Volume 15 Number 19 1987 Nucleic Acids Research

Sequence-specific recognition, photocrosslinking and cleavage of the DNA double helix by an oligo-[α]-thymidylate covalently linked to an azidoproflavine derivative

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Received August 6, 1987; Revised and Accepted September 10, 1987

ABSTRACT

A 3-azidoproflavine derivative was covalently linked to the 5'-end of an octathymidylate synthesized with the [α]-anomers of the nucleoside. Two target nucleic acids were used for this substituted oligo-[α]-thymidylate : a 27-mer single-stranded DNA fragment containing an octadeoxyadenylate sequence and a 27-mer duplex containing eight contiguous A.T base pairs with all adenines on the same strand. Upon visible light irradiation the octa-[α]-thymidylate was photocrosslinked to the single-stranded 27-mer. Chain breaks were induced at the crosslinked sites upon piperidine treatment. From the location of the cleavage sites on the 27-mer sequence it was concluded that a triple helix was formed by the azidoproflavine-substituted oligo-[α]-thymidylate with its complementary oligodeoxyadenylate sequence. When the 27-mer duplex was used as a substrate cleavage sites were observed on both strands after piperidine treatment of the irradiated sample. They were located at well defined positions which indicated that the octathymidylate was bound to the $(dA)_8.(dT)_8$ sequence in a parallel orientation with respect to the $(dA)_8$-containing strand. Specific binding of the [α]-octathymidylate involved local triple strand formation with the duplex $(dA)_8.(dT)_8$ sequence. This result shows that it is possible to synthesize sequence-specific molecules which specifically bind oligopurine-oligopyrimidine sequences in double-stranded DNA via recognition of the major groove hydrogen bonding sites of the purines.

INTRODUCTION

The regulation of gene expression involves the recognition of nucleic acid base sequences or nucleic acid structures by regulatory proteins or regulatory nucleic acids (see references 1 and 2 for reviews). The recognition occurs at the level of either double-stranded DNA (e.g., transcription regulation by repressors or activators) or single-stranded RNA (e.g., regulation of mRNA translation or splicing of pre-mRNA). Synthetic oligonucleotides and anti-sense RNAs can be used to artificially control gene expression (2). In most of these experiments complex formation between a messenger RNA and the oligonucleotide or the anti-sense RNA was responsible for translation arrest. There are additional mechanisms which may be involved such as prevention of mRNA transfer from the nucleus to the cytoplasm (3) or inhibition of splicing events (4). Short oligonucleotides could also interfere with DNA transcription

provided they are targeted to the unpaired DNA region which is created by RNA polymerase bound to a promoter (5). Since unpaired regions in DNA and accessible regions in mRNAs are usually very limited in size it is necessary to increase the affinity of the short complementary oligonucleotides for their target sequence. This can be achieved by covalent linkage of an intercalating agent to either end of the oligonucleotide (6-10).

The use of synthetic oligodeoxynucleotides **in vivo** is limited by their sensitivity to nucleases. Using the synthetic [α]-anomers of nucleosides instead of the natural [β]-anomers make oligodeoxynucleotides resistant to nucleases (11-14). Therefore oligo-[α]-deoxynucleotides represent interesting tools for the artificial control of gene expression **in vivo**. The efficiency of mRNA translation arrest should be notably increased if oligonucleotides could induce irreversible damages in their target sequence. This can be achieved by covalent attachment of reagents which can be chemically activated to form crosslinks or induce chain breaks in the complementary sequence (see references 15 and 16 for reviews). We have recently developed reagents which can be photochemically activated to create either crosslinks or photosensitized damages in the target sequence of the oligonucleotide (17). In all cases studied until now the target was a single-stranded nucleic acid (either RNA or DNA). Targeting irreversible reactions to specific sequences on a double-stranded DNA or RNA might open new ways for, **e.g., in vivo** site-specific mutagenesis or gene inactivation. Here we show that an oligo-[α]-deoxythymidylate covalently linked to an azidoproflavine derivative can recognize a stretch of A.T base pairs provided all adenines are on the same strand. This recognition involves local formation of a triple helix where thymines of the oligo-[α]-deoxythymidylate are hydrogen bonded to the adenines in the major groove of the DNA double helix. The azidoproflavine derivative can be excited by light to induce crosslinking reactions to either strand of the target duplex. These crosslinked species undergo cleavage reactions under alkaline conditions.

