DNA Hybridization

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Mismatch-Binding Ligands Function as a Molecular Glue for DNA**

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Single-stranded DNA (ssDNA) hybridizes with another ssDNA unit having the complementary base sequence. The high sequence specificity in the hybridization is one of the most important properties of DNA as a genetic material, and also characterizes DNA as the unique component of the molecular architecture.[1–5] The formation of a double-stranded DNA (dsDNA) molecule of fully matched base sequences is highly energetically favorable and proceeds spontaneously at a temperature below the melting temperature (T_m) of the duplex, and therefore it is difficult to turn the hybridization on and off under isothermal conditions. Studies toward controlling or modulating the DNA hybridization with photochemical[6–9] and electronic reactions[10,11] of chemically modified oligonucleotides have been reported. Herein, we describe an approach to turn on duplex formation of two natural, unmodified ssDNA sequences that do not spontaneously hybridize with each other by a mismatch-binding ligand (MBL).

Recent studies on the binding of an MBL to the $(CGG)_n$ trinucleotide repeat revealed a novel mode of ligand binding to a mismatched DNA duplex.[12,13] The naphthyridine carbamate dimer (**NC**) selectively binds to the 5′-CGG-3′/5′-CGG-3′ sequence (CGG/CGG), which involves a G–G mismatch flanked by two C–G base pairs, with a 2:1 **NC**/DNA stoichiometry. The binding of **NC** to the CGG/CGG sequence induced two cytosines to be out of the π stack, as evidenced by the selective cleavage at the unpaired cytosine triggered by the addition of hydroxylamine.[12] We anticipated that the flipped-out cytosine in the **NC**-bound CGG/CGG triad could be substituted with other nucleotide bases such as thymine, and therefore **NC** could stabilize the 5′-TGG-3′/5′-TGG-3′ (TGG/TGG) sequence that consists of three contiguous T–G, G–G, and G–T mismatches (Figure 1). As the hybridization of two ssDNA molecules to produce the TGG/TGG sequence would be energetically unfavorable, these ssDNA sequences could be adhered by binding of **NC** to the TGG/TGG sequence.

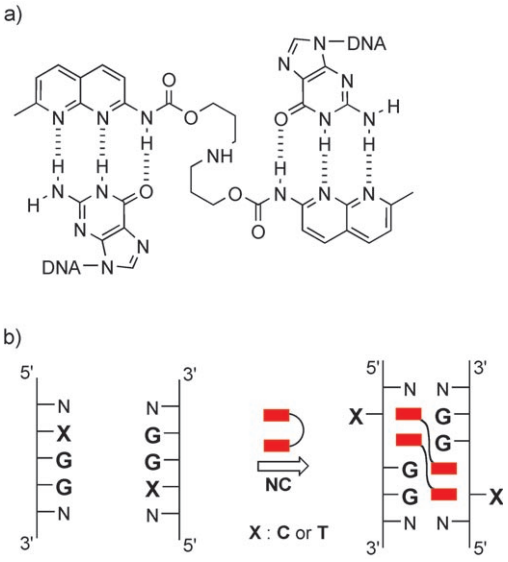

Figure 1. a) **NC** and its hydrogen-bonding pattern to a guanine–guanine mismatch. b) Schematic representation of the binding of **NC** to the XGG/XGG sequence. Red rectangles: naphthyridine rings.

The binding of **NC** was first investigated for 5′-TGG-3′/5′-CGG-3′ (TGG/CGG) where one cytosine of the CGG/CGG sequence was substituted with thymine. The 11-mers 5′-d(CCCATGGTCCG)-3′ (**T1**) and 5′-d(CGGACGGTGGG)-3′ (**C1**; 5 μM) produced a duplex (**T1**/**C1**) containing a TGG/CGG sequence with a T_m of 35.7 °C in sodium cacodylate buffer. In the presence of **NC**, the T_m of the **NC**-bound **T1**/**C1** was 71.1 °C, which shows an increase in T_m of 35.4 K (Figure 2). The transition from dsDNA to ssDNA is monophasic, regardless of the presence of **NC**. The transition occurred somewhat less cooperatively in the presence of **NC**. The melting kinetics may not be as simple as those in the

