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Nitroxide-labeled guanine as an ESR spin probe for structural study of DNA

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Abstract—A guanine derivative with a covalently linked nitroxide spin label has been devised. The spin label was incorporated into oligodeoxynucleotides by post-synthetic modification. The local environment of a variety of G-rich DNA is detectable by ESR using this spin probe.

1. Introduction

Electron spin resonance (ESR) has been widely used to investigate the organizational and dynamic properties of biomolecules, using persistent nitroxide spin probes for several decades.[1] The sensitivity and selectivity of the spin probe to microenvironmental change make it possible to detect differences in local conformational mobility and other structural properties of DNA.[2] Recently, several important studies on probing the formation of a variety of DNA structures, such as loops, duplex DNA, and triplex DNA, have been reported by Hopkins and co-workers,[3] Bobst et al.[4] and Gannett et al.[5] who utilized nitroxide-labeled probes covalently linked to uracil 5-position through a short tether.

G-rich DNA sequences are known to adopt various structures, such as B-DNA duplex, Z-DNA duplex, and quadruplex, which are biologically significant.[6] However, there is currently no reliable method that can probe the local environments of G-rich DNAs using a spin-labeled technique. If a spin-labeled guanine base is devised, it becomes possible to monitor the local environments of G-rich sequences easily and precisely.

Here, we report a guanine derivative that is equipped with a nitroxide spin label. Oligodeoxynucleotides (ODN) containing a guanine base to which 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) was covalently linked (^{TEMPO}G) were synthesized, and duplex and quadruplex structures were probed by ESR spectroscopy using this TEMPO-labeled ODN.

2. Synthesis of ^{TEMPO}G-containing ODN

We initially synthesized ODNs containing ^{TEMPO}G. The TEMPO unit **1** was post-synthetically incorporated into a 2-fluorohypoxanthine (^{F}I)-containing ODN 5′-d(TCGGTCGCTT^{F}ICCCGAGTGT)-3′ (**ODN1**(**^{F}I**)) (Fig. 1a). The preparation of **ODN1**(**^{F}I**) was accomplished by conventional solid-phase DNA synthesis.[7] **ODN1**(**^{F}I**) on the solid support was incubated with **1** (3 M) in water at 60 °C for 24 h, and then purified by HPLC. The composition of the ODN was proved to be consistent with a TEMPO-modified ODN, **ODN1**(**^{TEMPO}G**), by MALDI-TOF mass spectrometry (calcd 6269.16 for [(M−H)$^-$], found 6269.40) and enzymatic digestion with snake venom phosphodiesterase, nuclease P1 and alkaline phosphatase. An example of an HPLC chart after enzymatic digestion is shown in Figure 1b.

Keywords: G-rich DNA; Nitroxide spin label; Quadruplex; Artificial nucleoside.
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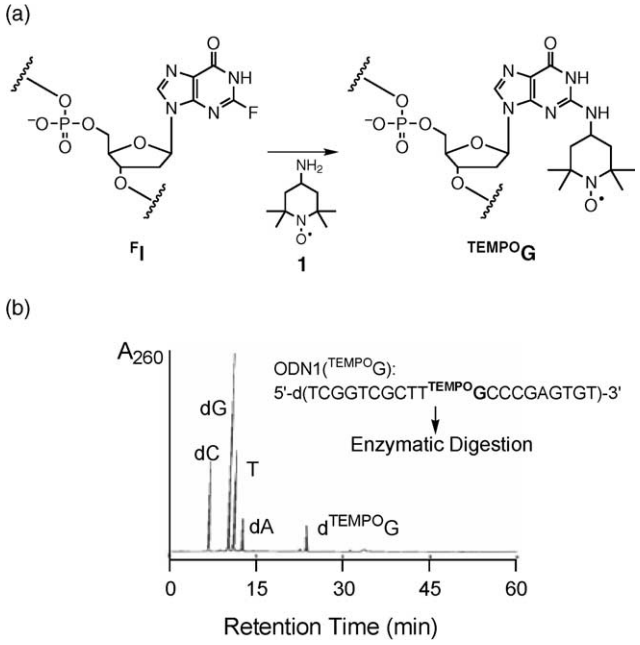

