Pergamon

Tetrahedron Letters 41 (2000) 10041–10045

TETRAHEDRON LETTERS

Site selective generation of guanine radical cation in duplex DNA: modulation of the direction of hole transport

Kazuhiko Nakatani*, Chikara Dohno and Isao Saito*

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

Received 17 October 2000; accepted 18 October 2000

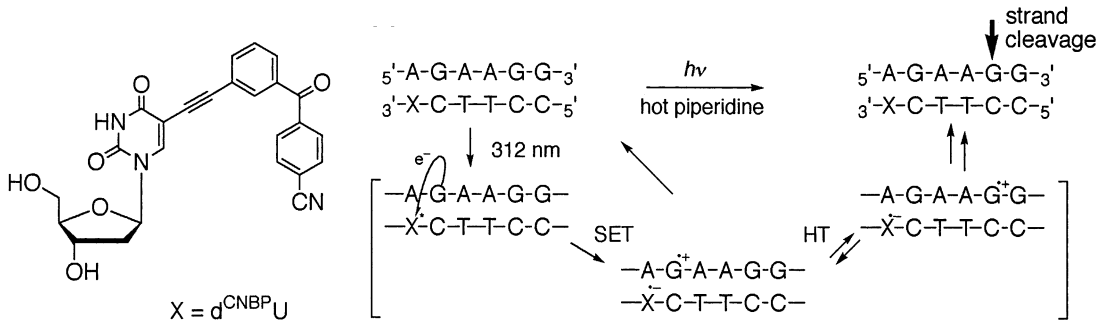
Abstract

Photoinduced electron transfer between cyanobenzophenone substituted 2′-deoxyuridine ($d^{CNBP}U$) and GG sequence on the same strand of duplex DNA was examined. It was found that 5′-$GT^{CNBP}U$-3′ was a most effective sequence for the generation of guanine radical cation, showing that DNA oligomers containing $d^{CNBP}U$ are widely usable for the studies for DNA-mediated charge transport. By using 5′-$GT^{CNBP}U$-3′ sequence, we observed a remarkable one direction hole transport. © 2000 Elsevier Science Ltd. All rights reserved.

DNA-mediated charge transport (CT) has been intensively studied since oxidative DNA damage causes mutations, aging and diseases.[1–11] Oligodeoxyribonucleotides (ODNs) tethered to strongly electron accepting chromophores have been used for such studies, and guanine (G) base is used as an intrinsic electron donor.[2–7] We have synthesized ODNs containing cyanobenzophenone substituted 2′-deoxyuridine ($d^{CNBP}U$) as an electron-accepting pyrimidine nucleoside and examined the photoreactions of the duplex containing $d^{CNBP}U$.[12] It was found that guanine radical cation (hole) is generated site selectively at the G in the sequence of $C^{CNBP}U/AG$ by single electron transfer (SET) from G to photoexcited $d^{CNBP}U$ in the complementary strand (Scheme 1).[7] The hole thus generated migrates to distal GG sites via hole transport (HT) and is eventually trapped by oxygen or water to give piperidine labile sites.[13] The efficiency of hole generation was highly sensitive to the sequences flanking the G residue. We report here that hole can be selectively generated at the G of the $GT^{CNBP}U$ sequence in the same strand. This finding significantly expands the flexibility of designing sequences of $d^{CNBP}U$-containing oligomers, since hole can be site selectively generated either on the same strand containing $d^{CNBP}U$ or on the complementary strand by selecting two sequences of $GT^{CNBP}U/AAC$ and $C^{CNBP}U/AG$, respectively.

* Corresponding authors. E-mail: saito@sbchem.kyoto-u.ac.jp

PII: S0040-4039(00)01816-5



Scheme 1.

