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Hg^{II} Ion Specifically Binds with T:T Mismatched Base Pair in Duplex DNA

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Abstract: Metal-mediated base pair formation, resulting from the interaction between metal ions and artificial bases in oligonucleotides, has been developed for its potential application in nanotechnology. We have recently found that the T:T mismatched base pair binds with Hg^{II} ions to generate a novel metal-mediated base pair in duplex DNA. The thermal stability of the duplex with the T-Hg-T base pair was comparable to that of the corresponding T:A or A:T. The novel T-Hg-T base pair involving the natural base thymine is more convenient than the metal-mediated base pairs involving artificial bases due to the lack of time-consuming synthesis. Here, we examine the specificity and thermodynamic properties of the binding between Hg^{II} ions and the T:T mismatched base pair. Only the melting temperature of the duplex with T:T and not of the perfectly matched or other mismatched base pairs was found to specifically increase in the presence of Hg^{II} ions. Hg^{II} specifically bound with the T:T mismatched base pair at a molar ratio of 1:1 with a binding constant of $10^6\,\text{M}^{-1}$, which is significantly higher than that for nonspecific metal ion–DNA interactions. Furthermore, the higher-order structure of the duplex was not significantly distorted by the Hg^{II} ion binding. Our results support the idea that the T-Hg-T base pair could eventually lead to progress in potential applications of metal-mediated base pairs in nanotechnology.

Keywords: Hg^{II} ions · isothermal titration calorimetry · mismatched base pair · nucleic acids · thermodynamics

Introduction

The interactions between metal ions and nucleic acids have attracted considerable interest not only for their involvement in biological processes, such as RNA folding[1] and enzymatic activity of ribozymes,[2] but also for their wide variety of potential applications in nanotechnology including the design of biomolecular nanomachines and nanodevices.[3] Metal-mediated base pairs involving interactions between metal ions and artificial bases in synthetic oligonucleotides have been extensively developed for their potential applications in nanotechnology.[4] Metal ions have been placed between two artificial bases in duplex oligonucleotides, as shown by structural analyses of such complexes.[5] However, we and other groups have found an alternative method for generating metal-mediated base pairs in duplex DNA, based on the binding between a metal ion and natural bases.[6] Only Hg^{II} ions and no other metal ions have been found to bind with the thymine–thymine (T:T) mismatch in duplex DNA to form T-Hg-T (Scheme 1 a). The binding of the Hg^{II} ion stabilizes the duplex with the T:T mismatched base pair.[6a,b] The thermal stability of the duplex DNA with the T-Hg-T base pair is comparable to that of the corresponding

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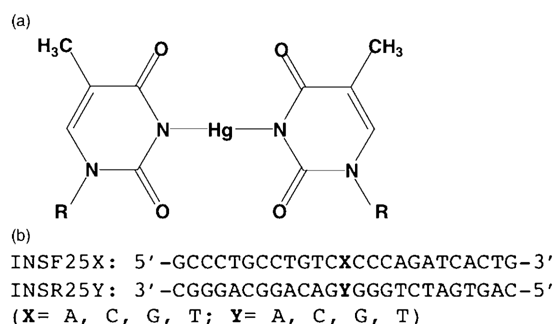

Scheme 1. a) Structural formula of the T-Hg-T base pair. b) Oligonucleotide sequences of the target duplex INSF25X:INSR25Y.

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T:A or A:T base pair.[6b] Because artificial bases are more difficult to prepare due to time-consuming organic synthesis, T-Hg-T base pair formation is more convenient than base pair formation between metal ions and artificial bases. However, the mechanistic explanations for Hg^{II} ion-mediated stabilization of duplex DNA with the T:T mismatched base pair and T-Hg-T formation are not clearly understood. Therefore, here, we have expanded our previous research to explore the specificity and thermodynamic properties of the interaction between the Hg^{II} ion and the T:T mismatched base pair.

The interaction between Hg^{II} ions and mismatched base pairs in duplex DNA or the corresponding perfectly matched DNA was analyzed by UV melting experiments, isothermal titration calorimetry (ITC),[7] and circular dichroism (CD) spectroscopy. UV melting analyses indicate that the Hg^{II} ion is able to significantly stabilize the duplex with the T:T mismatched base pair, but it is unable to stabilize duplexes that are perfectly matched or have other mismatches. Only the duplex with the T:T mismatch was specifically stabilized by the addition of Hg^{II} ions. In addition, ITC analyses demonstrate that the Hg^{II} ion specifically binds with the T:T mismatched base pair at a molar ratio of 1:1 with a binding constant of nearly $10^6\,M^{-1}$. The magnitude of the observed binding constant was significantly larger than those previously reported for the nonspecific interaction between metal ions and DNA.[8] The specific binding between Hg^{II} and the T:T mismatched base pair was driven by both a negative enthalpy change and a positive entropy change. CD spectroscopy showed that the higher-order structure of the duplex was not significantly distorted by the binding of Hg^{II} ions. The specific binding between Hg^{II} and the T:T mismatch would support further research in potential applications of metal-mediated base pairs in nanotechnology.

