J|A|C|S
COMMUNICATIONS

Published on Web 05/21/2002

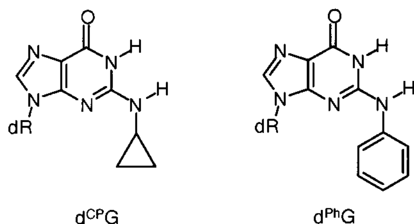
N^2-Phenyldeoxyguanosine: Modulation of the Chemical Properties of Deoxyguanosine toward One-Electron Oxidation in DNA

Kazuhiko Nakatani,*[†,‡,§] Chikara Dohno,[†,‡] and Isao Saito*[†,‡]

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, and CREST and PRESTO, Japan Science and Technology Corporation (JST), Kyoto 606-8501, Japan

Received February 1, 2002

Owing to the remarkable structures produced by a π-stacked array of nucleotide base pairs, DNA has been envisaged as a novel medium of charge transport.[1] Experimental[2−7] and theoretical[8−11] studies have shown that DNA actually mediates charge transport for some distances. A guanine radical cation (hole) produced by one-electron oxidation of DNA can migrate to a remote guanine site by successive hopping between neighboring guanines. The overall efficiency of the charge transport could be primarily determined by the rates of hole hopping and trapping. We have recently demonstrated the termination of charge transport by increasing the hole trapping rate at the site of N^2-cyclopropyldeoxyguanosine (d^{CP}G),[7a] in which cyclopropane ring opening functioned as a radical trapping device. These results imply that substitution of the exocyclic amino group of deoxyguanosine (dG) with functional groups could be an intriguing tool to modulate the reactivity of dG toward one-electron oxidation. We here report that incorporation of N^2-phenyldeoxyguanosine[12] (d^{Ph}G) into duplex DNA dramatically suppressed oxidative decomposition, not only at d^{Ph}G, but more importantly, at dGs remote from the modified guanine.



The DNA oligomers containing d^{Ph}G and complementary strands used for the studies are listed in Table 1. All d^{Ph}G-containing ODNs were synthesized from corresponding ODNs containing 2-fluoroinosine by substitution of fluorine with aniline.[13] d^{Ph}G was incorporated in the ODN **^{Ph}GG1** probe by replacing G_{15} of the G_{15}G doublet in the 21-mer ODN **GG1**. Complementary ODN **^{CNBP}U1** contains cyanobenzophenone-substituted uridine (d^{CNBP}U) as a photoinducible one-electron oxidant,[7] whereas ODN **T1** contained thymidine in place of d^{CNBP}U. In duplexes of **GG1**/**^{CNBP}U1** and **^{Ph}GG1**/**^{CNBP}U1**, the guanine radical cation was site selectively produced at G_8 by one-electron transfer to photoexcited d^{CNBP}U.[7] ODN **GG5** contained five GG doublets with identical neighboring sequences. The ^{Ph}GG site was embedded in **^{Ph}GG5(8)** and **^{Ph}GG5(16)** by replacing G_8 and G_{16} of **GG5**, respectively with ^{Ph}G. ODNs **GG4** and **^{Ph}GG4(8)** lacked the G_{16}G site of **GG5** and **^{Ph}GG5(8)**, respectively, by replacing them with the T_{16}A sequence.

Table 1. Oligomers Used for the Studies[a]

