Note

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Water-proton relaxivities of DNA oligomers carrying TEMPO radicals

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5-Uridine derivative carrying a TEMPO radical (UST) was prepared and its single strand (ssUST) and a double strand (dsUST) with its complementary strand were obtained. Similarly, single strands carrying two and five radicals (ssUST2 and ssUST5, respectively) and the corresponding double strands (dsUST2 and dsUST5) were prepared. Their electron paramagnetic resonance (EPR) spectra showed typical anisotropic broadening in the high field line. The rotational correlation times, τ_R, estimated by analyzing the EPR spectra are 1.1×10^{-10}, 5.9×10^{-10}, and 14×10^{-10} s for UST, ssUSTm, and dsUSTm, respectively. The water-proton relaxivities, r_1 and r_2, at 25 MHz, 0.59 T, and 25 °C, also increased in the same order and the r_1 values were 0.26, 0.41, and 0.56 $mM^{-1}\,s^{-1}$ for UST, ssUSTm, and dsUSTm, respectively. The r_1 values of 1.00 and 2.06 $mM^{-1}\,s^{-1}$ for dsUST2 and dsUST5, respectively, were obtained.

Supporting information may be found in the online version of this article.

Keywords: NMR; ^{1}H; EPR; MRI; nucleic acid; TEMPO radical

Introduction

The paramagnetic species strongly affect the relaxation time of the protons of water molecules surrounding them and are used to enhance the contrast in magnetic resonance imaging (MRI).[1] Recently, the larger size molecules[2–4] (polymers, dendrimers, and assemblies) carrying paramagnetic species have been studied intensively for the improvements of their relaxation time and the additional function such as the specificity to a tissue or organ.[3] In these studies, the system consisting of the nitroxide radical and the protein[4] showed high water-proton relaxivities and suggested that although the contribution of the outer sphere translation was dominant for the relaxation time,[5] the contribution of the inner sphere including the parameters of the rotational reorientation time, the electron relaxation time, and the exchange time for water molecule could not be ignored and the water molecule bounded on the surface of protein played an important role in determining the relaxation time. For the design of a macromolecule with the high water-proton relaxivity, we considered the introduction of a paramagnetic species to a large and rigid molecule collecting many water molecules. Although DNA helix in solution is a semiflexible macromolecule, this time, a combination of DNA oligonucleotide and stable radical, 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO), as a main framework and a paramagnetic species, respectively, was selected as a fundamental model. The DNA derivative can vary systematically in the molecular size in order of a nucleic acid monomer, a single strand of oligonucleotide incorporating monomer, and its double strand with a complementary strand (Fig. 1). In addition, the structure of DNA double strand binding the water molecules, and the dynamics of its water molecules have been investigated in detail by X-ray crystallography[6] and various NMR methods,[7] respectively. TEMPO radical with spin quantum number $S = 1/2$ has some disadvantages such as the intrinsically low relaxivities (0.2 $mM^{-1}\,s^{-1}$ at 25 MHz and 25 °C) compared with that for paramagnetic inorganic metal ion chelates; 5.5 for GdDTPA [Gd(diethylenetriamine-*N*, *N*, *N*′, *N*″, *N*″-pentaacetate][8] with $S = 7/2$, and the ease of bioreduction *in vivo*, while it may be advantageous for specific targeting, which is strongly required at the present stage, owing to their chemical flexibility and feasible preparation.

In this study, the length and rigidity of the linker between TEMPO and nucleic acid may affect the rotational correlation time for the relaxivities. First, a DNA nucleotide carrying TEMPO through a relatively flexible linker and its oligomers and their relaxivities, r_1 and r_2 of longitudinal and transverse relaxivities, respectively, were prepared. The rotational correlation time was investigated by pulse NMR spectrometry (25 MHz and 0.59 T) and X-band electron paramagnetic resonance (EPR) spectrometry, respectively.

