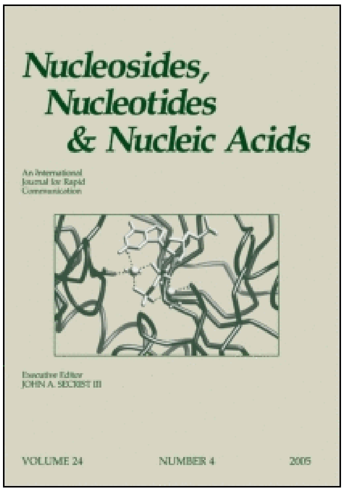

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PHOTOINDUCED CROSSLINKING OF DOUBLE-HELICAL DNA BY PSORALEN COVALENTLY LINKED TO A TRIPLE HELIX-FORMING OLIGONUCLEOTIDE UNDER NEAR-PHYSIOLOGICAL CONDITIONS

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□ *Stable triplexes have been generated under near-physiological conditions by the introduction of the C and T base analogues 3-methyl-2-aminopyridine-2′-deoxyriboside and 5-(3-aminoprop-2-ynyl)- ′- deoxyuridine into psoralen-conjugated triplex-forming oligonucleotides. After irradiation with UV light at 365 nm, photo-induced cross-linking of the TFO to double-helical DNA was observed by UV-melting analysis and fluorescence measurements.*

Keywords Psoralen; triplex; crosslinking

INTRODUCTION

Triplex-forming oligonucleotides (TFOs) bind in a sequence-specific manner to the major groove of double-stranded DNA (dsDNA) and can be used to modulate gene expression and as molecular tools to cause DNA strand cleavage.[1] However, the affinity of TFOs constructed from normal bases is relative low under physiological conditions due to the requirement for protonation of cytosine in the third strand.[2] Triplex formation can be improved by covalent attachment of reactive groups to the TFOs. Psoralens are bifunctional photo-crosslinking agents that have been used as probes of nucleic acid structure and function. When attached to the termini of TFOs, psoralens intercalate into the neighbouring duplex and stabilize the

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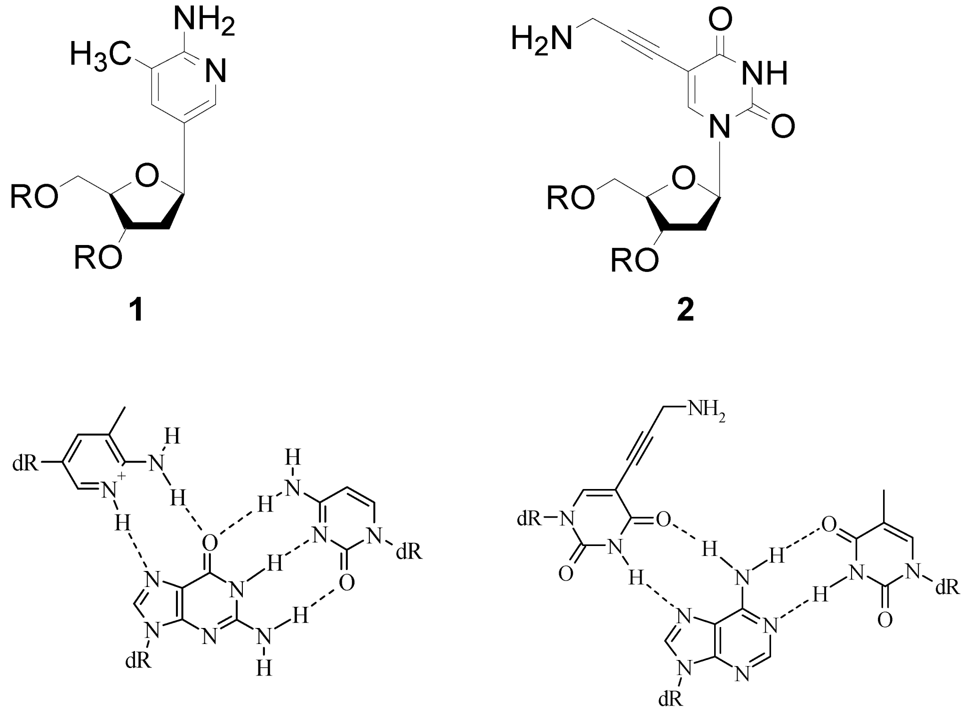

1 **2**

1·GC **2**·AT

FIGURE 1 1) Methyl-2-aminopyridine-2′-deoxyriboside; 2) 5-(3-aminoprop-2-ynyl)-2′-deoxyuridine.

triple-stranded complex. Irradiation at 365 nm induces a [2+2] cycloaddition reaction between the intercalated psoralen and pyrimidine bases in the double strand to form adducts with one or both duplex strands, principally at TpA steps. In our previous work, we have studied the DNA binding and photochemical crosslinking properties of TFOs that contain an internal psoralen or two psoralens, one at the 5′-end and one at the 3′-end, tethered to a uracil at pH 5.0.[3] In this work, modified bases 3-methyl-2-aminopyridine (**M**, Figure 1),[4] which recognises GC, and 5-propargylamino dU (**P**, Figure 1),[5] which recognizes AT were introduced into psoralen-conjugated TFOs. UV-melting analysis and fluorescence techniques were used to study the photoreaction properties of the psoralen TFO under near-physiological conditions.

METHODS

Attachment of psoralen to TFO

The attachment of Psoralen at the 5′-end of TFOs was realized using 4′-(methoxyhexyl)-4,5′, 8-trimethylpsoralen phosphoramidite (Pso C6) from Glen Research Inc. (Figure 1) in the final step of oligonucleotide assembly.

