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Conformational organizations of G-quadruplexes composed of $d(G_4T_n)_3G_4$

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ABSTRACT

Structural polymorphism is one of the important issues with regard to G-quadruplexes because the structural diversity may significantly affect their biological functions in vivo and their physical property in nano-material. A series of oligonucleotides with four repeat guanines sequence $[d(G_4T_n)_3G_4$ $(n = 1–6)]$ were designed. In this study, the effects of loop length on the formation of structures of G-quadruplex were investigated through the result of CD (circular dichroism) and 20% non-denatured polyacrylamide gel electrophoresis. Our studies demonstrate that the length of loop in 100 mM KCl solution could predict the conformation of G-quadruplex.

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Certain DNA sequences that contain tandem repeats of guanines are able to fold into more complex structures known as G-quadruplexes.[1–5] The basic repeating unit in these structures, G-quartet, is a square co-planar array of four guanine bases. Each base is both the donor and acceptor of two Hoogsteen hydrogen bonds with its neighbors. G-Quadruplexes is further stabilized by the existence of a cations especially potassium ion in the center of the tetrads.[6–8] In general, G-quadruplexes exhibit a great deal of polymorphism. For example, they can be formed from one, two or four separate strands of DNA and can display a wide variety of topologies as the consequence of various possible combinations of strand orientation as well as variations in loop size and sequence.[8] Moreover, G-quadruplex can be formed by two or more stacked G-tetrads.[2,5] The combination of the number of stacked G-tetrads, the polarity of the strands and the length of the loops would be expected to lead to diversified G-quadruplex structures.[8–11]

In the past years, G-quadruplexes have received considerable attention owing to its unique spatial arrangement which is not only related to its nano-technological applications but also involved in different biological processes such as gene regulation, immunoglobulin switch and telomere maintenance.[12–17] It could be important to predict the G-quadruplex structure based on G-rich sequence as well as to design a sequence that will adopt a desired G-quadruplex sturcture.[8] Therefore, manipulation of the polymorphism of G-quadruplex that has the potential to form needed conformations is helpful for further understanding of their roles in cellular processes and application of nano-technology.

Herein, we report the effects of loop length on the formation of structures of G-quadruplex $[d(G_4T_n)_3G_4$ $n = 1,2,3,4,5,6]$. From our recent studies, we discovered that some conformations of the G-quadruplex could be manipulated conveniently by varying loop lengths of the tetraplex structures.

Table 1 shows the G-rich sequences used in our studies. In order to investigate the effect of loop length on the structural polymorphism of G-quadruplex, different loop length of the G-rich sequences $[d(G_4T_n)_3G_4$, $(n = 1–6)]$ were designed. It has been known that G-rich sequences could form various forms of G-rich quadruplexes under different conditions and some of the possible conformations are exemplified in Figure 1.[18,19] Among the oligonucleotide sequences used in our studies, oligonucleotide 4 is 3.5 repeats of Oxytricha telomere sequence, which contains four T in a row. Under physiological-like condition, this sequence presumably forms chair formation of G-quadruplex.[22]

It is well known that circular dichroism spectroscopy (CD) is a very useful method for examining the secondary structures of

Table 1
Sequences of oligonucleotides used in our studies

Name of oligonucleotides	Sequences of oligonucleotide
Oligonucleotide 1	5′ GGGGTGGGGTGGGGTGGGG 3′ (19-mer)
Oligonucleotide 2	5′ GGGGTTGGGGTTGGGGTTGGGG 3′ (22-mer)
Oligonucleotide 3	5′ GGGGTTTGGGGTTTGGGGTTTGGGG 3′ (25-mer)
Oligonucleotide 4	5′ GGGGTTTTGGGGTTTTGGGGTTTTGGGG 3′ (28-mer)
Oligonucleotide 5	5′ GGGGTTTTTGGGGTTTTTGGGGTTTTTGGGG 3′ (31-mer)
Oligonucleotide 6	5′ GGGGTTTTTTGGGGTTTTTTGGGGTTTTTTGGGG 3′ (34-mer)

