# Chemistry of Sequence-Dependent Remote Guanine Oxidation: Photoreaction of Duplex DNA Containing Cyanobenzophenone-Substituted Uridine

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There has been much debate on the efficiency of charge transport (CT) mediated by DNA π-stack, especially with regard to the distance and sequence dependencies.[1] Earlier studies of DNA-mediated CT employed oligodeoxynucleotides (ODNs) that were tethered to electron acceptors such as Rh(III) intercalator,[2] anthraquinone derivative,[3] stilbene,[4] acridine,[5] and ethidium intercalator[6] by flexible linkers, with guanine (G) base as an intrinsic electron donor. Recently, improved ODNs that provide more accurate donor−acceptor distance within DNA duplex have been reported.[7,8] We have recently developed ODNs that contain a strong electron-accepting chromophore at predetermined sites without perturbing base stack in B-form duplex by incorporating cyanobenzophenone substituted 2′-deoxyuridine (d^{CNBP}U).[9,10] We
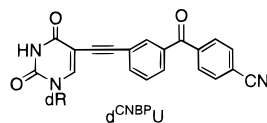
have examined the photoreactions of a series of oligomer duplexes containing both d^{CNBP}U and GG hole trap,[11] which were separated by various intervening base sequences. We herein report that G radical cation (G$^{•+}$) was site-selectively generated at the G of the core d(AG)/d(C^{CNBP}U) sequence, and the hole migrated over 24 Å to the remote GG site via a successive hole-hopping between guanine bases.

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(1) For recent reviews, see: (a) Holmlin, R. L.; Dandliker, P. J.; Barton, J. K. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2714−2730. (b) Beratan, D. N.; Priyadarshy, S.; Risser, S. M. *Chem. Biol.* **1997**, *4*, 3−8. (c) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1154.
(2) (a) Núñez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85−97. (b) Hall, D. B.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 5045−5046.
(3) (a) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762−12771. (b) Armitage, B.; Ly, D.; Koch, T.; Frydenlund, H.; Orum, H.; Batz, H. G.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12320−12325.
(4) Lewis, F. D.; Wu, T.; Zhang, Y.; Letsinger, R. L.; Greenfield, S. R.; Wasielewski, M. R. *Science* **1997**, *277*, 673−676.
(5) Fukui, K.; Tanaka, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 158−161.
(6) (a) Hall, D. B.; Kelley, S. O.; Barton, J. K. *Biochemistry* **1998**, *37*, 15933−15940. (b) Kelley, S. O.; Barton, J. K. *Chem. Biol.* **1998**, *5*, 413−425.
(7) (a) Giese, B.; Wessely, S.; Spormann, M.; Lindemann, U.; Meggers, E.; Michel-Beyerle, M. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 996−998. (b) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950−12955.
(8) Kelley, S. O.; Barton, J. K. *Science* **1999**, *283*, 375−381.
(9) Nakatani, K.; Dohno, C.; Saito, I. *J. Org. Chem.* **1999**, *64*, 6901−6904.
(10) (a) Nakatani, K.; Dohno, C.; Nakamura, T.; Saito, I. *Tetrahedron Lett.* **1998**, *39*, 2779−2782. (b) Nakatani, K.; Fujisawa, K.; Dohno, C.; Nakamura, C.; Saito, I. *Tetrahedron Lett.* **1998**, *39*, 5995−5998.
(11) (a) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686−12687. (b) Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373−7374. (c) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406−6407. (d) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.

Table 1. d^{CNBP}U- and GG-Containing Oligomers^a

^{a} X represents d^{CNBP}U. The A-X base pair and the GG stite are shown in boldface and red.

