Chemical ligation of oligodeoxynucleotides by X-irradiation and its application to regulation of G-quadruplex formation

Kazuhito Tanabe,* Taiga Mizoe, Masaaki Sugiura, Kana Okada and Sei-ichi Nishimoto

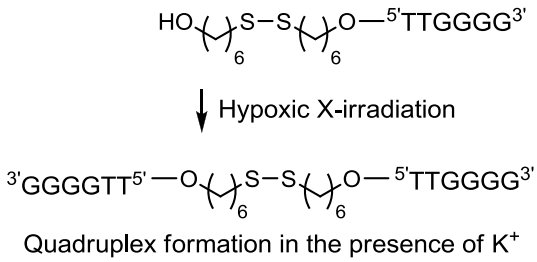
Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Katsura Campus, Nishikyo-ku, Kyoto 615-8510, Japan

*corresponding author

Phone: +81-75-383-2505 FAX: +81-75-383-2504

e-mail: tanabeka@scl.kyoto-u.ac.jp

Graphical Abstract



Abstract

We demonstrated radiolytic ligation of oligodeoxynucleotides (ODNs) possessing disulfide bond and its application to regulation of DNA quadruplex formation. G-rich hexamer ODNs had poor ability to form quadruplex, while X-irradiation of the ODNs induced interstrand exchange reaction at disulfide bond to form ligated 12 mer ODNs, leading to the ready formation of quadruplex due to the entropic effect. Since complexation of the ligated ODNs with hemin in the presence of K^+ showed strong soret band absorption and also catalyzed the H_2O_2-mediated oxidation of luminol, it appears that the quadruplex formed from ligated ODNs showed a function similar to native DNA quadruplex.

DNA or RNA, which contains guanine-rich sequences, can form G-quadruplexes that appear to play a significant role in gene function.[1] The formation of this unique structure often occurs within human genome centromeres and teromeres, and therefore G-quadruplex research is underway on topics ranging from fundamental structural chemistry to therapeutic advance.[2–8] G-quadruplexes exhibit a marked heterogeneity of higher order structures. As such, they may be composed of one, two, or four parallel or antiparallel strands, depending on the number of G-rich segments, the strand length, and the type and concentration of monovalent cations present, respectively.[9–17] Since the function and feature of G-quadruplex are related with their conformation, the regulation of their structure is essential for understanding the properties of G-quadruplex in the biological systems.

Here, we demonstrate regulation of G-quadruplex formation by X-irradiation. X-irradiation is a useful exogenous trigger for the activation of many biomolecules because radiation-induced chemical reactions can be regulated spatially and temporally.[18] Recently, we showed effective functionality of disulfide bond for radiolytic ligation and cyclization of oligodeoxynucleotides (ODNs).[19–22] A mechanistic

studies revealed that disulfide bond in ODNs undergoes reduction by reducing reactive species such as hydrated electrons (e_{aq}^-) or hydrogen atoms ($H^\bullet$), which are generated by the radiolysis of water molecules,[23] to form the thiyl radical intermediate for the succeeding disulfide exchange into ligation and cyclization.[20] In the present study, we extended our work on radiolytic disulfide exchange in ODNs to regulation of G-quadruplex formation. Our strategy for increasing the formation efficiency of quadruplex is to radiolytically ligate the two ODNs possessing G-rich sequences. The overall free energy of quadruplex formation is expected to be more favorable for dimerized ODNs, because two dimerized-ODNs should form quadruplex with more favorable entropy than four individual ODNs. Therefore, we prepared and characterized the reactivity of G-rich ODN hexamer with disulfide bond at strand end (ODN 1). We found that ODN 1 dimerized upon hypoxic X-irradiation via a strand exchange reaction at disulfide bond to generate 12 mer ODNs (ODN 2), and this 12 mer ODNs readily formed G-quadruplex.

