Structural Characteristics of Enantiomorphic DNA: Crystal Analysis of Racemates of the d(CGCGCG) Duplex

Mitsunobu Doi, Masatoshi Inoue, Koji Tomoo, Toshimasa Ishida, Yoshiaki Ueda, Masao Akagi, and Hidehito Urata*

Osaka University of Pharmaceutical Sciences 2-10-65 Kawai, Matsubara, Osaka 580, Japan

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Chirality has been closely related to life itself since crucial bioorganic molecules are chiral. The racemization of the chiral unit generally reduces its biological activity. This may be attributed to an unfavorable change in the highly-ordered structures. Recent research into unnatural chirality has focused on the chemical synthesis of the D-amino acid polypeptide.[1] The pure D-polypeptide may have activities and/or functions similar to those of its substrate enantiomer and fold to a mirror-image structure of the natural L-polypeptide.[2] The racemization has been also applied to DNA which was naturally composed of D-deoxyribose. The unnatural DNA enantiomer is composed of L-deoxyribose and shows a negative circular dichroism spectrum against the natural enantiomer.[3] It suggests that the structure of L-DNA is the mirror-image of natural D-DNA. The application of the mirror-image structure might give the crystallographic advantage in the phase problem[2] and allow us to solve the structures of macromolecules which have very important biological functions. This paper reports the first application of racemization in X-ray analysis of an oligonucleotide and the three-dimensional structure of a racemic duplex containing both the natural left-handed and the unnatural *right-handed* Z-forms.

The chemically synthesized L-d(CGCGCG)[4] has been co-crystallized with equimolar D-enantiomer.[5] The crystal contained two symmetry-related duplexes per unit cell. The structure was solved by the molecular replacement method[6] using natural Z-DNA[7] as the search probe. Figure 1 shows the conformation of the racemic DNA (thick line). Pairing between D- and L-strands had been shown to be energetically unfavorable in solution[8] and was not observed in the crystal. Molecular structure accounts for the only interaction between the enantiomeric duplexes in the crystal. Homochiral strands pair only with complementary strands having the same chirality. The D-DNA molecule forms a left-handed duplex which is similar to the natural Z-DNA form. The D-enantiomer of the racemate was compared with the natural D-d(CGCGCG) (spermine and magnesium forms; ref 7) as shown in Figure 1. The overall structures of the duplexes are similar with root-mean-square deviation (rmsd) of 0.774–0.839. The end regions, which are restricted by contacts to symmetry-related molecules, show larger deviations. The intermolecular forces induce a slight change in the local conformation of the terminal groups. The D-molecules are crystallographically translated to the right-handed L-DNAs by centrosymmetry operations, and both enantiomers show exactly the mirror-image structure of each other. Therefore, the right-handed Z-DNA has a zigzag backbone which is very similar to that of natural Z-DNA. This structural feature, which would be conserved in solution, provides a model to aid in interpretation of the spectra.[3]

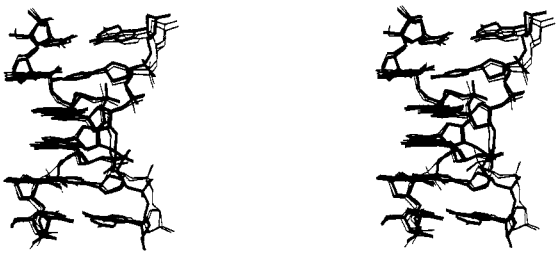

Figure 1. Molecular structure and superposition. The racemic DNA (thick lines) was fitted against the magnesium and spermine forms[7] (thin lines). Molecular fitting was calculated for all 240 non-hydrogen atoms of d(CGCGCG) by the least-squares method. The root-mean-square deviation (rmsd) of distances between corresponding atoms was monitored. The rmsds were 0.839 and 0.774 Å for the magnesium and spermine forms, respectively. The fitting was performed for the D-deoxyribose hexanucleotide (left-handed) model of the unit cell.

The crystal packing diagram is shown in Figure 2. The double helix of natural D-d(CGCGCG) lies parallel to the crystallographic *c*-axis and is continuously packed in an end-to-end fashion.[7]

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(2) (a) Mackay, A. L. *Nature* **1989**, *342*, 133. (b) Petsko, G. A. *Science* **1992**, *256*, 1403. (c) Moffat, A. S. *Science* **1992**, *256*, 309.

(3) Urata, H.; Shinohara, K.; Ogura, E.; Ueda, Y.; Akagi, M. *J. Am. Chem. Soc.* **1991**, *113*, 8174.

(4) Urata, H.; Ogura, E.; Shinohara, K.; Ueda, Y.; Akagi, M. *Nucleic Acids Res.* **1992**, *20*, 3325.

