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Bioorganic & Medicinal Chemistry Letters 14 (2004) 6031–6034
Bioorganic & Medicinal Chemistry Letters

Fluorescence quenching of parallel-stranded DNA bound ethidium bromide: the effect of 7-deaza-2′-deoxyisoguanosine and 7-halogenated derivatives

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Received 11 August 2004; revised 22 September 2004; accepted 24 September 2004

Abstract—Parallel stranded (ps) duplexes were constructed by incorporating 7-deaza-2′-deoxyisoguanosine (**1a**) or its 7-halogenated analogs **1b**,**c** in place of 2′-deoxyisoguanosine. UV and T_m analyses prove the high affinity of ethidium bromide (EB) to these modified duplexes. Steady-state fluorescence measurement shows that the fluorescence is quenched when EB is bound to ps duplexes containing compounds **1a**–**c**. The quenching effect depends on the 7-substituent of the nucleobase.

Parallel stranded (ps) DNA can be constructed from oligonucleotides incorporating isoguanine–cytosine or guanine–isocytosine base pairs instead of the canonical guanine–cytosine pair.[1] The dA–dT pair shows ambiguous base pairing properties and is accepted in anti-parallel stranded (aps) DNA as well as in ps DNA.[2] The interest in the structure and biophysical properties of ps DNAs has increased considerably in the last few years as they may offer new opportunities for designing new oligonucleotide hybridization probes, anti-sense constructs or interference RNA mimics.[3] Efforts have been undertaken towards the synthesis of novel nucleosides in order to increase the duplex stability of ps DNA.[4] Recently, our laboratory reported on the synthesis of 7-deaza-2′-deoxyisoguanosine (**1a**)[5] and its analogs **1b** and **1c** bearing halogen-substituents at C-7 with the potential to enhance the hydrophobic character of DNA bases with substituents protruding in the major groove of DNA and to strengthen dipole–dipole stacking interaction beneficial to duplex stability.[6] Further application of the modified nucleosides **1a**–**c** present in ps DNA focused our interest to molecules such as ethidium, which are bound to ps and aps DNA and can be used as site-specific reagent for foot printing reactions.[7] Meanwhile, the photoinduced redox activities of the nucleosides **1a**–**c** in oligonucleotide duplexes need to be elucidated because radical migration and damage of DNA have crucial implications with respect to carcinogenesis and mutagenesis.[8] Ethidium bromide (EB), an intercalator, can serve as an ideal candidate to realize those goals as it is used widely for various biochemical and biophysical applications both in aps DNA and in ps DNA.[2,9] Normally, the fluorescence of EB will be increased strongly when the EB molecule is bound to duplex DNA,[10] while the excited state of EB undergoes oxidative quenching by electron transfer when 7-deaza-2′-deoxyguanosine **2** (Scheme 1) replaces the canonical DNA constituent 2′-deoxyguanosine.[11] Thus, the changes in the fluorescence intensity of duplex bound EB can be used to decipher the redox activities of some modified nucleotides. Herein, we studied the photophysical properties of EB, when bound to ps duplexes containing compounds **1a**–**c** as well as 2′-deoxyisoguanosine (**3**). The structure dependent quenching abilities of compounds **1a**–**c** to the EB fluorescence are used to evaluate redox activities of the modified nucleosides. In addition, the stabilizing effect of the incorporation of compounds **1a**–**c** into ps duplexes was investigated as well.

