Specific interactions between silver(I) ions and cytosine–cytosine pairs in DNA duplexes[†]

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Very specific binding of the Ag(I) ion unexpectedly stabilized DNA duplexes containing the naturally occurring cytosine–cytosine (C–C) mismatch-base pair; because the C–C pair selectively binds to the Ag(I) ion, we developed a DNA-based Ag(I) sensor that employed an oligodeoxyribonucleotide containing C–C pairs used for Ag(I) binding sites.

Recently, synthetic oligodeoxyribonucleotides containing artificial bases have been used to form metal-mediated base pairs in which the hydrogen bonds of Watson–Crick (W–C)-type base pairs in natural DNA were replaced by metal–base bonds.[1] The artificial bases have been synthesized, incorporated into DNA strands, and used for capturing metal ions such as Cu(II),[1b,c] Ag(I),[1d–g] *etc.* In DNA duplexes, the metal ions have been believed to be placed between the modified base pairs, as indicated by a crystal structure of a metal–DNA complex.[1c] We previously reported an alternative method for generating metal-mediated base pairs in DNA duplexes using only naturally occurring thymine–thymine (T–T) mismatched base pairs. In DNA duplexes, T–T mismatches selectively captured Hg(II) ions and the metal-mediated T–Hg(II)–T base pairs stabilized the DNA duplex.[2] We report the formation of a novel, metal-mediated base pair, and the selective capture of Ag(I) ions by cytosine–cytosine (C–C) mismatches to form C–Ag(I)–C base pairs in DNA duplexes.

Fig. 1a shows thermally induced transition profiles of DNA duplexes (5′-$(A)_{10}C(A)_{10}$-3′ and 5′$(T)_{10}C(T)_{10}$-3′) containing a C–C mismatch in the presence of several metal ions. A comparison of the melting temperature (T_m)[3] for the duplex containing the C–C mismatch in the presence of Ag(I) ions (39 °C, – -□- - in Fig. 1a) with the T_m of the duplex in the absence of Ag(I) (31 °C) reveals that the Ag(I) ion efficiently stabilized duplex formation. Approximately one equivalent of Ag(I) was sufficient to fully stabilize duplex formation under these conditions (see ESI[†]).

Addition of Ag(I) ions did not alter the shape of the transition profile for normal duplexes containing A–T and G–C pairs. Other metals known to bind to nucleic acids,[4,5] such as Hg(II), Cu(II), Ni(II), Pd(II), Co(II), Mn(II), Zn(II), Pb(II), Cd(II), Mg(II), Ca(II), Fe(II), Fe(III), and Ru(III) had no notable effects on the thermal transition profiles (see ESI[†]). Note that Hg(II) ions, which exhibited significant stabilizing effects in DNA duplexes containing T–T pairs,[2] showed no effects on the denaturation profile (–■– in Fig. 1a). Detailed analysis revealed that Cu(II) slightly altered the transition profile of duplexes containing the C–C mismatch, although T_m values in the presence and absence of Cu(II) were similar (–△– in Fig. 1a). The effects of Ag(I) on the C–C pairs surpassed the effects of other metals and appeared to be highly specific.

The effect of solution pH on the metal-induced stabilization of the duplexes was also examined. Shown in Fig. 1b, the C–Ag–C mismatched duplex was stabilized considerably by the presence of Ag(I) between pH 5 and 9, but was destabilized outside of this range regardless of the Ag(I) content.

In the absence of Ag(I), the C–C mismatch duplex was particularly stable around pH 5, most likely caused by protonation of

