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Thermodynamic study of hybridization properties of heterochiral nucleic acids

Hidehito Urata,[a,*] Hana Shimizu,[a] Hidekazu Hiroaki,[b] Daisuke Kohda,[c] and Masao Akagi[a,*]

[a] *Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan*
[b] *Science of Biological Supramolecular Systems, Graduate School of Integrated Science, Yokohama City University, 1-7-29 Suehiro-chou, Tsurumi-ku, Yokohama 230-0045, Japan*
[c] *Division of Structural Biology, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

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Abstract

Heterochiral DNA and RNA heptamers, which contained an unnatural L-nucleotide, were synthesized, and thermodynamic analyses of their hybridization properties with complementary DNA and RNA strands were systematically conducted by UV melting experiments. The results clearly demonstrated that the incorporation of an L-ribonucleotide into the RNA strand leads to more significant destabilization of the duplexes than that of an L-deoxyribonucleotide into the DNA strand, regardless of whether the complementary strand is DNA or RNA. The destabilization of the duplexes by the substitution of D-thymidine with L-thymidine in the DNA strand is entropically driven, whereas that by the substitution of D-uridine with L-uridine in the RNA strand is enthalpically driven. The thermodynamic characteristic that the stability of homochiral duplex is far superior to that of heterochiral duplex is much more remarkable in RNA than in DNA. Thus, RNA might have been a self-replicating system superior to DNA to exclude the chiral antipode.

Keywords: Chirality; DNA; Duplex stability; Heterochiral; RNA; Thermodynamic

The chirality of molecules plays an important role in structure formation, specific ligand recognition, and catalysis [1]. Usually, optical isomers are mutually exclusive in chiral environments. Living bodies are chiral machineries consisting of D-nucleic acids and L-amino acids. This means that chiral selection and amplification would have been achieved during prebiotic chemical evolution of biomolecules [2–4], since optical isomers of mononucleotides and amino acids are equally produced by non-asymmetric syntheses. The incorporation of some L-deoxynucleotides into D-DNA sequences somewhat decreases the duplex stability with complementary DNA and RNA sequences [5–12]. Although the properties of homochiral L-RNAs have been reported by several groups [13–17], the effects of incorporation of L-ribonucleotides into D-RNA strands on duplex structure and stability have not yet been reported other than heterochiral ApAs [18]. Considering the processes of the chiral selection and amplification in the chemical evolution of nucleic acids, it is important to systematically examine the physicochemical properties of heterochiral nucleic acids because non-enzymatic oligomerization of racemic mononucleotides on an RNA template [19–22] or a clay mineral yields both homo- and heterochiral oligomers [23,24]. Furthermore, L-thymidine has been shown to have an inhibitory effect against HIV-1 reverse transcriptase by being incorporated into DNA strands by the enzyme after phosphorylation into L-dTTP [25,26], although conflicting results have been reported for L-dNTPs [27]. As a result, DNA–DNA or DNA–RNA duplexes containing L-nucleotides in the DNA strand are formed. The uptake of antiviral L-dNTPs into the DNA strand may be controlled by the physicochemical properties of the resulting heterochiral nucleic acids.

* Corresponding authors. Fax: +81-72-690-1005.
E-mail addresses: urata@gly.oups.ac.jp (H. Urata), akagi@gly.oups.ac.jp (M. Akagi).

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Here, we report the effects of incorporation of an L-nucleotide into DNA and RNA heptamers on the stability of the duplexes with complementary DNA and RNA sequences.

Materials and methods

Synthesis of oligonucleotides. L-Thymidine and its 5′-*O*-dimethoxytrityl-3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite derivative were synthesized according to the previously reported procedure [28,29]. L-Uridine was synthesized from L-ribose [30] using the method for the corresponding D-isomer [31,32], and L-uridine was converted to its 5′-*O*-dimethoxytrityl-2′-*O*-*tert*-butyldimethylsilyl-3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite derivative by the literature procedure [33]. Reagents for the DNA synthesizer other than L-thymidine and L-uridine phosphoramidites were purchased from Applied Biosystems Japan (Tokyo, Japan) and Glen Research (Virginia, USA). Ribo- and deoxyriboheptanucleotides were synthesized on an Applied Biosystems model 392 automated DNA–RNA synthesizer.

