RNA recognition by the 2′-structural isomer of DNA

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Formation of chimeric duplexes is investigated between DNA bearing exclusively 2′,5′-phosphodiester linkages and DNA or RNA bearing 3′,5′-linkages; 2′-isomeric-DNA yields a hybrid duplex with RNA of the same stability as the corresponding all 3′,5′-linked DNA–RNA duplex.

DNA or RNA bearing exclusively 2′,5′-phosphodiester bonds associates with itself through formation of purine–pyrimidine Watson–Crick pairs.[1–3] This finding raises the possibility that 2′,5′-linked RNA, in conjunction with 3′,5′-linked RNA, could have made a positive contribution to molecular evolution on the primitive Earth. Indeed, 2′,5′-linked RNA has been shown recently to oligomerize activated mononucleotides in the presence of divalent metal ions.[4,5] 2′,5′-Linked RNA is also the product of mononucleotide oligomerization with natural, 3′,5′-linked nucleotide templates, depending on conditions.[6,7] As part of a continuing effort to explore molecular recognition of isomeric nucleic acids,[1,8,9] we report here a comparison of thermodynamic stabilities for chimeric and homogeneous duplexes from all possible pairings of two 2′-isomeric-DNA strands and the corresponding strands of 3′,5′-linked DNA or RNA.

Synthesis and characterization of isomeric DNA strands **1** and **2** used in this study have been described previously (Table 1).[1b] To assess whether 2′-isomeric-DNA would associate with complementary strands of DNA or RNA, temperature *vs.* UV absorbance profiles were obtained. Cooperative transitions result from a combination of 2′-isomeric-DNA and complementary RNA strands [Fig. 1(*a*) and (*b*)], consistent with recognition by means of Watson–Crick base-pairing. Also given in Fig. 1(*a*) and (*b*) are control experiments showing thermal profiles of component single strands for each chimeric duplex. Whereas thermal profiles for 2′-isomeric-DNA strands are virtually featureless, profiles for the RNA single strands indicate structure. However, this structure is clearly absent from

Table 1 All values determined with samples containing 1 mol dm^{-3} NaCl, 10 mol dm^{-3} sodium phosphate, 0.1 mmol dm^{-3} EDTA at pH 7, and a 5 μmol dm^{-3} total concentration of oligonucleotides (except in the case of single-stranded oligomers where a concentration of 2.5 μmol dm^{-3} was employed)

Entry	Oligonucleotide	T_m/°C	$-\Delta°37$/ kcal mol^{-1}[b]
1	5′-dCCGGCCGCGCGC-2′ **1** 2′-dGGCCGGCGCGCG-5′ **2**	42.4[a]	9.44[a]
2	5′-dCCGGCCGCGCGC-3′ **3** 3′-dGGCCGGCGCGCG-5′ **4**	76.8[a]	19.52[a]
3	5′-rCCGGCCGCGCGC-3′ **5** 3′-rGGCCGGCGCGCG-5′ **6**	92.2	23.0
4	5′-dCCGGCCGCGCGC-2′ **1** 3′-rGGCCGGCGCGCG-5′ **6**	72.6	17.9
5	5′-rCCGGCCGCGCGC-3′ **5** 2′-dGGCCGGCGCGCG-5′ **2**	74.2	18.9
6	5′-dCCGGCCGCGCGC-2′ **1** 3′-dGGCCGGCGCGCG-5′ **4**	<0	—
7	5′-dCCGGCCGCGCGC-3′ **3** 2′-dGGCCGGCGCGCG-5′ **2**	<0	—
8	5′-dCCGGCCGCGCGC-3′ **3** 3′-rGGCCGGCGCGCG-5′ **6**	75.3	19.0
9	5′-rCCGGCCGCGCGC-3′ **5** 3′-dGGCCGGCGCGCG-5′ **4**	74.8	18.6

[a] Values taken from ref. 1*b*. [b] 1 cal = 4.184 J.

