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Biochemistry

Sequence-specific binding and photocrosslinking of α and β oligodeoxynucleotides to the major groove of DNA via triple-helix formation

[*p*-azidophenacyl/DNA cleavage/1-(2′-deoxy-α-D-ribofuranosyl)thymine/triple-stranded nucleic acids]

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ABSTRACT **A photocrosslinking reagent (*p*-azidophenacyl) was covalently linked to an octathymidylate synthesized with either the natural (β) anomer of thymidine or the synthetic (α) anomer. The oligothymidylate was further substituted by an acridine derivative to stabilize the hybrid formed with a complementary octadeoxyadenylate sequence via intercalation. A single-stranded 27-mer containing a $(dA)_8$ sequence and a 27-mer duplex containing a $(dA{\cdot}dT)_8$ sequence were used as targets. Upon UV irradiation, photocrosslinking of the octathymidylate to its target sequence was observed, generating bands that migrated more slowly in denaturing gels. In the 27-mer duplex, both strands were photocrosslinked to the octathymidylate. Upon alkaline treatment of the irradiated samples, cleavage of the 27-mers was observed at specific sites. These reactions were analyzed at different salt concentrations. The location of the cleavage sites allowed us to demonstrate the following. (*i*) Both α and β oligothymidylates can recognize a DNA double helix containing an oligo(dA)·oligo(dT) sequence; the oligothymidylate binds to the major groove of DNA in a parallel orientation with respect to the adenine-containing strand of the DNA double helix. (*ii*) α oligothymidylates form helices with a complementary single-stranded oligodeoxyadenylate; the two strands have a parallel orientation independently of whether or not an intercalating agent is attached to the oligothymidylate. (*iii*) At low salt concentration, β oligothymidylates form a double helix with an oligodeoxyadenylate in which, as expected, the two strands are antiparallel; at high salt concentration, a triple helix is formed in which the second oligothymidylate is oriented parallel to the adenine-containing strand. These results show that it is possible to recognize an oligopurine·oligopyrimidine sequence in a DNA double helix via local triple-helix formation and to target photochemical reactions to specific sequences in both double-stranded and single-stranded nucleic acids.**

The recognition of double-stranded DNA by proteins plays a central role in the regulation of gene expression (see ref. 1 for review). On the basis of recent discoveries on the mechanisms of base-sequence recognition by proteins, it has become possible to synthesize oligopeptides that bind selectively to double-stranded DNA (2). There are several drugs that can achieve sequence-specific recognition; however, even for the most specific DNA ligands, there is an important degeneracy in the binding sequence (3). Oligonucleotides provide another possibility for the specific recognition of certain base-pair sequences in the double helix via local triple-helix formation. It has been known for a long time that poly(dA) and poly(dT) can form both double and triple helices, depending on salt concentration and temperature (4). The formation of triple helices by poly[d(A-G)]·poly[d(C-T)] has also been described (5). Both Watson–Crick and Hoogsteen base pairings are involved, with one cytosine being protonated in the C·G·C triplex. Polypurine·polypyrimidine sequences in the genome adopt an unusual structure that makes them hypersensitive to the action of single-strand-specific nucleases (6). Here we demonstrate that a stretch of A·T base pairs in a double-stranded DNA can be selectively recognized by oligothymidylates. Attachment of a *p*-azidophenacyl group to the terminal (3′ or 5′) phosphate allows photocrosslinking to either strand of the double helix under UV excitation ($\lambda > 300$ nm). Crosslinks can be converted to chain breaks under alkaline conditions (7). Substituting an acridine derivative at the other end of the oligothymidylate stabilizes the complex via intercalation. We have used oligothymidylates synthesized with either the natural (β) anomer or the unnatural (α) anomer of thymidine. The most active oligonucleotide for the recognition and cleavage of the DNA duplex is an α oligothymidylate with the *p*-azidophenacyl group and the intercalating agent attached to the 3′ and 5′ ends, respectively. We also show that a β oligothymidylate covalently linked to an acridine derivative and a *p*-azidophenacyl group forms both a double and a triple helix with a single-stranded oligodeoxyadenylate sequence. α oligothymidylates form double helices with their complementary oligodeoxyadenylate sequence in which the two strands adopt a parallel orientation.

