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G-quadruplex as a new class of structural entities for directing the formation of circular oligodeoxyribonucleotides†

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It has been demonstrated for the first time that G-quadruplex is capable of acting as a template for directing the sequence-specific formation of certain circular oligodeoxyribonucleotides with high efficiency.

G-quadruplex, a spatial arrangement of four-stranded guanine-rich sequences of nucleic acids, has received much interest[1] over the last decade owing to its special biological significance and structural distinctiveness.[2–5] This unusual conformation of DNA was, for example, proposed to exist *in vivo* in the immunoglobulin switch region,[2] at the ends of telomeres[3] and in the promoter region of *c-myc* and other oncogenes.[4] In addition, certain catalytic DNA entities such as porphyrin-metalating deoxyribozyme, HD RNA-cleaving DNA enzyme and ATP-utilizing kinase deoxyribozyme[6] utilize G-quadruplex as stable structural cores to sustain their catalytic domains and activities. Moreover, the self-assembly of G-rich sequences of oligodeoxyribonucleotides can constitute rod-shaped liquid crystals of cholesteric and hexagonal phases[7] and may serve as the scaffold of artificial ion channels and ion carriers.[8] Herein we report for the first time that G-quadruplex can act as a template for directing the sequence-specific formation of circular oligodeoxyribonucleotides *via* self-recognition and mutual-templating processes. This newly identified property of G-quadruplex accordingly further exemplifies the versatility of functions and roles that the four-stranded structural entity can play.

G-quadruplex is a structural assembly composed of two or more stacks of G-quartets in which the four guanines are arranged in a square planar array.[1] The adjustable nature of strand stoichiometry[5] would allow these structures of G-quadruplex to be formed by association of either one [*e.g.* $d(G_3T_2AG_3T_2AG_3T_2AG_3)$, 3.5 human telomere], two [*e.g.* $[d(G_4T_4G_4)]_2$, Oxy-1.5], or four oligomeric molecules [*e.g.* $[d(TGGGGT)]_4$, see **1**, **2** and **3** in Fig. 1a].[9] Sequence 1 (5′ pGGTTAGGGGTTAGG 3′) (**4** in Fig. 1b) was accordingly chosen for our studies with the anticipation that a dimeric complex of this sequence would be formed with its polarity arrangement in an alternating antiparallel fashion (**5** in Fig. 1b). In addition, within this conformational form of the G-quadruplex, the two pairs of 3′ hydroxyl and 5′ phosphate termini would be aligned in close proximity, guided mutually by the opposite strands, providing an ideal structural precursor of the corresponding circular oligonucleotides.[10–13]

Accordingly, the linear sequence of 14-mer, pGGTTAGGGGTTAGG (sequence 1), was incubated in a pH 6.2 buffer to allow the designed structure of G-quadruplex to generate (**4** to **5** in Fig. 1b). The 5′-terminal phosphate within the dimeric complex was then activated by addition of *N*-cyanoimidazole[10] to facilitate the formation of a phosphodiester bond with its adjacent 3' hydroxyl group (**5** to **6** in Fig. 1b). Analysis of the reaction products was subsequently carried out *via* denaturing polyacrylamide gel electrophoresis. As shown on the autoradiograms (Fig. 2a), a new product (Lane 7) was generated in 64% yield with its rate of mobility shift between those of sequence 1 (14-mer, Lane 1) and a linear 10-mer (5′ pAAGAGGAGAA 3′, Lane 10) which was identified as the corresponding circular oligodeoxyribonucleotide generated from sequence 1. The efficiency of this circularization reaction was found to be dependent on the concentration of sequence 1: circularization at 10 nM (Lane 3), 100 nM (Lane 4), 1 μM (Lane 5), 10 μM (Lane 6) and 25 μM (Lane 7) of the linear precursor proceeded in 27, 45, 54, 61, and 64% yield, respectively. In addition, substitution of all of the guanines for adenines within sequence 1 gave no reaction (Lane 9) under the standard circularization condition, illustrating that the presence of guanine is a prerequisite for the circularization process, presumably because it is essential for the formation of the G-quadruplex.

Exonuclease VII is an exodeoxyribonuclease that hydrolyzes nucleotides from both the 3′ and 5′ ends of single-stranded linear DNA. Circular oligodeoxyribonucleotides are known to resist degradation by this enzyme[13] due to the absence of open termini within their structures. In order to confirm the circular nature of its phosphate-sugar backbones, the newly formed product (Lane 7 in Fig. 2a) was purified *via* denaturing polyacrylamide gel electrophoresis and digested by this exodeoxyribonuclease. As shown in Fig. 2b, there was no degradation product observed in this digestion reaction (Lane 4). As a positive control, the linear precursor, pGGTTAGGGGTTAGG, was incubated with exonuclease VII, which gave rise to a lower molecular-weight product (Lane 2) in

