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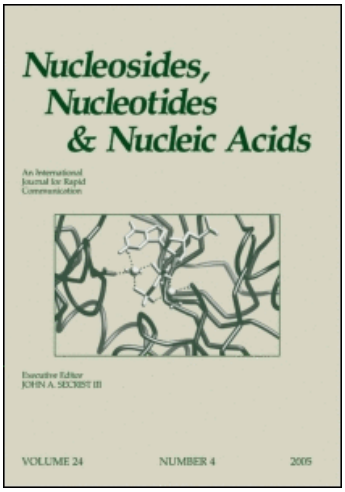

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STRUCTURAL STUDIES OF HETEROCHIRAL DNA/DNA, RNA/RNA, AND DNA/RNA DUPLEXES

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□ *Using DNA and RNA heptanucleotides containing an unnatural* L*-nucleotides as well as the complementary strands, effects of the introduction of an* L*-nucleotide on the structure of DNA/DNA, RNA/RNA, and DNA/RNA duplexes were investigated by circular dichroism experiments and RNase H-mediated RNA strand cleavage reaction. The results suggested that the substitution of the central* D*-nucleotide with an* L*-nucleotide in the duplexes causes the significant structural alterations as the duplex structures change to conformations with more B-form similarities.*

Keywords DNA; RNA; DNA/RNA hybrid duplex; Duplex structure; Homochiral; Heterochiral

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INTRODUCTION

Living bodies are chiral machineries consisting of D-nucleic acids and L-amino acids. Usually, enantiomers are mutually exclusive in such chiral environments and thus the chirality of biomolecules plays important roles in structure formation, specific ligand recognition, and catalysis.[1] Since both enantiomers of mononucleotides and amino acids would have been equally produced by prebiotic non-asymmetric syntheses, chiral selection and amplification must have been achieved during prebiotic chemical evolution of biomolecules.[2–4] Condensation of racemic monomers is thought to yield complex stereo-isomers of polypeptides and polynucleotides. Indeed, non-enzymatic oligomerization of racemic mononucleotides on an RNA template[5–8] or on a clay mineral yielded a complex mixture of both homo- and heterochiral oligomers.[9,10] Therefore, the comparative study for the physicochemical properties of homochiral- and heterochiral nucleic

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This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.
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acids would be useful for considering the processes of the chiral selection and amplification in the chemical evolution of nucleic acids. The physicochemical and biochemical properties of homochiral L-DNA and heterochiral DNA duplexes[11–20] as well as those of homochiral L-RNAs[21–25] have well established. However, there are few reports on the effects of the incorporation of L-ribonucleotides into D-RNA strands on the secondary structure formation and stability.[26–28]

Here, we report the effects of the incorporation of an L-nucleotide into the DNA or the RNA strand in DNA/DNA, RNA/RNA, and DNA/RNA duplexes on the duplex structure by using RNase H-mediated cleavage reaction as well as circular dichroism (CD) experiments.

EXPERIMENTAL SECTION

General

Synthesis of heptanucleotides has been reported previously.[28] *E. coli* RNase H was purchased from TOYOBO. Reverse-phase HPLC was performed on a column of μBondasphere 5C18 100 Å (3.9 × 150 mm, Waters) with a linear gradient of acetonitrile in 50 mM triethylammonium acetate, pH 7.0 by a Shimadzu LC-10A HPLC system.

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Measurement of CD Spectra

The concentrations of oligonucleotide solutions were calculated by using equation and coefficients described by Borer.[29] In this calculation, the coefficients of the heterochiral dimer units were assumed to be the same as the corresponding homochiral dimer units. Each complementary pair of 7-mers was mixed and dissolved in a buffer containing 1 M NaCl, 10 mM sodium phosphate (pH 7.5) at 6 μM duplex concentration. The solutions (4 ml) containing duplexes were heated at 80°C and cooled gradually to room temperature. CD spectra were measured at 0°C on a JASCO J-820 spectropolarimeter.

