The Reactivity of 4,5′,8-Trimethylpsoralen with Oligonucleotides Containing AT Sites†

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ABSTRACT: Pyrimidine bases of duplex DNA, of appropriate sequence context, are photoreactive toward 4,5′,8-trimethylpsoralen in the presence of long-wavelength UV light. It is generally believed that a 5′-AT site is less photoreactive with psoralen than a 5′-TA site. We have compared the reactivities of these two sites using oligonucleotide duplexes of different sequence context and found that 5′-TA and 5′-AT sites are equally reactive in certain sequences. The presence of alternating pyrimidine and purine (5′-PyATPu-3′) bases in oligonucleotide duplexes optimizes the reactivity of 4,5′,8-trimethylpsoralen in the 5′-AT sites.

Psoralens are naturally occurring linear furocoumarins and are used with long-wave UV light as an effective treatment for skin ailments such as psoriasis. Psoralens such as 4,5′,8-trimethylpsoralen (TMP; Figure 1)[1] intercalate into double-stranded nucleic acids and, in the presence of 365-nm UV light, undergo a photochemical reaction with the pyrimidine residues to form photoadducts. The photoreaction occurs between the 5,6-double bond of a thymine residue and the 4′,5′-furan double bond or the 3,4-pyrone double bond of TMP to form a [2 + 2] cycloaddition product (Song & Tapley, 1979; Kanne et al., 1982). The photoadduct is known as a furan-side monoadduct or a pyrone-side monoadduct depending on which ring of the furocoumarin has reacted. Since, in a pyrone monoadduct, the coumarin nucleus is destroyed, it does not absorb in the 365-nm region. Only a furan-side monoadduct can undergo further photochemical reaction to form an interstrand cross-link. Because of these photochemical properties, psoralens have been found to be valuable for the study of the structure of nucleic acids (Ussery et al., 1993; Cimino et al., 1985). Psoralen monoadducts and interstrand cross-links are good examples of bulky DNA lesions and, therefore, are useful substrates for studying both procaryotic and eucaryotic DNA repair mechanisms (Sancar & Tang, 1993; Yeung & Grossman, 1990; van Houten, 1990).

Psoralens are most photoreactive in TA-rich DNA sequences. The order of reactivity is 5′-TATATA > 5′-TAT > 5′-ATA > 5′-TA (Sage et al., 1987; Yeung et al., 1988; Zen et al., 1986). Other dinucleotide sequences that show weak photoreactivity toward TMP include 5′-AT, 5′-TG, and 5′-GT sites (Esposito et al., 1988). The 5′-TATATA sequences are more reactive because they contain multiple psoralen-reactive sites. An additional reason is the fact that such sites contain alternating purine–pyrimidine sequences (Dall'Acqua et al., 1978; Gia et al., 1992). Another example of alternating purine–pyrimidine sequences which are very photoreactive with TMP is the thymine residues present within the 5′-GTAC duplex (Jones & Yeung, 1990; Sage & Moustacchi, 1987; Gamper et al., 1984; Esposito et al., 1988).

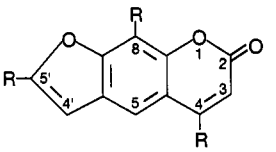

FIGURE 1: Structure of 4,5′,8-trimethylpsoralen (TMP). R = CH_3.

Because it is believed that the 5′-TA site is more reactive than the 5′-AT site (Esposito et al., 1988; Gamper et al., 1984; Kanne et al., 1982), the model sequences used to understand psoralen photoreaction and repair have so far focused on DNA sequences containing a single psoralen-reactive 5′-TA site. As a result, the psoralen cross-link at the 5′-AT site has been neglected in structural studies. The assumption that 5′-AT sites are less reactive is incompatible with our ability to isolate TMP cross-links at 5′-AT sites in good yield from restriction fragments (Jones & Yeung, 1990; Ramaswamy & Yeung, 1994). We observed that the 5′-AT sites tested in literature for TMP photoreactivity contain the 5′-PuATPy-3′ sequence (Esposito et al., 1988; Gamper et al., 1984). We reasoned that the 5′-AT site may be more reactive if it is in the 5′-PyATPu-3′ sequence, similar to its environment within a 5′-TATA psoralen-reaction hot spot. The experiments reported here were designed to test whether 5′-AT sites in an appropriate sequence context can be equal or more reactive toward TMP than 5′-TA sites.

