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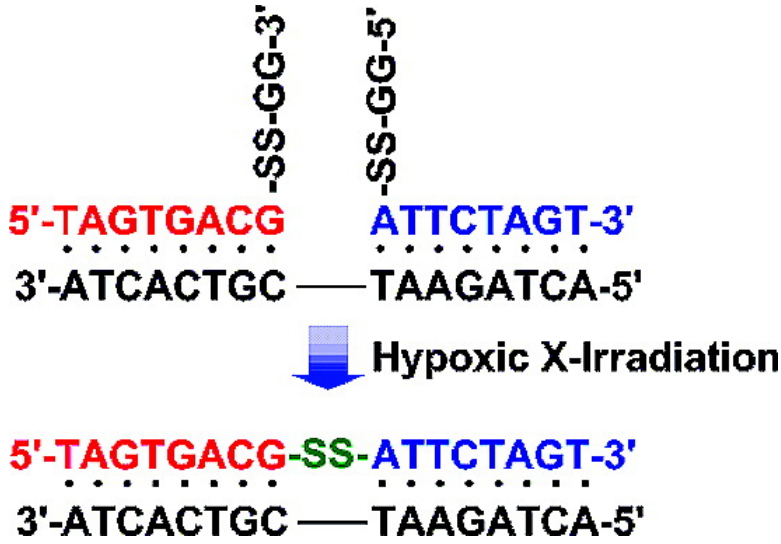

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One-Electron Reductive Template-Directed Ligation of Oligodeoxynucleotides Possessing a Disulfide Bond

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Disulfide bridges are one of the key regulators for conservation of well-defined structures of supramolecules. Disulfide bonds in the supramolecules such as proteins,[1] DNA architecture,[2] or polymers[3] are formed between two thiol groups to stabilize their folded forms. The higher-order structure of the supramolecule as created by such a folding feature is integral to the specific functions.

Disulfides, thiols, and their related radicals have been shown to induce unique reactions, including formation or cleavage of covalent bonding under reduction conditions. For example, the disulfide radical anion ($RSSR^{-\bullet}$) generated by one-electron reduction of a disulfide decomposes into a sulfide anion (RS^-) and thiyl radical ($RS^\bullet$).[4] Sulfide anions tend to undergo disulfide exchange reactions in the presence of disulfide compounds,[5] while the thiyl radical abstracts a hydrogen atom to form thiols.[6] In view of these reaction characteristics, it is essential to characterize the reactivity of the disulfide bond in the functional supramolecules.

High-energy ionizing radiation is an effective tool to generate a representative reducing species of hydrated electrons (e_{aq}^-) in a diluted aqueous reaction system, thereby inducing one-electron reductive formation or cleavage of chemical bonds under certain conditions.[7,8] Therefore, the ionizing radiation may be an appropriate extrinsic trigger for activation of disulfides in aqueous solution.

We attempted to characterize a radiolytic reaction of disulfide bonds on oligodeoxynucleotides (ODNs) that show unique one-electron reduction characteristics as identified by electrochemical techniques.[2b] This study illustrates that hypoxic X-irradiation of diluted aqueous solution induces highly regioselective ligation of two types of ODN strands possessing a disulfide bond in the presence of a complementary template ODN.

The ODNs possessing a disulfide bond were prepared by a standard automated DNA synthesis, as summarized in Figure 1. We initially performed one-electron reduction of dinucleotides bearing G bases (ODN 1) by X-radiolysis of an argon-purged aqueous solution[9] containing excess amount of 2-methyl-2-propanol. Reducing hydrated electrons are thus generated as the major active species along with a minor amount of hydrogen atoms (•H) under these radiolysis conditions, where oxidizing hydroxyl radicals (•OH) may be effectively scavenged by 2-methyl-2-propanol.[10] Figure 2a,b shows representative reaction profiles of the radiolytic reduction of ODN 1 under hypoxic conditions. Hypoxic irradiation of ODN 1 produced two new products (ODN 1a and ODN 1b), the yields of which increased with increasing radiation dose (Figure S1). Molecular weights of both products are identical to that of the starting material ODN 1, as identified by MALDI-TOF MS.[11] These results suggest that both products have chemical structures possessing a disulfide bond, similar to the original ODN 1, but not identical in structure. In contrast to hypoxic X-radiolysis, the reaction of ODN 1 was less efficient upon X-radiolysis under aerobic conditions, where a significant amount of starting material ODN 1 was recovered (Figure 2c). In view of the well-documented evidence that molecular oxygen efficiently captures the reducing species e_{aq}^- to form superoxide anion radical ($O_2^{-\bullet}$)[12] and thereby inhibits reduction, the hypoxic radiolysis of ODN 1 is most likely to occur via one-electron reduction by e_{aq}^- almost exclusively under hypoxic conditions.

