

## Mercury<sup>II</sup>-Mediated Formation of Thymine–Hg<sup>II</sup>–Thymine Base Pairs in DNA Duplexes

Yoko Miyake,<sup>†</sup> Humika Togashi,<sup>†</sup> Mitsuru Tashiro,<sup>‡</sup> Hiroshi Yamaguchi,<sup>§</sup> Shuji Oda,<sup>§</sup> Megumi Kudo,<sup>§</sup> Yoshiyuki Tanaka,<sup>\*,§</sup> Yoshinori Kondo,<sup>§</sup> Ryuichi Sawa,<sup>||</sup> Takashi Fujimoto,<sup>‡</sup> Tomoya Machinami,<sup>‡</sup> and Akira Ono<sup>\*,†,#</sup>

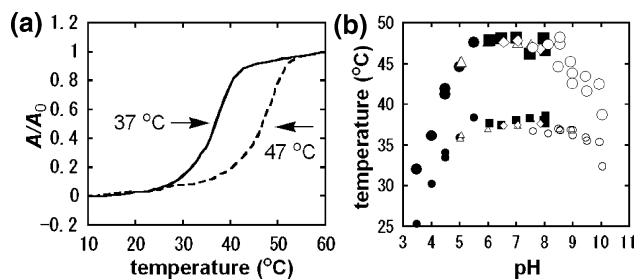
Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji, Tokyo 192-0397 Japan, Department of Chemistry, College of Science and Technology, Meisei University, Hino, Tokyo 191-8506, Japan, Tohoku University, Aobayama, Aoba-ku, Sendai, Miyagi 980-8578 Japan, Microbial Chemistry Research Center, 3-14-23 Kamiosaki, Shinagawa, Tokyo 141-0021, Japan, and Department of Applied Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rookakubashi, Kanagawa-ku, Yokohama-shi, Kanagawa-ken 221-8686, Japan

Received September 27, 2005; E-mail: akiraono@kanagawa-u.ac.jp; tanaka@mail.pharm.tohoku.ac.jp

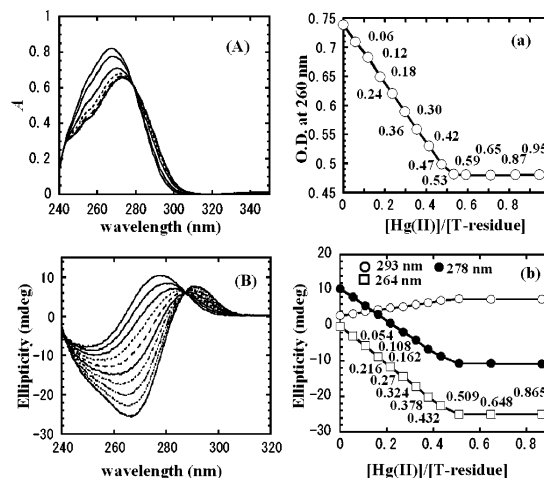
The interactions of metal ions with nucleic acids, nucleosides, and nucleobases have been investigated extensively.<sup>1</sup> Owing to the high toxicity of mercury, its behavior in cells has been of prime interest, and DNA–mercury interactions have been recognized as involving the denaturing of DNA duplex structures.<sup>1–7</sup> In this report, contrary to existing studies, we describe the stabilization of DNA duplexes via the formation of a Hg<sup>II</sup>-mediated base pair, thymine–Hg<sup>II</sup>–thymine (T–Hg<sup>II</sup>–T).

Figure 1a shows the thermally induced transition profiles of a DNA duplex, (A)<sub>10</sub>T(A)<sub>10</sub>–(T)<sub>21</sub>, containing the T–T mispair, in the presence and absence of Hg<sup>II</sup> ions. The transition profiles shifted to higher temperatures in the presence of Hg<sup>II</sup> (from 37 to 47 °C). Further comparison of the *T*<sub>m</sub> (47 °C) of the duplex containing the T–T mispair in the presence of Hg<sup>II</sup> ions (Figure 1a) with the *T*<sub>m</sub> (44 °C) of the control duplex, 5′-(dA)<sub>21</sub>-3′/5′-T<sub>21</sub>-3′, revealed that the Hg<sup>II</sup> ion-mediated T–Hg–T pair was more stable than the Watson–Crick (WC) A–T pair. Other metals known to bind to nucleic acids,<sup>1,2</sup> such as Ag<sup>I</sup>, Cu<sup>II</sup>, Ni<sup>II</sup>, Pd<sup>II</sup>, Co<sup>II</sup>, Mn<sup>II</sup>, Zn<sup>II</sup>, Pb<sup>II</sup>, Cd<sup>II</sup>, Mg<sup>II</sup>, Ca<sup>II</sup>, Fe<sup>II</sup>, Fe<sup>III</sup>, and Ru<sup>III</sup>, did not induce any notable effects on the thermal transition profiles (Supporting Information). Consequently, the stabilizing effect of Hg<sup>II</sup> on the T–T mispair surpassed the effects of other metals and appeared to be highly specific. A study using solutions at various pH values revealed that the addition of Hg<sup>II</sup> stabilized duplex formation over the entire pH range tested (Figure 1b).