EXPERIMENTAL PRECEDURES

Preparation of Oligonucleotide Duplexes. Methods for the preparation of ^{32}P-labeled DNA duplexes from oligonucleotides have been described previously (Yeung et al., 1988).

Kinetics of Psoralen–DNA Photoadduct Formation. A mixture of ^{32}P-labeled oligonucleotide duplex (10 μg, 308 μM nucleotide residues) and TMP (5 μg/mL, 22 μM) in 100 μL of 5 mM Tris-HCl, pH 7.6, 0.2 mM EDTA, and 50 mM NaCl was incubated at room temperature for 15 min. The reaction mixture was irradiated at 25 °C by a 500-W filtered light source (365-nm wavelength) at 130 J m^{-2} s^{-1} (Yeung et al., 1988). Ten-microliter aliquots were withdrawn after each time point and dried. To each dried aliquot was added tracking dye containing bromophenol blue and xylene cyanol FF (20

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[1] Abbreviations: bp, base pair(s); Pu, purine; Py, pyrimidine; TMP, 4,5′,8-trimethylpsoralen.

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A    5' G A C G T A C G T C  3' TY 235
     3' C T G C A T G C A G  5' TY 235

B    5' G A C G A T C G T C  3' TY 246
     3' C T G C T A G C A G  5' TY 246

C    5' G A C C A T G G T C  3' TY 245
     3' C T G G T A C C A G  5' TY 245

D    5' A G C C A T C T G C  3' TY 247
     3' T C G G T A G A C G  5' TY 248
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FIGURE 2: Sequence of the synthetic oligonucleotides used in this study. A–C: The oligonucleotides TY235, TY246, and TY245 are self-complementary. D: TY247/248 oligonucleotides can pair to form a duplex.

μL), and the sample was heated at 90 °C for 1 min. The cross-linked DNA and the monoadduct DNA were resolved from any unreacted material by electrophoresis in a 25% polyacrylamide gel containing 7 M urea at 49 °C. The gel was dried under vacuum. The bands were visualized by autoradiography. The radioactivity corresponding to the bands of starting material, monoadduct, and cross-link in each gel lane was measured by use of an AMBIS two-dimensional radioactivity densitometer (AMBIS Inc., CA). The percent photoadduct formed at each time point was calculated as: (monoadduct counts + cross-link counts)/total counts in each lane × 100.

RESULTS AND DISCUSSION

To test the comparative reactivity of the 5′-TA verus 5′-AT site, we have synthesized a set of oligonucleotides containing one single 5′-TA or 5′-AT site (Figure 2). The sequences of TY235, TY246, and TY245 (Figure 2A–C) oligonucleotides are self-complementary. The TY247/TY248 duplex (Figure 2D) contains a nonpalindromic DNA sequence. The sequence of TY235 (Figure 2A) contains a 5′-TA site within an alternating purine–pyrimidine sequence (5′-GTAC), and therefore, it is very photoreactive with TMP. The sequence of TY245 (Figure 2C) contains a 5′-AT site within an alternating pyrimidine and purine sequence at the center (5′-CATG).

The time course of photoreactivity of TMP with various oligonucleotides is shown in Figure 3 (A–D, lanes 1–6), and the plots of total photoadduct formed versus time for these oligonucleotide duplexes are shown in Figure 4. Comparison of the photoreactivity of the 5′-GTAC site (Figure 3A) versus the 5′-GATC site (Figure 3B) toward TMP revealed that the amount of photoadduct formed dropped almost 3-fold when the 5′-TA site is changed to a 5′-AT site within the same flanking sequence. However, when the 5′-GATC sequence in TY246 is changed to a 5′-CATG (TY245; Figure 2C), to conform to our alternating pyrimidine–purine prediction, the photoreactivity was restored (Figures 3C, 4). The amount of psoralen photoadduct formed from TY245 DNA duplex reaction with TMP is more than that formed from TY235 oligonucleotide duplex (Figure 4). The incomplete conversion of the monoadducted oligonucleotides to cross-linked oligonucleotides at longer irradiation times may indicate the formation of some 3,4-monoadducts which cannot lead to cross-links.