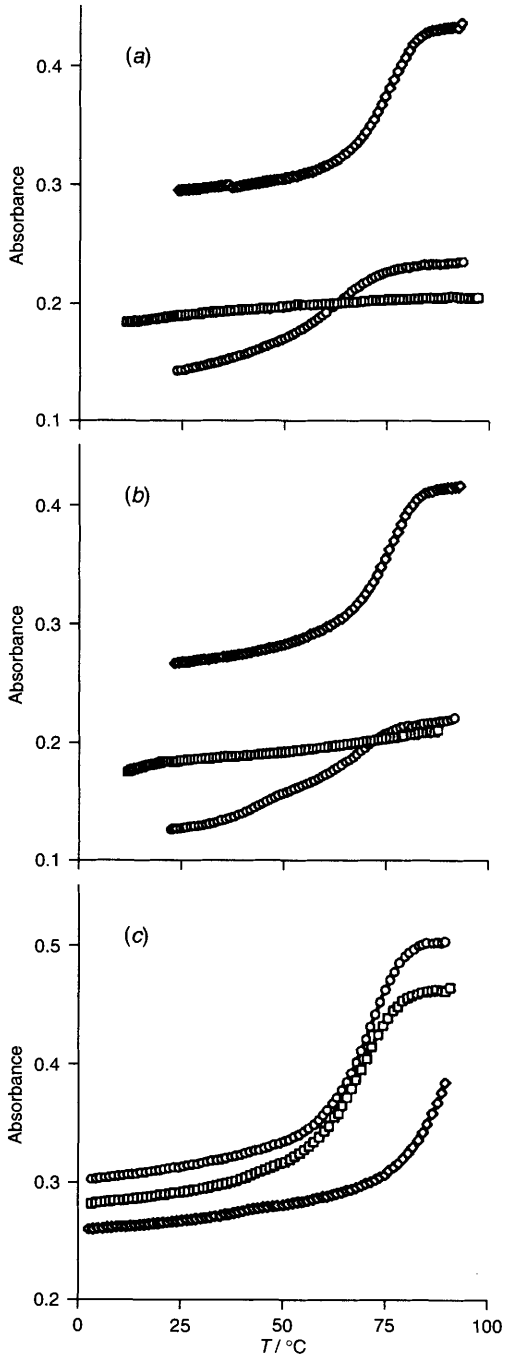

Fig. 1 UV absorbance profiles (280 nm) for: (*a*), 1 and 6 (◇, mixture of complementary strands), 1 (□, single-strand) and 6 (○, single-strand); (*b*), 2 and 5 (◇), 2 (□) and 5 (○); (*c*), 5 and 6 (◇), 4 and 5 (□), and 3 and 6 (○)

the chimeric duplex profiles, since the transitions derived from RNA single-strands alone occur at a temperature discernibly lower than the duplex transitions. Variation of the concentration of RNA single strands showed a clear effect on transition temperature, demonstrating that their structures derive from equilibria other than unimolecular. Parallel experiments with complementary DNA gave no discernible transitions (Table 1, entries 6 and 7), consistent with no strand association in this case.

Free energies of association were determined for 2′-isomeric-DNA–RNA duplexes along with other duplexes, and are summarized in Table 1. Values for $\Delta G°$ were derived by non-linear regression of temperature *vs.* UV absorbance profiles of the duplexes.[10] Comparison of free energy values in Table 1 shows that 2′-isomeric-DNA–RNA duplexes are approximately isoenergetic with DNA–RNA duplexes (entries 4 and 5 *vs.* 8 and 9). In addition, the duplex of lowest stability derives from strands of purely 2′-isomeric-DNA (entry 1), whereas the duplex composed of RNA strands is most stable (entry 3).

To investigate the molecular basis for the observed stabilities, circular-dichroism spectra of the duplexes were determined, and are presented in Fig. 2. 2′-Isomeric-DNA–RNA chimera adopt an A-form helix similar to the RNA–RNA duplex [Fig. 2(*a*) and (*b*)]. To complete the structural picture of 2′-isomeric-DNA, the CD spectrum of the fully isomeric duplex was also determined [Fig. 2(*b*)], and found to fall into the same A-form structural category.

The remarkable two-fold greater stability of the 2′-isomeric-DNA–RNA duplexes **1/6** and **5/2** relative to the parent 2′-isomeric-DNA duplex **1/2** (Table 1, entries 4, 5 and 1) is consistent with the adoption of A-form structures by these two systems. Preorganization or increased base stacking on the part of the RNA single-strands **5** and **6**, or both, could lead to the observed stability differences. The absence of 2′-isomeric-DNA complexation with natural DNA may be understood based on the reluctance of the latter to assume an A-type conformation, and the inability of the isomeric structure to override this preference.