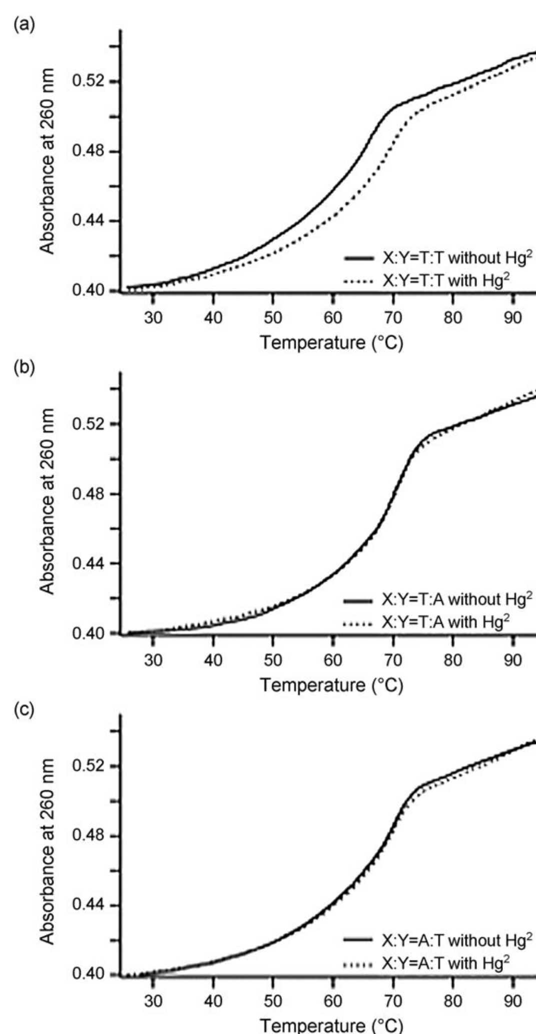

Figure 1. UV melting profiles of: a) the mismatched base pair duplex, INSF25T:INSR25T, and the perfectly matched duplexes b) INSF25T:INSR25A, and c) INSF25A:INSR25T, with or without $Hg(ClO_4)_2$. DNA duplexes (1 μM) at pH 6.8 in buffer A (see the Experimental Section) with or without $Hg(ClO_4)_2$ (1 μM) were melted at a scan rate of 0.2 °C min^{-1} with detection at 260 nm. The cell path-length was 1 cm.

Results

UV melting analyses of mismatched and perfectly matched duplex DNA either with or without Hg^{II} ions: The thermal stability of a series of duplex DNA molecules (1 μM), INSF25T:INSR25T, INSF25T:INSR25A, and INSF25A:INSR25T (INSF25X:INSR25Y, 5′-GCCCTGCCTGTC**X**CC-CAGATCACTG-3′/3′-CGGGACGGACAG**Y**GGGTCT-AGTGAC-5′; Scheme 1b), was examined in buffer A (see the Experimental Section) either with or without $Hg(ClO_4)_2$ (1 μM) by UV melting experiments (Figure 1). Without $Hg(ClO_4)_2$, the T_m values of INSF25T:INSR25A (70.4 °C) and INSF25A:INSR25T (70.1 °C) with perfectly matched base pairs were significantly higher than the T_m value of INSF25T:INSR25T (65.7 °C) with the T:T mismatch (Figure 1 and Table 1). The T_m of INSF25T:INSR25A (70.7 °C) and INSF25A:INSR25T (70.1 °C) with $Hg(ClO_4)_2$ were not significantly different to values obtained without $Hg(ClO_4)_2$ (Figure 1b, 1c and Table 1). In contrast, the addition of $Hg(ClO_4)_2$ increased the T_m of INSF25T:INSR25T by about 4 °C (65.7 °C→69.8 °C; Figure 1a and Table 1). The increase in the T_m of INSF25T:INSR25T in the presence of Hg^{II} was achieved at a molar ratio of $[Hg^{II}$ ion]/[INSF25T:INSR25T] = 1. These results indicate that the thermal stability of the duplex DNA with the T:T mismatched base pair was significantly increased by the addition of Hg^{II} ions at a molar ratio of 1.

To examine the specificity of the stabilization by Hg^{II} ions, we measured the T_m values of a series of duplexes with 16 different base pairs, INSF25X:INSR25Y (X:Y = A:A, A:C, A:G, A:T, C:A, C:C, C:G, C:T, G:A, G:C, G:G, G:T, T:A, T:C, T:G, and T:T; Scheme 1b) in buffer A with or without 1 μM $Hg(ClO_4)_2$ by UV melting experiments (Table 1). Without Hg^{II} ($T_m(-Hg^{II})$), the T_m values of the duplexes with perfectly matched base pairs (X:Y = A:T, C:G, G:C, and T:A) were significantly higher than those

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Table 1. Melting temperatures of duplexes [1 μM; INSF25X:INSR25Y (X:Y = A:A, A:C, A:G, A:T, C:A, C:C, C:G, C:T, G:A, G:C, G:G, G:T, T:A, T:C, T:G, and T:T)] at pH 6.8 in sodium cacodylate-cacodylic acid (10 mM) and $NaClO_4$ (100 mM) with or without $Hg(ClO_4)_2$ (1 μM) obtained from UV melting experiments.

X:Y	$T_m(-Hg^{II})$ [°C]	$T_m(+Hg^{II})$ [°C]	ΔT_m [°C][a]
A:A	66.6 ± 0.3	65.8 ± 0.4	−0.8
A:C	67.3 ± 0.1	66.9 ± 0.2	−0.4
A:G	69.6 ± 0.3	69.7 ± 0.3	0.1
A:T	70.1 ± 0.3	70.1 ± 0.7	0
C:A	65.5 ± 0.1	64.9 ± 0.1	−0.6
C:C	64.7 ± 0.1	63.9 ± 0.3	−0.8
C:G	72.2 ± 0.2	71.9 ± 0.4	−0.3
C:T	64.1 ± 0.3	64.5 ± 0.2	0.4
G:A	68.2 ± 0.3	68.5 ± 0.3	0.3
G:C	74.5 ± 0.3	74.1 ± 0.5	−0.4
G:G	69.6 ± 0.1	68.9 ± 0.2	−0.7
G:T	68.1 ± 0.2	67.8 ± 0.3	−0.3
T:A	70.4 ± 0.4	70.7 ± 0.1	0.3
T:C	65.7 ± 0.2	65.8 ± 0.4	0.1
T:G	68.1 ± 0.2	68.9 ± 0.1	0.8
T:T	65.7 ± 0.2	69.8 ± 0.3	4.1