Digestion of Duplexes with RNase H

Heptanucleotide duplex solutions (0.2 mM) containing 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT and 20 mM HEPES (pH 8.0) were cooled at 0°C. After addition of RNase H (2 μl, 10 units/μl), the reactions were incubated at 0°C. Aliquots of the solutions were periodically withdrawn into microtubes containing 30 mM EDTA (20 μl) and distilled water (75 μl). The solutions were filtered through Ultrafree-MC 10000NMWL (Millipore) and were analyzed by a reverse-phase HPLC. The DNA strands of the duplexes were used as the internal standard.

RESULTS AND DISCUSSION

The sequences of the heptanucleotides used are shown below. The lower strands contain D- or L-thymidine for DNA strands and D- or L-uridine for RNA strands at the central residue.

upper strand	lower strand
d7: d(C-G-T-A-C-G-C)	dT_D: d(G-C-G-T-A-C-G)
r7: r(C-G-U-A-C-G-C)	dT_L: d(G-C-G-**T**-A-C-G)
	rU_D: d(G-C-G-U-A-C-G)
	rU_L: d(G-C-G-**U**-A-C-G)

T: L-Thymidine
U: L-Uridine

The oligonuleotides were mixed by all combinations of the upper and lower strands, and their duplex structures were investigated by circular dichroism (CD) experiments. Generally, the CD spectrum of B-form DNA is conservative, consisting of a positive and a negative band on either side of the absorption maximum, whereas those of A-RNA and A-DNA show a large positive band near the absorption maximum, which is termed nonconservative. Figure 1 shows the CD spectra of the duplexes. The DNA/DNA duplex (d7/dT_D) showed a typical spectrum for B-form DNA, whereas the RNA/RNA duplex (r7/rU_D) showed that for A-form RNA (Figure 1A). The

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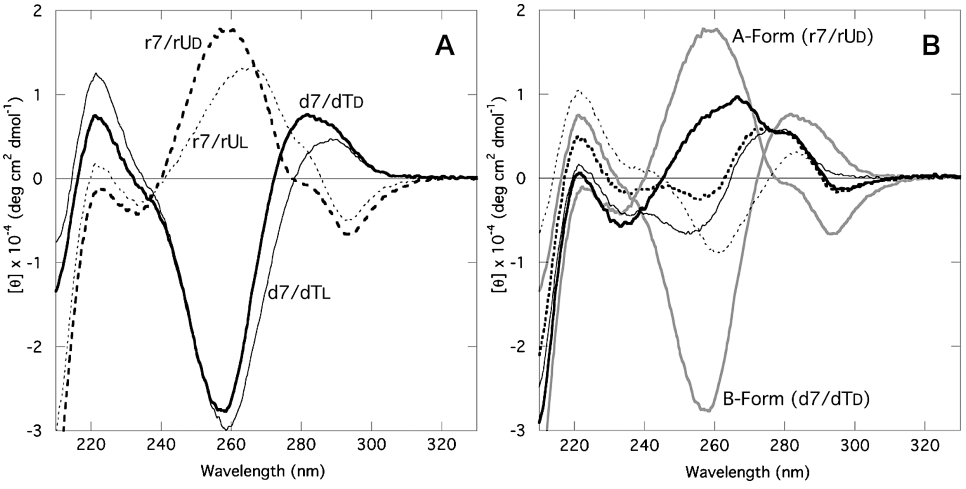

FIGURE 1 Circular dichroism (CD) spectra of duplexes at 0°C. Samples contained 6 μM duplex in 1 M NaCl, 10 mM sodium phosphate (pH 7.5). (A) The spectra of the homochiral- and the heterochiral DNA/DNA, d7/dT_D (thick solid line), d7/dT_L (thin solid line); and RNA/RNA duplexes, r7/rU_D (thick dotted line), r7/rU_L (thin dotted line) are shown. (B) The spectra of the homochiral, d7/rU_D (thick solid line), r7/dT_D (thick dotted line); and the hetereochiral DNA/RNA hybrid duplexes, d7/rU_L (thin solid line), r7/dT_L (thin dotted line) are shown. The spectra of the homochiral DNA/DNA (d7/dT_D) and RNA/RNA duplexes (r7/rU_D) are also shown in gray solid lines for comparison.

