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PREPARATION, CHARACTERIZATION AND DNA PHOTOCLEAVAGE OF DIAZAPYRENE-TETHERED OLIGOTHYMIDYLATES

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Abstract : Several diazapyrene-tagged oligodeoxynucleotides were synthesized and characterized. The interaction of these modified oligomers with complementary strands indicated that introduction of the diazapyrene moiety increased the stability of the duplex as compared to that of native oligomers. DNA cleavage experiments with diazapyrene-tagged oligomers induced localized scission at the duplex region.

As part of our effort to design functionalized antisense DNA oligomers[2] having the ability to produce site-specific DNA cleavage,[3] we synthesized several oligodeoxynucleotides[4] containing viologen[5] derivatives attached covalently to the phosphorous backbone. Several of these derivatives perturbed duplex formation, but failed to induce DNA cleavage. Lack of cleavage may be due to steric hindrance induced by the energetically favored orthogonal conformation[6] of the two viologen pyridinium rings. To overcome this unfavorable interaction, the 2,7-diazapyrenium dication[7] (DAP^{2+}) was chosen as a constrained viologen analogue having a planar π-framework which contains viologen as a built-in unit. We describe herein the preparation of diazapyrene-tagged oligodeoxynucleotides and their DNA photocleavage.

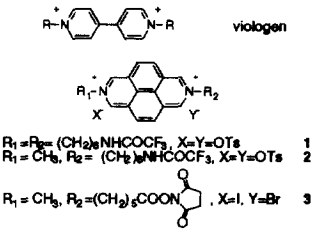
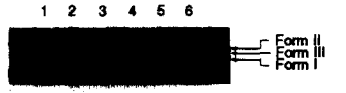


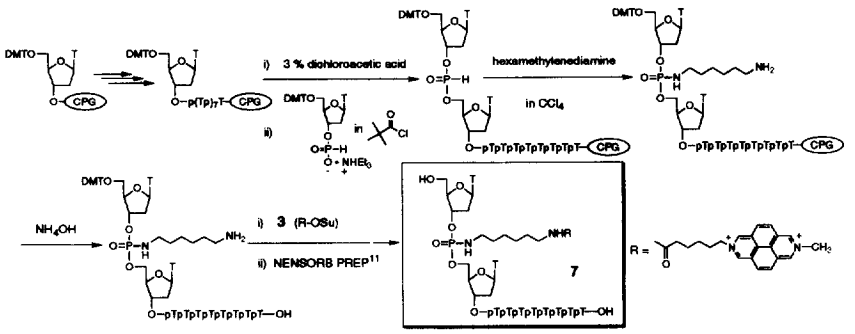
Figure 1. Photo-induced DNA cleavage of pBR322 plasmid DNA with **2**



Each solution (20 μl, 0.4 μg pBR322 plasmid DNA in the presence of **2** in 10 mM Tris-HCl containing 1 mM EDTA (pH 7.6)) was irradiated at 254 nm at 4 °C for 20 min and analysed by agarose gel electrophoresis (1 %, 100 V). Lane 1 : DNA control ; Lane 2 : 100 μM of **2** + DNA ; Lane 3 : 10 μM of **2** + DNA ; Lane 4 : 1 μM of **2** + DNA ; Lane 5 : 100 nM of **2** + DNA; Lane 6: 100 μM of **2** + DNA, unirradiated.

DAP^{2+} derivatives **1**~**3**[8] bearing aliphatic alkyl amino linker arms were prepared from 2,7-diazapyrene.[9] Photocleavage experiments of pBR322 plasmid DNA[10] with **2**, demonstrated the conversion of supercoiled cDNA into nicked DNA, suggesting a preferable interaction of the DAP^{2+} with cDNA (Figure 1). A second experiment using the EcoRI-BamHI fragment from pBR322 plasmid DNA, revealed that DAP^{2+} derivatives **1** and **2** had a 10^2 to 10^3-fold increase in ability to induce photocleavage as compared to the viologen derivatives (data not shown).