[a] $\Delta T_m = T_m(+Hg^{II}) - T_m(-Hg^{II})$.

with mismatches (X:Y = A:A, A:C, A:G, C:A, C:C, C:T, G:A, G:G, G:T, T:C, T:G, and T:T). Addition of Hg^{II} (T_m-($+Hg^{II}$)) increased the T_m value of the duplex with the T:T mismatched base pair by about 4 °C. The T_m values of duplex DNAs with perfectly matched (X:Y = A:T, C:G, G:C, and T:A) or with other mismatched base pairs (X:Y = A:A, A:C, A:G, C:A, C:C, C:T, G:A, G:G, G:T, T:C, and T:G) did not significantly change by the addition of Hg^{II} ions. These results indicate that only the duplex DNA with the T:T mismatched base pair was specifically stabilized by the addition of Hg^{II} ions.

ITC analyses of the interaction of Hg^{II} ions with mismatched and perfectly matched duplex DNAs: To explore the mechanism of specific stabilization of the T:T mismatched base pair by Hg^{II} ions, we examined the thermodynamic properties of the interaction between $Hg(ClO_4)_2$ and duplexes INSF25T:INSR25T, INSF25T:INSR25A, and INSF25A:INSR25T (Scheme 1 b) in buffer A at 25 °C and pH 6.8 by ITC (Figure 2).[7] Figure 2 a shows a typical ITC profile of the interaction between $Hg(ClO_4)_2$ and INSF25T:INSR25T at 25 °C and pH 6.8. An exothermic heat pulse was observed after $Hg(ClO_4)_2$ was injected into INSF25T:INSR25T. The magnitude of each peak decreased gradually with each new injection, and a peak was still observed at a molar ratio of [Hg^{II} ion]/[INSF25T:INSR25T] = 2. The area under each peak was integrated, and the heat of the dilution of $Hg(ClO_4)_2$ measured in a separate experiment by injecting $Hg(ClO_4)_2$ into the same buffer was subtracted from the integrated values. The corrected heat was divided by the moles of injected solution, and the resulting values were plotted as a function of a molar ratio of [Hg^{II} ion]/[INSF25T:INSR25T] (Figure 2 d, ●). The resultant titration plot was sigmoidal and indicates that the Hg^{II} ion specifically bound with INSF25T:INSR25T. The ITC profile of the interaction between $Hg(ClO_4)_2$ and INSF25T:INSR25A at 25 °C and pH 6.8 is shown in Figure 2 b. Although an exothermic heat pulse was observed after each injection of $Hg(ClO_4)_2$ into INSF25T:INSR25A, the magnitude of each peak did not significantly change with each new injection. This is in sharp contrast with the ITC profile observed for the interaction between $Hg(ClO_4)_2$ and INSF25T:INSR25T (Figure 2 a). The interaction between $Hg(ClO_4)_2$ and INSF25A:INSR25T at 25 °C and pH 6.8 also shows a similar ITC profile (Figure 2 c). The titration plots (Figure 2 d) obtained from Figure 2 b (■) and Figure 2 c (▲), which were obtained in the same way as that obtained from Figure 2 a (●), were not sigmoidal; this indicates that Hg^{II} ions nonspecifically bound with INSF25T:INSR25A and INSF25A:INSR25T.

The nonspecific binding of Hg^{II} ions with INSF25T:INSR25A and INSF25A:INSR25T as judged from the ITC titration plots (Figure 2 d) suggests that Hg^{II} might bind with the phosphate backbone of the perfectly matched duplexes in a nonspecific manner due to the attraction between the positive charge of Hg^{II} and the negative charge of the DNA phosphate backbones. On the other hand, the specific binding between Hg^{II} and INSF25T:INSR25T as judged from the ITC titration plot (Figure 2 d) suggests that Hg^{II} can specifically bind with the T:T mismatched base pair of this duplex, in addition to the nonspecific binding with the phosphate backbone. Thus, the net heat derived from the specific binding between the Hg^{II} ion and the T:T mismatch should be estimated by subtraction of the heat observed for either INSF25T:INSR25A or INSF25A:INSR25T from that observed for INSF25T:INSR25T. Based on these considerations, in order to analyze the thermodynamic parameters of the specific binding between Hg^{II} and T:T, the ITC profile observed for INSF25T:INSR25A (Figure 2 b) was subtracted from that observed for INSF25T:INSR25T (Figure 2 a) to obtain Figure 2 e. Also, the ITC profile observed for INSF25A:INSR25T (Figure 2 c) was subtracted from that observed for INSF25T:INSR25T (Figure 2 a) to obtain Figure 2 f. The area under each peak in Figure 2 e was integrated and the integrated values were divided by the moles of the injected solution. The resulting values were plotted as a function of the molar ratio of [Hg^{II} ion]/[duplex DNA] (Figure 2 g). The resultant titration plot was fitted to a sigmoidal curve by a nonlinear least-squares method. The stoichiometry, n, the binding constant, K_a, and the enthalpy change, ΔH, for the specific binding between the Hg^{II} ion and the T:T mismatched base pair were obtained from the fitted curve. The Gibbs free energy change, ΔG, and the entropy change, ΔS, were calculated from the equation, $\Delta G = -RT\ln K_a = \Delta H - T\Delta S$, where R is the gas constant and T is the temperature. The titration plot (Figure 2 h) and the thermodynamic parameters for the specific binding between Hg^{II} and the T:T mismatch were also obtained from Figure 2 f in the same way.

