Structural stability and length of I-motif of G-quartet in DNA octaplex

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INTRODUCTION))) Recent human genome project revealed that exons encoding proteins count only a few percent, while introns 29% and repetitive sequences 50%. There are known two types of repetitive sequences, interspersed and tandem. For example, the former is known as LINE and SINE. The latter example is found in a human telomeric sequence d(TTAGGG)n, which is relevant to a cell death[1]. Another example is single triplet repeat, which is related to some diseases[2]. Eight repeats of d(ccGAGGGGAgg) is found in VNTR of adjacent to the human pseudoautosomal telomere. X-Ray analyses of a simplified sequence d(gcGA[G]$_n$Agc) with n=1 (**G1**) revealed that the eight DNA strands form an octaplex with *I*-motif of G-quartets, and that it split into two quadruplexes at higher potassium concentration. Based on these structural properties, Kondo *et al.* proposed a slippage mechanism to explain polymorphism of the repeat in human genomes[3]. It is necessary to confirm that similar behavior occur even in a longer sequence with n>1. In this study, we started X-ray analyses of the sequences with n=2, 3 and 4 (hereafter, referred as **G2**, **G3**, **G4**, respectively).

MATERIALS AND METHODS))) DNA fragments **G2**, **G3** and **G4** were synthesized on a DNA synthesizer and purified by HPLC. Excess amount of mixed salts were removed by gel filtration chromatography. Crystallization conditions were surveyed at 277K by the hanging-drop vapor diffusion method, using a Kondo Screen and Mini Screen for nucleic acids. X-Ray diffraction data of **G2Co** crystal were taken at 100K using synchrotron radiation at PF in Tsukuba. Recorded patterns are processed by the Program HKL2000. In a similar way, X-ray data of **G2Mg** crystal were collected at SPring-8 in Nishiharima. Diffraction patterns were processed by the program DENZO.)))

RESULTS AND DISCUSSION))) Crystal data of **G2Co** and **G2Mg** are different, suggesting the cationic effect. In general, cobalt hexamine cations induce a hexagonal packing, in which three base-intercalated duplexes are associated, as seen in the **G1Co** crystal[4]. It is difficult to find an octaplex formation in the **G2Co** crystal. On the other hand, the **G2Mg** crystal is obtained under condition containing Mg^{++} cation, but the condition also contains Na^{+} cation, which stabilizes G-quartet formation, as well as K^{+} cation. Therefore, it is expected that DNA fragments of **G2** can form an octaplex in this crystal form. Based on the symmetry of **G2Mg** crystal, the octaplexes may be stacked along the c axis. The octaplex has an axial length of 39Å. When eight octaplexes are arranged according to the space group symmetry in the unit cell, two residues at both ends might be flipped out into solvent region. Higher resolution data will make it possible to discuss the detailed structure.

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