substitution of the central D-nucleotide with an L-nucleotide in r7/rU_D causes a significant red shift in the spectrum (r7/rU_L), and this phenomenon seems to be due to the conformational shift of the duplex to a conformation with some B-form similarities. RNA and A-form DNA have the substantial tilt of bases and the longer distance between the base pairs and the helix axis compared with B-form DNA. Johnson and Tinoco suggested that the differences of CD spectra between the A- and B-form are due to the tilt of bases which can be seen only in the A-form.[30] Later, Moore et al. suggested that the spectral difference is caused by the longer distance between the base pairs and the helix axis in the A-form than in the B-form.[31] Therefore, the present result may suggest that the substitution of the central residue with an L-nucleotide in the A-form duplex leads to the some dissolution of the tilt of bases or the some shortening of the base pair–helix axis distance.

Figure 1B shows the spectra of DNA/RNA hybrid duplexes as well as the typical A- and B-form duplexes. DNA/RNA hybrid duplexes have been reported to adopt the A-form or an intermediate duplex structure between the A- and B-forms.[32] Although the spectral intensity of the homochiral hybrid duplexes (d7/rU_D and r7/dT_D) is significantly decreased compared with the DNA/DNA and RNA/RNA duplexes, those are likely to adopt an intermediate structure between the A- and B-form duplexes. The spectrum of d7/rU_D is closer to that of the A-form duplex (r7/rU_D) than to that of r7/dT_D, which seems to be the truly intermediate spectrum between the A- and B-forms. This difference between these spectra can be explained by the purine base contents of the DNA strands in the duplexes, since DNA/RNA hybrid duplexes that have a higher purine content in the DNA strand adopt more B-like structures.[33] In the present case, the purine content of the DNA strand in r7/dT_D is 57%, whereas that in d7/rU_D is 43%; therefore, r7/dT_D should have the duplex structure with more B-form similarities compared with d7/rU_D. The substitution of the central D-nucleotide residue with an L-nucleotide in the homochiral hybrid duplexes causes the significant red shift of the CD spectra as well. The spectrum of d7/rU_D changed to resemble that of r7/dT_D closely by the substitution (d7/rU_L). Similarly, the spectrum of r7/dT_D changed to be close to that of the B-form duplex (d7/dT_D) by the substitution (r7/dT_L) in spite of its weakened spectral intensity. These results suggest that the structures of the hybrid duplexes shift to conformations with more B-form similarities by the substitution of the central D-residue with the L-nucleotide.

To confirm the above results, we employed RNase H as a probe for the duplex structure. RNase H recognizes a DNA/RNA hybrid duplex and specifically cleaves the RNA strand.[34] Reid and coworkers suggested that this enzyme recognizes the intermediate duplex structure between the A- and B-forms, especially the width of the minor groove.[31] We carried out the cleavage reaction of the hybrid duplexes by RNase H at 0°C, because the duplexes have enough stability at this temperature.[28] Although the salt

