3'-side of the thymidine; 5'-[T[HMT][T]-3', thymidine–HMT pyrone-side monoadduct with HMT on the 3'-side of the thymidine; T-HMT–T, thymidine–HMT thyminidime diadduct.

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**FIGURE 1:** Photoreaction kinetics of HMT monoadducts in double-stranded DNA.
The photoreaction kinetics at very low reactant concentrations can be analyzed by gel electrophoresis. These and above results indicate that XL, D1, D2, and D3 are DNA-HMT cross-links. Upon photoreversal at 254 nm, these purified adducts yielded, as expected, MFP-A and B as gel electrophoresis. These and above results indicate that XL, D1, D2, and D3 are DNA-HMT cross-links. In addition, photoreversal of D1 and D2 yielded bands running slightly slower than B. When similar photoreversal was performed on XL, D1, D2, and D3 with labeled A strand, only A and MFP-A were produced in all cross-links. These results suggest that there is photodamage on the B strand in D1 and D2. But the possibility that damage on the A strand, especially in the case of D1, where photoreversal yielded no damaged DNA when the label was on either A strand or B strand, cannot be ruled out because of the possibility of photoreversal of the damage at 254 nm, especially pyrimidine dimers, and the limited resolution by gel electrophoresis. The mechanism of the damaged product formation is unknown. These products could be produced by direct absorption of the DNA bases or more probably by energy transfer from the excited psoralen moiety to the pyrimidine bases, especially at wavelengths ≥334 nm, where DNA bases have little absorption. It has been shown that the triplet states of the furan-side monoadducts of several psoralen derivatives with thymine as well as those of coumarin and 4',5'-dihydropsoralen can be quenched by nucleic acid bases (Bensasson et al., 1980; Land & Truscott, 1979; Blais et al., 1985), suggesting that energy transfer between these adducts and nucleic acid bases can occur.

The bands of the reactant and the products, such as those in Figure 2, were excised and quantified by 32P Cerenkov counting in order to determine the concentration of each reactant and product. The plots of the concentrations as a function of irradiation dose at 302.2 and 334 nm are shown in Figure 3, where D stands for the total damaged cross-links running slower than XL on the gel. Again, it can be seen that only XL and D were produced at 334 nm, whereas photoreversal of MFP-A to yield A was a competing process at 302.2 nm. With increasing dose at 302.2 nm, XL and D initially increased and then decreased due to the photoreversal of the cross-links. The production of A was sigmoidal. Initially A was produced only from the photoreversal of MFP-A. As more XL and D were formed, a significant amount of A was produced from the photoreversal of XL and D, since it is known that about 30% of the time photoreversal of cross-links at this wavelength occurs at the furan end (Cimino et al., 1986), which in our case yields A. Consequently, the rate of A formation is increased. The initial rate constants for the
analyzed as described above, and the results are presented in Table 4.

Initial photoreversal occurs at wavelengths crossed-link (Cimino et al., 1986). A detectable amount of

Table I: Initial Rate Constants of MFu-A Cross-Linking

<table>
<thead>
<tr>
<th></th>
<th>25 °C,</th>
<th>4 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>$k^i$</td>
<td>2020</td>
<td>2530</td>
</tr>
<tr>
<td>$k_{XL}$</td>
<td>870</td>
<td>1090</td>
</tr>
<tr>
<td>$k_{RE}$</td>
<td>1150</td>
<td>1440</td>
</tr>
<tr>
<td>$k_{D}$</td>
<td>0.43</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*Abbreviations: MFu-A = 5'-GAAGC[T(HMT)]ACGAGC-3'; B = 5'-GCTCCTAGCTTC-3'; C = 5'-TCGTAAGCTT-3'; $k^i$ is total photoreversal rate constant of MFu-A [L/(einstein.cm)]; $k_{XL}$ is total photo-cross-linking rate constant [L/(einstein.cm)]; $k_{RE}$ is photoreversal rate constant [L/(einstein.cm)]; $k_D$ is rate constant of damaged cross-link formation [L/(einstein.cm)]. Estimated precision in rate constants is ±20%.

Figure 3: (A) C/C0 vs. irradiation dose for the photo-cross-linking of MFu-A. All the irradiation conditions and abbreviations are identical to those described in Figure 2. The ratios of concentrations of the reactant (MFu-A) and the products (A, XL, and D) to the initial concentration of MFu-A plotted in this figure were determined by exciting the bands from the photo-cross-linking gel and measuring the 32P counts of each band with a scintillation counter. The relative concentrations of A, XL, and D shown in (B) are the actual value for each product multiplied respectively by 2, 4, and 4 as indicated in the figure for the convenience of presentation.