We have examined the photoreactions and subsequent piperidine-induced cleavage of 22-mer ODNs **1**–**5** containing a probe sequence of 5′-GGTGX-3′ (ODN **1**), 5′-GGTGTX-3′ (ODN **2**), 5′-GGTTX-3′ (ODN **3**), 5′-GGTTGTX-3′ (ODN **4**), and 5′-GGTTATX-3′ (ODN **5**), as shown in Table 1, where X represents $d^{CNBP}U$. ^{32}P-5′-end-labeled ODN containing $d^{CNBP}U$ was annealed with the complementary strand and the duplex was photoilluminated at 312 nm. Piperidine labile sites were determined by denaturing PAGE. Cleavage efficiency was represented by a relative band intensity to the sum of total DNA bands. Sequences and the sites of strand cleavage are summarized in Fig. 1.

Table 1
d^{CNBPU} and GG-containing oligomers[a]

ODN 1:	5′-ATA	TAC	ATT	GGT	G**X**T	TGA	GTA	T-3′
ODN 2:	5′-ATA	ACA	TTG	GTG	T**X**T	TGA	GTA	T-3′
ODN 3:	5′-ACA	TTG	GTT	GGT	T**X**T	TGA	GTA	T-3′
ODN 4:	5′-ATA	CAT	TGG	TTG	T**X**T	TGA	GTA	T-3′
ODN 5:	5′-ATA	CAT	TGG	TTA	T**X**T	TGA	GTA	T-3′

[a] **X** represents $d^{CNBP}U$.

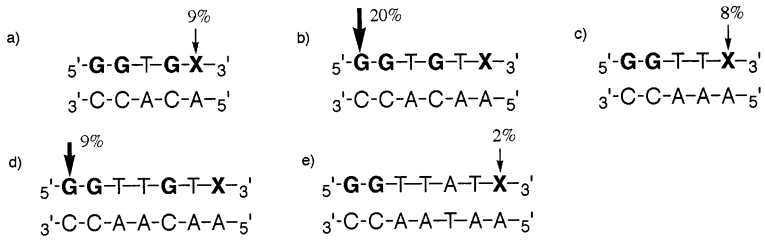


Figure 1. Sites of strand cleavage of 22-mer ^{CNBP}U (**X**)-containing ODNs by 312 nm irradiation and hot piperidine treatment. Only partial sequences of ODNs (a) **1**; (b) **2**; (c) **3**; (d) **4**; (e) **5** were shown for clarity. The most efficient cleavage site was indicated by an arrow with the intensity of the cleavage band relative to the sum of total DNA bands. Bold arrows indicate the strand cleavage occurred at distal GG site. Photoirradiation was carried out for 1 h for **1** and **5** or 2 h for **2**–**4**

For the GGTGX (Fig. 1(a)) sequence, negligible G oxidation was observed in addition to the moderate cleavage at X induced by only hot piperidine.[14] In marked contrast, cleavage of ODNs possessing GGTGTX (Fig. 1(b)) and GGTTGTX (Fig. 1(d)) occurred selectively at 5′ G of the

distal GG that are five and six base pairs apart from X, respectively. However, with one additional intervening thymine base incorporated between X and G as GGTTX (Fig. 1(c)), cleavage at the GG was completely suppressed. These results indicate that G must be located in two base pairs apart from X for efficient hole generation and forward hole migration in the same strand of X. Since almost no cleavage band was observed at the distal GG site in the GGTTATX sequence (Fig. 1(e)), which contained adenine instead of G in ODN **4**, the efficient hole migration occurred only when the sequence was GTX, neither at ATX or TTX. When T in the GTX sequence was replaced by purine base (G or A), strand cleavage selectively occurred at the G in the GPuX, showing no hole migration to the remote GG site (data not shown).

