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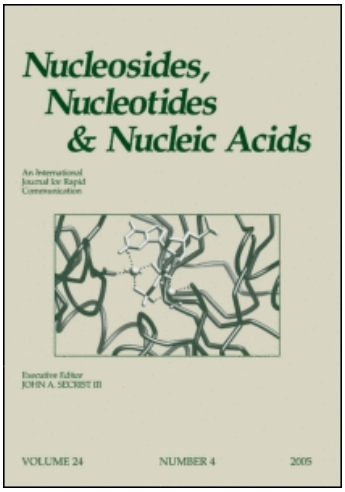

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PARALLEL AND ANTIPARALLEL DNA: FLUORESCENCE QUENCHING OF ETHIDIUM BROMIDE BY 7-DEAZAPURINES

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▫ *The fluorescence quenching of ethidium bromide caused by 7-deaza-2′-deoxyguanosine or 7-deaza-2′-deoxyisoguanosine is observed in DNA with parallel or anti-parallel chain orientation.*

Keywords 7-Deazapurines, Ethidium Bromide, Fluorescence, Quenching

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INTRODUCTION

Parallel stranded (*ps*) DNA may offer new opportunities for designing novel oligonucleotide hybridization probes, antisense constructs or interference RNA mimics. Thus, it is desirable to study the biophysical properties of this DNA.[1,2] The cationic dye ethidium bromide (EB) can intercalate into helical double-stranded oligonucleotides and polynucleotides and is used as nucleic acid stain.[3] Normally, the fluorescence of EB will be increased when the molecule is bound to duplex DNA,[4] while the fluorescence will be quenched when 7-deaza-2′-deoxyguanosine (c^7G_d, **1**, Scheme 1) replaces 2′-deoxyguanosine in anti-parallel (*aps*) DNA.[5,6] This manuscript reports on the properties of the ethidium bromide (EB) fluorescence when bound to *ps*-or to *aps*-duplexes containing 7-deazapurine nucleosides.

RESULTS AND DISCUSSION

The duplexes used in this study are shown in Table 1. The *aps*-duplex was designed from two single strands of alternating $d(GT)_6$ and $d(CA)_6$, which form the stable hybrid **5·6** with a T_m of 56°C. A similar stability is observed for duplex containing nucleoside **1** (**9·6**), while incorporation of nucleosides **3** into duplex **8·6** slightly stabilized the duplex (ΔT_m = 3°C). The *ps* duplex **5·7** containing

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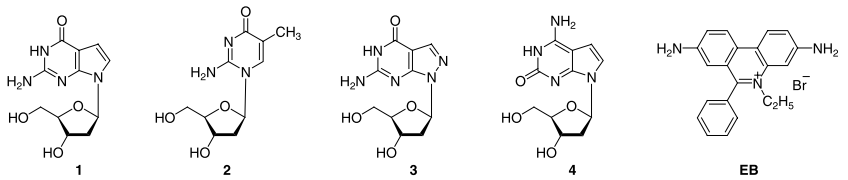

SCHEME 1 Modified nucleosides structures and EB.

compound **2** instead of dC is less stable than **5·6**. The T_m values of the *ps*-duplexes **9·7** and **10·11** are generally lower than their *aps*-counterparts due to the reverse WC-base pair formation of dA–dT. When EB is presented, the T_m values of *aps*-as well as *ps*-duplexes are increased about 1°C by the intercalating of EB.

As shown in Figure 1, the absorption maximum of free EB in buffer solution is at 481 nm. By the addition of DNA, no matter which duplex is used, an evident decrease of the absorbance of EB is observed. The UV maximum of EB in duplex DNA is shifted from 481 nm to about 516 nm, which is typical for intercalated EB. Similar changes of bound EB absorption of duplexes **8·6**, **9·6**, **9·7**, and **10·11** with their corresponding *aps* and *ps*-duplexes (**5·6**, **5·7**) are observed indicating that EB binding is almost base pair independent.

In the unmodified *aps*-duplex **5·6**, the fluorescence of EB increases strongly compare to that of unbound EB. Replacement of the dG-residues by the pyrazole [3,4-d] pyrimidine nucleoside **3** does not change the fluorescence intensity of bound EB, while in the *aps*-duplex **9·6**, compound **1** quenches the fluorescence totally. As in the *ps*-duplex **5·6**, the EB fluorescence is enhanced evidently in *ps*-duplexes even

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TABLE 1 T_m Values and Photophysical Properties of Duplexes Containing EB[a]

