Selective Binding of RNA, but Not DNA, by Complementary 2′,5′-Linked DNA

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We have been interested in the properties of an isomer of normal DNA based on 3-deoxyribose instead of the normal 2-deoxyribose.[1−5] Related studies have been described by Switzer,[6] and Turner has reported the properties of 2′,5′-linked RNA.[7] We have synthesized a series of 3′-deoxynucleoside phosphoramidite derivatives[1] and oligomerized them[2] using solid-phase synthesis into isoDNA molecules in which the connecting phosphodiester linkage is 2′,5′ instead of the natural 3′,5′-linkage (Figure 1). We demonstrated that hexadecamers containing mixed sequences of A* and T* units (the asterisk refers to the 3′-deoxy analogs) do not hybridize with their complementary strands containing either T and A units or T* and A* units.[2] However, under high-salt conditions an A* hexadecamer hybridizes with a T* hexadecamer but in a triple helix with two pyrimidine strands and one purine strand.[3]

More recently we have synthesized G* and C* phosphoramidite monomers.[4] We used them to prepare an isoDNA oligopurine strand (containing A* and G*) and a complementary isoDNA oligopyrimidine strand (containing T* and C*) and studied their duplex and triplex association.[5] We have now prepared the first examples of isoDNAs containing mixed sequences of the four nucleobases (A*, C*, G*, and T*) and have studied the binding of these isoDNA molecules to normal complementary RNA and DNA.

We prepared two complementary hexadecamer DNA sequences (**1** and **2**) and the analogous 2′,5′-linked isoDNA analogs (**1*** and **2***). Using *in vitro* transcription with T7 RNA polymerase,[8] we also synthesized a 24-mer RNA sequence (**3**) that was designed to be complementary to molecules with sequence **2** and to mimic a cellular RNA target. The properties of the complexes formed between complementary strands were assessed by UV-melting experiments and circular dichroism (CD) spectroscopy.

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    5'       ACAAGCTTGCATGCCT      3'(or 2')1 (1*)
3'(or 2')    TGTTCGAACGTACGGA      5'       2 (2*)
    5'  GGAGACAAGCUUGCAUGCCUGCAU   3'       3
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The RNA strand **3** possesses considerable self-structure in the absence of a complementary strand, exhibiting two cooperative melting transitions at 33 and 56 °C at pH 7.0 and 1.0 M NaCl (10 mM phosphate buffer, 0.1 mM EDTA, 4.0 μM total strand concentration). Under the same conditions, the duplex of **3** with equimolar **2** showed a single cooperative transition at 66.5 ± 0.5 °C,[9] while the duplex of **3** with isoDNA **2*** melts at 61.5 ± 0.5 °C (Figure 2). Under the same conditions with low salt (0.2 M), the two transitions (Figure 3) are at 59.7 ± 0.5 °C (**2·3**) and 53.6 ± 0.5 °C (**2*·3**). The hyperchromicities and shapes of the melting profiles are similar under both conditions. Furthermore, the melting temperatures are concentration-dependent. The CD spectra of the two complexes are virtually

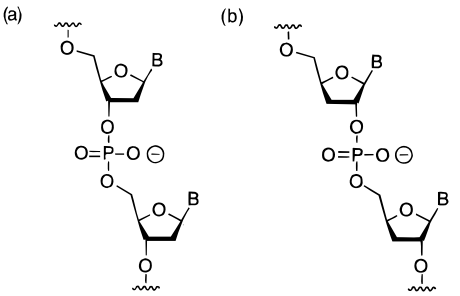

Figure 1. Structures for (a) natural 3′,5′-linked DNA and (b) unnatural 2′,5′-linked isoDNA (B = nucleobase).