Figure 1. Sequences and structure of oligodeoxynucleotides.

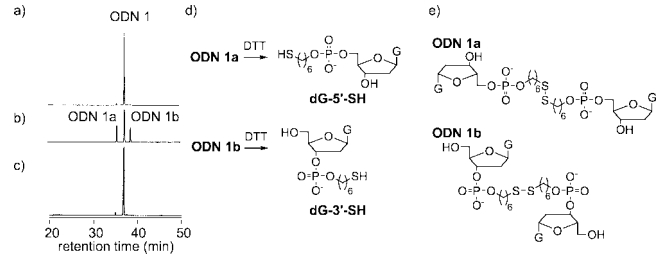

Figure 2. (a−c) HPLC profiles for the one-electron reduction of ODN 1 (50 μM) upon X-radiolysis of aqueous solution containing 2-methyl-2-propanol (5 mM): (a) before irradiation, (b) X-irradiation (15 Gy) under hypoxic conditions, (c) X-irradiation (15 Gy) under aerobic conditions. (d) Treatment of radiation products ODN 1a and 1b by DTT. (e) Chemical structures of ODN 1a and ODN 1b.

To identify the reaction products, both ODN 1a and ODN 1b were treated with dithiothreitol (DTT), which is a well-known reduction agent to convert disulfide into monomeric thiols (Figure 2d). The HPLC analysis revealed that ODN 1a and 1b produced their respective single products, which were identified as the 2′-deoxyguanosine derivatives possessing a monomeric thiol group at the 5′-side (dG-5′-SH) and 3′-side (dG-3′-SH), respectively.[13] These results strongly indicate that a couple of dG-5′-SH products formed a disulfide bond at the 5′-thiol group into ODN 1a, while dG-3′-SH products formed a similar disulfide bond at the 3′-thiol group into ODN 1b. Thus, hypoxic X-irradiation induced ligation of ODN 1 into two types of modified ODNs. The *G* values[10] were estimated as 2.42 and 2.13 μmol/J for the formation of ODNs 1a and 1b, respectively. Since the *G* value for the formation of e_{aq}^- in the radiolysis of a diluted aqueous solution is evaluated as 280 nmol/J, the formation of ODNs 1a and 1b should occur as a turnover reaction process. Considering the reactivity of disulfides and sulfide anions,[5] it is not surprising that the observed ligation of ODN 1 is an efficient reaction process. Thus, the disulfide bond in ODN 1 undergoes one-electron reduction to form $GSSG^{-\bullet}$ radical anions that decompose into sulfide anion RS^- and thiyl radical $RS^\bullet$,[14] the latter of which is further one-electron reduced into sulfide anion RS^- under steady generation of e_{aq}^-. The resulting RS^- could be a nucleophile for the disulfide bond to induce an interchange reaction into formation of a new disulfide bond along with regeneration of a sulfide anion. The multiple turnovers of the nucleophilic sulfide anion account for the efficient ligation of ODN

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1 (Figure S3). Similar X-radiolytic reductive ligation reactions with high efficiency under hypoxic conditions were confirmed for ODNs 2−4 with their respective base sets, indicating that the influence of base sequence on the present ligation reaction is small (see Table S1).

To identify the side reactions potentially caused by a residual hydroxyl radical and H atom as well as 2-methyl-2-propanol, we conducted hypoxic radiolysis of normal dinucleotides (5′-dTT-3′ and 5′-dGG-3′). The nucleobase and 2′-deoxyribose moieties showed much less reactivity to undergo a negligible amount of side reactions under the present conditions with a lower dose of X-ray (Figure S4). On the other hand, the disulfide bond turned out to play a critical function in the radiolytic ligation reaction.

