NMR Study of a Heterochiral DNA: Stable Watson–Crick-Type Base-Pairing between the Enantiomeric Residues

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Watson and Crick discovered a right-handed, double-helical model of DNA[1] which is a homochiral polymer consisting of D-deoxyribose as the only chiral unit. The homochirality should be an important factor for higher-order structures and functions of DNA. Recently, we reported the structure and properties of the mirror-image homochiral hexanucleotide, L-d(CGCGCG),[2] which consists of unnatural L-deoxyribose. Here, we use NMR spectroscopy to investigate the structure of the heterochiral dodecadeoxynucleotide, d(CGCGAATTCGCG), which has a single "chiral defect" at the G4 residue, whose sugar moiety has an unnatural L-chirality. Such heterochiral states may have existed in the process of prebiotic homochiral selection on the origin of life.[3-6] In addition, these studies may give new insight on the chemistry of antisense and antigene applications of L-oligonucleotides[7] which have nearly complete resistance to nucleases. Analyses of the data demonstrate the G4 residue to form stable Watson–Crick-type base-pairing with the natural C9 residue and to have *S*-type sugar geometry[8] and a low *anti* glycosyl conformation in a right-handed B-form duplex.

The ^{1}H NMR spectra of heterochiral (left) and parental (right) dodecamers in the exchangeable imino resonance region are shown in Figure 1. Six resonances were observed for both 12-mer duplexes at low temperature, and the chemical shifts of each

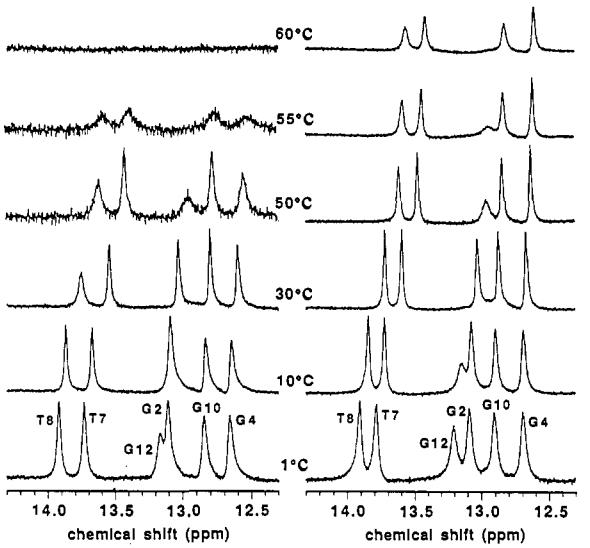

Figure 1. ^{1}H NMR spectra of the exchangeable imino resonance region for the heterochiral 12-mer (left) and parental natural 12-mer (right) as a function of temperature. Syntheses of L-nucleosides and oligonucleotides were described previously.[2b] Samples contained about 2.5 mM dodecanucleotide duplex in 0.1 M sodium chloride, 0.1 mM EDTA, and 10 mM sodium phosphate, pH 7.1 in 20% D_2O. The spectra were obtained with a 1–1 pulse sequence[18] to suppress the H_2O signal. Imino resonances were assigned by 1D NOE experiments at 1 °C.

imino resonance for the heterochiral 12-mer were quite similar to those of the corresponding resonances for the parental duplex. The spectral patterns of both 12-mers were also identical on 1D NOE experiments (not shown). Even at higher temperature, the G4H1 resonance of the heterochiral 12-mer was observable until the other imino resonances disappeared. The G4–C9 base-pairing should thus be significantly stable, although the overall duplex stability is somewhat decreased. The results strongly suggest that the L-deoxyguanosine residue (G4) of the heterochiral 12-mer forms stable Watson–Crick base-pairing, and stacking geometry of the G4–C9 with the neighboring base-pairs is quite similar to that of the parental 12-mer, which has a right-handed B-form conformation in solution.[10] Analysis of the NOESY and DQF-COSY spectra also supports the overall structure of the heterochiral 12-mer to be a right-handed B-form.[11,12]

Glycosyl conformations can be generally identified by analysis of intraresidual NOEs between a base proton and sugar protons.[10,15] The base proton of the G10 residue (G10H8) gives NOEs to its own sugar protons in the order in strength of H2′ > H2″ > H3′ ≥ H1′ ≫ H4′ as can be seen in Figure 2a. Similar results were obtained for the C9 residue, which forms base-pairing with the unnatural G4 residue (Figure 2b). The nucleotide residues other than the G4 residue thus have an *anti* glycosyl

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(8) Each torsion angle of an L-nucleoside has a sign opposite that of the corresponding torsion angles of a natural D-nucleoside which has the same conformation. Thus, the relations between the pseudorotation angle[9] and the sugar puckering of L-nucleosides shift by 180° from those of D-nucleosides which have the same sugar puckering. The notations "*N*-conformer" and "*S*-conformer" for L-nucleosides are thus reciprocals of those for natural nucleosides. For instance, the *S*-conformer implies a conformation around C2′-endo for natural D-nucleosides but around C3′-endo for L-nucleosides. Here, we use the same notation for L-nucleoside conformation as for natural D-nucleoside to avoid confusion on the comparison of their conformations.