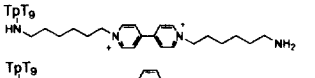
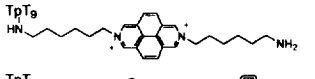
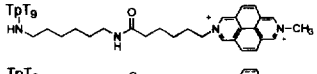
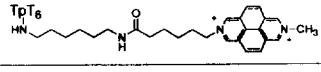
Scheme 1.



DAP^{2+}-tagged thymidine decamers **6** and **7**, and heptamer **8** were prepared using standard protocol for H-phosphonate chemistry,[12] as shown in Scheme 1, and were isolated as a diastereomeric mixture by reverse phase HPLC.

The modified oligomers and their cation radicals exhibited characteristic UV-visible absorption spectra.[8] The presence of the phosphoramidate linkage was confirmed by ^{31}P-NMR.[13] For example, **8** showed two peaks of equal height at ca. 11 ppm and a broad peak at -0.58 ppm in a 2 : 5 ratio due to the diastereomeric phosphoramidate and the native phosphodiester, respectively. HPLC analysis of enzymatic digests[14] of the modified oligomers with snake venom phosphodiesterase and alkaline phosphatase, showed formation of the corresponding monomeric nucleoside and the DAP^{2+}-tagged dimers,[15] indicating resistance of the phosphoramidate bond toward nucleases.[16]

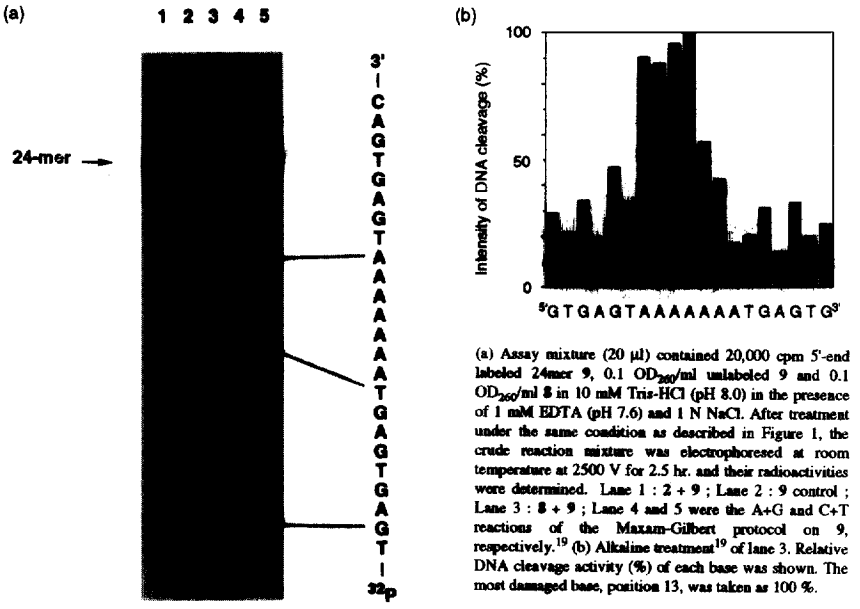
Table 1. Melting temperature of duplexes **4** ~ **8**[(a)]

Strands		Complementary Strand	NaCl (M)	Tm (°C)
T_{10} (native)	**4**	poly dA (equimolecular)	0	5
			0.1	24
			1.0	41
TpT$_9$ 	**5**	poly dA (equimolecular)	0	5
			0.1	22
			1.0	38
TpT$_9$ 	**6**	poly dA (equimolecular)	0	11
			0.1	28
			1.0	43
TpT$_9$ 	**7**	poly dA (equimolecular)	0	11
			0.1	31
			1.0	45
TpT$_8$ 	**8**	poly dA (equimolecular)	0.1	8
			1.0	24

(a) "p" = natural phosphodiester linkage ; Absorbance measured in 10 mM Tris-HCl (pH 7.0).