Experimental

Synthesis

5-(2-Carboxyethyl)-2′-deoxyuridine[9] and 4-amino TEMPO were prepared by the procedure reported in the literature. 1-acetoxy-2, 2, 6, 6-tetramethyl-4-amino-piperidine was prepared from 4-oxo-TEMPO by protection with acetyl moiety and then reductive amination with $NaBH_3CN$. The synthetic procedures and characterizations for the compounds and oligmers shown in Scheme 1 have been described in Supporting Information (S1).

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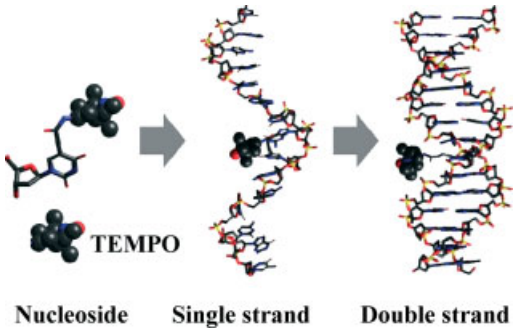
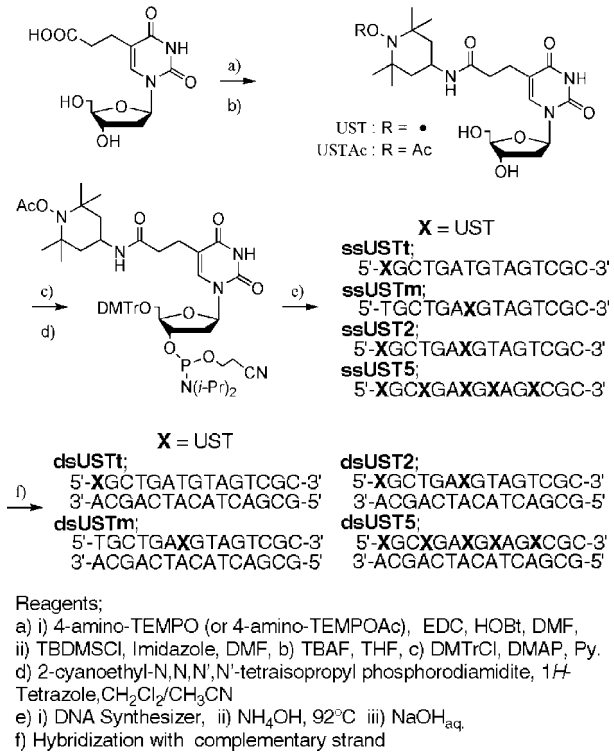


Figure 1. Change of molecular size of nucleic acid monomer and DNA strands carrying TEMPO.



Scheme 1. Preparation route.

Electron paramagnetic resonance (EPR)

The EPR spectra were recorded on a Bruker Biospin EMX EPR X-band (9.4 GHz) spectrometer at room temperature. Solutions (*ca* 0.1 mM) of **UST**, **ssUST**, and **dsUST** in 10 mM phosphate buffer were used as samples.

^{1}H NMR

The spin–lattice and spin–spin relaxation times, T_1 and T_2, respectively, were obtained on a JEOL JNM-MU25RAN spectrometer (25 MHz, 0.59 T) equipped with a temperature controller. The solutions (0.1–3.0 mM) of **UST, ssUST**, and **dsUST** in 10 mM phosphate buffer were used as samples and were measured at 25 °C.