MATERIALS AND METHODS

The synthesis of [α]-octathymidylate was previously described (14,17). The [α]-anomer of thymidine was purchased from Sigma and oligonucleotide synthesis was carried out in solution using the phosphotriester procedure. A thiophosphate group was added to the 5'-end of the octathymidylate as previously described (17,18). This thiophosphate group was reacted with 3-azido-6-(3-bromopropylamino)-acridine to obtain the azidoproflavine-substituted oligonucleotide which will be referred to as N_3Pf-[α]-$(dT)_8$ (see Figure

1). The azido derivative was prepared from 3-amino-6-(3-bromopropylamino)-acridine(18) as follows. The disulfate of 3-amino-6-(3-bromopropylamino)-acridine (0.5 g ; 0.89 mmol) was dissolved in ethanol (110 ml). To the solution, a water-concentrated hydrochloric acid mixture (5:1 v/v ; 60 ml) was added with stirring and the resulting solution was cooled in an ice salt bath at 0°C. Then, sodium nitrite (80 mg ; 1.16 mmol) was added in small aliquots. The diazotation was allowed to proceed at low temperature for 1 h. All further operations were performed in the dark. To the deep purple solution thus obtained, sodium azide (63 mg ; 0.97 mmol) in water (10 ml) was added slowly. The solution was stirred for one hour in an ice bath and then for an additional hour at room temperature. The solution was stored overnight at 0°C. 3-azido-6-(3-bromopropylamino)-acridine precipitated as the hydrochloride. It was filtered, washed with water and dried. A first fraction of pure material was thus obtained (160 mg). The filtrate was concentrated under reduced pressure to precipitate a second fraction of material (70 mg) containing traces of impurities. Overall yield : 63 % (monohydrochloride, monohydrate). mp 165°c (dec,N_2). ^1H NMR (60MHz,DMSO-d_6, HMDS as internal reference) : δ = 9.10 (1H,s,$C_9\underline{H}$) ; 8.45 (1H,broad s,$N\underline{H}$) ; 8.35-7.80 (2H,m) ; 7.60-7.00 (3H,m) ; 6.70 (1H,s,$C_5\underline{H}$) ; 3.75 (t,J=7 Hz,N-$C\underline{H}_2$ or $C\underline{H}_2$-Br) ; 3.45 (t,J=7 Hz,$C\underline{H}_2$-Br or N-$C\underline{H}_2$) ; 2.15 (2H,m,CH_2-$C\underline{H}_2$-CH_2). MS (FAB,thioglycerol,acetic acid 1 %, positive ions), m/z : 358 [$M^+(^{81}Br)+1$] ; 356 [$M^+(^{79}Br)+1$] ; 332 [$M^+(^{79}Br)-N_2+3H$]. Analysis, calculated for $C_{16}H_{14}BrN_5$, 1HCl, $1H_2O$: C,46.79 ; H,4.17 ; N,17.05 ; Br,19.46 ; found : C,46.51 ; H,4.08 ; N,16.83 ; Br,19.99. IR (KBr) : 2120 cm^{-1}. UV (ethanol), λ_{max} (ε) : 461 (15000), 370 (10500) ; 286 (25000) ; 263 nm (25500).

The two complementary 27-mer oligodeoxynucleotides used as targets for N_3Pf-[α]-$(dT)_8$ were synthesized with a Pharmacia automatic synthesizer. Their sequences are shown on figure 1. One of them contained a $(dA)_8$ sequence and will be referred to as 27mer-$(dA)_8$. The other contained a $(dT)_8$ sequence and will be abbreviated as 27mer-$(dT)_8$. They were purified by polyacrylamide gel electrophoresis and reverse phase chromatography. 5'-end labeling was achieved by T^4 polynucleotide kinase and γ-^{32}P-ATP (Amersham).