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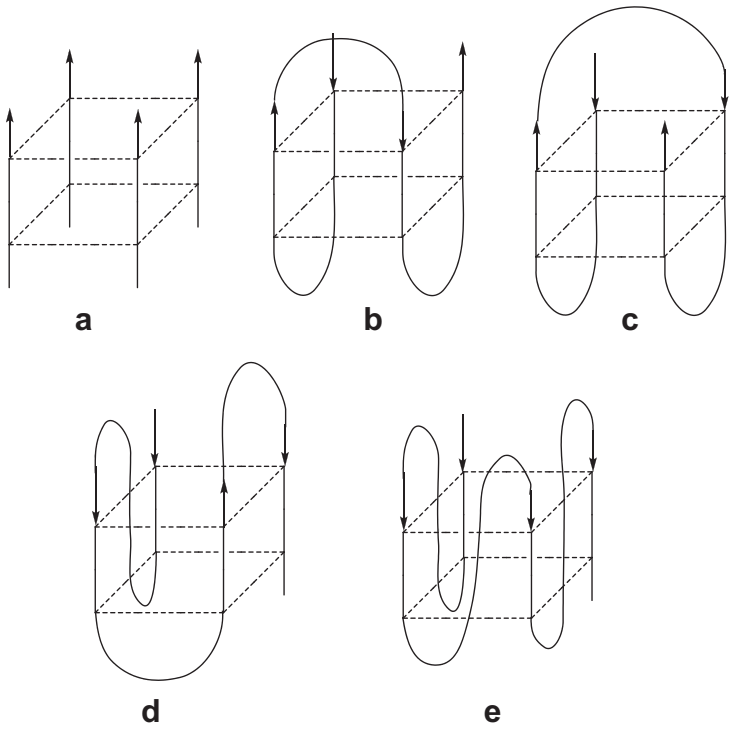

Figure 1. Schematic diagrams of the possible conformations of G-quadruplex formed by some G-rich sequences. (a) Four stands parallel structure, (b) chair structure, (c) basket structure, (d) anti-parallel structure and (e) propeller structure.

DNA and is particularly useful for determining between anti-parallel and parallel-stranded topologies associated with G-quadruplexes.[20] A G-quadruplex composed of all parallel structures is known to display a maximum absorptions at 260 nm and a minimum absorptions at 240 nm while an anti-parallel conformation gives rise to its maximum and minimum absorptions at 295 nm and 260 nm, respectively.[21,22] In our studies, the properties of G-quadruplex formed by each designed G-rich sequences are consequently examined using CD spectrometer and the obtained spectra are then compared with the CD spectra of the quadruplexes of known structures.[21] Besides, non-denaturing polyacrylamide gel electrophoresis (PAGE) is performed to evaluate the effect of variation of loop length on corresponding the G-quadruplexes structure.

The CD spectrum of oligonucleotide 4 [$d(G_4T_4)_3G_4$], which is compose of 3.5 Oxytricha sequence, is shown in Figure 2. The structure of this G-rich sequence, as expected, shows a positive absorption near 290 nm and a negative absorption near 260 nm, which is known to be the characteristics of anti-parallel structures of G-quadruplex.[19,21] This observation is consistent with the results reported earlier using same the sequence in the presence of 100 mM K^+, in which basket type anti-parallel G-quadruplex structure was formed (Fig. 1c).[22] Our PAGE analysis shows that only one band was produced (Fig. 3). This observation of ours is consistent with the suggestion that only one type of G-quadruplex structure is formed. When the number of T in the G-rich sequence is reduced to three [oligonucleotide 3, $(G_4T_3)_3G_4$], this resultant oligonucleotide gives a CD spectrum similar to that of oligonucleotide 4 (Fig. 2), except for that it shows a less significant negative band near 260 nm. This spectrum (Fig. 2) resembles the spectrum for chair type of anti-parallel structure reported previously (Fig. 1b).[21] In addition, two bands of oligonucleotide 3 were observed through the PAGE analysis (Fig. 3) in our studies. It is speculated that the major band is chair type structure while second band is predicted to be basket type anti-parallel structures.

The PAGE of oligonucleotide 2 [$(G_4T_2)_3G_4$] shows three bands, one of which exhibits relatively lower mobility shift than the others two (in Lane 3, Fig. 4). The low mobility band could be resulted from the G-quadruplexes that are composed of two or more oligonucleotide 2 arranged in parallel fashion (Fig. 1a). In addition, two positive absorptions were observed near 260 nm and 290 nm in the CD spectrum of oligonucleotide 2, respectively with significantly higher intensity near 260 nm (Fig. 2). The high intensity near 260 nm is the characteristics of four stranded parallel structure. It is expected that the low intensity is corresponding to the mixture of chair type anti-parallel structure and basket type anti-parallel structure that are formed by some amount of this oligonucleotide. As the number of T in the loop is further reduced to one [oligonucleotide 1, $(G_4T_1)_3G_4$], this G-rich sequence shows only one absorption near 260 nm in the CD spectrum, indicating that parallel stranded G-quadruplex structures are formed (Fig. 2). The CD spectrum of oligonucleotide 1 (Fig. 2) is comparable to the spectrum reported in literature in which the four stranded parallel structures are present. Our PAGE analysis of oligonucleotide 1 shows a smear broad band with significantly low mobility which indicate that the G-quadruplexes with diversified stoichiometry are generated in the mixture (Fig. 4).