Figure 1. Synthesis of ^{TEMPO}G-containing DNA. (a) Synthetic scheme for the post-synthetic incorporation of 4-aminoTEMPO **1** into ^{F}I-containing ODN. (b) HPLC profile of ^{TEMPO}G-containing ODN, **ODN1(^{TEMPO}G)**, after enzymatic digestion by snake venom phosphodiesterase, nuclease P1, and alkaline phosphatase (100 mM triethylammonium acetate, pH 7.0, 0–60% acetonitrile over 60 min).

3. ^{TEMPO}G-labeled duplex

Prior to the ESR study of TEMPO-modified DNA, we examined the structure of the 20-mer duplex **ODN1(^{TEMPO}G)/ODN1′** (Fig. 2a). In the CD spectrum of **ODN1(^{TEMPO}G)/ODN1′**, a negative peak at 250 nm and a positive peak at 280 nm were observed (Fig. 2b). This spectrum indicates that **ODN1(^{TEMPO}G)/ODN1′** still maintains a B-DNA duplex form.[6] The stability of **ODN1(^{TEMPO}G)/ODN1′** was examined by monitoring the melting temperature (T_m) of the duplex. The T_m of **ODN1(^{TEMPO}G)/ODN1′** was 78.7 °C, 1.3 °C higher than **ODN1(G)/ODN1′**, suggesting that the incorporation of TEMPO into the guanine base does not thermally destabilize the duplex. To confirm the location of the TEMPO unit in **ODN1(^{TEMPO}G)/ODN1′**, we examined a molecular model of the duplex. Energy-minimized structures of the duplex show that the TEMPO unit incorporated into DNA is located at the center of the minor groove of the duplex (Fig. 2c). Hence this spin probe does not significantly perturb the B-DNA duplex form of **ODN1(^{TEMPO}G)/ODN1′**.

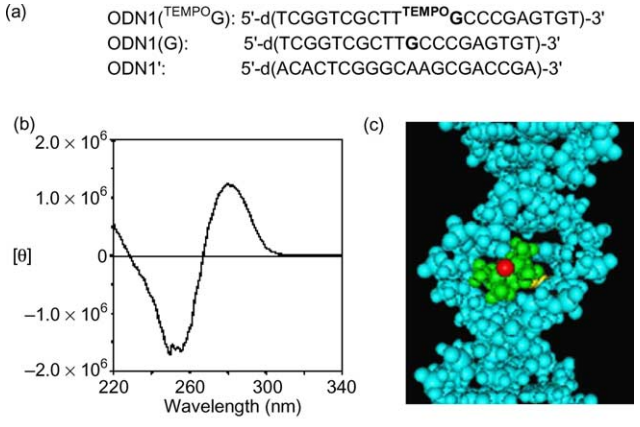

Figure 2. (a) Sequences of oligodeoxynucleotides used in this study. (b) CD spectra of the ^{TEMPO}G-containing duplex, **ODN1(^{TEMPO}G)/ODN1′**. The 3 μM duplex in 50 mM sodium phosphate (pH 7.0) was measured at 25 °C. (c) Molecular model of the ^{TEMPO}G-containing duplex, **ODN1(^{TEMPO}G)/ODN1′**. The model was obtained from minimization by Spartan '02. The base and TEMPO units of ^{TEMPO}G in the duplex are shown in yellow and green, respectively. The oxygen atom of TEMPO is shown in red.

