Modulation of DNA-Mediated Hole-Transport Efficiency by Changing Superexchange Electronic Interaction

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Received November 24, 1999
Revised Manuscript Received April 18, 2000

While DNA-mediated charge transport (CT) has been experimentally verified on DNAs containing various electron donor−acceptor systems,[1−7] the efficiency and mechanism of CT still remain unclear.[2,8,9] One mechanism assuming instantaneous delocalization of base radical cation (hole) over all DNA bases by superexchange[10] has recently been acknowledged to be less likely in long-range hole transport (HT); however, the hole can travel a long distance by a consecutive hopping process between neighboring guanines (Gs).[1a,2c,4b,7] The basis of the hopping mechanism is that a guanine radical cation ($G^{\bullet+}$) cannot oxidize adenine (A) due to the higher ionization potential (IP) of A compared with that of G, but can oxidize another G. We previously reported that (i) the IP of G is highly dependent on the flanking sequences and (ii) stacked Gs such as G doublet (GG) and triplet (GGG) possessing much lower IPs than that of isolated G can serve as an effective hole trap.[11] As a consequence, when a hole donor is a radical cation of G triplet $(GGG)^{\bullet+}$, an isolated G cannot be a hole acceptor due to the large free energy required for the process,[4b] but may act as a bridged base lowering the IP of the bridge between two G triplets. It is actually predicted by electron transfer theory that lowering the IP of a bridge increases the electronic coupling for the superexchange interaction between donor and acceptor.[8,9] To know more closely the effect of IP of bridged bases on HT efficiency, we have examined HT between two G triplets separated by a bridge of TT*B*TT containing a bridged base (*B*) of A, 7-deazaA (^{z}A), G, or 7-deazaG (^{z}G). We herein report for the first time that the efficiency of DNA-mediated HT markedly increases with decreasing IP of the bridged base. Furthermore, ^{z}G was shown to be an extremely efficient trap in HT through the DNA π-stack.

Table 1. Sequences of Oligomers Used for HT Experiments[a]

1	3′-M-**X**C ACAC CCAA TAAC CC-V-5′
2	5′-N-A**G$_2$**TGT**G$_6$GG**TT **A**TT**G$_{14}$GG**-W-3′
3	5′-N-A**G$_2$**TGT**G$_6$GG**TT**^{z}A**TT**G$_{14}$GG**-W-3′
4	3′-M-**X**C ACAC CCAA CAAC CC-V-5′
5	5′-N-A**G$_2$**TGT**G$_6$GG**TT **G**TT**G$_{14}$GG**-W-3′
6	5′-N-A**G$_2$**TGT**G$_6$GG**TT**^{z}G**TT**G$_{14}$GG**-W-3′

[a] X represents $d^{CNBP}U$. X, G_2, two G triplets, and bridged bases are shown in bold face. M = TAAATA, N = ATTTAT, V = AATAATA, W = TTATTAT.

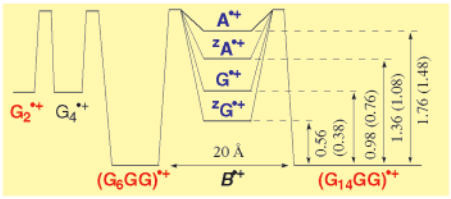

Figure 1. Schematic illustration of energy diagram for HT from $G_2^{\bullet+}$ to $(G_{14}GG)^{\bullet+}$ via putative $B^{\bullet+}$ with estimates of free energy change (ΔG, eV).[14] The numbers in parentheses are the difference of IPs (eV) between GGG and *B*.

The modulation of HT efficiency by changing the IP of a bridged base was investigated on a 29-mer duplex containing a probe sequence of 5′-A_1G_2T GTG_6 GGT T*B*T $TG_{14}G$ G-3′ with a bridged base (*B*) of A (ODN **2**), ^{z}A (ODN **3**), G (ODN **5**), or ^{z}G (ODN **6**) (Table 1). Upon photoirradiation of the duplex, the hole was site-selectively generated at G_2 by a single electron transfer to a photoexcited cyanobenzophenone-substituted 2′-deoxyuridine ($d^{CNBP}U$) opposite A_1 in the complementary strand (ODNs **1** and **4**), and then irreversibly migrated to a proximal G triplet (G_6GG).[7,12] A distal G triplet ($G_{14}GG$) is separated from a proximal G_6 triplet by five base pairs via TT*B*TT with a bridged base *B* in the middle. The calculated IPs of GGG, A, ^{z}A, G, and ^{z}G at B3LYP/6-31G(d) were 4.17, 5.66, 5.25, 4.93, and 4.55 eV, respectively.[13,14] A schematic illustration of the energy diagram for HT from $G_2^{\bullet+}$ to $G_{14}GG$ is shown in Figure 1.