Keywords: DNA; Ethidium bromide; 7-Deazapurine nucleosides; Fluorescence quenching.
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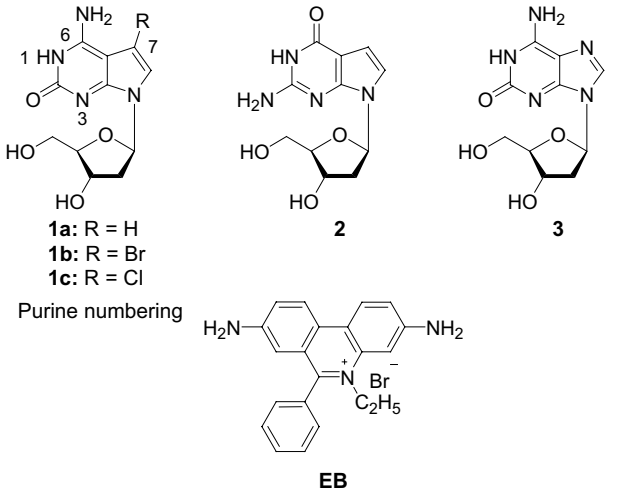
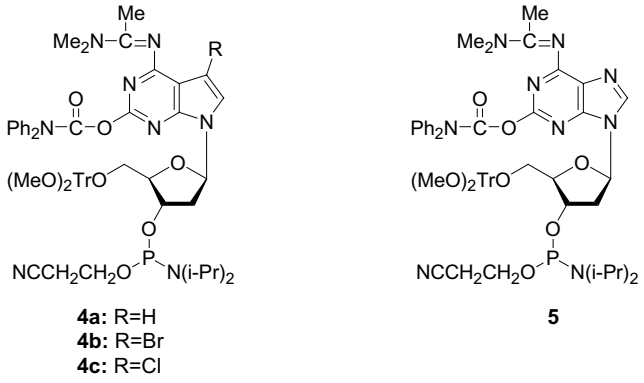
doi:10.1016/j.bmcl.2004.09.071



Scheme 1. Structure of modified nucleosides and ethidium bromide.

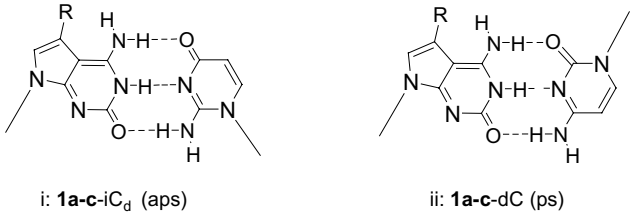


Scheme 2. Structure of phosphoramidites **4a**–**c** and **5**.

The nucleosides **1a**–**c** and **3** were converted into phosphoramidites **4a**–**c** and **5** (Scheme 2), respectively, as reported,[12] which are employed in the synthesis of base-modified oligonucleotides. The oligonucleotides **6**–**12** were prepared in a 1-μmol scale (DMT-on mode) on an ABI 392-08 synthesizer employing phosphoramidite chemistry. Purification of the oligonucleotides was performed as described.[12b] The oligomers were characterized by MALDI-TOF mass spectra (Supplementary data). The aps duplex was designed from two single strands of alternating $d(GT)_6$ and $d(CA)_6$, which form the stable hybrid **6·7** with a T_m of 56 °C. The corresponding ps **8·9** duplex containing 2′-deoxyisoguanosine instead of dG is less stable showing a T_m value of 45 °C.

The various duplexes and the T_m values are summarized in Table 1. As can be seen, ps duplex **10·9** containing the 7-deazapurine nucleoside **1a** shows almost the identical stability as the parent duplex **8·9**, while the incorporation of the 7-halogenated derivatives stabilized the duplexes significantly with $\Delta T_m = 2.5$ °C per modification for **1b** and $\Delta T_m = 2.0$ °C per modification for **1c**. The most probable base pair motifs for **1a**–**c** in the duplexes are shown in Scheme 3. When EB is present in the duplex solution the T_m value of all ps duplexes as well as aps duplex in our experiment is increased by about 1 °C. It means that intercalator (EB) is bound to the base-modified ps duplexes.

Next, the absorption properties of EB in different duplexes were studied. As shown in Figure 1, the maximum absorption of free EB in buffer solution is located at 481 nm. With the addition of DNA, no matter, which duplex is in presence, evident decrease in the absorbance of EB is observed. The hypochromism in the absorption



Scheme 3. Base pairs of ps or aps DNA containing compounds **1a**–**c**.