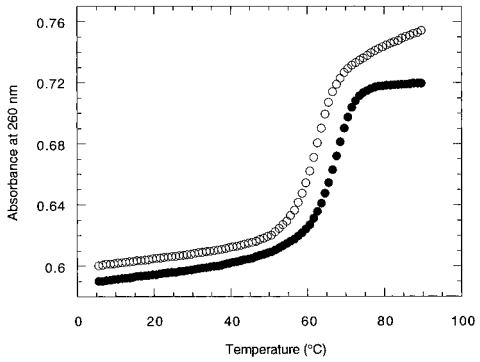

Figure 2. Melting profiles for DNA/RNA (**2·3**, closed circles) and isoDNA/RNA (**2*·3**, open circles) duplexes under high-salt conditions. The buffer was 10 mM phosphate, 1.0 M NaCl, 0.1 mM EDTA in H_2O at pH 7.0. The concentration of each strand was 2.0 ± 0.1 μM. The profile was recorded at 260 nm with a temperature ramp of 1.0 °C/min.

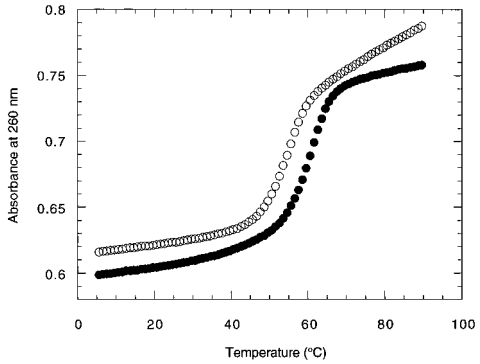

Figure 3. Melting profiles for DNA/RNA (**2·3**, closed circles) and isoDNA/RNA (**2*·3**, open circles) duplexes under low-salt conditions. The buffer was 10 mM phosphate, 200 mM NaCl, 0.1 mM EDTA in H_2O at pH 7.0. The concentration of each strand was 2.0 ± 0.1 μM. The profile was recorded at 260 nm with a temperature ramp of 1.0 °C/min.

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(9) The melting temperature (T_m) was determined by fitting the absorbance vs temperature profiles as described: Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601−1620. Melting profiles were measured three or more times per complex; T_m values for all complexes were reproducible to within ±0.5 °C.

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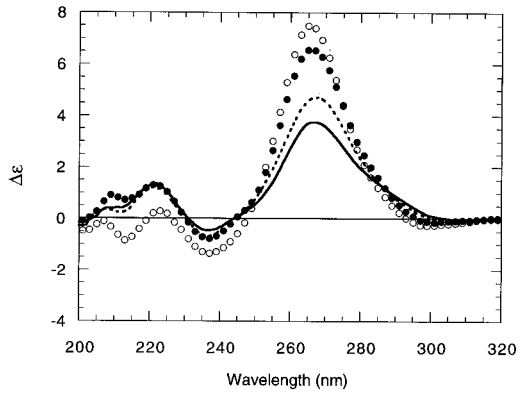

Figure 4. CD spectra of DNA/RNA (**2·3**, closed circles) and isoDNA/RNA (**2*·3**, open circles) duplexes under high-salt conditions at 27 °C. The conditions are the same as in Figure 2. The average CD spectra of the component single strands (**2** + **3**, solid line; **2*** + **3**, dashed line) are shown for comparison.

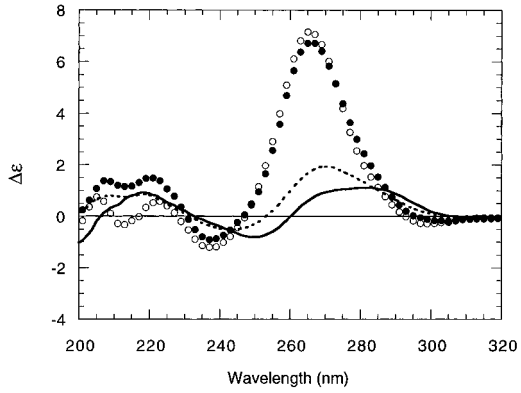

Figure 5. CD spectra of DNA/RNA (**2·3**, closed circles) and isoDNA/RNA (**2*·3**, open circles) duplexes under low-salt conditions at 27 °C. The conditions are the same as in Figure 3. The average CD spectra of the component single strands (**2** + **3**, solid line; **2*** + **3**, dashed line) are shown for comparison.