The oligonucleotide N_3Pf-[α]-$(dT)_8$ was mixed with the target sequence (either single-stranded or double-stranded) at 20°C and the solutions were cooled down to 0°C. After 30 minutes incubation the samples were irradiated at 0°C with a HBO-200 W lamp using a pyrex filter to remove wavelengths shorter than 300 nm. The irradiated samples either before or after alkaline treatment (1 M piperidine, 90°C, 20 minutes) were loaded on denaturing polya-

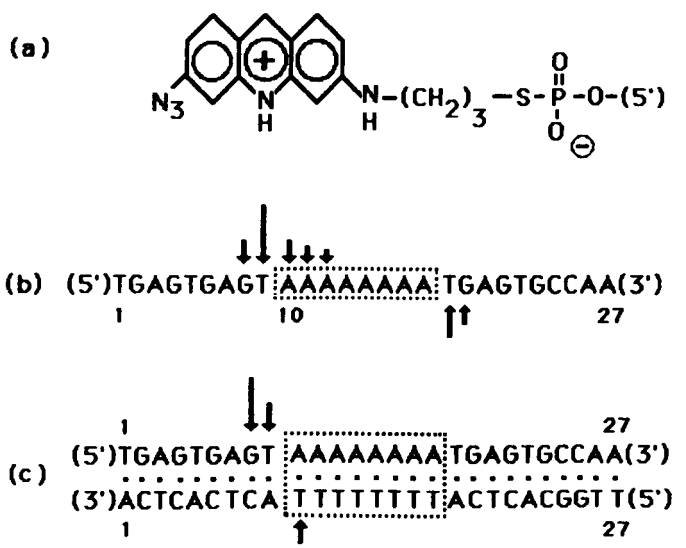

Figure 1 : (a) Structure of the azidoproflavine derivative covalently linked to the 5'-thiophosphate of the oligo-[α]-thymidylate used in the present studies. (b) Sequence of the 27mer-$(dA)_8$ single-stranded DNA fragment. (c) Sequence of the 27mer-duplex. In (b) and (c) the arrows indicate the sites of cleavage observed after irradiation of the single strand and the double strand in the presence of N_3Pf-[α]-$(dT)_8$ followed by piperidine treatment. The target sequence is boxed by a dotted line.

crylamide gels (20 % acrylamide, 0.5 % bis-acrylamide, 7 M urea). The gels were autoradiographed at -70°C using either Kodak (X-OMat) or Fuji(X-ray) films and an intensifying screen. For quantitative measurements bands were cut out of the gels and counted for radioactivity.

RESULTS

1. Site-specific photocrosslinking and cleavage of single-stranded DNA.

The 27mer-$(dA)_8$ single-stranded DNA fragment was ^{32}P-labeled at its 5'-end and used as a target for site-directed photochemical reactions using N_3Pf-[α]-$(dT)_8$ as a photosensitizer (figure 1). The irradiated mixture was loaded on a denaturing polyacrylamide gel at neutral pH. After electrophoresis and autoradiography new bands were revealed which migrated more slowly than the intact 27mer-$(dA)_8$ fragment (figure 2). Irradiation of azidoderivatives generate intermediate species (19) which are highly reactive towards nucleic

