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Crystal structures of d(CGAA) and d(CGAAGC): Parallel-stranded duplexes and their dimer

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We found the first example of CGAA forming a parallel-stranded right-handed double helix in the X-ray structure of the DNA d(GCGAAAGCT) (N6.5) [1]. The remaining AGCT forms a normal anti-parallel duplex. The parallel part is formed through homo base-pair formations, $C:C^+$, G:G, A:A and A:A ($^+$ indicates hemi-protonation). To examine whether the sequence CGAA can form such a parallel duplex without the anti-parallel part, and to survey a possibility of longer parallel duplex formation, the crystal structures of d(CGAA) (T5.5 at pH5.5, T6 at pH6, T7.5 at pH7.5) and d(CGAAGC) (H7 at pH7) have been determined at 1.4_5, 1.2, 1.0_5 and 2.5 Å resolutions, respectively. In the T5.5 crystal, the asymmetric unit contains two parallel duplexes, which are formed through homo base-pair formations similar to those in the N6.5 crystal, and the two duplexes are stacked on each other to form an infinitely long column. These structural features are retained in the T6 crystal, though the unit cell dimensions are quite different. Surprisingly, the same type of parallel duplexes is also formed in the T7.5 crystal. This means that the parallel duplex is stabilized by accepting a proton in the central hole of the $C:C^+$ pair even at neutral pH. Furthermore, the most unexpected finding in the H7 crystal is that the two parallel strands form a duplex between the d(CGAA) parts while the remaining d(CG) residues participate in forming an anti-parallel duplex so that the two parallel duplexes are associated to form a tetramer. In any of the parallel duplexes, the $C^{1'}...C^{1'}$ atomic distance between the paired nucleotides is shortest at $G_2:G_2$ and longest at $A_4:A_4$. Between them, $A_3:A_3$ has an intermediate distance. Assuming that $G_5:G_5$ in d(CGAAGC) also has the same as $C^{1'}...C^{1'}$ distance as that of $G_2:G_2$, a distortion in the backbone conformation between the fourth and the fifth residues might arise. This distortion may have to be released in order to form a long parallel duplex that is comprised of homo-base pairs. For this, it may be necessary to insert one or two A residues as spacers.

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Incorporation of a Disaccharide Nucleoside into the Backbone of Double Stranded DNA: Crystallization and Preliminary X-Ray Diffraction

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Systematic cleavage of double stranded DNA at specific sites requires the use of restriction enzymes, which produce dsDNA fragments with either blunt or sticky ends. Alternatively, the use of an incorporated disaccharide nucleoside - by inserting a ribose between the 3'-position and the 5'-position of two consecutive nucleotides - can be considered as a chemical (non-enzymatic) method for this site-specific cleavage of DNA [1].

The already determined NMR-structure of the Dickerson dodecamer with insertion of an extra ribose in the phosphodiester linkage between T*8(20) and C9(21) showed only minor changes in the sugar phosphate backbone, except from the modified site itself [1].

The same sequence d(CGCGAATT*CGCG) with * indicating the incorporated ribose was crystallized by the hanging-drop vapor-diffusion method. A 96.3% complete data set to 2.6 Å resolution was collected at EMBL beamline BW7b of the DESY synchrotron in Hamburg. The crystals belong to the primitive orthorhombic crystal system with cell parameters a = 41.515 Å, b = 57.625 Å and c = 81.389 Å. Inspection of the systematic absences in the (00l) direction indicated that $P222_1$ is the correct space group.

The unit cell and space group differ from the Dickerson dodecamer, which suggests a new crystal packing motif for an oligonucleotide dodecamer sequence. Structure determination by MR is currently in progress with the Dickerson dodecamer [2] as a MR model. However, as the unit cell and space group assignment already indicated a possible significant different structure, alternatively SAD phasing is performed on the anomalous signal of the P-atoms [3].

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