Enantio- and Meso-DNAs: Preparation, Characterization, and Interaction with Complementary Nucleic Acids

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Received June 7, 1993⊛

Abstract: Enantio-DNAs (DNA having 2-deoxy-L-*erythro*-pentose, the enantiomer of natural 2-deoxy-D-ribose, as the sugar backbone) and meso-DNAs (DNA having an alternating sequence of L-sugars and D-sugars) were prepared by the use of an automated DNA synthesizer. The characteristics of the products were analyzed, focusing on enantio- and meso-dodecadeoxyadenylic acids (designated as L-dA_{12} and LD-dA_{12}, respectively). Both L-dA_{12} and LD-dA_{12} were resistant to the action of phosphodiesterases, though LD-dA_{12} was decomposed very slowly by snake venom phosphodiesterase. The affinity of these dodecamers for their complementary natural nucleic acids, poly(U) and poly(dT), was analyzed by the UV-mixing curve and melting-temperature measurement methods. Both L-dA_{12} and LD-dA_{12} showed affinity for their complementary nucleic acids. L-dA_{12} showed high selectivity for poly(U) over poly(dT), and a UV-mixing curve analysis suggested that the interaction mode was triplex formation. LD-dA_{12} showed moderate selectivity for poly(U) over poly(dT). L-dT_{12}, the counterpart of L-dA_{12}, did not show any detectable interaction with its complementary natural nucleic acid.

Introduction

In natural nucleic acids, the higher-order structure is mainly determined by the chirality of the sugar-backbone units. Organisms in our world utilize only D-sugars, but not their enantiomers, probably for reasons closely related to the origin and the evolution of life. Nevertheless, molecular modeling studies suggest that an L-sugar backbone can locate nucleobases appropriately for complementary base-pairing with natural nucleic acids by Watson–Crick or Hoogsteen base-pairing.[1-5] This raises the possibility that L-sugar-containing nucleic acids might function as pseudo-DNA/pseudo-RNA.[6,7]

A huge number of nucleic acid derivatives and analogs have been synthesized,[6,7] including (i) oligomers derivatized with a specific functional group, such as photoreactive psoralens, intercalative acridines, alkylating agents, and EDTA, as an oxygen/Fe-dependent DNA-cleaving functional group, (ii) oligomers having modified phosphodiester linkages, such as phosphoramidite, phosphorothioate, and methylphosphonate, and (iii) oligomers with an α-deoxyribose backbone instead of a natural β-deoxyribose backbone. Some oligopeptides bearing nucleobases have been also prepared as pseudo-DNA.[8-10]

L-Sugar-containing nucleic acids [enantio-DNA (DNA having 2-deoxy-L-*erythro*-pentose, the enantiomer of natural 2-deoxy-D-ribose, in the backbone) and meso-DNA (DNA having an alternating sequence of L-sugar and D-sugar)] would be another possible simple approach for molecular design of a pseudo-DNA. It was anticipated that they would act as antisense/antigene oligonucleotides. In addition, as enzymes generally recognize only naturally occurring stereoisomers, it would be expected that enantio-DNA would not be a substrate for nucleases, whose oligonucleotide-degrading activity imposes a severe restriction upon antisense/antigene strategies.[6,7]

Though a few papers have appeared on enantio-oligonucleotides,[1-5,11-15] little has been established about the nature of the interactions of enantio-oligonucleotides with natural RNA or DNA. Recently, we have reported the preparation of a hexamer of enantio-deoxyadenylic acid [L-$(dAp)_5dA$, designated as L-dA_6] and its interaction with the complementary polymers/poly(U) (RNA-type) and poly(dT) (DNA-type).[1-3] L-dA_6 was quite resistant to nucleases and could bind to complementary RNA but not to complementary DNA.[1] The mode of interaction of L-dA_6 with poly(U) was suggested to be triplex formation, on the bases of both UV-mixing curve experiments and *ab initio* calculations for molecular modeling studies.[1,4] However, whether the RNA-type selectivity shown by L-dA_6 means that enantio-DNA recognizes only its complementary RNA or that the melting temperature of the L-dA_6/poly(dT) complex is lower than 0 °C is not known. In view of this, we studied the behavior of an enantio-oligodeoxyadenylic acid other than L-dA_6, i.e., the dodecamer L-dA_{12}. In addition, we have prepared enantio-dodecadeoxynucleotides bearing nucleobases other than adenine, i.e., L-dT_{12}, L-dC_{12}, and L-dG_{12}, and meso-DNA [$(L\text{-}dA\cdot D\text{-}dA)_6$, designated as LD-$dA_{12}$]. In this paper, we describe the preparation, the resistance to nucleases, and the interaction with natural nucleic acids of enantio- and meso-dodecadeoxyribonucleotides.