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(11) Analysis of the COSY data indicates that the all-nucleotide residues adopt an *S*-type sugar puckering.[8] Particularly, the G4 residue has no cross-peaks between H2″ and H3′ and between H3′ and H4′ (Figure S1). Thus, a sugar puckering of the G4 residue is C2′-endo to C4′-endo.[13]

(12) In the NOESY spectrum (mixing time, 150 ms), complete sequential connectivities between base H8/H6 and sugar H1′ were observed,[14] including the C3–G4–A5 step (Figure S2), which suggests that the overall structure of the heterochiral 12-mer is B-form and that the local structure around the G4 residue is not significantly different from that of the B-form. On the other hand, H8/H6–H2′/H2″ connectivities were disrupted at the G4–A5 step (Figure S3). This suggests the flipped sugar orientation of the G4 residue (Figure 3) but not the local distortion of the helix, because a unique NOE between G4H2′ and H1′ of its 5′-flanking C3 residue was observed (Figure S5).

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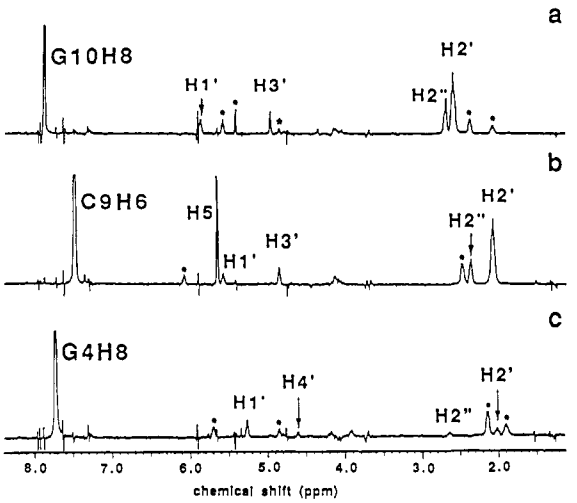

Figure 2. One-dimensional cross sections of the NOESY spectrum (150-ms mixing time) of the heterochiral 12-mer at the frequency of G10H8 (a), C9H6 (b), and G4H8 (c) resonances. Asterisks indicate the interresidual NOEs. No NOE between G4H8 and G4H3′ was observed.

conformation, as seen in normal B-DNA. In the case of the G4 residue, the base proton gives the strongest NOE to H1′ and weak NOEs to H2′ and H2″. A unique NOE was observed at H4′ and no NOE at H3′ (Figure 2c). Thus, the relative intensities of NOEs which G4H8 gives to its own sugar protons are in the order of H1′ > H2′ > H2″, H4′ ≫ H3′. The dihedral angle for O4′–C1′–N9–C4 (χ), which explains these relative NOE intensity data between G4H8 and the sugar protons, should be around 180°, a low *anti* glycosyl conformation.[16] Thus, the present results strongly suggest that an L-nucleotide residue in natural sequence forms stable Watson–Crick-type base-pairing with an *S*-type sugar puckering[8] and low *anti* glycosyl conformation without significant distortion of the right-handed B-form structure. A characteristic NOE network for the C3–G4–A5 step of the heterochiral 12-mer is summarized in Figure 3. On the basis of reciprocal conformational features between enantiomers,[2] it follows that the enantiomer of the heterochiral 12-mer forms a left-handed B-form duplex (namely mirror-image B-form), in which the natural G4 residue between the L-sequences adopts the low *anti* glycosyl, *S*-type sugar conformations. Thus, if natural DNA can form a non-Z-form left-handed helix by the aid of certain factors, the

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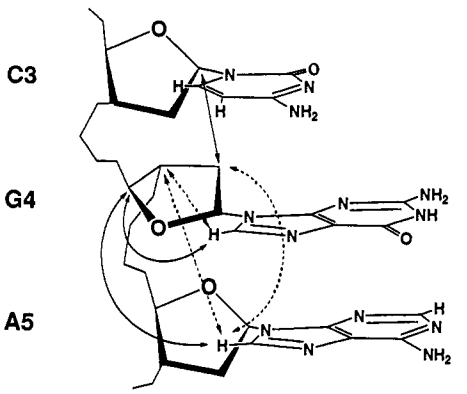

Figure 3. Schematic drawing of the C3–G4–A5 step of the heterochiral 12-mer. Broken arrows indicate NOEs which were not observed for the heterochiral 12-mer but are generally observable for regular B-form DNA. Solid arrows indicate unique NOEs observed only for the heterochiral 12-mer.

nucleotide residues may adopt the low *anti*, *S*-conformations, as seen in a left-handed B-DNA model proposed by Sasisekharan *et al.*[17]

It has been considered that even a single chiral defect destroys the secondary structure in a considerable part of the DNA chain.[6] However, the present results indicate that such a chiral defect permits a duplex structure without any disruption of base-pairing, and the L-nucleotide residue in the duplex has the unusual low *anti* glycosyl conformation, which may be one of the characteristics of hybridization between natural DNA and an L-oligonucleotide. This would be useful for design of a novel antisense molecule resistant to enzymatic degradation *in vivo*.

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Supplementary Material Available: COSY plot showing scalar couplings between H2′/H2″ and H1′,H3′ and expanded NOESY plots of the heterochiral 12-mer (7 pages). Ordering information is given on any current masthead page.

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