identical under high-salt (Figure 4) and low-salt (Figure 5) conditions: the spectra of both complexes differ significantly from those of their parent single strands, and both spectra exhibit a major absorption at 265 nm and parallel fine structure at lower wavelengths. Thus, isoDNA forms complexes with complementary RNA that are almost as strong as those in normal DNA/RNA duplexes, and the two heteroduplexes are structurally similar, as judged by their CD spectra.

In contrast, duplexes between complementary isoDNA sequences **1*** and **2*** are considerably less stable than those of their natural DNA counterparts **1** and **2**. For example, at pH 7.0 and 1.0 M NaCl, the duplex between the 3′,5′-linked oligomers **1** and **2** exhibits a single melting transition at 68.3 ± 0.5 °C, whereas the isoDNA complex (**1*·2***) displays a less cooperative transition at 25.5 ± 0.5 °C. The complexes between DNA and complementary mixed-sequence isoDNA are even less stable, with both the **1·2*** and **1*·2** complexes displaying uncooperative transitions centered near 14 °C.

We have also examined the susceptibility of isoDNA and its complexes to enzymatic hydrolysis.[10] As Table 1 shows, many nucleases that cleave DNA are unable to cleave isoDNA, but not all. Because cellular and serum exonucleases and intracellular endonucleases pose the greatest threat for degradation of antisense oligonucleotides,[11,12] the stability of isoDNA to these common nucleases suggests that the 2′,5′-linkage may confer adequate stability *in vivo*. However, isoDNA/RNA heteroduplexes are not substrates for RNase H, an enzyme that cleaves the RNA strand of normal RNA/DNA duplexes.[13] Thus, although our melting and CD experiments suggest that the global structures of the heteroduplexes are similar, the structure of the isoDNA/RNA complex is not sufficient for recognition by RNase H.

Table 1. Nuclease Digestion of isoDNA **2***

nuclease	hydrolysis of isoDNA **2***[a]
klenow 3′−5′ exonuclease (*Escherichia coli*)	−
exonuclease III (*E. coli*)	−
DNAse I (bovine pancreas)	−
micrococcal nuclease (*Streptomyces aureus*)	−
mung bean nuclease	++
nuclease S1 (*Aspergillus oryzae*)	++
nuclease P1 (*Penicillium citrinum*)	++
nuclease BAL-31 (*Aspergillus espejiani*)	+

[a] Key: ++, rapid degradation (>50% degradation in <15 min); +, slow degradation (10−50% degradation in 5 h); −, resistant (<10% degradation in 5 h). DNA **2** was completely degraded by all nucleases in <15 min.

Thus isoDNA forms a heteroduplex with RNA that is almost as stable as the comparable normal DNA/RNA duplex, yet binds only weakly (if at all) with complementary isoDNA and DNA sequences. Such binding selectivity has previously been observed with 2′,5′-linked RNA molecules,[14] 2′,5′-linked formacetal DNA,[15] and 2′,5′-linked DNA oligoadenylates.[16] The isoDNA is also resistant to a number of exo- and endonucleases that hydrolyze normal DNA. Clearly, the structural basis for the stability of complexes of isoDNA with RNA and the instability of its complexes with complementary DNA or isoDNA require further investigation. However, the observed binding selectivity, combined with the stability to common nucleases, makes isoDNA an excellent candidate for diagnostic and therapeutic antisense applications.[17,18]

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Supporting Information Available: Protocols for isoDNA and RNA synthesis and purification, procedures for the biophysical and biochemical characterization of isoDNA and its complexes, additional melting profiles, and enzymatic digestion data (17 pages). See any current masthead page for ordering and Internet access instructions.

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(18) Based on the Ph.D. thesis of Terry L. Sheppard, Columbia University, 1995.