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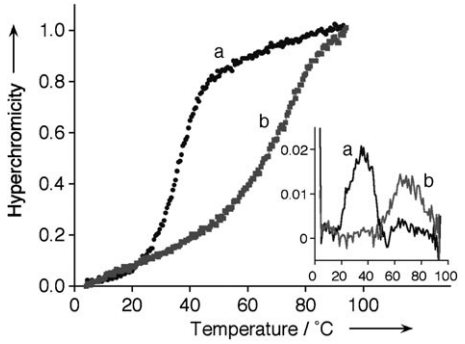


Figure 2. Thermal denaturation profile of **T1** and **C1** (5 μM each) in the absence (a) and presence (b) of **NC** (100 μM). The absorbance at 260 nm was measured in sodium cacodylate buffer (10 mM, pH 7.0) containing 0.1 M NaCl. The temperature was increased from 4 to 94 °C at a rate of 1 °C min^{-1}. The absorbance was measured with an interval of 1 °C. Measurements were carried out at least three times. The average of three data sets of denaturation profiles was used for the plots. Inset: differential plots of the melting profiles.

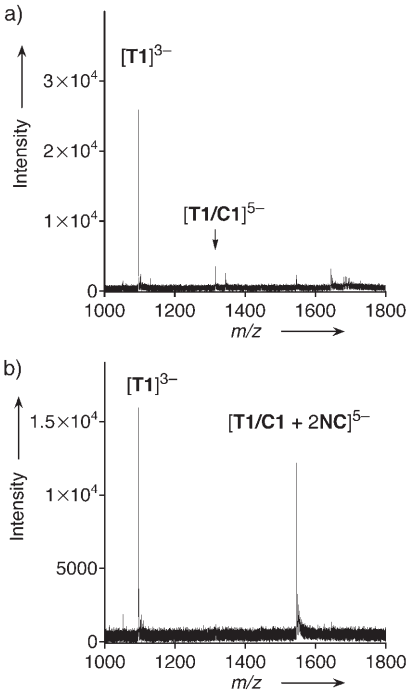
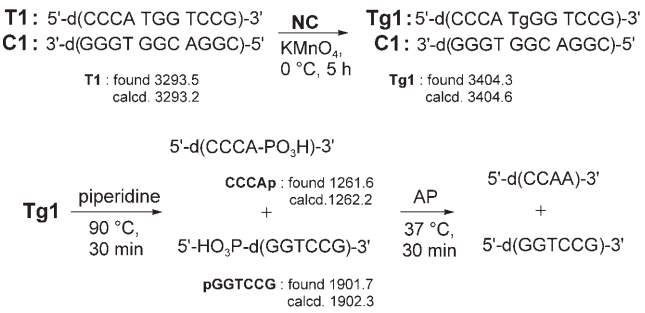


Figure 3. CSI-TOF MS of **T1** and **C1** in the a) absence and b) presence of **NC** (40 μM). Samples contained 20 μM of each strand in 50% aqueous methanol and ammonium acetate (100 mM). Ions in the range of m/z from 1000 to 1800 are shown for clarity. The sample solution was cooled at −10 °C during the injection with a flow rate of 0.5 mL h^{-1}.

absence of **NC**, because this transition involves the dissociation of four components, two DNA strands (**T1** and **C1**) and two **NC** molecules. The thermal denaturation profiles showed that in the temperature range between 45 and 55 °C, **T1** and **C1** were present as single-stranded forms in the absence of **NC** but as an **NC**-bound duplex form in the presence of **NC**.