Table 1. T_m values and photophysical properties for 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(GT)$_6$-3′ (**6**)	586.6	528.0	516.0	56	57
3′-d(CA)$_6$-5′ (**7**)	(214.7)	(212.3)			
5′-d(**3**T)$_6$-3′ (**8**)	587.2	527.8	516.0	45	46
5′-d(CA)$_6$-3′ (**9**)	(227.2)	(226.8)			
5′-d(**1a**T)$_6$-3′ (**10**)	587.0	527.8	515.0	46	47
5′-d(CA)$_6$-3′ (**9**)	(17.7)	(17.8)			
5′-d(**1b**T)$_6$-3′ (**11**)	586.8	528.0	516.5	61	62
5′-d(CA)$_6$-3′ (**9**)	(52.9)	(53.8)			
5′-d(**1c**T)$_6$-3′ (**12**)	587.0	528.0	516.0	58	59
5′-d(CA)$_6$-3′ (**9**)	(85.0)	(84.9)			

[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; 20–80 °C; increase 1 °C min^{-1}) using 5.0 μM concentration of each single strand.

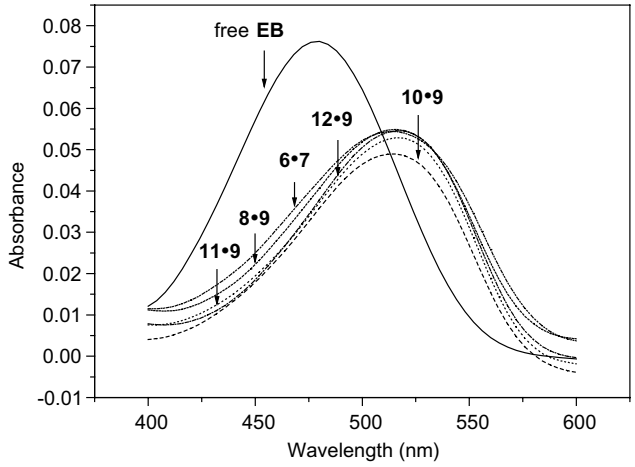


Figure 1. UV absorption spectra for free EB (17.0 μM) and in the presence of excess of duplexes (40.0 μM). Measurements were performed in buffer solution shown in Table 1.

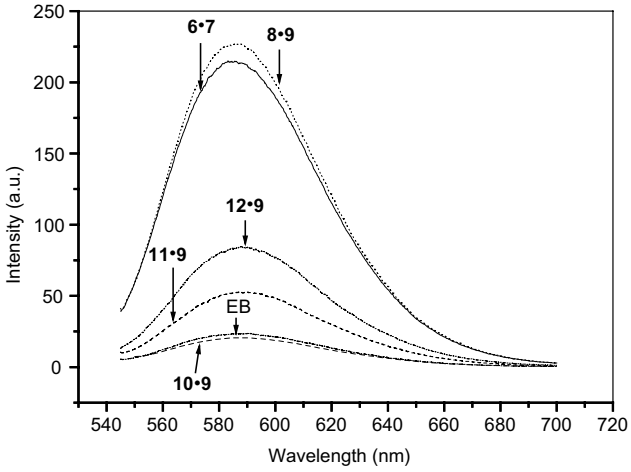


Figure 2. Fluorescence emission spectra for free EB and EB bound to DNA. The EB concentration was 0.85 μM (buffer see Table 1). The DNA (5.00 μM) was added in excess so that more than 95% of EB was bound.

spectra is due to the interaction between the electronic state of the intercalating chromophore and those of the DNA bases.[13] Because the strength of this electronic interaction is expected to decrease with the cube of the distance of separation between the chromophore and the DNA bases,[14] the observed large hypochromism suggests a close proximity of the EB molecules to the DNA bases. In addition to the decrease in absorption intensity, the peak position of the EB absorption band in duplex solution is shifted from 481 to around 516 nm, which is a typical alteration for intercalated EB.[11a] The similar changes of EB absorption between duplexes (**10·9**, **11·9** and **12·9**) and their parent aps and ps-duplexes (**6·7**, **8·9**) support that EB binding properties to these duplexes are uniform.