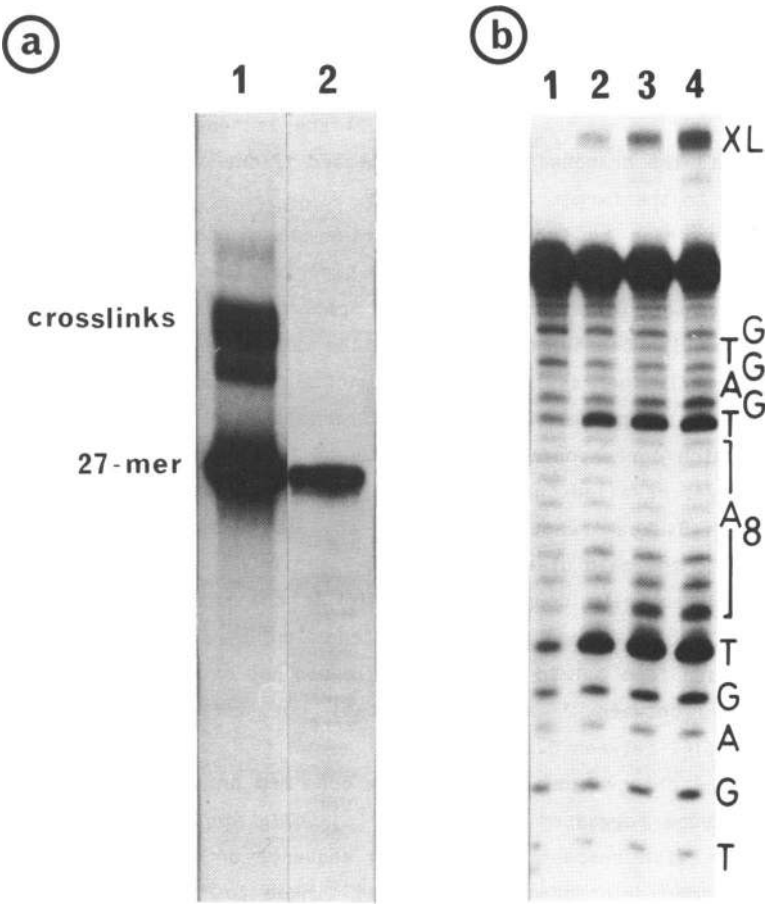

Figure 2 : (a) Crosslinks formed upon irradiation of N_3Pf-[α]-$(dT)_8$ with the 5'-end labeled 27mer-$(dA)_8$ (see sequence on figure 1). Lane 1 is the irradiated mixture loaded on a denaturing polyacrylamide gel at pH 8. Lane 2 is the 27mer marker together with faint bands corresponding to a (G + A) sequence. Irradiation was carried out at 0°C with 10 nM of 27mer and 0.5 μM N_3Pf-[α]-$(dT)_8$ in a pH 7 buffer containing 10 mM Na phosphate and 0.1 M NaCl.

(b) Autoradiogram of a polyacrylamide gel after electrophoresis of irradiated mixtures of N_3Pf-[α]-$(dT)_8$ and 5'-end labeled 27mer-$(dA)_8$ further treated by piperidine at 90°C for 20 minutes. Part of the sequence of the 27mer is shown on the right. XL indicates crosslinks which have not been cleaved by piperidine treatment. Lanes 1,2,3 and 4 correspond to increasing concentrations of N_3Pf-[α]-$(dT)_8$: 0.075, 0.25, 0.75 and 2.5 μM, respectively. The 27 mer-$(dA)_8$ concentration was 5 nM. Irradiation in the absence of N_3Pf-[α]-$(dT)_8$ did not give rise to any reaction but piperidine treatment revealed bands at all guanines with intensities similar to those shown in lane 1.

acid bases (20,21). Therefore the slowly migrating bands were most likely due to crosslinked species in which the oligo(dT)$_8$ was covalently attached to the 27-mer via its proflavine moiety. These photocrosslinked species should have a retarded migration on the gels due to i) the increased number of nucleotide units and ii) the branched structure expected if the oligo(dT)$_8$ is crosslinked at or close to the target (dA)$_8$ sequence.

In order to further characterize the photocrosslinked species the irradiated samples were treated with 1 M piperidine at 90°C for 20 minutes. We previously showed that photocrosslinked species induced upon irradiation of a p-azidophenacyl-substituted oligonucleotide could be cleaved under alkaline conditions (17). The results presented on figure 2b show that the crosslinked species nearly completely disappeared after alkaline treatment and new bands appeared which corresponded to fragments of the 27-mer. A comparison with a sequencing gel revealed that the main cleavage reaction occurred at T-9, followed by T-18. Weak reactions occurred at A-10, G-8 and G-19. The bands corresponding to the main cleavage sites (T-9 and T-18) were cut out from the gel and the radioactivity counted. The ratio of the radioactivity in these two bands (T-9/T-18) remained approximately constant (≈ 3.0) when NaCl concentration increased from 0.1 to 1 M. This ratio did not change with the duration of irradiation (between 1 and 10 minutes) nor with the concentration of N_3Pf-[α]-(dT)$_8$ (between 0.25 and 2.5 μM)

