Volume 17 Number 1 1989 Nucleic Acids Research

Psoralen covalently linked to oligodeoxyribonucleotides: synthesis, sequence specific recognition of DNA and photo-cross-linking to pyrimidine residues of DNA

Uwe Pieles and Uwe Englisch

Abteilung Chemie, Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Strasse 3, 3400 Göttingen, FRG

Received August 19, 1988; Revised and Accepted November 29, 1988

ABSTRACT

The psoralen derivative 4,5',8-trimethylpsoralen was covalently linked to the 5'-terminus of an 18mer oligodeoxyribonucleotide in the course of solid phase synthesis using phosphoroamidite chemistry. The derivative was introduced as a phosphitylation compound in the last cycle of the oligomer synthesis. The reagent was prepared by 4'-chloromethylation of 4,5',8-trimethylpsoralen, introduction of a linker by ethanediol and phosphitylation with chloro-[(ß-cyanoethoxy)-N,N-diisopropylamino]-phosphine. After oxydation and deprotection the 5'-psoralen modified oligodeoxyribonucleotide was characterised by HPLC.

Hybridisation of the psoralen-modified oligomer to a complementary single stranded 21mer followed by irradiation at 350 nm revealed a photo-cross-linked double-stranded DNA fragment analysed on denaturing polyacrylamide gels. The cross-link could be reversed upon irradiation at 254nm.

INTRODUCTION

The regulation of gene expression depends on the proper recognition of primary or secondary DNA or RNA structures by specific proteins or nucleic acids (1). In the past few years it was shown that synthetic oligodeoxyribonucleotides and anti-sense RNAs complementary to these structures can inhibit translation of the genetic information (2,3). DNA transcription can be blocked in vitro by modified oligodeoxyribonucleotides (4,5). The modification of the oligodeoxyribonucleotides used in those studies was either a covalently bound acridine derivative (4) or a psoralen derivative site specifically engineered to a DNA fragment (5). Psoralens (furocoumarins) are a class of naturally occuring heterocyclic compounds which can intercalate between basepairs of double-stranded nucleic acids. When exposed to long-wavelength UV intercalated psoralen becomes covalently attached to DNA via a cyclobutane linkage to pyrimidine bases, predominantly to thymine.

Since psoralen is bifunctional, the photoreaction can lead to monoadducts and diadducts as well, the latter ones are interstrand cross-links (6-8). The covalent bonds of the psoralen-nucleic acid linkage are photoreversible by irradiation at 254nm (9). Psoralen modified DNA is used in the elucidation of nucleic acid structure and nucleic acid/protein interactions (10,11).

In order to site specifically target DNA sequences for the photoreaction with psoralen, we have synthesized an oligodeoxyribonucleotide derivatized at the 5'-end with 4'-(hydroxyethoxymethyl)-4,5',8-trimethylpsoralen. We tested its ability to hybridize to complementary single-stranded DNA and studied the photo-cross-linking upon UV irradiation.

EXPERIMENTAL

Materials

4,5',8-trimethylpsoralen was purchased from Sigma, Munich, F.R.G., protected deoxynucleotide phosporoamidites from Merck, Darmstadt, F.R.G., deoxynucleotides from Biomol, Ilvesheim, F.R.G., and [γ-^{32}P]ATP from Amersham-Buchler, Braunschweig, F.R.G.. T4 polynucleotide kinase (10 U/μl), snake venom phosphodiesterase (3 U/μl), calf spleen phosphodiesterase (4 U/μl) and alkaline phosphatase (20 U/μl) were purchased from Boehringer, Mannheim, F.R.G.. Solid phase oligodeoxyribonucleotide synthesis was carried out on a SAM ONE Synthesizer (Biosearch, New Brunswick, NJ, USA). The modified oligodeoxyribonucleotides were analysed and purified by reverse phase chromatography on HPLC (Du Pont 850 Liquid chromatograph including gradient system, heated column compartment and uv-spectrophotometric detector (Du Pont, Bad Nauheim, F.R.G)). The columns used (analytical scale: 4.6 x 250 mm column, preparative scale: 21.2 x 250 mm column) were packed with ODS Hypersil C_{18} reversed phase material from Shandon (Astmoor, G.B.).

Synthesis and Characterisation of the Psoralen-modified Oligodeoxyribonucleotide

Chloromethylation of 4,5',8-trimethylpsoralen (I $\rightarrow$II): 1.38 g (5.74 mmol) 4,5',8-trimethylpsoralen were chloromethylated as described by Isaacs et al. (12).
Yield: 1.05 g (63 %).