(5) The D- and L-d(CGCGCG) fragments (0.5 mM each) were dissolved in the buffer solution containing 1.5 mM $MgCl_2$, 1 mM spermine, and 15 mM sodium cacodylate buffer (pH 7). The mixed solution (10 μL) was equilibrated against 1 mL of 20% methyl-2,4-pentanediol by the hanging-drop method. The specimen, with approximately 0.01 × 0.05 × 0.2 mm³ dimension, was mounted in a thin glass capillary with a small amount of the mother liquor. Lattice parameters were obtained from a least-squares fit of 20 reflections ($2\theta \leq 25°$) on a Rigaku AFC-5 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.5418 Å); triclinic, a = 29.302(6), b = 23.215(4), and c = 23.184(7) Å, α = 72.96(2), β = 90.60(2), and γ = 90.60(2)°, V = 15077(6) Å³, and Z = 2. Three standard reflections were recorded for every 100 measurements during data collection and showed no significant decay of crystal. A total of 2930 unique reflections were collected out to $2\theta_{max}$ = 40 in $\omega/2\theta$ mode with scan width (1.1 + 0.15 tan θ), in which 2273 non-zero reflections were observed. The statistical distributions of the intensities clearly showed that the crystal was centrosymmetric, and the space group was finally determined as $P\bar{1}$.

(6) An initial model was built from the natural Z-DNA (ref 7). The rotation function was calculated for the model by the program X-PLOR (Brünger, A. T. *X-PLOR Version 3.0, A System for Crystallography and NMR*; Yale University: New Haven, CT, 1992) with 8–4-Å resolution, and the resulting rotation angles were monitored by the calculation of the Patterson correlation (PC) function (Brünger, A. T. *Acta Crystallogr.* **1990**, *A46*, 46). A translation search was done in the unit cell with use of the rotated model with the highest PC value, 0.9633 (the secondary peak had the PC value 0.8704). The X-PLOR program chose the solution with the highest coefficient, 0.605, from 1000 peaks with correlation coefficients 0.134–0.605 (σ = 0.055 and mean = 0.380). The best solution gave an R value of 0.48. The rotated and translated model was used as the starting model for refinement. The structure was refined as a rigid body followed by conjugate-gradient minimization against 10–2.2-Å resolution data (both programs used standard protocols supplied with the X-PLOR package). Simulated annealing (Brünger, A. T.; Krukowski, A.; Erickson, J. *Acta Crystallogr.* **1990**, *A46*, 585) was carried out prior to the calculation of electron density maps. Difference density maps with coefficients ($2F_o - F_c$) and ($F_o - F_c$) at 2.2-Å resolution were displayed on a Silicon Graphics IRIS/INDIGO-R4000 computer system using TURBO-FRODO (*TURBO-FRODO Version 4.2*, BioGraphics: Faculté Médecin Nord, Marseille, Cedex 20, 1992). The omit maps were also calculated to establish the base pair sequence and the zigzagging backbone with use of the X-PLOR program. The $F_o - F_c$ map was examined for possible solvent sites, and a total of 38 water molecules were included in subsequent refinement. The conjugate-gradient minimization finally reduced the R value to 0.199 for 816 significant reflections with $F > 3\sigma(F)$. The root-mean-square deviations (rmsd) from the final cycle of the structural refinement were 0.019 and 3.76 for all distances and angles, respectively.

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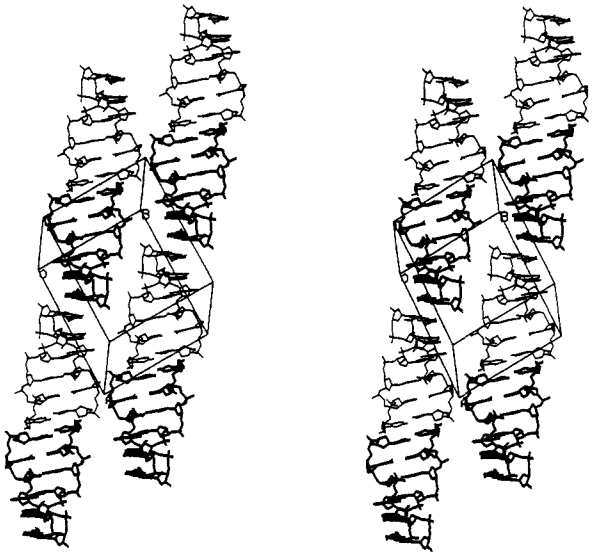

Figure 2. Packing diagram of racemic DNA. The right-handed duplex of L-enantiomers (thick line) and left-handed duplex of D-enantiomers (thin line) of d(CGCGCG) are related by centro-symmetry. Alphabet characters indicate the crystallographic axis, and the square box represents the unit cell boundaries.

Contrary to that, the racemic DNA molecules interact at the end of the strand, where D- and L-enantiomers interact with each other. There is a small gap between the terminal deoxyriboses of the symmetry-related molecules. This gap is slightly larger than that observed at every sixth residue of the natural duplex chain and results in discontinuities in the chain of the racemic DNA. In the crystal, the molecular packing subsequently makes a pseudohelix which contains half-a-turn of right-handed and half-a-turn of left-handed enantiomer structures (Figure 2). The entire diagram shows the side-by-side backbones along the helix axis.

Replacement of D- for L-deoxyribose results in Z-DNA with a right-handed helix while maintaining the property of a zigzag backbone. The deoxyribose is a common unit in all types of DNA structure, and replacement of one enantiomer with the other might enable the right-handed A/B/C-DNA to convert to the mirror-image helix.

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Supplementary Material Available: Tables of coordinates with the PDB format for racemic d(CGCGCG) (3 pages); tables of observed and calculated structure factors (5 pages). Ordering information is given on any current masthead page.