Table 2 summarizes the thermodynamic parameters for the specific binding between Hg^{II} and T:T, which were ob-

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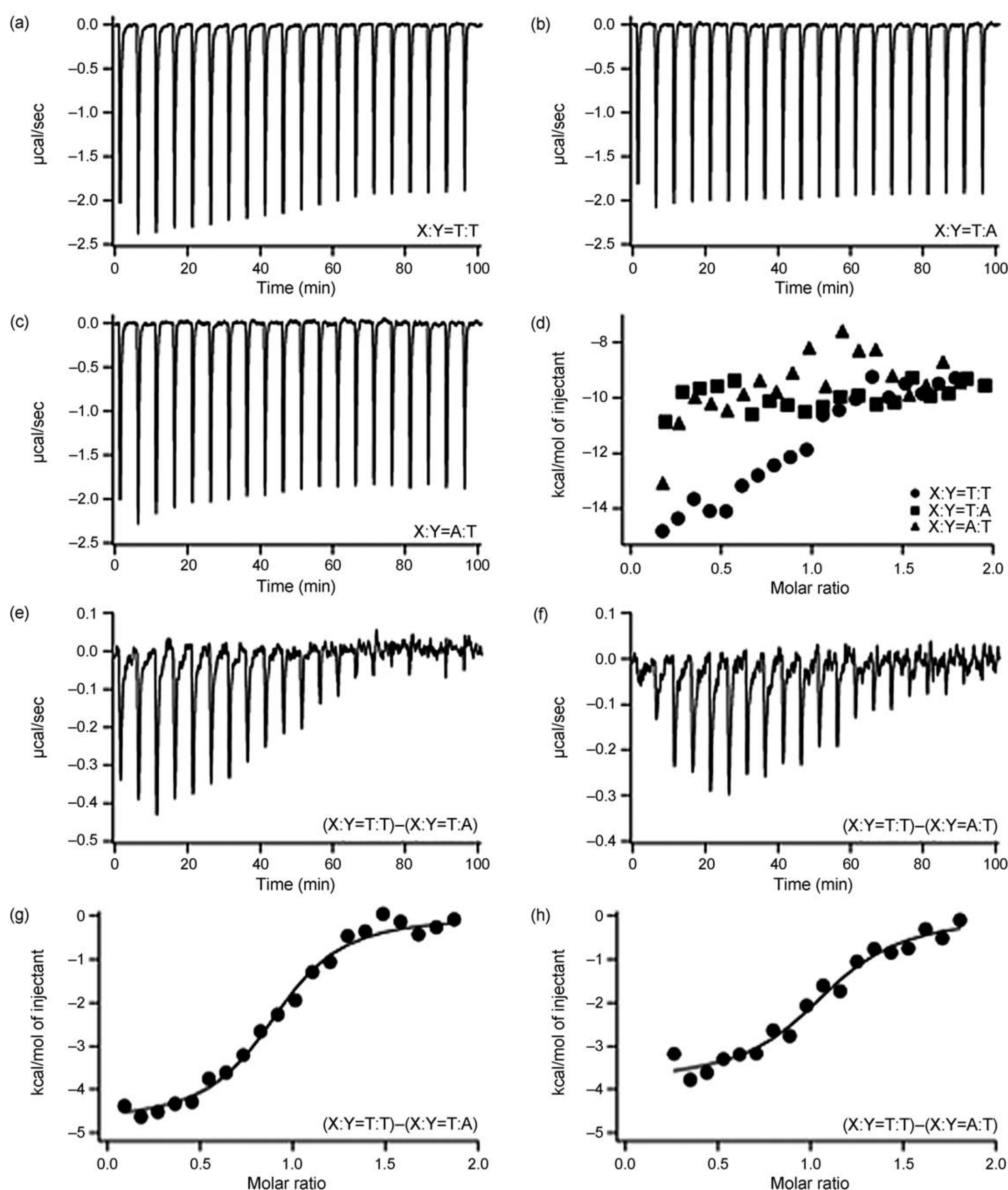

Figure 2. Thermodynamic analyses of the interaction of Hg^{II} ions with the mismatched duplex INSF25T:INSR25T and perfectly matched duplexes INSF25T:INSR25A and INSF25A:INSR25T. Typical ITC profile of the interaction between $Hg(ClO_4)_2$ and: a) INSF25T:INSR25T, b) INSF25T:INSR25A, and c) INSF25A:INSR25T at 25 °C and pH 6.8 in buffer A (see the Experimental Section). $Hg(ClO_4)_2$ solution (1 mM in buffer A) was injected 20-times in 5 µL increments into INSF25T:INSR25T, INSF25T:INSR25A, and INSF25A:INSR25T solutions (40 µM in buffer A). Injections were administered over 12 s at 5 min intervals. d) Titration plots against the molar ratio of [Hg^{II} ion]/[duplex DNA], obtained from the ITC profiles in a)–c). e) ITC profile for the specific binding between Hg^{II} ions and the T:T mismatched base pair, obtained by subtracting the ITC profile observed for INSF25T:INSR25A from that of INSF25T:INSR25T. f) ITC profile for the specific binding between Hg^{II} ions and the T:T mismatch, obtained by subtracting the ITC profile observed for INSF25A:INSR25T from that of INSF25T:INSR25T. g) Titration plot against the molar ratio of [Hg^{II} ion]/[duplex DNA], obtained from the ITC profile in (e). h) Titration plot against the molar ratio of [Hg^{II} ion]/[duplex DNA], obtained from the ITC profile in (f). g), h) Data were fitted by a nonlinear least-squares method.

tained from Figure 2g and 2h. The thermodynamic parameters obtained from Figure 2g and 2h are quite similar in magnitude. The obtained value of n is nearly 1; this indicates that the Hg^{II} ion binds with the T:T mismatched base pair at a molar ratio of 1:1. Although the sign of ΔH is negative, the sign of ΔS is positive. Because both the observed negative ΔH and positive ΔS are favorable for the specific binding between the Hg^{II} ion and the T:T mismatched base pair,

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Table 2. Thermodynamic parameters for the specific binding between Hg^{II} ions and the T:T mismatched base pair at 25 °C and pH 6.8 in sodium cacodylate-cacodylic acid (10 mM) and $NaClO_4$ (100 mM) obtained from ITC measurements.