^{1}H-NMR ($CDCl_3$, tetramethylsilane) 2.6 - 2.7 (9H, m, C4,5',8-methyl); 4.8 (2H, s, CH_2Cl); 6.3 (1H, s, C3-H); 7.6 (1H, s, C5-H) ppm.

Introduction of the linker (II → III): 360 mg (1.3 mmol) 4'-chloromethyl-4,5',8-trimethylpsoralen were suspended in 8 ml ethandiole. The mixture was heated to 100°C until the psoralen derivative was dissolved completely. The solution was stirred at that temparature for additional 10 min and after cooling to room temperature, 10 ml H_2O were added. The solution was then extracted three times with 5 ml dichloromethane. The pooled organic phases were dried over sodium sulfate and, after filtering, concentrated to dryness. The crude product was purified by silica gel chromatography using a dichloromethane/methanol gradient (increasing methanol concentration in 0.5 % steps).

Yield after recrystallization from acetonitrile: 310 mg (78 %). M.p. 152°C; C calc. 67.54, found 67.20; H calc. 5.96, found 6.19. ^{1}H-NMR ($CDCl_3$, tetramethylsilane) 1.6 (1H, s, -OH), 2.5 (6H, s, $-CH_3$), 2.57 (3H, s, $-CH_3$), 3.6 - 3.8 (4H, m, ethylene), 6.26 (1H, s, C3-H), 7.6 (1H, s, C5-H) ppm.

Phosphitylation of 4'-(hydroxyethoxymethyl)-4,5',8-trimethylpsoralen (III → IV): 310 mg (1.19 mmol) of III were dissolved in dry dichloromethane and 424 mg (3 mmol) diisopropylethylamine were added under moisture protection. Then 310 mg (1.31 mmol) chloro-[(ß-cyanoethoxy)-N,N-diisopropylamino]-phosphine were slowly dropped into the solution and the mixture stirred for 30 min at room temperature. The reaction was stopped by addition of 5 ml half saturated aqueous sodium hydrogencarbonate, the dichloromethane phase was washed twice with half saturated sodium hydrogencarbonate and dryed over sodium sulfate. The purification of the product was carried out by silica gel chromatography. Dichloromethane/ diethylamine (10:1, v/v) and n-hexane/triethylamine (10:1, v/v) were used as elution solvents in a ratio of 1:1. Yield: 419 mg (85 %).

^{31}P-NMR ($CDCl_3$, H_3PO_4): 149.54 (s) ppm.

Coupling of 4,5',8-trimethylpsoralen-4'-[(methyloxyethoxy)-O-[(ß-cyanoethoxy)-N,N-diisopropylamino]-phosphine] to the oligodeoxyribonucleotide (IV → VI): An 18mer (1 µmol scale) oligodeoxyribonucleotide was synthesized on a DNA-synthesizer using

nucleoside phosphoroamidites (V) as described (13). The psoralen-phosphoroamidite (IV) was coupled to 5'-end of the 18mer in the last cycle of the synthesis without changing any parameters of the standard procedure. The coupling yield of the psoralen-phosphoroamidite was in the range of 99 %. The psoralen-modified oligodeoxyribonucleotide was deprotected by ammonia treatment (5 ml conc. NH_3, 55°C, 6h), evaporated to dryness and redissolved in 1.5 ml water.

Purification and Analysis of the Psoralen-Modified Oligodeoxyribonucleotide

HPLC: The purification was carried out by reversed phase chromatography on ODS-Hypersil C_{18} columns (14). The solvent A was 50 mM triethylammonium acetate, pH 7.0. The solvent B was 70 % CH_3CN in 50 mM triehylammonium acetate, pH 7.0. The flow rate was 1.5 ml/min, the temperature 37°C; the gradient 15 - 60 % solvent B in 45 min. On the analytical scale 0.5 A_{260} units were applied to the column.

Enzymatic digestion of the psoralen-modified oligodeoxyribonucleotide with snake venom phosphodiesterase and calf intestine alkaline phosphatase: In a total assay volume of 50 µl (100 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$) 0.5 A_{260} units of the oligodeoxyribonucleotide were incubated with 3 units of snake venom phosphodiesterase and 4 units of calf intestine alkaline phosphatase for 30 min. (14) and the products analysed on HPLC under the conditions described above except a different gradient: 0 - 25 % solvent B in 50 min., when 14 % B were reached, the gradient was changed to 14 - 100 % B in 30 min.

NMR: All NMR spectra were recorded on a Bruker WP200 SY spectrometer operating at a frequency of 81.01 MHz with quadrature detection and ^1H broad-band decoupling when ^{31}P-NMR data were recorded and operating at a frequency of 200 MHz for recording ^1H-NMR-spectra.