In the presence of Hg<sup>II</sup> ions, the duplexes containing consecutive T–T mispairs (5′-GTGACCATTTCAGTG-3′/5′-CA CTGCAT-TTGGTCAC-3′ and 5′-d(GTGACCATTTGCAGT G)-3′/5′-CAC-TGCATTTGGTCAC-3′) were significantly stabilized, depending on the number of T–T mispairs (Supporting Information). The formation of T–Hg<sup>II</sup>–T pair(s) in the duplexes was also detected by electrospray ionization mass spectrometry (ESI-MS; Supporting Information). This indicates that T–Hg<sup>II</sup>–T pairs are stable enough to give molecular ion peaks of the corresponding duplexes. Figure 2A shows the absorption spectra of 5′-d(T<sub>3</sub>)-3′ in the presence of various concentrations of Hg<sup>II</sup> ion. The optical density decreased as the Hg<sup>II</sup> ion concentration increased (Figure 2A). A clear transition point at a [Hg<sup>II</sup>] to [thymine residues] ratio of around 1:2 was revealed by plotting the optical density against the Hg<sup>II</sup> concentration (Figure 2a), indicating the formation of complexes containing one Hg<sup>II</sup> and two thymine residues (T–Hg<sup>II</sup>–T pairs).



**Figure 1.** (a) Relative absorbance,  $A = [(A_{260} - A_{100°C}) / (A_{60°C} - A_{100°C})]$  at 260 nm versus temperature for a mixture of 5′-d(A)<sub>10</sub>T(A)<sub>10</sub>-3′ and 5′-T<sub>21</sub>-3′. Each solution contained 1 μM oligomer in 10 mM Mops, 100 mM NaNO<sub>3</sub>, pH 7.1. Solid line (—), in the absence of metal; dotted line (---), in the presence of 2 μM Hg(ClO<sub>4</sub>)<sub>2</sub>. The *T*<sub>m</sub> of the duplex 5′-(dA)<sub>21</sub>-3′/5′-T<sub>21</sub>-3′ is 44 °C and was unaffected by the addition of 2 μM Hg(ClO<sub>4</sub>)<sub>2</sub>. The temperature ramp was 1 °C min<sup>-1</sup>. (b) *T*<sub>m</sub> at 260 nm versus pH for the mixtures. Each solution contained 1 μM oligomer, 2 μM Hg(ClO<sub>4</sub>)<sub>2</sub>, and 100 mM NaNO<sub>3</sub> in an appropriate buffer [(10 mM): solid circles, Na-acetate; solid squares, Na-cacodylate; open triangles, Na-phosphate; open diamonds, Na-Mops; open circles, Na-borate] in the absence (small font) or presence of metal ions (large font).



**Figure 2.** (A) Absorption response of 5′-d(T<sub>3</sub>)-3′ on addition of Hg<sup>II</sup> ions. (a) The optical density (at 260 nm) versus Hg<sup>II</sup> ion concentration. (B) CD spectra of 5′-d(T<sub>3</sub>)-3′ on addition of Hg<sup>II</sup> ions. (b) Ellipticity versus Hg<sup>II</sup> ion concentration. Each solution contained 30 μM oligomer in 10 mM Mops, 20 mM NaNO<sub>3</sub>, pH 7.1. Each spectrum was measured at 20 °C.

Consequently, these results indicate the formation of a double helical structure consisting of only T–Hg–T pairs. The CD spectra (Figure 2Bb)<sup>8</sup> and ESI-MS results (Supporting Information) also indicate the formation of the double helical structure.<sup>9</sup>

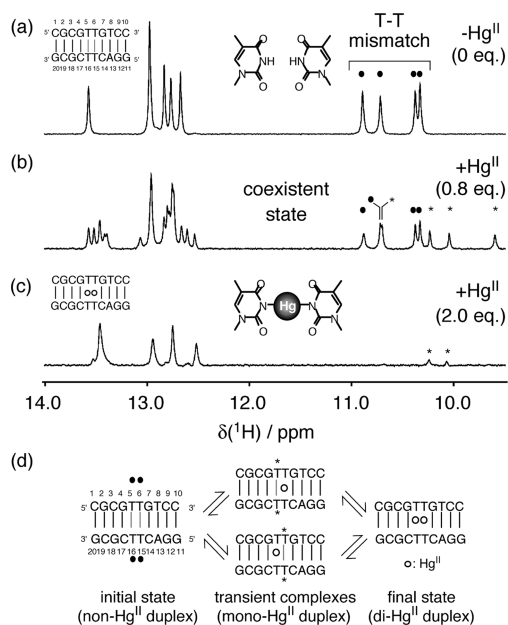
<sup>†</sup> Tokyo Metropolitan University.

<sup>‡</sup> Meisei University.

<sup>§</sup> Tohoku University.

<sup>||</sup> Microbial Chemistry Research Center.

<sup>#</sup> Kanagawa University.



