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Sequence Dependence of Thermodynamic Stability of Heterochiral DNA

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Abstract: Several heterochiral dodecadeoxynucleotides (**2-6**) containing an unnatural L-enantiomer of D-deoxyribose were synthesized and their thermodynamic stability for duplex formation was investigated. The results suggested that substitution of L-deoxyribose for natural D-deoxyribose somewhat decreases the duplex stability, depending on the site that incorporates the L-deoxynucleoside.
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Oligodeoxynucleotides (ODNs) have shown great promise as therapeutic agents for inhibiting gene expression owing to their ability to bind sequence-specifically to target nucleic acids.[1] However, one of the limiting factors in the success of this strategy is the rapid degradation of unmodified ODNs by cellular nucleases. It is thus important to improve the stability of ODNs against the action of nucleases. To this end, various modifications of ODNs have been reported[2-6] and recently, some groups have focused upon ODNs containing unnatural L-deoxyribose.[7-11] Unnatural L-ODNs are highly resistant to degradation by nucleases.[7,8,12] In contrast, the hybridization properties of L-ODNs toward complementary natural DNA and RNA sequences are ambiguous. L-$(dAp)_5$dA has been found to hybridize with natural D-poly(rU) rather than D-poly(dT),[7] and an acridine conjugate of L-$d(Ap)_4$ has been reported to form double and triple helices with poly(rU) and poly(dT), respectively.[8] However, oligomers of L-dU showed no UV detectable base-pairing with D-poly(dA).[9] Similarly, oligomers of L-dT failed to form complexes with complementary deoxy- or ribo-D-homopolynucleotide.[10] Furthermore, L-ODNs containing all four natural base residues did not interact with complementary D-DNA or RNA sequences.[11] Accordingly, we and others have independently focused upon heterochiral ODNs having both D- and L-deoxynucleosides.[13-16] Damha *et al.* found heterochiral ODNs to show significant resistance toward exonuclease digestion.[13] We investigated the structure of the heterochiral self-complementary dodecadeoxynucleotide, d(CGCGAATTCGCG) (**3**, **G** denotes the L-deoxyguanosine residue) and found stable Watson-Crick base-pairing to be formed between D- and L-nucleotide residues.[14] Such unexpected base-pairing between D- and L-nucleotide residues in a heterochiral ODN is supported by the work of Blommers *et al.*[15]

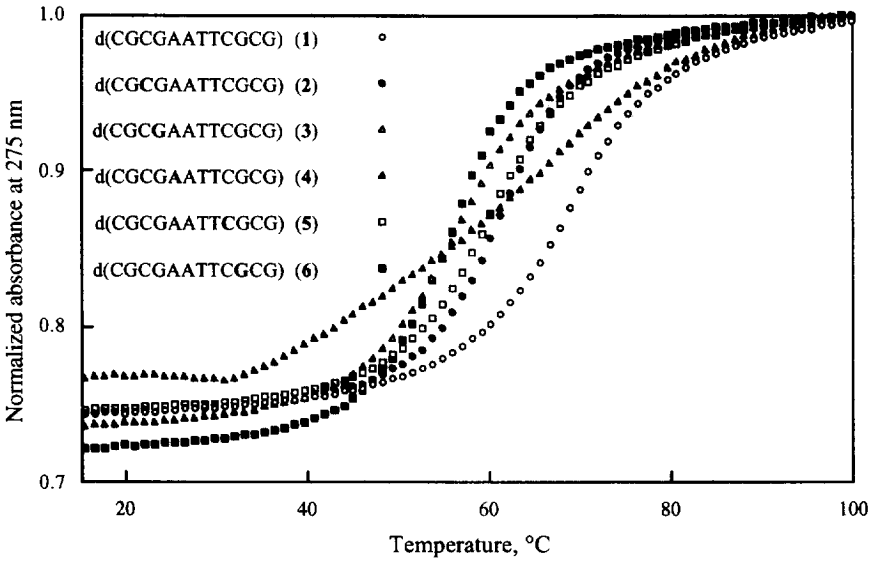

Figure 1. UV melting profiles of natural (**1**) and heterochiral ODNs (**2-6**). Samples contained 9 μM strand in 1 M NaCl, 10 mM $NaHPO_4$, pH7.5. Bold letters denote L-nucleotide residues.