Measurement of melting curves. The concentrations of oligonucleotide solutions were calculated by using equation and coefficients described by Bore [34]. The coefficients of the heterochiral dimer units were assumed to be the same as the corresponding homochiral dimer units. Each pair of 7-mers was mixed and dissolved in a buffer containing 1 M NaCl and 10 mM sodium phosphate (pH 7.5) at duplex concentrations of 6, 30, 60, and 120 μM. The solutions (0.4–4 ml) containing duplexes were heated at 80 °C and cooled gradually to room temperature. Melting curves were measured at least twice at 260 nm on a JASCO Ubest-55 spectrophotometer. The temperature was raised at a rate of 0.5 °C min^{-1} and the T_m values were obtained by the first-derivative plots of the melting curves.

Determination of thermodynamic parameters. The reciprocal of the melting temperature ($1/T_m$) was plotted against $\ln(C_T/4)$, where C_T is the total oligonucleotide concentration, as shown in Fig. 3. Since the relationship between $\Delta H°$ and $\Delta S°$ is expressed by the equation, $1/T_m = (R/\Delta H°)\ln(C_T/4) + \Delta S°/\Delta H°$, in which R is the gas constant ($= 1.987$ cal mol^{-1} deg^{-1}), for non-self-complementary duplexes, thermodynamic parameters were calculated as follows: $\Delta H° = R/(\text{slope})$ and $\Delta S° = \Delta H°$ (y-intercept). The free energy ($\Delta G°$) at temperature T (kelvin) was calculated using the equation $\Delta G° = \Delta H° - T\Delta S°$.

Results and discussion

The sequences of the synthesized oligonucleotides are shown in Fig. 1. The oligonucleotides were mixed by all combinations of template strands and complementary strands, and their duplex stabilities were investigated. Typical melting profiles of the above homo- and heterochiral duplexes are shown in Fig. 2. All 7-mer pairs show cooperative transition, thus forming a duplex structure. The decreases of the T_m value by substituting

complementary strand	template strand
d7: d(C-G-T-A-C-G-C)	dTD: d(G-C-G-T-A-C-G)
r7: r(C-G-U-A-C-G-C)	dTL: d(G-C-G-**T**-A-C-G)
	rUD: r(G-C-G-U-A-C-G)
	rUL: r(G-C-G-**U**-A-C-G)

Fig. 1. Sequences of heptanucleotides. Bold letters represent L-nucleotide residues.

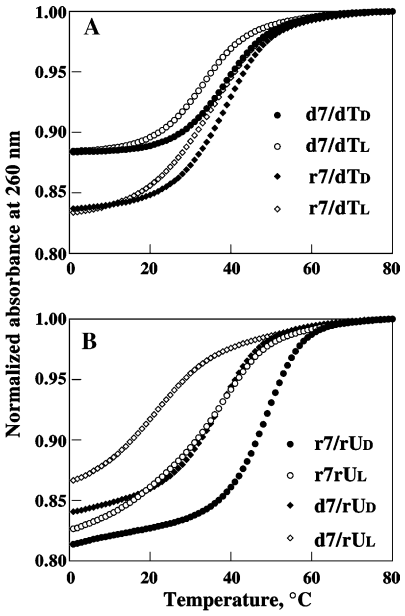

Fig. 2. Melting profiles of homochiral (closed symbols) and heterochiral (open symbols) duplexes (6 μM). (A) Duplexes contain D- or L-thymidine in the template DNA strand. (B) Duplexes contain D- or L-uridine in the template RNA strand.

D-thymidine with L-thymidine in the DNA strand are 5.7 and 3.3 °C with the DNA and RNA complements, respectively. In contrast, substitution of D-uridine with L-uridine in the RNA strand led to much greater decreases of the T_m value of 10.8 and 16.8 °C with the RNA and DNA complements, respectively. The results suggest that the destabilization of the duplexes by the substitution in the RNA strand is much greater than that in the DNA strand, regardless of the type of complementary strand.

To support the above findings, the thermodynamic parameters for duplex formation were determined by van't Hoff plots (Fig. 3), assuming a two-state model

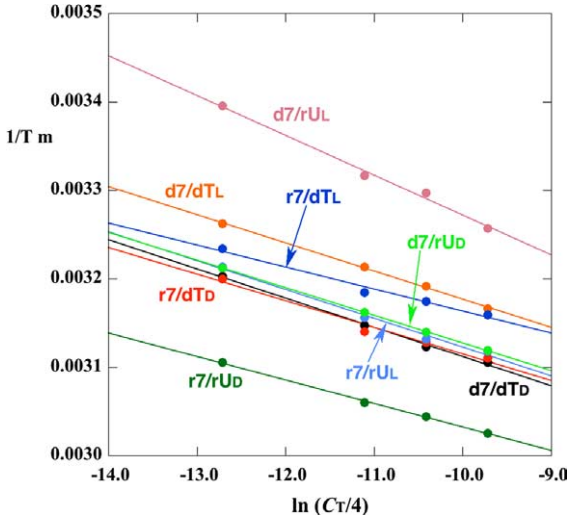

Fig. 3. Reciprocal T_m versus $\ln(C_T/4)$ plots for the duplexes.