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⊛ Abstract published in *Advance ACS Abstracts,* October 1, 1993.

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Experimental Section

L-Deoxynucleoside–Cyanoethyl Phosphoramidites. The enantiomers of 2′-deoxynucleosides (L-dN) were synthesized as described previously.[1,2,16] Each L-dN was converted to the corresponding L-deoxynucleoside–cyanoethyl phosphoramidite according to the usual method (Figure 1).[17-20] Briefly, L-dT, L-dA, and L-dC were treated with dimethoxytrityl chloride (1 equiv) in the presence of 4-(dimethylamino)pyridine in dry pyridine. For L-dA and L-dC, dimethoxytritylated nucleosides were trimethylsilylated and then benzoylated. For L-dG, isobutylation with isobutyric anhydride in dry pyridine was employed after trimethylsilylation with hexamethyldisilazane. The isobutylated L-dG was treated with dimethoxytrityl chloride. All of these protected L-dNs were obtained in moderate yields, as reported for the preparations of the corresponding D-isomers,[17-20] gave analysis values consistent with those expected, and showed the same ^{1}H-NMR spectra and melting points as those of the corresponding known D-isomers. Protected L-dN (2 mmol) was dissolved in dry tetrahydrofuran (10 mL), and diisopropylethylamine (8 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (4 mmol) were added to the solution. After being stirred at room temperature for 2 min, the solution was filtered and the filtrate was evaporated. The resulting residue was dissolved in 100 mL of ethyl acetate saturated with argon gas, and the solution was washed successively with ice–water and brine, dried over sodium sulfate, filtered, and then evaporated. The residue was separated by silica gel column chromatography (eluted with a mixture of ethyl acetate and methylene chloride, the silica gel was prewashed with the solvent containing 10% triethylamine). The separated fraction was evaporated and dried by repeated coevaporation using dry toluene containing 2% triethylamine, and the residue was further dried under vacuum. The resulting amorphous solid was directly used as the reagent for synthesis of enantio- and meso-DNAs in an automated DNA synthesizer. The structure and the purity of the obtained L-deoxynucleoside–cyanoethyl phosphoramidites were confirmed by measuring the ^{1}H-NMR spectra in $CDCl_3$ (the spectra were indistinguishable from those of the corresponding known D-isomers).

Preparation of Enantio- and Meso-DNAs. L-Deoxynucleoside–cyanoethyl phosphoroamidites were dissolved in dry acetonitrile, and the solutions were filtered through a 0.2-μm disk filter. The filtrate and/or a commercially available acetonitrile solution of the D-isomers was used in a DNA-Synthesizer Model 391 PCR-MATE equipped with a 1-μm CPG-column (Applied Biosystems Ltd.),[21] and oligomers containing L-dN were synthesized according to the instructions supplied with the machine. For preparation of enantio-DNAs, prepared oligomers were dissolved in 3 mL of 0.1 M ammonium bicarbonate (pH 9) containing 2 units of phosphodiesterase I (*Crotalus atrox* venom) and incubated for 1 h at 37 °C to remove the D-isomer unit at the 3′-end (see text). Then, the mixture was evaporated. Oligodeoxynucleotides were separated by HPLC using a Polygosil 60-5C_{18} column (0.8 cm o.d. × 25 cm) eluted with 0.1 M triethylammonium acetate containing 8–12% acetonitrile. The fraction containing the desired oligodeoxynucleotide was collected and evaporated. The residue was desalted by flash column chromatography using a silica gel ODS-Q3 column (1.8 cm o.d. × 15 cm) eluted with water containing 0–20% acetonitrile. Quantification of the obtained oligonucleotides was performed by comparison of the UV absorption with that of the corresponding D-isomer, whose extinction coefficient was determined after complete enzymatic hydrolysis to monomers.

Incubation with Phosphodiesterase. Oligodeoxynucleotides were dissolved in a buffer [0.1 M ammonium acetate (pH 6.5) for phosphodiesterase II (0.25 unit/mL, bovine spleen, Pharmacia) and 0.1 M ammonium bicarbonate (pH 9) for phosphodiesterase I (0.3 unit/mL, *C. atrox* venom, Pharmacia)] to give a final concentration of 0.09 mM. The mixture was incubated at 37 °C, and the reaction was monitored by UV-absorption measurement.

