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N1···N3 Hydrogen Bonds of A:U Base Pairs of RNA Are Stronger than Those of A:T Base Pairs of DNA

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RNA and DNA are different structurally and functionally, and much effort has gone into elucidating the physical and chemical bases of these differences. Although it is well accepted that hydrogen bonds are central to the structure and function of nucleic acids, it is not yet clear whether significant differences exist between RNA and DNA hydrogen bonds. Amplitudes of fast purine librations were found to be similar in homologous RNA and DNA duplexes.[1,2] Imino proton exchange comparisons show that base-pair lifetimes of RNA A:U base pairs are shorter than those of DNA A:T base pairs.[3] However, base-pair kinetics are not exclusively a function of hydrogen bonding but also of base stacking interactions.[4] Recently, it was shown in non-base-paired mononucleotides that the difference in pK_a values of rA and rU is less than that for dA and dT, from which it was inferred that RNA hydrogen bonds can be stronger than those of DNA.[5] Trans-hydrogen-bond scalar couplings, which reflect the extent of electron delocalization across the hydrogen bond, have been measured in RNA and DNA duplexes and triplexes.[6−9] However, the RNA and DNA sequences were not homologous, and a comparison of the distribution of $^{2h}J_{NN}$ values did not reveal any differences.

Deuterium isotope effects are well established as quantitative gauges of hydrogen bond strength, where larger absolute values of isotope effects are indicative of stronger hydrogen bonds.[11−17] Recently, we showed that the ^{13}C2 resonance of an adenosine residue will experience a ^{13}C2−N1···^{1,2}H3−N3 trans-hydrogen-bond deuterium isotope effect on the chemical shift, defined as $^{2h}\Delta^{13}C2 = \delta^{13}C2\{^1H3\} - \delta^{13}C2\{^2H3\}$.[10] $^{2h}\Delta^{13}$C2 values were shown to be indicators of hydrogen bond strength and should be less sensitive to other effects such as base stacking as compared to imino proton chemical shifts.[10] Here, we compare $^{2h}\Delta^{13}$C2 values obtained from RNA A:U base pairs to those of A:T base pairs of homologous DNA sequences studied earlier.[10]

Five self-complementary and isotopically unenriched RNA dodecamers (r[CGCGAAUUCGCG]$_2$, r[CGUUUUAAAACG]$_2$, r[CGAAAAUUUUCG]$_2$, r[CGUAUAUAUACG]$_2$, and r[CGCG-UAUACGCG]$_2$, referred to as **r1**, **r2**, **r3**, **r4**, and **r5**, respectively (Dharmacon, Inc.)) were dissolved in an isomolar solvent mixture of H_2O and D_2O to allow measurement of $^{2h}\Delta^{13}$C2. Homologous RNA and DNA sequences are **r1** and **d1**, **r2** and **d2**, and so on. Sample buffer conditions, experiment setup, and data processing were identical to those described earlier.[10] As a result of slow imino hydrogen exchange with solvent, ^{13}C2−N1···^{1,2}H3−N3 trans-hydrogen-bond isotope effects split the ^{13}C2 resonances of rA residues into doublets (Figure 1). The upfield and downfield doublet components correspond to the protonated and deuterated H3 isotopomers, respectively,[10] and are separated by an amount $^{2h}\Delta^{13}$C2. Adequate sensitivity and resolution can be achieved using a ^{1}H, ^{13}C TROSY-HSQC experiment (at 14.1 T) to correlate adenosine ^{1}H2 and ^{13}C2 resonances.[10,18,19]

A comparison of $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ values shows that 12 of 14 measured trans-hydrogen-bond isotope splittings are larger