When the number of T increase in the loop from four to five, [oligonucleotide 5, $(G_4T_5)_3G_4$] the corresponding oligonucleotide shows two maximum absorptions in its CD spectrum (Fig. 2). The absorption at 290 nm has a higher intensity than the one at 260 nm. The mild positive absorption might be attributed by formation of G-quadruplexes structure that contain parallel and one anti-parallel strands, as illustrated in Figure 1d. One band was found in our PAGE analysis which indicates that only one type of quadruplex structure was produced (Fig. 5). In addition, when oligonucleotide 6 [$(G_4T_6)_3G_4$] positive absorptions near 260 nm and a 290 nm absorption, respectively, which is consistent with the suggest that this G-quadruplex structure contain propeller type paral-

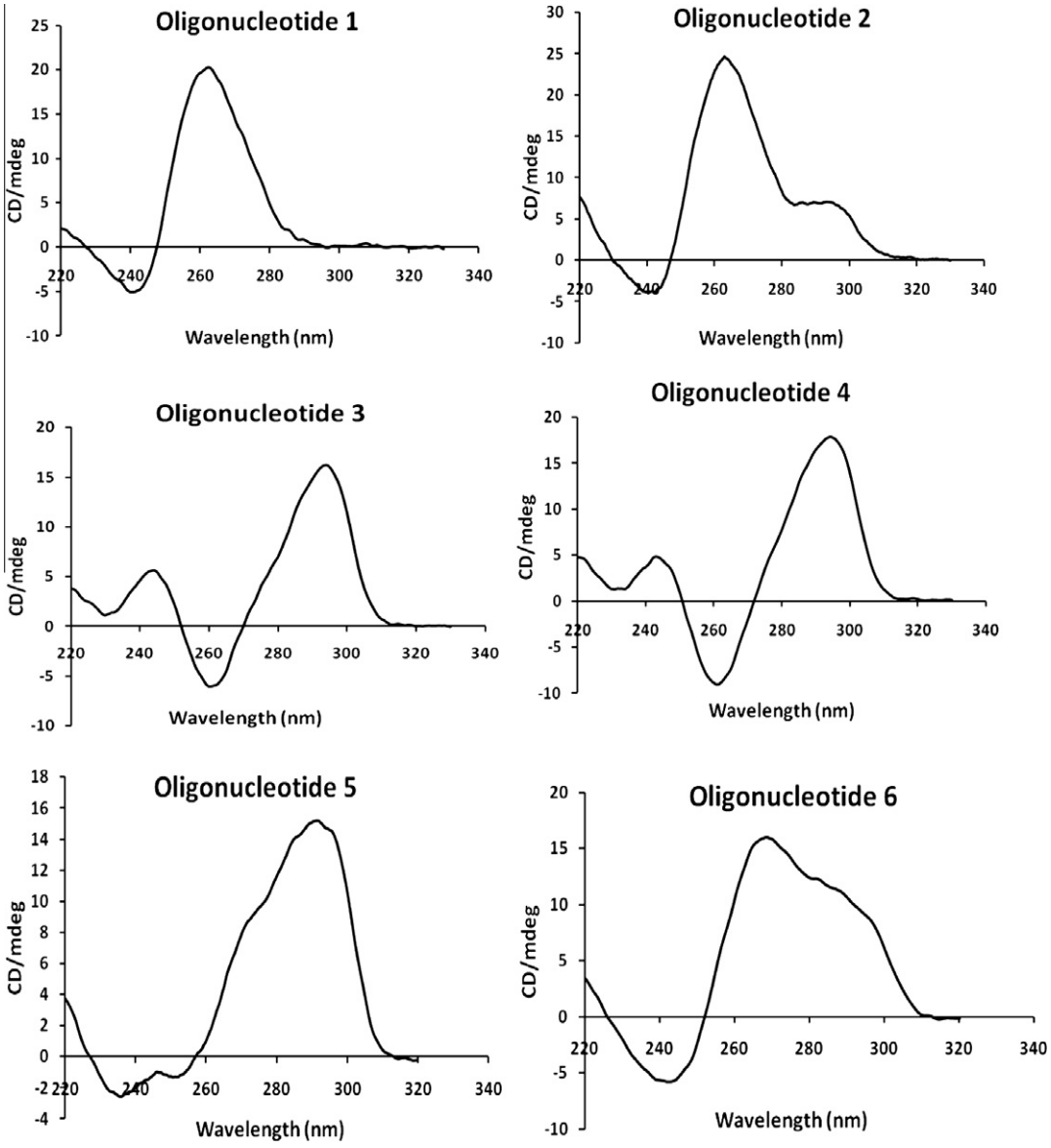

Figure 2. CD spectra of oligonucleotides used in the current studies [d(G_4T_n)$_3G_4$ (n = 1–6)]. Each oligonucleotides in a solution containing 20 mM Tris–HCl and 100 mM KCl was kept at 90 °C for 10 min followed by allowing it to cool down to room temperature over a period of 1 h. The CD spectra were record at room temperature.

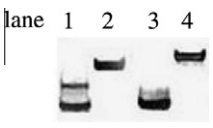

Figure 3. Electrophoretic analysis of oligonucleotide 3 and oligonucleotide 4. The oligonucleotide samples were analyzed using 20% polyacrylamide non-denaturing gel electrophoresis which was run at 4 °C, for 24 h (100 V and 15 W). Sybr Gold was used to stain the oligonucleotides after gel electrophoresis. Lane 1: 1 μM of oligonucleotide 3 pre-incubated with 100 mM KCl; Lane 2, oligonucleotide 7 (GGTG GTGGTGGTGGTGGTGGTT); Lane 3: 1 μM of oligonucleotide 4 pre-incubated with 100 mM KCl; Lane 4, oligonucleotide 8 (GGTGTGTGGTGTGTGGTGTGTGGTG TGT).

