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# Long-Distance Radical Cation Hopping in DNA: The Effect of Thymine–Hg(II)–Thymine Base Pairs

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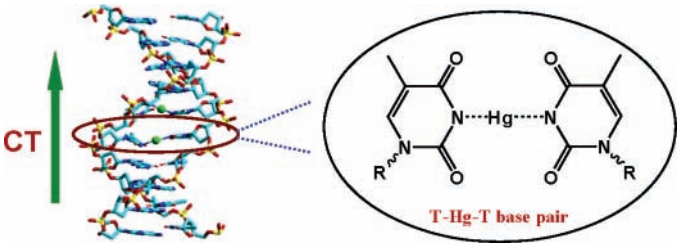
## ABSTRACT




**Thymine–Hg(II)–thymine base pairs have been incorporated in an oligonucleotide duplex to study their effect on DNA-mediated charge transport. The introduction of a formally charged Hg atom inside the DNA base core does not significantly alter the charge hopping and trapping properties, as discussed in this paper. Hg(II) replaces the protons normally found on thymines within the complex and acts like a "big proton" in terms of its role in DNA charge transport.**

Ono and co-workers recently demonstrated that the addition of $Hg(NO_3)_2$ to duplex DNA oligomers that contain thymine−thymine (T−T) mismatches results in its deprotonation and the formation of mercuric ion containing oligomers having thymine−Hg(II)−thymine (T−Hg−T) base pairs with the structure shown in Figure 1.[1] DNA melting and circular dichroism (CD) studies revealed that formation of the T−Hg−T base pairs increases the duplex stability when compared with the corresponding oligomers that contain A−T base pairs or the mismatched T−T base pairs, without affecting the B-form of DNA. These findings have importance in the design of metal-containing DNA duplexes for possible application in DNA-based nanoelectronics.[2−12] Also, the possibility of using such thymine−Hg complexes as a Hg(II) sensor has been considered.[4,13,14]

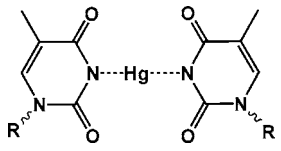


**Figure 1.** Structure of the thymine−Hg(II)−thymine complex.

(1) Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; Machinami, T.; Ono, A. *J. Am. Chem. Soc.* **2006**, *128*, 2172−2173.
(2) Di Ventra, M.; Zwolak, M. In *DNA Electronics*; Nalwa, H. S., Ed.; American Scientific Publishers: Stevenson Ranch, California, 2004; Vol. 2, pp 475−493.
(3) Taniguchi, M.; Kawai, T. *Phys. E* **2006**, *33*, 1−12.
(4) Ono, A. *Macromol. Chem. Phys.* **2006**, *207*, 1629−1632.
(5) Stanca, S. E.; Ongaro, A.; Eritja, R.; Fitzmaurice, D. *Nanotechnology* **2005**, *16*, 1905−1911.
(6) Wettig, S. D.; Wood, D. O.; Aich, P.; Lee, J. S. *J. Inorg. Biochem.* **2005**, *99*, 2093−2101.
(7) Tanaka, K.; Clever, G. H.; Takezawa, Y.; Yamada, Y.; Kaul, C.; Shionoya, M.; Carell, T. *Nat. Nanotechnol.* **2006**, *1*, 190−195.
(8) Richter, J. *Phys. E* **2003**, *16*, 157−173.
(9) Krizanovic, O.; Sabat, M.; Beyerlepfnur, R.; Lippert, B. *J. Am. Chem. Soc.* **1993**, *115*, 5538−5548.
(10) Tabata, H.; Cai, L. T.; Gu, J. H.; Tanaka, S.; Otsuka, Y.; Sacho, Y.; Taniguchi, M.; Kawai, T. *Synth. Met.* **2003**, *133*, 469−472.
(11) Zimmermann, N.; Meggers, E.; Schultz, P. G. *Bioorg. Chem.* **2004**, *32*, 13−25.
(12) Weizman, H.; Tor, Y. *J. Am. Chem. Soc.* **2002**, *124*, 1568−1569.

