Interaction of psoralen-derivatized oligodeoxyribonucleoside methylphosphonates with synthetic DNA containing a promoter for T7 RNA polymerase

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ABSTRACT

The interaction of 4'-N(2-aminoethyl)aminomethyl-4,5',8-trimethylpsoralen-modified oligonucleoside methylphosphonates with synthetic ds-DNA containing a T7 RNA polymerase promoter was studied. The oligomers effectively crosslinked with either coding or noncoding ss-DNA when irradiated at 365 nm, but not with ds-DNA. The extent of the crosslinking reaction, which was complete within 16 min: (a) reached its maximum at an oligomer concentration of 3 μ M; (b) remained constant below the T_m of the duplex and then rapidly decreased; and (c) appeared to depend upon the sequence surrounding the psoralen crosslinking site. An oligomer crosslinked to the template strand inhibited transcription by T7 RNA polymerase whereas an oligomer crosslinked to the non-template strand had only a small inhibitory effect. Oligomers did not crosslink to ds-DNA undergoing transcription nor did they inhibit the transcription reaction.

INTRODUCTION

Psoralens, such as 8-methoxypsoralen and 4'-aminomethyltrimethylpsoralen, are a class of furocoumarins which are capable of intercalating with base pairs in double stranded regions of DNA and RNA. Upon irradiation with long wavelength ultraviolet light, a photocycloaddition reaction can occur between the 5,6 double bonds of pyrimidine bases and the 3,4 double bond of the pyrone ring or the 4',5' double bond of the furan ring to give cyclobutane monoadducts (1,2). If the psoralen has intercalated into a site containing pyrimidines suitably positioned in both strands of the nucleic acid, two cycloadditions can occur resulting in formation of a psoralen crosslink between the two strands.

The ability of psoralens to form both monoadducts and crosslinked diadducts with double stranded nucleic acids has been used to explore the secondary structure of RNA (2-4). In

addition to these structural studies, psoralens have also been used to study the structure of the transcription complex formed by RNA polymerase and DNA (5). These studies have been carried out using free psoralens as well as psoralens linked to thymidine residues of oligonucleotides. The latter type molecule allows the psoralen to be precisely positioned at a predetermined site in the DNA undergoing transcription (6). This technique has also been used to examine the repair of psoralen monoadducts in DNA (7-9).

We have recently described the synthesis of psoralen-derivatized oligonucleoside methylphosphonates and their interactions with single-stranded DNA and messenger RNA (10-12). Methylphosphonate oligomers derivatized with 4'-N(2-aminoethyl)-aminomethyl-4,5',8-trimethylpsoralen, (ae)AMT, through a phosphoramidate linkage at the 5'-end of the oligomer are able to crosslink with single-stranded nucleic acids in a sequence specific manner when irradiated at 365 nm. The extent of crosslinking is primarily dependent upon the ability of the oligomer to bind to its complementary site on the nucleic acid. In addition to providing a means of effectively linking the methylphosphonate oligomer to the nucleic acid at low oligomer concentrations, a feature which enhances its potential utility as an antisense reagent, psoralen crosslinking also provides a potential means for exploring nucleic acid secondary structure. Thus we found, for example, that oligomers whose mRNA binding sites were in single-stranded, nuclease sensitive regions of the mRNA, crosslinked 10- to 30-fold more effectively than those whose binding sites were in nuclease insensitive regions of the mRNA (12). Because oligonucleoside methylphosphonates are taken up intact by cells in culture, psoralen-derivatized oligomers may find applications for studying and controlling nucleic acid structure and function in living cells. In this paper we further explore and characterize the interaction of (ae)AMT-derivatized oligonucleoside methylphosphonates with synthetic DNA and study the effect of the oligomers on this DNA when it is undergoing transcription. Our results suggest that the in addition to the stability of oligomer binding, the extent of crosslinking is also influenced by the nucleotide sequence

surrounding the psoralen crosslinking site. It appears that although oligomers crosslinked to DNA prior to transcription can prevent transcription, DNA actively undergoing transcription is refractory to oligomer binding and subsequent crosslinking.

