# Comparison of 3′,5′- and 2′,5′-linked DNA duplex stabilities by electrospray ionization mass spectrometry

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**Two mutually complementary strands of 2′,5′-linked DNA form a unique noncovalent complex observed by electrospray ionization mass spectrometry; by the latter method, the double-stranded state of a 2′,5′-linked DNA duplex is less stable than the natural DNA duplex of identical base composition.**

Nucleic acids figure centrally in the transfer of biological information by way of noncovalent interactions within complementary base-pairs. Natural nucleic acids, however, are not unique in their noncovalent behaviour as evidenced by arrays of nucleic acid bases with unnatural backbones mimicking the parent ribose phosphodiester system. Prebiotically abundant variants of present-day, natural nucleic acids may have contributed to the evolution of biological systems by participating in multiple genetic takeovers[1] with replicating molecules of increasing fitness, leading ultimately to an 'RNA world.'[2] Among the oligomers synthesized that could be considered pre-RNA world candidates are so-called peptide nucleic acids (PNA),[3] homo-RNA,[4] pyranosyl-RNA[5] and 2′,5′-linked RNA.[6–8] A few of the attractive features of these systems include, respectively: the structural simplicity of PNA, an efficient route to hexose sugars from simple precursors in the case of homo-RNA,[9] the possibility of isomerization to natural RNA for pyranosyl- and 2′,5′-linked RNA, and an abiotic route to oligomers from monomers for 2′,5′-linked RNA.[10] Ultimately the plausibility of these alternative structures rests on their performance in an abiotic setting. Furthermore, attributes of candidate structures need also be weighed against where they might fit in an evolutionary time-line and the attendant degree of synthetic complexity attainable.

To date the stabilities of nucleic acid complexes with alternative backbones have been compared to natural ones by solution- and solid-phase methods. In the case of 2′,5′-linked oligonucleotides, methods include UV-monitored thermal denaturation, differential scanning calorimetry, gel shift and NMR spectroscopy.[6–8] Determination of the specificity of noncovalent interactions is not always straightforward by these methods. Electrospray ionization mass spectrometry (ESI-MS) has found recent use to determine noncovalent associations between biological macromolecules,[11] including several investigations of natural DNA duplexes.[12–15] Here we report a comparison of natural DNA and 2′,5′-linked DNA duplex stabilities in the gas phase using ESI-MS, including an examination of the specificity of interactions between component strands.

Two 2′,5′-linked DNA dodecamers, 5′-dCCGGCCGCG-CGC-2′ **1** and 2′-dGGCCGGCGCGCG-5′ **2**, and their natural counterparts were studied. Synthesis of these oligomers and characterization of their solution-phase duplex stabilities in both low and high concentrations of sodium chloride have been described.[6b] To facilitate ESI-MS analysis, duplex formation by these oligomers was determined in aqueous ammonium acetate (results not shown). Stabilities were found to be equivalent to the same concentration of sodium chloride by UV absorbance *vs.* temperature denaturation profiles. A Finnegan MAT TSQ 7000 triple-quadrupole mass spectrometer equipped with a home-built microspray emitter was used to obtain spectra.[16] Spectrometer parameters were set by an autotune procedure, and an *m/z* range mode up to 4100 *m/z* was used at unit mass resolution.

In Fig. 1(*a*) is shown a negative mode ESI mass spectrum for a 1 : 1 mixture of 2′,5′-linked DNA oligomers **1** and **2**. The spectrum was acquired using a capillary interface temperature of 90 °C, and a sample mixture of 50 μmol dm$^{-3}$ single strands in 10 mmol dm$^{-3}$ ammonium acetate. Duplex peaks have been assigned with charge states ranging from 4− to 6−. The most abundant duplex peak, with a charge state of 5−, falls at 1459.6 *m/z* and corresponds to 7303 Da. This mass is consistent with the calculated mass of the heteroduplex formed by **1** and **2** of 7296.8 Da. Calculated masses of the putative homoduplexes are, respectively, 7216.7 and 7376.8 Da. As demonstrated by the spectrum inlayed in Fig. 1(*a*), neither possible homoduplex is observed. Fig. 1(*b*) shows the ESI mass spectrum for a 1 : 1 mixture of natural DNA corresponding in sequence to **1** and **2**. In contrast to the behavior of 2′,5′-linked DNA, natural DNA