5′-^{32}P-End-labeled oligomers **2**, **3**, **5**, and **6** were annealed with their complementary strands. Duplexes **1**/**2**, **1**/**3**, **4**/**5**, and **4**/**6** were photoirradiated at 312 nm for 1 h. G oxidation sites were determined by densitometric assay of the cleavage bands after

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(14) Free energy changes (ΔG) for HT from $(GGG)^{\bullet+}$TT*B*TTGGG to GGGTT$B^{\bullet+}$TTGGG were estimated from total energy change (ΔE) obtained from the following equation:

$$(GGG)^{\bullet+} + B \rightarrow (GGG) + B^{\bullet+} + \Delta E$$

ΔGs (eV) were 1.76, 1.36, 0.98, and 0.56 for A, ^{z}A, G, and ^{z}G, respectively.

10.1021/ja994113y CCC: $19.00
Published on Web 06/06/2000

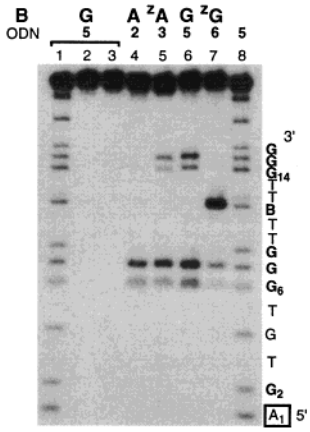


Figure 2. An autoradiogram of the denaturing sequencing gel for photoreactions of duplexes **1**/**2**, **1**/**3**, **4**/**5**, and **4**/**6**. 5′-^{32}P-End-labeled ODNs **2**, **3**, **5**, and **6** were hybridized to the complementary strand **1** or **4** (2 μM, strand concentration) in 10 mM sodium cacodylate at pH 7.0. Duplexes were irradiaded at 312 nm with transilluminator at 0 °C for 1 h under atmospheric conditions. After piperidine treatment (90 °C, 20 min), ODNs were electrophoresed through a denaturing 15% polyacrylamide/7 M urea gel. Lanes 1−3, 6, and 8, ODN **5**; lane 4, ODN **2**; lane 5, ODN **3**; lane 7, ODN **6**; ODNs in lanes 3−7 were photoirradiated; all ODNs except in lane 3 were heated with piperidine; lanes 1 and 8, Maxam−Gilbert G + A sequencing reactions for ODN **5**. Partial base sequences of ODNs were shown on the side. d^{CNBP}U was located opposite A_1 shown with a box.

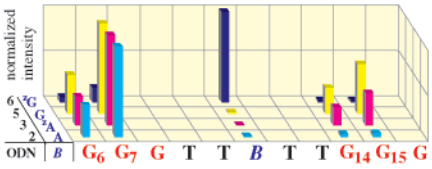
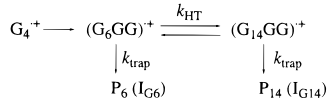


Figure 3. Graphical illustration of normalized intensities of cleavage bands at G_6, G_7, B, G_{14}, and G_{15} for oligomers **2**, **3**, **5**, and **6**. Data represents average of three data sets. Intensities are normalized so that the strongest cleavage is 1.00. I_{G14}/I_{G6} for ODNs **2**, **3**, and **5** was 0.05, 0.42, and 0.59, respectively.

hot piperidine treatment on the PAGE shown in Figure 2. As note in our previous communication,[7] the G oxidation increases linearly with irradiation time. Normalized intensities of the cleavage bands are graphically shown in Figure 3. G oxidation of ODN **2** having adenine as a bridged base occurred selectively at G_6 and G_7 in a proximal G_6 triplet (lane 4). Cleavage intensities decreased in the order middle G (G_7) ≫ 5′G (G_6), indicating a typical one-electron oxidation at the TGGGT sequence.[15] Band intensity at G_{14}GG relative to that at G_6GG (I_{G14}/I_{G6}) was only 0.05, confirming previous observations that HT through five AT base pairs proceeds with extremely low efficiency.[4b,7] In sharp contrast, cleavage at G_{14} was observed for ODNs **3** and **5** containing ^{z}A and G as a bridged base, respectively (lanes 5 and 6). I_{G14}/I_{G6} was 0.42 for ODN **3** (B = ^{z}A) and increased to 0.59 for ODN **5** (B = G). The trajectory of HT dramatically changed when ^{z}G was incorporated into the bridge between two G triplets. Intensive cleavage of ODN **6** occurred selectively at ^{z}G but not at all at the G_{14} triplet (lane 7, Figure 2).[16] Furthermore, the cleavage at G_6 of ODN **6** was significantly suppressed compared with those of ODNs **2**, **3**, and **5**, indicating that ^{z}G not only terminates HT but also effectively drags a hole into its own site.[17]