In order to get further insight into the remote G oxidation of ODNs containing $GT^{CNBP}U$ sequence, we next examined the photoreactions of 27-mer ODN **6** containing $G_{12}TX$ sequence in the middle and two GG sites of G_8G_9 and $G_{18}G_{19}$ (Fig. 2). The complementary ODN **7** also

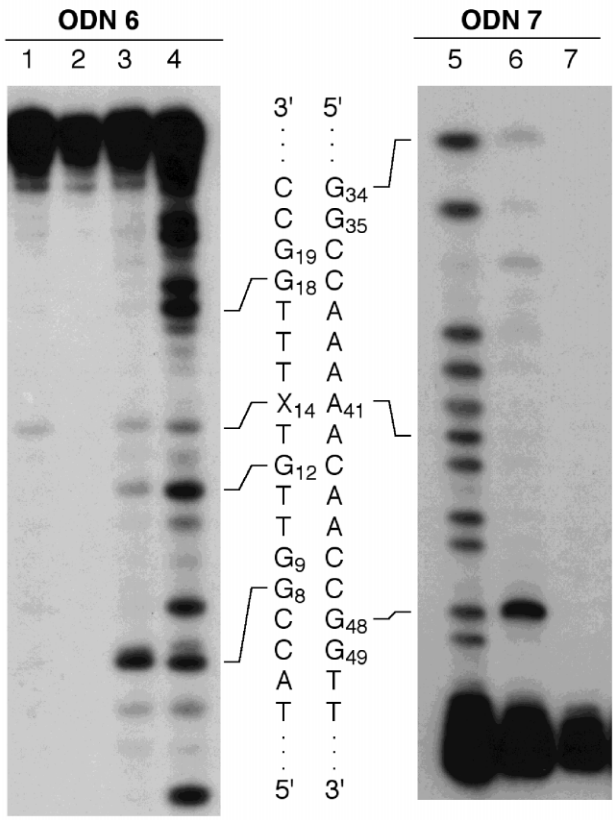
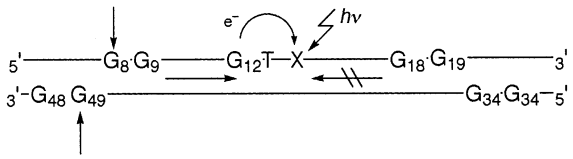


Figure 2. Autoradiograms of denaturing sequencing gels for the photoreaction of the duplex of 5′-d(ATT AAC CGG TTG TXT TTG GCC AAT TAT)-3′ (ODN **6**) and 5′-d(TAT TAA CCG GTT TTT GTT GGC CAA TTA)-3′ (ODN **7**). ODNs **6** and **7** were separately 5′-^{32}P-end labeled and hybridized to the complementary strand (5 μm, strand concentration) in 10 mM sodium cacodylate at pH 7.0. Hybridization was achieved by heating the sample at 90°C for 5 min and slowly cooling to room temperature. The duplex was illuminated at 312 nm with transilluminator at 0°C for 2 h. After piperidine treatment (90°C, 20 min), the samples were recovered by ethanol precipitation and suspended in denaturing loading buffer and electrophoresed through a denaturing 15% polyacrylamide 7 M urea gel. Lanes 1–4, ODN **6**; lanes 5–7, ODN **7**; ODNs in lanes 2, 3, 6 and 7 were photoirradiated; ODNs in lanes 1–3, and 6 were heated with piperidine; lanes 4 and 5, Maxam–Gilbert G+A sequencing reactions. Partial base sequences of both oligomers were shown in the middle. For clarity, the lanes for ODN **7** were shown upside down

possessed two GG sites ($G_{34}G_{35}$ and $G_{48}G_{49}$). Each strand was ^{32}P-5′-end labeled, annealed with the complement and then photoilluminated. As is clear from Fig. 2, strand cleavage of ODN **6** selectively occurred at G_8 of the G_8G_9 step, but no G cleavage occurred at the $G_{18}G_{19}$ step (lane 3). This is probably because $G_{18}G_{19}$ is located in six base pairs apart from G_{12}, where hole is initially generated. The hole migration through five AT base pairs proceeds with extremely low efficiency as described previously.[6,7] G oxidation of ODN **7** was observed only at G_{48} of the $G_{48}G_{49}$ step (lane 6). Preferential oxidation of G_8G_9 and $G_{48}G_{49}$ steps is rationalized by initial one-electron oxidation of G_{12}, followed by a subsequent hole migration to the G_8G_9 and then hopping to $G_{48}G_{49}$ sites (Scheme 2). In contrast, hole migration to $G_{18}G_{19}$ and $G_{34}G_{35}$ is unfeasible because of the long distance between G_{18} and G_{12}.