Materials and Methods

γ -[^{32}P]-ATP and α -[^{32}P]-ATP were purchased from Amersham Inc. T4 polynucleotide kinase and T7 RNA polymerase were purchased from United States Biochemical Corp. Polyacrylamide gel electrophoresis was carried out on 16cm x 18cm x 0.75mm gels containing 15% acrylamide and 7M-urea. The gels were run in TBE buffer which contains 0.089 M (hydroxymethyl)aminomethane (Tris), 0.089 M boric acid, and 0.2 mM ethylenediamine tetraacetic acid (EDTA) (13). Oligodeoxyribonucleoside methylphosphonates, their 4'-(aminoethyl)aminomethyl-4,5',8-trimethylpsoralen [(ae)AMT] derivatives and the oligodeoxyribonucleotide 57-mers were synthesized as previously described (11). The (ae)AMT-derivatized oligomers were purified by polyacrylamide gel electrophoresis. The isolated yields of the derivatized oligomers were 35%-45%. Each oligomer was stored in a 25 % ethanol solution at -20°C .

Crosslinking 4'-(aminoethyl)aminomethyl-4,5',8-trimethylpsoralen-derivatized oligonucleoside methylphosphonates with single-stranded DNA A 5 μl solution of 0.15 μM γ -[^{32}P]-labeled ssDNA 57-merI or II and 10 μM (ae)AMT-derivatized methylphosphonate oligomer in buffer containing 10 mM-Tris and 0.1 mM-EDTA, pH 7.5, was preincubated in a borosilicate glass test tube at 37°C for 5 min. The solution was then irradiated at 365 nm for 0 to 60 min at an intensity of $0.83 \text{ J}/\text{cm}^{-2} \text{ m}^{-1}$ in a thermostated water bath using a long wavelength ultraviolet lamp (Ultraviolet Product Inc.). The reaction mixture was then subjected to gel electrophoresis on a 15% polyacrylamide gel containing 7M urea. The wet gels were autoradiographed at -80° and the autoradiograms were scanned with an LKB ultrascan XL laser densitometer to quantitate the extent of photocrosslinking.

In vitro transcription Transcription reactions were carried out in a solution containing 40 mM-Tris (pH 7.6) 10 mM-magnesium chloride, 20 mM sodium chloride, 5 mM-dithiothreitol, 1 mM-NTP,

0.5 μM DNA template, 0 or 10 μM (ae)AMT-methylphosphonate oligomer and $1\mu\text{g}/\mu\text{l}$ T7 RNA polymerase in a total volume of 10 or 20 μl . [^{32}P]-Labeled transcripts were prepared by adding 1 μCi of α -[^{32}P]-ATP or 1 μCi α -[^{32}P]-UTP to the reaction mixture without changing the total concentration of the NTPs. The reactions were initiated by addition of T7 RNA polymerase and were incubated for 60 min at 37°C . The reactions were stopped by adding 10 mM EDTA and heating for 2 min at 90°C . The reactions were then analyzed by polyacryl-amide gel electrophoresis on a 15% gel containing 7-M urea. After autoradiography of the gels, the extent of polymerization was quantitated by densitometry. Each of the transcription experiments described below was carried out at least 4 times and gave essentially the same results.

RESULTS AND DISCUSSION

The general structure of the (ae)AMT-derivatized oligonucleoside methylphosphonates is shown in Figure 1. The (ae)AMT group is linked to the 5'-end of the oligomer via a phosphoramidate linkage which is readily formed by reaction of 4'-N(2-aminoethyl)aminoethyl-4,5',8-trimethylpsoralen with 5'-phosphorylated methylphosphonate oligomer in the presence of a water soluble carbodiimide (11). This linkage is resistant to hydrolysis by exo- and endonucleases (12). Treatment with acid cleaves the N-P bond resulting in formation of the original 5'-phosphorylated oligomer (11). The 5' terminal nucleotide unit is linked to the rest of the oligomer via a phosphodiester linkage.

(ae)AMT-derivatized oligomers complementary to single-stranded DNAs I and II were prepared. The sequences of the oligomers and the DNAs are shown in Figure 2. DNA I and II form a duplex which contains a 17 bp region corresponding to the consensus sequence for the T7 RNA polymerase promoter. We have previously studied the interaction of psoralen-derivatized oligonucleoside methylphosphonates complementary to single-stranded 35mers whose nucleotide sequences correspond to nucleotides 23-57 of I and II (11). Methylphosphonate oligomers 2 and 4 are complementary to two regions within the promoter sequence of the template strand (II) of the DNA duplex, whereas oligomers

Figure 1: Structure of oligonucleoside methylphosphonates derivatized with 4'-N(2-aminoethyl)-4,5',8-trimethylpsoralen.