Our experiments described here clearly show that (i) HT through a bridge of five AT base pairs proceeds with extremely low efficiency, (ii) HT is effectively mediated when the bridge contains ^{z}A or G, (iii) cleavage intensities at the proximal G triplet are much higher than those at the distal G triplet, (iv) HT efficiency significantly increases by lowering the IP of the bridged base, and (v) HT is terminated at the site of ^{z}G. Stronger cleavage at the proximal G_6 triplet than at the distal G_{14} triplet observed for ODNs **2** and **3** indicates that the rate (k_{trap}) for trapping of $(GGG)^{\bullet+}$ with oxygen eventually giving piperidine labile products (P_6 and P_{14}) exceeds the rate (k_{HT}) for HT (Scheme 1).[7] Assuming

Scheme 1. Kinetic Scheme for Hole Hopping



a very weak directional preference of HT between two G triplets, the rate for HT relative to hole trapping for ODNs **2**, **3**, and **5** would be estimated by the I_{G14}/I_{G6} value. Lowering the IP of a bridged base by 0.32 eV (from ^{z}A to G) increased I_{G14}/I_{G6} 1.4-fold (Figure 3). These results clearly show that HT efficiency is sensitively modulated by IPs of the bridged base, suggesting that HT between two G triplets with bridges of TT^{z}ATT and TTGTT proceeds via a superexchange mechanism. Lowering the IP of the bridged base increased the electronic coupling for the superexchange interaction between the two G triplets.[18] Further lowering the IP at the bridged base, by replacing G with ^{z}G, resulted in HT from $(GGG)^{\bullet+}$ actually inducing oxidation of the bridged base, ^{z}G.[19] These results show that the efficiency of HT through DNA π-stack is highly sequence dependent.[20]

Acknowledgment. We thank Professor Michel-Beyerle (Technical University Munich) and Dr. Imahori (Osaka University) for valuable discussions.

Supporting Information Available: Autoradiography for photooxidation of duplex **4**/**6** with riboflavin, and HPLC profile of photoreaction of ODN containing ^{z}G (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA994113Y

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(16) HPLC analysis of the nucleoside mixture of photoirradiated duplex d(GTCCACXATC)/d(GATAGT^{z}G GAC) after heating with piperidine showed a complete disappearance of ^{z}G, whereas more than 80% of X (d^{CNBP}U) was recovered unchanged together with almost quantitative recovery of A, C, G, and T (see Supporting Information). This indicates that ^{z}G is actually oxidized and decomposed to a piperidine labile site under the photoirradiation conditions.

(17) These results were further supported by the observation that one-electron oxidation of duplex **4**/**6** by an external oxidizing agent such as photoexcited riboflavin produced a similar cleavage pattern as observed for lane 7 in Figure 2 (see Supporting Information).

(18) In addition to ΔG term, reorganization energy (λ) of a bridged base radical cation would also affect electronic coupling. Large increase of the HT efficiency by replacing A with ^{z}A may suggest extra effects of decreased λ for ^{z}A$^{\bullet+}$.

(19) Our calculations show that ^{z}G is not a better thermodynamic sink for HT than G triplet, suggesting that the selective cleavage of ODN **6** at ^{z}G is most likely due to a kinetic factor, e.g., the trapping rate of ^{z}G$^{\bullet+}$ leading to a piperidine labile site would be significantly higher than that for $(GGG)^{\bullet+}$.

(20) One reviewer questioned whether hole migration between two GGGs on the complementary strand could be trapped by ^{z}G, because 8-OxoG was not an efficient hole trap when the hole migration took place on the complementary strand.[2b] While we proposed a different mechanism for efficient hole trapping by ^{z}G from that by 8-OxoG,[19] hole-trapping efficiency by ^{z}G may be different between the migration from strand to complementary strand and the migration along a strand.