Scheme 2. Schematic representation of the one direction hole transport shown in Fig. 2. Guanine radical cation is site selectively generated at G_{12}, followed by a subsequent hole migration only to G_8G_9 and $G_{48}G_{49}$ sites. The vertical arrows indicate the site of strand cleavage

We showed that $GT^{CNBP}U$ is a very useful sequence for generating a hole at predetermined G site in duplex DNA. We also observed a remarkable one direction hole transport. The results reported here clearly indicated that $d^{CNBP}U$-containing oligomers are widely usable for the studies of DNA-mediated charge transport.

References

1. For reviews, see: (a) Núñez, M. E.; Barton, J. K. *Curr. Opin. Chem. Biol.* **2000**, *4*, 199–206. (b) Grinstaff, M. W. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3629–3635. (c) Beratan, D. N.; Priyadarshy, S.; Risser, S. M. *Chem. Biol.* **1997**, *4*, 3–8.
2. (a) Núñez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85–97. (b) Arkin, M. R.; Stemp, E. D. A.; Pulver, S. C.; Barton, J. K. *Chem. Biol.* **1997**, *4*, 389–400. (c) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731–734.
3. (a) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253–260. (b) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353–8358. (c) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762–12771.
4. (a) Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *Nature* **2000**, *406*, 51–53. (b) Lewis, F. D.; Wu, T.; Liu, X.; Letsinger, R. L.; Greenfield, S. R.; Miller, S. E.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 2889–2902. (c) 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. Engl.* **1998**, *37*, 158–161.
6. (a) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950–12955. (b) Bixon, M.; Giese, B.; Wessely, S.; Langenbacher, T.; Michel-Beyerle, M. E.; Jortner, J. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11713–11716.
7. (a) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **1999**, *121*, 10854–10855. (b) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5893–5894.
8. (a) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6014–6019. (b) Kelley, S. O.; Barton, J. K. *Science* **1999**, *283*, 375–381. (c) Kelley, S. O.; Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 9861–9870.

9. (a) Berlin, Y. A.; Burin, A. L.; Ratner, M. A. *J. Phys. Chem. A* **2000**, *104*, 443–445. (b) Grozema, F. C.; Berlin, Y. A.; Siebbeles, L. D. A. *Int. J. Quant. Chem.* **1999**, *75*, 1009–1016. (c) Jortner, J.; Bixon, M.; Langenbacher, T.; Michael-Beyerle, M. E. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12759–12765.
10. (a) Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635–638. (b) Fink, H.-W.; Schönenberger, C. *Nature* **1999**, *398*, 407–410.
11. Steenken, S. *Biol. Chem.* **1997**, *378*, 1293–1297.
12. (a) Nakatani, K.; Dohno, C.; Saito, I. *J. Org. Chem.* **1999**, *64*, 6901–6904. (b) Nakatani, K.; Dohno, C.; Nakamura, T.; Saito, I. *Tetrahedron Lett.* **1998**, *39*, 2779–2782.
13. (a) Yoshioka, Y.; Kitagawa, Y.; Takano, Y.; Yamaguchi, K.; Nakamura, T.; Saito, I. *J. Am. Chem. Soc.* **1999**, *121*, 8712–8719. (b) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686–12687. (c) Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373–7374. (d) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845–846. (e) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063–7068. (f) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406–6407.
14. Only hot piperidine treatment of $d^{CNBP}U$-containing oligomers without photoirradiation produced a weak cleavage band at the modified base.