Supporting information

Photoreactivity of the linker region of two consecutive G-quadruplexes formed by human telomeric DNA

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CD spectra and general photoreaction procedure. All oligonucleotides with HPLC purification were purchased from Japan Bio Services Co., LTD. (Saitama, JAPAN). CD spectra of oligonucleotides (5μM) in Li^+ solution (20mM lithium cacodylate, pH 7.0, 80 mM LiCl), Na^+ solution (20 mM lithium cacodylate , pH 7.0, 100 mM NaCl) and K^+ solution (20 mM lithium cacodylate, pH 7.0, 100 mM KCl) accordingly were measured at 4°C in 0.5-nm steps from 340 to 220 nm using JASCO J-805LST Spectrometer in a 1-cm quartz cuvette. The same concentration of oligonucleotides and buffer solutions were used in photoreaction. Irradiation under 302 nm was performed using a 3UV™ Transilluminator (UVP) at 0°C for 15 min. After irradiation, the samples were subjected to heating at 95°C for 15 min. Finally, each sample was analyzed by denaturing gel (12%) in 1×TBE buffer at room temperature for 1h. The gels were imaged by FUJIFILM FLA-3000.

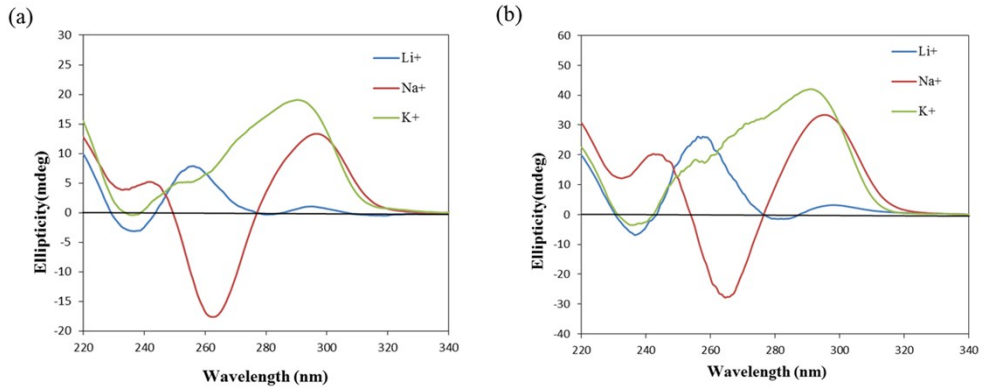

Fig. S1 CD spectra of ODN 1 (a) and ODN 2 (b) in Li^+ (blue), Na^+ (red), and K^+ (green) solutions at 4°C. DNA samples (5 μM) were prepared in Li^+ solution (20 mM lithium cacodylate, pH 7.0, 80 mM LiCl), Na^+ solution (20 mM lithium cacodylate, pH 7.0, 100 mM NaCl), and K^+ solution (20 mM lithium cacodylate, pH 7.0, 100 mM KCl), respectively.

Mechanism of photoreaction in duplex DNA. Take the hot-spot sequence 5'-GAA^{Br}U^{Br}U-3' for example. Since guanine has the lowest oxidative potential among A, T, G, and C bases, ^{Br}U traps the electron from G and forms an anion radical under 302 nm UV irradiation. After release of Br^- and C1' hydrogen abstraction, the resulted C1' radical is oxidized to the C1' cation by the electron-donated radical cation residue G•$^+$. Then the C1' cation reacts with H_2O and produces 2'-deoxyribonolactone with the release of adenine. The sites of 2'-deoxyribonolactone can

be detected as DNA cleavage bands after heating cleavage. The intervening adenines in the hot-spot sequence are quite important as they prevent rapid electron back transfer and increase the lifetime of anion radical formed by ^{Br}U. The whole mechanism was shown in Fig. S2.

