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Site-specific self-cleavage of G-quadruplexes formed by human telemetric repeats

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ABSTRACT

It is demonstrated in our investigations that certain G-quadruplex structures formed by human telomeric repeats could perform self-cleaving actions. Our further studies verify that these reactions are site-specific and undergo hydrolytic pathways.

Human telomere consists of repetitive stretches of TTAGGG at the end of chromosomes of human cells that prevents the ends of chromosomal DNA strands from destruction during the course of replication.[1,2] The average length of human telomere varies between 5 and 15 kilo-bases depending on the tissue type and several other factors.[3] At the very end of human telomeric DNA, however, there is a single-stranded 3′ overhang of 75–300 nucleotides, which is essential for telomere maintenance and capping.[4,5] In vitro studies in the past illustrated that these single-stranded human telomeric repeats could fold into G-quadruplex structure,[6–10] a four stranded assembly of DNA sustained by Hoogsteen hydrogen bonding between guanines as well as by other types of physical interactions.[11–15] It was demonstrated in our previous studies,[16] on the other hand, that certain G-quadruplex structures formed by artificially designed and non-biologically relevant guanine-rich oligonucleotides could carry out self-cleaving actions. In order to examine whether self-cleaving activity of biologically relevant G-quadruplex structures could exist as well, certain oligonucleotides containing human telomeric repeats (TTAGGG) were examined very recently in our lab. Here we report that some G-quadruplex structures formed by human telomeric repeats are capable of performing site-specific self-cleaving actions under certain physiological-like conditions.

Figure 1 shows a schematic diagram of a DNA self-cleaving process of G-quadruplex formed by a DNA segment of human telomeric repeats uncovered in our lab recently. To facilitate the formation of a G-quadruplex assembly in a unimolecular fashion, a 25-mer oligonucleotide (5′ TTAGGGTTAGGGTTAGGGTTAGGGT 3′) containing four human telomeric repeats (TTAGGG) was designed at the initial stage of our investigations. Upon generation of tetraplex assemblies in the presence of potassium ions by this guanine-rich oligonucleotide, a premixed solution of $MgCl_2$ and histidine[16] was introduced to activate the DNA self-cleaving process. Unlike the non-biologically relevant oligonucleotides reported in our previous studies in which a cleavage site occurs within external loops,[16] the new self-cleavage reaction of telomeric repeats takes place at the end of the corresponding columnar structure of G-quadruplex (Fig. 1).

5′ ^{32}P-labeled oligonucleotide 1 (see Fig. 1 and Table 1 for its sequence) was accordingly prepared from its non-phosphorylated precursor in the presence of γ-^{32}P-ATP and T_4 polynucleotide kinase during our investigations. Formation of G-quadruplex assembly by this telomeric repeats was accomplished through incubation of a mixture of 80 mM KCl and 20 nM oligonucleotide 1 at room temperature for 2 h. Self-cleavage reaction of the G-quadruplex assembly formed by oligonucleotide 1 was further activated through addition of a premixed solution of $MgCl_2$ and histidine to the oligonucleotide 1-containing mixture. The final cleavage products were next analyzed through polyacrylamide gel electrophoresis and the resultant autoradiogram from these studies is illustrated in Figure 2. As seen in lane 3, a fast moving band (Band 2) was generated when the self-cleavage reaction of oligonucleotide 1 was allowed to continue for 10 h, which moved as fast as a dimeric nucleotide (lane 4). These results indicate that the strand