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|---|---|
| DNA(1) | 5'-AQ-AA TTGG$_1$TTGG$_2$TTGG$_3$TTGG$_4$TTGG$_5$TTGG$_6$TTAT AT*-3'<br>3'-TT AACC AACC AACC AACC AACC AACC AATA TA- 5' |
| DNA(2) | 5'-AQ-AA TTGG$_1$TTGG$_2$TTGG$_3$TTGG$_4$TTGG$_5$TTGG$_6$TTAT AT*-3'<br>3'-TT AACC AACC TTCC AACC AACC AACC AATA TA- 5' |

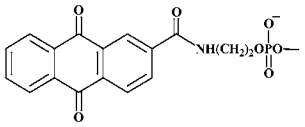


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**Figure 2.** Structure of regular and mismatched DNA duplexes used in this study: * indicates the presence and location of a $^{32}$P radiolabel.

Long-distance radical cation transfer in DNA duplexes has been studied over the past decade, and a hopping mechanism has been revealed.[15−18] Numerous experiments have been designed to explore base sequence effects[19] and the effect of unnatural nucleotides[20] on charge transfer. In an experiment of special relevance to the current study, we assessed the results of incorporating a positively charged pyridinium nucleoside in duplex DNA on charge transport.[21] Not unexpectedly, hopping is perturbed when the migrating radical cation encounters this positively charged species.

Incorporation of the T−Hg−T complex in a duplex provides a unique system for investigating the effect of a formally charged metal in the central DNA core on radical cation hopping. In this regard, Voityuk recently reported the results of a theoretical investigation of the effect of T−Hg−T base pairs on DNA-mediated charge transport.[22] These quantum calculations indicate that the valence orbitals of the mercury ions do not participate significantly in mediating the electronic coupling for radical cation transfer. Interestingly, however, the calculations do reveal that T−Hg−T base pairs may play a role in an excess electron-transfer process in DNA in that the electronic coupling ($V_{da}$) of GC donor and acceptor sites separated by two or more T−Hg−T base pairs differs from that separated by canonical A−T base pairs.[22] We report herein studies designed to assess experimentally the effect of T−Hg−T base pairs on radical cation transport in DNA.

The duplex oligonucleotides shown in Figure 2 were prepared by standard means for use in the analysis of the effect of T−Hg−T base pairs on radical cation hopping.[23,24] Both DNA(1) and DNA(2) have an anthraquinone photosensitizer (AQ) linked to a 5′-terminus. Irradiation of the AQ at 350 nm introduces a radical cation into the duplex.[23] DNA(1) contains a fully complementary $(TTGG)_6$ segment that has been shown previously to effectively promote regular long-distance radical cation transfer.[24] In contrast, the $(TTGG)_6$ segment of DNA(2) is not fully complementary, as it contains tandem T−T mismatches that are located between the $GG_2$ and $GG_3$ steps. Any perturbation that results from the mismatches, or their conversion to T−Hg−T base pairs, that affects either the rate of charge hopping or trapping in these sequences will be directly reflected in the relative amounts of strand cleavage observed at the GG steps.

DNA(1) has a melting temperature ($T_m$) of 56 °C in a 10 mM sodium phosphate buffer solution (pH 7.0), whereas DNA(2), which is identical except for the presence of two T−T mismatches, not unexpectedly, is destabilized with a $T_m$ of 49 °C (see Figure 3). Circular dichroism studies show that DNA(1) and DNA(2) both have overall B-form helix structures. The melting behavior of DNA(1) is unaffected by the addition of Hg(II) up to a mole ratio of ca. 4:1, as shown in Figure 3A. On the other hand, addition of Hg(II) to a solution of DNA(2) results in a remarkable increase in $T_m$. The extent of the $T_m$ increase depends sensibly upon the concentration of Hg(II) and reaches a plateau of ca. 60 °C at a ratio of one Hg(II) ion for each T−T mispair ($\Delta T_m \sim$ 15 °C), and this value changes little with further increase in Hg(II) concentration (see Figure 3B). This finding is in agreement with the formation of T−Hg−T base pairs at T−T mismatches as reported by Ono and co-workers.[1] The CD spectra of DNA(1) and DNA(2) are largely unaffected by the addition of Hg(II) ions at the concentrations employed in this work, which indicates that the formation of the T−Hg−T base pairs does not cause a gross structural change to the DNA.