CD analysis of duplexes **4** ~ **8** with poly dA showed B-form structures (data not shown) . The thermal stability of the modified oligomers was determined from their melting curves (Tm, Table 1). Decamer **6** and **7** complexed with the poly dA and showed higher Tm values at various salt concentrations relative to **4** or **5**, suggesting that both the cationic nature and the planar π-system of DAP^{2+} contributed to duplex stabilization.

Figure 2. DNA cleavage by **8**



(a) Assay mixture (20 μl) contained 20,000 cpm 5'-end labeled 24mer **9**, 0.1 OD_{260}/ml unlabeled **9** and 0.1 OD_{260}/ml **8** in 10 mM Tris-HCl (pH 8.0) in the presence of 1 mM EDTA (pH 7.6) and 1 N NaCl. After treatment under the same condition as described in Figure 1, the crude reaction mixture was electrophoresed at room temperature at 2500 V for 2.5 hr. and their radioactivities were determined. Lane 1 : **2** + **9** ; Lane 2 : **9** control ; Lane 3 : **8** + **9** ; Lane 4 and 5 were the A+G and C+T reactions of the Maxam-Gilbert protocol on **9**, respectively.[19] (b) Alkaline treatment[19] of lane 3. Relative DNA cleavage activity (%) of each base was shown. The most damaged base, position 13, was taken as 100 %.

Finally, we examined the interaction of the DAP^{2+}-tagged oligomer **8**[17] with a short, synthetic fragment containing a poly dA run (**9**[18], Figure 2a). Polyacrylamide gel electrophoresis (PAGE) analysis indicated a photoinduced covalent attachment occurred between these two species upon irradiation (Lane 3, for conditions see Figure 1). Although no photocleavage of the 24-mer by **8** alone was observed, alkaline-induced DNA scission of the photoadduct of **8** and **9** suggested that **8** interacted with **9** in a site-selective manner (Figure 2b). The mechanism of this selectivity is currently unknown and will be the subject of a subsequent study.

In conclusion, DAP^{2+} was successfully incorporated into oligodeoxynucleotides, and the DAP^{2+}-modified oligomer interacted selectively with it's complementary strand and induced DNA scission localized to the duplex region. DAP^{2+}-tagged oligodeoxynucleotides are potentially promising candidates as functionalized DNA oligomers possessing selective DNA cleavage properties. More detailed investigations on the function of the DAP^{2+}-tagged oligonucleotides (e.g., the optimum condition of DAP^{2+}-induced cleavage) are in progress.

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References and Notes :

(1) Current address : Laboratory of Medicinal Chemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

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(8) Compounds **1~3** exhibited characteristic absorption at 320 and 420 nm in the UV-visible spectra and were readily photoreduced using visible light in the presence of EDTA to generate the cation radical ($DAP^{+\bullet}$), with absorption at around 450 and 650 nm, similar to that obtained electrochemically from DAP^{2+}.[6]

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(10) pBR322 plasmid DNA was prepared according to the previously reported procedure. Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning 2nd Ed.*, pp1.21-1.52, Cold Spring Harbor Lab. Press: New York.

(11) The removal of undesirable oligomers and excess **3**, and the detritylation of the final product were performed on a NENSORB^{TM} PREP cartridge.

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(15) DAP^{2+}-modified dimers were also synthesized using a standard H-phosphonate protocol, and were purified by reverse phase HPLC and characterized by UV and ^{31}P-NMR spectra (data not shown).

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(17) Due to insuficient amounts of decamers **6** and **7**, heptamer **8** was used for this experiment.

(18) 24-mer **9**, $^{5'}$TGAGTGAGTA$_7$TGAGTGAC$^{3'}$, was prepared manually by solid-phase DNA synthesis.

(19) Alkaline treatment was carried out according to Maxam, A.M.; Gilbert, W. *Methods in Enzymology*, Vol. 65, p. 499, Academic Press: New York, **1980**. Briefly, a mixture of **8** and **9** in 10 % aqueous piperidine was incubated at 90 °C for 30 min and concentrated under vaccuum. This residue was separated using 15 % PAGE and analyzed using a UltraScan XL Densitometer.

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