Photoreactions were carried out with a variety of duplexes consisting of d^{CNBP}U-containing 22-mer ODNs and their complementary strands. The GG step was incorporated into GGTTG*A* (ODN **1**), GGTTGT*A* (ODN **3**), *A*GTTGG (ODN **5**), and *A*TG-TTGG (ODN **7**) sequences, where *A* (shown in italic) forms a Watson−Crick base pair with d^{CNBP}U (Table 1). Photocleavage sites of ODNs **1**, **3**, **5**, and **7** were summarized in Figure 1. For clarity, the sites of strand cleavage are shown by the number of bases separated from the A-^{CNBP}U base pair with "plus" (toward 5′ side) and "minus" (toward 3′ side) signs. The cleavage efficiency was determined from the band intensity relative to the sum of total DNA band intensities.[12] Remarkable strand cleavage was observed only for ODN **5** at the G of the position −4. Highly selective cleavage occurred at 5′G of the GG step, suggesting that the G cleavage proceeded via G$^{•+}$.[11] The efficiency of 5′G oxidation of GG steps was sensitive to the position of the single G (shown in underlined bold face) proximal to the d^{CNBP}U. The cleavage efficiency decreased about one-tenth when the single G moved one base pair away from position −1 (ODN **5**) to position −2 (ODN **7**). Only weak cleavage was observed for ODNs **1** and **3** containing the single G at positions +1 and +2, respectively. In a control experiment, photoirradiation of a duplex possessing a d(ATTGG)/d(CCAA^{CNBP}U) sequence containing no such proximal single G resulted in no cleavage of the GG step,[12] indicating that direct single electron transfer (ET) to the photoexcited d^{CNBP}U from Gs that are more than three bases away from the A-^{CNBP}U base pair is unfeasible. On the basis of these results, it is apparent that the single G proximal to d^{CNBP}U of the d-(A*G*TTGG)/d(CCAAC^{CNBP}U) sequence was indispensable and oxidized to G$^{•+}$ via single ET to adjacent photoexcited ^{CNBP}U. The hole thus generated would undergo migration to the remote GG site. Since the intervening 5′TT3′ sequence between the single G and the remote GG sites was the same for ODNs **5** and **7**, the cleavage intensity at 5′G of the GG step directly reflects the apparent efficiency of G$^{•+}$ formation in both systems (positions −1 and −2).

Having established that radical cation is most effectively generated at the single G in the core d(AG)/d(C^{CNBP}U) sequence, we have examined the distance and sequence dependency of hole migration through the duplex DNA. Oligomer duplexes used for this purpose contained the core d(AG)/d(C^{CNBP}U) sequence and a GG hole trap which was seven base pairs apart from the initially generated G$^{•+}$ center. The intervening base sequences were designed so as to have two GG steps in the same strand (ODNs **9/10**) or one in an opposite strand (ODNs **11/12**). Other sequences consisted of two single Gs instead of one GG step (ODNs **13/14**) or only AT base pairs (ODNs **15/16**). Strand cleavage of ODN

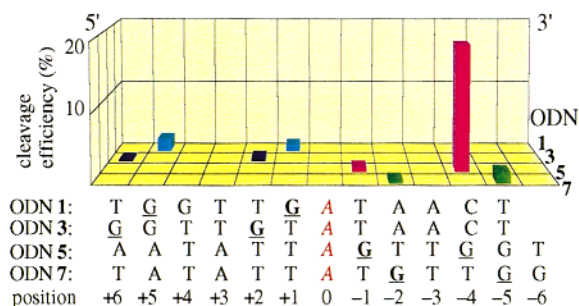(12) See Supporting Information for experimental data.

Figure 1. Photocleavage of GG-containing oligomers complementary to the corresponding d^{CNBP}U-containing strands. d^{CNBP}U was located opposite to the A at position 0 (shown in red). Partial sequences of ODNs are shown, and the sites of strand cleavage are underlined. Single G's proximal to the d^{CNBP}U-A base pair are shown in boldface. Efficiencies at the major cleavage sites were 18.5% (at position −4, ODN **5**), 1.9% (at position +5, ODN **1**), and 1.5% (at position −5, ODN **7**).
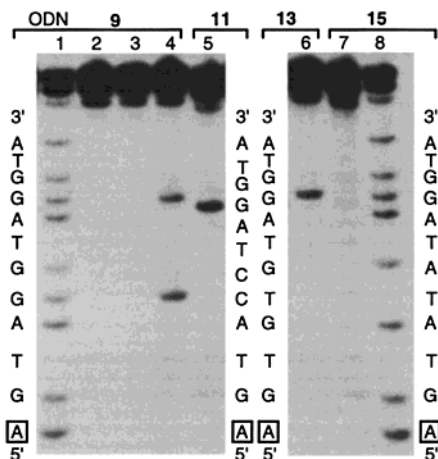

Figure 2. Autoradiograms of the denaturing sequencing gel for photoreactions of duplexes **9/10**, **11/12**, **13/14**, and **15/16**. Lanes 1−4, ODN **9**; lane 5, ODN **11**; lane 6, ODN **13**; lanes 7 and 8, ODN **15**. ODNs in lanes 3−7 were photoirradiated; all ODNs except that in lane 3 were heated with piperidine. Lanes 1 and 8, Maxam−Gilbert G + A sequencing reactions for ODNs **9** and **15**, respectively. Partial base sequences of oligomers are shown on the side. d^{CNBP}U is located opposite to the A shown with a box.

