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Helical structure of heterochiral RNA dimers: helical sense of ApA is determined by chirality of 3′-end residue

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Heterochiral ApAs (ALpAL and ALpAL) have been synthesized and investigation of their helical structures by means of spectroscopic techniques indicates that the chirality of the 3′-end residue is the primary factor for determining the helical sense of ApA.

Nucleic acids are homochiral polymers that exclusively use D-(deoxy)ribose as the sugar unit. Natural DNA primarily forms a right-handed double helix, the so called B-form DNA, and the helical structure of L-DNA is its mirror image.[1] The structures of natural RNA and L-RNA are also mirror images of each other.[2] Thus, the chirality of homochiral nucleic acids is the primary factor for determining their helical sense, although the glycosyl conformation of each nucleotide residue is also an important factor.[3] Chiral homogeneity is thought to be essential for the formation of higher order structures of biopolymers and their interaction with specific ligands. In fact, the optical isomers of some biomolecules have been shown to have reciprocal specificity for their specific chiral ligands.[4] However, there is little information about the structure and properties of heterochiral nucleic acids,[5] especially heterochiral RNAs. Ts′o and coworkers reported the properties of unnatural homochiral L-(ApA), which adopts a left-handed helix alone and forms a right-handed triplex with D-poly(U), which is less stable than natural D-(ApA).[6] The formation of the less stable right-handed triplex of L-(ApA) with poly(U) should be due to the nature of L-(ApA) to form the left-handed helix. In this case, the L-chirality of the ribose residues contributes to the formation of the left-handed helix. However, it remains unknown whether there is a structural element primarily responsible for the helical sense of ApA or not and, if yes, which ribose this would be. This is an important problem in elucidating the basis of right-handedness of nucleic acids and designing novel helix-forming molecules. Here, we report the structure, in particular the helical sense, of heterochiral ApA dinucleoside monophosphates.

The synthesis of L-ribose will be reported elsewhere. L-Adenosine was synthesized from L-ribose according to the conventional methods for synthesizing the corresponding D-isomer.[7] The four homochiral and heterochiral ApAs were synthesized by the phosphotriester method.[8] The chemical structures of the dimers were characterized by enzymatic digestion experiments with nuclease P1, RNase T_2 and snake venom phosphodiesterase (SVPD). The dimer D-(ApA) was digested by all three enzymes; however, the dimer L-(ApA) was as expected almost completely resistant to digestion by any of the enzymes under the same conditions, although slight degradation was observed in the reaction with SVPD. The heterochiral dimer ADpAL was digested by nuclease P1 and RNase T_2, while the dimer ALpAD was digested only by SVPD.† These results support the chemical structures of the dimers.

To obtain information on the helical structure of the dimers, CD experiments were conducted. The results are shown in Fig. 1. The natural dimer D-(ApA) shows a typical conservative CD spectrum by exciton interaction, with positive and negative bands at 270 and 252 nm, respectively. On the other hand, the spectrum of L-(ApA) shows the opposite sign but the same intensity as that of D-(ApA). The positive sign of the long-wavelength component of the conservative bands indicates a right-handed helical twist of the bases (transition moment of the bases)[9] when the bases are nearly perpendicular to the helix axis. These results suggest that D-(ApA) has a right-handed helical sense and L-(ApA) is its mirror image, as reported.[6] In the case of the heterochiral dimer, the two heterochiral dimers ADpAL and ALpAD also exhibit spectra that are symmetrical to each other. Although ALpAD and ADpAL show somewhat different spectra from D- and L-(ApA) respectively, the spectra are still considered to be conservative. Thus, it is very likely that ALpAD has a right-handed helical sense, while ADpAL has a left-handed helical sense. This means that the helical sense of the dimers is primarily determined by the chirality of the 3′-end residue, considering the chemical structures of ALpAD and ADpAL.