(Figure 1)

The ODNs possessing a disulfide bond at the strand end (ODN 1) was prepared by standard automated DNA synthesis.[24] The structure and sequence of ODN 1 are shown in Figure 1. We initially conducted reduction of ODN 1 by means of X-irradiation in argon-purged aqueous solution containing 2-methyl-2-propanol.[25] The scavenger 2-methyl-2-propanol was added to prevent side reactions derived by oxidizing hydroxyl radicals, which are one of the active species generated by the radiolysis of water molecule. Thus, reducing e_{aq}^- and $H^\bullet$ act as the major active species under these conditions for radiolysis.[23] Figure 2 shows a representative profile for the radiolytic reduction of ODN 1. X-irradiation to ODN 1 under hypoxic conditions gave a single new product, the yield of which increased as a function of increasing radiation dose. To identify the product, we purified it by HPLC, and analyzed with mass spectrometry. The molecular weight of the product was identical to the 12 mer ODNs possessing a disulfide bond (ODN 2),[24] confirming that hypoxic X-irradiation of ODN 1 induced an exchange reaction of disulfide bond to form ligated ODN 2. The G values were estimated to be 80.7 nmol/J for the decomposition of ODN 1 and 21.6 nmol/J for the

formation of ODN 2. To verify the reaction mechanism in detail, we next performed a similar radiolytic reduction of ODN 1 under aerobic conditions. As shown in Figure 2, both formation of ODN 2 and decomposition of ODN 1 were markedly suppressed, in contrast to the efficient ligation upon hypoxic irradiation. There are many reactions showing that molecular oxygen captures both e_{aq}^- and $H^{\bullet}$,[23,26] and thereby it is reasonable to conclude that these reactive species are key for the radiolytic ligation of ODNs possessing a disulfide bond.

(Figure 2)

To confirm that ligated product, ODN 2, formed G-quadruplex in the presence of K^+, we followed the absorption of ODNs in the presence of hemin,[27] Since it is well-documented that the Soret band of hemin is enhanced on binding of a DNA quadruplex.[28,29] As shown in Figure 3A, hemin in the presence of ODN 1 showed a weak Soret band at 402 nm, illustrating minimal hemin-quadruplex interaction. On the other hand, Soret band absorption increased dramatically when the hypoxically

X-irradiated ODN 1 was added to the hemin. These results strongly indicate that ODN 2 produced by X-irradiation of ODN 1 efficiently formed quadruplex, which interacts with hemin strongly, while ODN 1 hardly formed quadruplex. We also confirmed that addition of Na^+ instead of K^+ resulted in a slight increase of absoption intensity. K^+ preferably attributed to the quadruplex formation of ODN 2, consistent with the previous results.[30]

(Figure 3)

The K^+-stabilized quadruplexes have been shown to bind hemin, resulting in a complex that mimics the horseradish peroxidase and catalyzes the H_2O_2-mediated oxidation of luminol to generate chemiluminescence (CL) emission.[31,32] In light of the above properties, further attempt was made to verify the function of quadruplex formed from X-irradiated ODN 1 by monitoring CL emissions.[33] X-irradiated ODN 1 was added to the solution containing hemin, K^+ and H_2O_2, and then emission spectra were immediately measured. As shown in Figure 4, addition of ODN 1 without X-irradiation

led to only slight emission. It is striking that strong CL was observed when the hypoxically irradiated ODN 1 was added to luminol. These results clearly indicate that quadruplex formed from ODN 2, which were generated from X-irradiation of ODN 1, exhibited a catalytic function similar to that of naturally occurring DNA quadruplexes.

(Figure 4)

In conclusion, we have characterized the radiolytic ligation and quadruplex formation of ODNs possessing a disulfide bond at the strand end. X-irradiation of hexamer ODN 1 induced a strand exchange reaction at disulfide bond to form a ligated product, ODN 2. The reaction proceeded in a hypoxia selective manner, because molecular oxygen captured the reactive species, e_{aq}^- and $H^\bullet$. ODN 2 formed quadruplex in the presence of K^+ and Na^+, while ODN 1 had poor quadruplex formation capability, indicating that ligation facilitated the quadruplex formation due to the entropic effect. The quadruplex consisting of ODN 2 bound to hemin to form the complex that catalyzed the oxidation of luminol mediated by H_2O_2. Thus, formation and function of DNA quadruplex were

successfully regulated by X-irradiation.

Acknowledgement: We thank Dr. Hiroshi Harada (Group of Radiation and Tumor Biology, Career-Path Promotion Unit for Young Life Scientists, Kyoto University) for X-irradiation experiments. This work is partly supported by the Strategic Promotion Program for Basic Nuclear Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References and Notes

(1) Xu, Y. *Chem. Soc. Rev.* **2011**, *40*, 2719.

(2) Li, Y.; Li, X.; Ji, X.; Li, X. *Biosens. Bioelectron.* **2011**, *26*, 4095.