MATERIALS AND METHODS

Chemical Synthesis. The two 27-mer oligodeoxynucleotides whose sequences are shown in Figs. 1–3 were synthesized with a Pharmacia automatic synthesizer using phosphoramidite chemistry. They were purified by polyacrylamide gel electrophoresis followed by reverse-phase chromatography. They will be referred to as 27-mer(A_8) and 27-mer(T_8) to indicate that they contain either an A_8 or a T_8 sequence.

The synthesis of the α oligothymidylate $(\alpha Tp)_7\alpha T$ was achieved by the phosphotriester method previously applied to β oligonucleotides but using 1-(2′-deoxy-α-D-ribofuranosyl)thymine (α-thymidine, Sigma) as the starting material (8). A thiophosphate group was introduced either at the 5′ end or at the 3′ end of the α oligomer by using bis(cyanoethyl) thiophosphate as an intermediate (9). The terminal

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Abbreviations: $N_3\phi$- and -ϕN_3, *p*-azidophenacyl thiophosphate at the 5′ and 3′ ends of oligodeoxynucleotides; Acr, acridine derivative at the 5′ or 3′ end of oligodeoxynucleotides.

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thiophosphate reacted with *p*-azidophenacyl bromide (Pierce) to give the *p*-azidophenacyl thiophosphate derivative of the oligonucleotide (abbreviated as $N_3\phi$). The same procedure was applied to α and β oligothymidylates. Purification was achieved by high-performance liquid chromatography on polyanion HR 5/5 columns (Pharmacia).

Previous work (10–12) showed that the stability of oligonucleotide complexes with complementary sequences could be strongly increased by covalent attachment of an intercalating agent 2-methoxy-6-chloro-9-aminoacridine (abbreviated as Acr) to the 5′ or 3′ end of an oligonucleotide. The intercalating agent provides additional binding energy without perturbing the specificity of the oligonucleotide towards its complementary sequence. We applied the same chemical strategy to α oligothymidylates. The following compounds were synthesized: $N_3\phi$-βT_8-Acr, $N_3\phi$-αT_8, and Acr-αT_8-ϕN_3. Each oligomer is written in the 5′→3′ direction from left to right with respect to the oligothymidylate sequence. For example, in Acr-αT_8-ϕN_3 the octathymidylate was synthesized with the α anomer of thymidine, the acridine derivative (Acr) was attached to the 5′ phosphate (via a pentamethylene linker), and the *p*-azidophenacyl group ($-\phi N_3$) was linked to a 3′ thiophosphate.

Photocrosslinking and Cleavage. The two 27-mers were 5′-end-labeled by use of bacteriophage T4 polynucleotide kinase and [γ-^{32}P]ATP (Amersham). The experiments using the single-stranded 27-mer(A_8) were carried out at a concentration of 10 nM (strand concentration) and oligothymidylate concentrations ranging from 0.5 to 5 μM. In the experiments involving the 27-mer duplex, the two 27-mers were mixed at 20°C, then heated to 80°C and slowly cooled to 0°C. A slight excess (2 nM) of the 27-mer(T_8) was used in order to avoid the presence of free 27-mer(A_8). The duplex concentration was 10 nM. All solutions were made in a pH 7.0 buffer containing 10 mM sodium phosphate and varying concentrations of sodium chloride.

Irradiation was carried out at 0°C with a high-pressure mercury HBO Osram 200-W lamp. A glass plate was used to remove wavelengths below 300 nm. A study as a function of irradiation time indicated that maximum cleavage was obtained after 5 min of irradiation. This duration of irradiation was used in all further experiments. The irradiated samples were then treated with 1 M piperidine at 90°C for 20 min. Electrophoresis was carried out in 20% polyacrylamide denaturing gels and autoradiographed with Fuji films at −70°C using intensifying screens.