At last, the fluorescence properties of EB in base-modified duplexes were characterized. Table 1 compiles intensities of steady-state fluorescence of EB in duplexes. In the presence of template duplexes **6·7** and **8·9**, the EB fluorescence is enhanced greatly compared to that of free EB. The maxima of the emission are around 587 nm. The excitation maximum of EB is shifted from 480 (free EB) to 527 nm (duplex bound EB). When compounds **1a**–**c** are replacing iG_d in the ps duplex **8·9**, interestingly, the strong fluorescence of the EB/DNA complex is quenched tremendously compared to that of the EB bound to unmodified DNA (Fig. 2). Furthermore, the quenching effect depends on the halogen substituents in the 7-position of compound **1a**. Without 7-halogen substituents, compound **1a** quenches the fluorescence almost totally, while the 7-bromo or 7-chloro derivative shows less quenching ability in the order of **1b**→**1c**.

Generally, the fluorescence quenching of an intercalator in DNA duplexes can be attributed to the energy transfer[15] or electron transfer process.[11b–d] In our case, because of the lack of spectral overlap between the fluorescence of photoexcited EB and the absorption of the modified nucleosides **1a**–**c**, a quenching via energy transfer can be ruled out. Thus, the possible mechanism of quenching of the EB fluorescence by compounds **1a**–**c** in ps duplexes is the electron transfer from the nucleosides to EB^*, which has also been found in the case of 7-deazaguanosine modified aps duplexes.[11b] To support this hypothesis, we measured the oxidation potentials of **1a**–**c** and **3** by CV spectra. As shown in Table 2, the oxidation potential of compound **3**, which is about +1.30 V versus normal hydrogen electrode (NHE), is similar to that of natural $dG^{+/0}$ (+1.29 V)[16] and higher than that of $EB^{*/0}$ (+1.20 V).[11b] Thus, in the duplex **8·9**, the electron transfer process did not happen between EB excited state and nucleoside **3**, and the normal fluorescence enhancement of binding EB was observed. While for compounds **1a**–**c**, the lower oxidation potentials (about +0.95 V) would lead to the oxidation of modified nucleosides by EB excited state in duplexes **10·9**, **11·9** and **12·9**. Therefore, the fluorescence of binding EB was quenched. Normally, the quenching effect of nucleosides depends on the oxidation potentials. However, nucleosides **1a**–**c** shows different quenching ability despite of their nearly identical oxidation potentials. It might be due to the 7-substituents intervene the reaction pathway differently for nucleosides **1a**–**c** radical cation, which is formed during the electron transfer process.[17]

Table 2. Reduction potentials for the nucleosides **1a**–**c**, **3** and EB^*

Compound	Potential versus NHE[a] (V)
$\mathbf{1a}^{+/0}$	+0.93
$\mathbf{1b}^{+/0}$	+0.95
$\mathbf{1c}^{+/0}$	+0.97
$\mathbf{3}^{+/0}$	+1.30
$EB^{*/0}$	+1.2[b]

[a] Potentials were obtained in 0.1 M phosphate buffer, pH 7.0 with a normal three electrode configuration consisting of a glassy carbon working electrode, Ag/AgCl (3.0 M KCl solution) electrode and platinum auxiliary electrode.

[b] Ref. 11b.

In conclusion, ps duplexes incorporating nucleosides **1a**–**c**, which are much more easily oxidized than their purine counterpart isoG_d, quenched the fluorescence of binding EB strongly. The quenching ability of compounds **1a**–**c** to the EB fluorescence depends on the different 7-substituents. Further studies of quenching effect of modified nucleosides to EB fluorescence in duplexes are still under way.

Acknowledgements

We thank Dr. Yang He for the synthesis of the oligonucleotides. We also thank Monika Dubiel and Elisabeth Michalek for their kind help in the preparation of this manuscript. In addition we also thank Burghard Lutter for assistance in carrying out the CV measurements. Financial support by Roche Diagnostics GmbH is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.09.071.

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