UV-Mixing Curves. A solution of synthesized oligodeoxynucleotide (0.09 mM in 10 mM Tris–HCl (pH 7.4) containing 10 mM $MgCl_2$) and a solution of natural homopolynucleotide (0.09 mM in the same buffer) were mixed in various ratios. The mixture was heated to 80 °C for 5 min and then slowly cooled to room temperature. It was subsequently cooled to 0 °C for 2 h, and the UV absorption was measured. In each case, the optical density of a 0.09 mM solution of synthesized oligodeoxynucleotide was assigned the value of 1.0. The absorption ratio (A/A_0, the vertical scale in Figure 4) of the mixture containing synthesized oligodeoxyribonucleotide and natural homopolynucleotide in the molar nucleotide-units ratio of x:y ($x + y = 1$) was defined as $A/A_0 = A_{obs}/(xA_{oligo} + yA_{poly})$, where A_{obs} is the observed absorption of the mixture and A_{oligo} and A_{poly} are the absorptions of the 0.09 mM solutions of synthesized oligodeoxynucleotide and natural homopolynucleotide, respectively.

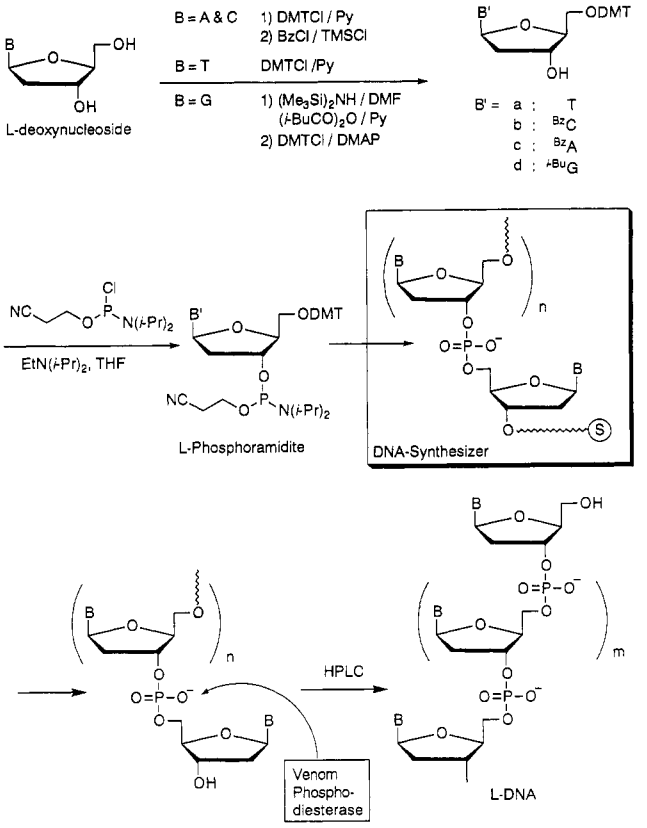

Figure 1. Synthesis of enantio- and meso-DNAs.

Melting-Temperature Measurement. The solutions of synthesized oligodeoxynucleotide and natural homopolynucleotide (both prepared as described above) were mixed in the molar nucleotide-units ratio of 1:2. The mixture was heated and cooled as described above. The UV absorption of the mixture at gradually increasing temperature (typically, 0.5 °C per min) was monitored by using a UV spectrophotometer equipped with a thermocontroller (handy cooler, TRL-108H, Tomasz Ltd.). The results of melting-temperature determinations were reproducible with experimental errors of less than ±3 °C. Values obtained in one set of typical experiments are presented in the text.

CD Spectra. The CD spectra of the samples were measured by using a JASCO J-600 spectropolarimeter equipped with a TRL-108H thermocontroller. For the measurement of complex formation upon mixing synthesized oligodeoxynucleotide and natural homopolynucleotide, samples were prepared as described in the section on melting-temperature measurements.

Nuclease S1 Assay. The solutions of synthesized oligodeoxynucleotide and natural homopolynucleotide (both prepared as described in the section on UV-mixing curves) were mixed in the molar nucleotide-units ratio of 1:1. The mixture was heated and cooled as described above. Then Nuclease S1 (470 units/mL) was added, and incubation was started by warming the mixture at 30 °C. The reaction was monitored by UV-absorption measurement.

Results and Discussion

Preparation of Enantio- and Meso-dodecadeoxyribonucleotides. The L-deoxynucleotide–cyanoethyl phosphoramidites prepared as described in the Experimental Section were used directly in the automatic DNA synthesizer for preparation of oligonucleotides