Photoreversal ($k_{RE}$) and for XL and D formation ($k_{XL}$ and $k_D$, respectively) were determined from these plots. The experiments were repeated at wavelengths ranging from 248 to 379 nm to obtain the action spectra for the photoreversal and photo-cross-linking. Figure 4 shows the initial rate constants for the photoreversal ($k_{RE}$) and the total cross-link formation ($k_{XL} = k_{XL} + k_D$) as a function of wavelength. Essentially no photoreversal occurs at wavelengths ≥313 nm, although a detectable amount of A was produced after prolonged irradiation at 313.2 nm, which was due to photoreversal of the cross-link (Cimino et al., 1986).

Two series of experiments were done to determine the dependence of the reaction rate on the stability of the double helix. In the first, the irradiation temperature was raised from 4 to 25 °C, and in the second, the oligonucleotide C was used as the complement of MFu-A. The photoreaction data were analyzed as described above, and the results are presented in Table 1. When the irradiation temperature was changed from 4 to 25 °C without altering other conditions, the photoreaction rate constants were slightly decreased, though the differences were not significant compared to the experimental error (see data in the first and second columns for each wavelength). The ratio of $k_D/k_{XL}$ at 25 °C was also smaller than that at 4 °C at all wavelengths, indicating that fewer damaged cross-links (D) were produced at the higher temperature. Data in the second and third columns for each wavelength show that the rate constants when C was used as the complement were smaller than those obtained with B as the complement. The results from these two series of experiments indicate that the photoreactions are less efficient in less stable helices. Also shown by these experiments is that the ratio of $k_D/k_{XL}$ was 0, or no damaged cross-links (D) were produced, at all wavelengths when C was used as the complement.

To ensure that all MFu-A were in double-stranded state under the conditions used to obtain the action spectra shown in Figure 4, the salt concentration of the irradiation buffer and/or the DNA concentrations were increased, and the samples were irradiated at 334 nm and analyzed as described above. The results are shown in Table II. Upon changing the MgCl2 concentration in the irradiation buffer from 10 to 20 mM or increasing the DNA concentrations by a factor of five, or both, the photoreversal rate constants as well as the ratio of $k_D/k_{XL}$ remained the same. Since these changes favor double-stranded DNA formation, the results indicate that

![Figure 4: Action spectra for the photoreversal and photo-cross-linking of T-HMT furan-side monoadduct in double-stranded MFu-A:B. $k_{XL}$ is the initial rate constant for the total cross-link formation, and $k_{RE}$ is the initial rate constant for the formation of the unmodified oligonucleotide A, i.e., the photoreversal. $k_{RE}$ is zero at wavelengths ≥313 nm.](image-url)
under the original conditions all the Mβ-A was double-stranded.

Tessman et al. (1985) have reported that a thymidine-8-methoxysoralen furan-side monoadduct in double-stranded calf thymus DNA can be converted to a pyrone-side monoadduct by direct photoisomerization upon irradiation at 341.5 nm. To test whether the T-HMT furan-side monoadduct in a double helix can photoisomerize to the pyrone-side monoadduct, 5'-32P-labeled oligonucleotide C was used as the complement of Mβ-A in photoass-linking experiments. Any adduct by direct photoisomerization upon irradiation at 334 nm.

The quantum yield above 300 nm. The light intensity and are one-photon processes.

The reciprocity of the photoreactions was confirmed by performing under the same conditions used for Mβ-A. All of the Mβ-A should be double stranded under these conditions on the basis of the thermodynamic parameters for the double-helix formation by Mβ-A and B (Shi & Hearst, 1986).

The photo-cross-linking samples were treated as above, and an autoradiogram of the time courses of the irradiation at 297 nm is shown in Figure 5. With increasing irradiation dose, the amount of Mβ-A decreased. The loss of Mβ-A was predominantly photoreversal. The amount of XL formed was only a few percent, and it decreased after prolonged irradiation (compare lane 5 to lanes 2-4). When this XL was isolated and photoreversed at 254 nm, it gave Mβ-A and A (data not shown), indicating that in XL the HMT is attached to A through its pyrone end and attached to B through its furan end and that the formation of XL was not due to contamination of Mβ-A with Mβ-A. The bands migrating slightly slower than A represent damaged DNA. These same bands were also observed in the photoreversal of Mβ-A in its single-stranded state [see the preceding paper, (Shi & Hearst, 1987)]. The amount of material in each band was quantified by exciting the band from the gel and counting it in a scintillation counter. The photoreaction rate constant(s) could then be determined. The experiments were repeated at different wavelengths in both absorption bands of the T-HMT pyrone-side monoadduct [see Figure 4 of the preceding paper (Shi & Hearst, 1987)]. It was found that photoreversal was the predominant reaction and that only a few percent of XL was formed at all wavelengths. Thus, the total photoreaction rate constant (i.e., the rate constant for the consumption of Mβ-A) is approximately equal to the photoreversal rate constant of Mβ-A in the double-stranded helix. The photoreaction rate constant (kR) was calculated from the rate constant (kR) of the reverse reaction (kR = kA + kB).