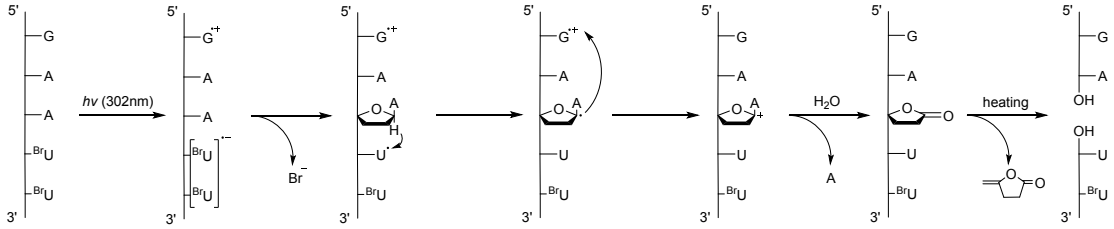

Fig. S2 Proposed mechanism of photoreaction of hot-spot sequence.

Heating cleavage of the photoreaction products of ODN 1 in K^+ and Na^+ solutions. The photoreactions of ODN 1 in Na^+ and K^+ solutions were performed and the photoreaction products (before and after heating cleavage) were analyzed by gel. A new cleavage band was observed after heating cleavage of photoreaction products in Na^+ solution while no new band was observed in K^+ solution. Therefore, deoxyribonolactone as a C1' oxidation product was formed in Na^+ solution but not in K^+ solution. There were two bands just below the original DNA in Na^+ and K^+ solutions. These bands might be other photoreaction products which could not be cleaved by heating at 95°C (for example, erythrose-containing hexameric product as a C2' oxidation product). Since the photoreaction method in this manuscript was based on the heating cleavage of deoxyribonolactone products, the heating cleavage bands were mainly discussed.

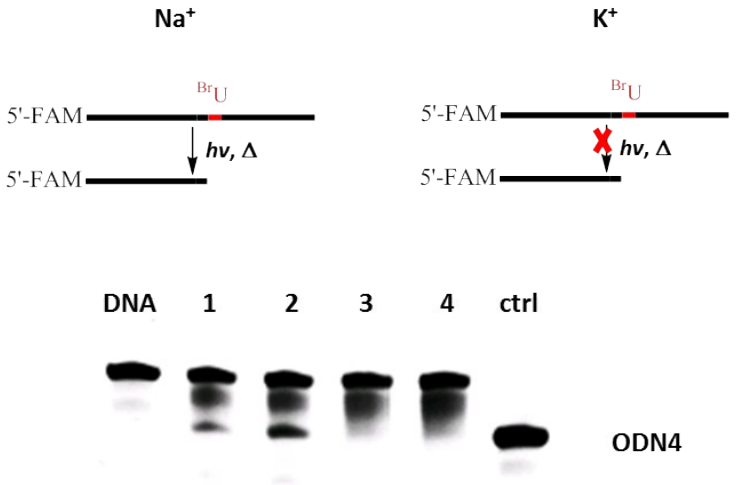

Fig. S3 Gel analysis of the photoreaction of ODN 1 in Na^+ solution before (band 1) and after (band 2) heating cleavage and in K^+ solution before (band 3) and after (band 4) heating cleavage. ODN 4 was used as the reference DNA.

Hybrid-1 and hybrid-2 structures in K^+ solution. NMR structure of hybrid-1 and hybrid-2 G-quadruplexes and their loop structures were shown in Fig. S4. The ^{Br}U was directly stacked with G-tetrad in the loop structure of hybrid-1 while in the loop structure of hybrid-2 the ^{Br}U was away from G-tetrad. Therefore, the photoreaction in K^+ solution was quite different from the photoreaction of diagonal loop of antiparallel G-quadruplex in Na^+ solution.

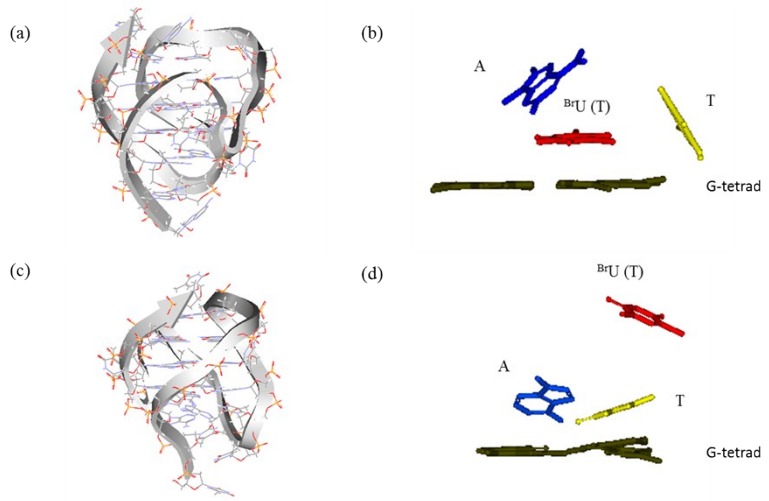

Fig. S4 NMR structures of hybrid-1 (a) and hybrid-2 (c) structures in K^+ solution (PDB code 2HY9 and 2JPZ) and a stacking model of thymine (yellow), 5-bromouracil (red), and adenine (blue) in the second loop region.

