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Duplex with non-WC pairings: Crystal structure of d(gcGAGGGAgc)

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DNA fragments with the sequence d(gcGAGAgc) (G1) easily form a base-intercalated duplex, which is the basic unit for further association to form a quadruplex and an octaplex depending on potassium concentration [1]. In the octaplex, the eight G_5 residues form two G-quartets through the direct N^1-H ...O^6 and N^2-H ...N^7 hydrogen bonds.
Between, above and below the two G-quartets, potassium ions are bound to the O^6 atoms of the G_5 residues. To examine the stability of longer octaplexes, several G residues were added in the central part of G1 for the present study. Electrophoresis experiments have shown that as the number of G residues increases at the center, octaplex formation becomes more stable. The DNA fragment d(gcGAGGGAgc) was crystallized in two forms: $P2_12_12_1$ and $P2_1$. In the latter form obtained at higher cobalt-hexamine concentration, the two fragments in the asymmetric unit form a duplex, in which the two strands are aligned in an anti-parallel fashion. At both ends of the duplex, two Watson-Crick (WC) type G_1:C_{10} and C_2:G_9 pairs are followed by a sheared-type G_3:A_8 pair. These parts are the same as those of the base-intercalated duplexes [2,3,4]. In the remaining part, however, the association mode of the two strands is quite different from that of the base-intercalated duplex. Surprisingly, it is found that the subsequent A_4 residue also forms a sheared-type pair with the G_7 residue and that the central two G residues form G_5:G_6 pairs through the N^1-H ...O^6 and N^2-H ...N^7 hydrogen bonds. These pairing modes comprise just half of the G-quartet. However, the alignment of the two phosphate backbones is anti-parallel, different from that of the octaplex, which is parallel. Two A:GxG:A crossings occur at both side of the central two G:G pairs. It could be concluded that the major part of the present duplex is formed by non-WC pairings. It is interesting to examine whether the central sequence d(GAGGGA) can form such a non-WC duplex without two WC pairs at both ends. Electrophoresis patterns on a native gel containing 20mM potassium show that all of d(GAG$[G]_n$GA) (where n=1-4) form not only duplexes, but also multiplexes such as quadruplexes, octaplexes, and so on. Crystallizations of those multiplexes are in progress.

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Cyclohexene Oligonucleotides: Structure of the L-CeNA sequence GTGTACAC

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Here we present the structure of the left-handed Cyclohexene Nucleic Acid (L-CeNA) sequence GTGTACAC. The left-handed building blocks contain a cyclohexene ring instead of the normal ß-D-2'-deoxyribose and were synthesized using phosporamidite chemistry [1]. These new oligonucleotides may be useful for antisense therapy, being stable against enzymatic degradation, having enhanced binding properties towards RNA sequences and inducing RNaseH activity [2] [3]. Crystals were obtained at 289K by hanging drop vapour-diffusion. Diffraction data were collected at EMBL (beamline X11) [4] in the range 20 to 1.48 Å. The collected data were processed to 1.53 Å with Denzo and Scalepack. The space group is R32 (hexagonal setting a=b=41.455 Å; c=65.580 Å) with half a L-CeNA double helix in the asymmetric unit. The sequence was refined using SHELXL (R = 15.81% for 1694 reflections with F>4σF including 31 water molecules). The double helix has characteristics of both the A- and B- type family, with 12 residues per turn. The left-handed duplex forms continuous helices. This end-to-end stacking of helices less than one turn long gives rise to statical disorder, mapping every guanine on an adenine and every thymine on a cytosine. As a consequence the structure was refined in two parts with half occupancy. These two parts are connected by a non-crystallographic 2-fold axis. The cyclohexene ring adopts the $^3H_2$-conformation mimicking the C3'-*endo* furanose ring. Other helical parameters and hydratation will be discussed.

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