Supplementary Information

Title:

Site specific self-cleavage of certain assemblies of G-quadruplex

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DETAILED LEGEND OF FIGURE 2:

Fig. 2. Polyacrylamide gel electrophoretic analysis of self-cleavage of DNA visualized by autoradiography. Oligonucleotide 1 was labeled with [γ-^{32}P] ATP at its 5$^{'}$ end in the presence of T_4 polynucleotide kinase followed by purification via polyacrylamide gel electrophoresis (20%). The slice of band containing ^{32}P-labled Oligonucleotide 1 was cut out from the polyacrylamide gel and kept at an elution buffer (5 mM HEPES, pH 7.0, 5 mM NaCl and 5 mM histidine) for 3 hour followed by purification with gel filtration chromatography (NAP-25, GE Healthcare) eluted with the same elution buffer. The obtained Oligonucleotide 1 (~20 nM) in 5 mM HEPES (pH 7.0), 5 mM NaCl and 5 mM histidine was then kept at 20 ^{0}C for 12 hr. KCl was then added and the resultant solution was further adjusted to contain in 5 mM HEPES (pH 7.0), 5 mM NaCl, 5 mM KCl, 5 mM histidine and ~10 nM Oligonucleotide 1, which was further maintained at 20 ^{0}C for additional 12 hr. Self-cleavage reactions of Oligonucleotide 1 was initiated next by mixing a premixed solution of $MgCl_2$ and L-histidine with other reaction components and the resultant mixture [5 mM HEPES (pH 7.0), 5 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, 5 mM L-histidine and ~5 nM Oligonucleotide 1] was further kept at 34 ^{0}C for different time periods. The self-cleavage reaction products were analyzed via 20% polyacrylamide gel electrophoresis after the reactions were stopped by addition of loading buffers followed by placing the reaction mixtures on ice.

DETAILED LEGEND OF FIGURE 5:

Figure 5. Polyacrylamide gel electrophoretic analysis of internally ^{32}P-labeled Oligonucleotide 1 (5' TGGGGTTAGGGGAA-^{32}p-AAGGTTAGGGGTTAGG 3') in its self-cleavage reactions. A 16-mer oligonucleotide, 5'AAGGTTAGGGGTTAGG 3', was labeled with [γ-^{32}P] ATP at its 5' end in the presence of T_4 polynucleotide kinase. The purified 5' phosphorylated 16-mer was further ligated with a 14-mer, 5' TGGGGTTAGGGGAA 3', on the template of 5' CCTAACCTTTTCCCCTAA 3' in the presence of T_4 DNA ligase. The produced internally ^{32}P-labeled Oligonucleotide 1 was further purified with 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. Lane 1: internally ^{32}P–labeled Oligonucleotide 1 alone; Lane 2 to 3: self-cleavage reactions lasting for 0 and 2 hr; Lane 4: a 17-mer (5' *p-AAAGGTTAGGGGTTAGG 3') alone; Lane 5: a 16-mer (5' *p-AAGGTTAGGGGTTAGG 3') alone; Lane 6: a 15-mer (5' *p-AGGTTAGGGGTTAGG 3') alone; Lane 7: a 14-mer (5' *p-TGGGGTTAGGGGTT 3').

