Communication

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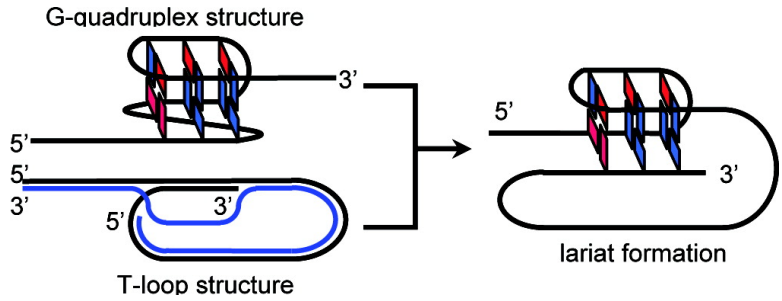

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Stable Lariat Formation Based on a G-Quadruplex Scaffold

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Human telomeres have guanine-rich sequences at the end of chromosomes.[1] They play an important role in genome stability and cell growth by protecting chromosome ends.[2] The human telomere DNA, ~5−8 kbp long, consists of tandem repeats of the sequence TTAGGG, with a single-stranded 3′ overhang of 100−200 nt.[3] The 3′ overhang strand is proposed to encroach into the double-stranded region of the telomeric tract. This structure, called a T-loop, is thought to participate in the protection of the chromosome end in the case of mammals.[4] In normal cells, each cell division results in a 50−200 bp loss of the telomere, which induces senescence and finally apoptosis.[5] In contrast, cancer cells maintain the telomere length to achieve immortality. Reverse transcriptase, called telomerase, is activated in 80−85% of cancer cells and extends the telomeric sequence.[6] It is known that human telomere sequences can fold in a variety of ways to form G-quadruplexes in vitro, and small molecules stabilizing the G-quadruplex, such as telomestatin, effectively inhibit telomerase activity.[7] Therefore, human telomeric G-quadruplex is a potential target for tumor chemotherapy. Recently, three groups including our group determined the topology of the human telomeric G-quadruplex in K^+ solution, having two lateral loops and one external loop.[8] This structure contains the (3 + 1) hybrid G-quadruplex topology, in which three strands are oriented in one direction and one is in the opposite direction.

Although T-loop structure and G-quadruplex structure are proposed to form in the same telomere region, only we and Patel speculate on the possible relationship between the two structures.[8a,9] Therefore, critical evidence is demanded. Patel and colleagues suggested that the interstrand G-quadruplex formation in a sodium ion solution may participate in T-loop formation. Here we have examined the interstrand G-quadruplex structure in the more physiological potassium ion solution. Then we examined a long lariat structure having the same G-quadruplex formation as a model of the T-loop structure.

We first examined the formation of an interstrand G-quadruplex with d(GGGTTAGGGTTAGGGT) (ODN1) and d(TAGGGT) (ODN2). Figure 1 shows the CD spectra of ODN1 and ODN2 in 100 mM K^+ ion solution with mole fraction variation. The mixture of ODN1 and ODN2 showed a strong positive Cotton effect at 290 nm with negative signals near 255 and 235 nm, which are characteristic of the hybrid G-quadruplex structure.[8a] In clear contrast, ODN2 alone showed the CD spectra of an essentially unstructured single strand. Figure 1b shows the Job plot of the CD cotton effect monitored at 290 nm. A clear inflection point around 50% indicates a 1:1 stoichiometry for the formation of interstrand G-quadruplex by ODN1 and ODN2. FRET experiments using FAM-attached ODN1 and TAMRA-attached ODN2 further confirmed the formation of the interstrand G-quadruplex formation (Figure 1S).