TABLE 1 Oligonucleotide sequences. Oligonucleotides **3**·**4** form the duplex that contains the target site for the TFO **5**. Pso indicates 5′-psoralen (4′-(methoxyhexyl)-4, 5′, 8-trimethylpsoralen), M and P correspond to 3-methyl-2-aminopyridine-2′-deoxyriboside and 5-(3-aminoprop-2-ynyl) -2′-deoxyuridine, respectively

Duplexes	
5’-TCA**TA**GAGAA**TA**AAGA**TA**TAGGC-3’	3
3’-AGT**AT**CTCTT**AT**TTCT**AT**ATCCG-5’	4
TFOs	
5′-**Pso**-TMTMTPGPTPMT-3′	5

Duplex and TFOs

A 23-mer duplex (**3**·**4**) was the target for TFO **5** (Table 1, in which the 12-base pair TFO binding site is underlined). In TFO **5**, M and P correspond to 3-methyl-2-aminopyridine-2′-deoxyriboside and 5-(3-aminoprop-2-ynyl)-2′-deoxyuridine, respectively.

Experimental Methods

Triplexes composed of psoralen-linked TFOs and duplex target were irradiated with UV light at 365 nm for up to 60 mins and the photo-products were analyzed using UV-melting and fluorescence methods.

RESULTS AND DISCUSSION

UV-Melting Studies

Triplex **5**·**3**·**4** gave two transitions, the lower of which (42 °C) was due to triplex melting and the higher (69°C) to duplex melting (Figure **2**). The triplex was then irradiated with UV light (365 nm, for up to 60 minutes at 0°C) and subjected to further melting studies. The melting curve was totally transformed. After irradiation, the transition due to dissociation of the TFO disappeared. A minor transition, corresponding to that of the residual duplex, was present around 67°C and a new high temperature transition was observed, which presumably reflects melting of the crosslinked triplex.

Fluorescence Studies

Further proof of cross-linking between the intercalated psoralen and its target duplex can be obtained from fluorescence measurements.[6] TFO **5**, containing a 5′-attached psoralen, exhibited a weak fluorescence emission spectrum between 380 nm and 600 nm with a maximum at 460 nm (Figure 3). Upon hybridization with the target duplex **3**·**4**, the intensity of

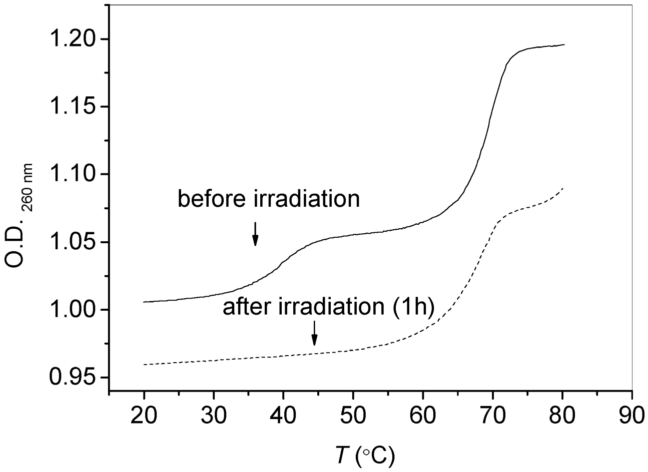

FIGURE 2 UV-melting profiles for triplexes **5·3·4.** The experiment was performed in 10 mM sodium phosphate, 200 mM NaCl, 1 mM EDTA, pH 7.0. The duplex concentration was 1 μM and the TFO concentration was 10 μM.

the psoralen fluorescence dropped slightly, while the λ_{max} shifted from 460 nm to 450 nm due to fluorescence quenching of the excited state of the intercalated dye by the DNA bases. After irradiation at 365 nm to promote crosslinking, the fluorescence at 450 nm declined and a new intense peak appeared at 380 nm due to formation of the monoadduct which contains the highly fluorescent coumarin nucleus. Further irradiation produced a decline in fluorescence intensity due to the conversion of the mono-adducts to

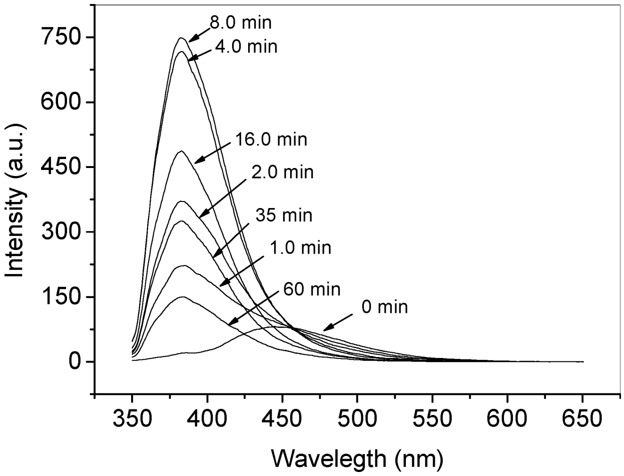

FIGURE 3 Fluorescence emission spectra of triplex **5·3·4** after various emission times. The experiment was performed in 10 mM sodium phosphate, 200 mM NaCl, 1 mM EDTA, pH 7.0. The duplex concentration was 5 μM with a TFO concentration of 1 μM. The inset shows the time-dependent fluorescence intensity at 380 nm.

bis-adducts and a consequent loss of conjugation. This result corroborates our previous fluorescence experiments with the other psoralen modified triplexes.[3]

CONCLUSION

Our research demonstrates that by using psoralen derivatives in combination with nucleotide analogues, efficient triplex-directed double-stranded psoralen photoadduct formation can be achieved under near-physiological conditions.

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