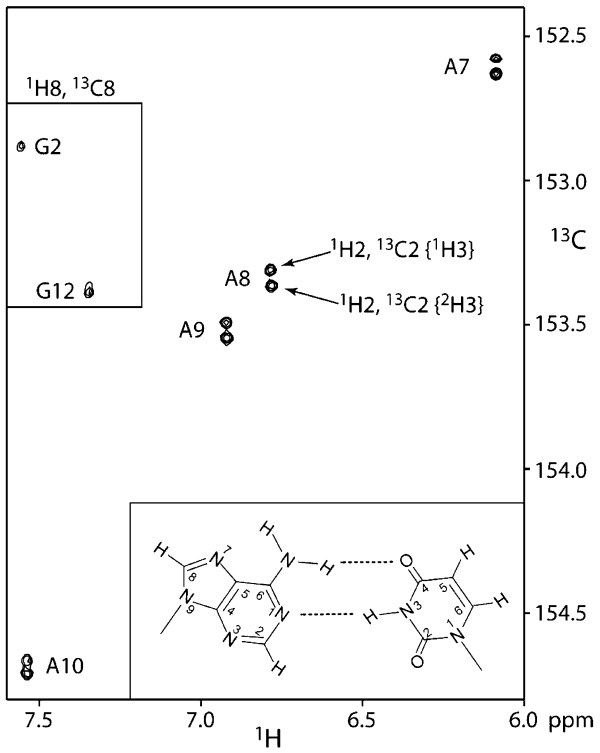

Figure 1. The ^{1}H, ^{13}C TROSY-HSQC spectrum of **r2** collected at 14.1 T (600 MHz ^{1}H frequency) and 25 °C. The spectrum was collected in approximately 21 h. Acquisition parameters, data processing, and analysis of all RNA spectra are identical to those used for the DNA spectra.[10] The ^{1}H2, ^{13}C2 correlations appear as doublets, split by $^{2h}\Delta^{13}$C2 along the ^{13}C dimension and further shifted along the ^{1}H dimension by $^{3h}\Delta^1$H2. The inset is a diagram of an A:U base pair.

in RNA than in DNA (Figure 2). Only A5 of **r1** and **d1** have $|^{2h}\Delta^{13}C2_{RNA}| < |^{2h}\Delta^{13}C2_{DNA}|$, the reason for which is not clear. The RNA and DNA sequences studied here and earlier were chosen to sample a reasonable variation in base-pair angles (e.g., tilt and propeller). For example, poly A-tract DNA (**d1** and **d3**) is characterized by strong base-pair propeller, whereas TA repeats have weaker propeller (**d4** and **d5**);[20−22] poly A-tract RNA (**r1** and **r3**) have reduced tilt angles relative to UA repeats (**r4** and **r5**).[23] At UpA or TpA steps, magnitudes of $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ are in general smaller, indicating that these hydrogen bonds are weaker in both RNA and DNA (Table 1).

The virtually consistent discrepancy between $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ observed across the several different sequences investigated here suggests that N1···N3 hydrogen bonds of A:U base pairs in RNA duplexes are stronger than those of A:T base pairs in DNA. Previous studies have shown excellent linear correlations between the natural logarithm of intramolecular $^2\Delta^{13}$C values and hydrogen-bond energies.[13,24,25] It is reasonable to assume that $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ have an identical dependence upon

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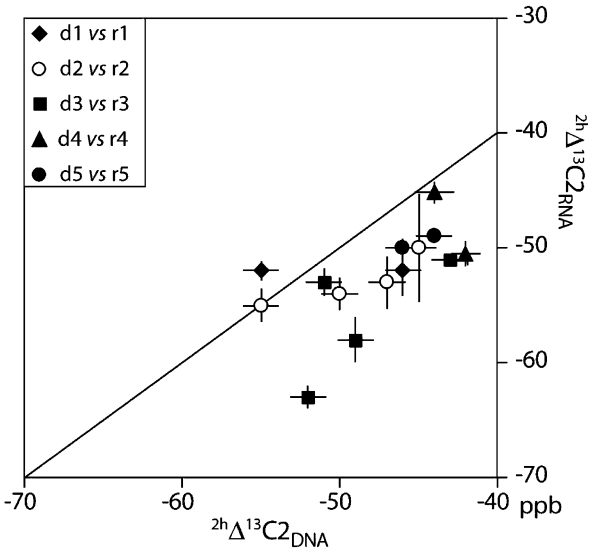

Figure 2. A plot of $^{2h}\Delta^{13}C2_{RNA}$ versus $^{2h}\Delta^{13}C2_{DNA}$. The solid line is the diagonal. Error bars are shown as horizontal and vertical lines.

