Pyrimidine–pyrimidine base pairs stabilized by silver(I) ions†

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Received 27th September 2010, Accepted 26th October 2010
DOI: 10.1039/c0cc04091f

In the presence of Ag^I ions, the C–T and m^5iC (5-methylisocytosine)–T base pairs showed comparable stability to the C–Ag^I–C base pair, and the m^5iC–C base pair was highly stabilized by the synergetic effect of Ag^I coordination and possible hydrogen bonding.

DNA forms a double-stranded structure through the formation of A–T and G–C Watson–Crick base pairs.[1] The selectivity of the hydrogen bonding between bases is essential for the replication and expression of genetic information. Recently, several types of artificial base pairing systems formed by non-Watson–Crick type hydrogen bonding,[2] hydrophobic interaction based on shape complementarity,[3] and metal coordination[4] have been reported. These non-natural artificial bases form base pairs to stabilize duplex structures. Among them, base pairs of the metal coordination type can be used as a functional switching device that is dependent on the presence or absence of metal ions. It has been reported that T–T, U–U and C–C mismatch base pairs are effectively stabilized by the addition of Hg^{II} and Ag^I ions, respectively (Fig. 1).[5–7] Ono and Togashi applied the metal-ion-mediated base pairing system to detect Hg^{II} ions.[8]

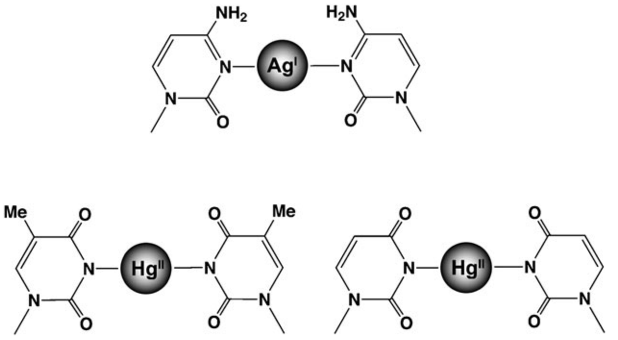
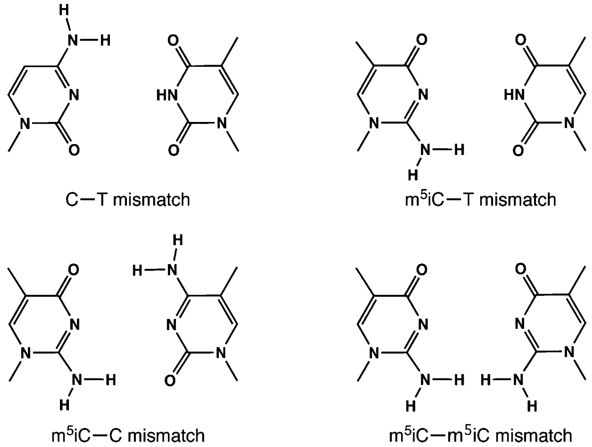


Fig. 1 Structures of C–Ag^I–C, T–Hg^{II}–T and U–Hg^{II}–U base pairs.

In T–Hg^{II}–T and C–Ag^I–C base pairs, hydrogen bonding cannot take place due to the non-complementarity of the T–T and C–C base pairs. Recently, Müller and Polonius, however, reported a novel type of base pair involving both hydrogen bonding and metal coordination through the Hoogsteen edge.[9] Silver(I) ion has the ability to deprotonate thymine in oligonucleotides and binds to the N^3 atom of deprotonated thymine to afford an Ag^I–thymine complex without any charge, and this complex binds to the N^7 position of 1-deazaadenine.[9] This suggests that the Ag^I–thymine complex has affinity toward an aromatic tertiary nitrogen atom. Here, we describe the effects of Ag^I ions on the stability of oligodeoxynucleotides (ODNs) containing a pyrimidine–pyrimidine mismatch base pair consisting of cytosine, thymine, and 5-methylisocytosine (m^5iC) (Fig. 2).