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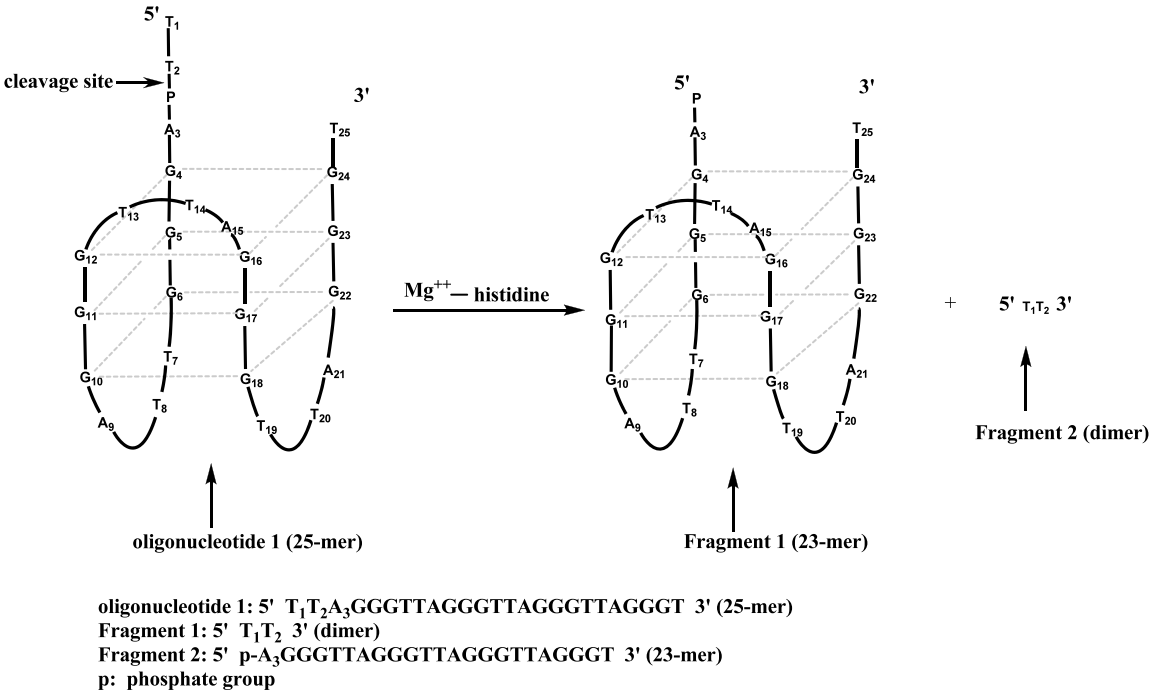

Figure 1. Schematic diagram of a newly uncovered self-cleaving process of G-quadruplex formed by human telomeric repeats in our studies.

Table 1
Sequences of oligonucleotides used in our studies

Name of oligonucleotides	Sequences of oligonucleotide
Oligonucleotide 1	5′ TTAGGGTTAGGGTTAGGGTTAGGGT 3′ (25-mer)
Oligonucleotide 1–1	5′ TTAGGGTTAG-^{32}p-GGTTAGGGTTAGGGT 3′ (25-mer)
Oligonucleotide 1–2	5′ TT-^{32}p-AGGGTTAGGGTTAGGGTTAGGGT 3′ (25-mer)
Oligonucleotide 2	5′ ^{32}p-GGTTAGGGTTAGGGT 3′ (15-mer)
Oligonucleotide 3	5′ TTAGGGTTAG 3′ (10-mer)
Oligonucleotide 4	5′-CCCTAACCCTAACCCT 3′ (16-mer)
Oligonucleotide 5	5′ ^{32}p-AGGGTTAGGGTTAGGGTTAGGGT 3′ (23-mer)
Oligonucleotide 6	5′ AGTGATGCATT 3′ (11-mer)
Oligonucleotide 7	5′-CCTAACCCTAATGCATCACT 3′ (20-mer)
Oligonucleotide 8	5′ AGTGATGCATT-^{32}p-AGGGTTAGGGTTAGGGTTAGGGT 3′ (34-mer)
Oligonucleotide 9	5′-CCTAACCCTAATGCATCACT 3′ (20-mer)
Oligonucleotide 10	5′ TTAAGGTTAGGGTTAGGGTTAGGGT 3′ (25-mer)
Oligonucleotide 11	5′ TTAGGGTTAGAGTTAGGGTTAAGGT 3′ (25-mer)
Oligonucleotide 12	5′ TTAGGGTGGGTGGGTGGGT 3′ (19-mer)
Oligonucleotide 13	5′ TTAGGGTTGGGTTGGGTTGGGT 3′ (22-mer)
Oligonucleotide 14	5′ TTAGGGTTATGGGTTATGGGTTATGGGT 3′ (28-mer)
Oligonucleotide 15	5′ TTAGGGTTATTGGGTTATTGGGTTATTGGGT 3′ (31-mer)
Oligonucleotide 16	5′ GTTAGGGTTAGGGTTAGGGTTAGGGT 3′ (26-mer)

scission of oligonucleotide 1 probably occurs between T_2 and A_3 within the sequence of oligonucleotide 1 (Fig. 1).