(3) Xu, Y.; Suzuki, Y.; Komiyama, M. *Angew. Chem. Int. Ed.* **2009**, *48*, 3281.

(4) Miyoshi, D.; Sugimoto, N. *Biochimie* **2008**, *90*, 1040.

(5) Miyoshi, D.; Karimata, H.; Wang, Z. –M.; Koumoto, K.; Sugimoto, N. *J. Am. Chem. Soc.* **2007**, *129*, 5919.

(6) Virgilio, A.; Esposito, V.; Randazzo, A.; Mayol. L.; Galeone, A. *Nucleic Acids Res.* **2005**, *33*, 6188.

(7) Krishnan-Ghosh, Y.; Stephens, E.; Balasubramanian, S. *J. Am. Chem. Soc.* **2004**, *126*, 5944.

(8) Lyonnais, S.; Gorelick, R. J.; Mergny, J. –L.; Cam, E. L.; Mirambeau, G. *Nucleic Acids Res.* **2003**, *31*, 5754.

(9) Phan, A. T.; Kuryavyi, V.; Luu, K. N.; Patel. D. J. *Nucleic Acids Res.* **2007**, *35*, 6517.

(10) Dai, J.; Punchihewa, C.; Ambrus, A.; Chen, D.; Jones, R. A.; Yand, D. Z. *Nucleic*

Acids Res. **2007**, *35*, 2240.

(11) Phan, A. T.; Luu, K. N.; Patel, D. J. *Nucleic Acids Res.* **2006**, *34*, 5715.

(12) Matsugami, A.; Xu, Y.; Noguchi, Y.; Sugiyama, H.; Katahira, M. *FEBS J.* **2007**, *274*, 3545.

(13) Luu, K. N.; Phan, A. T.; Kuryavyi, V.; Lacroix, L.; Patel, D. J. *J. Am. Chem. Soc.* **2006**, *128*, 9963.

(14) Xu, Y.; Noguchi, Y.; Sugiyama, H. *Bioorg. Med. Chem.* **2006**, *14*, 5584.

(15) Parkinson, G. N.; Lee, M. P.; Neidle, S. *Nature* **2002**, *417*, 876.

(16) Kato, Y.; Ohyama, T.; Mita, H.; Yamamoto, Y. *J. Am. Chem. Soc.* **2005**, *127*, 9980.

(17) Wang, Y.; Pate. D. J. *J. Mol. Biol.* **1993**, *234*, 1171.

(18) Spinks, J. W. T.; Woods, R. J. Introduction to Radiation Chemistry, 3rd ed.; Wiley-interscience: New York, 1990.

(19) Tanabe, K.; Asada, T.; Ito, T.; Nishimoto, S. *Bioconjugate Chem.* **2012**, *23*, 1909.

(20) Tanabe, K.; Matsumoto, E.; Ito, T.; Nishimoto, S. *Org. Biomol. Chem.* **2010**, *8*,

4837.

(21) Tanabe, K.; Kuraseko, E.; Yamamoto, Y.; Nishimoto, S. *J. Am. Chem. Soc.* **2008**, *130*, 6302.

(22) Tanabe, K.; Asada, T.; Nishimoto, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7045.

(23) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.

(24) The formation of ODN 1 and ODN 2 was identified by measurement of ESI-TOF mass spectrometry. (ODN 1: calcd 1094.34, found 1094.21 $[M–2H]^{2-}$; ODN 2: calcd 1370.57, found 1370.91 $[M–3H]^{3-}$.

(25) **Radiolytic reduction of ODN 1.** To establish hypoxia, aqueous solutions of ODN1 (40 μM) containing 2-methyl-2-propanol (50 mM) were purged with argon for 10 min and then irradiated in a sealed glass ampoule at ambient temperature with an X-ray source (4.0 Gy min^{-1}). After the irradiation, the solution was immediately subjected to HPLC analysis.

(26) Tanabe, K.; Zhang, Z.; Ito, T.; Hatta, H.; Nishimoto, S. *Org. Biomol. Chem.* **2007**, *5*, 3745.

(27) **Identification of quadruplex formation by measurement of UV absorption spectra.** To measure the UV spectra, ODN 1 (2.5 μM) was X-irradiated under hypoxic conditions, and then the measurement of UV-vis spectra of X-irradiated ODN 1 were performed in the presence of 5 μM hemin containing 1mM KCl in Tris-Ac buffer (pH 8.0) at 25 ˚C.