**Figure 3.** The imino proton region of the 1D  $^1\text{H}$  NMR spectra of DNA– $\text{Hg}^{\text{II}}$  complexes. One-dimensional  $^1\text{H}$  NMR spectra were recorded at 23 °C on a JEOL ECA600 spectrometer, with a spectral width of 15 024 Hz digitized into 16 384 complex points, and 128 scans were averaged. The spectra were processed with an exponential window function to give a line-broadening of 3 Hz. (a) A 1D  $^1\text{H}$  NMR spectrum in the absence of  $\text{Hg}^{\text{II}}$ . (b) In the presence of  $\text{Hg}^{\text{II}}$  (0.8 equiv to the duplex). (c) In the presence of  $\text{Hg}^{\text{II}}$  (2.0 equiv to the duplex). (d) The equilibrium system between the duplex and  $\text{Hg}^{\text{II}}$ .

According to reports from the 1960s, protons are released when  $\text{Hg}^{\text{II}}$  ions bind to thymine residues in DNA.<sup>2</sup> Later, Katz proposed the formation of mercury–thymine (1:2) complexes in denatured DNA.<sup>3</sup> Coincidentally, the mercury–thymine (1:2) complex had been used in nucleoside synthesis procedures,<sup>10</sup> the so-called “mercury method”, a traditional synthetic method for coupling glycosyl halides and bases. The elemental analysis of this complex showed that two HCl molecules disappear during the complex formation process. Consequently, it had been assumed that thymine residues bind to  $\text{Hg}^{\text{II}}$  through covalent N– $\text{Hg}$  bonds. The crystal structure of a 2:1 complex of 1-methylthymine– $\text{Hg}^{\text{II}}$  supports this idea.<sup>6</sup> However, no definitive conclusion has been drawn for the arrangement of T– $\text{Hg}^{\text{II}}$ –T base pairs in nucleic acids until now.

To confirm the dissociation of the imino protons of thymine residues when T– $\text{Hg}^{\text{II}}$ –T pairs form in DNA duplexes,  $^1\text{H}$  NMR spectroscopy of the duplex– $\text{Hg}^{\text{II}}$  complexes was performed. Of the sequences we examined, the duplex 5′-CGCGTTGTCC-3′:5′-GGACTTCGCG-3′ (the two T–T mismatches are in bold) gave the most clearly interpreted spectrum.<sup>11</sup> In the absence of  $\text{Hg}^{\text{II}}$ , four imino proton resonances of the T–T mismatches (10.4–11.1 ppm) were observed, in conjunction with those of the WC base pairs (12.5–13.5 ppm; Figure 3a). However, with the addition of 2.0 molar equiv of  $\text{Hg}^{\text{II}}$ , the imino proton resonances of the T–T mismatches almost disappeared (Figure 3c), suggesting that the imino protons of the T–T mismatches in the  $\text{Hg}^{\text{II}}$ -free duplex were substituted with  $\text{Hg}^{\text{II}}$  ions. Interestingly, the imino proton resonances from the  $\text{Hg}^{\text{II}}$ -free and  $\text{Hg}^{\text{II}}$ -bound states were observed separately, under  $\text{Hg}^{\text{II}}$ -unsaturated conditions (Figure 3b and Supporting Information). This indicates that the  $\text{Hg}^{\text{II}}$  association and dissociation processes occur slowly relative to the NMR time scale. Combining the data, we conclude that  $\text{Hg}^{\text{II}}$  binds directly to N3 of thymidine in place of the imino proton and bridges two thymidine residues to form the T– $\text{Hg}^{\text{II}}$ –T pair (a scheme in Figure 3c). In the T– $\text{Hg}^{\text{II}}$ –T, the

mercury nuclei are neutral as suggested by the previous reports.<sup>6,10</sup> Since the van der Waals radius of mercury is  $\sim 1.44$  Å and the base pair spacing in DNA duplex is  $\sim 3.4$  Å, it is suggested that mercury can be incorporated without largely altering the double helical structure.

Recently, synthetic oligodeoxyribonucleotides containing artificial bases have been used to form metal-mediated base pairs in which the hydrogen bonds in W–C-type base pairs in natural DNA are replaced by metal–base bonds.<sup>12</sup> In this paper, we report an alternative method for generating metal-mediated base pairs in DNA duplexes using only naturally occurring pyrimidine bases. Namely, T–T mismatches in DNA duplexes selectively capture  $\text{Hg}^{\text{II}}$  ions, and the metal-mediated T– $\text{Hg}^{\text{II}}$ –T base pairs form in DNA duplexes. The natural thymine bases shown to form stable metal-mediated base pairs are of prime interest as the phenomenon may affect many scientific fields.<sup>13–15</sup>

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**Supporting Information Available:** The thermal denaturation profiles of duplexes containing T–T mismatches in the presence of various metal ions; ESI-MS profiles and NMR spectra of the DNA– $\text{Hg}^{\text{II}}$  complexes; and UV–vis and CD spectra of T<sub>5</sub> in the presence of  $\text{Hg}^{\text{II}}$ . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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