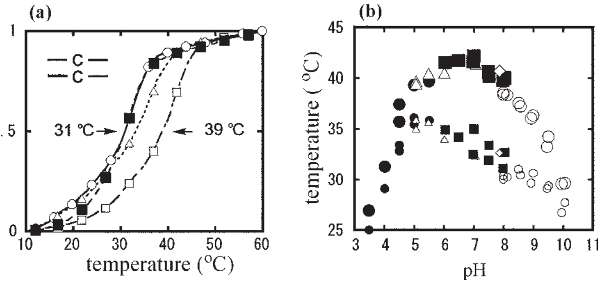

Fig. 1 (a) Relative absorbance, $A = [(A_{t\,°C} - A_{10\,°C})/(A_{60\,°C} - A_{10\,°C})]$, at 260 nm *vs.* temperature for a mixture of 5′-d$(A)_{10}C(A)_{10}$-3′ and 5′-$(T)_{10}C(T)_{10}$-3′. Each solution contained 1 μM of oligomer in 10 mM Mops, 100 mM $NaNO_3$, pH 7.1. -○- in the absence of metal, – -□- - $AgNO_3$ (2 μM), –■– $Hg(ClO_4)_2$ (2 μM), –△– $CuCl_2$ (2 μM). The T_m values of the 5′-$(dA)_{21}$-3′/5′-T_{21}-3′ duplexes, and 5′-d$(A)_{10}G(A)_{10}$-3′/5′-$(T)_{10}C(T)_{10}$-3′ were 44 °C and 46 °C , and were not affected by the addition of $AgNO_3$ (2 μM). (b) T_m at 260 nm *vs.* pH for the mixtures. Each solution contained 1 μM of oligomer, 2 μM $AgNO_3$, and 100 mM $NaNO_3$ in the appropriate buffer (10 mM); ● acetate, ■ cacodylate, △ phosphate, ◇ Mops, ○ borate in the absence (small size) or presence of metal ions (large size).

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[†] Electronic supplementary information (ESI) available: The thermal denaturation profiles of the duplex containing the C–C mismatch pair in the presence of various metal ions, ESI-MS profiles of the DNA–Ag^I complexes and fluorescence emission spectra of the sensor. See DOI: 10.1039/b808686a

the cytosine bases[6] in acidic solution and the generation of positive charges that thereby stabilized the duplex formation. In the presence of Ag(I), this characteristic pH effect was diminished due to C–Ag(I)–C complex formation.

Ag(I)-titration experiments were performed using 1D ^{1}H NMR spectroscopy (Fig. 2). New peaks were generated in the imino-proton region following the addition of Ag(I) ions, and the intensities of these features increased with Ag(I) ion concentration. The spectrum acquired in Ag(I)-saturated solution (1.2 eq) (Fig. 2a, bottom) differed from that of the Ag(I)-free DNA duplex (Fig. 2a, top). This spectrum was the result of Ag(I)–DNA complex formation, and indicated that the stoichiometry of the complex was 1 : 1. It was therefore reasonable to conclude that one C–C mismatch captured one Ag(I) ion. Note that at lower Ag(I) concentrations (0.4 eq), the imino-proton resonances from Ag(I)-free and Ag(I)-complexed DNA duplexes were independently observed (Fig. 2a, middle). This indicated that the proton exchange rate between Ag(I)-free and Ag(I)-complexed DNA duplexes was slow relative to the timescale of the NMR measurement. Such slow exchange phenomena were also observed over the entire range of the 1D ^{1}H NMR spectra. For example, the methyl-proton regions of the spectra are shown in Fig. 2b. In general, exchange rates of metal association–dissociation processes with DNA/RNA molecules are fast relative to the timescale of the NMR measurement.[7d] The slow exchange rate observed in Fig. 2 therefore suggests that the Ag(I) ion binds between the C–C base pair. A similar slow exchange was observed in the NMR study of T–Hg(II)–T formation.[2]