In this paper, we describe the thermodynamic properties in the duplex formation of several heterochiral ODNs, each possessing an L-nucleotide residue at a different site. L-Nucleosides were syntheized from L-arabinose and heterochiral ODNs were synthesized by a β-cyanoethylamidite method.[12,17]

Figure 1 shows the sequences of natural (**1**) and heterochiral ODNs (**2-6**) and their melting profiles. When the natural 12-mer (**1**) showed a simple sigmoidal profile, the heterochiral 12-mer (**4**) indicated a non-cooperative gradual melting pattern. The 12-mer (**4**) would thus appear likely to have a more complex melting pathway (*e.g.* duplex ⇔ hairpin ⇔ single strand[18]). On the other hand, the heterochiral 12-mers other than **4** showed a simple sigmoidal melting pattern as well as the natural 12-mer in spite of the significant decrease of melting temperature (Tm). The extent of decrease of Tm by substitution of the L-deoxyribose residue for the D-deoxyribose residue ($\Delta Tm \fallingdotseq 10°C$) was less than that by introducing a base pair mismatch ($\Delta Tm \geqq 20°C$).[19] The L-nucleotide residue in 12-mers other than **4** would thus appear quite likely to form base-pairing with the complementary natural residue. The present heterochiral system should be useful for thermodynamic evaluation of base-pairing between D- and L-nucleotides. The CD spectra of **1-6** supported the results of the UV-melting experiments. The temperature-dependent CD spectra of 12-mers other than **4** showed isoelliptical points suggesting a simple two-state transition (data not shown).

Thermodynamic parameters for duplex formation of the 12-mers were determined by van't Hoff plots

of the strand concentration dependence of Tm [20] and the results are shown in Table 1. Free-energy change in duplex formation for heterochiral 12-mers at 25°C in the 1M NaCl buffer was shown to be less than that for natural 12-mer by as much as 2-4 Kcal/mol. The double-helix of the heterochiral 12-mers is thus shown to be somewhat destabilized. This destabilization depends on the particular site that incorporates an L-nucleotide. Comparable -ΔΔG° for **3** and **6** suggests double-helix destabilization by substitution of an L-nucleotide for a natural nucleotide is not dependent on the kind of 3'-neighboring base of the L-nucleotide residue. On the other hand, the kind of 5'-neighboring base of the L-nucleotide residue may be essential for the duplex stability. The destabilization effect for the Pu-**Py** sequence (**2**; -ΔΔG°= 2.2 Kcal/mol) is much less than that for the Py-**Py** sequence (**5**; -ΔΔG°= 3.4 Kcal/mol) and is the strongest for the Py-**Pu** sequences (**3**, **6**; -ΔΔG°= 4.0~4.1 Kcal/mol) (**Py** and **Pu** indicate L-pyrimidine and L-purine nucleotides, respectively). Antisense molecules having Pu-**Py** sequences should thus prove useful for improving the hybridization properties of heterochiral oligonucleotides.

In the present self-complementary dodecanucleotide sequence, the average -ΔΔG° per D-L base pair is 1.7 Kcal/mol (1.1- 2.05 Kcal/mol), suggesting that the 12-mer no longer forms the double-helix when six residues of the 12-mers replace the D-sugar with L-sugar, in which each base pair is a D-L pair. Homochiral L-ODNs consisting only of the L-deoxyribose sugar would thus not likely form a double-helix with natural DNA sequences and this is consistent with earlier observations.[7,9-11] The thermodynamic investigations described here show possibilities of heterochiral ODNs to serve as antisense ODNs.

Table 1. Thermodynamic parameters for duplex formation of natural and heterochiral 12-mers at 25°C[a].

Sequence[b]	-ΔH° (Kcal/mol)	-ΔS° (cal/Kmol)	-ΔG° (Kcal/mol)	-ΔΔG° (Kcal/mol)	Tm (°C)
d(CGCGAATTCGCG)(**1**)	106	288	20.1	—	67.8
d(CG**C**GAATTCGCG)(**2**)	104	289	17.9	2.2	60.5
d(CGC**G**AATTCGCG)(**3**)	99	278	16.1	4.0	55.7
d(CGCGAATT**C**GCG)(**5**)	96	267	16.7	3.4	58.9
d(CGCGAATT**C**G**C**G)(**6**)	99	277	16.0	4.1	55.3

[a]Samples contain 1M NaCl, 10 mM sodium phosphate, pH 7.5. [b]Bold letters denote L-nucleotide residues.

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