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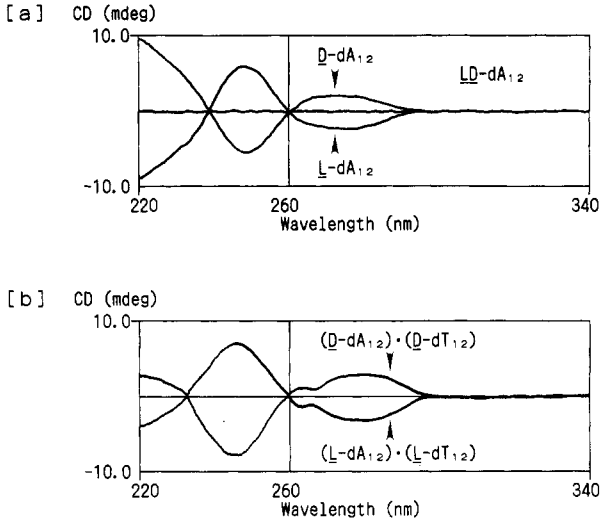

Figure 2. CD spectra of (a) L-dA_{12}, D-dA_{12}, and LD-dA_{12} and (b) the L-dA_{12}/L-dT_{12} complex and the D-dA_{12}/D-dT_{12} complex.

(Figure 1). The use of the commercial CPG column resulted in oligodeoxynucleotides bearing a D-deoxynucleotide unit at the 3′-terminal. This D-deoxynucleotide unit could be removed by digestion of the obtained oligodeoxynucleotide with snake venom phosphodiesterase (Figure 1). Therefore, for the synthesis of L-dA_{12}, for example, we first prepared (L-dA_{12})(D-dA) in the automated DNA synthesizer and then the oligodeoxynucleotide was treated with snake venom phosphodiesterase to give L-dA_{12}, which was purified by HPLC (the purity was at least 95%). The structure was confirmed by comparison of the CD spectrum with that of the corresponding natural D-dodecadeoxyribonucleotide; the CD spectra should be in a mirror-image relation (Figure 2). Of course, the mixture of L-dA_{12} and L-dT_{12} thus obtained should form a left-handed duplex which is the mirror image of the natural D-$(dA)_{12}$·D-$(dT)_{12}$ right-handed duplex, and this was confirmed by their CD spectra (Figure 2).

Other deoxyoligonucleotides including meso-dodecadeoxyribonucleotides and L-oligodeoxynucleotides of various lengths and various base sequences could be prepared by basically the same method. Meso-dodecadeoxyribonucleotides showed flat CD spectra, as expected (Figure 2).

Resistance to the Action of Phosphodiesterases. L-dA_{12} was resistant to both bovine spleen and snake venom phosphodiesterases, as expected; essentially no decomposition of L-dA_{12} was observed during incubation of the compound with the enzymes, though the corresponding natural form of the dodecamer (D-dA_{12}) was completely hydrolyzed under the same conditions (Figure 3a). Other enantio-dodecadeoxyribonucleotides (L-dT_{12}, L-dC_{12}, and L-dG_{12}) were also resistant to these phosphodiesterases (data not shown). Addition of L-oligodeoxynucleotides to the incubation mixtures of natural DNA with phosphodiesterases did not affect the rate or the products of enzymatic hydrolysis of the DNA. The results suggest that enantio-DNA can not be an efficient substrate for the phosphodiesterases and it does not inhibit the hydrolytic function of the enzymes.

Meso-dodecadeoxyribonucleotides were also resistant to both bovine spleen and snake venom phosphodiesterases, though they were slowly decomposed. For example, LD-dA_{12} was completely resistant to bovine spleen phosphodiesterase, but it was slowly decomposed by snake venom phosphodiesterase (Figure 3b). HPLC analysis of LD-dA_{12} incubated with snake venom phosphodiesterase for 24 h showed partial hydrolysis to 4–10-mers of meso-deoxyadenylic acid (data not shown).

Triplex Formation with Natural Nucleic Acids. To investigate the interaction of enantio- and meso-DNAs with natural homopolynucleotides, we initially measured UV-mixing curves by

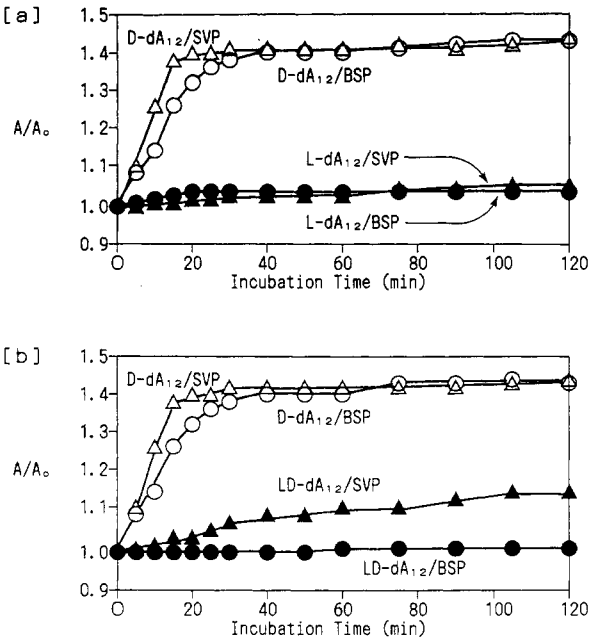

Figure 3. Resistance of enantio- and meso-DNAs to phosphodiesterases (time courses of hydrolysis): (a) L-dA_{12} and D-dA_{12} incubated with bovine spleen and snake venom phosphodiesterases and (b) LD-dA_{12} and D-dA_{12} incubated with bovine spleen and snake venom phosphodiesterases; SVP = snake venom phosphodiesterase and BSP = bovine spleen phosphodiesterase.