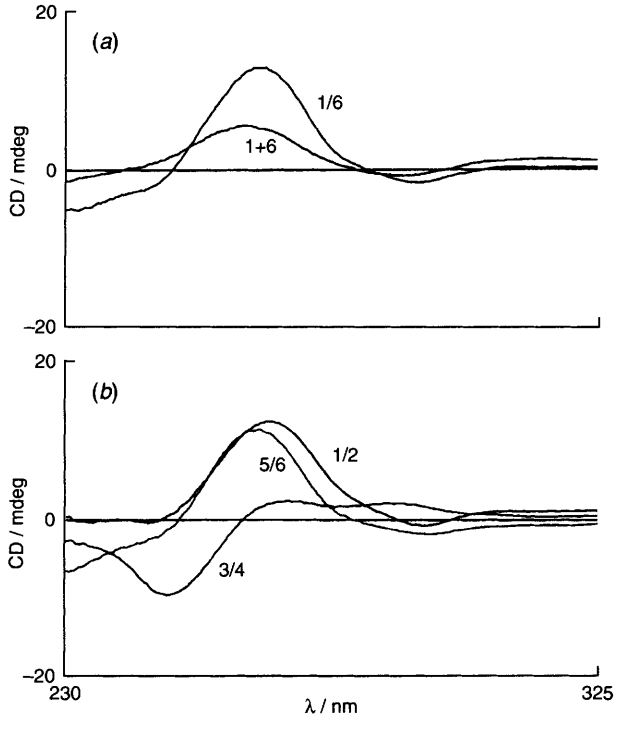

Fig. 2 CD spectra for mixtures of complementary strands (/) and the summation of separate spectra for single-strands (+). All spectra were recorded at 10 °C. The CD spectra for 2/5 and 2+5 (not shown) were found to be essentially identical to those of 1/6 and 1+6 shown in Fig. 1(*a*).

Several nucleic acids complexes bearing 2′-isomeric strands have been characterized in the past. Chimeric triple helical complexes formed from strands of 2′,5′- and 3′,5′-linked RNA have been reported,[11–13] and extended to include 2′,5′-DNA.[14] In one case a chimeric duplex between 2′,5′- and 3′,5′-linked RNA strands was observed,[13] but a comparison of its stability with a purely 3′,5′-linked RNA duplex through T_m measurements was complicated by single-strand self-association phenomena as noted by the authors.

Chimeric duplexes derived from purely 2′,5′- and 3′,5′-linked strands of nucleic acids can rival in stability those of their homogenous 3′,5′-linked counterparts as shown in the present work. Our data also suggest that a 2′,5′-linked oligomer would prefer template-directed formation of a 3′,5′-linked product over a 2′-isomeric one, based on primer stabilities alone. A tendency toward formation of 3′-linkages from 2′-linkage precursors would have merged the evolutionary fate of 2′-isomeric nucleic acids with that of modern nucleic acids.

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Footnote

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References

1 (*a*) H. Hashimoto and C. Switzer, *J. Am. Chem. Soc.*, 1992, **114**, 6255; (*b*) K.-E. Jung and C. Switzer, *J. Am. Chem. Soc.*, 1994, **116**, 6059; (*c*) H. Robinson, K.-E. Jung, C. Switzer and A. H.-J. Wang, *J. Am. Chem. Soc.*, 1995, **117**, 837.
2 J. P. Dougherty, C. J. Rizzo and R. Breslow, *J. Am. Chem. Soc.*, 1992, **114**, 6254; R. Z. Jin, W. H. Chapman, A. R. Srinivasan, W. K. Olson, R. Breslow and K. J. Breslauer, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 10 568.
3 R. Kierzek, L. He and D. H. Turner, *Nucleic Acids Res.*, 1992, **20**, 1685.
4 G. Ertem and J. P. Ferris, *Nature*, 1996, **379**, 238.
5 T. P. Prakash and C. Switzer, unpublished results.
6 T. Wu and L. E. Orgel, *J. Am. Chem. Soc.*, 1992, **114**, 7963, and references cited therein.
7 G. F. Joyce, *Cold Spring Harbor Symposium on Quantitative Biology*, 1987, **LII**, 41.
8 R. Bandaru, H. Hashimoto and C. Switzer, *J. Org. Chem.*, 1995, **60**, 786.
9 C. Roberts, R. Bandaru and C. Switzer, *Tetrahedron Lett.*, 1995, **36**, 3601.
10 C. E. Longfellow, R. Kierzek and D. H. Turner, *Biochemistry*, 1990, **29**, 278.
11 A. M. Michelson and C. Monny, *Biochem. Biophys. Acta*, 1967, **149**, 107.
12 I. Hirao, Y. Ishido and K.-I. Miura, *Nucleic Acids Res., Sym. Ser.*, 1983, **12**, 193.
13 P. A. Giannaris and M. J. Damha, *Nucleic Acids Res.*, 1993, **21**, 4742.
14 R. Alul and G. D. Hoke, *Antisense Research and Development*, 1995, **5**, 3.

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