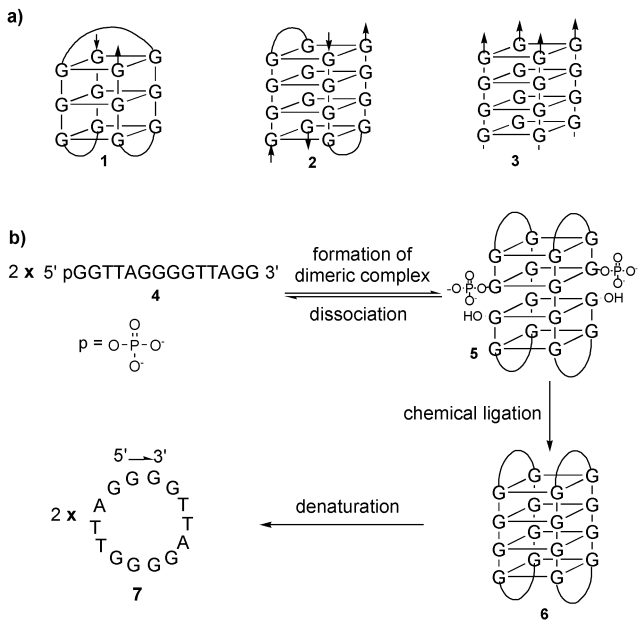

Fig. 1 (a) Schematic representation of G-quadruplex formed unimolecularly (**1**), bimolecularly (**2**) and through the association of four strands of oligodeoxyribonucleotides (**3**). (b) Diagrammatic illustration of our strategy for fabricating circular oligodeoxyribonucleotides on the structural basis of G-quadruplex.

† Electronic supplementary information (ESI) available: experimental procedures. See http://www.rsc.org/suppdata/cc/b2/b208075n/

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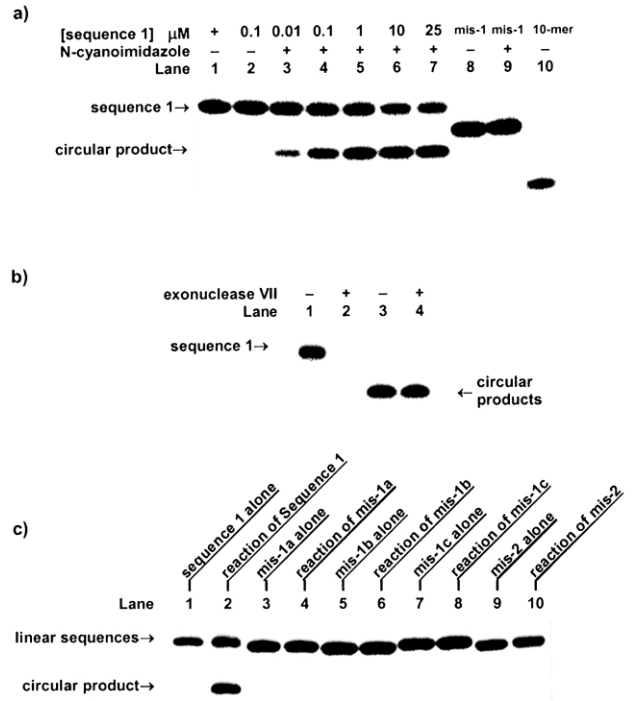

Fig. 2 Formation of circular oligodeoxyribonucleotides on the structural basis of G-quadruplex and product analysis.

quantitative yield. These results indicated that there is an absence of open termini within the newly formed products.

If the designed self-assembly of G-quadruplex (**5** in Fig. 1) is indeed an intermediate structure, any deviation from this conformation should have an effect on the circularization course. In order to examine the effect of this conformation-dependency, four 'mismatched' sequences were prepared in which one or two guanines of sequence 1 were replaced with cytosine, thymine or adenine (mis-1a, 5′ p**C**GTTAGGGGTTAGG 3′; mis-1b, 5′ p**T**GTTAGGGGTTAGG 3′; mis-1c, 5′ p**A**GTTAGGGGTTAGG 3′; mis-2, 5′ p**A**GTTAGGGTTAG**A** 3′). As shown in Fig. 2c, under the same reaction conditions as that described for sequence 1 (Lane 2), none of the four "mismatched" sequences showed any sign of the formation of a circularization product (Lanes 4, 6, 8 and 10).

It should be noted that our studies demonstrated that certain linear oligodeoxyribonucleotides possessing different sequences and lengths than sequence 1 (pGGTTAGGGGTTAGG, 14-mer) could also be circularized through the intermediary structure of G-quadruplex. These linear sequences included pGG**TT**GGGG**TT**GG (12-mer), pGG**TTT**GGGG**TTT**GG (14-mer), pGG**TTTT**GGGG**TTTT**GG (16-mer), pGG**TTTTT**GGGG**TTTTT**GG (18-mer), pGG**ATA**GGGG**ATA**GG (14-mer), pGG**TCT**GGGG**TCT**GG (14-mer), and pGG**TAA**GGGG**TAA**GG (14-mer). However, there was an absence of circular product observable when pGG**T**GGGG**T**GG (10-mer) was used as the linear precursor. In addition, it should be pointed out that the circularization course of sequence 1 was a pH-dependent process. The yields of the circular product from sequence 1 increased with decreasing the pH values of the corresponding buffer solutions from 7.5 to 5.5. In the course of formation of the phosphodiester bond of DNA activated by *N*-cyanoimidazole, the imidazole group connected to the intermediary structure of the *N*-cyanoimidazole-phosphate complex acted as a leaving group.[14] An acidic environment might consequently facilitate the dissociation of this imidazole group from the corresponding intermediary structure thus accelerating the rate of this circularization reaction. It is for certain that alternative causes of this pH-dependency are also possible.

In conclusion, besides the advanced solid phase methodology,[15] the self-assembled entities of double[16] and triple[17] helices and i-motif[18] could act as templates for directing the formation of some circular oligodeoxyribonucleotides. Unlike these previously identified templating systems, the structure of each G-quartet is organized exclusively through four identical Hoogsteen pairings.[19] In view of this structural uniqueness, G-quadruplex could accordingly stand for a new class of self-assembled entities that are capable of supporting the formation of certain circular oligodeoxyribonucleotides structurally.

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