To further confirm the helical sense of the dimers, hybridization experiments of the dimers with D-poly(U) were conducted. The homochiral dimers D-(ApA) and L-(ApA) were shown to interact with D-poly(U) to form a complex with

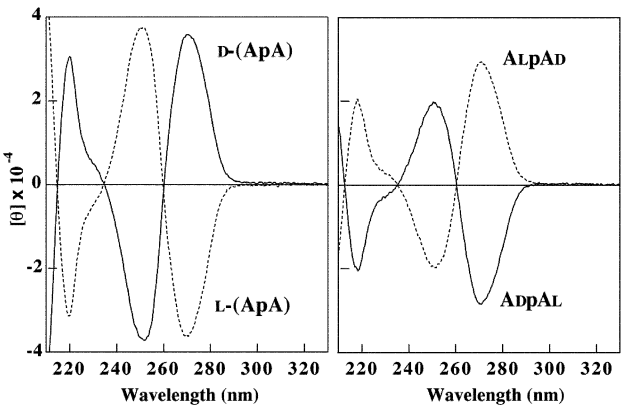

Fig. 1 CD spectra of ApAs at 0 °C. (a) Solid and broken lines represent CD spectra of homochiral D- and L-ApAs, respectively. (b) Solid and broken lines represent CD spectra of heterochiral ADpAL and ALpAD, respectiveely. Samples contained 40μM ApA in 0.1 M NaCl, 10 mM sodium phosphate, pH 1.0.

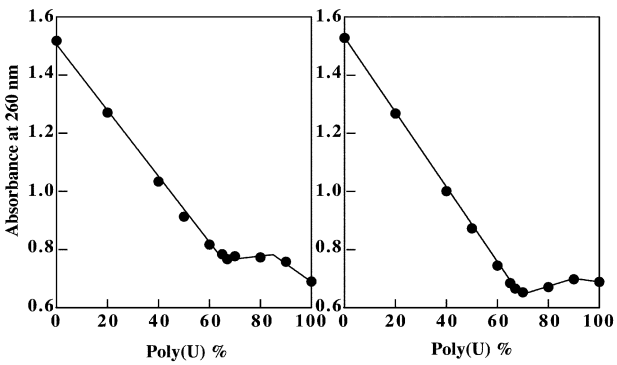

Fig. 2 UV mixing curves of ADpAL (left) and ALpAD (right) with D-poly(U) in 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5 at −5 °C. Total nucleotide concentration is 120 μM.

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1A:2U stoichiometry.[6] First, UV mixing experiments were performed to determine the stoichiometry of the interaction between the heterochiral dimers and D-poly(U). It was clearly shown that both dimers form the 1:2 complex with D-poly(U) (Fig. 2). Therefore, in the same manner as the homochiral dimers, the heterochiral dimers also form the triplex structure. The UV melting profiles of the complexes are shown in Fig. 3. The homochiral D- and L-dimers form relatively stable and unstable triplexes with D-poly(U), respectively, as reported by Ts'o and coworkers.[6] Although the triplex of ALpAD with D-poly(U) shows the stability similar to that of D-(ApA), the triplex of ADpAL with D-poly(U) is significantly destabilized. Furthermore, all the complexes retain the right-handed triplex as revealed by their CD spectra (Fig. 4). These results strongly confirm the conclusion that ADpAL and ALpAD themselves

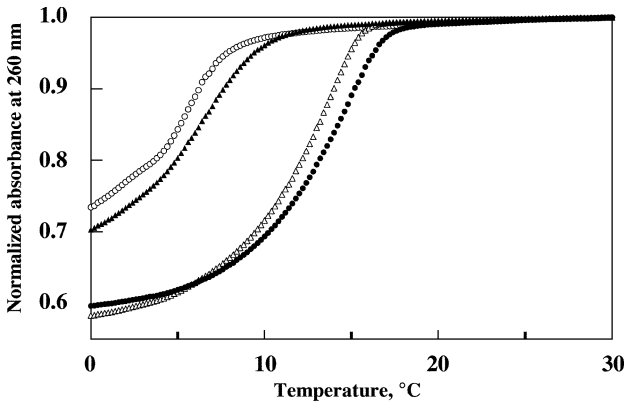

Fig. 3 UV melting profiles of the triplexes of D-(ApA) (closed circles), L-(ApA) (open circles), ADpAL (closed triangles) and ALpAD (open triangles) with D-poly(U). Total nucleotide concentration is 120 μM in 10 mM $MgCl_2$ 10 mM Tris-HCl, pH 7.5. The T_m values in the text were determined from the first-derivative plots of the profiles.