the method of continuous variations (Figure 4).[22] The UV-mixing curves for interaction of L-dA_{12} with poly(U) at 0 and 20 °C show maximum hypochromicity (ca. 25%) at approximately a 1:2 molar ratio (35% L-dA_{12}) of L-dA_{12} to poly(U), which indicates U·A·U triple helix formation.[23,24] The profile of the UV-mixing curve, as well as the value of the maximum hypochromicity, was very close to that for the interaction of the natural dodecamer (D-dA_{12}) with poly(U). In addition, the shape of the CD spectrum of the triplex formed from L-dA_{12} with poly(U) (molar nucleotide-units ratio of 1:2, Figure 5) is quite similar to that of the natural right-handed U·A·U triplex. Therefore, the L-dA_{12}/poly(U) triplex is expected to take the right-handed conformation which is macroscopically similar to that of the natural right-handed triplex, and we tentatively concluded that the interaction mode of L-dA_{12} with poly(U) is right-handed triplex formation. There is no doubt that enantio-DNA tends to form a left-handed helix (a mirror image of a natural DNA helix, Figure 2 for the L-dA_{12}/L-dT_{12} left-handed duplex). Therefore, the right-handed triplex formation of the L-dA_{12}/poly(U) complex suggests that the tendency of L-dA_{12} (enantio-DNA with purine bases) to form a left-handed helix is overwhelmed by the tendency of the two poly(U) chains (natural RNA with pyrimidine bases) to form a right-handed helix. It would be interesting to examine whether or not the tendency of enantio-DNA with pyrimidine bases to form a left-handed helix is overwhelmed by the tendency of natural RNA with purine bases to form a right-handed helix.

L-dA_{12} did not show any hypochromicity when mixed with poly(G), poly(A), or poly(C), as was also the case with D-dA_{12}, indicating that the triple helix formation is complementary base-specific.

UV-mixing curve analyses for the interaction of L-dC_{12} or L-dG_{12} with poly(dG) or poly(dC) did not give easily interpretable results, perhaps because of the tendency of guanylic acid oligomers to form higher-order structures by themselves. However, the

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(24) Addition of a small amount of L-dA_{12} (<10%) to poly(U) generated weak hyperchromicity owing to destruction of the self-stacking conformation of poly(U).