Irradiation of an AQ-containing DNA duplex at 350 nm results in excitation of the AQ and its consequent injection

(13) Tang, Y. L.; He, F.; Yu, M. H.; Feng, F. D.; An, L. L.; Sun, H.; Wang, S.; Li, Y. L.; Zhu, D. B. *Macromol. Rapid Commun.* **2006**, *27*, 389−392.
(14) Wang, Z.; Zhang, D. Q.; Zhu, D. B. *Anal. Chim. Acta* **2005**, *549*, 10−13.
(15) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747−8748.
(16) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8353−8358.
(17) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(18) O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471−11483.
(19) Joseph, J.; Schuster, G. B. *J. Am. Chem. Soc.* **2006**, *128*, 6070−6074.
(20) Joy, A.; Guler, G.; Ahmed, S.; McLaughlin, L. W.; Schuster, G. B. *Faraday Discuss.* **2006**, *131*, 357−365.
(21) Onyemauwa, F. O.; Schuster, G. B. *Org. Lett.* **2006**, *8*, 5255−5258.
(22) Voityuk, A. A. *J. Phys. Chem. B* **2006**, *110*, 21010−21013.
(23) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(24) Liu, C.-S.; Hernandez, R.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 2877−2884.

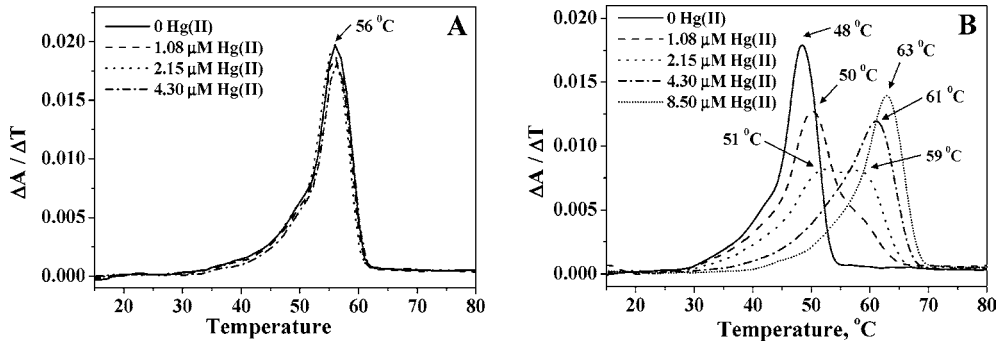


**Figure 3.** First derivative plots of the DNA melting curves for (A) DNA(1) and (B) DNA(2) in the presence of various amounts of $Hg^{2+}$ salt (added as $Hg(ClO_4)_2$) in 10 mM phosphate buffer (pH 7.0). The duplex DNA concentration is 1 $\mu$M in all cases.

of a radical cation into the DNA by the one-electron oxidation of an adjacent base. The radical cation thus formed can hop through the duplex until it is trapped (at GG steps in the current case) by a reaction with $O_2$ and/or $H_2O$.[23,25] The products of these reactions result in DNA strand scission at the "damaged" site upon subsequent treatment of irradiated samples with piperidine. The pattern of strand cleavage that results is analyzed by polyacrylamide gel electrophoresis (PAGE) to reveal the extent and distribution of cleavage at the individual GG steps and by phosphorimagery to yield quantitative results. Under single-hit conditions, the amount of strand cleavage observed is proportional to the amount of reaction at each location. Figure 4 shows the PAGE results from irradiation of DNA(1) and DNA(2) in solutions that contain various concentrations of Hg(II).

As expected, UV irradiation of DNA(1) results in detectable strand cleavage at each of its six GG steps with reaction occurring primarily at the 5′-G (see Figure 4). The addition of Hg(II), up to a maximum concentration of four times that of the DNA duplex, results in no measurable change in the location or the yield of strand cleavage as shown in Figure 5.

The cleavage pattern for DNA(2) is strikingly different from that of DNA(1). Introduction of the tandem T−T mismatches between $GG_2$ and $GG_3$ reduces significantly the yield of strand cleavage observed at $GG_2$ without affecting measurably the amount of strand cleavage at $GG_3$ or any other GG step in the duplex (see Figures 4 and 5). The influence of these mismatches is surprisingly local, which indicates that they affect the probability that the radical will be trapped irreversibly at $GG_2$ but do not, to a significant extent, affect the rate of radical cation hopping. This result is especially meaningful because the rate of radical cation hopping from one GG step to the next is comparable with that of its trapping by reaction with $O_2$ or $H_2O$ ($k_{hop}/k_{trap}$ = 10); i.e., the semilog plot (Figure 5) has a slope of −0.02 $Å^{-1}$.[19,24]

The effect of the inclusion of Hg(II) ions at the T−T mismatches of DNA(2) is also shown in Figures 4 and 5. The behavior of the duplex when it contains T−Hg−T base pairs is not measurably different from the case when these sites are simple tandem T−T mismatches. These results indicate that for these sequences replacement of two adjacent T−A base pairs with tandem T−T mismatches or with tandem T−Hg−T base pairs does not affect the rate of radical cation hopping through the duplex to an extent great enough to reduce the amount of strand cleavage detected at GG steps that occur after the mismatch or the metal-containing base pairs. On the other hand, the rather small change to $k_{trap}$ at $GG_2$ that the mismatch or T−Hg−T base pairs cause is reflected in the reduced yield of strand cleavage at that site.