Our hypothesis that 5′-TA or 5′-AT sites in an alternating pyrimidine–purine sequence context are very reactive toward TMP would also call for the 5′-TATG (= 5′-CATA) and 5′-TATA sequences to be able to restore the TMP reactivity

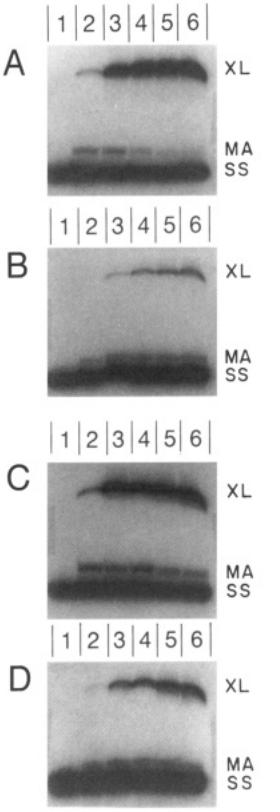

FIGURE 3: Photoreactivity of TMP with oligonucleotide duplexes. 5′-^{32}P-labeled DNA duplexes (10 μg) were reacted with TMP in the presence of 365-nm UV light in 100 μL of 5 mM Tris-HCl, pH 7.6, 0.2 mM EDTA, and 50 mM NaCl buffer. Ten microliters of the sample was withdrawn at different time points for electrophoretic analysis in a 25% polyacrylamide gel containing 7 M urea, 50 mM Tris-borate, and 1 mM EDTA, pH 8.3. The panels correspond to sequences presented in Figure 2. Lanes 1–6 represent the TMP photoreactions at 0, 1, 5, 10, 20, and 45 min, respectively. XL = cross-link; MA = monoadduct oligonucleotide, 3,4-MA or 4′,5′-MA; SS = single stranded.

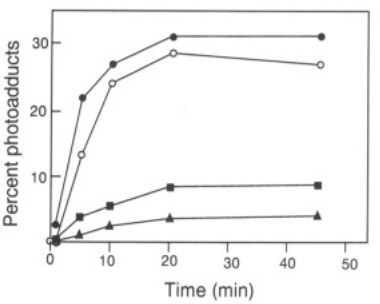

FIGURE 4: Kinetics of TMP reactivity with DNA duplexes. The radioactivity in each lane of the gels shown in Figure 3A–D was quantified using an AMBIS two-dimensional radioactivity densitometer. The photoreactivity of TMP toward the DNA duplexes corresponding to TY235, TY245, TY246, and TY247/Ty248 is represented by hollow circles, filled circles, filled squares, and filled triangles, respectively.

of the 5′-AT site. These sequences were not tested in the current study because these are already known to be hot spots of psoralen photoreaction (Sage et al., 1987). Thus, we conclude that the 5′-AT site in a 5′-PyATPu sequence is a preferred site for TMP photoreaction.

To test whether the alternating PyPuPyPu rule requires all four bp or whether 5′-PyAT or 5′-ATPu would be sufficient to confer higher psoralen reactivity, we made the DNA duplex which possesses both sequence contexts (Figure 2D). In the top strand (TY247), there is a 5′-CAT sequence, and in the bottom strand (TY248), there is a 5′-ATG sequence. The

results in Figures 3D and 4 show that neither sequence context is sufficient to restore the maximum photoreactivity of the 5′-AT site.

It is known that TMP photobinds with the B-form DNA more efficiently than with the A- and Z-form DNA (Cole, 1970; Sinden & Koechel, 1987; Esposito et al., 1988). Esposito et al. (1988) showed by circular dichroism analysis that the conformation of DNA duplexes varies in a sequence-dependent manner. They reasoned that subtle changes in local twist angles could contribute to overall DNA conformation and, hence, affect the reactivity of TMP toward 5′-TA sites in different DNA sequences. Similarly, the observed sequence-dependent reactivity of TMP toward 5′-AT sites can be due to variations in the conformation of different DNA sequences.

