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Formation of Silver(I)-Mediated DNA Duplex and Triplex through an Alternative Base Pair of Pyridine Nucleobases

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Bioinspired bottom-up strategy is currently being explored for artificial molecular devices.[1] As a highly functionalized biopolymer, DNA provides a particularly promising structural basis for renewing or controlling the building block, and the resulting artificial strands could possibly be assembled noncovalently in various ways. We and others have recently reported that natural hydrogen-bonded base pairs in DNA can be replaced by metal-mediated base pairs.[2−6] The generation of such additional base pairs would lead not only to an expansion of the genetic alphabet but also to novel DNA structures and functions based on the controlled spacing of the metallo-base pairs along the helix axis.

Along this strategy we first reported metal-mediated base pairing with a phenylenediamine-, a catechol-, or an aminophenol-bearing nucleoside in a square-planar or a tetrahedral fashion.[4] To extend the coordination mode of base pairing, we chose combination of a pyridine-bearing *β*-*C*-nucleoside (**1**)[7] and a Ag(I) ion which can form complex with pyridine in a linear (bis),[8] a trigonal (tris),[9] or a tetrahedral (tetrakis)[10] fashion (Chart 1). To examine how metal ions influence on the thermodynamical behavior of double- and triple-stranded DNA, 21 nucleotide-long DNA oligomers, $dT_{10}\mathbf{P}T_{10}$ (**2**) and $dA_{10}\mathbf{P}A_{10}$ (**3**), containing a pyridine nucleobase (**P**) in the middle of the sequence were synthesized, and their melting temperatures (T_m) were examined by UV-monitored thermal denaturation. In the absence of transition metal ions, the duplex **2·3** showed a melting temperature of 34.2 °C (Figure 1a), whereas a natural-type oligoduplex, $dT_{21}{\cdot}dA_{21}$ (**4·5**), in which the **P**-**P** base pair is replaced by an A−T base pair, melted at 46.5 °C (Figure 1e). Thus, in the absence of metal ions, the **P**-**P** base pair destabilizes the duplex structure as a mispair.

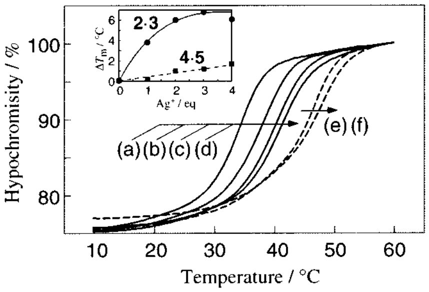
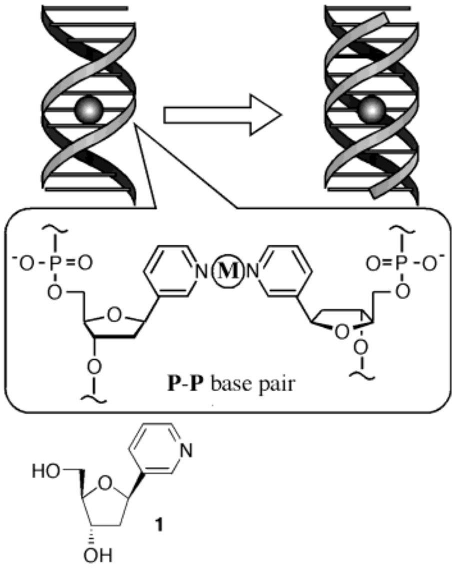


Figure 1. Melting curves of the duplexes **2·3** (a−d) and **4·5** (e and f) at different concentrations of $AgNO_3$, [**2·3**] = [**4·5**] = 1.2 μM in 10 mM Mops (pH 7.0), 100 mM $NaNO_3$. [Ag(I)] = (a), (e) 0 μM, (b) 1.2 μM, (c) 2.4 μM, and (d), (f) 3.6 μM. Inset, effect of Ag(I) concentrations on ΔT_m.

Chart 1



2 d(5'-TTTTTTTTTT**P**TTTTTTTTTT-3')
3 d(5'-AAAAAAAAAA**P**AAAAAAAAAA-3')
4 d(5'-TTTTTTTTTTTTTTTTTTTTT-3')
5 d(5'-AAAAAAAAAAAAAAAAAAAAA-3')
6 d(5'-CGCGAA**P**TTCGCG-3')

Addition of Ag(I) ions raised the melting temperature of the duplex **2·3** to 41.0 °C (ΔT_m = + 6.8 °C) at a 3 mol ratio of Ag(I) ions (3.6 μM), where [**2·3**] = 1.2 μM (Figure 1). In contrast, this metal-dependent high stabilization of duplex was only slight in the natural DNA duplex **4·5** (ΔT_m = + 1.2 °C when [Ag(I)] = 3.6 μM). This result suggests that Ag(I) ions interact preferentially with the pyridine moiety and consequently stabilize the pyridine-bearing duplex, whereas without the pair of pyridine nucleobases, Ag(I) ions bind preferably to the heteroatoms of natural nucleobases.[11] Other evidence to verify the generation of a Ag(I)-mediated base pair inside the DNA was obtained by ^{1}H NMR study using a self-complementary duplex of a pyridine-containing oligomer, d(5′-CGCGAA**P**TTCGCG-3′) (**6**) ([duplex (**6·6**)] = 0.77 mM). Only aromatic proton signals for pyridine moieties appeared at higher field and broadened upon addition of equimolar amount of Ag(I) ions ([Ag(I)] = 0.77 mM), and the spectrum no longer changed by additional Ag(I) ions (see Supporting Information). These results indicate that the **P**-**P** base pairs in the duplexes are induced by 2:1 complexation with Ag(I) ions and the resulting duplex is significantly stabilized.