C–T mismatch

m^5iC–T mismatch

m^5iC–C mismatch

m^5iC–m^5iC mismatch

Fig. 2 Structures of pyrimidine–pyrimidine mismatch base pairs.

2′-Deoxy-5-methylisocytidine, a more stable derivative of 2′-deoxyisocytidine, was synthesized from thymidine according to the literature procedure,[10] with slight modifications. 2′-Deoxy-5-methylisocytidine was protected with di(*n*-butyl)-formamidine (dbf)[11] for the 2-amino group and with 4,4′-dimethoxytrityl group (DMT) for the 5′-hydroxyl group, and then converted into the phosphoramidite derivative.[11] The ODNs used in this study were synthesized on an automated DNA synthesizer (Table 1).

Fig. 3 shows the UV melting curves of oligonucleotide duplexes in the absence and presence of metal ions. In the absence of metal ions (Fig. 3a), some of the curves are non-sigmoidal and the T_m values were calculated by curve fitting with the Meltwin program.[12] Duplexes other than duplexes 2 and 4 showed similar T_m values (Table 1). The exceptional T_m values for duplexes 2 and 4 may be attributed to their non-sigmoidal transitions.

As reported by Ono and coworkers,[5,7] the T–T and C–C mismatch base pairs (duplexes 1 and 2) showed significant stabilization when 1 equivalent of Hg^{II} and Ag^I ions, respectively, was added (Fig. 3b, Table 1). It has been reported that although Ag^I ion is able to deprotonate thymine to form an Ag^I–thymine complex,[9] it does not stabilize a T–T mismatch base pair.[5,13] To examine the binding affinity of the Ag^I–thymine complex to cytosine, the effects of Ag^I ions on the stability of the C–T mismatch base pair (duplex 3) were evaluated. As shown in Fig. 3 and Table 1, the addition of Ag^I

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† Electronic supplementary information (ESI) available: Synthetic details of the duplexes containing m^5iC and supplementary melting curves. See DOI: 10.1039/c0cc04091f

Table 1 Synthesized duplexes containing a pyrimidine–pyrimidine base pair mismatch and their melting temperatures (T_ms)[a]

d(GA CGT **X** CTA CG)

(CT GCA **Y** GAT GC)d

	X–**Y**	T_m/°C	T_m/°C (+ metal ion)	ΔT_m/°C
Duplex 1	T–T	27.6	45.7[b]	18.1
Duplex 2	C–C	31.7	36.5[c]	4.8
Duplex 3	C–T	27.9	37.3[c]	9.4
Duplex 4	m^5iC–T	19.7	32.2[c]	12.5
Duplex 5	m^5iC–C	26.6	45.5[c]	18.9
Duplex 6	m^5iC–m^5iC	26.6	36.2[c]	9.6

[a] Samples contained 5 μM duplex, 1 M $NaClO_4$, and 10 mM MOPS, pH 7.1. T_m values were calculated by using the Meltwin program.[12] [b] T_m value in the presence of 1 equivalent of Hg^{II} ions. [c] T_m value in the presence of 1 equivalent of Ag^I ions.