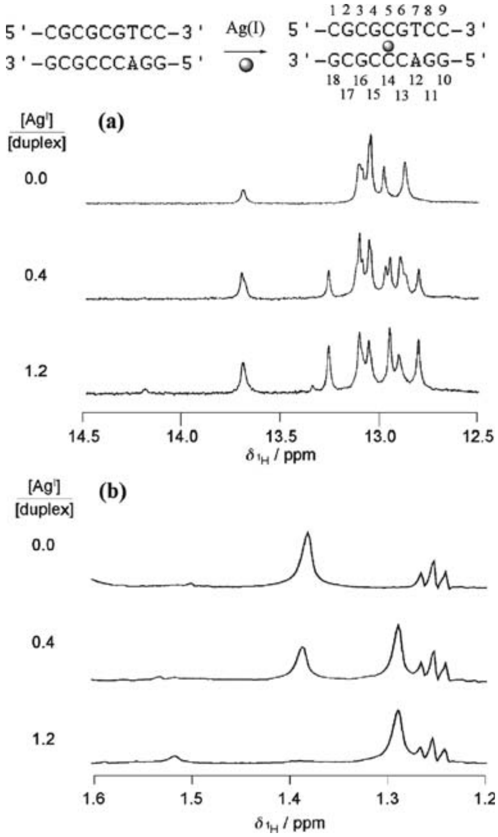

Fig. 2 Imino-proton region (a) and methyl-proton region (b) of 1D ^{1}H NMR spectra of a DNA duplex with a single C–C mismatch. Molar ratios ([Ag(I)]/[duplex]) are indicated on the left side of each spectrum. The sequence of the DNA duplex is indicated at the top of the figure. Solution conditions are 2.0 mM DNA duplex, 100 mM $NaNO_3$, 1 mM Na-cacodylate pH 6.0 and various concentrations of Ag(I) in aqueous solution ($D_2O : H_2O = 1 : 9$). 1D ^{1}H NMR spectra were recorded on a JEOL ECA600 spectrometer at 23 °C with a spectral width of 18 678 Hz digitized into 16 384 points, and 128 scans were averaged. Each spectrum was processed with an exponential window function to give a line-broadening of 1.0 Hz.

The results of electrospray ionization mass spectroscopy (ESI-MS) support the binding of a silver ion to a C–C pair in the DNA duplex (see supporting information†).

The interactions of metal ions with nucleic acids, nucleosides, and nucleobases have been extensively investigated.[1,4,5,7] Ag(I), like Hg(II), binds to base moieties selectively rather than to the phosphate and sugar groups in nucleic acids.[4,5] In the metal-mediated T–Hg(II)–T pair, the mercury ion interposed itself between the thymine residues by forming covalent bonds with the N3 of each thymine residue.[2,7a] Analogous to the T–Hg(II)–T pair, Ag(I) may bind between the N3 nitrogen atoms of cytosine in the C–C mismatch pairs. A similar binding mode was proposed for Ag(I) and artificial pyridine nucleosides in DNA duplexes.[1e] Such linear coordination geometries were observed in the monovalent metal-binding sites of metalloregulatory proteins.[8]

Both thymine and cytosine have been shown to form specific metal-mediated pairs. The formation of the metal ion-mediated T–Hg(II)–T and C–Ag(I)–C base pairs in DNA duplexes can be used to synthesize new materials, such as DNA wires containing metal ions at desired sites,[1a] and molecular sensors for detecting metal ions[9] or redox environments.[10] Similar to the development of mercury sensing using T–T pair–Hg(II) binding, we constructed a DNA-based sensor for Ag(I) ions, as shown in Fig. 3. The oligodeoxyribonucleotide (ODN) based sensor, **D–ODN–F**, consists of an ODN carrying a fluorescent moiety (fluorescein, **F**) and a quencher (dabcyl, **D**) at the 3′- and 5′-ends, respectively.[11] The ODN sequence is divided into two parts: a cytosine-rich, silver-binding sequence and the linker sequence. A proposed mechanism for detecting Ag(I) ions using **D–ODN–F** is shown schematically in Fig. 3. In the absence of Ag(I) ions, the ODN exists as a random coil. In the presence of Ag(I) ions, silver-mediated base pairs (C–Ag(I)–C) are formed between C-residues in two Ag ion-binding sequences, leading to the formation of a hairpin structure. In the hairpin structure, the termini of the ODN are brought into close proximity to each other, thereby enhancing the fluorescence resonance energy transfer (FRET) between the **F** and **D** moieties and dramatically quenching the fluorescence emission.