RESULTS

Photocrosslinking of Azidophenacyl-Substituted α and β Octathymidylates to a Single-Stranded 27-mer. Derivatized octathymidylates were irradiated at 0°C in the presence of a 5′-labeled 27-mer containing an octadeoxyadenylate sequence. On denaturing 20% polyacrylamide gels bands were revealed that migrated more slowly than the 27-mer (Fig. 1). The number and the distribution of photocrosslinked species changed with NaCl concentration (Fig. 1). The band intensity corresponding to the photocrosslinked species observed at low salt was increased at high salt concentration. No cleavage of the 27-mer was observed when the samples were kept at pH 7.0. The slowly migrating bands of the irradiated samples at pH 7 are due to photocrosslinking of the octathymidylates to the 27-mer. Photocrosslinking of an octathymidylate to the 27-mer should produce branched species due to the location of the target octadeoxyadenylate sequence in the center of the 27-mer. The migration of such branched species in a denaturing gel is expected to depend on the crosslinking site. In addition, the positively charged acridine dye attached at the end of the octathymidylate retards the migration.

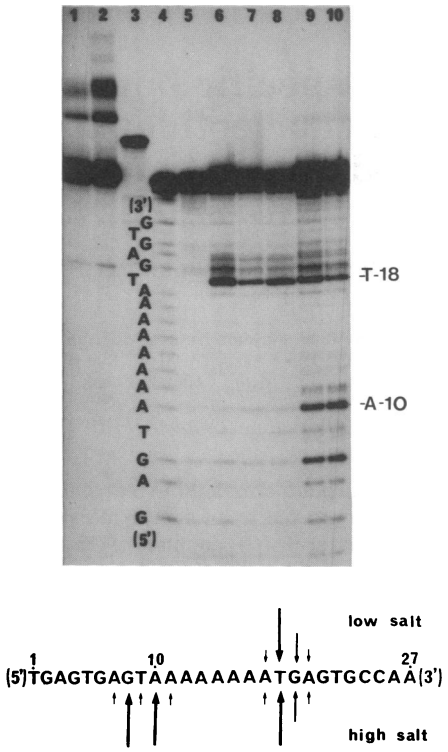

FIG. 1. (*Upper*) Salt dependence of photocrosslinking and cleavage of the single-stranded 27-mer(A_8) by $N_3\phi$-βT_8-Acr. The 27-mer (10 nM) was mixed with the octathymidylate and UV irradiated as indicated in *Materials and Methods*. Lanes 1 and 2: samples were loaded at neutral pH; the NaCl concentration in the irradiation buffer was 0.25 M (lane 1) or 1 M (lane 2). Photocrosslinked species appear as slowly migrating bands above the 27-mer band (the band below is an impurity present in unirradiated 27-mer). Lane 3: a 34-mer used as a length marker. Lane 4: G + A sequence of the 27-mer. Lane 5: control 27-mer irradiated in the absence of oligothymidylate and treated with piperidine. Lanes 6–10: irradiated samples were treated with 1 M piperidine at 90°C for 20 min; NaCl concentration was 0.1 M (lane 6), 0.2 M (lane 7), 0.25 M (lane 8), 0.5 M (lane 9), or 0.75 M (lane 10). Less radioactivity was loaded in lanes 7 and 8 than in lanes 6, 9, and 10. (*Lower*) Cleavage sites observed after UV irradiation and alkaline treatment of the 27-mer(A_8) sequence in the presence of $N_3\phi$-βT_8-Acr. Arrows above the 27-mer sequence indicate the photocrosslinked bases at low (0.1 M) NaCl concentration; arrows below correspond to high (0.75 M) NaCl concentration.