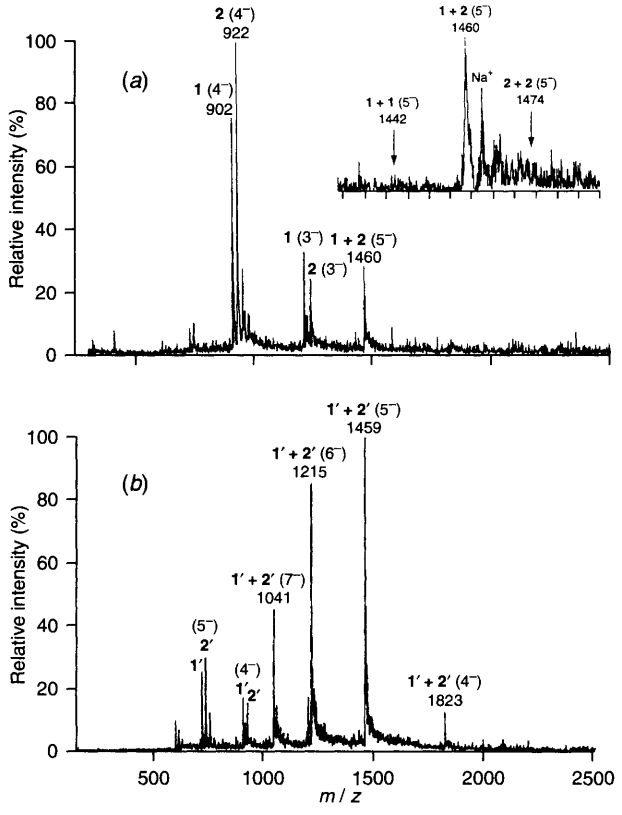


**Fig. 1** (*a*) ESI mass spectrum of 2′,5′-linked DNA strands **1** and **2** with a total strand concentration of 50 μmol dm$^{-3}$ in 10 mmol dm$^{-3}$ ammonium acetate at a capillary temperature of 90 °C. (*b*) ESI mass spectrum of 3′,5′-DNA strands corresponding to **1** and **2** using the same conditions.

gives an ESI mass spectrum dominated by duplex. Additionally, there is a greater population of higher charge states for natural DNA compared to 2′,5′-linked DNA for both the intact duplex and individual strands.

A qualitative measurement of duplex stability can be obtained by the comparison shown in Fig. 1, and further amplified by variation of the ESI interface capillary temperature.† Temperature dependencies of duplex stabilities for 3′,5′- and 2′,5′-linked DNA dodecamers in 10 mmol $dm^{-3}$ ammonium acetate are summarized in Fig. 2. Marked dependencies are observed for both systems. The population of double-stranded state is seen to be approximately three times greater for natural DNA versus 2′,5′-linked DNA at 90 °C, the lowest temperature assayed, with this ratio increasing to 12:1 at the highest temperature of 130 °C where comparative data are available. In separate experiments the 2′,5′-linked DNA duplex stability was also shown to depend on the solution ionic strength, where a five-fold increase in salt concentration to 50 mmol $dm^{-3}$ was seen to quadruple the amount of duplex observed (data not shown).

The absence of detectable homodimerization in the ESI mass spectra of 2′,5′-linked DNA strands is consistent with heterodimer formation through Watson-Crick base-pairing. Relative stabilities of the 2′,5′- and 3′,5′-linked duplexes determined by ESI-MS agree qualitatively with solution-phase stability measurements,[6b] as the free energy of association in solution was observed in the 2′,5′-case to be less by half. Thus, the present results provide additional evidence of the utility of ESI-MS to characterize macromolecules. Finally, the high degree of specificity found in the interaction of 2′,5′-linked strands of DNA provides new insight into the fidelity of this alternative genetic material.

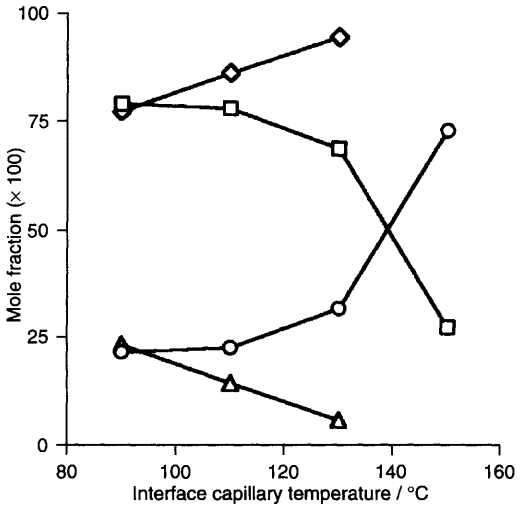


**Fig. 2** Comparison of the thermal stability of 2′,5′-linked DNA and 3′,5′-linked DNA duplexes by electrospray mass spectrometry. In all cases sample solutions contained 10 mmol $dm^{-3}$ ammonium acetate and total single strand concentrations of 50 μmol $dm^{-3}$. 2′,5′-linked DNA: ◊ = single-stranded **1** and **2**; △ = double-stranded **1** and **2**. 3′,5′-DNA: □ = double-stranded **1′** and **2′**; ○ = single-stranded **1′** and **2′**.

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## Footnote

† It should be noted that the residence time of the electrosprayed droplets in this region is short (*ca.* $1 \times 10^{-3}$ s), and the actual temperature attained during transit of the capillary is substantially less than the reported capillary temperature.

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