The observation that cleavage occurred on both sides of the (dA)$_8$ target sequence suggested either that [α]-(dT)$_8$ was bound in two different orientations with respect to the (dA)$_8$ sequence or that a triple helix was formed. Using p-azidophenacyl covalently linked to [α]-(dT)$_8$ we previously showed that a double helix was formed with the (dA)$_8$ sequence in which the two strands had a parallel orientation (17). Covalent attachment of proflavine might have perturbed this interaction in such a way that a fraction of the [α]-(dT)$_8$ was bound in an anti-parallel orientation with respect to the (dA)$_8$ sequence. However this seemed unlikely as covalent attachment of another intercalating agent, namely, 2-methoxy-6-chloro-9-aminoacridine, did not induce such an inversion (17). In order to determine whether a triple helix could form we used absorption spectroscopy to determine the stoichiometry of the association between N_3Pf-[α]-(dT)$_8$ and oligo(dA)$_8$. As shown on figure 3 complex formation between the two octadeoxynucleotides was accompanied by a hypochromism in the visible and the near-UV bands of azidoproflavine. A stoichiometry of 2 oligo(dT)$_8$ per oligo(dA)$_8$ was obtained both in 0.1M and 1 M NaCl. In contrast a 1:1 stoechiometry was obtained when poly(rA) was used

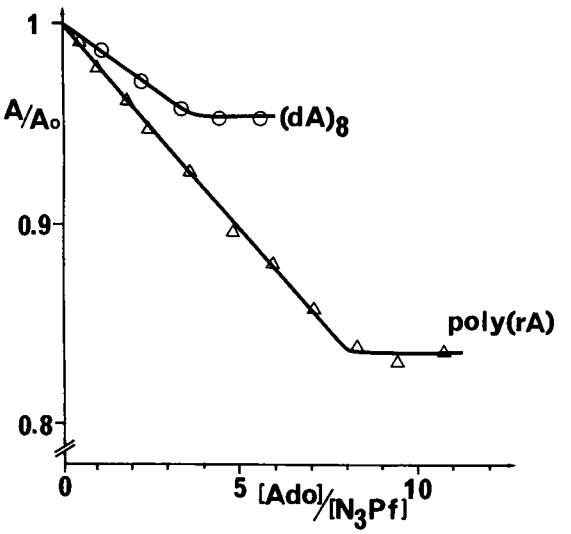

Figure 3 : Relative absorbance of 10 µM N_3Pf-[α]-$(dT)_8$ at 394 nm upon addition of increasing concentrations of either oligo$(dA)_8$ (o) or poly(rA) (Δ). [Ado]/[N_3Pf] is the ratio of adenine to azidoproflavine concentrations. Absorbance measurements were carried out at 5°C in a pH 7 buffer containing 10 mM Na cacodylate and 0.1 M NaCl.

instead of oligo$(dA)_8$ (figure 3). These results indicated that at 0°C N_3Pf-[α]-$(dT)_8$ formed a triple helix with a $(dA)_8$ sequence in which each adenine was hydrogen-bonded to two thymines as observed in poly(dA).2 poly(dT) (22,23). Therefore the photocrosslinking and cleavage reactions observed at T-9 and T-18 reflect the formation of a triple helix in which the two oligo$(dT)_8$ have an anti-parallel orientation with respect to each other. Previous studies have shown that the double helix formed by [α]-$(dT)_8$ with $(dA)_8$ involves a parallel orientation of the two strands (17). Therefore the second [α]-$(dT)_8$ in the triple helix should be bound in an antiparallel orientation with respect to the $(dA)_8$ strand (see figure 5).

Site-specific cleavage of a double-stranded DNA by N_3Pf-[α]-$(dT)_8$

Adenine can form hydrogen bonds with two thymines. The results reported above showed that two [α]-$(dT)_8$ could bind to a $(dA)_8$ sequence. It was therefore of interest to determine whether [α]-$(dT)_8$ could bind to a natural DNA double helix containing eight A.T base pairs with all adenines on the same strand. We used a 27-mer duplex as a target for N_3Pf-[α]-$(dT)_8$. A slight excess of the 27mer-$(dT)_8$ strand was used in order to ensure that no free 27mer-$(dA)_8$ was present in the solution. The duplex and N_3Pf-[α]-$(dT)_8$