GG1:	5'-**X** AG$_8$TGTGTA $\underline{G}_{15}\underline{G}$ **V**-3'
^{Ph}GG1:	5'-**X** AG$_8$TGTGTA$^{Ph}\underline{G}_{15}\underline{G}$ **V**-3'
T1:	3'-**Y** TC ACACAT C C **W**-5'
^{CNBP}U1:	3'-**Y** ^{CNBP}UC ACACAT C C **W**-5'
GG5:	5'-**X** A $\underline{G}_8\underline{G}TA\underline{G}_{12}\underline{G}$TA $\underline{G}_{16}\underline{G}TA\underline{G}_{20}\underline{G}TA\underline{G}_{24}\underline{G}$ **V**-3'
^{Ph}GG5(8):	5'-**X** A$^{Ph}\underline{G}_8\underline{G}TA\underline{G}_{12}\underline{G}$TA $\underline{G}_{16}\underline{G}TA\underline{G}_{20}\underline{G}TA\underline{G}_{24}\underline{G}$ **V**-3'
^{Ph}GG5(16):	5'-**X** A $\underline{G}_8\underline{G}TA\underline{G}_{12}\underline{G}TA^{Ph}\underline{G}_{16}\underline{G}TA\underline{G}_{20}\underline{G}TA\underline{G}_{24}\underline{G}$ **V**-3'
c-GG5:	3'-**Y** T C CATC CAT C CATC CATC C **W**-5'
GG4:	5'-**X** A $\underline{G}_8\underline{G}TA\underline{G}_{12}\underline{G}$TA TA TA$\underline{G}_{20}\underline{G}TA\underline{G}_{24}\underline{G}$ **V**-3'
^{Ph}GG4(8):	5'-**X** A$^{Ph}\underline{G}_8\underline{G}TA\underline{G}_{12}\underline{G}$TA TA TA$\underline{G}_{20}\underline{G}TA\underline{G}_{24}\underline{G}$ **V**-3'
c-GG4:	3'-**Y** T C CATC CAT AT ATC CATC C **W**-5'

[a] **X** = ATTTAT; **Y** = TAAATA; **V** = TATTT; and **W** = ATAAA.

c-GG5 and **c-GG4** are complementary strands to **GG5** and **GG4**, respectively. The oxidation potential of d^{Ph}G measured by cyclic voltammetry in DMF containing 0.1 M $LiClO_4$ was 0.70 V (vs Ag/Ag^+, cf. G 0.67 V). The melting temperature of the d^{Ph}G-containing 10-mer duplex d(GAT AGT ^{Ph}GGA C)/d(GTC CAC TAT C) was 3.7 °C higher than the corresponding normal G-containing duplex (50 μM base concentration, 100 mM NaCl). CD spectra of the duplex showed a typical B-form structure (Figure S1).

Photoirradiation of duplex **GG1**/**^{CNBP}U1** at 312 nm for 60 min and subsequent piperidine treatment (90 °C, 20 min) produced a distinct cleavage band at the 5′ side G of the G_{15}G doublet (Figure 1a, lane 2). In marked contrast, the cleavage at $^{Ph}G_{15}$ in duplex **^{Ph}GG1**/**^{CNBP}U1** (lane 3) was considerably weaker than the cleavage at G_{15} in **GG1**/**^{CNBP}U1** (lane 2). The band intensity of G_{15} relative to intact full length **GG1** was 0.31, whereas the relative band intensity of $^{Ph}G_{15}$ of **^{Ph}GG1** was only 0.05. Suppression of the decomposition of d^{Ph}G by one-electron oxidation was further confirmed by riboflavin-sensitized oxidation of duplex **^{Ph}GG1**/**T1**. While strong cleavage occurred at G_{15} of **GG1**/**T1** (Figure 1b, lane 1), only a faint band was observed at $^{Ph}G_{15}$ of **^{Ph}GG1** (lane 2). We separately confirmed that strand cleavage of single-stranded d^{Ph}G-containing oligomer d(ATT TAT AGT AGT AGT A^{Ph}GT ATT T) actually occurred at d^{Ph}G by the riboflavin-sensitized oxidation and subsequent piperidine treatment (Figure S2). The efficiency of the cleavage at d^{Ph}G was comparable to that at dG. Nucleoside analysis of d^{Ph}G-containing DNA by HPLC showed that 68% of d^{Ph}G remained intact in the duplex after 1 h of irradiation in the presence of riboflavin, whereas only 36% of d^{Ph}G remained intact in the single strand (Figure S3).

Oxidative decomposition of the d^{Ph}G-containing duplex was suppressed not only at d^{Ph}G but also remarkably at the GG sites

* Corresponding author. E-mail: nakatani@sbchem.kyoto-u.ac.jp.
† Kyoto University.
‡ CREST.
§ PRESTO.