We next investigated the change of ESR signal of ^{TEMPO}G caused by a structural change in **ODN1-(^{TEMPO}G)**.[8] ESR spectra of ^{TEMPO}G nucleoside, single-stranded **ODN1(^{TEMPO}G)**, and duplex **ODN1(^{TEMPO}G)/ODN1′** are summarized in Figure 3. The hyperfine coupling constant, a_N can provide information about the micropolarity of the microenvironment surrounding the nitroxide probe.[9] A less polar environment produces a smaller value of a_N due to a lower spin density at nitrogen. The ESR results show that the ESR spectrum of single-stranded **ODN1(^{TEMPO}G)** exhibits a characteristic three-line pattern with a typical hyperfine coupling constant, $a_N = 17.0$ G, and is closely identical with that of ^{TEMPO}G nucleoside ($a_N = 17.1$ G). a_N values for the spin probe in the duplex **ODN1(^{TEMPO}G)/ODN1′** (16.3 G) are lower than for the single-stranded state in bulk water, which reflects the reduced micropolarity around the nitroxide in the minor groove of the duplex.

The rotational correlation times τ_c were calculated from the ESR spectra according to Kivelson's equation,[10,11] which is reflective of the probe mobility and is used to monitor changes in the microviscosity experienced by the probe. The τ_c of single-stranded **ODN1(^{TEMPO}G)** was 0.56 ns, which is much larger than that of ^{TEMPO}G

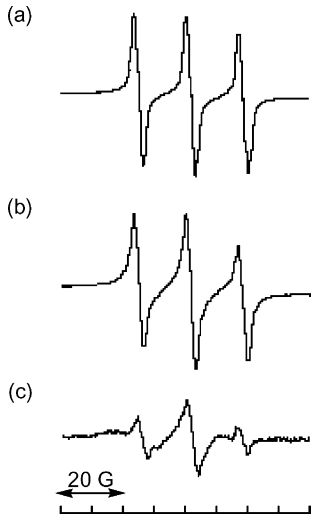

Figure 3. ESR spectra of (a) ^{TEMPO}G nucleoside, (b) **ODN1(^{TEMPO}G)**, and (c) **ODN1(^{TEMPO}G)/ODN1′** in 50 mM sodium phosphate (pH 7.0). The spectra were measured at 25 °C at a concentration of 100 μM.