(28) Li, T.; Shi, L.; Wang, E.; Dong, S. *Chem. Eur. J.* **2009**, *15*, 1036.

(29) Li, T.; Wang, E.; Dong, S. *Anal. Chem.* **2010**, *82*, 1515.

(30) Sen, D.; Gilbert, W. *Nature* **1990**, *344*, 410.

(31) Li, T.; Du, Y.; Wang, E. *Chem. Asian J.* **2008**, *3*, 1942.

(32) Liu, X.; Freeman, R.; Golub, E.; Willner I. *ACS Nano* **2011**, *5*, 7648.

(33) **H_2O_2-mediated oxidation of luminol and measurement of chmiluminescence.** ODN 1 was X-irradiated (0 Gy or 650 Gy) under hypoxic conditions, and then the measurement of luminenscence spectra were performed. Briefly, solution of hemin (0.5 μM) / X-irradiated ODN 1 (0.25 μM) containing KCl (2 mM) and luminol (5 mM) were added to a cuvette. To the resulting solution, H_2O_2 solution (30 mM) was quickly added, and then the light emission was measured

immediately. Measurements were performed in 10mM Tris-Ac buffer.

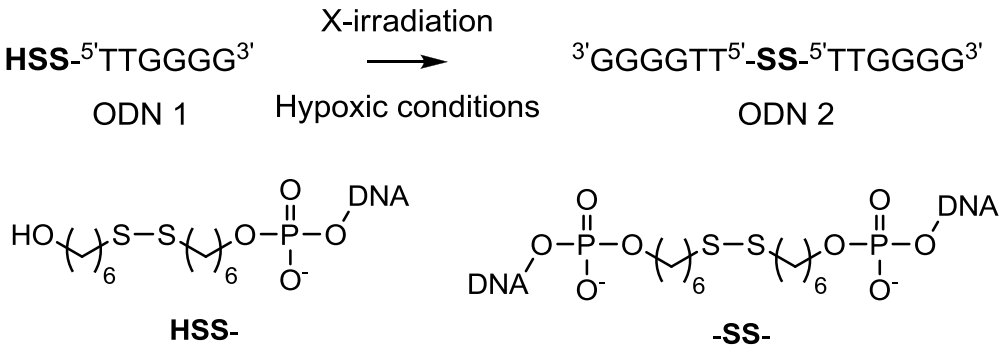


Figure 1. Sequences and structure of oligodeoxynucleotides used in this study and chemical ligation upon hypoxic X-irradiation.

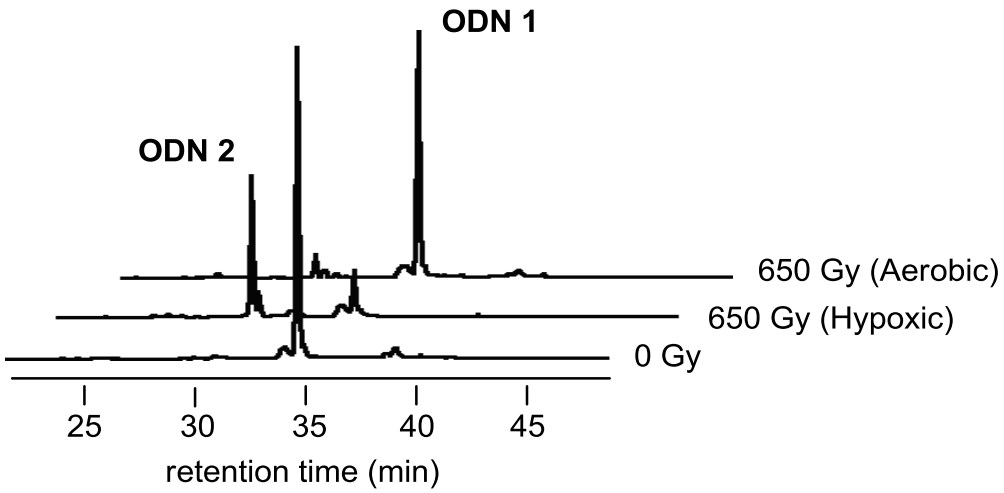


Figure 2. HPLC profiles for reaction of ODN 1 (40 μM) in the X-radiolysis of aqueous solution containing 2-methyl-2-propanol (50 mM) under hypoxic or aerobic conditions.