It should be noted that Ag(I) promoted **P**-**P** base pairing inside the DNA even at the micromolar order despite relatively low affinity between Ag(I) and pyridine in aqueous media.[12] Hence, the complex formation of Ag(I) and pyridine in the artificial DNA was reinforced

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cooperatively by surrounding hydrogen-bonded and stacked base pairs in hydrophobic environment inside the duplex.

Addition of other transition metal salts such as $CuCl_2$, $NiCl_2$, K_2PdCl_4, and $Hg(ClO_4)_2$ showed almost no significant effect (Table 1). Thus Ag(I) ions can be specifically incorporated into the DNA.

Table 1. Melting Temperatures of the Duplex **2·3** with Various Transition Metal Ions[a]

metal ion (metal source)	T_m/°C	ΔT_m/°C
none	34.2	–
Ag^+ ($AgNO_3$)	38.0	+3.8
Ag^+ ($AgNO_3$) 3 equiv[b]	41.0	+6.8
Cu^{2+} ($CuCl_2$)	33.7	−0.5
Ni^{2+} ($NiCl_2$)	34.3	+0.1
Pd^{2+} (K_2PdCl_4)	34.0	−0.2
Hg^{2+} ($Hg(ClO_4)_2$)	33.7	−0.5

[a] [Duplex] = 1.2 μM in 10 mM Mops (pH 7.0), 100 mM $NaNO_3$. Unless otherwise noted, [metal ion] = 1.2 μM. [b] [Ag(I)] = 3.6 μM.

Homopyrimidine and homopurine oligonucleotides are well-known to form a triplex under some specific conditions.[13] A mixing-curve study (Job plots) was carried out to examine triplex formation between **2** and **3** (Figure 2A). The mixture of **2** and **3** at 0 °C in the presence of Ag(I) ions reached a minimum absorbance at a mol fraction of 66% **2** to 34% **3** (Figure 2A). This ratio clearly indicates that a triplex consisting of two strands (**2**) and one strand (**3**) is formed in each solution. From comparison of UV-monitored thermal denaturation curves of the 2:1 mixture of **2** and **3** in the absence or presence of Ag(I) ions (Figure 2B (a) and (b), respectively), triplex **2·3·2** was proven to be stabilized by Ag(I) ions (15.5 °C → 17.5 °C; $\Delta T_m = +2.0$ °C when [Ag(I)] = 4.8 μM), whereas natural triplex **4·5·4** was destabilized by Ag(I) ions added under the same condition (28.2 °C → 25.8 °C; $\Delta T_m = -2.4$ °C, see Figure 2B (c) and (d)). This result implies that three nitrogen donors of the pyridyl moieties may coordinate to the Ag(I) center of triplex **2·3·2**, leading to the formation of a Ag(I)-mediated base triplet in the DNA triplex.

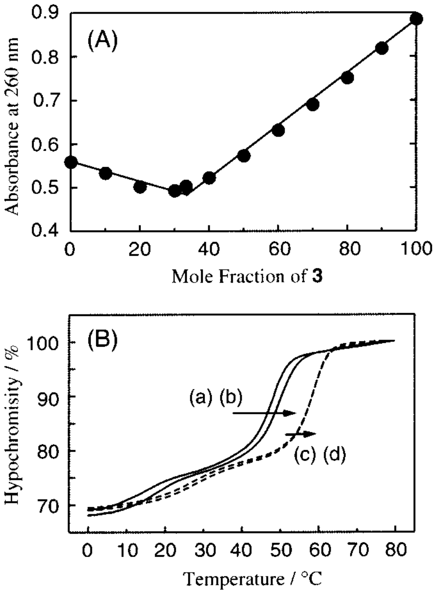

Figure 2. (A) Job plots of absorbance at 0 °C for **2** and **3** at 260 nm. [**2**] + [**3**] = 3.6 μM (constant), [Ag(I)] = 4.8 μM in 10 mM Mops (pH 7.0), 500 mM $NaNO_3$. (B) Melting curves of the triple-stranded **2·3·2** (a and b) and **4·5·4** (c and d) in the absence (a and c) or presence (b and d) of $AgNO_3$. [Triplex] = 1.2 μM in 10 mM Mops (pH 7.0), 500 mM $NaNO_3$, where [Ag(I)] = 0 μM for (a) and (c), and 4.8 μM for (b) and (d).

In this report, we accomplished a Ag(I)-mediated base pair or a base triplet in a double- or triple-stranded DNA, respectively, by introducing a pair of pyridine nucleobases in the middle of the sequence. As a result, the incorporated Ag(I) complex significantly stabilized the DNA duplex and triplex. Metal alignment along the DNA helix axis is now underway toward construction of functionalized nanomaterials such as molecular wires or magnets.

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Supporting Information Available: ^{1}H NMR data for a self-complementary duplex of pyridine-containing oligomer, d(5′-CGC-GAA**P**TTCGCG-3′)$_2$ (**6·6**) with or without Ag(I) ions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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