lel structure (Fig. 2).[21] Furthermore, one band is observed from our PAGE analysis (Fig. 5), which is the indicated that only one type of structure is formed of oligonucleotide 6.

In conclusion, it is demonstrated in our studies that loops length have great influence on the conformation of G-quadruplex adopted by some G-rich sequences. When the number of T in the loop is five

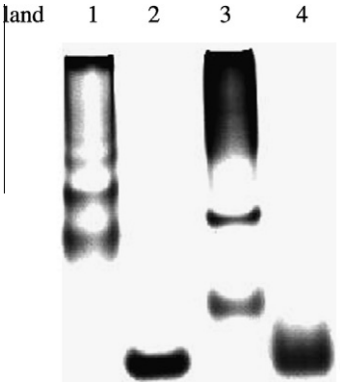

Figure 4. Electrophoretic analysis of oligonucleotide 1 and oligonucleotide 2. The procedures as described in Figure 3 were used except the oligonucleotide 3 and oligonucleotide 4 were replaced with oligonucleotide 1 and oligonucleotide 2. Lane 1: 1 μM of oligonucleotide 1 pre-incubated with 100 mM KCl; Lane 2, oligonucleotide 9 (GGAGTGGAGTGGAGTGGAG); Lane 3: 1 μM of oligonucleotide 2 pre-incubated with 100 mM KCl; Lane 4, oligonucleotide 10 (GGGTGGGTGGGTGGGTGGTGTG).

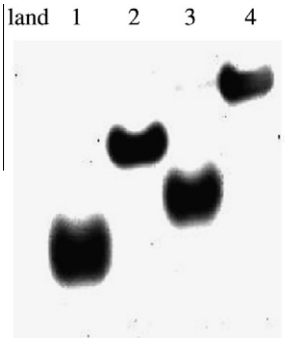

Figure 5. Electrophoretic analysis of oligonucleotide 5 and oligonucleotide 4. The procedures as described in Figure 3 were used except the oligonucleotide 3 and oligonucleotide 4 were replaced with oligonucleotide 5 and oligonucleotide 6. Lane 1: 1 μM of oligonucleotide 5 pre-incubated with 100 mM KCl; Lane 2, oligonucleotide 11 (GGTGTGTTGGTGTGTTGGTGTGTTGGTGTGT); Lane 3: 1 μM of oligonucleotide 6 pre-incubated with 100 mM KCl; Lane 4, oligonucleotide 12 (GGTTGGTT GGTTGGTTGGTTGGTTTGGTTGGTTT).

or more, the corresponding G-rich sequence would start to adopt external looped structures. It is our expectation that the outcomes of the current studies could be helpful for predicting the possible conformation of G-quadruplex formed by some G-rich sequences and could be useful for designing therapeutic agents that target different DNA conformations.

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