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X-RAY STRUCTURE OF D(GCGAAAGCT), PARALLEL-STRANDED DNA DUPLEX WITH HOMO BASE PAIRS

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Every duplex structure of nucleic acids has always been observed as an anti-parallel aligned helix in X-ray analyses, except for dinucleotides, triplexes and quadruplexes. Although several NMR studies suggested existence of parallel-stranded duplexes, the detailed structures are not yet defined in crystalline state. Recently we have solved an X-ray structure of d(GCGAAAGCT) as a part of serial studies on thermostability of hairpin DNA, and found interestingly that a half of the sequence forms a parallel aligned duplex, while the other half forms an anti-parallel duplex. Single crystals obtained at pH 6.0 diffracted to 2.5 Å at 100 K with synchrotron radiation of SPring8 (space group $I4_122$, a = 53.4, c = 54.0 Å). The structure was solved by the MAD method using anomalous effect of cobalt atoms (data collected at PF, Tsukuba), and refined to R = 21.5% (R_{free} = 27.0%). Electron density maps show that the first G residues are disordered in the unit cell. The subsequent four residues CGAA form a right-handed helix with those of the counter strand along the crystallographic two-fold axis, the two strands being in parallel to each other. The homo base pairs, C+:C, G:G, A:A, A:A, are formed, the first pair being hemi-protonated, the second in the minor groove sites, the third in the major groove sites, and the fourth between the Watson-Crick sites. The remaining halves of the two strands spread out in the opposite directions, and each strand (AGCT residues) is associated to form another duplex with a neighboring strand in anti-parallel alignment by forming the normal Watson-Crick pairs.

Keywords: PARALLEL DNA, HOMO BASE PAIRS, PARALLEL-STRANDED HELIX

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X-RAY STRUCTURE OF D(GCGAAGC), SWITCHING OF PARTNER FOR G:A PAIR IN DUPLEX FORM

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DNA fragment of d(GCGAAGC) shows extraordinarily high thermostability, for which a mini-hairpin structure has been reported from NMR study. To investigate the structure in crystalline state, X-ray analysis of the heptamer has been performed. Single crystals diffracted to 1.6 Å at 100 K with synchrotron radiation at PF (space group $P2_12_12_1$, a = 48.7, b = 48.9, c = 63.8 Å). The structure was solved with MAD technique, and refined to R = 21.5% (Rfree = 25.0%). Two heptamers are associated to form a duplex having a molecular two-fold symmetry. In the asymmetric unit, there are three duplexes, of which the structures are very similar to each other. At the both ends of each duplex, two Watson-Crick G:C pairs constitute the stem region. In the central part, two sheared G:A pairs are formed between G3 and A4 (the counter strand), and A4 and G3 (the counter strand) through the two hydrogen bonds (N3...N6-H and N2-H...N7). The most interesting points are that the adenine moieties of A5 stay inside the duplex and intercalated between the A4 and G6 residues. The G:A pairs are tilted largely to make the open space between C2 and G3 narrow, so that G3 is also close A5. In the NMR structure, the sheared G:A pairs occur between G3 and A5. When superimposed the two structures, a characteristic difference is found in the tilt angle of G3 for forming the sheared G:A pair. Under high salt concentration for crystallization, switching the partner of the sheared pair might be a trigger for conversion between mini-hairpin and duplex.

Keywords: DNA, SHEARED G:A PAIR, BULGE-IN DUPLEX

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STRUCTURAL IMPLICATIONS FOR SUBSTRATE BINDING BY *PYROCOCCUS FURIOSUS* DNA PRIMASE

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Primases initiate DNA replication by synthesizing RNA oligonucleotides on single-stranded DNA, which subsequently serve as primers for the replicative DNA polymerase(s). They have been extensively characterized in various systems and can be divided in a eukaryotic/archaeal (prim-type) and a bacterial/bacteriophage (DnaG-type) family. We have determined the structure of the catalytic subunit of *Pyrococcus furiosus* DNA primase (Pfu-prim) in three different crystal forms by Multiwavelength anomalous dispersion (MAD, using selenium and an intrinsic zinc-ion as anomalous scatterers) and Molecular Replacement (MR). Pfu-prim is folded into two globular domains, where the larger domain contains all of the residues conserved within eukaryotes and archaea. The highly variable small domain might mediate the interaction of the catalytic subunit with an accompanying subunit, which has recently been identified. The active site is formed by three absolutely conserved aspartate residues close to a proposed DNA-binding cleft. These active site residues are shown to be in a similar three-dimensional arrangement as in various DNA polymerases, although the overall enzyme topologies are completely different. In the initial MAD-structure, the zinc-ion is located within a loop in close proximity to the active site, whereas both MR-structures lack the zinc ion due to different crystallization conditions and the loop becomes unstructured. However, the overall structures are highly similar, which implies a functional rather than a structural role for the zinc ion. Based upon these findings, we present a theoretical model for substrate binding by Pfu-prim.

Keywords: DNA REPLICATION, ZINC-BINDING, ARCHAEA

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STRUCTURE OF THE RPA TRIMERIZATION CORE

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Human single-stranded (ss) DNA-binding protein, replication protein A (RPA) binds DNA in at least two different modes - initial (8-10 nucleotides (nt) mode) and stable (30 nt mode). Switching from 8 to 30 nt mode is associated with a large conformational change. Here we report the 2.8 Å structure of the RPA trimerization core comprising the C-terminal DNA-binding domain of subunit RPA70 (DBD-C), the central DNA-binding domain of subunit RPA32 (DBD-D), and the entire RPA14 subunit. All three domains are built around a central OB-fold and flanked by a helix at the C-terminus. Trimerization is mediated by three C-terminal helices arranged in parallel. The OB-fold of DBD-C possesses unique structural features; embedded zinc-ribbon and helix-turn-helix motifs. Taken together with available biochemical results, our data indicate that switching from 8-10 nt to 30 nt mode is mediated by DNA binding with the trimerization core.

Keywords: SSDNA BINDING MULTIHELICAL INTERFACE ZINC COORDINATION