If a self-cleavage reaction of oligonucleotide 1 indeed takes place as shown in Figure 1, a second oligonucleotide fragment of 23-mer should be generated besides the observed dimer (lane 3 in Fig. 3). With the aim of visualizing the second cleavage fragment, oligonucleotide 1 containing ^{32}P between G_{10} and G_{11} (oligonucleotide 1–1, see Table 1 for its sequence) was prepared in our lab. As shown in Figure 3, when oligonucleotide 1–1 was incubated under our standard reaction conditions, a new band was generated which moved as fast as a 23-mer molecular weight marker (lane 5 in Fig. 3). The above observations are consistent with the suggestion that the strand scission of oligonucleotide 1 occurs between T_2 and A_3 within oligonucleotide 1 (Fig. 1).

In order to determine which of the two cleaved fragments (23-mer and dimer) hold the corresponding phosphate group when the DNA self-cleaving process takes place, oligonucleotide 1 containing radiolabeled phosphorus (^{32}P) between T_2 and A_3 (5′ TT-^{32}p-AGGGTTAGGGTTAGGGTTAGGGT 3′, oligonucleotide 1–2) was synthesized and examined during our investigations. As shown in Figure 4, only one new band was observable from the self-cleaving process of oligonucleotide 1–2, which corresponds to a 23-mer fragment (5′ *p-A_3GGGTTAGGGTTAGGGTTAGGGT 3′) whereas no dimeric product (5′ TT 3′) was generated. These results are the sign that the corresponding phosphate group is covalently linked to the 23-mer fragment rather than to the dimeric fragment of 5′ T_1T_2 3′ after the self-cleavage reaction takes place as illustrated in Figure 1. In addition, it is worth to note that unlike the DNA self-cleavage reactions reported previously in which strand scission occurs within one of the loops,[16] the self-cleavages in the newly discovered reactions take place in the non-loop regions (Fig. 1). These observations could be taken as the indication that the structures of G-quadruplex could be more labile chemically than previously expected. Moreover, the time course of the self-cleavage reactions

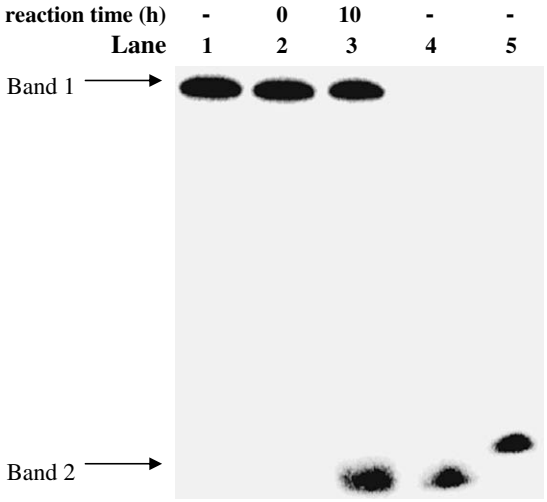

Figure 2. Polyacrylamide gel electrophoretic analysis of self-cleavage of oligonucleotide 1 visualized through autoradiography. 5′ ^{32}P-labeled oligonucleotide 1 (20 nM) in 10 mM histidine (pH 7.2) and 80 mM KCl was incubated at 23 °C for 2 h to allow it to form G-quadruplex structure. Self-cleavage reactions of this telomeric repeats was activated next by mixing a solution of $MgCl_2$ and L-histidine with the rest of reaction components and the resultant mixture [10 mM histidine (pH 7.2), 10 mM $MgCl_2$, 80 mM KCl, and 5 nM oligonucleotide 1] was further kept at 27 °C for different time periods. Lane 1: 5′ ^{32}P-labeled oligonucleotide 1 alone; lane and lane 3: reactions lasting for 0 and 10 h, respectively; lane 4 and lane 5: dimeric and trimeric molecular weight markers obtained from restriction enzyme hydrolysis.

