Supporting Information

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

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Experimental Section

General Methods

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry of oligonucleotides were obtained on JEOL LMS-ELITE MALDI-TOF MASS spectrometer with 2',3',4'-trihydroxyacetophenone as matrix. The oligonucleotides were purchased from Invitrogen. The reagents for DNA synthesizer such as A, T, G, C and thiol-modifier C6 S-S were purchased from Glen Research. Calf intestinal alkaline photphatase (AP), nuclease P1 (P1) and phosphodiesterase I were purchased from PROMEGA, YAMASA and ICN, respectively. High-performance liquid chromatography (HPLC) was performed with Shimadzu 6A, HITACHI D-7000 or HITACHI L-2400 HPLC system. Sample solutions were injected on a reversed phase column (Inertsil ODS-3, GL Sciences Inc., ϕ 4.6 mm $\times$ 150 mm or 10 mm $\times$ 150 mm). The solvent mixture of 0.1 M triethylamine acetate (TEAA), pH 7.0 and 100% acetonitrile was delivered as mobile phase at a flow rate of 0.6 mL or 3.0 mL / min at 25 $^{\circ}$C. The column eluents were monitored by the UV absorbance at 254 nm or 260 nm. Rigaku RADIOFLEX-350 was used for X-radiolysis. Gel electrophoresis was carried out on a ATTO AE-6155 apparatus. All aqueous solutions were prepared using purified water (YAMATO, WR600A).

Synthesis of oligodeoxynucleotides. ODNs bearing disulfide bond were synthesized by the conventional phorphoramidite method using an Applied Biosystems 392 DNA/RNA synthesizer. Synthesized ODNs were purified by reversed phase HPLC on a Inertsil ODS-3 column (10 × 250 mm, elution with a solvent mixture of 0.1 M triethylammonium acetate (TEAA), pH 7.0, linear gradient over 40 min from 0% to 30% acetonitrile at a flow rate 3.0 mL/min). Mass spectra of ODNs purified by HPLC were determined with MALDI-TOF mass spectroscopy (acceleration voltage 21 kV, negative mode) with 2',3',4'-trihydroxyacetophenone as matrix, using T_8 ($[M–H]^-$ 2370.61) and T_{17} ($[M–H]^-$ 5108.37) as an internal standard; **ODN 1**, 5'-dG-$(CH_2)_6$-SS-$(CH_2)_6$-dG-3', *m/z* 923.62 (calcd for $[M–H]^-$ 923.87); **ODN 2**, 5'-dA-$(CH_2)_6$-SS-$(CH_2)_6$-dA-3', *m/z* 891.33 (calcd for $[M–H]^-$ 891.87); **ODN 3**, 5'-dT-$(CH_2)_6$-SS-$(CH_2)_6$-dT-3', *m/z* 873.22 (calcd for $[M–H]^-$ 873.85); **ODN 4**, 5'-dC-$(CH_2)_6$-SS-$(CH_2)_6$-dC-3', *m/z* 842.83 (calcd for $[M–H]^-$ 843.82); **ODN 5**, 5'-dCATAGTGACG-$(CH_2)_6$-SS-$(CH_2)_6$-dGG-3', *m/z* 4038.08 (calcd for $[M–H]^-$ 4037.86); **ODN 6**, 5'-dGG-$(CH_2)_6$-SS-$(CH_2)_6$-dATTCTAGTTGAGAGC-3', *m/z* 5583.00 (calcd for $[M–H]^-$ 5583.84); **ODN 7**, 5'-dCTAAGTGACG-$(CH_2)_6$-SS-$(CH_2)_6$-dATTCTAGTTGAGAGCG-3', *m/z* 8039.61 (calcd for $[M–H]^-$ 8039.41).

Radiolytic Reduction. To establish hypoxia, aqueous solutions of **ODNs** (50 μM) in 10 mM phosphate Na buffer (pH 7.0) containing 100 mM NaCl and 5 mM 2-methyl-2-propanol were purged with argon for 10 min and then irradiated in a sealed glass ampoule at ambient temperature with an X-ray source (5.0 Gy min^{-1}). After the irradiation, the solution was immediately subjected to HPLC or gel electrophoresis analysis.

For the PAGE analysis, the samples were loaded onto 20% polyacrylamide and 7 M urea sequencing gel and electrophoresed at 55 W for approximately 150 min. After the electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ mL). The gels were analyzed by autoradiography with the ATTO densitograph software library (version 3.0). The intensity of the spots was determined by volume integration to estimate the amount of products.