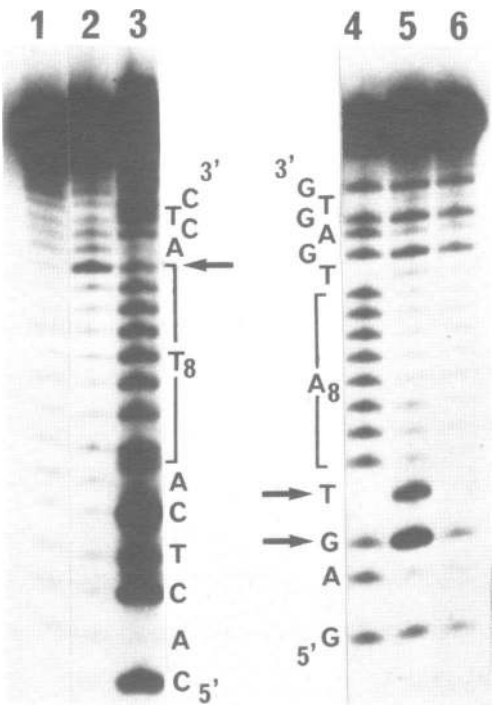

Figure 4 : Cleavage of the two strands of the 27 mer-duplex (10 nM in each strand) after irradiation at 0°C in the presence of 50 µM N_3Pf-[α]-$(dT)_8$ in a pH 7 buffer containing 10 mM Na phosphate and 1 M NaCl followed by piperidine treatment at 90°C for 20 minutes. Horizontal arrows indicate the main cleavage sites.
Left : 5'-end labeled 27mer-$(dT)_8$ strand. Lane 1 : control (irradiation in the absence of N_3Pf-[α]-$(dT)_8$). Lane 2 : irradiation in the presence of N_3Pf-[α]-$(dT)_8$. Lane 3 : (C + T) sequence.
Right : 5'-end labeled 27mer-$(dA)_8$ strand. Lane 4 : (G + A) sequence. Lane 5 : irradiated in the presence of N_3Pf-[α]-$(dT)_8$. Lane 6 : irradiated in the absence of N_3Pf-[α]-$(dT)_8$. (The weak bands at guanines arise from piperidine treatment).

were mixed and irradiated at 0°C at wavelengths longer than 300 nm. After irradiation the samples were treated by 1M piperidine at 90°C for 20 minutes as described above for single-stranded 27mer-$(dA)_8$. Two separate experiments were carried out with either the $(dA)_8$-containing strand or the $(dT)_8$-containing strand 5'-end-labeled by polynucleotide kinase. The results are shown in figure 4 together with the (C+T) sequence of the 27mer-$(dT)_8$ strand and the (G + A) sequence of the 27mer-$(dA)_8$ strand. The number assigned to each base pair are shown on figure 1. Note that the $(dA)_8$-containing strand is numbered star-

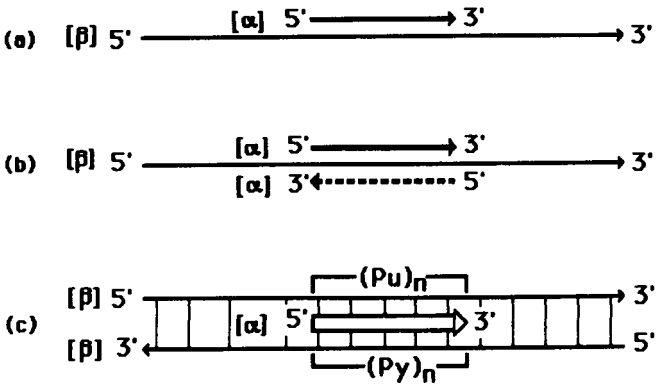

Figure 5 : Schematic representation of (a) a double helix with parallel strands formed by an oligo-[α]-deoxynucleotide with its complementary [β]-sequence, (b) a triple helix formed by an oligo-[α]-thymidylate with a complementary oligo-[β]-deoxyadenylate sequence and (c) a local triple helix formed by an oligo-[α]-pyrimidine with a double-stranded DNA fragment (natural [β]-anomers) containing an oligopurine-oligopyrimidine sequence.

