Basis for the Right-Handed Helical Sense of Double-Stranded DNA: Formation of the Right-Handed Helix by L-Oligonucleotides Fixed in Low-*anti* Glycosyl Conformation

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Since the discovery of a right-handed double-helical model of DNA by Watson and Crick,[1] it has been found that double helical DNA can adopt several major conformations (A-, B-, and Z-forms), depending on the environment and sequences.[2] However, DNA rarely adopts the A-form in solution, and the left-handed Z-form[3] is formed in alternating pyrimidine−purine sequences only under stresses such as high salt, polyamine, and negative superhelicity of double-stranded DNA.[4−6] Thus, DNA essentially forms the right-handed B-form in solution. It was originally thought that D-chirality of the deoxyribose moiety is responsible for the right-handedness of DNA. In fact, we have already shown that the DNA hexamer, d(CGCGCG), containing L-deoxyribose (L-DNA) forms a left-handed B-form double helical structure under low salt conditions.[7] However, it is still unknown why D-deoxyribose is responsible for the right-handness of DNA. Uesugi et al. reported the structure of D-homooligonucleotides consisting of 6,2′-*O*-cyclouridine and 8,2′-*S*-cycloadenosine, whose glycosyl torsion angles are restricted in a high *anti* (*sc*) conformation, and they concluded based on the results of CD and ^{1}H NMR experiments that the high *anti*-fixed D-oligonucleotides form a left-handed double helix.[8] Thus, D-deoxyribose apparently permits a left-handed structure of DNA which is different from the Z-form, as well as the right-handed structures. In this paper, we describe the basis for the right-handed helical sense of double-stranded DNA.

We have elucidated the structure of an L-nucleotide residue in a natural double-stranded oligonucleotide, in which the L-nucleotide residue adopts a low *anti* (*ap*) glycosyl conformation to form the stable Watson-Crick base-pairing,[9a] and this result has been supported by another research group.[9b] This means the possibility that L-DNA with the low *anti* glycosyl conformation forms a right-handed helix. On the basis of the hypothesis that glycosyl conformation could be one of the major factors determining the helicity of double-stranded DNA, we designed novel carbocyclic L-nucleoside analogues (**2**, **3**) whose glycosyl conformation is restricted to the low *anti* conformation (Scheme 1). We have already reported the synthesis of racemic compounds carbocyclic 6,6′-*O*-anhydro-6,6′-dihydroxy-2′-deoxyuridine **2** and carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxyadenosine **3**.[10,11] Synthesis of the L-like enantiomer of **2** and **3** was carried out similarly by using chiral (−)-olefin **1** as a starting material, whose

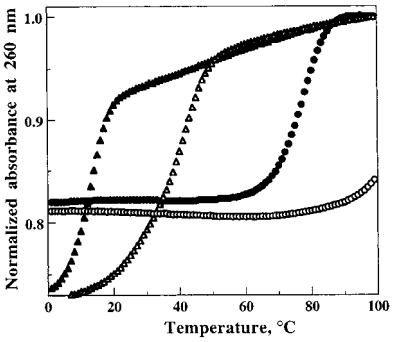
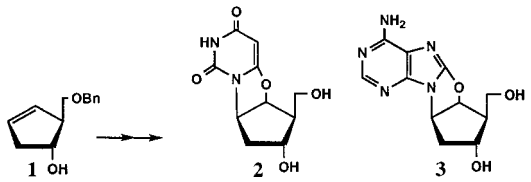


Figure 1. UV-melting profiles of the D1−D2 (triangles) and C1−C2 (circles) duplexes. The open and closed symbols represent the melting profiles of each duplex (4 μM) in 10 mM sodium phosphate (pH 7.0) containing 1 M NaCl and 0 M NaCl, respectively. The temperature was raised at a rate of 0.5 °C/min.

Scheme 1



(+)-enantiomer was reported to be a useful chiral synthon for syntheses of carbocyclic D-nucleoside analogues.[12] The optical purity of (−)-olefin **1** was estimated to be more than 97% ee by chiral HPLC analysis.[13] Finally, compounds **2** and **3** were converted to phosphoramidite derivatives according to conventional methods.[14] Then we synthesized oligonucleotides (C1−D2) on an applied biosystems model 392 DNA synthesizer. Cleavage from the support and deprotection were effected by treatment with concentrated aqueous ammonia for 2 h at room temperature,[15] and purification was carried out on reversed-phase HPLC. The structure of the modified oligonucleotides, C1 and C2, was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra.[17]

First, we measured the UV-melting profiles to evaluate the ability of formation of a duplex structure for the C1−C2 strands (Figure 1). The C1−C2 annealed strands showed only slight rise of the baseline near 100 °C and we could not observe definite transition of duplex to random coil for the C1−C2 annealed

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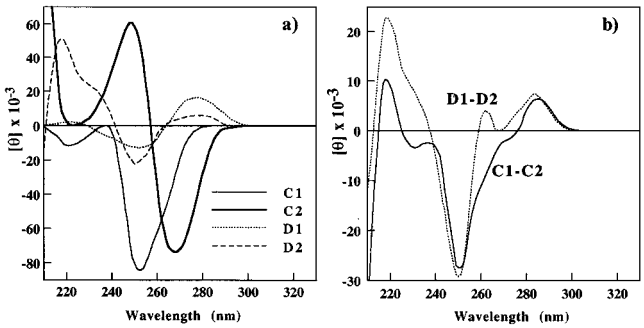

Figure 2. CD spectra of oligonucleotides under single-strand (a) and double-strand (b) conditions at 1 °C. Samples contained 1 M NaCl, 10 mM sodium phosphate, pH 7.0. (a) Solid, bold, dotted, and broken lines are the spectra of C1, C2, D1, and D2, respectively. (b) Solid and dotted lines are the spectra of the C1−C2 duplex and D1−D2 duplex, respectively.