Time-dependent photoreaction. The photoreactions of ODN 1 in Na^+ solution (a) , ODN 2 in Na^+ solution (b), and ODN 3 in K^+ solution (c) were performed in different irradiation time periods (5, 10, 15, 20 and 30 min). Fig. S5 (d) showed the calculated efficiency under different irradiation time periods.

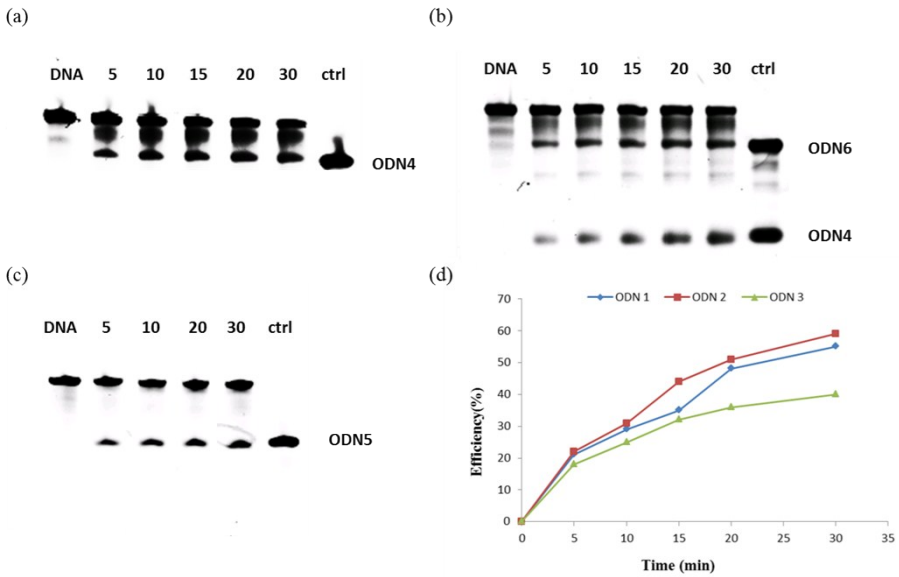

Fig. S5 Gel analysis of time-dependent photoreaction of ODN 1 in Na^+ solution (a), ODN 2 in Na^+ solution (b), and ODN 3 in K^+ solution (c). (d) Efficiency of photoreaction calculated from gel analysis.

Molecular modeling. The models of dimer structure were generated based on the hybrid units (PDB code 2HY9 and 2JPZ) and antiparallel unit (PDB code 143D) using the Discover software (MSI, San Diego, CA). For the dimeric model in K^+ solution, hybrid-1 and hybrid-2 units were manually linked together and the stacking of two G-quadruplex units was maximized. The final sequence for dimer model in K^+ solution was 5'-AGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTT-3'. K^+ ions were placed between two adjacent G-tetrads and one additional K^+ ion was added in the linker region. In the case of dimeric

model in Na^+ solution, two antiparallel units were connected and the stacking of two units was maximized similarly. The final sequence for dimer model in Na^+ solution was 5'-AGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'. Na^+ ions were added in the center of each G-tetrads and another Na^+ was added in the linker region. All G-tetrads in these two models were fixed during the simulation in order to maintain the stability of the whole structure. After manual structure construction, the two dimeric models were pre-minimized by Charmm27 force field and solvated. Solvation was performed by explicit periodic boundary model with 7Å minimum distance from the boundary. K^+ ions were used as the counterions for the model in K^+ solution and Na^+ ions were used as the counterions for the model in Na^+ solution. Then molecular dynamics simulations were performed by the standard dynamics cascade in Discovery software. The obtained dimeric models were minimized to the stage where the RMS was less than 0.001 kcal/mol Å as shown in Fig. 4.