Design of a Hole-Trapping Nucleobase: Termination of DNA-Mediated Hole Transport at N^2-Cyclopropyldeoxyguanosine

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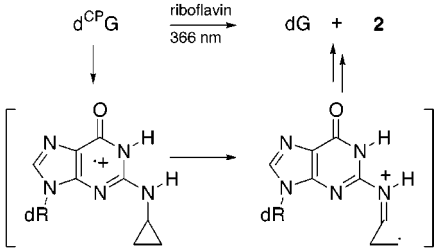
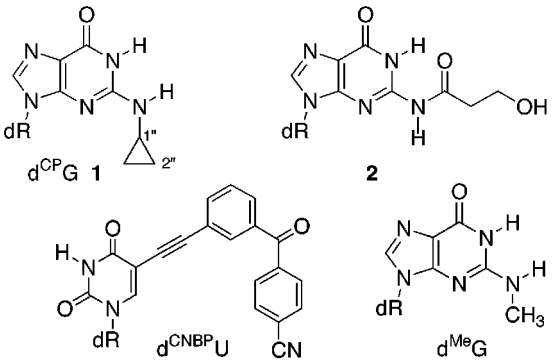
While the mechanism of DNA-mediated hole transport is a subject of controversy,[1−6] it is now evident from remote guanine (G) oxidation of duplex DNAs containing a tethered oxidant that G radical cation (hole) migrates a distance through the DNA π-stack.[5−9] Due to ionization potentials of GG and GGG lower than that of single G,[2d,10,11] these stacked G sites function as a thermodynamic sink of holes eventually producing piperidine labile sites. In principle, hole migration from hole donor to acceptor competes with hole trapping by water and/or oxygen. Therefore, overall efficiency of hole transfer is primarily determined by the rates of hole migration and hole trapping. When the rate of hole trapping is much slower than that of hole migration, equilibration of hole between donor and acceptor can be achieved.[8d] While the rate of hole migration can be attenuated by changing the potential energy gap between hole donor and acceptor,[7c] the modulation of hole-transport efficiency by changing the rate of hole trapping has never been demonstrated. We report a novel hole-trapping nucleoside N^2-cyclopropyl-2′-deoxyguanosine, **1** ($d^{CP}G$), which possesses a cyclopropyl group on N^2 as a radical-trapping device. One-electron oxidation of $d^{CP}G$ by photoexcited riboflavin induces homolytic cyclopropane ring opening as evidenced by the formation of N^2-(3-hydroxypropanoyl)dG, **2**. With the use of a $d^{CP}G$-containing duplex, we have demonstrated that $d^{CP}G$ efficiently terminates DNA-mediated hole transport at its own site.

Scheme 1





It is known that radical cations of cyclopropylamine[12] and *N*-alkyl- and *N*-arylcycloprpylamines[13] rapidly undergo homolytic cyclopropane ring opening to produce β-iminium carbon radicals. The rate of homolytic ring opening of the cyclopropylamine radical cation is believed to be larger than that of the corresponding ring opening of the neutral *N*-alkylcycloprpylaminyl radical (7.2×10^{11} s^{-1}).[14] While the magnitude of the rate is unknown, the cyclopropane ring opening of $d^{CP}G$ radical cation is expected to be rapid. We first examined one-electron oxidation of $d^{CP}G$ with photoexcited riboflavin in aqueous solution.[15] $d^{CP}G$ was rapidly consumed by photoirradiation at 366 nm in the presence of riboflavin, producing two major products after subsequent incubation of the photoirradiated mixture (Figure S1). These products were identified as dG and N^2-(3-hydroxypropanoyl)dG **2** by ^{1}H NMR and high-resolution FABMS. Comparison of ^{1}H NMR spectra of **2** with those of the authentic sample unambiguously confirmed the structure.[16] Formation of dG by incubating the photoirradiated mixture suggested that one-electron oxidation of **1** activated transformation of the cyclopropyl group at N^2 to a group being highly susceptible to hydrolysis (Scheme 1).[17]