10.1021/ja025781d CCC: $22.00 © 2002 American Chemical Society

Figure 1. Autoradiograms of denaturing sequencing gels for photoreactions of $d^{Ph}G$-containing oligomer duplexes. Photoirradiated ODNs were heated with piperidine and electrophoresed through a denaturing 15% polyacrylamide/7 M urea gel. Partial base sequences of ODNs were shown on the side. $d^{CNBP}U$ was located opposite A shown with a box. (a) Duplexes were irradiated at 312 nm for 60 min. Lane 1, Maxam−Gilbert A+G sequencing reactions of **GG1**; lane 2, **^{CNBP}U1/GG1**; lane 3, **^{CNBP}U1/^{Ph}GG1**. (b) Duplexes were irradiated at 366 nm in the presence of riboflavin. Lane 1, **T1/GG1**; lane 2, **T1/^{Ph}GG1**; lane 3, A+G reactions of **GG1**. (c) Duplexes were irradiated at 366 nm in the presence of riboflavin. Lane 1, A+G reactions of **GG5**; lane 2, **GG5/c-GG5**; lane 3, **^{Ph}GG5(16)/c-GG5**; lane 4, **^{Ph}GG5(8)/c-GG5**; lane 5, **^{Ph}GG4(8)/c-GG4**; lane 6, **GG4/c-GG4**; lane 7, A+G reactions of **GG4**.

Figure 2. Band intensities (%) of GG sites relative to the intact full length bands obtained for the photoirradiated duplexes of (a) lanes 2, 3, and 4 and (b) lanes 5 and 6 shown in Figure 1c.

that were distant from $d^{Ph}G$ (Figure 1c). Band intensities at GG sites of lanes 1−5 in Figure 1c were shown in Figure 2. While strand cleavage of photoirradiated **GG5** in the presence of riboflavin occurred at all GG sites with comparable efficiency, the cleavage of GG sites of **^{Ph}GG5(8)** was suppressed at $G_{12}G$ and $G_{16}G$ in addition to $^{Ph}G_8G$ (Figure 2a). In contrast to $G_{12}G$, cleavage at $G_{24}G$ was only weakly suppressed compared with the cleavage in **GG5**, showing that the efficiency of suppression of strand cleavage decreased with an increase in distance from $d^{Ph}G$. Distance dependency of cleavage suppression was clearly shown in the oxidation of **^{Ph}GG5**(**16**) that contained $d^{Ph}G$ in the middle of five GG sites. The efficiency of the cleavage was considerably reduced at all four GG sites in addition to the ^{Ph}GG site. Significant insights into the mechanism of cleavage suppression by $d^{Ph}G$ were obtained by the riboflavin-sensitized oxidation of **GG4** and **^{Ph}GG4(8)**. Strand cleavage of **GG4** was observed at all four GG sites, whereas the cleavage of **^{Ph}GG4**(**8**) was strongly suppressed at G_8G and $G_{12}G$ sites, but not at all at $G_{20}G$ and $G_{24}G$ sites (Figure 2b). The $G_{12}G$ and $G_{20}G$ sites in **^{Ph}GG4(8)**/**c-GG4** were separated by six intervening A−T base pairs, and the rate of hole transport between two sites was expected to be much smaller than that between G_8 and G_{12} (e.g., $2.5 \times 10^6\ s^{-1}$ for $G^{\bullet+}TTG \rightarrow GTTG^{\bullet+}$) and the estimated rate of hole trapping with water at the G site (e.g., $6 \times 10^4\ s^{-1}$).[5b,6] Thus, it is apparent that the efficiency of suppression of the oxidative decomposition at the given G sites in $d^{Ph}G$-containing duplex increased upon increasing the rate of hole transfer to $d^{Ph}G$.[14]

Our studies showed that (1) substitution of an exocyclic amino group of dG is effective in modulating the chemical properties of dG toward one-electron oxidation and (2) decomposition of the guanine radical cation was effectively suppressed near $d^{Ph}G$. These results indicate that $d^{Ph}G$ is a prototype of nucleosides functioning as an intrinsic antioxidant of duplex DNA toward one-electron oxidation.