9 occurred selectively at 5′G of both proximal and distal GG steps (GG)$_p$ and (GG)$_d$, respectively (Figure 2, lane 4). The relative band intensity [GG]$_d$/[GG]$_p$ was 0.84 in an average of seven separate experiments. Cleavage at (GG)$_d$ of duplex **11/12**, where (GG)$_p$ was in the opposite strand (ODN **12**), was roughly 2-fold more efficient than that observed for duplex **9/10**, possessing two GG steps and the G$^{•+}$ center in the same strand (lane 5 vs lane 4).[13] In a separate experiment, we have confirmed the cleavage at 5′G of the GG step in opposite strand ODN **12**.[12] The presence of G in the intervening sequence was essential for hole migration over 24 Å by comparing the cleavage at the GG step of ODN **13** (lane 6) with that of ODN **15** (lane 7). Thus, the intervening

5′T*G*T*G*TA3′ sequence in ODN **13** is able to mediate hole migration, whereas the 5′T*A*T*A*TA3′ sequence in ODN **15** does not.[14] In a separate experiment, we have confirmed that the hole migration from the G$^{•+}$ to the remote GG step through four intervening AT base pairs is extremely difficult.[12] These observations are consistent with recent results reported by Giese and coworkers that (i) long-range CT in duplex DNA proceeds via a successive hole hopping process between G bases and (ii) the efficiency of each CT process rapidly decreases with increasing the number of AT base pairs separating individual G bases.[3b,7,15,16]

To know the chemistry of G oxidation at the remote GG site, a reaction mixture obtained by photoirradiation of duplex d(GTC-CACXATC)/d(GATAGTGGAC) followed by enzymatic digestion (0 °C, 12 h) was carefully analyzed by HPLC.[12] A comparison of the the peak areas of each nucleoside before and after photoirradiation relative to adenine added as an internal standard shows that approximately one molecule of total five dGs was consumed during this photoreaction.[17,18] The product eluted at 3.4 min was identified as 2-aminoimidazolone (dIz),[11b,19] by
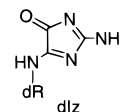
photodiode array assay and the comigration with the authentic sample on HPLC.[20] This is the first direct observation that dIz was produced as a major detectable product in the remote oxidation of the GG step in duplex DNA.[21]

In addition to base sequence dependency, base stack would also be an important factor for the CT efficiency.[8] As exemplified by the present remote GG oxidation, the present d^{CNBP}U-incorporated DNA generates G$^{•+}$ site selectively in a duplex without perturbing π-stack by photoirradiation and thus can serve as a valuable tool for studying DNA-mediated charge transport. Moreover, dIz was confirmed to be a major oxidation product in the remote guanine oxidation, as previously demonstrated in one-electron oxidation of ODNs by exogenous photosensitizers.[10,11b,19]

Supporting Information Available: Autoradiograms of denaturing PAGE for the photoreactions of duplex ODNs **1/2**−**7/8**, **11/12**, and **17** containing d(ATTGG)/d(CCAA^{CNBP}U) sequence, and experimental details for investigating distant dependency of hole migration and HPLC analysis of G oxidation products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

(14) Despite the formation of G$^{•+}$ next to the A-^{CNBP}U base pair in the photoirradiation of ODNs **15/16**, only very weak cleavage was observed at this G. This suggests that the initially formed G$^{•+}$ may be rapidly quenched by back electron transfer from ^{CNBP}U radical anion prior to the degradation of G$^{•+}$, leading to piperidine-sensitive product dIz.

(15) Jortner, J.; Bixon, M.; Langenbacher, T.; Michel-Beyerle, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12759−12765.

(16) Harriman, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 945−949.

(17) Heating the photoirradiated duplex with piperidine produced d(GAT-AGT) and d(GAC), confirming the selective oxidation of 5′G of the GG step.

(18) After photoreaction, ca. 70% of d^{CNBP}U was recovered unchanged, suggesting that there exists a path for the benzophenone anion radical to revert to d^{CNBP}U.

(19) (a) Raoul, S.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Morin, B.; Weifeld, M.; Cadet, J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 371−381. (b) Vialas, C.; Pratviel, G.; Claparols, C.; Meunier, B. *J. Am. Chem. Soc.* **1998**, *120*, 11548−11553.

(20) Authentic dIz was obtained by photooxidation of dG with riboflavin.[11b,19a]

(21) Under the conditions of the photoreaction and the HPLC analysis, formation of another G oxidation product, 8-oxodG, was not observed.

(13) Relative band intensities at (GG)$_d$ were 4.3% for ODN **9** and 8.7% for ODN **11**.