#### m04.o04

# 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<sub>5</sub> residues form two G-quartets through the direct N<sup>1</sup>-H ...O<sup>6</sup> and N<sup>2</sup>-H ...N<sup>7</sup> hydrogen bonds.

Between, above and below the two G-quartets, potassium ions are bound to the O<sup>6</sup> atoms of the G<sub>5</sub> 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<sub>1</sub>:C<sub>10</sub> and C<sub>2</sub>:G<sub>9</sub> pairs are followed by a sheared-type G3:A8 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 G7 residue and that the central two G residues form G<sub>5</sub>:G<sub>6</sub> pairs through the N<sup>1</sup>-H ...O<sup>6</sup> and N<sup>2</sup>-H ...N<sup>7</sup> 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  $d(GAG[G]_nGA)$  (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.

#### m04.005

## 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 lefthanded building blocks contain a cyclohexene ring instead of the normal B-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>4 $\sigma$ F 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 <sup>3</sup>H<sub>2</sub>-conformation mimicking the C3'-endo furanose ring. Other helical parameters and hydratation will be discussed.

- Gu P., Schepers G., Rozensi J., Van Aerschot A., Herdewijn P., Oligonucleotides, 2003, 13, 479-489.
- [2] Wang J., Verbeure B., Luyten I., Lescrinier E., Froeyen M., Hendrix C., Rosemeyer H., Seela F., Van Aerschot A., Herdewijn P., J. Am. Chem. Soc., 2000, 122, 8595-8602.
- [3] Verbeure B., Lescrinier E., Wang J., Herdewijn P. Nucleic Acids Research, 2001, 29 4941-4947.
- [4] Robeyns K., Herdewijn P. and Van Meervelt L., Acta Cryst. F61, 2005, 585-586.

Kondo J., Adachi W., Umeda S., Sunami T. and Takénaka A. (2004) Nucl. Acids Res., 32, 2541-2549.

<sup>[2]</sup> Kondo J., Umeda S., Fujita K., Sunami T. and Takénaka A. (2004) J. Synchrotron Rad., 11, 117-120.

<sup>[3]</sup> Sunami T., Kondo J., Hirao I., Watanabe K., Miura K. and Takénaka A. (2004) Acta. Crystallogr., D60, 90-96.

<sup>[4]</sup> Sunami T., Kondo J., Hirao I., Watanabe K., Miura K. and Takénaka A. (2004) Acta. Crystallogr., D60, 422-431.