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Editor's Choice

Noncovalent Assembly of TEMPO Radicals Pair-wise Embedded on a DNA Duplex

Hiroshi Atsumi,[1] Kensuke Maekawa,[1] Shigeaki Nakazawa,[2] Daisuke Shiomi,[2]
Kazunobu Sato,[2] Masahiro Kitagawa,[3] Takeji Takui,[2] and Kazuhiko Nakatani*[1]
[1]*Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research (ISIR), Osaka University, Ibaraki 567-0047*
[2]*Departments of Chemistry and Materials Science, Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585*
[3]*Department of System Innovation, Graduate School of Engineering Science, Osaka University, Toyonaka 560-8531*

(Received March 12, 2010; CL-100236; E-mail: nakatani@sanken.osaka-u.ac.jp)

We have synthesized and characterized a new G–G mismatch binding ligand containing a TEMPO radical. The two mismatch binding ligands cooperatively bound to the CGG/CGG triad and an apparent binding constant was in the order of $10^5\,M^{-1}$. The tumbling of the ligand on a DNA duplex was ten times slower than that of the unbound ligand. Asymmetric addressability of two TEMPO radicals on DNA is shown.

The unique structural motifs and self-recognition properties of DNA can be exploited to generate self-assembling DNA nanostructures of specific two- and three-dimensional shapes.[1] DNA-based nanostructures also provide well-defined programmable templates with addressable and accessible base sequences, exploring the possibility to assemble auxiliary functional groups on multidimensional DNA nanostructures by sequence specific binding of ligand molecules. The quest for the addressable assembly of spin-bearing units on DNA is relevant to the current issues of the implementation of molecular-spin quantum computers.[2]

Along the line above, we have been interested in the assembly of stable organic radicals on the multi-dimensional DNA structure. While assembly of spins in three-dimensional space has been mainly studied by crystal engineering, the use of the DNA nanostructure can afford the assembly in a designed manner, allowing molecular *g*-engineering.[2] Toward this end, we have studied the non-covalent introduction of stable organic spins on to the DNA structure. The introduction of radicals on to the DNA structure has been achieved in a covalent fashion using automated DNA synthesis and newly developed polymerases.[3,4] In addition to these conventional approaches, our noncovalent approach could facilitate the introduction and further expand the potential of the DNA nanostructure as the template of addressable assemblage of molecular spins. Recently, we reported a construction of one-dimensional DNA structures holding multiple nitronyl nitroxide (NN) radicals by the ligand covalently carrying NN (NCD–NN). The parent molecule naphthyridinecarbamate dimer (NCD) selectively bound to the guanine–guanine (G–G) mismatch in the 5′-CGG-3′/5′-CGG-3′ sequence (Figure 1a, left).[5] NCD–NN was delivered to the duplex DNA having the G–G mismatch site and two 5′ single-stranded overhangs, of which sequence is complementary with each other. Upon mixing the DNA duplex and NCD–NN, the formation of one-dimensional DNA structure holding multiple NCD–NN was shown by PAGE analysis and CW ESR/pulse ELDOR spectroscopy. To further explore the potential of our approach, we have investigated the synthesis of NCD derivative

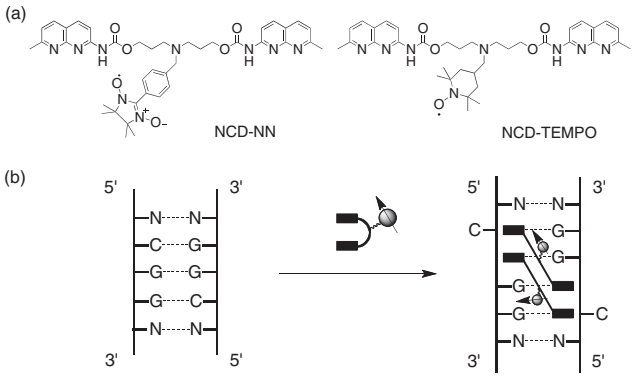

Figure 1. (a) Structures of NCD–NN (left) and NCD–TEMPO. (b) Schematic illustration of NCD–TEMPO binding to a CGG/CGG triad in DNA duplex.

Table 1. T_m values of the duplexes[a]

Y	$T_m(+)$/°C	ΔT_m/°C
C	39.2 (0.1)	−0.3 (0.6)
A	31.8 (1.1)	5.0 (1.5)
G	39.2 (1.7)	13.1 (3.0)
T	27.1 (1.6)	−2.9 (1.0)

[a]The Melting curves were measured for the 11-mer duplex (4.5 μM) of 5′(CTAA*CGG*AATG)-3′/5′(CATT*CYG*TTAG)-3′ in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol. $T_m(+)$ represents a melting temperature in the presence of NCD–TEMPO (45 μM). ΔT_m is an increase of T_m in the presence of ligand. All measurements were taken three times, and standard deviations are in the parentheses.

(NCD–TEMPO) holding another stable organic radical TEMPO on it (Figure 1a, right) and anticipated the binding of NCD–TEMPO to the CGG/CGG triad (Figure 1b). Here we report the chemical and spin properties of NCD–TEMPO.