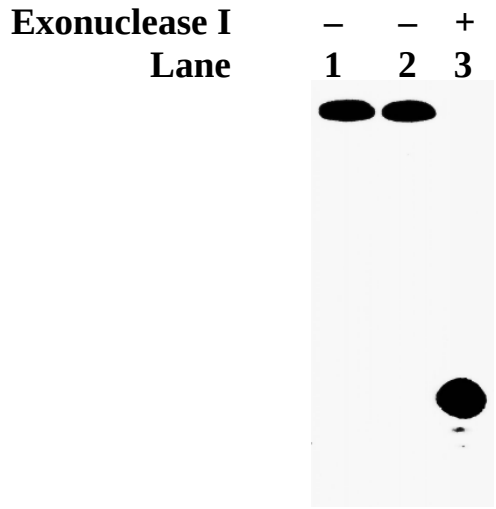

Fig. S1. Hydrolysis of Fragment 2 (see Fig. 1 for its sequence information) generated in the self-cleavage reaction of Oligonucleotide 1 by exonuclease I. The oligonucleotide fragment in Band 1 in Lane 3 in Fig. 5 was cut out, eluted and further purified using gel filtration chromatography (NAP-25, GE Healthcare). A mixture (40 μL) containing 1 x exonuclease I buffer, the purified ^{32}P-containing oligonucleotide fragment (Fragment 2) and 5 units of exonuclease I was incubated next at 37 °C for 30 min. Lane 1: Fragment 2 alone; Lane 2: a reaction mixture containing no exonuclease I and Lane 3: a reaction mixture containing 5 units of exonuclease I.

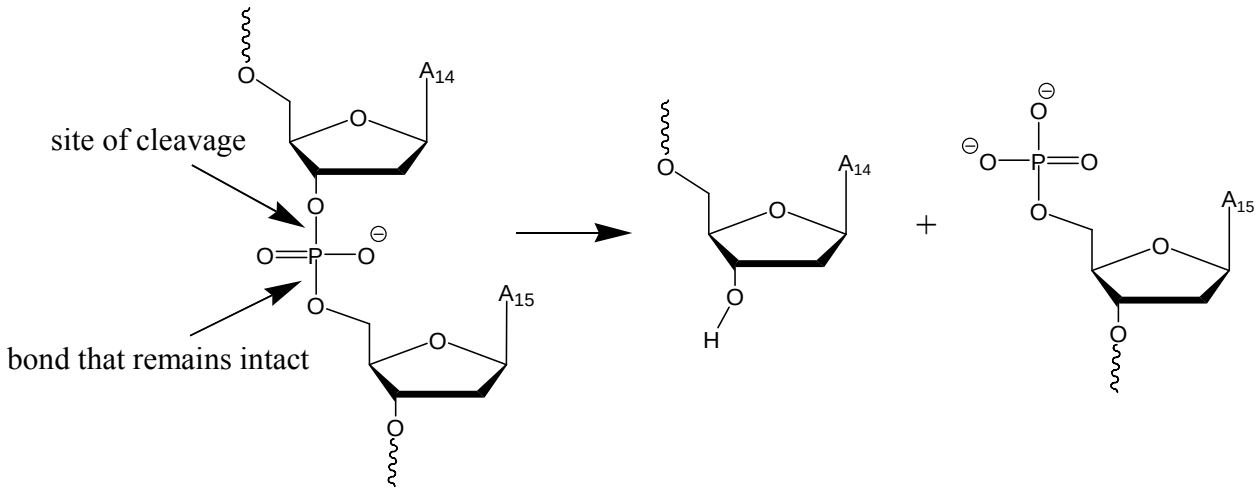

Fig. S2. Diagrammatic illustration of a possible self-cleaving reaction at one of the two phosphodiester bonds between A_{14} and A_{15} of Oligonucleotide 1.