Cleavage at the Photocrosslinked Sites Under Alkaline Conditions. Upon treatment of the UV-irradiated octathymidylate/27-mer mixtures with 1 M piperidine at 90°C for 20 min, the slowly migrating bands disappeared and new bands corresponding to cleaved products of the 27-mer were observed (Figs. 1 and 2). The cleavage sites were located at well-defined sites along the 27-mer. In the β octathymidylate ($N_3\phi$-βT_8-Acr) the azidophenacyl group was attached to the 5′ end. At 0.1 M NaCl, cleavage was observed on the 3′ side of the A_8 sequence with an efficiency decreasing in the order T-18 > G-19 > A-20. Therefore, as expected, the β oligothymidylate was bound in an antiparallel orientation with respect to the A_8 sequence. At high salt concentration, new bands were observed due to cleavage on the 5′ side of the A_8 sequence in addition to the bands (due to cleavage on the 3′ side) observed at low salt. Oligothymidylates are able to form triple helices with an oligodeoxyadenylate sequence—e.g., in poly(dT)·poly(dA)·poly(dT) (4). The formation of the triple helix is favored at high salt as a result of a decrease in the electrostatic repulsion between the negatively charged phosphodiester backbones. Photocrosslinking and cleavage

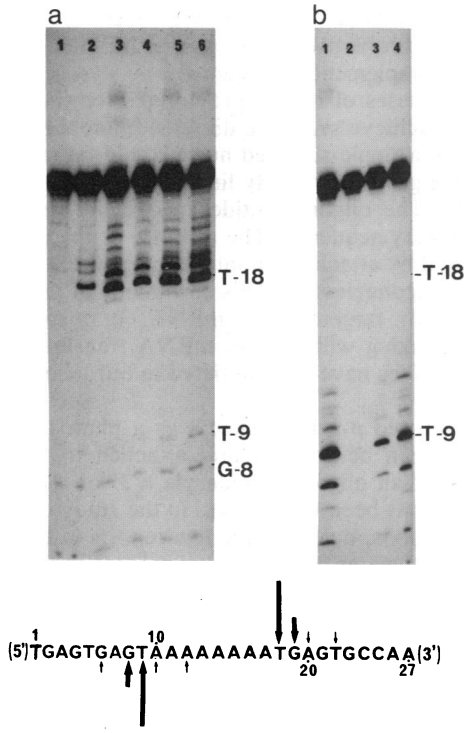

FIG. 2. (*Upper*) Salt dependence of photocrosslinking and cleavage of the 27-mer(A_8) sequence in the presence of Acr-αT_8-ϕN_3 (*a*) or $N_3\phi$-αT_8 (*b*). In *a*, lanes 1–3 correspond to oligothymidylate concentrations of 0.05, 0.5, and 5 μM, respectively. Salt concentration was 0.5 M NaCl. Lanes 4–6 correspond to an oligothymidylate concentration of 5 μM and NaCl concentrations of 0, 0.1, and 0.25 M, respectively. The pH 7 buffer contained 10 mM sodium phosphate. In *b*, lanes 1–4 correspond to an oligothymidylate concentration of 5 μM and NaCl concentrations of 0.5, 0, 0.1, and 0.25 M, respectively. (*Lower*) Main cleavage sites observed after photocrosslinking and alkaline treatment of the 27-mer(A_8) in the presence of Acr-αT_8-ϕN_3 (above the sequence) or $N_3\phi$-αT_8 (below the sequence).

on both the 5′ and the 3′ side of the 27-mer(A_8) sequence observed at high salt indicated that $N_3\phi$-βT_8-Acr was able to form a triple helix with the A_8 sequence of the 27-mer. One of the T_8 strands was antiparallel to the A_8 sequence as observed in the double helix and the second one was oriented parallel to the A_8 strand.