1 and **3** are complementary to a promoter and nonpromoter regions of the non-template strand (I) of the duplex. For each of these (ae)AMT-oligomers, the (ae)AMT group occurs opposite a thymidine residue in the complementary DNA strand.

To study crosslinking of the (ae)AMT-oligomers to I or II, the 57-mers were end-labeled with a [32 P]-phosphate group. Crosslinking was detected by the mobility shift change of I or II on a denaturing polyacrylamide gel after irradiation of the 57-mer with an (ae)AMT-oligomer at 365nm. For example, as shown in Figure 3, irradiation of a solution containing oligomer **1** and

Figure 2: Sequences of the single-stranded DNA target molecules and their complementary (ae)AMT-derivatized methylphosphonate oligomers. The ■ indicates the (ae)AMT group. The oligomers shown in parentheses indicate additional binding sites for methylphosphonate oligomer **2**.

Figure 3: Crosslinking of (ae)AMT-derivatized oligomers to ssDNA. A solution containing 10 μ M (ae)AMT-oligomer and 0.15 μ M ssDNA was irradiated at 365nm for 20 min at 4 $^{\circ}$ C and the reaction mixture was subjected to PAGE. Lane 1: [32 P]-I; lane 2: oligomer 1 crosslinked with [32 P]-I; lane 3: oligomer 2 crosslinked with [32 P]-II.

I or oligomer 2 and II for 16 min at 4 $^{\circ}$ C results in virtually quantitative conversion of the 57 mer to crosslinked product. In the case of 1 a single crosslinked adduct is formed whereas oligomer 2 results in the formation of one major and two minor adducts. Irradiation of the adducts with UV light at 254nm results in complete conversion to the starting 57-mers (data not shown). This behavior is consistent with the formation of a cyclobutane bridge between the (ae)AMT group of the oligomer and the thymine base of the 57-mer as a result of the photocycloaddition reaction (11,14).

Of the three adducts formed by 2, one has the same mobility as that formed by 1, whereas the other two have lower mobilities and hence higher molecular weights. This behavior suggests that the three adducts formed by 2 consist of one, two or three molecules of 2 crosslinked to II. It appears that the adduct produced in highest yield is that in which three molecules of 2 are crosslinked to one molecule of II. Examination of the sequence of II shows that in addition to the complementary binding site at nucleotides 16 to 23 (counting from the 3'-end of II), there are two additional potential binding sites at nucleotides 37 to 44 and 46 to 53. Binding between 2 and

Figure 4: Effect of temperature on crosslinking between oligomer 2 and ss DNA I. A solution containing 10 μM 2 and 0.15 μM [^{32}P]-II was irradiated at 365nm for 20 min at 20°C (lane 2), 30°C (lane 3) or 40°C (lane 4) and the reaction mixture was subjected to PAGE. Lane 1 contains [^{32}P]-II.

nucleotides 37 to 44 would result in the formation of 5 G-T base pairs and would position the (ae)AMT group opposite T residue #36. Binding of 2 to nucleotides 46 to 53 would result in formation of 2 G-T base pairs, 2 G-A base pairs (14) and an A-A mismatch at position #47. The (ae)AMT group would be positioned opposite T residue #45.

Previous studies have shown that inclusion of G-T base pairs in duplexes formed between ssDNA and psoralen-derivatized methylphosphonate oligomers results in lower extents of crosslinking (11). Although duplexes formed at positions 37 to 44 and 46 to 53 would contain less stable G-T and/or G-A base pairs, duplex formation may be favored at low temperature by the potential cooperativity between the two oligomers binding to adjacent sites on the 57-mer. This hypothesis is supported by the observed temperature effects on the crosslinking reaction as shown in Figure 4. At 20°C essentially two crosslinked products are observed: 57 mer crosslinked with one molecule of 2 and 57-mer crosslinked with three molecules of 2. Little or no product corresponding to two crosslinked molecules of 2 is observed. At temperatures above 30°C, only 57-mer crosslinked to one molecule of 2 is formed. Thus it appears low temperature favors formation

Figure 5: Effect of temperature on crosslinking between oligomer 1 and ssDNA I (—●—●—) or oligomer 2 and ssDNA II (—■—■—). The reaction conditions are the same as those given in Figure 4.

of complexes containing three molecules of 2, whereas at higher temperatures these complexes directly melt out with the formation of a duplex containing one molecule of 2.