Fluorescence: All fluorescence spectra were recorded on a Shimadzu spectrofluoro-photometer (Model RF-540, Shimadzu corporation, Kyoto, Japan) in the wavelength range from 200 nm to 600 nm for both excitation and emission spectra. The psoralen-modified oligodeoxyribonucleotide was dissolved in ultrapure water (HPLC quality) at a concentration of 1 µM.

Thermal denaturation data: T_m-values were recorded on a Gilford (Oberlin, Ohio, USA) spectrophotometer (2400-S) equipped with a thermoprogrammer (2527-S). Scan rates were 0.5°C/min. Data were collected at 260 nm in the temperature range from 20 - 80°C.
Hybridisation: A complementary 21mer oligodeoxyribonucleotide used as target was aswell synthesized automatically on a 0.2 mol scale. 5'-end labeling of this template (100 pmol) was performed by standard procedures using T_4 polynucleotide kinase (10 units) and [γ-^{32}P]ATP (15). A solution of 0.25 µM ^{32}P-labeled 21mer and 0.75 µM psoralen-modified 18mer in 15 µl water was incubated in a quartz glass test tube at 70°C for 5 min. and then cooled down to room temperature within 30 min.
Irradiation: Photoreactions were carried out using the Xenon discharge light source system LH 152 from Schoeffel (Trappenkamp, F.R.G.) equipped with a GM 250 high intensity quarter meter grating monochromator. The spectral accuracy is ± 6 nm and the energy focused to the sample is about 6 mW/cm^2 at 250 nm and 25 mW at 400 nm. The sample was irradiated at 360 nm for 0 - 60 min. at 20°C. For the photoreversion of the crosslinked duplex, probes were first irradiated at 360 nm for 20 min. and then irradiated at 254 nm for 0 - 30 min. After the photoreaction 10 µl formamide-dye mix (95 % deionized formamide, 0.1 % bromophenol blue) was added and the samples were loaded on a denaturing polyacrylamide gel (20 % acrylamide, 0.5 % bis-acrylamide, 7 M urea). The gels were autoradiographed using Kodak (X-OMat) films. The quantification of reaction products was carried out by density scanning the autoradiograms.

RESULTS

Synthesis of the Psoralen-modified Oligodeoxyribonucleotide

The psoralen derivative 4,5',8-trimethylpsoralen was modified in three reaction steps resulting in a psoralen phosphitylation compound suitable for solid phase oligodeoxyribonucleotide synthesis (Fig. 1). The first step, the chloromethylation of the 4'-position of 4,5',8-trimethylpsoralen (I) was carried out following the procedure published by J. E. Hearst and coworkers (12). The 4'-chloromethyl-4,5',8-trimethylpsoralen (II) was further modified by the introduction of a spacer using ethanediol,

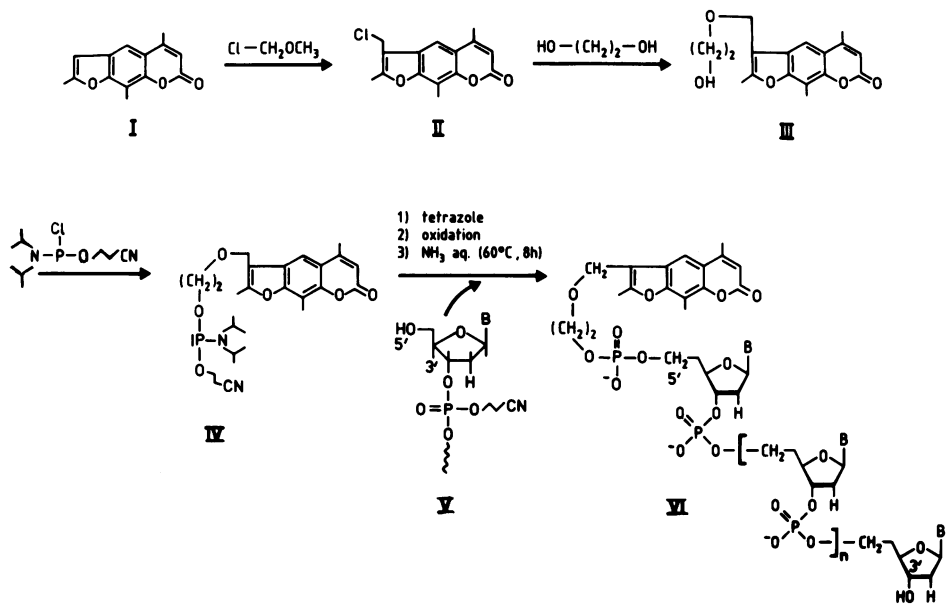

Fig. 1: General reaction scheme for the synthesis of 5'-psoralen modified oligodeoxyribonucleotides. The roman numbers refer to the educt, the intermediates and the endproduct of the synthesis.