The cold-spray ionization time-of-flight mass spectrometry (CSI-TOF MS)[12,14] of **T1** and **C1** showed ions corresponding to a 3− ion of a single-stranded form ([**T1**]$^{3-}$, m/z: found: 1096.2; calcd: 1096.2) and a 5− ion of a duplex form ([**T1/C1**]$^{5-}$, m/z: found: 1344.8; calcd: 1344.6; Figure 3a). On addition of **NC** to the duplex with a 2:1 molar ratio, the intensity of the ions corresponding to [**T1**]$^{3-}$ became weaker with the concomitant appearance of a new ion corresponding to the 5− ion of a 2:1 complex of **NC** and the duplex ([**T1/C1** + 2**NC**]$^{5-}$, m/z: found: 1546.3; calcd: 1545.9; Figure 3b). On increasing the concentration of **NC**, the intensity of the ion corresponding to [**T1/C1** + 2**NC**]$^{5-}$ became strong with a concomitant decrease of the intensity of [**T1**]$^{3-}$ (see Supporting Information). Complexes of **NC** bound to **T1/C1** with 1:1 and/or 3:1 stoichiometries were not detected. These results clearly showed that the binding of **NC** to the duplex **T1/C1** proceeded in an exclusive stoichiometry of 2:1. This stoichiometry of **NC** binding is the characteristic feature that is observed for the binding of **NC** to the CGG/CGG sequence[12] and the binding of the related MBL naphthyridine azaquinolone to the CAG/CAG sequence. The structure of the latter complex has been determined by NMR spectroscopy.[13] These findings suggested that the thymine in the **NC**-bound **T1/C1** would most likely be out of the π stack.

The T component in the extrahelical position could be preferentially oxidized with potassium permanganate ($KMnO_4$) compared to that in the intrahelical position.[15] The resulting thymine glycol (Tg) can be degraded with hot piperidine, eventually leading to strand cleavage.[16–18] The oxidation of **T1/C1** with $KMnO_4$ followed by treatment with hot piperidine was examined (Scheme 1). The duplex **T1/C1** (12.5 μM) did not react with $KMnO_4$ (0.2 mM) at 0 °C for



Scheme 1. The oxidation of **T1/C1** by $KMnO_4$ upon binding of **NC**. AP = alkaline phosphatase.

320 min, as judged by reversed-phase HPLC analysis (see Supporting Information). In contrast, the TGG-containing DNA **T1** was consumed by 70% in the presence of **NC** (40 μM) and converted into the 11-mer DNA 5′-d(CCCA-TgGGTCCG)-3′ (**Tg1**) which contained a Tg unit.[16] After isolation by HPLC, **Tg1** was treated with hot piperidine to give two products corresponding to oligomers 5′-d(CCCA)-PO_3H-3′ (**CCCAp**) and 5′-HO_3P-d(GGTCCG)-3′ (**pGGTCCG**). All DNA products were identified by MALDI-TOF mass spectrometry (Scheme 1). Although the duplex **T1/C1** contained two kinds of thymine residues, only that in the TGG/CGG sequence was reactive to $KMnO_4$ upon **NC** binding. These results clarified the finding that the T unit in the TGG/CGG sequence was in the extrahelical position in the **NC**-bound complex.

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Having confirmed that 1) **NC** could stabilize the duplex containing two contiguous T–G and G–G mismatches in **T1**/**C1** and 2) the T component was in the extrahelical position in the **NC**-bound TGG/CGG sequence, the binding of **NC** to the three contiguous mismatches T–G, G–G, and G–T in the TGG/TGG sequence was investigated. The 11-mers 5′-d(CCTTTGGTCAG)-3′ (**T2**) and 5′-d(CTGATGGAAGG)-3′ (**T3**) that had a 5′-TGG-3′ sequence were present as single-stranded forms at room temperature, as shown by the denaturation profile, because the possible duplex **T2**/**T3** should involve three contiguous mismatches (Figure 4a). At