The effect of temperature on the crosslinking reactions of 1 and 2 is further illustrated in Figure 5. In this experiment, the conversion of I or II to crosslinked product was monitored as a function of increasing temperature by polyacrylamide gel electrophoresis. The extent of crosslinking remains essentially unchanged over the temperature range 4 to 30°C and then begins to drop off rapidly at higher temperatures. However even at 50°C, the extent of crosslinking is approximately 70%. The similar temperature profiles suggest that these oligomers form duplexes with I and II of equal stability and is consistent with their potential to each form duplexes having 5 G-C base pairs. Although the T_m 's of the duplexes formed by 1 and I or 2 and II under conditions where the concentration of the (ae)AMT oligomers are in 70-fold excess of those of the 57 mers are not

Figure 6: Kinetics of crosslinking at 37°C between oligomer 1 and ssDNA I (—●—●—) or oligomer 2 and ssDNA II (—■—■—). The oligomer and ssDNA concentrations are the same as those given in Figure 4.

known, it would appear from these results that T_m 's are approximately 53°C.

The kinetics of crosslinking and the effect of oligomer concentration on the extent of crosslinking at 37°C were studied. As shown in Figure 6, both 1 and 2 have almost identical rates of crosslinking. The extent of crosslinking levels off after 20 min irradiation. A similar behavior was previously observed for psoralen-derivatized methylphosphonate 12 mers and 15 mers targeted against ssDNA (11). The lack of further crosslinking after 20 min, apparently is the result of inactivation of the (ae)AMT group due to photochemical degradation of the pyrone ring (1,11).

The extent of crosslinking during 20 min irradiation increases over the concentration range of 0.5 to 3 μ M for both oligomers 1 and 2. In these experiments the concentration of I or II is 0.15 μ M. The extent of oligomer crosslinking in these experiments depends upon the extent to which the oligomer is bound to the target, which in turn depends upon the binding equilibrium constant; the rate of the crosslinking reaction; and the rate of photochemical formation of inactive oligomer. It

seems reasonable that the starting oligomer and the inactive oligomer will have approximately the same binding constant with the 57 mer. Thus inactive oligomer, whose concentration increases during the course of the irradiation will effectively compete with original oligomer for binding to the 57 mer. The results suggest that binding of oligomer to the 57 mer is saturated above concentrations of 3 μ M and that the rates of crosslinking and photochemical inactivation are similar.

Crosslinking of oligomers 1 and 2 to I and II is essentially quantitative after 20 min irradiation at 4 °C as shown in Figure 5. In contrast, the extent of crosslinking of 3 to I is 50% whereas that of 4 to II is 30% under these conditions. Oligomers 1, 2, and 3 can each form duplexes having five G-C and three A-T base pairs whereas oligomer 4 will form a duplex having three G-C and five A-T base pairs. Although the difference in G-C and A-T base pairs for oligomer 4 versus that of 3 could explain the reduced level of crosslinking of 4, based on this criterion, it would appear that oligomers 1, 2, and 3 should exhibit the same extents of crosslinking. In addition, because the experiment was carried out at 4°C, which is well below the estimated T_m of the duplexes, one would expect all four oligomers to undergo equal extents of crosslinking.

Examination of molecular models suggests that the (ae)AMT group of oligomers 1-4 can interact with the target nucleic acid strand in two ways. The psoralen ring could stack on the terminal base pair of the duplex and intercalate between the 3'-terminal base and the T-residue of the target strand as illustrated in Figure 7a. In this model, the psoralen pyrone ring overlaps the thymine ring and is suitably positioned to undergo photocycloaddition to yield a cis-syn adduct, which is the same configuration as that found for photoadducts of 8-methoxypsoralen or 4'-hydroxymethyl-4,5',8-trimethylpsoralen with DNA (15-18). If we designate the target strand nucleoside complementary to the 5'-nucleoside of the oligomer as n, then the psoralen ring would intercalate between nucleosides n and n+1 and crosslinking would occur with nucleoside n+1. Alternatively the psoralen ring could intercalate between the terminal and next to last base pairs of the duplex as illustrated in Figure 7b. It

Figure 7: Stereodrawings of interaction of an (ae)AMT-derivatized oligomer with its target nucleic acid at the psoralen binding site. In each drawing the left hand strand is the ssDNA target having the sequence 5'---TpApT---3', and the right hand strand is the 5'-end of the (ae)AMT-oligomer having the sequence 5'-(ae)AMTpTpA---3'. The arrow indicates the position of the pyrone ring of the (ae)AMT group. In A the (ae)AMT is intercalated between nucleosides n and $n+1$ of the ssDNA strand, whereas in B the (ae)AMT is intercalated between nucleosides n and $n-1$ of the ssDNA strand.

appears that the aminoethyl linker arm is long enough to accommodate this mode of intercalation and that the pyrone ring would be suitably oriented to undergo photocycloaddition if a pyrimidine nucleoside were present at this position in the target strand. In this case the psoralen would intercalate between nucleoside n and $n-1$ and crosslinking could occur with nucleoside $n-1$. It appears unlikely, based on examination of models, that the psoralen ring is suitably oriented for crosslinking to nucleoside n if this nucleoside is a pyrimidine.