a reaction step which was newly developed during the course of this synthesis (II → III). 4'-(hydroxy-ethoxymethyl)-4,5',8-trimethylpsoralen was phosphitylated using chloro-[(ß-cyanoethoxy)-N,N-diisopropylamino]-phosphine (16). This newly developed phosphitylation step resulted with high yields (85 %) in the desired phosphoamidite compound 4,5',8-trimethyl-psoralen-4'-[(methyloxyethoxy)-O-[(ß-cyanoethoxy)-N,N-diisopropylamino]-phosphine] (IV). This psoralen-phosphoamidite could be used directly in solid phase oligodeoxyribonucleotide synthesis (IV → VI) under standard conditions. The stability of psoralen in the alkaline deprotection medium was proven by incubating psoralen at 60°C for 24 h, followed by chromatography and nmr analysis. The stability of the aromatic system under comparable alkaline conditions is also reported by other investigators (12,17). The coupling rates were in the order of 99 % and the synthesis could be scaled up to 200 A_{260} units.

The nucleotide sequence of the psoralen-modified 18mer

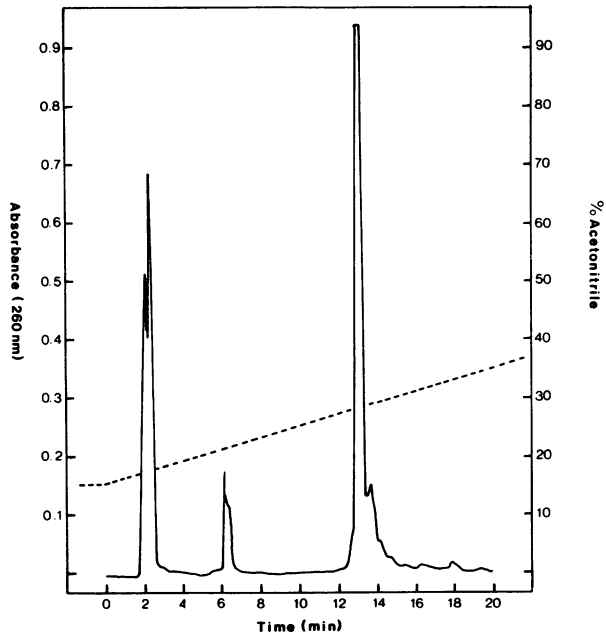

Fig. 2: Analysis of the crude psoralen-modified 18mer on a 4.6 x 250 mm column of ODS-Hypersil. 0.5 A_{260} units of the crude oligomer were applied to the column. Buffer A: 50 mM triethylammonium acetate (pH 7.0); buffer B: 50 mM triethylammonium acetate (pH 7.0), 70 % acetonitrile; gradient: 15 - 60 % buffer B in 45 min; flow 1.5 ml/min; temperature 37°C.

5'-Ps-T A G C C G C T A T C G G T T A G T-3' was designed in such a way that the psoralen molecule is linked to the terminal 5'-TpA sequence. This 5'-TpA site favors both monoadduct and cross-link formation with psoralen. The complementary single stranded 21mer 3'-G C C A T C G G C G A T A G C C A A T C A-5' which served as template has the same 5'-TpA site flanked by G's and C's. The rest of the sequence was chosen with respect to formation of a stable duplex.

Characterisation of the Psoralen-modified Oligodeoxyribonucleotide

The psoralen-modified oligodeoxyribonucleotide was purified by HPLC reversed phase chromatography (Fig. 2). The retention time of the psoralen-modified oligomer was about 13 min. The 5'-linked psoralen causes a hydrophobic shift of the oligomer on reversed phase HPLC similar to 5'-protecting groups like 4,4'-dimethoxy-