Table III: Quantum Yield of Mβ-A Cross-Linking

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>248</th>
<th>266</th>
<th>281.7</th>
<th>289.5</th>
<th>297</th>
<th>302.2</th>
<th>313.2</th>
<th>334</th>
<th>365.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ϕ (×10^−5)</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>1.2</td>
<td>1.4</td>
<td>1.7</td>
<td>2.4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Oligonucleotide B was used as the complement in the photoreaction (4 °C). See Table I for abbreviations and other reaction conditions.
reaction rate constants at 248 and 297 nm under these conditions are listed in the first column for each wavelength in Table IV. Compared to the photoreversal rate constant of the single-stranded Mpy-A in the fourth column for each wavelength, the photoreversal rate in a double-stranded helix is about twice as fast as that in a single-stranded helix. The enhancement of photoreversal under the photo-cross-linking conditions could be due to (1) photoreversal through a transient photo-cross-linking, i.e., through formation and photoreversal of XL, which would give mostly oligonucleotide A and Mpy-B (Cimino et al., 1986), (2) direct photoisomerization, i.e., the excited HMT group photoreversing at its pyrone end and simultaneously attaching to the thymidine residue in B through its furan end, (3) the irradiation buffer and/or temperature, (4) the electronic properties and steric constraints of the pyrone-side monoadduct in the double-stranded DNA, and (5) energy transfer from excited DNA bases in double-stranded DNA to the HMT group.

The first two possibilities were investigated by irradiating samples of Mpy-A in the presence of 32P-labeled complementary oligonucleotide C under the same conditions as above. The furan-side monoadducted C, if generated by either one of these, could be detected as a unique band on a polyacrylamide gel (Shi & Hearst, 1986). These experiments showed that the furan-side monoadducted C was generated at only a few percent even when most of the Mpy-A had been photoreversed. The photoreaction rate constants at 248 and 297 nm and the amount of XL formed were similar to those obtained when oligonucleotide B was used as the complement, and the rate constants are shown in the second column for each wavelength in Table IV. On the basis of these results, it is concluded that the photoreversal through transient cross-linking and direct photoisomerization may contribute to the enhancement of the photoreversal, but they have only a small effect and cannot explain the twofold enhancement. To test the third possibility, samples of Mpy-A were irradiated under the same conditions as above except no complement was added and the irradiated samples were analyzed to obtain the photoreversal rate constant. These results, shown in the third column for each wavelength in Table IV, show that the photoreversal rate constant of single-stranded Mpy-A in the buffer at 4 °C is identical with that in 0.1 mM EDTA at 24 °C. Thus, the enhancement of photoreversal is dependent on double-helix formation and independent of the irradiation temperature and buffer conditions. The effects of double-stranded DNA formation on the electronic properties and the steric constraints of the pyrone-side monoadduct and the possibility of energy transfer from excited DNA bases to the HMT group cannot be directly tested.

**DISCUSSION**

**Effects of Double-Helix Formation on the Photoreactions of Mpy-A.** Photoreversal is the only reaction that occurs when single-stranded Mmp-A is irradiated with UV light as described in the preceding paper. In the presence of complement B (5'-GCTCGTAGCTTC-3') or C (5'-TCGTAAGCT-3'), the photoreactions of Mmp-A become more complicated. Initially, there are two parallel reactions: photoreversal and photo-cross-linking of Mmp-A to its complement. The relative rate of photoreversal vs. photo-cross-linking is dependent on irradiation wavelength. As shown in Figure 4, except at wavelengths below 250 nm, photo-cross-linking is the favored reaction for the double-stranded Mmp-A:B, and the ratio of kRE/kRE increases as the irradiation wavelength increases. At wavelengths ≥313 nm, only photo-cross-linking occurs. Photo-cross-linking is still detectable at 379 nm. Due to the photoreversibility of the cross-link(s), unmodified oligonucleotide A will be the major product after prolonged irradiation at wavelength ≤313 nm.