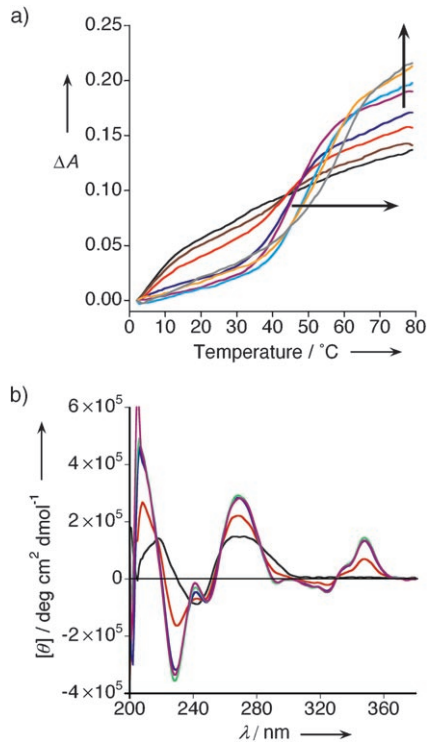

Figure 4. a) Thermal denaturation profile of **T2** and **T3** (5 μM each) with different concentrations of **NC** (0 (black), 2.5 (brown), 5 (red), 10 (blue), 20 (purple), 40 (cyan), 60 (yellow), and 100 μM (gray)). The vertical arrow indicates the increase in ΔA with increasing **NC** concentration. The horizontal arrow indicates the increase in melting temperature with increasing **NC** concentration. b) CD spectrum of **T2** and **T3** (5 μM each) measured in sodium cacodylate buffer (10 mM, pH 7.0) and NaCl (100 mM) at 25 °C in the absence (black) and presence of **NC** at 5 μM (red), 10 μM (blue), 15 μM (green), and 20 μM (purple).

increasing concentrations of **NC**, the transition from dsDNA to ssDNA became apparent and shifted toward a higher-temperature region. Notably, the T_m value for **T2** and **T3** reached 58.8 °C in the presence of 100 μM **NC**, thus showing the formation of a stable, **NC**-bound **T2**/**T3** duplex at room temperature.

The transition of ssDNA to dsDNA of **T2** and **T3** during titration with **NC** was monitored by circular dichroism (CD) spectroscopy (Figure 4b). Without **NC**, the CD spectrum of the mixture of **T2** and **T3** showed a positive band at 272 nm and a negative band at 250 nm. After addition of **NC**, the ellipticity of the positive band increased on increasing the amount of **NC** from one to four molar equivalents. In addition, the induced CD bands observed at 348 and 324 nm also changed their magnitude in response to the **NC** concentration. The change in CD involved the isodichroic points, and showed the transition of single-stranded **T2** and **T3** to the **NC**-bound duplex. The formation of the **NC**-bound duplex was further supported by CSI-TOF MS observations which indicated a 2:1 complex of **NC** with **T2**/**T3**. Again, the stoichiometry was exclusively 2:1. A selective strand cleavage of the 11-mer 5′-d(GCAATGGTTGC)-3′ (**T4**) at the T component in the TGG/TGG sequence was also confirmed by $KMnO_4$ oxidation upon binding of **NC** to the **T4**/**T4** duplex followed by heating with piperidine. In contrast, the protection of the other two T units from oxidation by $KMnO_4$ indicated that these components are in the intrahelical position (see Supporting Information).

The results described herein showed that **NC** could stabilize not only the two contiguous T–G and G–G mismatches in the TGG/CGG sequence, but also the three contiguous T–G, G–G, and G–T mismatches in the TGG/TGG sequence. By choosing an appropriate DNA sequence, two ssDNA molecules that do not spontaneously hybridize with each other could be adhered by the binding of **NC** to the TGG/TGG sequence. On the basis of **NC** binding to the CGG/CGG sequence, we used the TGG/TGG sequence in which the C component is substituted with another pyrimidine nucleotide base. In fact, the C component in the CGG/CGG sequence could be substituted with purine bases A and G, as evidenced by the CSI-TOF MS of the **NC**-bound complex for the AGG/AGG and GGG/GGG sequences. These results will be reported elsewhere. The molecule **NC** represents a new class of substances that function as molecular glue, not only in DNA hybridization but also in modulating the DNA secondary structure.

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