NCD–TEMPO was synthesized by the reductive amination of 4-formyl TEMPO with NCD. The thermal denaturation profiles of 11-mer DNA duplexes 5′-d(CTAA*CGG*AATG)-3′/5′-d(CATT*CYG*TTAG)-3′, where the symbol Y is C, A, G, and T, were measured to evaluate the thermodynamic stability of each duplex in the presence of NCD–TEMPO (Table 1). The presence of NCD–TEMPO led to an increase of melting temperature (T_m) by 13.1 °C for the duplex containing the G–G mismatch, whereas T_m increase was small for G–A mismatch

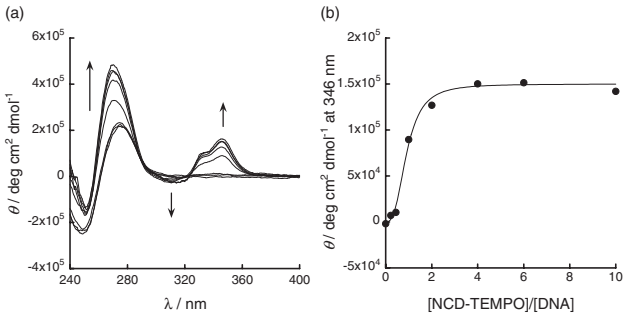

Figure 2. (a) CD spectra for the titration of the DNA duplex (4.5 μM) in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol with the concentration of NCD–TEMPO at 0, 1, 2, 4.5, 9.0, 18, 27, and 45 μM. (b) Concentration dependence of the molar ellipticity at 346 nm. The closed circles and the solid line denote the observed values and the theoretical curve fitted to the Hill equation, respectively.

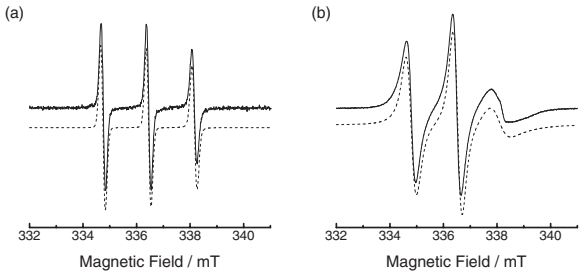

Figure 3. ESR spectra of NCD–TEMPO (200 μM) in a buffer containing 100 mM NaCl, 10 mM sodium cacodylate (pH 7.0), and 10% (v/v) methanol in the (a) absence and (b) presence of the 11-mer DNA duplex (100 μM) at 297 K. The dashed spectra denote the simulation with (a) an isotropic and (b) anisotropic rotational diffusion: (a) Microwave frequency, $\nu =$ 9.44525 GHz, the ^{14}N-hyperfine tensor, $\mathbf{A} = (23, 23, 98)$ MHz and the g tensor, $\mathbf{g} = (2.009, 2.006, 2.002)$, an isotropic correlation time, $\tau_c = 1.6 \times 10^{-10}$/s. (b) The simulation was carried out by superposing two hyperfine spectra with the 1:1 equivalent weight; $\nu = 9.44632$ GHz, $\mathbf{g} = (2.009, 2.005, 2.002)$. Two sets of the ^{14}N-hyperfine tensors, $\mathbf{A} = (23, 23, 98)$ and $(27, 27, 96)$ MHz and a single set of anisotropic correlation times with perpendicular and parallel components; $\tau_c' = (2.78, 0.83) \times 10^{-9}$/s, ten times larger than τ_c.

or not observed for G–T mismatch and G–C match duplexes. These results indicate that the conjugation of a TEMPO radical with NCD did not alter the mismatch selectivity for the binding.[6] Titration experiments in circular dichroism (CD) spectra with the 11-mer DNA containing the CGG/CGG triad showed the increase of the induced CD bands with a negative and positive cotton signal as the increase of the ligand concentration (Figure 2). The intensity of the induced CD band at 346 nm increased up to the [NCD–TEMPO]/[DNA] ratio of 2.0, and then remained unchanged up to the molar ratio of 10.0. This clearly indicates that NCD–TEMPO formed a 2:1 complex with CGG/CGG triad. The CD increase was much steeper than that observed for NCD–NN, suggesting that the cooperativity of the binding of two NCD–TEMPO is higher than that of NCD–NN.[5,7] The difference in the cooperativity might be due to the steric bulkiness of the radical moiety and the presence of an aryl group for NN. The affinity of NCD–TEMPO to the CGG/CGG triad was calculated by a least square fitting of the CD titration curve to the Hill equation ($R = 0.996$), yielding a Hill coefficient of $n_H = 2.8$ and an apparent binding constant (K_{app}) of $2.4 \times 10^5\,M^{-1}$.

The CW-ESR spectrum of NCD–TEMPO exhibited a typical hyperfine splitting pattern with three well-separated peaks attributed to a nitrogen nucleus on the radical moiety (Figure 3a). The spectrum of the radical solution containing the CGG/CGG duplex showed a line broadening, which is dependent on the m_I component of the nuclear spin I. The broadening reflects a slower tumbling of the radical moieties compared with that of NCD–TEMPO (Figure 3b). The spectral simulation of the radical solution in the presence of the duplex shows the presence of a pair of anisotropic ^{14}N-hyperfine tensors with a single set of anisotropic rotational diffusion coefficients, suggesting that two TEMPOs embedded on DNA are bound in nonequivalent environments. This is most likely due to the non-palindromic sequence of 5′-ACGGA-3′/5′-TCGGT-3′ at the ligand binding site. The bound fraction of 0.958 obtained by the ESR simulation is in good agreement with that of 0.961 derived from K_{app} obtained from the CD titration.

In summary, we have developed a new ligand holding a TEMPO radical and binding to the G–G mismatch. Two ligands cooperatively bound to the CGG/CGG triad in a 10^5 affinity and delivered two TEMPO radicals at the designated positions on the DNA duplex.

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