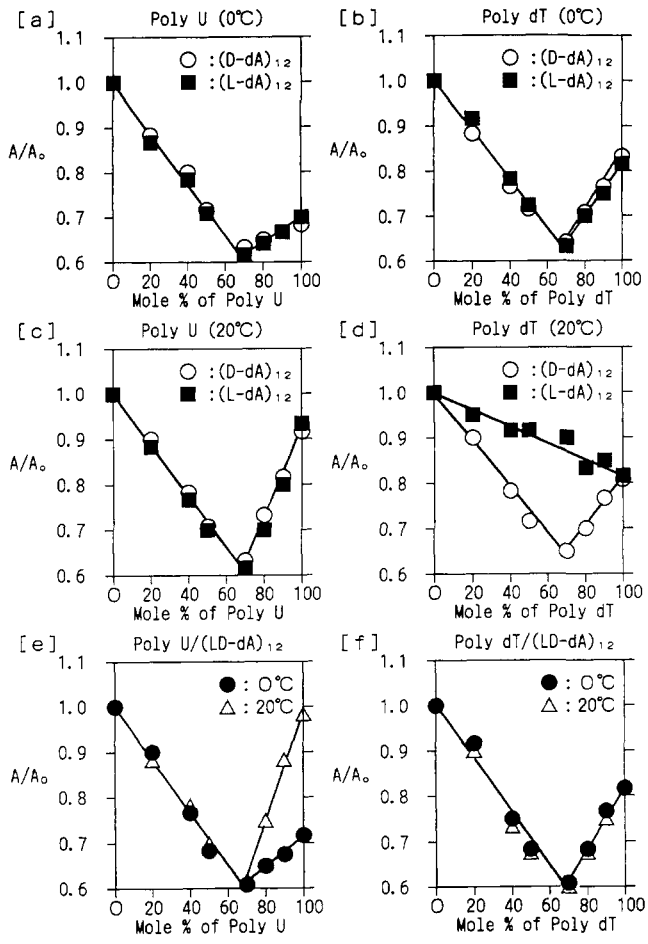

Figure 4. UV-mixing curves: (a) L-dA_{12} and D-dA_{12} mixed with poly(U) at 0 °C, (b) L-dA_{12} and D-dA_{12} mixed with poly(dT) at 0 °C, (c) L-dA_{12} and D-dA_{12} mixed with poly(U) at 20 °C, (d) L-dA_{12} and D-dA_{12} mixed with poly(dT) at 20 °C, (e) LD-dA_{12} mixed with poly(U) at 0 and 20 °C, and (f) LD-dA_{12} mixed with poly(dT) at 0 and 20 °C.

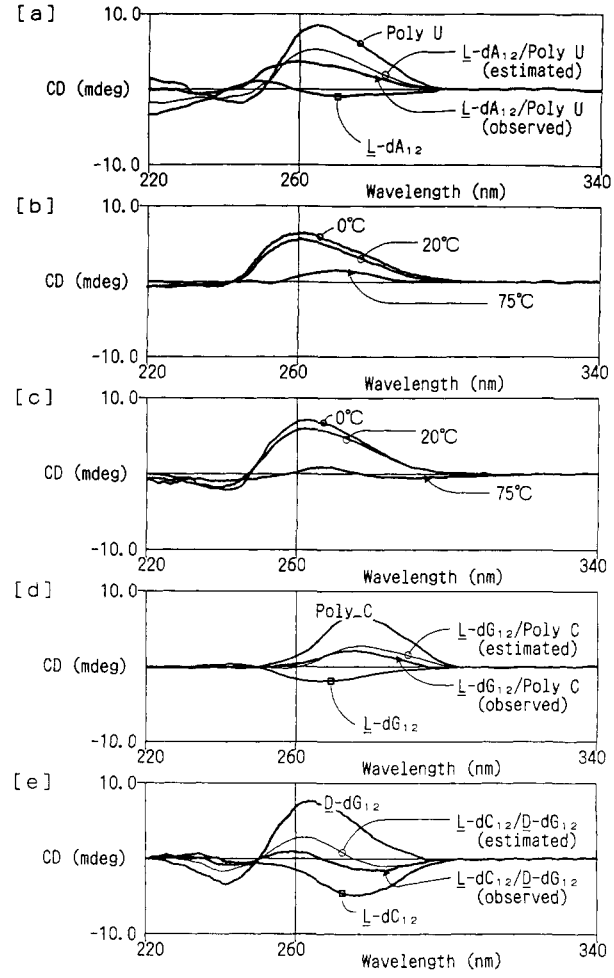

Figure 5. CD spectra of mixtures of enantio- or meso-DNAs and the natural homopolymer: (a) CD spectra of the L-dA_{12}/poly(U) complex, L-dA_{12}, and poly(U), (b) temperature dependency of the CD spectrum of the L-dA_{12}/poly(U) complex, (c) temperature dependency of the CD spectrum of the LD-dA_{12}/poly(U) complex, (d) the L-dG_{12}/poly(C) complex, and (e) the L-dC_{12}/D-dG_{12} complex.

CD spectra of L-dC_{12}/poly(G) and L-dG_{12}/poly(C) mixtures showed a temperature-dependent blue shift and a hypochromicity which are similar to those observed for the L-dA_{12}/poly(U) mixture (Figure 5). The results suggest that both enantio-oligocytidylic and enantio-oligoguanylic acids interact with their complementary natural nucleic acids, though the mode of interaction is not yet clear.

Natural RNA-Selectivity and Base-Dependency. L-dA_{12} also showed hypochromicity when mixed with complementary poly(dT) at 0 °C. However, it is particularly noteworthy that L-dA_{12} did not show any hypochromicity when mixed with poly(dT) at 20 °C, though natural D-dA_{12} did show apparent hypochromicity under the same conditions. The results strongly suggest that at 20 °C, L-dA_{12} specifically recognizes RNA (but not DNA) as having complementary base sequences but at 0 °C, L-dA_{12} interacts with both complementary RNA and DNA.