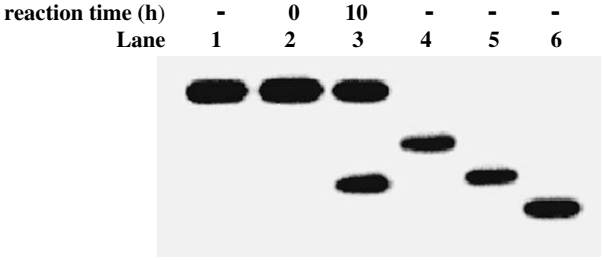

Figure 3. Polyacrylamide gel electrophoretic analysis of self-cleaving reactions of oligonucleotide 1–1 (5′ TTAGGGTTAG-^{32}p-GGTTAGGGTTAGGGT 3′). A 5′ ^{32}P-phosphorylated 15-mer, 5′ ^{32}p-GGTTAGGGTTAGGGT 3′ (oligonucleotide 2), was firstly ligated with a 10-mer, 5′ TTAGGGTTAG 3′ (oligonucleotide 3), on the template of 5′-CCCTAACCCTAACCCT 3′ (oligonucleotide 4) in the presence of T_4 DNA ligase to generate 5′ TTAGGGTTAG-^{32}p-GTTAGGGTTAGGGT 3′ (oligonucleotide 1–1). The generated internally ^{32}P-labeled oligonucleotide 1 was further purified through polyacrylamide gel electrophoresis (20%) and gel filtration chromatography (NAP-25, GE Healthcare). The same procedures as those for preparing samples loaded in lane 3 in Figure 2 was further carried out except that 5′ ^{32}P-labeled oligonucleotide 1 was replaced with the internally ^{32}P-labeled oligonucleotide 1–1. Lane 1: oligonucleotide 1–1 alone; lane 2 and lane 3: self-cleavage reactions lasting for 0 and 10 h, respectively; lane 4: a 24-mer (5′ *p-TAGGGTTAGGGTTAGGGTTAGGGT 3′) alone; lane 5: a 23-mer (5′ *p-AGGGTTAGGGTTAGGGTTAGGGT 3′) alone; lane 6: a 22-mer (5′ *p-GGGTTAGGGTTAGGGTTAGGGT 3′) alone.

of oligonucleotide 1–1 was examined in our studies. The yield of these DNA self-cleaving processes was increased when the reaction time was prolonged. In fact, the yield could reach up to 40% within 10 h (Fig. 5). Further studies on the factors that affect the DNA-cleaving reactions are still in progress in our lab in order to find out proper new reaction conditions under which the self-cleavage rates of oligonucleotide 1–1 could be optimized.

It is known that potassium ion is one of the preferable monovalent cations needed for stabilizing G-quadruplex structures of DNA.[11,17] Additional self-cleavage reactions of oligonucleotide 1 were accordingly carried out next in our studies in which concentration of potassium ion varied from 0 to 160 mM. As shown in Figure S2, there was no DNA cleavage detectable when potassium ion is absent in the corresponding reaction mixture (lane 1) while the maximum reaction yield was observed when K^+ concentration was set at 80 mM. These observations indicate that formation of G-quadruplex is a prerequisite for the self-cleavage reaction of oligonucleotide 1 and the reaction rates of these DNA-cleaving processes vary by the variation of K^+ concentrations. Moreover, temperature dependence of the self-cleavage reactions of oligonucleotide 1 was examined during our investigations. As it happens to many other enzymatic reactions, the rates of the self-cleavage reaction of oligonucleotide 1 decreased when reaction temperature decreased (lane 2 in Fig. S3). In addition, when temperature of the corresponding reactions increased from 27 to 48 °C, the self-cleaving reactivity of this oligonucleotide decreased dramatically (lane 4

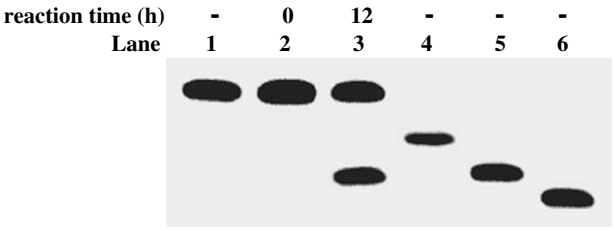

Figure 4. Determination of location of phosphate groups within DNA cleavage fragments through polyacrylamide gel electrophoresis. A 5′ ^{32}p-phosphorylated 23-mer (5′ ^{32}p-AGGGTTAGGGTTAGGGTTAGGGT 3′, oligonucleotide 5), was ligated with a 11-mer (5′ AGTGATGCATT 3′ oligonucleotide 6) on a template of 5′-CCTAACC CTAATGCATCACT 3′ (oligonucleotide 7, 20-mer) in the presence of T_4 DNA ligase. The produced oligonucleotide, 5′AGTGATGCATT-^{32}p-AGGGTTAGGGTTAGGGTTA GGGT 3′ (oligonucleotide 8, 34-mer), was further allowed to form a duplex assembly with an additional complementary sequence (oligonucleotide 9, 20-mer):

NsiI cutting site

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5' AGTG ATGCAT T-32P*-AGGGTTAGGGTTAGGGTTAGGGT 3'  (Oligonucleotide 8)
3' TCAC TACGTA A-----TCCCAATCC 5'                 (Oligonucleotide 9)
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NsiI cutting site