When the α oligomer Acr-αT_8-ϕN_3 was used, the main cleavage sites were observed on the 3′ side of the A_8 sequence (T-18 being the most reactive) even though the azidophenacyl group was attached to the 3′ end of the oligonucleotide (Fig. 2). This result unambiguously demonstrated that the α oligothymidylate formed a double helix with the A_8 sequence of the 27-mer in which the two strands were oriented parallel to each other. This conclusion was confirmed by the results obtained with $N_3\phi$-αT_8, where the *p*-azidophenacyl group was attached to the 5′ end. Cleavage sites were located on the 5′ side of the A_8 sequence (with T-9 being the most reactive site), again demonstrating that the two strands were parallel in the α/β hybrid (Fig. 2). In the case of the two α octathymidylates, increasing the salt or the oligonucleotide concentration did not lead to the appearance of new cleavage bands after photocrosslinking to the 27-mer and alkaline treatment (Fig. 2). This indicated either that no triple helix was formed or that the two octathymidylates in the triple helix adopted the same parallel orientation with respect to the A_8 sequence. Spectroscopic measurements (to be described elsewhere) showed that Acr-αT_8-ϕN_3 formed a double helix with octadeoxyadenylate both at low (0.1 M) and high (1 M) NaCl concentration. Therefore the results described in Fig. 2 at low and high NaCl concentrations reflect the properties of the double helix formed by the 27-mer with Acr-αT_8-ϕN_3. The α oligothymidylate substituted by the acridine derivative gave the most efficient reactions, in agreement with the stabilization expected from the stacking interactions involving the acridine ring and the base pairs of the mini-duplex structure (for example, compare lane 4 of Fig 2*a* to lane 2 of Fig 2*b*).

Photocrosslinking and Cleavage of a DNA Double Helix. A 27-mer duplex containing eight A·T base pairs was used as a target for oligothymidylates covalently linked to a *p*-azidophenacyl group. One of the strands will be referred to as 27-mer(T_8) and the other as 27-mer(A_8). A slight excess of 27-mer(T_8) was used in order to ensure that there was no free 27-mer(A_8) available to form a double helix with the octathymidylate. Upon UV irradiation, photocrosslinking and cleavage were observed at well-defined positions on both strands (Fig. 3). With Acr-αT_8-ϕN_3 the most reactive site