Profile	n	K_a [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]
Figure 2 g	0.89 ± 0.02	$(6.34 \pm 1.17) \times 10^5$	-7.91 ± 0.12	-4.76 ± 0.13	10.6 ± 0.84
Figure 2 h	1.06 ± 0.03	$(4.87 \pm 1.35) \times 10^5$	-7.76 ± 0.19	-3.85 ± 0.18	13.1 ± 0.65

the specific binding between Hg^{II} and T:T is driven by both the negative ΔH and positive ΔS. The magnitudes of the observed K_a and ΔG are significantly larger than those previously reported for the nonspecific interaction between metal ion and DNA;[8] this indicates that the Hg^{II} ion specifically bound with the T:T mismatched base pair.

CD spectroscopy of mismatched and corresponding perfectly matched duplex DNA either with or without Hg^{II}: To examine the effect of Hg^{II} ion binding on the higher-order structure of duplex DNA, CD spectra of mismatched (INSF25T:INSR25T; Scheme 1 b) and perfectly matched duplex DNAs (INSF25T:INSR25A and INSF25A:INSR25T; Scheme 1 b) were measured in buffer A either with or without $Hg(ClO_4)_2$ at 25 °C and pH 6.8 (Figure 3). The CD profiles of INSF25T:INSR25T, INSF25T:INSR25A, and INSF25A:INSR25T with $Hg(ClO_4)_2$ were quite similar to that observed without $Hg(ClO_4)_2$. This result indicates that there was no significant change in the higher-order structure of the duplex DNAs after addition of Hg^{II}. The higher-order structure of the mismatched base pair DNA was not significantly distorted by the binding of Hg^{II} ions.

Discussion

UV melting analyses have shown that the addition of Hg^{II} significantly increases the thermal stability of duplex DNA with the T:T mismatched base pair (Figure 1 and Table 1). However, the thermal stability of duplex DNA molecules with perfectly matched base pairs (X:Y = A:T, C:G, G:C, and T:A) or with other kinds of mismatches (X:Y = A:A, A:C, A:G, C:A, C:C, C:T, G:A, G:G, G:T, T:C, and T:G) were not significantly changed by the addition of Hg^{II} (Table 1). Thus, only the duplex DNA with the T:T mismatch was specifically stabilized by the addition of Hg^{II} ions. To examine the effect of Hg^{II} ions on the thermal stability of duplex DNA with a different base sequence context, we measured the T_m values of another series of duplexes, APMF25X:APMR25Y (5′-CTCAGATCCTGC**X**CTTCAAAAACAA-3′/3′-GAGTCTAGGACG**Y**GAAGTTTTTGTT-5′; X:Y = A:A, A:C, A:G, A:T, C:A, C:C, C:G, C:T, G:A, G:C, G:G, G:T, T:A, T:C, T:G, and T:T) in buffer A with or without 1 μM $Hg(ClO_4)_2$ by UV melting experiments (Table S1 in the Supporting Information). Similar to INSF25X:INSR25Y, only the duplex DNA with the T:T mismatch was specifically stabilized by the addition of Hg^{II} ions. We have previously reported that other metal ions, such as Mg^{II}, Ca^{II}, Mn^{II}, Fe^{II}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Ru^{III}, Pd^{II}, Ag^{I}, Cd^{II}, and Pb^{II}, do not show any notable effect on the thermal stability of duplex DNA with the T:T mismatched base pair.[6a,b] Thus, only the Hg^{II} ion was able to specifically increase the thermal stability of the duplex DNA with the T:T mismatch. These results clearly indicate that the stabilization is highly specific for Hg^{II} ions and the duplex DNA with the T:T base pair.

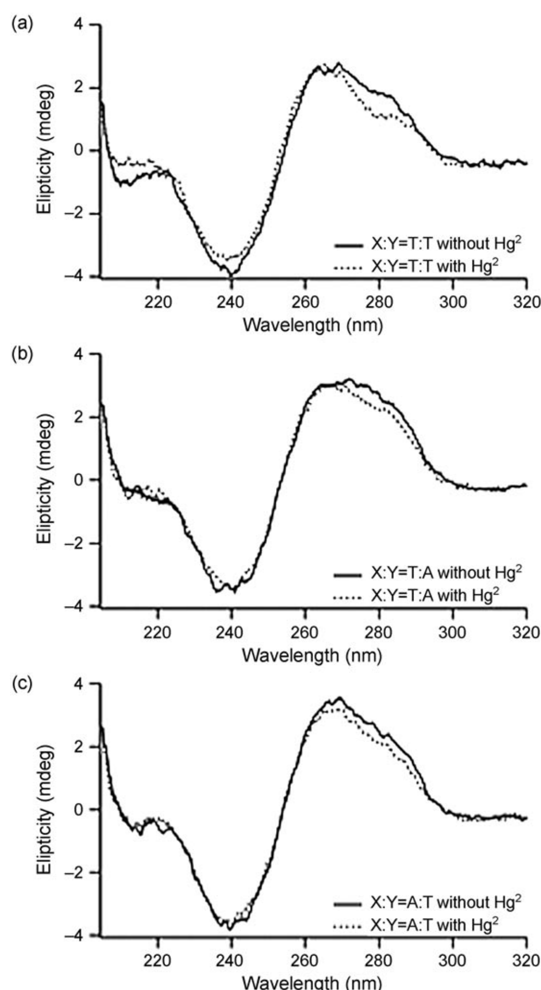

Figure 3. CD spectra of: a) the mismatched duplex INSF25T:INSR25T, and the perfectly matched duplexes, b) INSF25T:INSR25A, and c) INSF25A:INSR25T, with or without $Hg(ClO_4)_2$. DNA (1 μM) at 25 °C and pH 6.8 in buffer A (see the Experimental Section) with or without $Hg(ClO_4)_2$ (1 μM) was analyzed at a wavelength of 205–320 nm. The cell path-length was 1 cm.