On the basis of our results, Tomioka and Itai recently performed *ab initio* molecular modeling calculations on the conformation of the L-dA_{12}/poly(U) triplex.[4] They have found that Hoogsteen's U·A·U base-pairing with the second poly(U) chain being antiparallel to the first poly(U) chain is the most stable conformation. According to their molecular modeling study, the macroscopic conformation of the triplex containing enantio-poly(dA) is quite similar to that of the natural U·A·U triplex, with some difference between the conformation of the L-dA chain and that of the D-dA chain. They also suggested the existence of an intramolecular interaction which stabilizes the enantio-poly(dA)/poly(U) triplex, i.e., hydrogen-bonding and/or electrostatic interactions between the 2′-hydroxy group of poly(U) and the oxygen atoms at position 1′ and the 5′-phosphate group of the adjacent nucleotide unit. This stabilization might explain the high RNA-selectivity exhibited by L-dA_{12}.

LD-dA_{12} showed behavior similar to that of natural D-dA_{12} as far as analyzed by the UV-mixing curve method for interaction with poly(U) and poly(dT) at both 0 and 20 °C (Figure 4). LD-dA_{12} could be interpreted as showing intermediate characteristics between L-dA_{12} and D-dA_{12}, concerning the interaction with natural nucleic acids.

It should be noted that the characteristics of interaction of L-dA_{12} with its complementary natural nucleic acids were different from those of L-dT_{12} with its natural nucleic acids. Though L-dA_{12} interacts with poly(U) (triplex formation), its counterpart L-dT_{12} did not show any detectable interaction with poly(A) under the same incubation conditions. This difference between L-dA_{12} and L-dT_{12} might be interpreted in terms of the triplex-forming nature of an enantio-DNA/natural RNA mixture, i.e., the duplex formed between enantio-DNA and natural RNA would presumably be too unstable to allow its detection. This would lead to observation of only the enantio-DNA/natural RNA triplex. Triplex structure is known to be formed by Hoogsteen base-pairing. In the case of the triplex formed from enantio-poly(dA) and poly(U), Hoogsteen base-pairing was found to be the most stable mode of pairing by *ab initio* molecular modeling calculations.[4] A triplex structure involving the A·T·A-type composition has never been reported. Therefore, we suggest that though L-dA_{12} is able to form a triplex with macromolecular poly(U), L-dT_{12} is too short

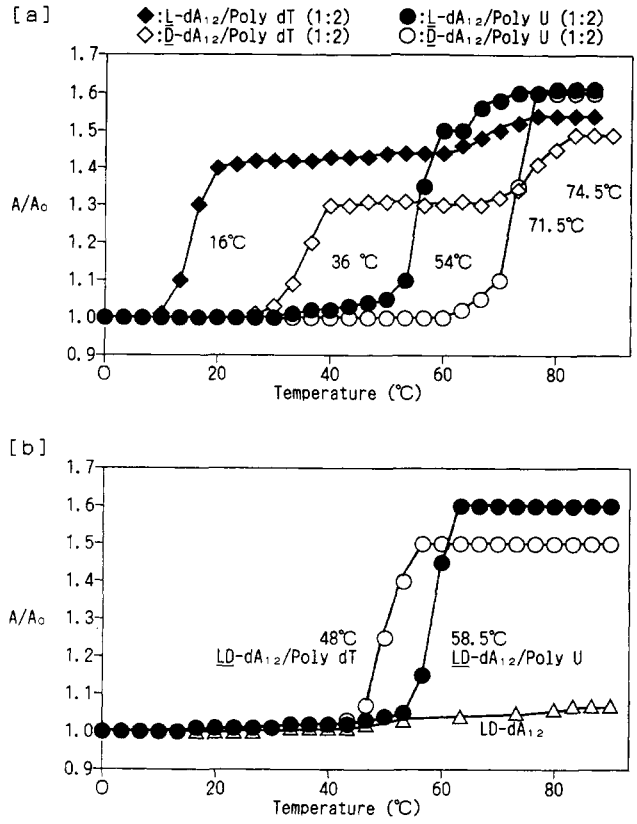

Figure 6. Melting-temperature profiles: (a) D-dA_{12}/poly(U), D-dA_{12}/poly(dT), L-dA_{12}/poly(U), and L-dA_{12}/poly(dT) and (b) LD-A_{12}/poly(U) and LD-A_{12}/poly(dT).

to form a stable triplex with poly(A). In fact, the UV-mixing curve for interaction of the much longer L-dT_{99} and poly(A) showed hypochromicity with a maximum (ca. 20%) at approximately a 2:1 molar ratio of L-dT_{99} and poly(A) (melting temperature of 32 °C), suggesting T·A·T-type triplex formation. Hypochromicity of ca. 20% was also observed on mixing L-dT_{99} and natural dodecariboadenylic acid (D-A_{12}) in the ratio of 2:1 (melting temperature of 21 °C), while no interaction was observed between L-dT_{99} and the deoxyoligomer D-dA_{12}. This result suggests that L-dT_{99} also prefers its complementary RNA-type oligomer over the DNA-type oligomer.[25]