The reason for psoralen reactivity at alternating purine–pyrimidine sequences may be the easy unwindability of TA or AT sites in these sequences, which allows more psoralen intercalation and photoreactivity than in other sites. For example, the easy unwindability of TATA or TAAT sites has been hypothesized to explain the biological significance of these sites at the origins of replication (Drew et al., 1985; McClellan et al., 1986; Quintana et al., 1982). On the other hand, not all TA sites show partial unwinding. For example, the crystal structure of an A-form sequence containing 5′-TA sites (5′-GTACGTAC) showed unwinding at the central 5′-CG step, but the TA steps remained wound (Takusagawa, 1990).

The sequence-dependent effect on nucleic acid structure, based on crystallographic and modeling data, has been recently discussed (Hunter, 1993). However, it is not clear how the nucleic acid structure for different sequences would be perturbed after psoralen intercalation. Once the psoralen is localized in the double helix, the photocycloaddition of excited states (singlet or triplet) of furan or pyrone ring double bonds toward the pyrimidine double bond must be determined by both steric and electronic factors of the environment. Details on how neighboring nucleic acid bases change the electronic environment of the psoralen and thymine bases are not known. It is very likely that favorable alignment of the psoralen molecule with the thymine residues will be influenced by neighboring bases. The crystal structures of 8-methoxypsoralen monoadducts formed at the 5′-AT and 5′-TA sites have been solved (Peckler et al., 1982). The structures of these monoadducts revealed that thymine and psoralen moieties are in a planar configuration and the interplanar angle between the psoralen molecule and the thymine residue in the isomers of 5′-AT and 5′-TA monoadducts ranged from 44° to 53°. It can be reasoned that the sequence-dependent variation in conformation of 5′-AT or 5′-TA sites may change the above interplanar angles between the thymine 5,6-double bond and psoralen and, hence, alter the photoreactivity.

Reports on psoralen-binding studies with DNA showed that sequences containing alternating GC or AT bases exhibit tighter binding constants than bulk DNA which should contain random sequences (Dall'Acqua et al., 1978). It is possible that the high affinity of psoralen locally at alternating purine and pyrimidine bases containing 5′-TA or 5′-AT sites can lead to high photochemical reactivity at these sites.

Our analysis of the sequence effect of psoralen reactivity at 5′-AT has concentrated on the central four bases containing the 5′-AT site. It is possible that distal sequences may also change the photoreactivity of 5′-AT sites with TMP as shown for the 5′-TA site (Esposito et al., 1988). An examination of their data shows that for all except in one case (5′-TTTAAA sequence), the 5′-TA site is more reactive when it is in the 5′-PuTAPy sequence context. It will require a more comprehensive study to test whether the TMP reactivity at the 5′-TA site is less dependent on the alternating purine–pyrimidine sequence context than that at the 5′-AT site.

Recent studies (Gia et al., 1992) on several monoadduct-forming psoralens (angelicin, 2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one) indicated that for the 5′-AT site in one sequence (5′-CGAATTG), the psoralen photoreaction paralleled the reaction at a 5′-TA site in another oligonucleotide sequence (5′-CGTTTAAACG). For two bifunctional psoralens (8-methoxypsoralen, 5-methoxypsoralen), however, the above 5′-AT site sequence showed several fold reduced photoreactivity than the 5′-TA site sequence (Gia et al., 1992). The sequences used in the above experiments contained nonalternating purines and pyrimidines. It is possible that the alternating PyPuPyPu sequence is not a crucial factor for the alignment of monofunctional psoralens. One would expect the reactivity of bifunctional psoralens like TMP with a 5′-AT site to also be influenced by the structure of the psoralen used. Our results indicate that by the choice of a proper sequence context (PyPuPyPu), the reactivity of a bifunctional psoralen like TMP at 5′-AT sites can be equal to the reactivity at 5′-TA sites.

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