RNA and DNA, which contain two GGAGG segments connected with UUUU or TTTT sequences, form entirely different quadruplex structures

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ABSTRACT

We determined solution structure of d(GGAGGTTTTGGAGG) (D14) in the presence of Na⁺ ions by NMR. Two molecules of D14 form a quadruplex structure with parallel and antiparallel strand alignments. The quadruplex is formed by four helical GGAGG segments and two diagonal TTTT loops at the top and bottom of the helix. This quadruplex structure is entirely different from that of r(GGAGGUUUUGGAGG) (R14), which has been previously determined by NMR. In the case of RNA, R14 forms an intra-strand parallel quadruplex with a UUUU loop at lateral position of the helix and two such molecules form a dimer by stacking. The factors determining the type of RNA and DNA quadruplexes are discussed.

INTRODUCTION

It is known that DNAs containing guanine-rich sequences readily form a quadruplex structure. The core of the G-quadruplex is formed by stacked planes of G-tetrads where four guanines associate through Hoogsteen-type hydrogen-bonding in a head-to-tail manner. The structure of DNA quadruplexes were extensively studied mainly because of telomere DNAs containing repetitive G-rich segments and potential inhibitory activity of quadruplex-forming DNA derivatives against cancer (1). During our research on model oligonucleotides for hammerhead ribozymes, we found that RNA and DNA oligomers containing repeating GGA sequences show properties characteristic of a G-quadruplex. NMR studies of d(GGA)₆ oligomers revealed that they form novel intra-strand parallel quadruplexes (2,3). We have recently determined an RNA quadruplex structure of r(GGAGGUGUUUGGAGG) (R14), where two GGAGG segments are connected by a UUUU linker, by NMR (4,5). R14 forms an intra-strand parallel quadruplex, which further dimerizes through stacking, similar to the case of d(GGAGG)₂ (2). In this paper, we report the structure of a corresponding DNA counterpart, d(GGAGGTTTTGGAGG) (D14), in the presence of Na⁺ ions as determined by NMR. D14 forms an intermolecular quadruplex with parallel and antiparallel strand alignments, which is entirely different from that of R14.

MATERIALS AND METHODS

D14 was synthesized on a DNA synthesizer (Applied Biosystems 391) using the solid-phase phosphoramidite chemistry and purified by polyacrylamide gel electrophoresis. NMR spectra were measured on a Bruker DRX-600 spectrometer. Measurement was performed mainly in 50 mM NaCl, sodium phosphate buffer (pH 6.5) at 15°C. Proton resonance assignment was performed based on the data of NOESY, DQF-COSY, TOCSY, and ¹H-¹³C HSQC experiments. Structure calculations were performed with program X-PLOR 3.8 using distance and dihedral angle constraints obtained from NMR data analysis with a simulated annealing protocol supplied.

RESULTS AND DISCUSSION

A schematic diagram for the D14 quadruplex structure is shown in Fig. 1 and a stereo view of the superposition of the 10 final structures is shown in Fig. 2. The quadruplex structure is formed by two molecules of D14. The core helix is composed of four GGAGG segments. Two TTTT loops connect diagonally two GGAGG segments at both ends of the core helix. The quadruplex contains both antiparallel and parallel strand alignments. The presence of an antiparallel alignment requires some dG residues to take on a syn conformation for formation of the G-tetrad. In the D14 quadruplex, G1, G4, G10 and G13 take on a syn conformation; these residues are indicated with black circles in Fig. 1. All dG and dT residues take on C2'-endo or C1'-exo sugar puckering conformation that belongs to S-type sugar puckering. The dA residues are located in the center of the core helix and between a pair of two stacked G-tetrads.
These residues are assumed to have more flexible conformation than the other residues. It turned out that the D14 quadruplex structure is entirely different from that of R14. R14 forms an intra-molecular parallel quadruplex. The G residues make two G-tetrad planes; the two A residues associate with one of the two tetrads forming a G4A2-hexad. Thus stacked G-tetrad and G4A2-hexad make up a core helix. The U residues make a lateral loop with respect to the core helix. R14 further forms a dimer through stacking between two hexad planes. It is surprising that RNA and DNA with very similar sequences form entirely different quadruplex structures. It is common that DNA easily forms a quadruplex containing antiparallel strand alignment. Since deoxynucleoside residues can adopt a syn glycosidic conformation, which is coupled with S-type sugar puckering conformation, more easily than ribonucleoside residues, D14 may be able to form the quadruplex structure containing antiparallel strand alignment even in the presence of Na⁺ ions. The stable nature of the TTTT loop in DNA quadruplexes may also contribute to formation of the D14 quadruplex. In the case of R14, the parallel intra-molecular quadruplex may be stabilized by the presence of 2'-OH groups that can provide additional hydrogen-bonding capacity, while the greater difficulty in adopting a syn conformation may make it less favorable to form a quadruplex containing antiparallel strand alignment.

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REFERENCES

Fig. 1 Schematic diagram for the D14 quadruplex structure.

Fig. 2 Stereo view of the superposition of the 10 final structures of D14.