The extent of crosslinking as a function of the nucleotide sequence at the psoralen binding site is shown in Table I. In addition to the psoralen binding sites for oligomers 1 through

Table I. Extent of Crosslinking of (ae)AMT-Derivatized Oligonucleoside Methylphosphonates to Single Stranded DNA^(a)

Oligomer	Binding Site ^(b)	% Crosslinking
<u>1</u>	5' - CAT - 3' - GT•	> 95%
<u>2</u>	5' - TAT - 3' - AT•	> 95%
<u>3</u>	5' - ACT - 3' - TG•	50%
<u>4</u>	5' - AGT - 3' - TC•	30%
<u>5</u>	5' - TTT - 3' - AA•	85%
<u>6</u>	5' - TTA - 3' - AA•	35%
<u>7</u>	5' - TAT - 3' - AT•	85%

- (a) Crosslinking was carried out under the following conditions: [ssDNA], 0.15 μ M; [oligomer], 10 μ M; temperature, 4°C; solvent, 10 mM Tris, 0.1 mM EDTA (pH 7.5) or H₂O; irradiation, 365 nm for 20 min.
- (b) The upper strand is the sequence of the ssDNA at the psoralen binding site and the lower strand corresponds to 5'-end of the (ae)AMT-derivatized oligomer. The • represents (ae)AMT. For oligomers 1 - 4, the ssDNA is 57 mer I or II. For oligomers 5-7, the ssDNA is a 35 mer III.

4, the binding sites for three other (ae)AMT-derivatized oligomers, 5 through 7 are shown. The extent of crosslinking of these latter oligomers to a single-stranded DNA 35 mer (III) whose sequence corresponds to nucleotides 23-57 of II was determined. The sequence of the 35 mer (III) and oligomers 5-7 are shown in Figure 2.

The greatest extent of crosslinking is observed for oligomers 1, 2, 5 and 7. In each case the nucleosides at n+1 and n-1 are pyrimidines and crosslinking could presumably occur at either position. However in the case of oligomer 1 the nucleoside at n-1 is deoxycytidine. Studies by Hearst and coworkers (20) and recent studies from our laboratory (12) suggest that the extent of crosslinking to a cytosine-containing nucleoside is approximately 15-fold less than to thymidine or uridine.

Based on these results, it would appear that crosslinking should occur primarily to nucleoside n+1 in the target strand for oligomer 1.

Lower extents of crosslinking are observed for oligomers 3, 4, and 6. In the case of 3 and 4, crosslinking should only occur at position n+1 whereas in the case of 6 crosslinking should only occur at position n-1 of the target strand. The observation that each of these oligomers does crosslink to the target strongly suggests that psoralen can crosslink to pyrimidine nucleotides at either the n+1 or n-1 position in the target strand and thus supports the intercalation models shown in Figures 7a and 7b. The reduced levels of crosslinking of 2, 3 and 6 relative to that of oligomers 1, 2, 5, and 7 could be explained by the reduced number of crosslinking sites in the target strand. However, the observation that 1 crosslinks >95% suggests that 3 and 4 should also be capable of extensive crosslinking to thymidine at n+1.

The terminal base pair of oligomer 1 is A-T whereas the terminal base pairs of oligomers 3 and 4 are G-C. Studies by Hearst and coworkers suggest that psoralen derivatives preferentially intercalate and photoreact at sites containing an A-T base pair as opposed to sites which contain a G-C base pair (2,21). The reduced extent of crosslinking by 3 and 4 could thus be due to the reduced ability of the (ae)AMT group to intercalate and/or photocyclize at a site terminating with a G-C base pair. More extensive studies on the influence of the sequence at the psoralen binding site on crosslinking will be required to test this possibility.