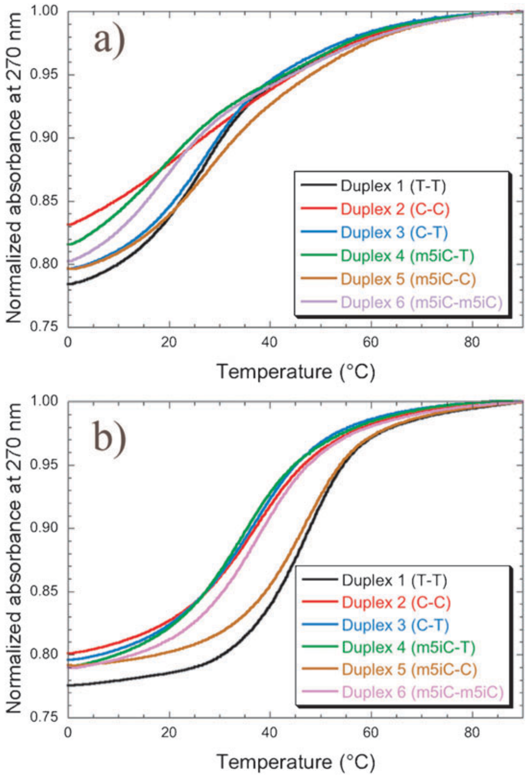

Fig. 3 UV melting curves of duplexes 1–6 in the absence (a) and presence (b) of 1 equivalent of $HgClO_4$ (duplex 1) or $AgNO_3$ (duplexes 2–6). Samples contained 5 μM duplex, 1 M $NaClO_4$, and 10 mM MOPS, pH 7.1.

ion resulted in a significant stabilization of the C–T mismatch base pair (ΔT_m = 9.4 °C), and the T_m value of duplex 3 is comparable to that of duplex 2 in the presence of Ag^I ions, although the ΔT_m value of the C–T mismatch pair (ΔT_m = 9.4 °C) was higher than that for the C–C mismatch pair (ΔT_m = 4.8 °C). It should be noted that 1 equivalent of Ag^I ions stabilized the C–T base pair and an excess of Ag^I ions did not result in further stabilization (Fig. S1, ESI†).

Artificial base m^5iC is a structural isoform of cytosine. Duplex 4 containing the m^5iC–T base pair was effectively stabilized by the addition of Ag^I ions (ΔT_m = 12.5 °C) and the effects were slightly larger than those of duplex 3, although duplex 3 was more stable than duplex 4 in the presence of Ag^I ions. The m^5iC–m^5iC mismatch base pair is a structural isoform of the C–C mismatch base pair and duplex 6 showed thermal stability (36.2 °C) that was analogous to that of duplex 2 (36.5 °C) in the presence of Ag^I ions. The relatively large difference between the ΔT_m values of duplexes 2 and 6 may be due to the non-sigmoidal transition in the melting process of duplex 2 in the absence of Ag^I ions.

The m^5iC–C base pair has two hydrogen bond donor–acceptor pairs. In the presence of Ag^I ions, duplex 5 showed a much higher T_m value (45.5 °C) than duplexes containing the other Ag^I-mediated base pairs (32–37 °C). Notably, this T_m value is comparable to that of the Hg^{II}-mediated T–T base pair (45.7 °C) and is higher than that of the A–T full-match sequence (X = A and Y = T, 45.0 °C) (not shown). This result suggests the possibility that the Ag^I coordination and hydrogen bonding synergistically contribute to the stabilization of the m^5iC–C mismatch base pair. As usual Ag–N bond length is 2.1–2.2 Å, the N^3 to N^3 distance of the m^5iC–Ag–C complex is approximately 4.2 Å when the N^3–Ag–N^3 bond angle is 180°. Thus, some deformation in the m^5iC–C base pair such as buckle or opening[14] may be caused to form the hydrogen bond.

We have found that the Ag^I–thymine complex is able to coordinate to cytosine and 5-methylisocytosine in ODNs. These C–Ag^I–T and m^5iC–Ag^I–T base pairs have roughly comparable thermal stability to the C–Ag^I–C base pair. Thus, the coordination of Ag^I ion would not be selective for the C–C mismatch base pair. In addition, we have found that the m^5iC–Ag^I–C base pair is highly stable compared to the C–Ag^I–C and C–Ag^I–T base pairs. This result demonstrated the synergetic effect of metal coordination and possible hydrogen bonding on the mismatch stabilization. These novel silver(I)-mediated base pairs described here were stabilized sufficiently by 1 equivalent of Ag^I ions (Fig. S1–S4, ESI†) and thus the K_d values for the binding of Ag^I ions to these base pairs would be less than 10 μM. Thus, Ag^I-coordinated thymine and Ag^I-coordinated 5-methylisocytosine may be useful as a recognition device for cytosine in ODNs. In particular, the m^5iC–Ag^I–C base pair may be a promising device for sensing Ag^I ions.

We thank Professor D. H. Turner and Dr J. A. McDowell who generously provided the Meltwin program for calculation of T_m values.

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