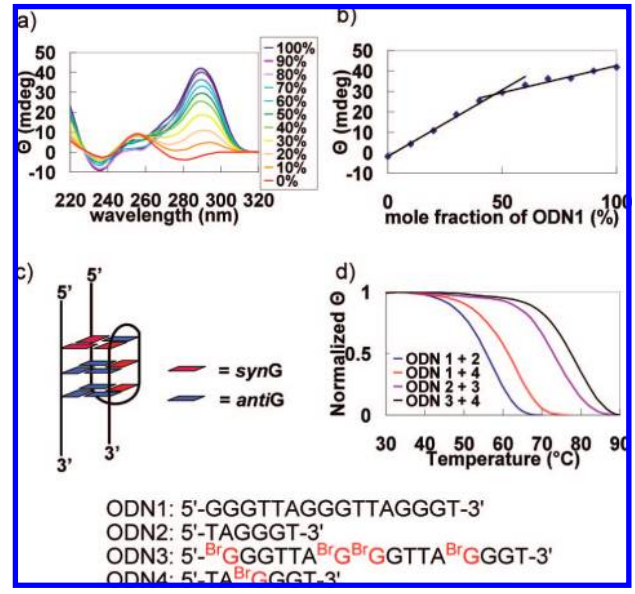

Figure 1. (a) CD spectra of ODN1 and ODN2 in the presence of 100 mM KCl at 25 °C. The total strand concentration of ODN 1 and 2 was 15 μM. The mole fraction of ODN1 is shown right next to the spectra. (b) Job plot of CD cotton effect monitored at 290 nm based on the result of Figure 1a. (c) Interstrand G-quadruplex formation and the sequence of oligonucleotides. Red and blue boxes represent guanine bases in *syn* and *anti* conformations, respectively. (d) CD melting curves for ODNs 1 + 2 (blue), ODNs 1 + 4 (red), ODNs 2 + 3 (purple), and ODNs 3 + 4 (black) monitored at 290 nm. Each strand concentration of oligonucleotides was 10 μM.

In the G-quadruplex structure, G residues adopt unique arrangements of *syn*/*anti* conformations around individual G-tetrads. To examine the *syn*/*anti* arrangement, G in the *syn* conformation was substituted with ^{Br}G based on the previously determined (3 + 1) hybrid G-quadruplex structure.[8a] The ^{Br}G substitution showed an increase in thermal stability upon increasing the number of ^{Br}G substituted in dG at putative *syn* conformations (Figure 1c). The results clearly demonstrate that the present interstrand G-quadruplex has the same *syn*/*anti* arrangement as those of the (3 + 1) hybrid G-quadruplex structure. The ^{Br}G substitution at all putative *syn* positions in ODN1 and ODN2 results in a significant increase in thermal stability. The T_m of the heterodimeric G-quadruplex formed by ODN3 and ODN4 was above 75 °C.

We assumed that intrastrand G-quadruplex formation may participate in the T-loop formation. To test this hypothesis, we prepared ODNs 5−10, in which ODN1 and ODN2 are linked with different numbers of thymines. The CD spectra of ODNs 5−7 showed a strong positive band at 290 nm with weak negative peaks near 250 nm, which are characteristic of the hybrid G-quadruplex structure (Figure 2S). The CD spectra of