ITC analyses revealed that Hg^{II} ions specifically bind with the T:T mismatched base pair in duplex DNA at a molar ratio of 1:1 (Figure 2 and Table 2). The observed specific stabilization of the duplex DNA with the T:T base pair by Hg^{II}

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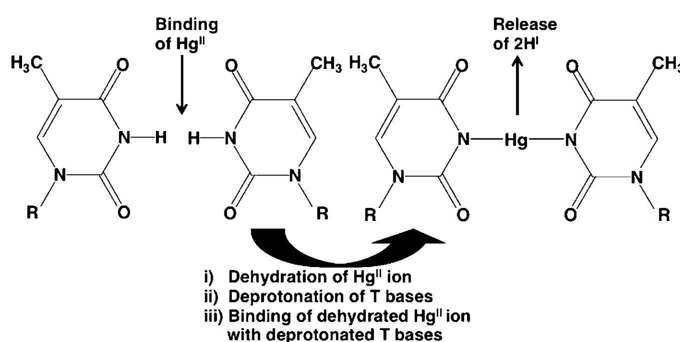
ions (Figure 1 and Table 1) could result from the specific binding of Hg^{II} with T:T. Hg^{II} ions are known to bind selectively with base moieties rather than with the phosphate or sugar groups in DNA.[6e–j] In particular, the Hg^{II} ion has a strong affinity for the N3 position of thymine bases.[6e–g] A covalent and linear N3-Hg-N3 bond was observed in the crystal structure of a 1:2 complex of Hg^{II} and 1-methylthymine.[6g] In the 1960s, Yamane and Davidson reported that protons are released when Hg^{II} ions bind with several natural DNA molecules.[6i] Katz proposed the possibility of the formation of a 1:2 complex between Hg^{II} ions and thymine bases in a double-stranded polynucleotide, $d(AT)_n$•$d(AT)_n$, with the release of protons.[6j] Also, a 1:2 complex of mercury and thymine was used in nucleoside synthesis procedures, the so-called "mercury" method, a traditional synthesis method for coupling glycosyl halides and bases.[9] Thus, it is assumed that Hg^{II} ions can bind with the T:T mismatch in duplex DNA through a covalent N3-Hg-N3 bond. We have previously analyzed the ^{1}H NMR spectra of duplex DNA containing the T:T mismatched base pair in the absence and presence of Hg^{II}.[6b] We found that the imino proton resonances of the T:T mismatched base pair disappeared in the presence of Hg^{II} ions; this suggests that the imino protons of the T:T mismatched base pair were substituted with Hg^{II} ions.[6b] We also previously examined the ^{15}N NMR spectra of the complex between Hg^{II} ions and duplex DNA with the T:T mismatch labeled with ^{15}N at the N3 position.[10] We found ^{15}N-^{15}N J coupling across the Hg^{II} ion with the coupling constant ($^{2}J_{NN}$) of 2.4 Hz.[10] This observation clearly demonstrated the N3-Hg-N3 bond formation in the T-Hg-T complex. Taken together, we conclude that the Hg^{II} ion specifically binds with the N3 positions of two thymine bases in place of the imino protons and bridges two thymine bases to form the T-Hg-T complex in duplex DNA (Scheme 1 a).

The K_a and ΔG for the specific binding between Hg^{II} ions and the T:T mismatch was nearly $10^{6}\,\mathrm{M}^{-1}$ and $-7.8\,\mathrm{kcal\,mol^{-1}}$, respectively (Table 2). The magnitudes of the observed K_a and ΔG are significantly larger than those previously reported for the nonspecific interaction between metal ions and DNA;[8] this supports the specific binding between Hg^{II} ions and T:T. The observed ΔG resulted from both the observed negative ΔH and positive ΔS (Table 2). The positive ΔS for the specific binding between Hg^{II} ions and the T:T mismatch measured by ITC (Table 2) includes a major contribution of a positive dehydration entropy change from the release of structured water molecules surrounding the Hg^{II} ion and the DNA, and a conformational entropy change from the conformational change of duplex DNA upon binding with Hg^{II} ions.[11] The CD spectra show that the higher-order structure of the duplex DNA was not significantly distorted by the specific binding of Hg^{II} ions (Figure 3); this suggests there was no significant contribution from a conformational entropy change to the observed positive ΔS (Table 2). Thus, the observed positive ΔS (Table 2) could mainly result from the positive dehydration entropy change from the release of structured water molecules surrounding the Hg^{II} ion and DNA. In fact, a positive dehydration entropy change of the Hg^{II} ions ($57\,\mathrm{cal\,mol^{-1}\,K^{-1}}$)[12] is similar in magnitude to the observed positive ΔS (Table 2). On the other hand, the negative ΔH for the specific binding between Hg^{II} ions and the T:T mismatched base pair measured by ITC (Table 2) reflects a major contribution from a positive dehydration enthalpy change of Hg^{II} ($439\,\mathrm{kcal\,mol^{-1}}$),[13] a positive deprotonation enthalpy change of the two thymine bases ($347\,\mathrm{kcal\,mol^{-1}}$)[14] upon Hg^{II} binding, an accompanying positive protonation enthalpy change of the cacodylate buffer ($0.47\,\mathrm{kcal\,mol^{-1}}$)[15] taking up the two protons released from the two thymine bases, and a negative binding enthalpy change from the N3-Hg-N3 bond formation in the T-Hg-T complex. Because only the sign of the binding enthalpy change upon N3-Hg-N3 formation was negative and the signs of the enthalpy changes from the other three contributions were positive, the observed negative ΔH (Table 2) might have been mainly driven by the negative binding enthalpy change from the N3-Hg-N3 bond formation. Based on this, we propose a possible scheme for the specific binding between Hg^{II} and T:T (Scheme 2). The Hg^{II} ion surrounded by structured water