Treatment of ODN by DTT. Aqueous solution of ODN (50 μM) was treated with 0.1 M dithiothreitol (DTT) in 50 mM phosphate buffer (pH 8.7) for 1 h at ambient temperature. After the reaction, the solution was immediately subjected to HPLC analysis.

Melting Temperature (T_m) Measurement. T_ms of the duplexes (1 μM, duplex concentration) were taken in a 10 mM phosphate Na (pH 7.0) containing 100 mM NaCl. Absorbance *vs* temperature profiles were measured at 260 nm using a JASCO V-530 UV/VIS spectrometer connected with ETC-505T temperature controller. The absorbance of the samples was monitored at 260 nm from 5 ˚C to 70 ˚C with a heating rate of 1 ˚C /min. From these profiles, first derivatives were calculated to determine T_m values.

Table S1. G-values (μmol/J) for the formation of ODN Xa and ODN Xb in the radiolysis of ODNs 1-4 under hypoxic conditions

	Starting Material (ODN X)			
	ODN 1	ODN 2	ODN 3	ODN 4
Product ODN Xa	2.42	2.29	2.07	1.81
Product ODN Xb	2.13	2.37	1.81	2.06

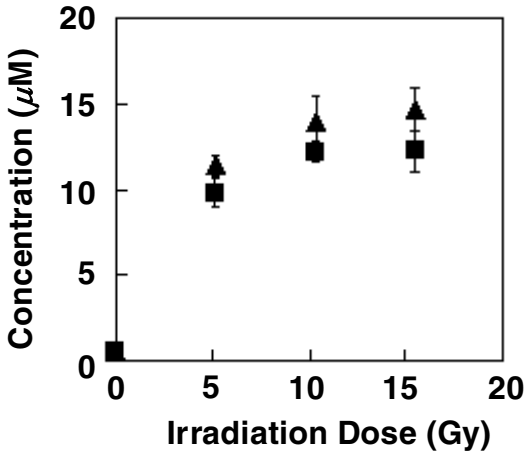

Figure S1. One-electron redution of ODN 1 (50 μM) in the X-radiolysis of Ar-purged aqueous solution containing 2-methyl-2-propanol (5 mM). Formation of ODN 1a (triangle); Formation of ODN 1b(square).

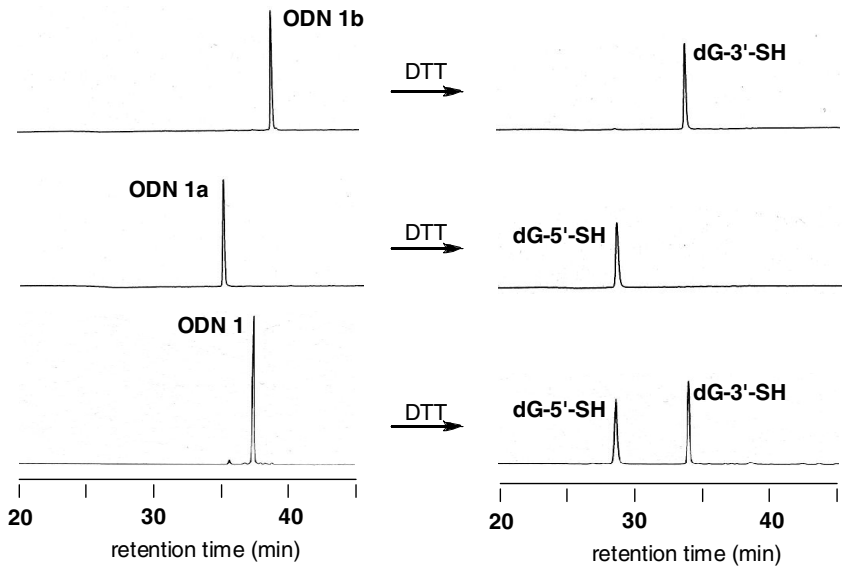

Figure S2. HPLC profiles for the treatment of ODN 1, ODN 1a and ODN 1b. Aqueous solutions of ODNs (50 μM) were treated with 0.1 M dithiothreitol (DTT) in 50 mM phosphate buffer (pH 8.7) for 1 h at ambient temperature. ODN 1a and 1b produced their respective single products, which were dG-5'-SH and dG-3'-SH, respectively, as identified by the HPLC analysis with overlap injection of authentic samples prepared by treatment of ODN 1 with DTT.