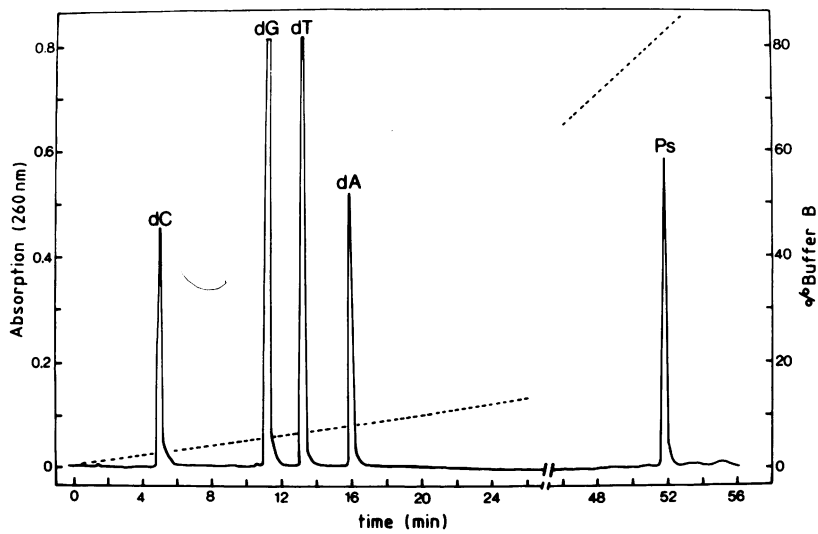

Fig. 3: Analysis of the digestion of the psoralen-modified 18mer using snake venom phosphodiesterase and alkaline phosphatase on a 4.6 x 250 mm column of ODS-Hypersil. Conditions are as described in the legend to Fig. 2 except a different nonlinear gradient: 0 - 25 % buffer B in 50 min.; from 14 % B on the gradient was changed to 14 - 100 % B in 30 min.

trityl-, which allows an easy purification. The enzymatic digestion of the oligodeoxyribonucleotide with snake venom phosphodiesterase and alkaline phosphatase resulted in a product mixture which on HPLC analysis showed a single peak beside the four deoxynucleoside peaks (Fig. 3). The single peak eluting after 52 min. represents 4'-(hydroxyethoxymethyl)-4,5',8-trimethyl-psoralen, as indicated by a parallel HPLC of the intact chromophore, by presence of an unaltered fluorescence spectrum (which would disappear upon lactone ring hydrolysis) and as expected by testing the stability in alkaline medium mentioned above.

The fluorescence excitation and emission spectra of the psoralen-modified oligodeoxyribonucleotide were recorded in aqueous solutions according to published procedures (18). The fluorescence properties of the psoralen residue of the modified oligodeoxyribonucleotide are very similar to those of other psoralen derivatives (6). The fluorescence excitation is maximized by the broad band centered at 330 nm, whereas the

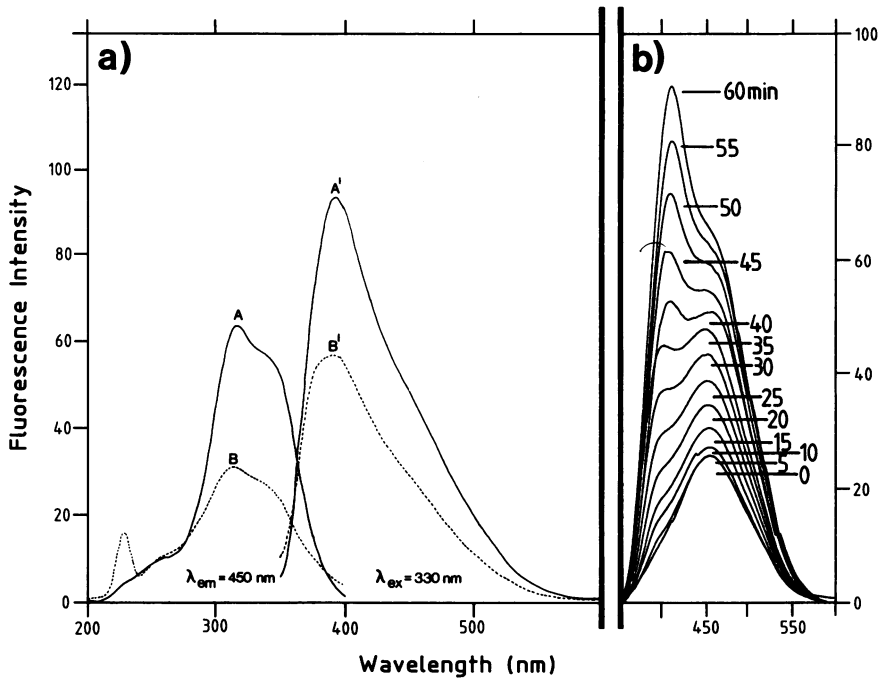

Fig. 4: a) Fluorescence emission spectra (right-hand curves) and excitation spectra (left-hand curves) of 1 μM free psoralen-modified 18mer (solid lines) and of 1 μM 18mer hybridized to 1 μM complementary 21mer (broken lines). Fluorescence was recorded with an excitation wavelength of 330 nm in the range from 350 - 600 nm. Excitation spectra were recorded for an emission wavelength of 450 nm in the range from 200 - 400 nm.
b) Time-dependent shift of the fluorescence emission spectrum of the free psoralen-modified 18mer recorded for 60 min.

fluorescence emission is observed from 360 to 560 nm with a maximum at 395 nm. As reported also by other investigators (18) upon exciting, the maximum shifts from 455 nm to 395 nm within minutes. This is probably due to the fluorescent monoadduct and reaches a maximum at about 10 min of irradiation under our conditions. On hybridisation to complementary oligodeoxyribonucleotides the fluorescence intensity is decreased significantly (Fig. 4). This decrease can be explained by the intercalation of the psoralen into the DNA.