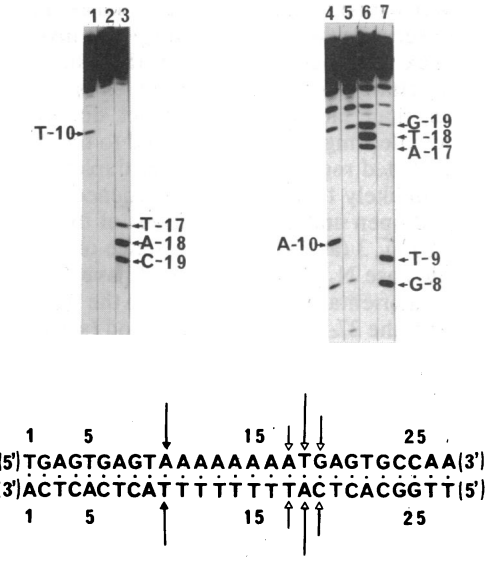

FIG. 3. (*Upper*) Cleavage under alkaline conditions of the duplex formed by the two 27-mers whose sequences are shown below the autoradiographs. The duplex (10 nM) with a slight excess of the 27-mer(T_8) (2 nM) was incubated for 30 min with octathymidylates (50 μM) bearing the *p*-azidophenacyl group, in a buffer containing 10 mM sodium phosphate (pH 7.0) and 1 M NaCl. The mixture was then irradiated and treated with 1 M piperidine at 90°C for 20 min. In lanes 1–3, the 27-mer(T_8) was 5′-end-labeled. Numbering of the bases starts from the 3′ end (see *Lower*). Lane 1: cleavage at T-10 in the presence of $N_3\phi$-βT_8-Acr. Lane 2: irradiated control in the absence of octathymidylate. Lane 3: cleavage at A-18 > C-19 > T-17 in the presence of Acr-αT_8-ϕN_3. In lanes 4–7, the 27-mer(A_8) was 5′-end-labeled. Numbering of bases starts from the 5′ end (see Fig. 1 *Lower*). Lane 4: cleavage at A-10 in the presence of $N_3\phi$-βT_8-Acr. Lane 5: irradiated control in the absence of octathymidylates (bands located at guanines are revealed by piperidine treatment). Lane 6: cleavage at T-18 > G-19 > A-17 in the presence of Acr-αT_8-ϕN_3. Lane 7: cleavage at G-8 > T-9 in the presence of $N_3\phi$-αT_8. (*Lower*) Sequences of the 27-mer duplex used in this study. Arrows indicate where cleavage occurs after photocrosslinking of *p*-azidophenacyl-substituted octathymidylates to the duplex 27-mer followed by cleavage under alkaline conditions. Open arrows refer to Acr-αT_8-ϕN_3 and filled arrows to $N_3\phi$-βT_8-Acr. In the α oligonucleotide the photocrosslinking group was attached to the 3′ end. Cleavage occurred on the 3′ side of the A_8 sequence. In the β oligonucleotide the photocrosslinking group was attached to the 5′ end. Cleavage occurred on the 5′ side of the A_8 sequence. Therefore, in both cases the octathymidylate was oriented (5′→3′) parallel to the A_8 sequence. The third oligonucleotide ($N_3\phi$-αT_8) cleaved at (G·C)-8 > (T·A)-9 >> (A·T)-10 (see lane 7 of *Upper*). The corresponding arrows are not presented on the sequence to avoid overlap with $N_3\phi$-βT_8-Acr.

was base pair (T·A)-18 followed by (G·C)-19 and (A·T)-17 (see Fig. 3 for the numbering of the base pairs in the 27-mer duplex). The photocrosslinking group (-ϕN_3) was covalently linked to the 3′ end of this α octathymidylate. Cleavage sites were observed on the 3′ side of the A_8 sequence, demonstrating that the α oligothymidylate was bound to the 27-mer double helix in a parallel orientation with respect to the A_8 strand. With $N_3\phi$-βT_8-Acr, whose photocrosslinking group was attached to the 5′ end, the most efficient cleavage occurred at base pair (A·T)-10, which is located on the 5′ side of the A_8 sequence. This behavior requires a parallel orientation of the β octathymidylate and 27-mer(A_8) sequences. In the case of $N_3\phi$-αT_8 the efficiency of cleavage decreased in the order (G·C)-8 > (T·A)-9 >> (A·T)-10, again in agreement with a parallel orientation of the octathymidylate and A_8 sequences. Of the three octathymidylates, Acr-αT_8-ϕN_3 gave the more efficient cleavage reactions.

As expected, the 27-mer(T_8) single strand, which did not contain any target for the octathymidylates, did not lead to any photocrosslinking and cleavage, indicating that the reactions observed when the 27-mer(T_8) was involved in the 27-mer duplex were due to binding of the *p*-azidophenacyl-substituted octathymidylates to the duplex structure. However, rather than binding to the intact double helix the oligothymidylates might have hybridized to the A_8 sequence in a locally melted region of the 27-mer duplex even though it was very unlikely that the $(A{\cdot}T)_8$ sequence of the 27-mer duplex would open under the conditions of the experiments described in Fig. 3 (at 0°C in the presence of 1 M NaCl). If this were the case $N_3\phi$-βT_8-Acr should have been bound in an antiparallel orientation with respect to the A_8 sequence as observed with the 27-mer(A_8) single strand (see above). The reverse orientation was observed. If a local triple helix had been formed by $N_3\phi$-βT_8-Acr with the A_8 sequence in an open "bubble," then cleavage should have occurred on both sides of the A_8 sequence. This was not observed. Therefore a local opening of the 27-mer double helix was excluded as a plausible explanation for targeted photocrosslinking.