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concentration in the cleavage experiments is significantly lower than that in the CD experiments. However, it is likely that the duplexes under RNase H cleavage conditions maintain the similar conformations essentially as those under the CD conditions, because no significant conformational change of a dodecanucleotide duplex has not been observed at salt concentrations in a range of 0.1–1 M NaCl.[35] Figure 2 shows the reversed-phase HPLC analyses of the RNase H-mediated cleavage reactions of the hybrid duplexes. Although the exact cleavage sites have not yet been determined, the cleavage of the RNA strands in the duplexes, $d7/rU_D$, $r7/dT_D$, and $d7/rU_L$ by RNase H were observed, whereas that in $r7/dT_L$ were not cleaved at all. Figure 3 shows the time course of the reaction. Within 6 h, the RNA strands in the duplexes $r7/dT_D$ and $d7/rU_L$ were completely cleaved, and 52% of the cleavage for that in $d7/rU_D$ was observed. Thus, the susceptibility of the hybrid duplexes toward RNase H is in the order of $r7/dT_D \approx d7/rU_L > d7/rU_D \gg r7/dT_L$. This result suggests that $r7/dT_D$ and $d7/rU_L$ form the proper duplexes to be easily recognized by RNase H, which are the truly intermediate structures between the A- and B-forms, and $d7/rU_D$ forms the duplex that is not very easily recognized by the enzyme; however, $r7/dT_L$ is no longer recognized by the enzyme. These are in good agreement with the results of the CD experiments. The CD experiments showed that $r7/dT_D$ and $d7/rU_L$ adopt the "intermediate" structures, which resemble closely each other, between those of the A- and B-forms, whereas $d7/rU_D$ have the conformation with more A-form similarities rather than the intermediate structure. $r7/dT_L$ showed the basically same structure as the B-form, although its CD intensity is significantly decreased. These results indicate that the substitution of the central D-nucleotide with an L-nucleotide in the duplexes causes the significant conformational shift to the conformations with more B-form similarities.

Previously, we have shown that the thermodynamic stabilities of the DNA/DNA, RNA/RNA, and DNA/RNA duplexes are decreased by the substitution of the D-nucleotide residue with an L-nucleotide, whose extent highly depends on whether the DNA strand or the RNA strand is substituted.[28] Namely, the substitution in the RNA strand causes the much greater duplex destabilization than that in the DNA strand. Thus, the introduction of an L-nucleotide is likely to cause the remarkable destabilization for the A-form structure, because in a DNA/RNA hybrid duplex, the DNA strand adopts the B-form, whereas the RNA strand adopts the A-form,[36] and the overall duplex structure is an intermediate conformation between the A- and the B-form.[32] Here, we have demonstrated that the substitutions of the central D-nucleotide with an L-nucleotide in duplexes containing the RNA strand lead to the significant conformational shift to the duplex structures with more B-form similarities. The remarkable destabilization for the A-form or A-like structures may lead to the conformational shift of the duplex toward the B-form.