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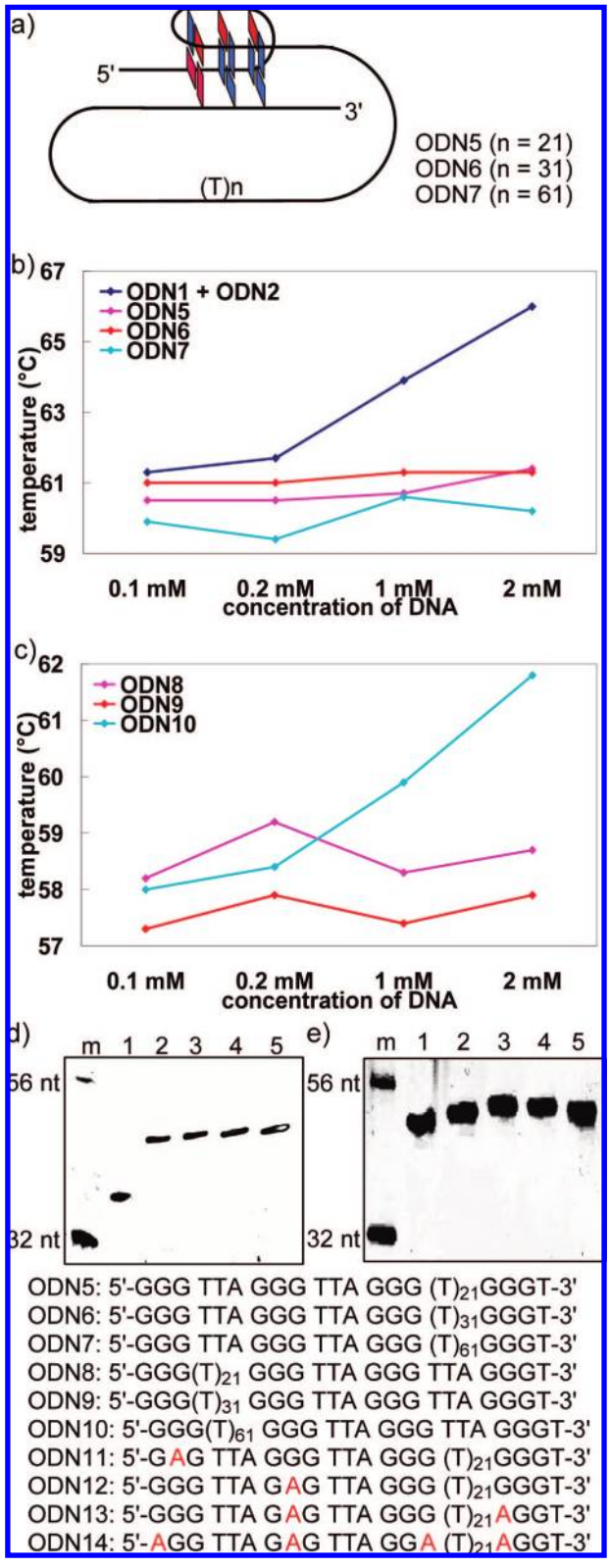

Figure 2. (a) Proposed intrastrand lariat model containing G-quadruplex formation. (b) T_m values in various base concentrations of ODN1 + ODN2 and ODNs 5−7. (c) T_m values in various base concentrations of ODNs 8−10. (d and e) 20% Nondenaturing PAGE analysis of ODN5 and its mutants (ODNs 11−14) at 4 °C in the presence of potassium ion (d) and in the absence of potassium ion (e) lane 1: ODN5; lanes 2−5: ODNs 11−14.

ODNs 5−7 were measured at 75 °C rather than at 25 °C. At 75 °C, the dominant CD band at 290 nm was shifted toward 275 nm, which is consistent with the unstructured single-strand DNA. A plot of T_m values in various concentrations of ODNs is shown in Figure 2b. The T_m values of ODNs 5−7 stayed constant, whereas those of ODN1 and ODN2 increased upon increasing the DNA concentration. These results suggest intramolecular G-quadruplex formation in ODNs 5−7. Interestingly, ODNs 8−10, having three G-tracts at the 3′ end, showed lower T_m values than those of ODNs 5−7 (Figure 2c). Moreover, the T_m value of ODN10 with long T linkers showed a dependence on the DNA concentration. These results indicate that the stable intramolecular lariat conformation contains three G-tracts at the 5′ end and one G-tract at the 3′ end. We then carried out nondenaturing PAGE experiments to examine the possible formation of a lariat (Figure 2d and e). Electrophoretic mobilities of ODN5 and its mutants ODNs 11−14 were analyzed by PAGE experiments. ODNs 11−14 contained the G to A mutation(s) to prevent G-quadruplex formation. In the presence of K^+, the ODN5 migrates faster than ODNs 11−14, whereas, in the absence of K^+, no mobility change was observed between ODN5 and other ODNs. These results clearly suggest that an intrastrand lariat can be formed using G-quadruplex formation in the presence of K^+.

To the best of our knowledge, this is the first evidence that G-quadruplex structure can stabilize a relatively large lariat structure. To date, it has been proposed that the T-loop structure is stabilized by the 3′ overhang strand encroaching into the double-stranded region. However, this model cannot explain the inhibition of telomerase activity by a G-quadruplex stabilizing molecule. Our results might indicate that not only strand encroachment but also G-quadruplex formation could stabilize the T-loop structure. Present results may possibly solve this puzzle, because the present model strikes a happy medium between the two structures of G-quadruplex and T-loop.

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Supporting Information Available: Experimental procedures and information on the FRET experiments and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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