[35,36]. Table 1 shows that ΔG° values for all duplexes at 25 °C are negative, indicating that their duplex formation is favorable at this temperature. The heterochiral duplexes are destabilized by +0.19 to +3.23 kcal mol^{-1} compared with the corresponding homochiral duplexes. Leonard et al. [37] reported that the A–C mismatch causes the free energy loss for duplex formation of +5.5 kcal mol^{-1} per mismatch compared with the A–T matched duplex. Because the free energy loss ($\Delta\Delta G^\circ$ values; +0.19 to +3.23 kcal mol^{-1}) by substituting D-nucleotide with L-nucleotide is much smaller than that (+5.5 kcal mol^{-1}) caused by the A–C mismatch, the present results suggest that the L-nucleotide residue of all the heterochiral duplexes retains the base-pairing with the complementary residue even in d7/rUL, which is the most destabilized duplex. The differences ($\Delta\Delta G^\circ$) in free energy changes for duplex formation by substituting D-nucleotide with L-nucleotide are much larger for the RNA strand (+2.96 to +3.23 kcal mol^{-1}) than for the DNA strand (+0.19 to +1.04 kcal mol^{-1}). This means that the substitution of D-nucleotide with L-nucleotide in the RNA strand more intrinsically decreases duplex stability than that in the DNA strand. Notably, the heterochiral DNA strand (dTL) is enthalpically more favorable for duplex formation compared with the parental D-homochiral DNA strand (runs 1/2 and 3/4) and the destabilization of the duplexes is entropically driven, independent of the type of complementary strand. In contrast, the heterochiral RNA strand (rUL) is entropically more favorable for duplex formation compared with the parental D-homochiral RNA strand (runs 5/6 and 7/8), and the destabilization of the duplexes is enthalpically driven. These thermodynamic features suggest that the heterochiral RNA strand is significantly distorted, although the heterochiral DNA strand is not quite distorted. The duplexes containing r7 as the complementary strand are more stabilized than the corresponding duplexes containing d7. This effect of the heterochiral duplexes is much more marked than that of the corresponding homochiral duplexes. Thus, homochiral RNA may have greater ability to accommodate the helical distortion of the heterochiral strands to form duplexes than homochiral DNA.

Since we evaluated the thermodynamic stability of only a single heptameric sequence, the effects of bases of the incorporated L-nucleotide and those flanking the L-nucleotide residue are not clarified. We have already reported the sequence dependence of the thermodynamic properties of heterochiral DNA duplexes, in which the differences ($\Delta\Delta G^\circ$) in free energy changes for duplex formation by substituting D-nucleotide with L-nucleotide vary in +1.1 to +2.0 kcal mol^{-1} per substitution [10]. However, the $\Delta\Delta G^\circ$ values (+2.96 to +3.23 kcal mol^{-1} per substitution) of heterochiral RNA described here are much larger than those of heterochiral DNA.

RNA duplexes are much more extensively hydrated than DNA duplexes and the 2′-hydroxy groups serve as a scaffold for hydrogen-bonding networks via water molecules in the minor groove [38]. Thus, the 2′-hydroxy groups play an important role in the enthalpic stabilization of RNAs. We found that the L-sugar moiety in heterochiral DNA has the flipped conformation, leading to the change of the relative position of each sugar atom [6]. Although the molecular structure of heterochiral RNA remains unknown, the present data suggest that the substitution of D-uridine with L-uridine in the RNA strand would cause the destruction of such hydrogen-bonding networks. However, the substitution of D-thymidine with L-thymidine in the DNA strand would not significantly affect the hydrogen-bonding networks because DNA does not have any free hydroxy groups on the sugar moieties. The energy of hydrogen bonds is 3 to 6 kcal mol^{-1} per bond; therefore, the $\Delta\Delta H^\circ$ values obtained by substituting of D-uridine with L-uridine in the RNA strand correspond to the loss of 3–4 hydrogen bonds (runs 5/6 and 7/8). The entropy–enthalpy compensation plot for the duplex formation shows a good linearity (Fig. 4) and the slope is 1.1, indicating that

Table 1
Thermodynamic parameters for duplex formation of homo- and heterochiral 7-mers at 25 °C[a]