Single-stranded DNAs I and II form a duplex which can be transcribed by T7 RNA polymerase to yield an RNA molecule 40 nucleotides in length. The ability of oligomers 1 and 2 to crosslink to duplex I+II during transcription was examined. Neither 1 or 2 crosslinked to the duplex formed by I and II upon irradiation at 365 nm (data not shown). Irradiation of a mixture of the duplex and either oligomer in the presence of nucleoside triphosphates and T7 RNA polymerase, conditions under which the duplex is transcribed, did not lead to any detectable crosslinking to either I or II.

Figure 8: Effect of (ae)AMT-oligonucleoside methylphosphonates **1** or **2** on transcription of duplex I+II by T7 RNA polymerase. The reaction conditions are described in Materials and Methods. In addition to unlabeled nucleoside triphosphates, α -[32 P]-ATP and RNA polymerase, the reaction mixtures contained the following components: Lanes 1 and 6: I+II incubated for 60 min; Lane 2: I+II irradiated for 10 min and incubated an additional 50 min; Lane 3: I+II and oligomer **2** incubated for 60 min; Lane 4: I+II and oligomer **1** irradiated for 10 min and incubated an additional 50 min; Lane 5: I+II and oligomer **2** irradiated for 10 min and incubated an additional 50 min; Lane 7: I and oligomer **1** preirradiated for 10 min and then incubated with RNA polymerase and nucleoside triphosphates for 50 min; Lane 8: II and oligomer **2** preirradiated for 10 min and then incubated with RNA polymerase and nucleoside triphosphates for 50 min; Lane 9: I and oligomer **1** preirradiated for 10 min and then incubated with II, RNA polymerase and nucleoside triphosphates for 50 min; Lane 10: II and oligomer **2** preirradiated for 10 min and then incubated with I, RNA polymerase and nucleoside triphosphates for 50 min. The arrow indicates the position of the RNA 40-mer transcript.

The effect of the oligomers on the transcription reaction itself were also investigated. The results of a typical experiment are shown in Figure 8. Lanes 1 and 6 show the position of the RNA transcript after incubation of duplex I+II with nucleoside triphosphates and T7 RNA polymerase for 60 min at 37°C. The band indicated by the arrow corresponds to the 40-mer RNA transcript. Additional longer transcripts were consistently observed near the top of the gel in all the experiments we performed. Irradiation of the transcription reaction mixture at

365nm does not have any effect on transcription of the duplex as shown in lane 2. Oligomer 2, which is complementary to the template strand of duplex I+II, did not affect the transcription reaction either alone or when irradiated at 365nm (lanes 3 and 4). Likewise oligomer 1, which is complementary to the non-template strand of duplex I+II, did not affect the transcription reaction upon irradiation (lane 5). Synthesis of RNA was inhibited approximately 70% if II was first crosslinked with oligomer 2 and subsequently annealed with I and the duplex transcribed (lane 10). A similar experiment in which the non-coding strand I was crosslinked with oligomer I prior to annealing with II and transcription gave essentially no inhibition of RNA synthesis (lane 9). No RNA synthesis was observed if I crosslinked with 1 or II crosslinked with 2 was incubated with RNA polymerase under the transcription conditions (lanes 7 and 8). This reflects the inability of the polymerase to transcribe the ssDNA.

The results of these experiments suggest that although the DNA strands of duplex I+II may exist in an open form during transcription (22-25), they are not accessible to oligomer binding. Possibly the bases of the separated strands are blocked from participating in hydrogen bonding interactions by the RNA polymerase. Inhibition of transcription can occur if oligomer 2 is precrosslinked to the coding strand of the duplex. In this case the oligomer is crosslinked to part of the promoter sequence and could affect polymerase binding by distorting the conformation of the duplex or by directly blocking binding of the polymerase to the duplex. Oligomer 1 precrosslinked to the non-template strand of the duplex had little or no effect on the transcription reaction. This result suggests that the oligomer does not sufficiently distort the duplex to prevent duplex formation at the promoter region, an apparent prerequisite to transcription since transcription does not occur on II alone. These results are also consistent with those of Shi *et al.* (7) in which they showed that psoralen monoadducts on the template strand prevent polymerase elongation but monoadducts on the non-template do not prevent transcription. The experiments described in Figure 8 also demonstrate

that irradiation of the reaction mixture and (ae)AMT-derivatized oligomers does not result in damage to RNA polymerase. This result suggests that during irradiation, the action of the (ae)AMT-derivatized oligomers is confined to their single-stranded target nucleic acids and that unwanted side reactions with cellular proteins resulting from UV irradiation are unlikely to occur.

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