Results and Discussion

Preparation route of 5-uridine nucleoside derivative carrying TEMPO radical (**UST**), its single strand (**ssUST**), and a double strand (**dsUST**) with its complementary strand is shown in Scheme 1. The **UST** was prepared by the coupling reaction of 5-(2-carboxyethyl)-2′-deoxyuridine[9] and 4-amino TEMPO with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and was obtained as a single crystal containing MeOH. Its molecular structure was analyzed by X-ray crystallography (Figure S2, Supporting Information). **USTAc**, in which the TEMPO radical is protected with acetyl moiety, was incorporated into the oligonucleotide (15 mer) by a standard amidite DNA synthesis.[10] Taking the stability of the double strand formation into account, the sequence with a relatively high melting temperature, T_m (T_m is 63.2 °C for an unmodified duplex) was selected. After cleavage with aqueous ammonia, the deprotection reaction of acetyl moiety with aqueous NaOH solution (*ca*. 0.5 M), followed by autoxidation, gave the single strand (**ssUST**) incorporating **UST.** The formation of radicals was followed by HPLC technique and the reaction was confirmed to be quantitative (Figure S1, Supporting Information). By using this procedure, two single strands incorporating **UST** at the terminal and the middle position of the sequence, **ssUSTt** and **ssUSTm**, respectively, were obtained. Similarly, the single strands incorporating two and five **UST** units, **ssUST2** and **ssUST5**, respectively, were prepared. After purification by a preparative HPLC, the single strands obtained were characterized by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy. Those single strands were mixed with their complementary strand to afford the corresponding double strands (**dsUST**). The values of T_m for the formation of double strand were determined from the temperature dependence of the absorption at 260 nm (Table 1 and Figure S3). T_m values of around 63 °C were obtained for all **dsUST** except **dsUST5**, whose T_m value was 53.2 °C. The circular dichroism (CD) spectra of **dsUST** showed the strong positive signal at ~280 nm and negative signal at ~250 nm typical to the B form (Figure S4, Supporting Information). These CD spectra were consistent with that for a natural double strand.

In order to estimate the rotational correlation times, τ_R, for **UST**, **ssUST**, and **dsUST**, the EPR spectra were measured at 25 °C. The EPR spectra of **UST**, **ssUSTm**, and **dsUSTm** are shown in Fig. 2. In the spectra, anisotropic broadening in the high field line was observed and became broader in the order **UST, ssUSTm**, and **dsUSTm**. The τ_R values estimated by analyzing the EPR spectra

Table 1. The values of MW, τ_R, r_1, r_2, and T_m for **UST, ssUST**, and **dsUST**

	MW	τ_R ($\times 10^{-10}$ s)	r_1[a] ($mM^{-1}s^{-1}$)	r_2[a] ($mM^{-1}s^{-1}$)	T_m (°C)
UST	486	1.1	0.26	0.28	–
Single strand DNA oligomer (15 mer)					
ssUSTm	4811	5.9	0.41	0.48	–
ssUSTt	4811	5.2	0.39	0.49	–
ssUST2	5022	–	0.82 (0.41)[c]	1.10 (0.55)[c]	–
ssUST5	5655	–	1.83 (0.37)[b]	1.92 (0.38)[b]	–
Double strand DNA oligomer (15 mer)					
dsUSTm	9358	14 (14)[b]	0.56	0.78	61.7
dsUSTt	9358	10 (10)[b]	0.54	0.87	63.9
dsUST2	9569	–	1.00 (0.50)[c]	1.39 (0.70)[c]	62.6
dsUST5	10 202	–	2.06 (0.41)[c]	2.52 (0.50)[c]	53.2

[a] 25 MHz, 0.59 T, and 25 °C.
[b] See text for the value within parenthesis.
[c] Number within parenthesis is the value per radical.

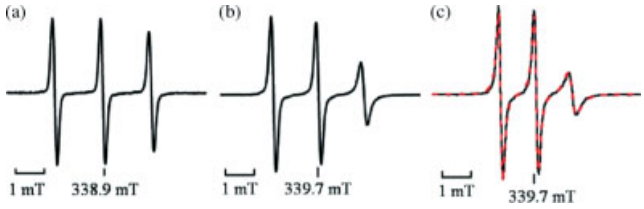


Figure 2. EPR spectra of **UST** (a), **ssUSTm** (b), and **dsUSTm** (c). Dash line (red) shows the simulation curve by Brownian diffusion model.