C1; L-d(cUcUcUcUcUcUcUcUcUcUcU) cU; **2**
C2; L-d(cAcAcAcAcAcAcAcAcAcAcA) cA; **3**
D1; D-d(TTTTTTTTTTTT)
D2; D-d(AAAAAAAAAAAA)

strands in 1 M NaCl solution. However, we were able to clearly observe a melting transition under low-salt conditions. The midpoint of the transition for D1−D2 is 15 °C, but that for C1−C2 is 80 °C under identical conditions. This can be explained by favorable entropic effects by means of covalent fixation of the glycosyl bonds for the C1−C2 duplex. The substantial stabilization of duplex structure is also observed in modified oligonucleotides whose sugar conformation is restricted by covalent bonds.[18] The duplex structure of C1−C2 was evaluated by measuring CD spectra. The results are shown in Figure 2. The spectra of C1 and C2 under single-stranded conditions are quite different from corresponding natural dodecanucleotides D1 and D2, respectively (Figure 2a). The modified L-like dodecamers C1 and C2 show much more intense ellipticity than natural dodecamers D1 and D2. This may be due to rigidity of glycosyl conformation for C1 and C2 by covalent fixation. A conservative CD band of C2 means degenerate exciton interaction between the bases, which reflects the stacking of bases in an asymmetric helix. A nonconservative CD band of C1 means nondegenerate exciton interaction between the bases related to base stacking. On the other hand, the spectrum of the C1−C2 duplex is very similar to that of the D1−D2 duplex which is well-known to adopt the right-handed B-form (Figure 2b).[19] The results of the CD spectra strongly suggest that the C1−C2 duplex forms a B-form-like right-handed double helical structure, despite its unnatural L-like chirality. This result is equivalent to natural D-oligonucleotides with the low *anti* glycosyl conformation forming a left-handed double helix. Such a low *anti* left-handed structure of DNA was predicted by potential energy and molecular mechanics calculations.[22,23]

Our results strongly suggest that a helical sense of DNA depends tightly on a glycosyl conformation as well as chirality of deoxyribose. In other words, a glycosyl conformation would be a major determinant of the helical sense of natural D-DNA. Figure 3 represents the means of relationship between a glycosyl conformation and the helical sense of natural D-DNA. The helical sense of double-stranded DNA changes left ⇒ right ⇒ left on varying the glycosyl conformation from high *anti* to low *anti*. However, there is steric repulsion between H8 of a purine base (or H6 of a pyrimidine base) and H2′ or O4′ of the sugar moiety, when nucleos(t)ides adopt the high *anti* or low *anti* conformation, respectively. Therefore, the conformation around the glycosyl bond is eventually restricted in narrower *anti* region other than both high *anti* and low *anti*. This unique restricted stability of the *anti* conformation would be one of the reasons why D-deoxyribose is responsible for the right-handness of double-stranded natural D-DNA.

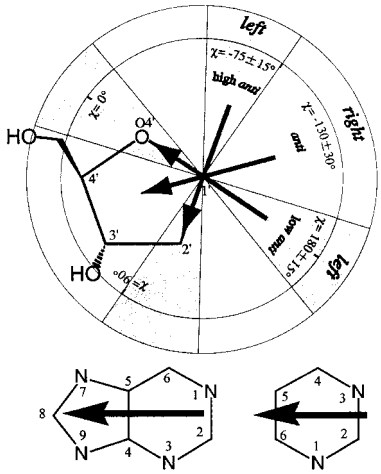

Figure 3. Schematic diagram representing a relationship between a glycosyl torsion angle and a helicity of double-stranded natural D-DNA. The arrows indicate the direction of the nucleobase ring, whose base proton (H8 for purines or H6 for pyrimidines) is positioned at the arrowhead as illustrated at the bottom. Sectors in the inner circle represents each glycosyl conformational limit defined by a dihedral angle of χ = O4′−C1′−N9−C4 for purines and χ = O4′−C1′−N1−C2 for pyrimidines. The sector opposite to that of each glycosyl conformational limit means the region where the H8 atom of the purine ring (or H6 atom of the pyrimidine ring) is located on each conformation. The *anti* region is shown by values taken from crystallographic data of A- and B-form DNA.[24] The outer circle represents the helicity of double-stranded natural D-DNA on each glycosyl conformation.

We have demonstrated that differential stability among the glycosyl conformations determines the helical sense of DNA, although D-deoxyribose itself has potential to permit both the right- and left-handed helices of DNA. The understanding of the mechanism determining the handness of double-stranded DNA would be useful for the prediction of the handness of novel helix-forming molecules.

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(19) The geometry of the cyclopentane ring of compounds **2** and **3** is estimated as an *N*-type conformation from their $J1'2' + J1'2''$ values.[10,20] Therefore, influences of fusion of the cyclopentane ring with the nucleobase on the duplex structure could not be negligible. However, influences of substitution of the methylene group for 4′-oxygen on the duplex structure would not be significant, because the CD spectra of carbocyclic $(dT)_{20}$ complexed with poly(dA) have been reported to basically be not different from $(dT)_{20}$·poly(dA).[21]

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