The efficiency of the photocrosslinking and cleavage reactions was higher at 1 M NaCl than at 0.2 M NaCl (results not shown). High ionic concentrations are known to favor the formation of triple helices by poly(dA) and poly(dT) as a result of a decrease in the electrostatic repulsion between the negatively charged polymers (4). The same behavior was observed for the β oligothymidylate covalently linked to the acridine derivative.

DISCUSSION

We are interested in developing sequence-specific nucleic acid-binding substances that could be used to selectively block gene expression (10–11). Previous work (13, 14) showed that oligodeoxynucleotides covalently linked to an intercalating agent inhibited translation of mRNAs containing the complementary sequence of the oligonucleotide. There are now several reports of specific effects on gene expression of oligodeoxynucleotides synthesized with either a phosphodiester (15–18) or a phosphonate backbone (19, 20). The synthesis of α oligodeoxynucleotides, which are resistant to nucleases, has also been described (21–25). Intercalating agents have been covalently attached to α oligodeoxynucleotides (24, 26). All these molecules bind to their target sequence in a reversible way. It is thus difficult to completely block biological functions. Therefore it seemed important to develop oligodeoxynucleotides that could be activated to modify the target sequence selectively and irreversibly. There have been several reports describing covalent modification of nucleic acids by reagents covalently attached to an oligonucleotide (see ref. 27 for review). It is also possible to induce strand breaks in the target nucleic acid by covalent linkage of metal complexes that generate hydroxyl radicals (27–33). Here we describe a photoactive system that can be used to photocrosslink the oligonucleotide to its complementary sequence.

The first series of results presented here show that it is possible to achieve sequence-directed photochemical modifications on a single-stranded nucleic acid target by using a photoactive group covalently linked to one end of an oligonucleotide. The oligonucleotide specifically recognizes its complementary sequence. The stability of the complex can be increased by attaching an intercalating agent at the other end of the oligonucleotide. This new family of molecules can be specifically targeted to a mRNA. It is expected that photocrosslinking will prevent mRNA translation. Studies along these lines have been initiated in our laboratories (13, 14, 18).

We have used *p*-azidophenacyl as a photoactive group. This group can be conveniently attached to the terminal nucleotide of an oligonucleotide via a 3′ or a 5′ thiophosphate (7). It has been widely used in the study of protein–ligand interactions, but there are few reports in the literature describing its use as a crosslinking reagent in nucleic acid–nucleic acid interactions. A dinucleotide substituted on its 5′ side by a *p*-azidophenacyl group was previously used as a primer for *Escherichia coli* RNA polymerase (34, 35). Upon UV irradiation short transcripts were crosslinked to the DNA template. This reaction was not further characterized and the nature of the photoadducts that are formed is not known. We have shown that upon piperidine treatment some of the photocrosslinks could be converted to chain breaks, which allowed us to identify the location of the crosslinked sites. It should also be noted that some of the crosslinks were unstable to heat and generated modified bases, which then led to a cleavage reaction under alkaline conditions (unpublished observations).

We have used the property of the photocrosslinking reaction followed by cleavage under alkaline conditions to characterize the orientation of the oligonucleotide with respect to its target sequence (Fig. 4). A β octathymidylate covalently linked to an acridine derivative forms both a double and a triple helix with a complementary single-stranded octadeoxyadenylate sequence. In the double helix the T_8 and A_8 chains are anti-parallel. In the triple helix the