The melting temperatures of duplexes formed after hybridisation of the unmodified 18mer and the psoralen-modified 18mer to the complementary 21mer were measured in ultrapure water at a

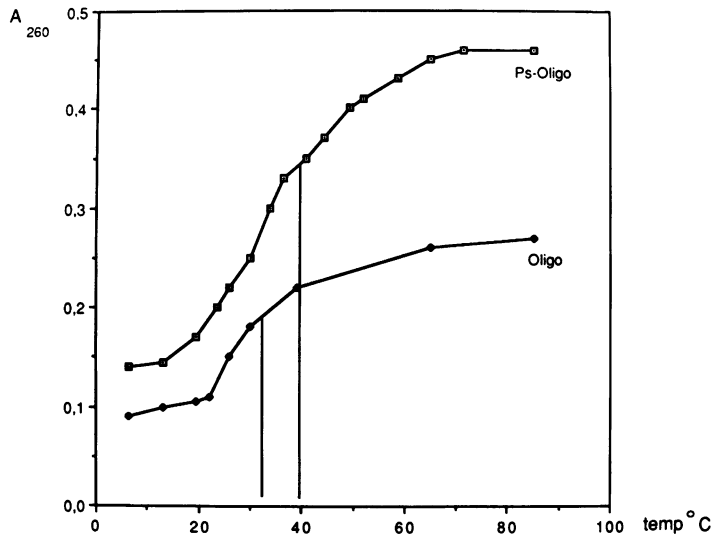

Fig. 5: Melting curves of the duplex-DNAs formed by hybridisation of the psoralen-modified 18mer (Ps-oligomer) and of the unmodified 18mer (oligomer) to the complementary 21mer at a total strand concentration of 2 μM in ultrapure water.

total strand concentration of 2 μM. Temperature denaturation data of the psoralen-modified oligodeoxyribonucleotide hybridized to the complementary 21mer revealed that the T_m increases from about 32°C for the unmodified double-stranded DNA to about 40°C for the double-stranded fragment intercalated by psoralen (Fig. 5).

Photocrosslinking of Double-Stranded DNA

In order to test the photocrosslinking ability of the 18mer psoralen-modified oligodeoxyribonucleotide, it was hybridized to a ^{32}P-labeled complementary 21mer oligodeoxyribonucleotide and the mixture irradiated at 350 nm. The irradiated sample was loaded on a denaturating polyacrylamide gel at neutral pH and after electrophoresis the products analysed by autoradiography (Fig. 6). The product of the photoreaction (Fig. 6, lane 2) migrated more slowly than the intact target strand (Fig. 6, lane 4). The hybridized but not irradiated fragment is dissociated under the denaturating conditions used and migrates with the target strand (Fig. 6, lane 3). Therefore the slowly migrating band is most likely due to a cross-linked species in which the 18mer was covalently attached to the 21mer via its psoralen moiety. The

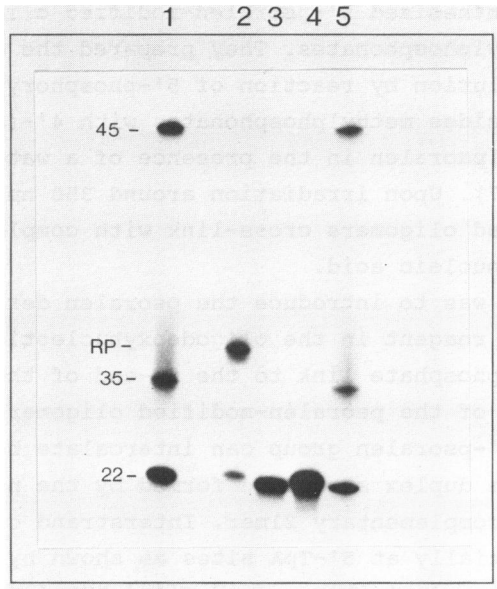

Fig. 6: Photo-cross-linking of the psoralen-modified 18mer to the single-stranded 21mer. A solution containing 0.25 μM 21mer and 0.75 μM psoralen-modified 18mer in water was irradiated at 350 nm for 20 min. at 20°C. The autoradiogram of the polyacrylamide gel electrophoresis of the reaction mixtures shows the following band pattern: lanes 1 and 5, length standards; lane 2, psoralen-modified 18mer and template 21mer irradiated at 350 nm; lane 3, psoralen-modified 18mer and template 21mer without irradiation; lane 4, 21mer irradiated at 350 nm.

extent of cross-linking reached a stable level of 75 % photoproduct after 20 min. with the Xenon discharge system used. The analysis of the photoreversion experiments (Fig. 7) shows that the photoadducts formed after irradiation at 350 nm for 20 min. almost completely disappeared after 10 min. of irradiation at 254 nm and that the template 21mer is regenerated.