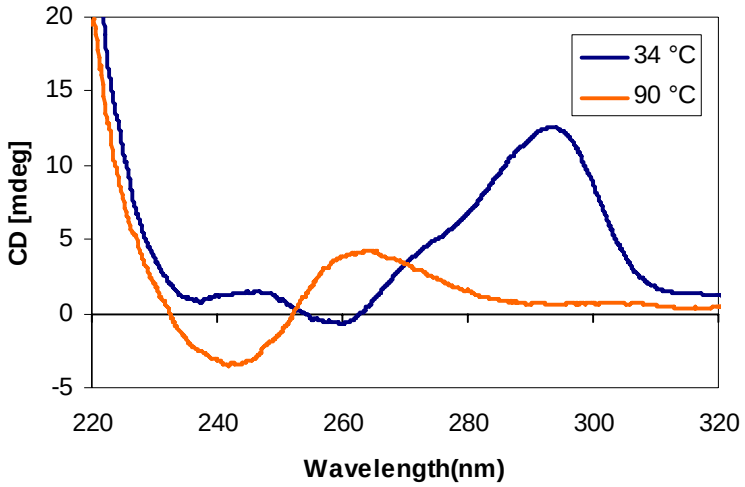

Fig. S3. CD spectroscopic analysis of Oligonucleotide 1. A mixture (pH 7.0) containing 5 mM HEPS, 5 mM NaCl, 5 mM KCl and 10 μM Oligonucleotide 1 was examined with a CD Spectropolarimeter at 34 ^{o}C (black) and 90 ^{o}C (red) respectively over an range of wavelengths from 220 nm to 330 nm.

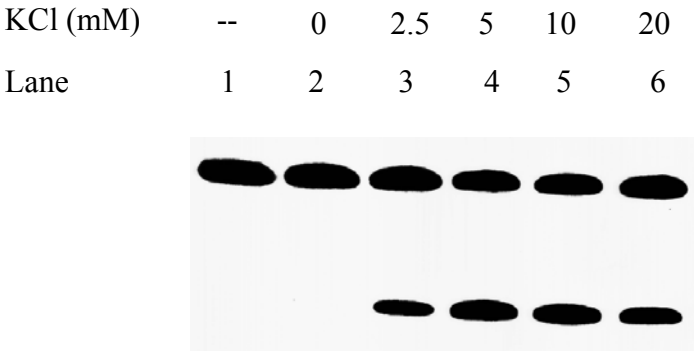

Fig. S4. Effect of potassium ion concentration on the self-cleavage reaction of Oligonucleotide 1. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that concentration of potassium chloride in the new experiments varied. Lane 1: Oligonucleotide 1 alone; Lane 2: 0 mM KCl; Lane 3: 2.5 mM KCl; Lane 4: 5 mM KCl; Lane 5: 10 mM KCl and Lane 6: 20 mM KCl.

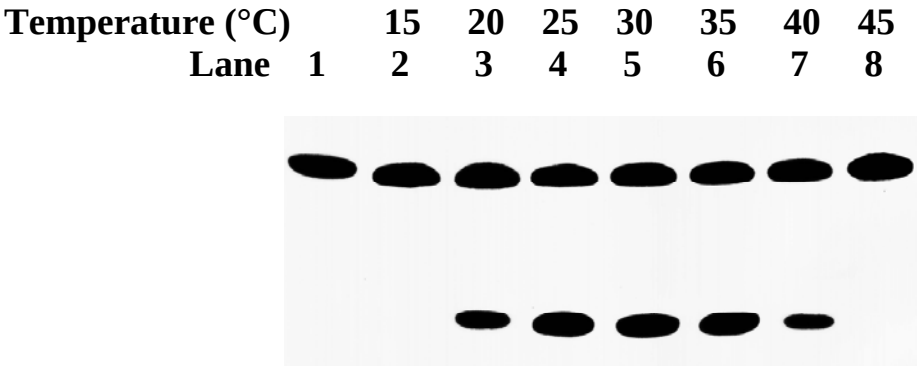

Fig. S5. Temperature dependence of self-cleavage reactions of Oligonucleotide 1. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that the new reaction mixtures were incubated at different temperatures. Lane 1: Oligonucleotide 1 alone. Reaction temperatures of the samples loaded in Lanes 2 to 8 were set at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C respectively.