In light of the above reaction characteristics, an attempt was also made to demonstrate radiolytic one-electron reduction of an ODN possessing a disulfide bond with a longer strand length. We performed radiolytic reduction of a mixture of ODNs 5 and 6 possessing disulfide bonds in the presence and absence of a complementary strand at 21 °C under hypoxic conditions.[15] The reaction profiles as monitored by polyacrylamide gel electrophoresis are shown in Figure 3. Hypoxic irradiation of ODNs 5 and 6 in the absence of complementary ODN 8 resulted in formation of various products such as 20mer ODN 5a and 30mer ODN 6a as well as 25mer ODN 7 as a consequence of random ligation reactions. In contrast, addition of complementary ODN 8 led to ligation of ODNs 5 and 6 into ODN 7 with high regioselectivity, the yield of which increased by up to 2.8-fold relative to the yield obtained in the absence of ODN 8. We confirmed the formation of ODN 7 by MALDI-TOF MS analysis after purification of the crude products.[16] These results indicate that cohybridization of ODNs 5 and 6 with ODN 8 into a duplex structure gives rise to close contact of ODNs 5 and 6, thereby leading to a specific disulfide bond interexchange to form a substantial amount of ODN 7.[17] In the separate experiments, we also determined the radiolytic reactivity of an equimolar mixture of ODN 5, ODN 6, and noncomplementary ODN 9. Upon hypoxic X-irradiation, random ligation occurred between ODNs 5 and 6, as in the absence of complementary ODN 8. These results show that interexchange of the disulfide bonds on a duplex derived from hybridization of ODNs 5 and 6 with ODN 8 could efficiently enhance the regioselective ligation to form ODN 7. Thus, control of radiolytic one-electron reduction of disulfide bonds in ODNs may lead to an efficient regioselective preparation of a prescribed ODN.

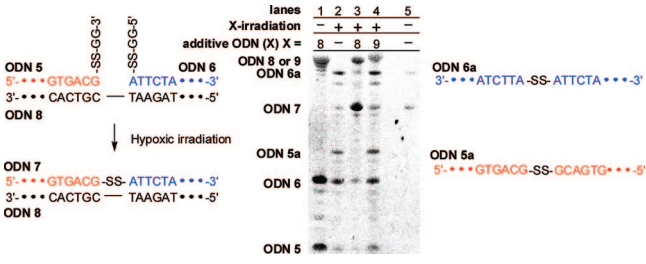

Figure 3. Denaturing gel electrophoresis for X-irradiated hypoxic solutions of ODNs 5 and 6 in the presence or absence of complementary ODN 8. After irradiation (0 or 200 Gy), the samples were electrophoresed, and then the gel was stained with ethidium bromide: lane 1, the sample consisting of ODNs 5, 6, and 8 before irradiation; lane 2, irradiation of ODNs 5 and 6; lane 3, irradiation of ODNs 5 and 6 in the presence of complementary ODN 8; lane 4, irradiation of ODNs 5 and 6 in the presence of noncomplementary ODN 9; lane 5, authentic sample of ODN 7. The yield of ODN 7: 18% (lane 2), 48% (lane 3), and 14% (lane 4).

In summary, we characterized one-electron reduction of a disulfide bond incorporated into ODNs. Hypoxic X-irradiation of dinucleotides in aqueous solution efficiently produced two forms of dinucleotides via intermolecular exchange of the disulfides and ligation that proceeded with a multiple turnover. In contrast to the efficient reaction by hypoxic X-irradiation, the reaction efficiency was dramatically decreased when irradiated under aerobic conditions. More remarkable was that the reductive ligation reaction between two types of ODNs with longer sequences in the presence of a template ODN strand could lead to the formation of a prescribed ODN in substantial amounts. Thus, radiolytic one-electron reduction of a disulfide bond can be used as an effective and easy method for regioselective template-directed preparation of a prescribed ODN. Further exploration of regulation of higher-order DNA structures by means of X-radiolytic reduction is in progress.