As shown in Fig. 3a, the fluorescence emission intensity of **D–ODN–F** decreased linearly with increasing Ag(I) concentration around 50 nM (Fig. 3b). Since the fluorescence emission of **ODN–F**, which did not carry the quencher, only slightly decreased upon addition of Ag(I) ions (see ESI†), the fluorescence emission was quenched by the proposed mechanism in the presence of Ag(I). In addition to high sensitivity, the system boasts high selectivity for Ag(I) ions. Fluorescence spectra of **D–ODN–F** in the absence and presence of other heavy metal ions are shown in Fig. 3c. Since the presence of each heavy metal ion did not greatly alter the emission of

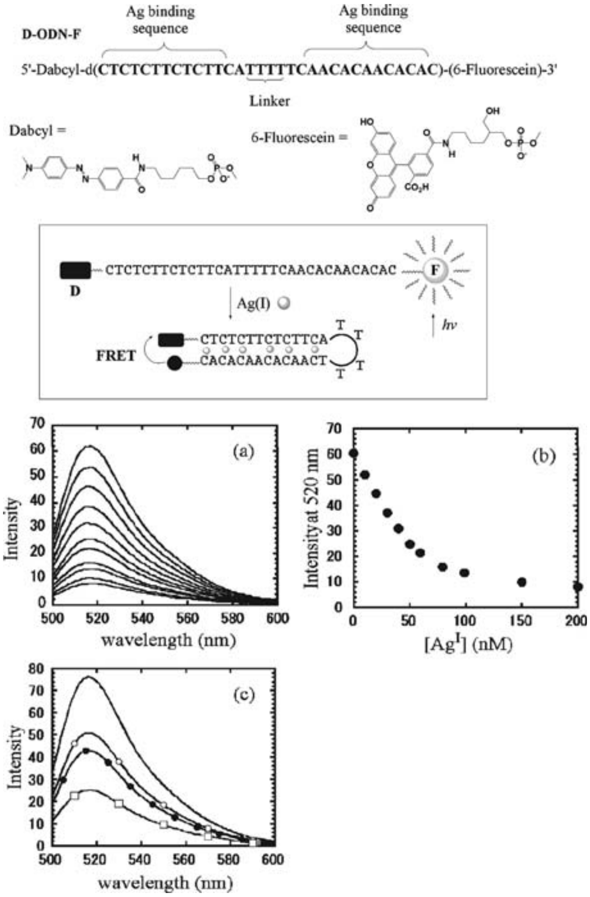

Fig. 3 Upper: structures of the ODN-based silver molecular sensor **D–ODN–F** and a schematic representation of the hairpin structure induced in **D–ODN–F** by Ag(I) ion-mediated C–Ag–C pair formation, which quenches the fluorescence. (a) Fluorescence spectra of **D–ODN–F** (10 nM) upon the addition of 0, 10, 20, 30, 40, 50, 60, 80, 100, 150, and 200 nM $AgNO_3$. The intensity of the fluorescence emission decreased as the Ag^I ion concentration increased. (b) Fluorescence emission intensity *vs.* Ag^I concentration. (c) A solution containing **D–ODN–F** (10 nM) in the absence or presence of metal ions. —in the absence of metal ions, —○— in the presence of 60 nM metals (Mix = Zn(II), Fe(II), Cu(II), Ni(II), Cd(II), Mn(II), Co(II), and Pb(II)), —●— 120 nM Mix, —□— 120 nM Mix + 60 nM Ag(I). A buffer solution of 3-(*N*-morpholino)propanesulfonic acid (10 mM, pH 7.0) 50 mM $NaNO_3$ was used.

D–ODN–F, a mixture of metal ions was used to estimate the effect of background metals. Upon adding a mixture containing 120 nM Zn(II), Fe(II), Cu(II), Ni(II), Cd(II), Mn(II), Co(II), and Pb(II), the emission intensity decreased (—●— in Fig. 3c). However, upon adding 60 nM Ag(I), the emission intensity decreased further (—□— in Fig. 3c). Thus, the sensor exhibited more sensitivity toward Ag(I) ions than toward the other metal ions (see ESI†).[12]

In conclusion, much like the previously reported mercury-mediated thymine pairs,[2] cytosine–cytosine base pair mismatches bind Ag(I) ions with high specificity in DNA duplexes. These results indicate that Ag(I) ions may accumulate in DNA duplexes composed of commercially available oligonucleotides with natural base residues. This phenomenon can be applied to a variety of scientific fields, including metal ion sensing, silver-coated DNA fabrication, DNA-templated Ag cluster formation, and the development of DNA nano-architectures.[13]

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