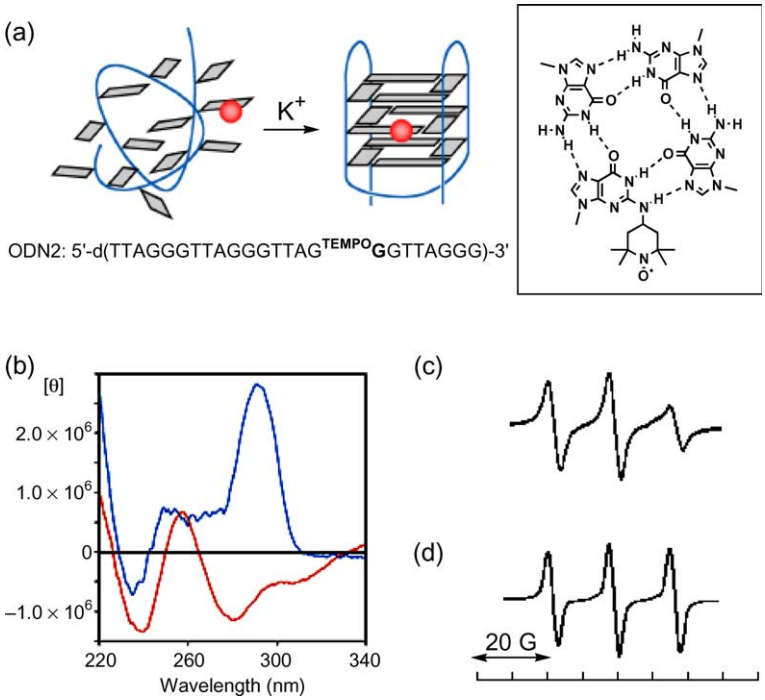

Figure 4. (a) Structural change of **ODN2** in the presence of potassium ion to an antiparallel quadruplex form. Red closed circles represent the sites where the nitroxide of ^{TEMPO}G is located. (b) CD spectra of **ODN2**. The 1 μM strand in 10 mM Tris–HCl and 1 mM EDTA (pH 8.0) was measured at 20 °C (red). The 7.9 μM strand in 2 mM sodium cacodylate and 0.1 mM EDTA (pH 7.2) in the presence of 70 mM potassium chloride was measured at 20 °C (blue). (c) ESR spectra of single-stranded **ODN2** in 10 mM Tris–HCl and 1 mM EDTA (pH 8.0). The spectra were measured at 25 °C at a concentration of 100 μM. (d) ESR spectra of quadruplex-formed **ODN2** in 2 mM sodium cacodylate and 0.1 mM EDTA (pH 7.2) in the presence of 70 mM potassium chloride. The spectra were measured at 25 °C at a concentration of 100 μM.

nucleoside (0.19 ns). The τ_c calculated for the duplex **ODN1**(^{TEMPO}**G**)/**ODN1**′ (2.39 ns) increased compared to that for the single-stranded state. Increase of τ_c is attributed to the motional restriction of the nitroxide group of TEMPO within duplex DNA.

4. ^{TEMPO}G-labeled quadruplex

We also examined ESR spectra for a G-quadruplex, one of the structures often observed in G-rich sequences.[13] We designed a fragment of human telomere sequence containing ^{TEMPO}G, **ODN2** (MALDI-TOF, calcd 7728.10 for [(M−H)$^-$], found 7728.74). The nitroxide group in **ODN2** protrudes to the outside of the groove when **ODN2** forms a quadruplex in the presence of potassium ion as shown in Figure 4a. The CD spectrum of **ODN2** in the presence of potassium ion showed a positive peak at 290 nm, which is characteristic for an antiparallel quadruplex structure (Fig. 4b). The T_m of this quadruplex was 60.5 °C, given by monitoring the absorbance change at 295 nm versus temperature.

Having established that **ODN2** forms an antiparallel quadruplex structure in the presence of potassium ion, we compared the ESR spectra of **ODN2** in the presence and absence of potassium ion (Fig. 4c and d). The hyperfine coupling constant a_N obtained from the ESR spectrum of the quadruplex in the presence of potassium ion was 17.0 G, which did not change from that of the single strand state in the absence of potassium ion ($a_N = 17.0$ G), suggesting that the change of micropolarity is small relative to the change in the structure. On the other hand, the rotational correlation time τ_c calculated from the quadruplex (0.32 ns) slightly decreased in comparison with that of the single strand state ($\tau_c = 1.56$ ns). The change in τ_c of **ODN2** suggests that the probe mobility increased, and is probably due to the wideness and shallowness of the groove and compactness of folding structure, characteristic of telomeric DNAs.[14]

5. Conclusion

In conclusion, a novel G nucleotide with a covalently linked nitroxide spin label has been devised. This spin label was readily incorporated into ODNs by a post-synthetic modification method. ESR data obtained from ^{TEMPO}G-containing DNA offers a useful knowledge of the local environments of a variety of G-rich DNA and the microenvironments around the nitroxide spin probe.

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10. τ_c may be regarded as the time needed for a molecule to rotate through an angle of π. As an approximation, from the ESR spectrum, the rotational correlation time can be calculated from the following equation:[11]

$$\tau_c = (6.6 \times 10^{-10}) \times W_0 \times [(h_0/h_{-1})^{1/2} + (h_0/h_{+1})^{1/2} - 2]$$

where W_0 represents the peak-to-peak line width of the ESR mid field line (in gauss) and h_{-1}, h_0 and h_{+1} are the peak-to-peak heights of the low-, mid-, and high-field lines, respectively. The constant 6.6×10^{-10} has been calculated for di-*tert*-butyl nitroxide,[12] but to a good approximation, it can be used for other nitroxide radicals as well. τ_c can be correlated with the microviscosity of the probe by the following relation:

$$\tau_c = 4\pi\eta\alpha^3/3kT$$

where α is the hydrodynamic radius of the probe, η is the viscosity, and k and T represent the Boltzmann constant and the temperature, respectively.

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