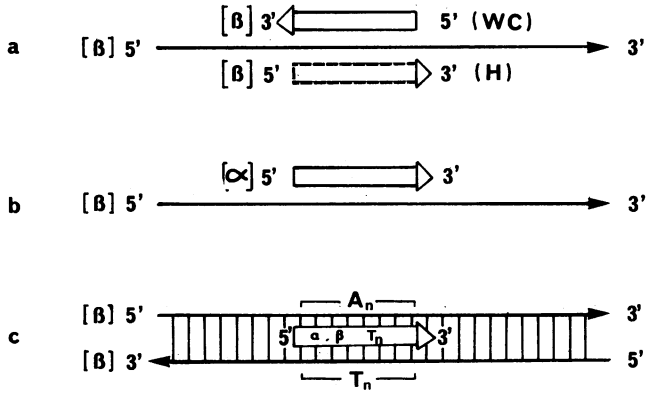

FIG. 4. Schematic representation of an oligothymidylate (thick arrow) bound to its complementary sequence. In *a* and *b* the target sequence is included in a single-stranded DNA fragment. In *c* it is included in double-stranded DNA. In *a* a β oligothymidylate can form an antiparallel double helix with Watson–Crick (WC) base pairing and a triple helix in which the second oligonucleotide is parallel to the target sequence and involves Hoogsteen base pairing (H, broken arrow). In *b* an α oligothymidylate forms a parallel double helix with its complementary sequence. In *c* an α or a β oligothymidylate binds in the major groove of the DNA double helix in a parallel orientation with respect to the adenine-containing strand.

second T_8 chain adopts a parallel orientation with respect to the A_8-containing strand. This result is in agreement with fiber diffraction (36) and hydrodynamic studies (37) on triple-stranded polynucleotides [poly(dT)·poly(dA)·poly(dT) and poly(rU)·poly(rA)·poly(rU)] that suggested that the two polypyrimidine strands have an antiparallel orientation. The α octathymidylates form a double helix with the complementary octadeoxyadenylate sequence in which the two strands adopt a parallel orientation, independently of whether or not the α octathymidylate is covalently attached to an intercalating agent. Due to its symmetric sequence, an oligothymidylate can bind in either orientation with respect to an octadeoxyadenylate sequence. The photocrosslinking reactions indicate that the α oligomer adopts a parallel orientation.

The second series of results presented here show that an oligothymidylate can selectively recognize a sequence of A·T base pairs in a double helix, provided that all the purines are on the same strand. In the triple-stranded structure, the oligothymidylate is oriented parallel to the adenine-containing strand independently of whether it is synthesized from the natural β anomers or the synthetic α anomers (Fig. 4). A similar conclusion was recently reached by using a *p*-azidoproflavine-substituted oligothymidylate (38). Recognition occurs via the major groove of the double helix, where thymine can form Hoogsteen (or reverse Hoogsteen) hydrogen bonds with the adenine of an A·T (Watson–Crick) base pair (4–6). This behavior is reminiscent of that of regulatory proteins that recognize base-pair groups in the major groove (1). In contrast, many sequence-specific drugs bind to DNA via the minor groove (3).

Triple helices can be formed by cytosine-containing polydeoxynucleotides (5, 6). One of the cytosines must be protonated to form the triad $C{\cdot}G{\cdot}CH^+$. The protonated cytosine binds in the major groove of the duplex. We have begun investigating oligodeoxynucleotides containing both cytosines (or 5-methylcytosines) and thymines in order to determine the conditions under which any double-stranded DNA containing all purines on one strand and all pyrimidines on the other strand could be selectively recognized and modified (cleaved). Covalent attachment of an intercalating agent to the oligonucleotide stabilizes the complexes (9–11). This should allow short stretches of oligopyrimidine·oligopurine sequences to be chosen as targets for such oligo(pyrimidine deoxynucleotides). Substitution of a *p*-azidophenacyl group at one end of the oligonucleotide allows photocrosslinking to either strand of the target duplex. Conversion of crosslinks to chain breaks occurs under alkaline conditions. These reactions provide an interesting example of how to target (photo)chemical reactions to specific sites on a double helix. They could be useful in cleaving DNA at sites where no restriction site exists or in site-directed mutagenesis on double-stranded DNA. The use of α oligonucleotides, which are much more resistant to nucleases (21–26), makes it possible to contemplate *in vivo* applications. Local triple-helix formation might, for example, interfere with the control of transcription or replication of double-stranded DNA.

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