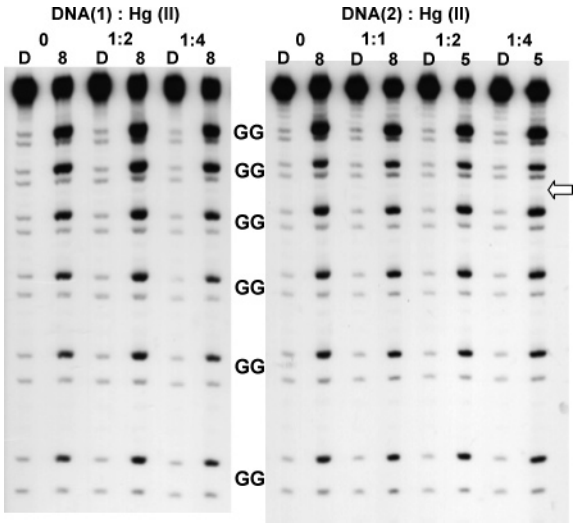


**Figure 4.** Autoradiograms of PAGE gels following the irradiation of DNA(1) and DNA(2) in the presence and absence of Hg(II) ions. The lanes labeled D are the dark controls, with no irradiation. The samples in the lanes labeled 8 were irradiated for 8 min. The ratios of DNA duplex to Hg(II) molar concentrations (0, 1:1, 1:2, and 1:4) are indicated above the relevant lanes. All samples were treated with piperidine (1 M) for 30 min at 90 °C after irradiation but before gel electrophoresis. GG indicates the position of GG steps, and the arrow shows the position of TT mismatches or T−Hg(II)−T base pairs in DNA(II).

(25) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692−9694.

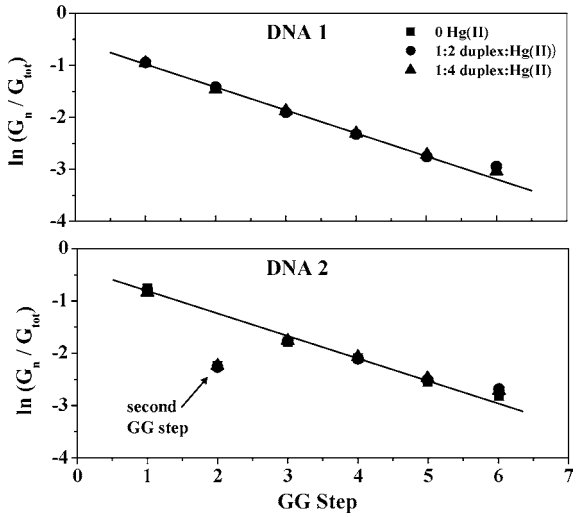


**Figure 5.** Semilog plots showing the ratio of DNA damage at different GG steps for DNA(1) and DNA(2) in the presence and absence of Hg(II) ions. Standard errors estimated from replicate measurements are less than 5% of the reported values. The slope of the line for DNA(1) is $-0.02$ Å$^{-1}$ based upon assignment of 3.4 Å per base pair.

Evidently, the introduction of Hg(II) ions into the core of a DNA duplex has a negligible effect on the radical cation hopping rate. This finding is consistent with Voityuk's prediction that the valence orbitals of Hg(II) ions do not participate essentially in mediating the electronic coupling for radical cation transfer. The computed difference in electronic coupling ($V_{da}$) between an A−T and a T−Hg−T base pair is too small to translate to an observable difference in charge-transfer processes in these systems.

In conclusion, we have incorporated a formally positively charged metal in a DNA duplex through the introduction of T−T mismatches followed by complexation with Hg(II). Charge-transfer results show that the introduction of a formally charged metal in this form does not significantly affect the charge hopping rates for radical cations in these duplex DNA oligomers. From a practical point of view, the Hg(II) that replaces the protons normally found at these thymines within the duplex core may be considered to be acting like a "big proton" in terms of its role in DNA charge transport.

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**Supporting Information Available:** General experimental procedures, mass spectra of oligonucleotide strands, and CD spectra of the duplexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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