Having established that **1** radical cation undergoes a very rapid cyclopropane ring opening, we examined the hole trapping by **1**

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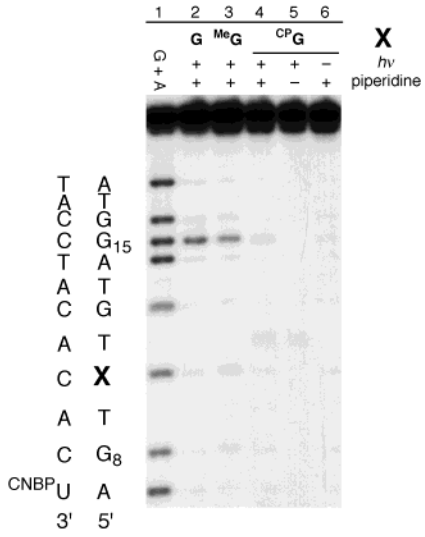

Figure 1. Autoradiogram of a denaturing sequencing gel for photoreactions of duplexes of ^{32}P-5′ end-labeled **G**−**P21**, ^{Me}**G**−**P21**, and ^{CP}**G**−**P21** hybridized to **C21**. Duplex ODNs were irradiated at 312 nm (0 °C, 15 min). After piperidine treatment (90 °C, 20 min), the samples were electrophoresed through a denaturing 15% polyacrylamide/7 M urea gel. Lane 1, Maxam−Gilbert G+A sequencing reactions; lane 2, **G**−**P21**; lane 3, ^{Me}**G**−**P21**; lanes 4−6, ^{CP}**G**−**P21**.

in DNA-mediated hole transport. We have used 21-mer probe ODNs d(ATT TAT AG_8T **X**TG TA**G$_{15}$** **G**TA TTT) containing G (**G**−**P21**), ^{Me}G (^{Me}**G**−**P21**), and ^{CP}G (^{CP}**G**−**P21**) as a base **X**. The complementary strand d(AAA TAC CTA CAC AC^{CNBP}U ATA AAT) (**C21**) contains cyanobenzophenone-substituted uridine (d^{CNBP}U) as a photoinducible one-electron oxidant.[9] Guanine radical cation was site selectively produced at G_8 in duplexes **G**−**P21**/**C21**, ^{Me}**G**−**P21**/**C21**, and ^{CP}**G**−**P21**/**C21** by single-electron transfer to photoexcited d^{CNBP}U.[9a] The hole is able to migrate up to G_{15}G through a bridge of d(T**X**TGTA)/d(TACACA). Probe ODNs ^{Me}**G**−**P21** and ^{CP}**G**−**P21** were obtained from 2-fluoroinosine containing 21-mer according to the reported method.[15] Photoirradiation of duplex **G**−**P21**/**C21** at 366 nm for 15 min followed by subsequent piperidine heating produced a distinct cleavage band at G_{15} selectively (Figure 1, lane 2). This indicates an efficient hole migration from G_8 to G_{15}. In marked contrast, G_{15} oxidation was significantly suppressed in ^{CP}**G**−**P21**/**C21** duplex, where d^{CP}G was incorporated into the bridge of d(T^{CP}GTGTA)/d(TACACA) (lane 4). Normalized band intensities at G_{15} relative to intact bands as determined by densitometry were 1.00 for **G**−**P21** (standard), 0.53 for ^{Me}**G**−**P21**, and 0.09 for ^{CP}**G**−**P21**. While incorporation of the methyl group at N^2 of dG resulted in a modest reduction of G_{15} oxidation via hole transport from G_8 to G_{15}, the incorporation of the cyclopropyl group was dramatically effective for the suppression of hole transport.