Ten units of NsiI (restriction enzyme) was next added to a mixture containing 1 μM duplex form of oligonucleotide 8 and oligonucleotide 9, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol and 50 mM Tris–HCl (pH 7.9), which was further kept at 37 °C for 2 h. A desired 25-mer oligonucleotide, 5′ TT-^{32}p-AGGGTTAGGGTTAGGGT-TAGGGT 3′ (oligonucleotide 1–2) was subsequently obtained through purification from mixture of this enzymatic reaction. The same procedures as those for preparing samples loaded in lane 3 in Figure 2 was further carried out except that 5′ ^{32}p-labeled oligonucleotide 1 was replaced with the internally ^{32}p-labeled oligonucleotide 1–2. Lane 1: oligonucleotide 1–2 alone; lane 2 and lane 3: reactions lasting for 0 and 12 h, respectively; lane 4: a 24-mer (5′ *p-TAGGGTTA GGGTTAGGGTTAGGGT 3′) alone; lane 5: a 23-mer (5′ *p-AGGGTTAGGGTTAGGGTTA GGGT 3′) alone; lane 6: a 22-mer (5′ *p-GGGTTAGGGTTAGGGTTAGGGT 3′) alone.

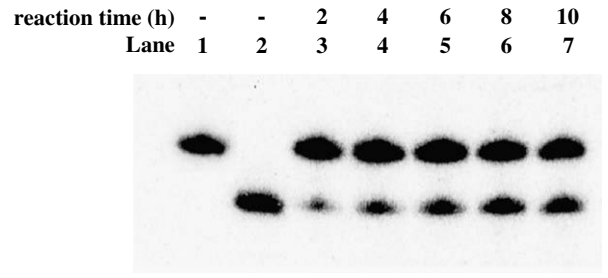

Figure 5. Time dependence of self-cleavage reactions of oligonucleotide 1–1 analyzed through polyacrylamide gel electrophoresis. The same procedures as those for preparing samples loaded in lane 3 in Figure 2 were used except that the reactions were stopped at different time intervals. Lane 1: oligonucleotide 1 alone; lane 2: a 23-mer (*p-AGGGTTAGGGTTAGGGTTAGGGT) alone. The times of reaction in lanes 3, 4, 5 and 6 were 2, 4, 6, 8 and 10 h, respectively.

to lane 6 in Fig. S3), which could be resulted from the dissociation of G-quadruplex structure at relatively high temperatures.

Similar to the DNA self-cleaving processes reported previously,[16] magnesium ion and histidine are required as cofactors for the newly discovered self-cleaving reactions of oligonucleotide 1. As shown in Figures S4 and S5, no self-cleavage product was detectable in the absence of either histidine (lane 1 in Fig. S5) or magnesium ion (lane 1 in Fig. S4) while the optimal yield of self-cleavage of oligonucleotide 1 was observed when concentrations of magnesium and histidine were both set at 10 mM (lane 3 in Fig. S4). In addition, when Mg^{2+} was replaced with Ca^{2+}, Sr^{2+}, Ba^{2+} and Ni^{2+} (lane 2 to lane 5 in Fig. S6), there was no self-cleavage products generated. The above observations are the indication that the co-existences of Mg^{2+} and histidine are essential for the self-cleaving reactions of oligonucleotide 1. Moreover, it was reported in literature[18] that magnesium diethylenetriamine complex could bind to phosphate group of DNA and further lead to the hydrolysis of the corresponding phosphordiester bonds. It accordingly is our expectation that the complex of Mg^{2+} and histidine could act in the same way as Mg^{2+}-diethylenetriamine complex does in the DNA self-cleavage processes of oligonucleotide 1 (Fig. S9).

Loop lengths of G-quadruplex are known to affect the stability of this tetraplex entity considerably.[19] Effect of loop size on the self-cleaving reaction of G-quadruplex was accordingly examined next in our studies. As show in Figure S7, self-cleavage products were observed when two or three nucleotides (oligonucleotide 13 and oligonucleotide 1) were present in the loops of the corresponding G-quadruplex structures while no cleavage product was found when one, four or five nucleotides (oligonucleotide 12, oligonucleotide 14 and oligonucleotide 15, for their sequence information, see Table 1) were present between each guanine tracts. These observations suggest that formation of proper G-quadruplex is the prerequisite for the DNA self-cleaving reactions. In addition, in order to examine whether addition of a nucleotide to 5′ end of oligonucleotide 1 could affect the corresponding self-cleaving reaction, a new 26-mer oligonucleotide (5′GTTAGGGTTAGGGTTAGGGTTAGGGT 3′, oligonucleotide 16) was subsequently examined. As shown in Figure S8, this new oligonucleotide displayed similar self-cleaving activity (lane 4 in Fig. S8) to that oligonucleotide 1 (lane 2 in Fig. S8), which is the sign that addition of a nucleotide at 5′ end of oligonucleotide 1 has little effect on the DNA self-cleaving reaction.