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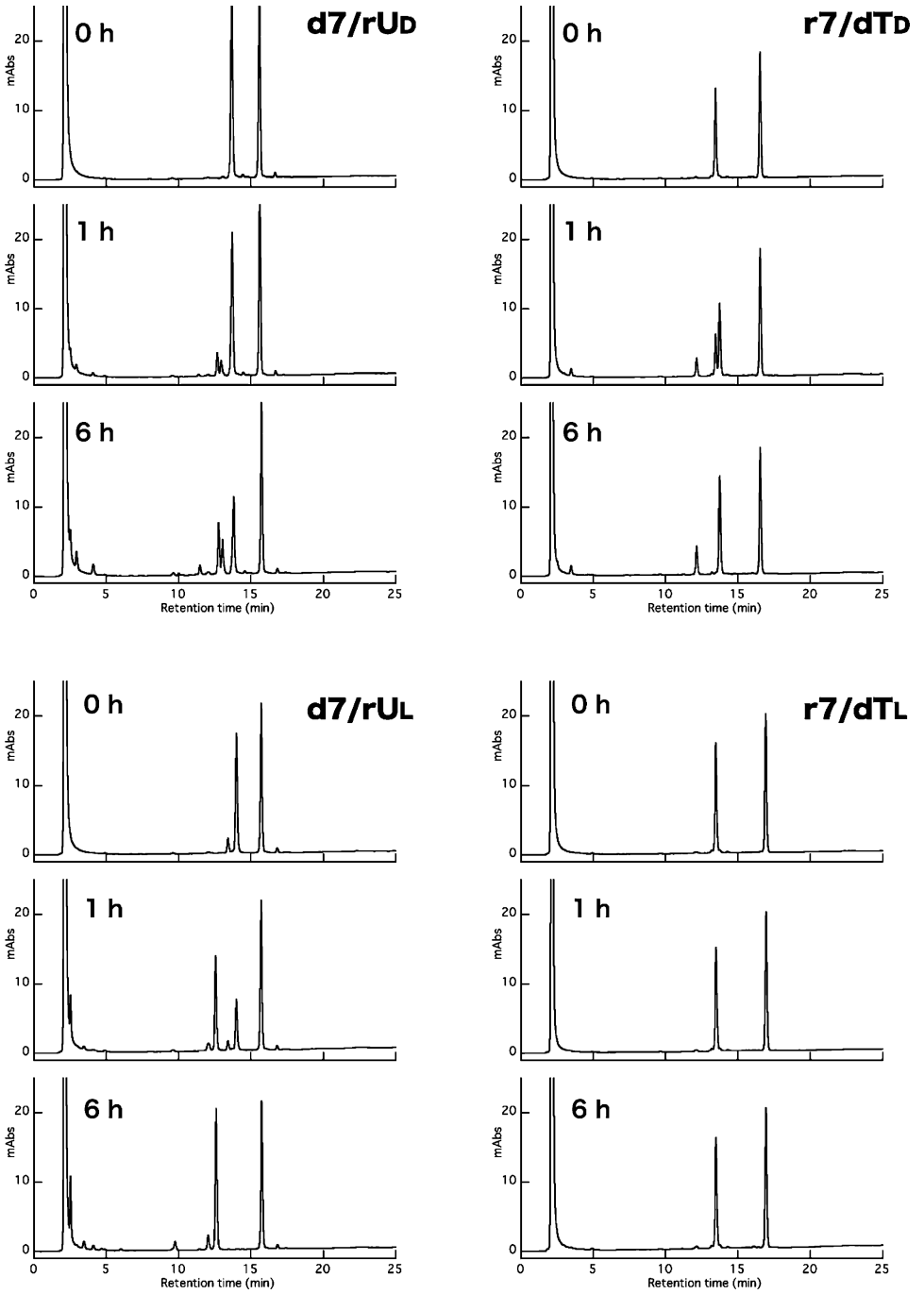

FIGURE 2 HPLC analyses of the reaction for the DNA/RNA hybrid duplexes with RNase H.

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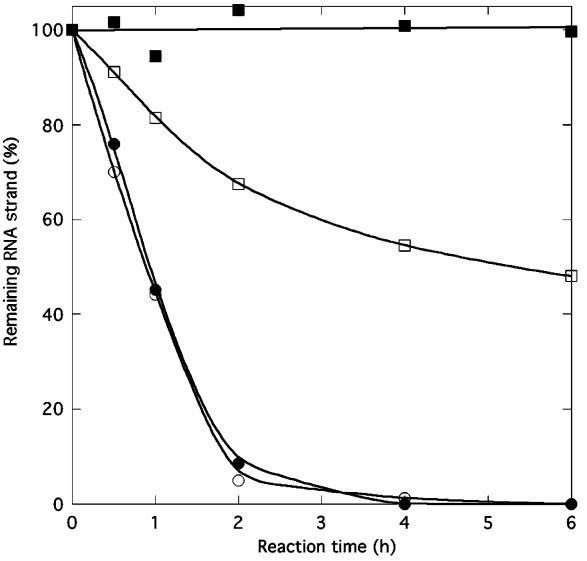

FIGURE 3 Time course of the reaction for the DNA/RNA hybrid duplexes, r7/dT_D (closed circle), d7/rU_L (open circle), d7/rU_D (open square), and r7/dT_L (closed square) with RNase H.

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These fundamental understanding for the structure and stability of heterochiral nucleic acids would be important for considering the chemical evolution of nucleic acids as well as for molecular design of novel functional molecules containing L-nucleotides.

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