DISCUSSION

Derivatives of psoralen are very powerful tools in the elucidation of nucleic acid secondary structure (6). In order to specifically target DNA sequences for the photoreaction with psoralen, Hearst and coworkers recently prepared psoralen-modified oligomers containing a site specifically engineered psoralen monoadduct (19). In another approach Miller and co-

workers have synthesized 5'-psoralen-modified oligodeoxyribonucleoside methylphosphonates. They prepared the derivatized oligomers in solution by reaction of 5'-phosphorylated oligodeoxyribonucleosides methylphosphonates with 4'-(aminoalkyl)-4,5',8-trimethylpsoralen in the presence of a water-soluble carbodiimide (17). Upon irradiation around 350 nm, both types of psoralen-modified oligomers cross-link with complementary sequences of a nucleic acid.

Our strategy was to introduce the psoralen derivative as a phosphitylation reagent in the oligodeoxynucleotide synthesis resulting in a phosphate link to the 5'-end of the oligomer.

The sequence of the psoralen-modified oligomers was designed such that the 5'-psoralen group can intercalate between the 5'-TpA sites of the duplex structure formed by the psoralen-modified 18mer with its complementary 21mer. Interstrand cross-links are formed preferentially at 5'-TpA sites as shown by photobinding of different psoralen derivatives to defined DNA fragments (20-23).

Upon irradiation of a ^{32}P-labeled 21mer and its complementary psoralen-modified 18mer with UV light at 350 nm, a new band appeared on the gel whose mobility was less than the 22mer template (Fig. 6, lane 2). This new band is the photoproduct formed. As the photo-cross-link can only occur between the 5'-TpA sites of the two strands proper binding of the psoralen-modified oligomer to the target nucleic acid is clearly demonstrated. The extent of photo-cross-linking increased during the first 20 min of irradiation to a stable level of about 75 %. Quantitative cross-linking was not observed even in the presence of a 100 fold excess of the modified oligomer. This finding suggests that the psoralen-modified oligomer is inactivated during the irradiation. A similar effect was observed with psoralen-modified methyl-phosphonate oligomers (17). The psoralen molecule is partly converted to a cross-linking inactive product when irradiated at 365 nm (24).

The photoreaction of pyrimidine-psoralen adducts at 350 nm is reversible at 254 nm (9). Irradiation of the 21mer/psoralen-modified oligomer photodiadduct at 254 nm resulted in a regeneration of the template 21mer. The photodiadduct disappeared almost completely within 10 min. (Fig. 7). This photoreversion is based on the presence of a thymine-psoralen-thymine diadduct which

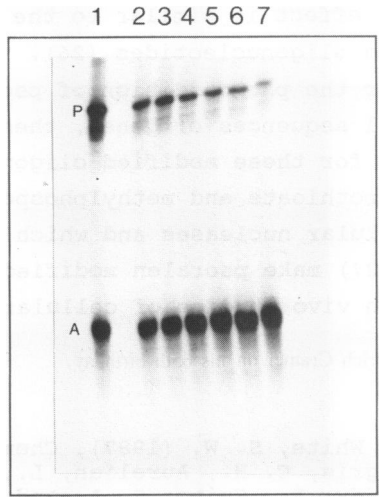

Fig. 7: Autoradiogram of the photoreversal experiment: the hybridized psoralen-modified 18mer and complementary 21mer were first irradiated at 360 nm for 20 min. to give the photodiadduct (P) and then irradiated at 254 nm for 0 - 10 min. releasing the free 21mer (A). Lane 1: photodiadduct; lane 2 to 6 correspond to irradiation times at 254 nm of 0.5, 1, 2, 3, 5 and 10 min.

is formed in a two step reaction after irradiation of the intercalated psoralen. In the first step the 4',5' double bond of the psoralen furan ring reacts with the 5,6 double bond of the terminal thymine base of the modified oligomer and in the second step the 3,4 double bond of the psoralen pyrone ring reacts with the 5,6 double bond of the thymine in the complementary strand to form the diadduct (25). In our system the intercalation is a prerequisite for the cross-linking reaction what is in contrast to the stacking of the psoralen ring upon the opposing thymidine ring in the system described by Miller and coworkers (17).