Supporting Information Available: Experimental procedures and detailed data of radiolytic reduction and treatment with DTT. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Burke-Gaffney, A.; Callister, M. E.; Nakamura, H. *Trends Pharmacol. Sci.* **2005**, *26*, 398. (b) Abdoul-Carime, H.; Cecchini, S.; Sanche, L. *Radiat. Res.* **2002**, *158*, 23.
(2) (a) Endo, M.; Majima, T. *Chem. Commun.* **2004**, 1308. (b) Gorodetsky, A. A.; Barton, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 6074.
(3) (a) Lee, Y.; Mo, H.; Koo, H.; Park, J.-Y.; Cho, M. Y.; Jin, G.-W.; Park, J.-S. *Bioconjugate Chem.* **2007**, *18*, 13. (b) Meng, Y. Z.; Liang, Z. A.; Lu, Y. X.; Hay, A. S. *Polymer* **2005**, *46*, 11117. (c) Endo, K. *Kobunshi* **2005**, *54*, 805.
(4) (a) Antonello, S.; Benassi, R.; Gavioli, G.; Taddei, F.; Maran, F. *J. Am. Chem. Soc.* **2002**, *124*, 7529. (b) Hoffman, M. Z.; Hayon, E. *J. Am. Chem. Soc.* **1972**, *94*, 7950.
(5) (a) Mathur, N. K.; Narang, C. K.; Williams, R. E. *Polymers as Aids in Organic Chemistry*; Academic Press: New York, 1980. (b) Ishiguro, M. *SHki no Kagaku Shuushoku*; Japan Scientific Societies Press: Tokyo, 1978.
(6) Wojcik, A.; Naumov, S.; Marciniak, B.; Brede, O. *J. Phys. Chem. B* **2006**, *110*, 12738.
(7) Spinks, J. W. T.; Wood, R. J. *Introduction to Radiation Chemistry*, 3rd ed.; Wiley-Interscience: New York, 1990.
(8) Tanabe, K.; Zhang, Z.; Ito, T.; Hatta, H.; Nishimoto, S. *Org. Biomol. Chem.* **2007**, *5*, 3745.
(9) Ar bubbling was employed to purge oxygen from an aqueous solution for radiolytic reduction. For comparison, X-irradiation of ODN 1 was also carried out in anoxic aqueous solution, as prepared by a freeze−thaw cycle technique. Thus, we confirmed that the yields of ODN 1a and ODN 1b obtained from anoxic irradiation were similar to those obtained in the present Ar-purged reaction system (see Figure S5).
(10) Radiolysis of a diluted aqueous solution at around pH 7.0 produces primary water radicals such as oxidizing hydroxyl radicals (•OH), reducing hydrated electrons (e_{aq}^-), and reducing hydrogen atoms (•H) with the G values of G(•OH) = 280 nmol/J, $G(e_{aq}^-)$ = 280 nmol/J, and G(•H) = 60 nmol/J. G values: the number of molecules produced per 1 J of radiation energy absorbed by the reaction system.
(11) MALDI-TOF mass; ODN 1, 5′-dG-$(CH_2)_6$-SS-$(CH_2)_6$-dG-3′, *m/z* 923.62 (calcd for $[M-H]^-$ 923.87); ODN 1a, *m/z* 923.47; ODN 1b, *m/z* 923.58.
(12) Similar effect of oxygen was observed for the activation of 5-fluoro-2′-deoxyuridine prodrugs. See: Tanabe, K.; Makimura, Y.; Tachi, Y. ; Imagawa-Sato, A. Nishimoto, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2321.
(13) The products (dG-5′-SH and dG-3′-SH) were identified by an HPLC analysis with overlap injection of authentic samples, which were prepared by treatment of ODN 1 by DTT. See Figure S2.
(14) In the present reactions with ODNs, we could not detect any possible products that may be derived from several reactions involving thiyl radical produced by splitting of $RSSR^-$, such as hydrogen abstraction and addition to pyrimidine bases, as reported previously.
(15) Melting temperatures of ODN 5/ODN 8 duplex and ODN 6/ODN 8 duplex were characterized to be 33.1 and 52.6 °C, respectively. In view of these thermal denaturation profiles, we carried out radiolytic reacitons at 21 °C.
(16) We confirmed the formation of ODN 7 in the X-radiolysis of ODN 5 and ODN 6 in the presence of ODN 8 by MALDI-TOF MS; *m/z* 8040.37 (calcd for $[M-H]^-$ 8039.41).
(17) Recent reports for similar template-directed chemical ligation of DNA: (a) Ihara, T.; Fujii, T.; Mukae, M.; Kitamura, Y.; Jyo, A. *J. Am. Chem. Soc.* **2004**, *126*, 8880. (b) Gartner, Z. J.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370. (c) Czlapinski, J. L.; Sheppard, T. L. *J. Am. Chem. Soc.* **2001**, *123*, 8618. (d) Xu, Y.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 9040. (e) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646.

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