

Mutants at the 4th and 5th positions of the sequence d(gcGXYAgc) suggest a variety of the DNA octaplex, Wataru Adachi,* Jiro Kondo, Kenta Mitomi, Tanashaya Ciengshin Tomoko Sunami and Akio Takénaka, *Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 226-8501, Japan.* E-mail: wadachi@bio.titech.ac.jp

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It is found that DNA octamer with the sequence d(gcGAGAgc) adopts the base-intercalated duplex, and further associated to form an octaplex in the condition containing potassium ion [1]. This octaplex is the largest multiplex found in nucleic acid structures so far. The eight G₅ residues form two G-quartets through the direct N1-H...O6 and N2-H...N7 hydrogen bonds. Between the two G-quartets, a potassium ion occupies the center to bind the eight O6 atoms of the G₅ residues. In addition, above and below the double G-quartets, two other potassium ions are bound to the four O6 atoms, respectively. To investigate the effects of point mutations at the 4th and 5th residues on the structure, crystal structures of two mutants with the sequence d(gcGGGAgc) and d(gcGAAAgc) have been determined by X-ray crystallography, and compared with that of d(gcGAGAgc). In the d(gcGAAAgc) crystal, eight DNA strands form a right-handed octaplex-assembly. This structure is similar to that of the octaplex found in the d(gcGAGAgc) crystal. In the central part of the d(gcGAAAgc) octa-assembly, the eight A₅ residues are bundled through water-mediated hydrogen bonds. This octa-assembly is slightly swollen at the central parts, when compared with that of d(gcGAGAgc). At the fourth residue of both assemblies, water molecules are bridged to the four A₄ residues through hydrogen bonds between the Watson-Crick sites. In the two peripheral parts, many water molecules are found around the helical axis of the octa-assembly. The third G₃ residues also seem to be assembled by mediation of water molecules in the major groove of the guanine bases. In their minor groove, the A₆ residues of the adjacent anti-parallel strands are bound to form sheared pairs through two hydrogen bonds. At the second and the first residues, the C₂ and G₁ bases are pointed to the outsides of the assembly, and form the Watson-Crick G:C pairs with the complementary bases of the adjacent anti-parallel strands. On the other side of G:C base pairs, water molecules occupy in the center of the assembly. On the surface of the d(gcGAAAgc) octa-assembly, hexahydrated magnesium ions are bound to the Watson-Crick site of the G₃ residues and major groove site of G₇ residues. These hydrogen-bonding networks are indispensable to stabilize both assemblies. Crystals of d(gcGGGAgc) were obtained from a solution containing calcium ion. The two DNA strands form a base-intercalated duplex, and the three duplexes are further associated to form a hexa-assembly similar to that of d(gcGAAAgc) containing hexaminecobalt [2]. Crystals are also obtained from a potassium containing solution.

[1] Kondo J., Umeda S., Sunami T. and Takénaka A. (2003) *AsCA'03/Crystal-23 conference (Broome)* Abstract, 82

[2] Sunami T., Kondo J., Hirao I., Watanabe K., Miura K. and Takénaka A. (2004) *Acta. Crystallogr.*, **D60**, 90-96