The ethoxy-group as a spacer between the psoralen molecule and the oligomer was optimal with respect to the extent of photo-cross-linking after intercalation of the modified oligomer. Similar results were obtained with psoralen-modified methylphosphonate oligomers, in which an aminoethyl linker was used (17).

The melting temperature of the duplex formed between the psoralen-modified oligodeoxyribonucleotide and the 21mer increase the T_m by about 8°C. This effect shows that the intercalated psoralen group contributes significantly to the stability of the

duplex. The observed effect is similar to the effect reported for acridine-modified oligonucleotides (26).

As 5'TpA sites for the photoaddition of psoralens are located in signal and control sequences of genes, these structures are preferential targets for these modified-oligomers. In addition the synthesis of phosphorothioate and methylphosponate oligomers which are resistant to cellular nucleases and which can pass the membran of mammalian cells (27) make psoralen modified oligomers a valuable probe for in vivo studies of cellular gene expression.

Dedicated to Professor Dr Friedrich Cramer on his 65th birthday

REFERENCES

1. Ollis, D. L. and White, S. W. (1987), Chem. Rev. 87, 981-995.
2. Miller, P. S., Agris, C. H., Aurelian, L., Blake, K. R., Murakami, A., Reddy, P., Spitz, S. A. and TS'O, P. O. P. (1985), Biochimie 67, 769-776.
3. Green, P.J., Pines, O. and Inouye, M. (1986), Annu. Rev. Biochem. 55, 569-597.
4. 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.
5. Shi, Y., Gamper, H. and Hearst, J. E. (1987), Nucl. Acids Res. 15, 6843-6854.
6. Cimino, G. D., Gamper, H. B., Isaacs, S.T. and Hearst, J. E. (1985), Annu. Rev. Biochem. 54, 1151-1193.
7. Turner, S. and Noller, H. F. (1983), Biochem. 22, 4159-4164.
8. Garrett-Wheeler E., Lockard, R. E. and Kumar A. (1984), Nucl. Acids Res. 12, 3405-3423.
9. Shi, Y. and Hearst, J. E. (1987), Biochem. 26, 3786-3792.
10. Courey, B. A. J., Plon, S. E. and Wang, J. C. (1986), Cell 45, 567-574.
11. Nielsen, P. E. (1987), Nucl. Acids Res. 15, 921-932.
12. Isaacs, S. T., Shen, C. K., Hearst, J. E. and Rapoport, H. (1977), Biochem. 16, 1058-1064.
13. McBride, L. J. and Caruthers, M. H. (1983) Tetrahedron Lett. 24, 245-248.
14. McLaughlin, L. W. and Piel, N. (1984) in "Oligonucleotide synthesis, a practical approach", 117-133, ed.: Gait, M. J., IRL Press, Oxford, UK.
15. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) Molecular cloning, 125-126, Cold Spring Harbor Laboratory Cold Spring Harbor, NY, USA.
16. Sinha, N. D., Biernat, J., McManus, J. and Köster, H. (1984), Nucl. Acids Res. 12, 4539-4547.
17. Lee, B. L., Murakami, A., Blake, K. R., Lin, S-B. and Miller, P. S. (1988), Biochem. 27, 3197-3203.
18. Johnston, B. H. and Hearst, J. E. (1981), Photochem. Photobiol. 37, 785-792.
19. Shi, Y-B. and Hearst, J. E. (1987), Biochem. 26, 3792-3798.
20. Sage, E. and Moustacchi, E. (1987), Biochem. 26, 3307-3314.

21. Yeung, A. T., Jones, B. K. and Chu, C. T. (1988), Biochem. 27, 3204-3210.
22. Gamper, H. B., Piette, J. and Hearst, J. E. (1984), Photochem. Photobiol. 40, 29-34.
23. Zhen, W., Buchardt, O., Nielsen, H. and Nielsen, P. E. (1986), Biochem. 25, 6598-6603.
24. Hearst, J. E. (1981), Annu. Rev. Biophys. Bioeng. 10, 69-86.
25. Peckler, S., Graves, B., Kanne, D., Rapoport, H., Hearst, J. E. and Kim, S. H. (1982) J. Mol. Biol. 162, 157-172.
26. Asseline, U., Delarue, M., Lancelot, G., Toulmé, F., Thuong, N. T., Montenay-Garestier, T. and Hélène, C. (1984), Proc. Natl. Acad. Sci. U.S.A. 81, 3297-3301.
27. Marcus-Sekura, C. J., Woerner, A., Shinozuka, K., Zon, G. and Quinnas, G. V. (1987), Nucl. Acids Res. 15, 5749-5763.