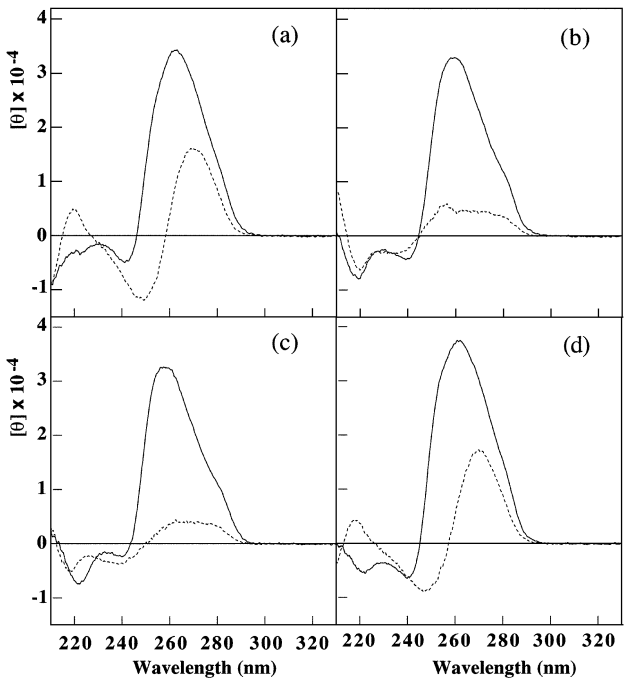

Fig. 4 CD spectra of the triplexes of D-(ApA) (a), L-(ApA) (b), ADpAL (c) and ALpAD (d) with D-poly(U). Samples contained 80 μM D-poly(U) and 40 μM ApA in 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5. Solid and broken lines represent spectra at −5 and 30 °C, respectively.

have the nature to form the left- and right-handed helical structures, respectively.

It should be noted that the T_m value (13.7 °C) of the ALpAD·2poly(U) complex is comparable to that (14.7 °C) of the D-(ApA)·2poly(U) complex, and these values are significantly higher than those of the L-(ApA)·2poly(U) (5.7 °C) and ADpAL·2poly(U) (6.6 °C) complexes. In addition, the CD spectrum of ALpAD·2poly(U) at −5 °C is very similar to that of D-(ApA)·2poly(U) (Fig. 4a,d). These results mean that the propensity of ALpAD to form the right-handed helical structure is similar to that of D-(ApA). On the other hand, L-(ApA)·2poly(U) and ADpAD·2poly(U) have a comparable thermal stability, and both triplexes show quite similar CD spectra (Fig. 4b,c). Therefore, dimers L-(ApA) and ADpAL have the similar propensity of resisting the formation of the right-handed helical structure.

In conclusion, our results clearly indicate that the chirality of the 3′-end residue is the primary factor for determining the helical sense of the dimers. This conclusion would be important for considering the basis of the right-handedness of nucleic acids and designing novel helix-forming molecules. The investigations of effects of L-sugars on the helical sense of longer oligomers and 5′-phosphorylated dimers as well as the structural basis for ALpAD to form the right-handed helical structure by using ^{1}H NMR techniques are under way.

Notes and references

† Molar extinction coefficients of D-(ApA), ADpAL and ALpAD were determined by measuring hyperchromicity after nuclease P1 or SVPD treatment as ε_{260} = 25,500, 26,200 and 26,400, respectively. That for L-(ApA) was assumed to be the same as that of D-(ApA)

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