ting from the 5'-end and the $(dT)_8$-containing strand from the 3'-end. It is clearly seen that cleavage reactions occurred on both strands. The main cleavage occurred at T-10 on the 27mer-$(dT)_8$ strand and at G-8 followed by T-9 on the 27mer-$(dA)_8$ strand. The location of these cleavage sites revealed that N_3Pf-[α]-$(dT)_8$ was bound to the $(dA)_8.(dT)_8$ sequence of the 27-mer duplex in a parallel orientation with respect to the $(dA)_8$-containing strand. The isolated 27mer-$(dT)_8$ single-stranded fragment did not give rise to any photoreaction with N_3Pf-[α]-$(dT)_8$ as expected from the lack of complementarity between the two sequences (results not shown). Therefore base modifications observed on the 27mer-$(dT)_8$ strand in the 27-mer-duplex was a convincing argument for N_3Pf-[α]-$(dT)_8$ binding to the duplex structure. There remained the possibility that N_3Pf-[α]-$(dT)_8$ was bound to the $(dA)_8$ sequence in a locally melted 27mer duplex. However it was very unlikely that the 27mer duplex was open in its central region at 0°C in the presence of 1M NaCl. Moreover had N_3Pf-[α]-$(dT)_8$ been bound to the $(dA)_8$ sequence in a locally single-stranded region, the main reaction would have been located at T-9 as observed with the 27mer-$(dA)_8$ single strand (figure 2) and not at G-8 as observed with the 27mer duplex.

All these observations demonstrate that N_3Pf-[α]-$(dT)_8$ binds to the $(dA)_8.(dT)_8$ sequence of the 27mer double-stranded DNA in a parallel orientation with respect to the $(dA)_8$-containing strand (figure 5) and that azidoproflavine is responsible for photocrosslinking reactions with cleavage occur-

ring at the crosslinked sites after piperidine treatment. There is a difference in reactivity between the two strands with the 27mer-$(dA)_8$ strand reacting more efficiently. This difference very likely arises from different orientations of the azidoproflavine ring with respect to the reacting bases. Further physicochemical studies are required to characterize the structure of the local triple helix.

DISCUSSION

Azidoproflavine covalently attached to the 3'-end of an oligo-[α]-deoxythymidylate is able to induce crosslink formation with a complementary sequence upon irradiation with light of wavelengths longer than 300 nm. Crosslinks can be converted to chain breaks upon treatment with piperidine at high temperature. The results summarized in figure 1 show that cleavage occurs at well-defined positions on both a single-stranded oligodeoxynucleotide and a double-stranded DNA fragment containing a stretch of A.T base pairs with all adenines on the same strand.

On the single-stranded DNA fragment photocrosslinking and cleavage occur on both sides of the target sequence. This has been ascribed to the formation of a triple helix where the two oligo-[α]-$(dT)_8$ strands have an anti-parallel orientation with respect to each other. Previous studies (17) have shown that an oligo-[α]-$(dT)_8$ can form a double helix with a $(dA)_8$ sequence in which the two strands have a parallel orientation, as shown on figure 5. The presence of azidoproflavine covalently linked to the 5'-end of the oligothymidylate appears to favor formation of a triple helix as does proflavine (18).

We have shown that [α]-$(dT)_8$ covalently linked to an azidoproflavine group could bind to a double helix containing a $(dA)_8.(dT)_8$ sequence. The [α]-dT_8 oligonucleotide is oriented <u>parallel</u> to the $(dA)_8$ strand as shown on figure 5. Complex formation results from the ability of thymine to form two hydrogen bonds with an adenine already engaged in a Watson-Crick base pair. Therefore recognition of the $(dA)_8.(dT)_8$ duplex structure involves the major groove of the double helix. The major groove is also involved in the recognition of base pair sequences by regulatory proteins (1) whereas the minor groove accommodates several sequence-specific DNA-binding drugs (24). Guanine can also form hydrogen bonds with two cytosines provided one of them is protonated. Triple helix formation by polyd(GA).polyd(CT) has previously been demonstrated (25). The results presented in the above study therefore open the way for the design of sequence-specific DNA-binding drugs which could

selectively recognize polypurine-polypyrimidine sequences in double-stranded DNA. The nuclease resistance of oligo-[α]-deoxynucleotides (13,14) make them valuable tools for **in vivo** studies. The covalent attachment of substituents which can be chemically or photochemically activated allows irreversible reactions to be targeted to complementary sequences. Polypurine-polypyrimidine sequences are often present in the control region of eukaryotic genes and exhibit hypersensitivity to S1 nuclease (26 and references therein). Such sequences constitute good targets for site-specific reagents which could interfere with gene expression. Work along this line is in progress in our laboratories.