Table 1. DIE Measurements on RNA and DNA Dodecamers[a]

	RNA $^{2h}\Delta^{13}C2$[b]	DNA $^{2h}\Delta^{13}C2$[c]	δ_{H3} (RNA)
r1, A5:U8	−52 ± 0.8	−55 ± 1.1	13.76
r1, A6:U7	−52 ± 2.1	−46 ± 1.1	14.02
r2, U3:A10	−50 ± 4.7	−45 ± 1.1	14.20
r2, U4:A9	−54 ± 1.3	−50 ± 1.1	13.76
r2, U5:A8	−55 ± 1.4	−44 ± 1.1	13.62
r2, U6:A7	−53 ± 2.2	−47 ± 1.1	12.86
r3, A3:U10	−58 ± 1.9	−49 ± 1.1	13.66
r3, A4:U9	−53 ± 1.1	−51 ± 1.1	13.61
r3, A5:U8	−63 ± 0.9	−52 ± 1.1	13.66
r3, A6:U7	−51 ± 0.0	−43 ± 1.1	13.96
r4, U3:A10	−45 ± 0.9	−44 ± 1.2	13.40
r4, A4:U9	N.A.[d]	−42 ± 0.8	13.12
r4, U5:A8	N.A.[d]	−44 ± 0.4	13.12
r4, A6:U7	−50 ± 1.0	−42 ± 0.9	13.11
r5, U5:A8	−50 ± 0.8	−46 ± 1.1	13.44
r5, A6:U7	−49 ± 0.1	−44 ± 1.1	13.14

[a] Units of $^{n}\Delta A = \delta_A\{^1H3\} - \delta_A\{^2H3\}$ are in ppb, and units of δ_{H3} are in ppm relative to internal DSS. [b] Shown here are values from two separate data sets and the uncertainties in the average values ($=^1/_2|x_1 - x_2|$). [c] Values and uncertainties for the equivalent A:T base pairs (DNA sequences **d1**, **d2**, **d3**, **d4**, and **d5**) are taken from Vakonakis et al.[10] [d] Not available due to resonance overlap.

hydrogen-bond strength and equal zero in the absence of a hydrogen bond. The average $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ values of −53 and −47 ppb then imply that an A:U N1···N3 RNA hydrogen bond is about 3% stronger than that of a DNA A:T hydrogen bond.

It may be that RNA hydrogen bonds are slightly shorter than those of DNA. However, to the best of our knowledge, there are no reports of hydrogen-bond length differences between A-form and B-form duplexes. We analyzed the highest resolution X-ray crystal structures available of RNA duplexes with A:U base pairs (PDB ID codes 434D, 1.16 Å resolution; 466D, 1.16 Å; 397D, 1.3 Å; 1QCO, 1.9 Å) and those of DNA duplexes with A:T base pairs (1D8G, 0.74 Å; 1ENN, 0.89 Å; 1EN8, 0.98 Å; and 1EN9, 0.98 Å). A comparison of N1···N3 distances of A:U and A:T base pairs of these RNA and DNA duplexes gave 2.821 ± 0.069 and 2.825 ± 0.032 Å, respectively. If any hydrogen-bond length differences exist, they are smaller than a few hundredths of an angstrom. Alternatively, any persistent differences in base-pair angles between the RNA and DNA studied here may be responsible for the different $^{2h}\Delta^{13}C2_{RNA}$ and $^{2h}\Delta^{13}C2_{DNA}$ values, as they would be expected to affect hydrogen-bond strength as well. Possible influences of the methyl group of thymine are not known but can be determined from measurements of $^{2h}\Delta^{13}C2_{RNA}$ in RNA duplexes containing 5-methyl-uridine residues. Ab initio calculations of $^{2h}\Delta^{13}C2$ as a function of A:T/U base-pair geometry, including base-pair dynamics,[26,27] should help resolve the physical bases of these observations.

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