Supporting Information Available: CD spectra, HPLC, and PAGE analysis of $d^{Ph}G$-containing DNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Eley, D. D.; Spivey, D. I. T. *Trans. Faraday Soc.* **1962**, *58*, 411−415.
(2) (a) Holmlin, R. E.; Dandliker, P. J.; Barton, J. K. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2714−2730. (b) Williams, T. T.; Odom, D. T.; Barton J. K. *J. Am. Chem. Soc.* **2000**, *122*, 9048−9049.
(3) Grinstaff, M. W. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3629−3635.
(4) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(5) (a) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636. (b) Giese, B.; Spichty, M. *Chem. Phys. Chem.* **2000**, *1*, 195−198.
(6) Lewis F. D.; Wu, T.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.
(7) (a) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2001**, *123*, 9681−9682. (b) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5893−5894. (c) Nakatani, K.; Dohno, C.; Saito, I. *Tetrahedron Lett.* **2000**, *41*, 10041−10045. (d) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **1999**, *121*, 10854−10855. (e) Nakatani, K.; Dohno, C.; Ogawa, A.; Saito, I. *Chem. Biol.* **2002**, *9*, 361−366.
(8) Beratan, D. N.; Priyadarshy, S.; Risser, S. M. *Chem. Biol.* **1997**, *4*, 3−8.
(9) (a) Bixon, M.; Jortner, J. *J. Am. Chem. Soc.* **2001**, *123*, 12556−12567. (b) Jortner, J.; Bixon, M.; Langenbacher, T.; Michel-Beyerle, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12759−12765.
(10) Berlin, Y. A.; Burin, A. L.; Ratner, M. A. *J. Am. Chem. Soc.* **2001**, *123*, 260−268.
(11) Grozema, F. C.; Berlin, Y. A.; Siebbeles, L. D. A. *J. Am. Chem. Soc.* **2000**, *122*, 10903−10909.
(12) Focher, F.; Hildebrand, C.; Freese, S.; Ciarrocchi, G.; Noonan, T.; Sangalli, S.; Brown, N.; Spadari, S.; Wright, G. *J. Med. Chem.* **1988**, *31*, 1, 1496−1500.
(13) (a) Acedo, M.; Fàbrega, C.; Aviño, A.; Goodman, M.; Fagan, P.; Wemmer, D.; Eritja, R. *Nucleic Acid Res.* **1994**, *22*, 2982−2989. (b) Adib, A.; Potier, P. F.; Doronina, S.; Huc, I.; Behr, J.-P. *Tetrahedron Lett.* **1997**, *38*, 2989−2992.

(14) These remarkable observations could be rationalized by assuming an annihilation process of the $d^{Ph}G$ radical cation that was prevented from decomposing, leading to the formation of a piperidine-labile site, as shown in the scheme below. The rate of annihilation of $d^{Ph}G$ radical cation (k_{ann}) should be much faster than the hole trapping rate at GG (k_{trap1}) and ^{Ph}GG (k_{trap2}) to suppress the decomposition at these sites. When the hole transfer to $d^{Ph}G$ overrode the hole trapping at the given G site (i.e., $k_{HT} \gg k_{trap1}$), the hole was selectively depleted at $d^{Ph}G$ by the annihilation process.

$$\rightleftarrows (GG)^{\cdot+} \underset{}{\overset{k_{HT}}{\rightleftarrows}} (^{Ph}GG)^{\cdot+} \rightleftarrows$$

$$(GG)^{\cdot+} \xrightarrow{k_{trap1}} P_1 \qquad (^{Ph}GG)^{\cdot+} \xrightarrow{k_{trap2}} P_2 \qquad (^{Ph}GG)^{\cdot+} \xrightarrow{k_{ann}} {}^{Ph}GG$$

P_1, P_2 denote pipeidine labile products.

Molecular modeling simulation showed that the phenyl group of $d^{Ph}G$ in duplex DNA was located just in the middle of the minor groove, suggesting a considerable increase of solvent-accessible surface upon phenyl substitution. The calculated spin density of the $d^{Ph}G$ radical cation was delocalized on both the purine and phenyl rings. Since suppression of $d^{Ph}G$ decomposition was specific for duplex DNA, the annihilation process most likely occurred in the vicinity of the phenyl ring in the minor groove. One plausible mechanism of the putative annihilation process may involve a back-electron transfer from superoxide radical anion to a $d^{Ph}G$ radical cation. In the case of riboflavin sensitization, the riboflavin radical anion is also conceivable as an electron donor.

JA025781D