The initial photoreversal rate constant kRE is larger than that for the single-stranded Mmp-A as reported in the preceding paper. As for Mmp-A in double-stranded Mmp-A:B, there are several possible causes for this enhanced photoreversal, which have not been tested. The kRE in Figure 4 overestimates the actual photoreversal rate constant, since cross-link formation and subsequent photoreversal are fast and 30% of this photoreversal yields unmodified oligonucleotide A (Cimino et al., 1986), which contributes to the apparent photoreversal rate.

The efficient photo-cross-linking of DNA-HMT furan-side monoadducts at wavelengths above 313 nm, where DNA bases do not absorb, enhances the usefulness of oligonucleotide hybridization probes. Cross-linkable HMT-monoadducted oligonucleotide probes facilitate solution hybridization formats, which are much more rapid and convenient than traditional hybridization methods. These same probes can be used to study hybridization kinetics and thermodynamics of the interactions between oligonucleotides and large nucleic acids (Cimino et al., submitted for publication; Gamper et al., submitted for publication).

**Quantum Yield of Photo-Cross-Linking of Mmp-A.** The accurate quantum yield for photo-cross-linking is difficult to determine due to the difficulty in measuring the extinction coefficient of the T-HMT furan-side monoadduct in the double-stranded Mmp-A:B. The quantum yield can be estimated approximately by using the photo-cross-linking rate constant kRE and the extinction coefficient of the isolated T-HMT furan-side monoadduct. Though the error in the quantum yield so calculated is unknown, the data in Table III clearly indicate that the quantum yield for photo-cross-linking of Mmp-A at wavelengths above 300 nm (2.4 x 10^-2) is much larger than the corresponding value for the photoreversal of the T-HMT furan-side monoadduct (7 x 10^-4, the preceding paper). The photoreversal of the single-stranded Mmp-A above 300 nm is even less efficient than that of the T-HMT furan-side monoadduct (see the preceding paper). This explains why at wavelengths ≥313 nm only photo-cross-linking was observed.

The quantum yields of photo-cross-linking of Mmp-A at wavelengths ≤281.7 nm are slightly larger than those at longer wavelengths. This difference could be due to either more...
efficient cross-linking of the monoadduct at these wavelengths or energy transfer from excited DNA bases to the HMT group in the monoadduct, especially around 260 nm, where DNA bases absorb efficiently. At wavelengths above 300 nm, DNA bases absorb little light, and only the absorption of the T-HMT furan-side monoadduct in the helix contributes to the photo-cross-linking. The quantum yield at wavelengths above 300 nm is similar to that observed for the photo-cross-linking of the thymidine-8-methoxypsoralen furan-side monoadduct in calf thymus DNA (Tessman et al., 1985).

Enhanced Photoreversal of MPy-A in Double-Stranded DNA. In contrast to MPy-A:B, UV irradiation of double-stranded MPy-A:B results mostly in photoreversal of MPy-A. Consequently, the photoreaction rate constant approximates the value of the photoreversal rate constant of MPy-A in the double helix. Results show that photoreversal of MPy-A in the double helix is more efficient than that in the single-stranded helix. Upon the formation of the double-stranded MPy-A:B, the electronic and steric constraints of the T-HMT pyrone-side monoadduct may change, thereby affecting the photoreversal of the monoadduct. As described above, energy transfer from excited DNA bases in the double-stranded DNA to the HMT group may also play a role in the enhancement of photoreversal. Two other mechanisms, though having only a small effect as described above, can also contribute to the enhanced photoreversal. These are photoreversal through a transient cross-link formation and direct photoisomerization. In the first case, the cross-link is formed initially and then photoreversed. Approximately 70% of the photoreversal of the cross-link will produce the furan-side monoadducted B and unmodified oligonucleotide A (Cimino et al., 1986), consequently increasing the apparent photoreversal rate constant of MPy-A. In the second case, the excited HMT group photoreverses at the pyrone end and simultaneously attaches to B through its furan end. This yields the same products as in the first case.

CONCLUSIONS

In this paper we have shown that the photoreactions of the HMT-monoadducted oligonucleotide in the double-stranded state are quite different from that in the single-stranded state. In the case of the pyrone-side monoadducted oligonucleotide, MPy-A, photoreversal is the predominant reaction even in a double helix, and very little cross-link is generated. The photoreversal of the monoadduct in the double-stranded DNA is enhanced compared to that in the single-stranded DNA. In contrast, UV irradiation of MPy-A in the double-stranded state produces predominantly cross-link, although the amount of cross-link decreases after prolonged irradiation at wavelengths ≤313 nm due to photoreversal of the cross-link. At wavelengths ≥313 nm, no photoreversal of MPy-A can be detected, and photo-cross-linking occurs with a quantum yield of 2.4 × 10^{-2}.

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