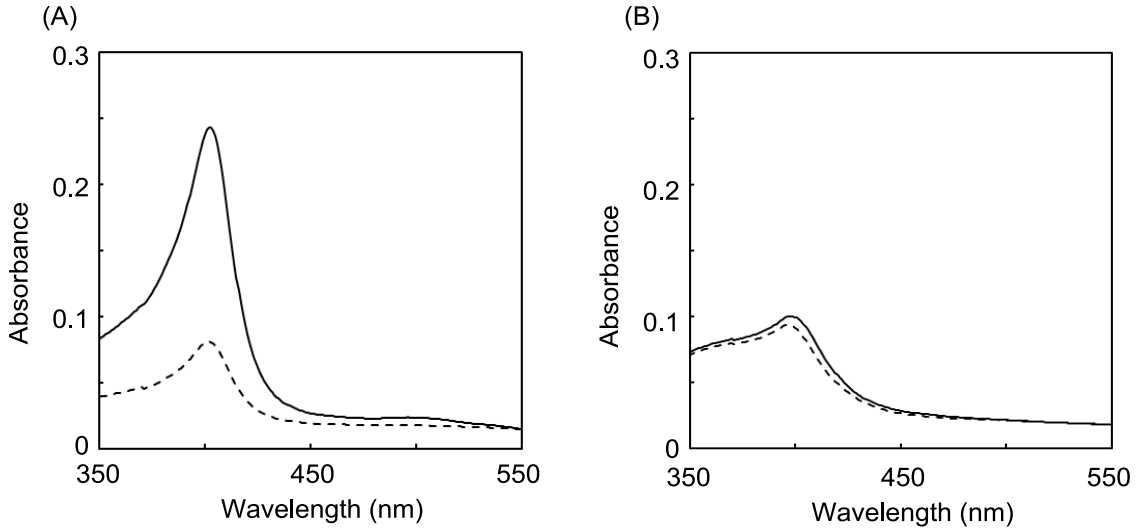


Figure 3. Analysis of hemin-quadruplex interactions by using UV/Vis absorption spectroscopy. An aqueous solution of ODN 1 (2.5 μM) containing 2-methyl-2-propanol (50 mM) was X-irradiated (0 Gy: dotted line, 650 Gy: solid line) under hypoxic conditions, and then hemin (5 μM) was added to the resulting solution. The measurement of absorption spectra was performed in Tris-Ac buffer (pH 8.0) at 25 ˚C in the presence of 1 mM KCl (A) or 1 mM NaCl (B).

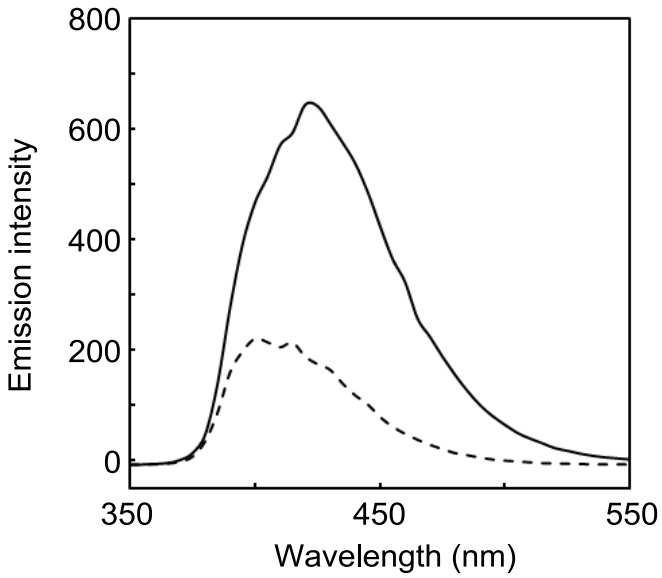


Figure 4. Chemiluminescence emission on the basis of the formation of G-quadruplex. An aqueous solution of ODN 1 (250 nM) was X-irradiated (0 Gy: dotted line, 650 Gy: solid line) under hypoxic conditions, and then hemin (500 nM), luminol (5 mM), H_2O_2 (30 mM) and KCl (2 mM) were added to the resulting solution. After the addition of these reagents, emission spectra were measured immediately.