With the aim of examining "mismatch" effect on the DNA self-cleaving process, additional two oligonucleotides were examined next during our investigations that hold the same sequence as oligonucleotide 1 except that one or two guanines are replaced with non-guanine nucleotides (oligonucleotide 10 and oligonucleotide 11 in Table 1). As shown in Figure 6, neither oligonucleotide 10 nor oligonucleotide 11 exhibited an observable self-cleaving activity under our standard reaction conditions (lane 4 and lane 6 in Fig. 6). This happened most likely because oligonucleotide 10 and oligonucleotide 11 might not be able to assemble themselves into proper G-quadruplex structures needed for DNA self-cleaving activity owing to the existence of mismatched guanine nucleotides within their sequences. In addition, CD spectroscopic analysis of oligonucleotide 1, oligonucleotide 10 and oligonucleotide 11 were carried out under our standard incubation condition. As shown in Figure S1, oligonucleotide 1-containing solution displayed a maximum absorption at 290 nm along with a shoulder around 270 nm, which is the sign of the formation of the G-quadruplex structures that possess propeller loops.[20–22] Different from the CD spectrum of oligonucleotide 1 (Fig. S1 a), on the other hand, a positive peak around 290 nm with no shoulder around 270 nm was observed from oligonucleotide 10, which is the indication that a chair type anti-parallel structure is formed by this oligonucleotide.[23,24] In addition, a two mismatched bases-containing oligonucleotide (oligonucleotide 11) was examined in our studies, which gave a positive peak around 250 nm in its CD spectrum, which is consistent with the suggestion that there was no G-quadruplex structure formed in the corresponding solution.[19,24] These CD spectroscopic data shown in Figure S1 suggest that formation of proper G-quadruplex structure is needed for the DNA self-cleavage process.

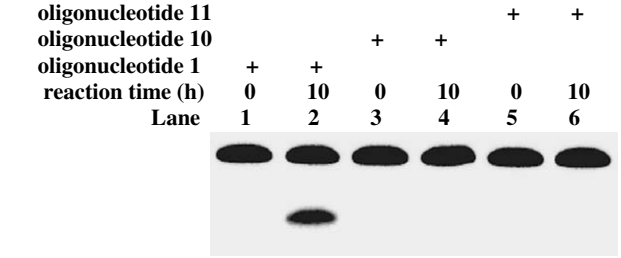

Figure 6. Polyacrylamide gel electrophoretic analysis of oligonucleotides containing mismatched bases. The same procedures as those for preparing samples loaded in lane 3 in Figure 2 were used except that oligonucleotide 1 was replaced with different oligonucleotides. lane 1 and lane 2: reactions of oligonucleotide 1 lasting for 0 and 10 h, respectively; lane 3 and lane 4: reactions of oligonucleotide 10 (5′ TTAAGGTTAGGGTTAGGGTTAGGGT 3′) lasting for 0 and 10 h respectively; lane 5 and lane 6: reactions of oligonucleotide 11 5′ TTAGGGTTAGAGTTAGGGTTAAGGT 3′) lasting for 0 and 10 h, respectively.

Systematic examinations of reaction conditions of oligonucleotide 1 including variation of cofactors such as amino acids and metal ions are, on the other hand, still in progress in our labs. It is our optimal expectation that certain physiological-like conditions could be found out ultimately under which highly effective self-cleavage of oligonucleotide 1 could take place. In addition, it is our hope that the observations presented in this report could inspire new research for the possible existence of self-cleavage of telomeres in vivo.

In conclusion, it is demonstrated in our recent studies that a certain G-quadruplex structure formed by telomeric repeats could perform self-cleavage actions in a site specific fashion. Different from those non-biologically relevant sequences reported previously,[16] cleaving site of the G-quadruplex assembly formed by telomeric repeats occurs on the top of the corresponding columnar structure. It is our expectation that our new results could motivate further exploration for new unrecognized property of G-quadruplexes.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.001.

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