Scheme 2. Possible scheme for the specific binding between Hg^{II} ions and the T:T mismatched base pair. The Hg^{II} ion surrounded by structured water molecules can be dehydrated, and the protons at the N3 position of the two thymine bases in T:T can be released. The dehydrated Hg^{II} ion can then bind with the two deprotonated thymine bases to form N3-Hg-N3.

molecules can be dehydrated with significant contribution of the positive dehydration entropy change. The protons at the N3 position of the two thymine bases in the T:T mismatched base pair can then be released. The dehydrated Hg^{II} ion can bind with the two deprotonated thymine bases to form the N3-Hg-N3 bond with significant contribution from the negative binding enthalpy change. Thymine deprotonation could be brought about at pH 6.8 in the present experimental conditions, below the pH at which thymine becomes deprotonated (the pK_a of the proton at N3 position of thymine is 9–10), because initial Hg^{II} ion binding to O4 and/or O2 positions of the canonical tautomer of thymine can acidify the proton at the N3 position.[16] The deprotonation could be brought about by Hg-OH, which is undoubtedly present at physiological pH, because $[Hg(H_2O)_x]^{2+}$ is a strong Lewis

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acid, followed by metal migration from O4 and/or O2 to N3 and reprotonation of O4 and/or O2.[16]

Conclusion

The present study has demonstrated that the combination of Hg^{II} ions and the T:T mismatch is highly specific in this metal-mediated base-pair formation. This work has also revealed that the binding affinity between the Hg^{II} ion and T:T mismatched base pair is significantly larger than that for nonspecific interactions between metal ions and DNA. The highly selective T-Hg-T formation involving the natural bases with the large binding affinity is more convenient than other metal-mediated base pairs with artificial bases due to the lack of time-consuming synthesis. Taken together, we conclude that T-Hg-T could be a key structure that could eventually lead to progress in potential applications of metal-mediated base pairs in nanotechnology.

Experimental Section

Preparation of oligonucleotides: The 25-mer complementary DNA oligonucleotides, INSF25X: 5′-GCCCTGCCTGTC**X**CCCAGATCACTG-3’ (**X** = A, C, G, T) and INSR25Y: 5′-CAGTGATCTGGG**Y**GACAGGCAGGGC-3’ (**Y** = A, C, G, T; Scheme 1 b) were synthesized with a DNA synthesizer by using the solid-phase cyanoethyl phosphoramidite method. These were purified with reverse-phase HPLC on a Wakosil DNA column. The concentration of all oligonucleotides was determined by UV absorbance. The purified complementary strands INSF25X and INSR25Y were annealed by being heated to 90 °C followed by gradual cooling to room temperature. The annealed sample was applied to a hydroxyapatite column (Biorad, Inc.) to remove unpaired single strands. The concentration of duplex DNA (INSF25X:INSR25Y) was determined by UV absorption considering the DNA concentration ratio of 1 OD = 50 $\mu g mL^{-1}$, with a M_w of 16500.

UV melting experiments: UV melting experiments were carried out on a DU-640 spectrophotometer (Beckman, Inc.) equipped with a Peltier-type cell holder; the cell path-length was 1 cm. The UV melting profiles were measured in buffer A (10 mM sodium cacodylate-cacodylic acid, pH 6.8, 100 mM $NaClO_4$) either with or without $Hg(ClO_4)_2$ (1 µM) at a scan rate of 0.2 °C min^{-1} with detection at 260 nm. The first derivative was calculated from the UV melting profile. The peak temperatures in the derivative curve were designated as the melting temperature, T_m. The concentration of duplex DNA (INSF25X:INSR25Y) used was 1 µM.

Isothermal titration calorimetry: ITC experiments were carried out on a VP ITC system (Microcal Inc., USA).[7] Duplex DNA (INSF25X:INSR25Y) solutions were prepared by extensive dialysis against buffer A. $Hg(ClO_4)_2$ was dissolved in the dialysis buffer. The $Hg(ClO_4)_2$ solution in buffer A was injected 20-times in 5 µL increments at 5 min intervals into the DNA solution without changing the reaction conditions. The heat for each injection was subtracted by the heat of dilution of the injectant, which was measured by injecting the $Hg(ClO_4)_2$ solution into the same buffer. Each corrected heat was divided by the moles of $Hg(ClO_4)_2$ injected and analyzed with Microcal Origin software supplied by the manufacturer.

CD spectroscopy: CD spectra were recorded at 25 °C and pH 6.8 in buffer A either with or without $Hg(ClO_4)_2$ (1 µM) on a JASCO J-720 spectropolarimeter interfaced with a microcomputer. The cell path-length was 1 cm. The concentration of the duplex DNA (INSF25X:INSR25Y) used was 1 µM.