ACKNOWLEDGMENTS

We wish to thank M. Chassignol (Orléans) for his contribution to oligonucleotide synthesis and M. Takasugi (Paris) for absorption measurements. This work was supported in part by Ministère de la Recherche et de l'Enseignement Supérieur (Contrat n° 86.T.0628), Ligue Nationale Française contre le Cancer, Fondation pour la Recherche Médicale and Rhône-Poulenc-Santé.

REFERENCES

1. Hélène, C. and Lancelot, G. (1982) Prog. Biophys. Mol. Biol. 39, 1-68.
2. Green, P.J., Pines, O. and Inouye, M. (1986) Ann. Rev. Biochem. 55, 569-597.
3. Kim, S.K. and Wold, B.J. (1984) Cell 42, 129-138.
4. Smith, C.C., Aurelian, L., Reddy, M.P., Miller, P.S. and T'so, P.O.P. (1986) Proc. Natl. Acad. Sci. USA 83, 2787-2791.
5. Hélène, C., Montenay-Garestier, T., Saison, T., Takasugi, M., Toulmé, J.J., Asseline, U., Lancelot, G., Maurizot, J.C., Toulmé, F., and Thuong, N.T. (1985) Biochimie 67, 777-783.
6. Asseline, U., Thuong, N.T., and Hélène, C. (1983), C.R. Acad. Sci. Paris, 297 (III) 369-372.
7. Asseline, U., Delarue, M., Lancelot, G., Toulmé, F., Thuong, N.T., Montenay-Garestier, T. and C. Hélène (1984) Proc. Nat. Acad. Sci. USA 81, 3297-3301.
8. Asseline, U., Toulmé, F., Thuong, N.T., Delarue, M., Montenay-Garestier, T. and Hélène, C. (1984) EMBO J. 3, 795-800.
9. Lancelot, G., Asseline, U., Thuong, N.T. and Hélène, C. (1985) Biochemistry 24, 2521-2529.
10. Lancelot, G. and Thuong, N.T. (1986) Biochemistry 25, 5357-5363.
11. Holy, A. (1973) Coll. Czech. Chem. Comm. 38, 100-114.
12. Sequin, U. (1974) Helv. Chem. Acta 57, 68-81.
13. Morvan, F., Rayner, B., Imbach, J.L., Thenet, S., Bertrand, J.R., Paoletti, J., Malvy, C. and Paoletti, C. (1987) Nucleic Acids Res. 15, 3421-3437.
14. Thuong, N.T., Asseline, U., Roig, V., Takasugi, M. and Hélène, C. (1987) Proc. Natl. Acad. Sci.USA 84, 5129-5133.
15. Knorre, D.G. and Vlassov, V.V. (1985) Progress in Nucleic Acid Research and Molecular Biology 32, 291-320.
16. Hélène, C. and Thuong N.T. (1987) in "Molecular mechanisms of carcinogenic

and antitumor activity", Chagas C. and Pullman B., Eds., Adenine Press, pp. 205-222.
17. Praseuth, D., Chassignol, M., Takasugi, M., Le Doan, T., Thuong, N.T. and Hélène, C. (1987) J. Mol. Biol. 196, 939-942.
18. Praseuth, D., Le Doan, T., Chassignol, M., Decout, J.L., Habhoub, N., Lhomme, J., Thuong, N.T. and Hélène C., submitted for publication.
19. Nielsen, P.E. and Buchardt, O. (1982) Photochem. Photobiol. 35, 317-323.
20. Hanna, M.M. and Meares, C.F. (1983) Biochemistry 22, 3546-3551.
21. Nielsen, P.E. (1985) Biochemistry 24, 2298-2303.
22. Riley, M., Maling, B. and Chamberlin, M.J. (1966) J. Mol. Biol. 20, 359-389.
23. Steely, H.T., Gray, D.M. and Ratliff, R.L. (1986) Nucleic Acids Res. 14, 10071-10090.
24. Zimmer, C. and Wähnert, U. (1986) Prog. Biophys. Mol. biol. 47, 31-112.
25. Lee, J.S., Johnson, D.A. and Morgan, A.R. (1979) Nucleic Acids Res. 6, 3073-3091.
26. Evans, T. and Estrafiadis, A. (1986) J. Biol. Chem. 261, 14771-14780.