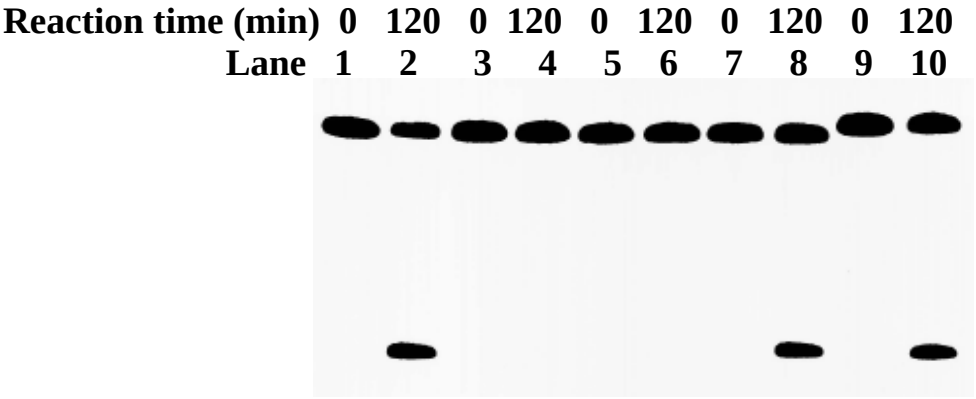

Fig. S6. Sequence dependence of self-cleavage reaction of G-quadruplexes. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that Oligonucleotide 1 was replaced with Oligonucleotide 2, Oligonucleotide 3, Oligonucleotide 4, Oligonucleotide 5, Oligonucleotide 6 and Oligonucleotide 7 (see Table S1) respectively. Lane 1 and Lane 2: reactions of Oligonucleotide 1 lasting for 0 and 120 min respectively; Lane 3 and Lane 4: reactions of Oligonucleotide 2 (5' TGGCGTTAGGGGAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 5 and Lane 6: reactions of Oligonucleotide 3 (5' TGGCGTTAGAGGAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 7 and Lane 8: reactions of Oligonucleotide 4 (5' TGGGGTTAGGGGAAAAGGTTTGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 9 and Lane 10: reactions of Oligonucleotide 5 (5' TGGGGTTAGGGGAAAAGGTTTTGGGGTTAGG 3') lasting for 0 and 120 min respectively.

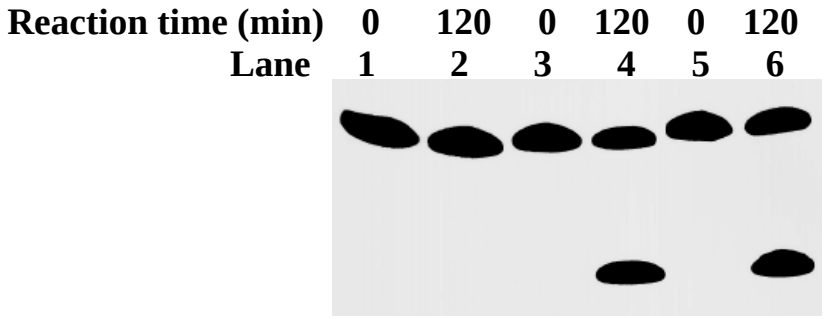

Fig. S7. Effect of side loop variation on the self-cleaving reactivity of G-quaduplex structures. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that Oligonucleotide 1 was replaced by Oligonucleotide 6 and Oligonucleotide 7 respectively. Lane 1 and Lane 2: reactions of Oligonucleotide 7 (5' TGGGGTTAGGGGAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 3 and Lane 4: reactions of Oligonucleotide 1 lasting for 0 and 120 min respectively; Lane 5 and Lane 6: reactions of Oligonucleotide 6 (5' TGGGGTTAGGGGAAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively;

Materials:

Sodium chloride, potassium chloride and HEPES were purchased from Sigma-Aldrich Pte Ltd (Singapore 118529) while the mixture solution of magnesium chloride and L-histidine was provided by 1st BASE Pte Ltd (Singapore 117610). All oligonucleotides used in these studies were customized products from Sigma Proligo in Singapore (Singapore 138667).