Stability of the Enantio-DNA/Natural Nucleic Acid Complex. The melting temperature (T_m) profiles for interaction of L-dA_{12} and LD-dA_{12} with homopolynucleotides were studied (Figure 6).[23] The T_m profiles of L-dA_{12} also demonstrated its selectivity for poly(U) over poly(dT). The T_m profile for the L-dA_{12}/poly(U) triplex indicated that the triplex is stable below 54 °C. The L-dA_{12}/poly(dT) triplex showed a biphasic T_m profile with the T_m values of 16 and 67.5 °C. Because L-dA_{12} was established to show no hypochromicity in the interaction with poly(dT) at 20 °C (Figure 4), the significant T_m value would be 16 °C, which

(25) When L-dT_{99} was mixed with a longer complementary homodeoxypolymer, poly(dA), hypochromicity with a maximum (ca. 20%) at approximately a 2:1 molar ratio of L-dT_{99} and poly(dA) was observed. Under the experimental conditions, the UV-mixing curve for interaction of D-dT_{12} and natural oligoadenylic acid analogs (D-dA_{12}, D-A_{12}, poly(dA), and poly(A)) showed hypochromicity with a maximum (20–25%) at approximately a 2:1 molar ratio of D-dT_{12} and oligoadenylic acid analogs.

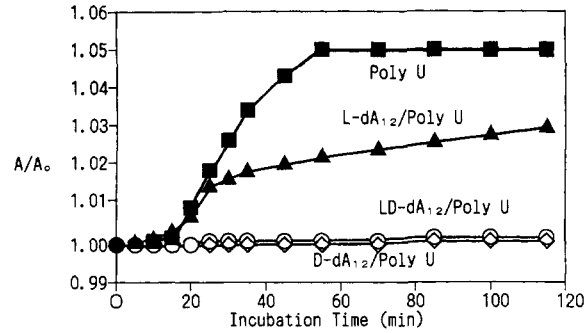

Figure 7. Nuclease S1 assay (time course of hydrolysis).

is much lower than the T_m value(s) for the L-dA_{12}/poly(U) triplex. The T_m curves for the interaction of LD-dA_{12} with homopolymers showed that LD-dA_{12} interacts with both poly(U) and poly(dT), with the single T_m values being respectively 58.5 and 48 °C under the experimental conditions used. Judging from the T_m values observed, LD-dA_{12} seems to interact with poly(U) more strongly than with poly(dT). The T_m curve of the D-dA_{12}/poly(U) triplex showed a single T_m of 71.5 °C, while that of the D-dA_{12}/poly(dT) triplex showed two values (74.5 and 36 °C). These results indicate that (i) L-dA_{12} possesses extremely high selectivity for its complementary RNA, poly(U), over the corresponding DNA, poly(dT), (ii) natural D-dA_{12} did not show remarkable selectivity, and (iii) LD-dA_{12} possesses intermediate selectivity between L-dA_{12} and D-dA_{12}. Such RNA-selectivity of these nonnatural DNAs could make them useful as biochemical research tools.

Our T_m measurements indicate that the affinity for poly(U) of dodecadeoxyadenylic acids decreased in the order of D-dA_{12} > LD-dA_{12} > L-dA_{12}. This order was also reflected in the Nuclease S1 assay (Figure 7). Addition of dodecadeoxyadenylic acids to the poly(U) solution protected poly(U) from enzymatic hydrolysis with Nuclease S1, and the protecting efficacy decreased in the order of D-dA_{12} = LD-dA_{12} > L-dA_{12}.

The determined melting temperatures of the L-dA_{12}/poly(U) triplex (T_m values were 54 and 68.5 °C) were consistent with the temperature-dependent change of its CD spectrum; a blue-shift of the CD spectrum was observed in the temperature range of 0–4 °C, but no shift was observed at 75 °C (Figure 5).

Conclusion

We have designed and prepared enantio- and meso-DNAs. These unnatural DNAs were obtained by using an automated DNA synthesizer, which is highly advantageous for efficient synthesis. The prepared enantio- and meso-DNAs showed high resistance to phosphodiesterases. Such resistance means that these unnatural DNAs could be valuable as lead compounds for developing antisense oligonucleotides. In this paper, enantio- and meso-homooligodeoxyribonucleotides have been shown to recognize their complementary natural nucleic acids with selectivity for RNA over DNA. Though homooligomers are expected to possess special properties which may not be found in DNAs with random nucleobase sequences, clarification of the nature of enantio-/meso-homooligomer interactions would be helpful in improving our understanding of the molecular recognition chemistry of L-sugar-containing nucleic acids. Detailed structural investigations using enantio- and meso-DNAs with various base sequences are continuing.