in terms of Kivelson's equation[11] are 1.1×10^{-10}, 5.9×10^{-10}, and 1.4×10^{-9} (1.4×10^{-9}) s for **UST, ssUSTm**, and **dsUSTm**, respectively. The value in the parenthesis was obtained from the simulation of EPR spectrum in terms of Stochastic Liouville equation (SLE), followed by Brownian diffusion model.[12] The value for **dsUSTm** was 13 times longer than the one for **UST**. Obtained τ_R values, which were close to those for analogous molecules reported previously,[13] indicate that the rotation of the molecule becomes slower with variance in the order: monomer, single strand, and double strand. The other sets of oligomers also showed similar tendencies and the τ_R values obtained by analyzing the EPR spectra are listed in Table 1. In the EPR spectra for the oligomer carrying plural **UST**s, the significant line broadening of the characteristic three-line signal was observed for **UST5** but not for **UST2** (Figure S5, Supporting Information). The observed line broadening due to the spin-spin dipolar interaction between the spin centers suggests that the electron relaxation time is shortened. Furthermore, the degree of line broadening in **ssUST5** was larger than that in **dsUST5**, indicating that the distance between the spin centers became long by the formation of the double strand.

The relaxation times, T_1 and T_2, were also measured at 25 °C by pulse NMR spectrometry (25 MHz and 0.59 T). The concentration dependence of T_1 for **UST**, **ssUSTm**, **dsUSTm**, **dsUST2**, and **dsUST5**, is shown in Figure S6 (Supporting Information). The relaxivities r_i ($i = 1$ and 2) obtained from the T_i *versus* concentration plots increased in order of **UST, ssUST**, and **dsUST** and the r_1 values are 0.26, 0.41 (0.39), and 0.56 (0.54) $mM^{-1}s^{-1}$ for **UST, ssUSTm** (**ssUSTt**), and **dsUSTm** (**dsUSTt**), respectively. Interestingly, the r_1 value for **dsUST** is two times higher than that for the corresponding monomer, **UST**, whose value was close to that for a parent radical, 4-hydroxy-TEMPO. The values for **USTm** and **USTt** were similar, indicating that the relaxivities were not affected by the difference in the position of radical centers. The observed increase in the relaxivities might be due to the restricted local motion of TEMPO. In the Lipari–Szabo theory[14] for a system with isotropic molecular tumbling, the effective rotational correlation time, τ_e, is given by $1/\tau_e = 1/\tau_r + 1/\tau_i$, where τ_r is the correlation time for the molecular tumbling and τ_i is the correlation time for the internal motion within the molecular frame. The observed EPR spectra might suggest that the τ_i is still dominant in this system. In the oligomer carrying plural **UST**s, on the other hand, the values of the relaxivity, r_1, increased with increasing the number of radicals and the r_1 values were 0.82 (0.41), 1.83 (0.36), 1.00 (0.50), and 2.06 (0.41) $mM^{-1}s^{-1}$ for **ssUST2**, **ssUST5**, **dsUST2**, and **dsUST5**, respectively. However, the values of the relaxivities per TEMPO for **ssUST5** and **dsUST5** are lower than those for the corresponding **dsUST**, which might be mainly attributed to the decrease in the electron relaxation time observed in EPR spectra. The r_2 values also showed similar tendency that the values increased in the same order. The physical data for **UST, ssUST**, and **dsUST** are summarized in Table 1.

In summary, a nucleoside monomer (**UST**), single strands (**ssUST**, **ssUST2**, and **ssUST5**), and the corresponding double strands (**dsUST, dsUST2**, and **dsUST5**) were prepared. The τ_R estimated by analyzing the EPR spectra became longer in order of **UST**, **ssUST**, and **dsUST**; and their relaxivities (r_1 and r_2) also increased in the same order. It is noted that the r_1 value of 0.56 mM^{-1} s^{-1} for **dsUSTm** is two times higher than that (0.28) for the parent molecule, 4-hydroxy-TEMPO. In the oligomer carrying plural **USTs**, on the other hand, the values of the relaxivity, r_1, increased with an increase in the number of radicals. In **dsUST5**, an r_1 of 2.06 $mM^{-1}s^{-1}$ was obtained.

To understand the motion of the DNA strand accompanying water molecules in detail, the effect of a linker and the DNA sequence dependence of the relaxivities are being investigated.

Supporting information

Supporting information may be found in the online version of this article.

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