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[1] a) D. E. Draper, *Biophys. J.* **2008**, *95*, 5489–5495; b) E. A. Aleman, R. Lamichhane, D. Rueda, *Curr. Opin. Chem. Biol.* **2008**, *12*, 647–654.
[2] a) J. K. Frederiksen, J. A. Piccirilli, *Methods* **2009**, *49*, 148–166; b) J. Schnabl, R. K. Sigel, *Curr. Opin. Chem. Biol.* **2010**, *14*, 269–275.
[3] a) E. Gazit, *FEBS J.* **2007**, *274*, 317–322; b) F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795–1799.
[4] a) E. Meggers, P. Holland, W. Tolman, F. Romesberg, P. Schultz, *J. Am. Chem. Soc.* **2000**, *122*, 10714–10715; b) H. Weizman, Y. Tor, *J. Am. Chem. Soc.* **2001**, *123*, 3375–3376; c) S. Atwell, E. Meggers, G. Spraggon, P. G. Schultz, *J. Am. Chem. Soc.* **2001**, *123*, 12364–12367; d) K. Tanaka, Y. Yamada, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 8802–8803; e) K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shiro, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 12494–12498; f) N. Zimmermann, E. Meggers, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 13684–13685; g) K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, *Science* **2003**, *299*, 1212–1213; h) M. Shionoya, K. Tanaka, *Curr. Opin. Chem. Biol.* **2004**, *8*, 592–597; i) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem.* **2007**, *119*, 6340–6350; *Angew. Chem. Int. Ed.* **2007**, *46*, 6226–6236.
[5] a) F. A. Polonius, J. Muller, *Angew. Chem.* **2007**, *119*, 5698–5701; *Angew. Chem. Int. Ed.* **2007**, *46*, 5602–5604; b) M. K. Schlegel, L. O. Essen, E. Meggers, *J. Am. Chem. Soc.* **2008**, *130*, 8158–8159.
[6] a) A. Ono, H. Togashi, *Angew. Chem.* **2004**, *116*, 4400–4402; *Angew. Chem. Int. Ed.* **2004**, *43*, 4300–4302; b) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173; c) I. Okamoto, K. Iwamoto, Y. Watanabe, Y. Miyake, A. Ono, *Angew. Chem.* **2009**, *121*, 1676–1679; *Angew. Chem. Int. Ed.* **2009**, *48*, 1648–1651; d) A. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, S. Oda, Y. Miyake, I. Okamoto, Y. Tanaka, *Chem. Commun.* **2008**, 4825–4827; e) R. M. Izatt, J. J. Christensen, J. H. Rytting, *Chem. Rev.* **1971**, *71*, 439–481; f) R. B. Simpson, *J. Am. Chem. Soc.* **1964**, *86*, 2059–2065; g) L. D. Kosturko, C. Folzer, R. F. Stewart, *Biochemistry* **1974**, *13*, 3949–3952; h) T. Yamane, N. Davidson, *Biochim. Biophys. Acta* **1962**, *55*, 780–782; i) T. Yamane, N. Davidson, *J. Am. Chem. Soc.* **1961**, *83*, 2599–2607; j) S. Katz, *Biochim. Biophys. Acta* **1963**, *68*, 240–253.
[7] T. Wiseman, S. Williston, J. F. Brandts, L. N. Lin, *Anal. Biochem.* **1989**, *179*, 131–137.
[8] a) H. Arakawa, R. Ahmad, M. Naoui, H. A. Tajmir-Riahi, *J. Biol. Chem.* **2000**, *275*, 10150–10153; b) A. A. Ouameur, S. Nafisi, N. Mohajerani, H. A. Tajmir-Riahi, *J. Biomol. Struct. Dyn.* **2003**, *21*, 561–565; c) A. A. Ouameur, H. Arakawa, R. Ahmad, M. Naoui, H. A. Tajmir-Riahi, *DNA Cell Biol.* **2005**, *24*, 394–401; d) J. Wu, F. Du, P. Zhang, I. A. Khan, J. Chen, Y. Liang, *J. Inorg. Biochem.* **2005**, *99*, 1145–1154; e) E. Stellwagen, Q. Dong, N. C. Stellwagen, *Biochemistry* **2007**, *46*, 2050–2058; f) K. Utsuno, *Chem. Pharm. Bull.* **2008**, *56*, 247–249; g) Y. Li, Y. L. Xia, Y. Jiang, X. P. Yan, *Electrophoresis* **2008**, *29*, 1173–1179.
[9] J. J. Fox, N. Yung, J. Davoll, G. B. Brown, *J. Am. Chem. Soc.* **1956**, *78*, 2117–2122.
[10] a) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, *J. Am. Chem. Soc.* **2007**, *129*, 244–245; b) Y. Tanaka, A. Ono, *Dalton Trans.* **2008**, 4965–4974.
[11] D. C. Rau, V. A. Parsegian, *Biophys. J.* **1992**, *61*, 260–271.
[12] a) D. R. Rosseinsky, *Chem. Rev.* **1965**, *65*, 467–490; b) G. Chillemi, G. Mancini, N. Sanna, V. Barone, S. Della Longa, M. Benfatto, N. V. Pavel, P. D′Angelo, *J. Am. Chem. Soc.* **2007**, *129*, 5430–5436.
[13] A. A. Rashin, B. Honig, *J. Phys. Chem.* **1985**, *89*, 5588–5593.

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[14] a) A. K. Chandra, M. T. Nguyen, T. Uchimaru, T. Zeegers-Huyskens, *J. Phys. Chem. A* **1999**, *103*, 8853–8860; b) Y. Q. Huang, H. Kenttamaa, *J. Phys. Chem. A* **2003**, *107*, 4893–4897.
[15] H. Fukada, K. Takahashi, *Proteins* **1998**, *33*, 159–166.
[16] T. van der Wijst, C. Fonseca Guerra, M. Swart, F. M. Bickelhaupt, B. Lippert, *Chem. Eur. J.* **2009**, *15*, 209–218.

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