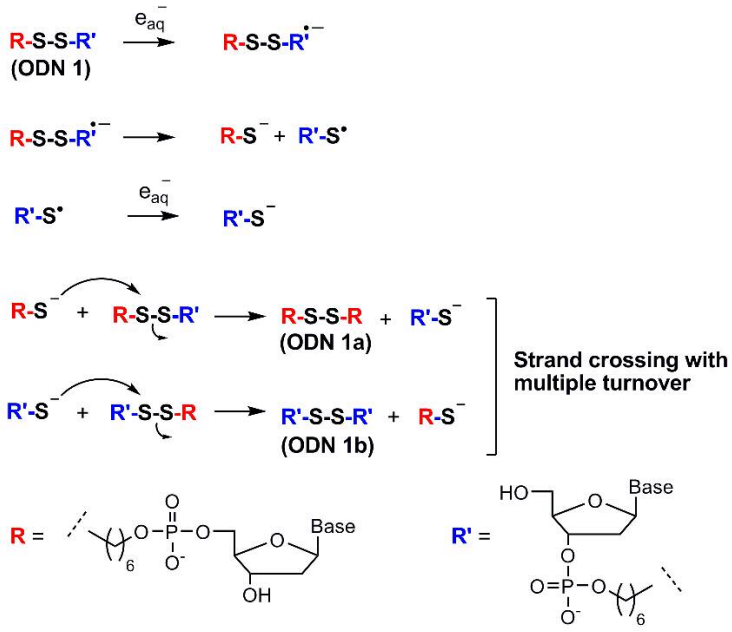

Figure S3. Plausible radiolytic reduction mechanism for efficient interexchange of disulfide bonds of dinucleotides.

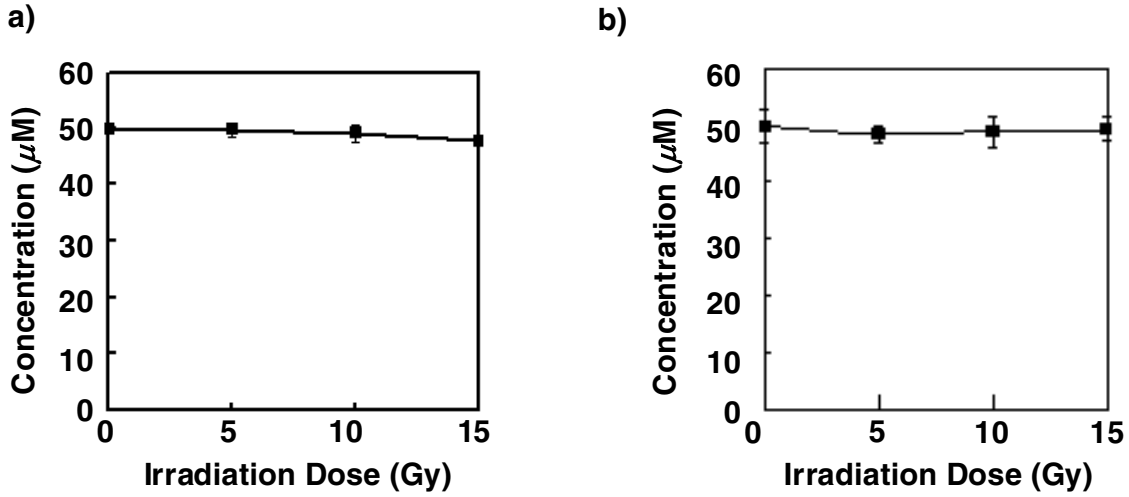

Figure S4. One-electron redution of normal dinucleotides (50 μM) in the X-radiolysis of Ar-purged aqueous solution containing 2-methyl-2-propanol (5 mM). a) Degradation of 5'-dTT-3'; b) Degradation of 5'-dGG-3'.

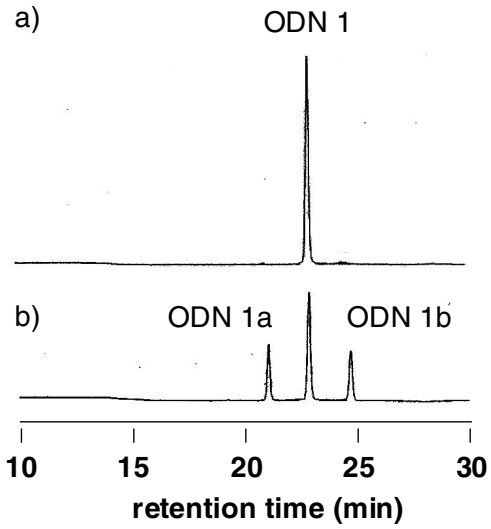

Figure S5. HPLC profiles for the one-electron reduction of ODN 1 (50 μM) upon X-radiolysis of aqueous solution containing 2-methyl-2-propanol (5 mM) under anoxic conditions prepared by a freeze-thaw cycle techniques; a) before irradiation, b) after irradiation (15 Gy).