Run	Duplex	T_m (°C)[b]	ΔH° (kcal mol^{-1})	$\Delta\Delta H^{\circ c}$ (kcal mol^{-1})	$T\Delta S^\circ$ (kcal mol^{-1})	$\Delta(T\Delta S^\circ)^c$ (kcal mol^{-1})	ΔG° (kcal mol^{-1})	$\Delta\Delta G^{\circ c}$ (kcal mol^{-1})
1	d7/dTD	39.2	−60.10	—	−49.82	—	−10.28	—
2	d7/dTL	33.5	−62.53	−2.43	−53.29	−3.47	−9.25	+1.04
3	r7/dTD	39.5	−66.21	—	−55.54	—	−10.67	—
4	r7/dTL	36.2	−79.68	−13.48	−69.20	−13.66	−10.48	+0.19
5	r7/rUD	49.0	−74.69	—	−61.57	—	−13.11	—
6	r7/rUL	38.2	−60.92	+13.76	−50.77	+10.80	−10.15	+2.96
7	d7/rUD	38.3	−63.63	—	−53.38	—	−10.25	—
8	d7/rUL	21.5	−44.02	+19.61	−37.00	+16.38	−7.02	+3.23

[a] Melting curves were obtained by measuring the absorbance change at 260 nm as a function of temperature, varying duplex concentration (6–120 μM) in 1 M NaCl, 10 mM sodium phosphate, pH 7.5. Temperature was raised at a rate of 0.5 °C min^{-1}. T_m values were determined from the first-derivative plots of the melting curves.

[b] Duplex concentration is 6 μM.

[c] $\Delta\Delta H^\circ$, $\Delta(T\Delta S^\circ)$, and $\Delta\Delta G^\circ$ represent the difference of each thermodynamic parameter from that of the corresponding homochiral duplex.

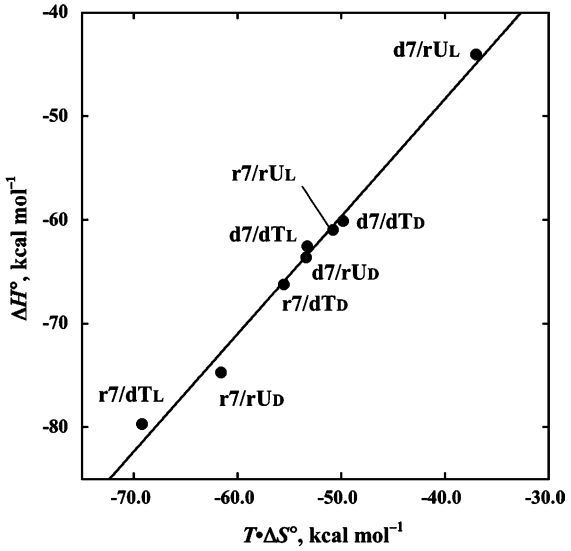

Fig. 4. Entropy–enthalpy compensation of the duplex formation.

$\Delta(T\Delta S^\circ)$ undercompensates for $\Delta\Delta H^\circ$. This means that the penalty in enthalpy is 10% greater than the benefit in entropy by the destruction of the hydrogen-bonding networks. Therefore, such thermodynamic characteristic also contributes to the destabilization of the duplex containing the heterochiral RNA strand.

The present results suggest that the homochirality of RNA strands contributes to the enthalpic stabilization of their duplex structures by promoting continuous hydration via the 2′-hydroxy groups in the minor groove. This means that the incorporation of the chiral antipode into the DNA strand does not appreciably influence its duplex structure and stability with the complementary strand compared with that into the RNA strand. This is also true even in DNA–RNA hybrid duplexes. This thermodynamic feature should make L-dNTPs relatively easier to be incorporated into DNA strands by reverse transcriptase. On the contrary, the duplex stability of RNA is more sensitive to the incorporation of the chiral antipode than that of DNA. Not only double-stranded RNAs but also single-stranded RNAs adjacent to the double-stranded region are more resistant to hydrolysis under weak alkaline conditions than single-stranded RNAs [39,40]. This implies that stable duplex-forming RNAs (homochiral RNAs) are more resistant to hydrolysis than heterochiral RNAs. Therefore, RNA might have been a self-replicating system superior to DNA to exclude the chiral antipode, namely chiral amplification, leading to homochirality in the process of the prebiotic chemical evolution.

In conclusion, we have shown that the incorporation of L-nucleotide into the RNA strand leads to more significant destabilization of the duplexes than that into the DNA strand, regardless of whether the complementary strand is DNA or RNA. These fundamental understanding for the structure, stability, and functions of heterochiral nucleic acids would be important for application of L-nucleic acids as a novel functional molecule and for considering the origins of chirality of molecules. The investigations of sequence effects on above thermodynamic characteristics are currently under way.

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