The melting temperature for ^{CP}G-containing 10-mer duplex d(GTC CAC TAT C)/d(GAT A^{CP}GT GGA C) is only 1.3 °C lower than that for the corresponding G-containing 10-mer duplex (50 μM base concentration, 100 mM NaCl). CD spectrum of the ^{CP}G-containing duplex shows a typical B-form structure (Figure S2). These observations imply that the disruption of the π-stack by incorporating d^{CP}G is not the reason for the suppression of hole transport. HPLC analysis of a nucleoside mixture obtained by enzymatic digestion of photoirradiated ^{CP}**G**−**P21**/**C21** clearly showed that d^{CP}G was completely consumed under the photoirradiation conditions used for the PAGE experiments, although other nucleosides, including d^{CNBP}U, remain largely unchanged (Figure 2). These results suggest that suppression of G_{18} oxidation in ^{CP}**G**−**P21**/**C21** is most likely due to the selective destruction of d^{CP}G. Calculated ionization potential at B3LYP/6-31G(d) level for N^9-methyl-^{CP}G/N^9-methyl-C base pair is only 0.13 eV lower than that for the normal G/C base pair, and 0.40 eV higher than that for the GG/CC doublet. The oxidation potential of d^{CP}G measured in water containing 0.1 M $LiClO_4$ was 0.93 V (vs SCE, cf. G 1.07 V). Therefore, it is unlikely that d^{CP}G functions as a thermodynamic sink in hole migration. On the basis of these observations, we propose that d^{CP}G effectively terminates hole transport through the DNA π-stack by a rapid and irreversible cyclopropane ring opening of its radical cation.

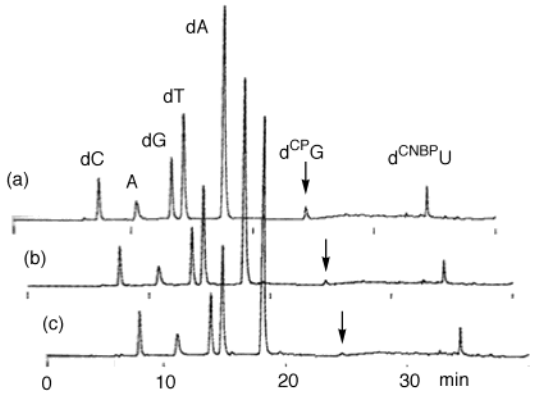

Figure 2. HPLC profiles of a nucleoside mixture obtained by enzymatic digestion of ^{CP}**G**−**P21**/**C-21** after photoirradiation for (a) 0 min, (b) 15 min, and (c) 30 min. The ODN solution (50 μM strand concentration) in sodium cacodylate buffer (pH 7.0) was irradiated at 312 nm at 0 °C for the indicated period. Photoirradiated oligomers were treated with alkaline phosphatase (33 u/mL), snake venom phosphodiesterase (0.1 u/mL), and P1 nuclease (33 u/mL) at 0 °C for 12 h. Adenine (A) was added as an internal standard.

The pseudo-first-order rate of trapping of $G^{\bullet+}$ by water was estimated to be 6×10^4 s^{-1}, which is significantly smaller than the rate for the hole migration between the two single Gs separated by two AT base pairs (2.5×10^6 s^{-1}).[8d] Discovery of a powerful hole-trapping nucleobase possessing a trapping rate faster than 2.5×10^6 s^{-1} would change the current view of hole transport. The studies described here clearly demonstrated that d^{CP}G can serve as a useful probe for gaining deeper insight into the kinetic aspects of hole transport through the DNA π-stack.

Supporting Information Available: Experimental protocol for the synthesis of **2**, HPLC analysis of the photoreaction of d^{CP}G, and CD spectra of ^{CP}G-containing duplex (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Authentic sample of **2** was synthesized by acylation at N^2 of dG with 3-benzyloxypropionyl chloride, followed by hydrogenolysis of the benzyl ether (see Supporting Information).

(17) For a review of reactions of phenylcyclopropane radical cation, see: Mizuno, K.; Ichinose, N.; Yoshimi, Y. *J. Photochem. Photobiol., C.* **2000**, *1*, 167−193.