Duplex	Emission (nm) (intensity)	Excitation (nm) (intensity)	Absorbance (nm)	T_m (°C)	
					EB binding
EB	590.0 (23.7)	486.8 (23.2)	481.0		
5′-d(G-T)$_6$-3′ **(5)**	586.6	528.0	516.0	56	57
3′-d(C-A)$_6$-5′ **(6)**	(191.0)	(191.1)			
5′-d(G-T)$_6$-3′ **(5)**	587.2	527.8	516.0	45	46
5′-d(**2**-A)$_6$-3′ **(7)**	(155.4)	(158.9)			
5′-d(**3**-T)$_6$-3′ **(8)**	588.0	528.0	515.0	59	60
3′-d(C-A)$_6$-5′ **(6)**	(190.8)	(191.0)			
5′-d(**1**-T)$_6$-3′ **(9)**	587.0	527.0	516.0	56	57
3′-d(C-A)$_6$-5′ **(6)**	(9.2)	(9.2)			
5′-d(**1**-T)$_6$-3′ **(9)**	586.6	527.0	514.0	42	43
5′-d(**2**-A)$_6$-3′ **(7)**	(16.7)	(17.3)			
5′-d(**4**-T)$_6$-3′ **(10)**	587.0	527.0	516.0	46	47
5′-d(C-A)$_6$-3′ **(11)**	(17.7)	(17.8)			

[a]Measurements were performed in buffer solution of 100 mM NaCl, 10 mM $MgCl_2$, 10 mM sodium cacodylate, pH 7.0. Melting temperatures (T_m values) were taken from the first derivative of the melting curve (A_{260} *vs.* temperature; 20 to 80°C; increase 1°C min^{-1}) using 5.0 μM concentration of each single strand.

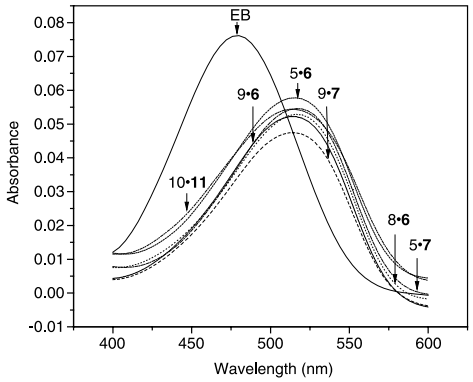

FIGURE 1 UV absorption spectra for free EB (17.0 μM) and in the presence of excess of duplexes (40.0 μM). Measurements were performed in buffer solution shown in Table 1.

when dC is replaced by compound **2**. But interestingly, this strong fluorescence of bound EB is quenched totally in the *ps*-duplexes **9·7** and **10·11**, which are modified by the nucleosides **1** and **4**, respectively (Figure 2).

Earlier, it has been postulated that the mechanism of quenching of the EB fluorescence by c^7G_d in aps duplexes results from a photo-induced electron transfer from c^7G_d to EB.[6] During this process the nucleoside becomes oxidized forming a radical cation. The electron is then transferred to the intercalated ethidium cation, which becomes a neutral non-fluorescent radical. This radical can transfer the electron back to the nucleoside radical cation. This is one possible reaction pathway. Others can lead to the oxidation of the nucleobase moiety.

In conclusion, it was observed that Watson-Crick as well as reverse Watson-Crick base pairs (Scheme 2) containing the 7-deazapurine the nucleoside **1** or **4** quench the fluorescence of duplex bound EB strongly. This is valid for *ps*-as well

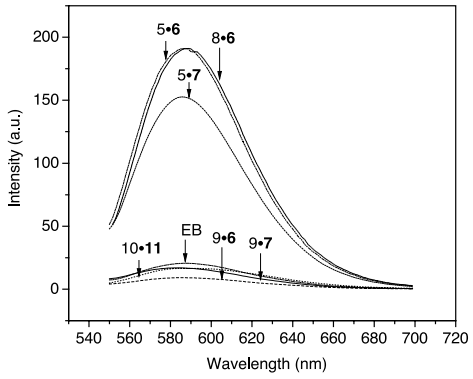

FIGURE 2 Fluorescence emission spectra for free EB and EB bound to DNA. The EB concentration was 0.85 μM (buffer see Table 1). The DNA (5.00 μM) was added in excess so that more than 95% of EB was bound.

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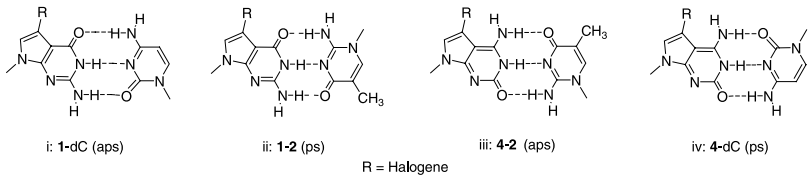

SCHEME 2 Base pair motifs of *ps* or *aps* DNA containing 7-deazaguanine or 7-deazaisoguanine.

as for *aps*-DNA. Recently, it was shown by our laboratory that 7-substituents of 7-deazapurine nucleosides alter the EB fluorescence quenching considerably.

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