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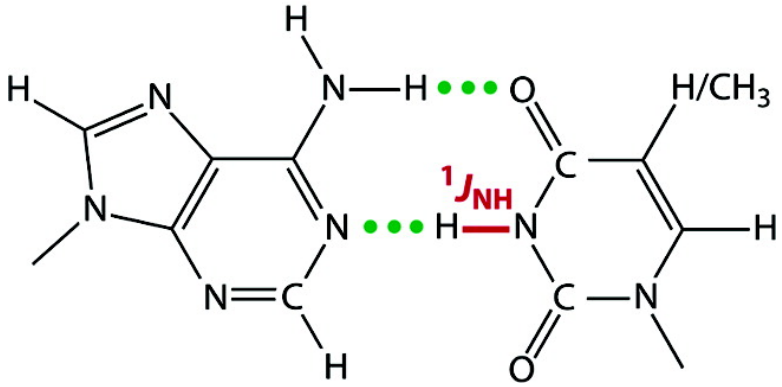
$$\left|{}^{1}J_{NH}(RNA)\right| < \left|{}^{1}J_{NH}(DNA)\right|$$



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$^1J_{NH}$ Values Show that N1···N3 Hydrogen Bonds Are Stronger in dsRNA A:U than dsDNA A:T Base Pairs

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Despite the well-established differences between RNA and DNA A- and B-form structures,[1] it is still debatable whether there are differences in the hydrogen-bond lengths or strengths of RNA and DNA. The highest resolved X-ray crystal structures reveal no differences in hydrogen-bond lengths within uncertainty.[2] It was shown in nonbase paired mononucleotides that the difference in pK_a values of rA and rU is less than that for values of dA and dT, from which it was inferred that RNA hydrogen bonds can be stronger than those of DNA.[3] However, it has been shown that pK_a values of nucleobases can shift significantly upon base pairing.[4−6] Trans-hydrogen bond deuterium isotope shifts of ^{13}C2 of adenines in Watson−Crick base pairs, $^{2h}\Delta^{13}$C2 (= δ^{13}C2{^{1}H3} − δ^{13}C2{^{2}H3}), of RNA were found to be more negative than in DNA, which implies that RNA hydrogen bonds are stronger.[2] However, recent ab initio calculations have suggested that the difference between RNA and DNA $^{2h}\Delta^{13}$C2 values merely reflects the chemical difference between uracil and thymine rather than any difference in hydrogen-bond strengths.[7]

As the evidence regarding the relative hydrogen-bond strengths of RNA and DNA are not conclusive, additional independent lines of evidence are needed. We propose to use one-bond ^{15}N−^{1}H *J*-coupling constants, $^1J_{NH}$, to help resolve this lingering question. Ab initio calculations have shown that $^1J_{NH}$ becomes less negative with decreasing hydrogen-bond length in DNA,[9] and in proteins $^1J_{NC'}$ have been shown to be sensitive to hydrogen bonding.[10] *J* couplings arise from the magnetic polarization of electrons by nuclear spins,[11] whereas $^{2h}\Delta^{13}$C2 are vibrational in origin,[12] so they are mutually independent observables. Measurement of $^1J_{NH}$ can be accomplished without isotopic enrichment, which makes the comparison of several RNA and DNA duplexes doable within reasonable budgetary limits.

$^1J_{NH}$ was measured for ^{15}N−^{1}H imino groups of five isosequential pairs of RNA and DNA duplexes at natural abundance ^{15}N by adapting the two-dimensional in-phase, anti-phase (IPAP[13]) technique to an ^{15}N-filtered, proton-detected, one-dimensional NMR experiment (Supporting Information), which allows the determination of doublet peak positions without increasing spectral complexity. Shown in Figure 1 is a plot of the isotropic chemical shifts of imino protons of RNA and DNA versus the corresponding $^1J_{NH}$ values, where it can be seen that the RNA $^1J_{NH}$ values are less negative than those of DNA. A pairwise comparison of isosequential RNA and DNA pairs shows that the difference in RNA and DNA $^1J_{NH}$ values is 0.4 ± 0.4 Hz (Supporting Information). Using the calculations by Barfield et al. for a Watson−Crick A:T base pair of a T·A:T trimer,[9] a change in $^1J_{NH}$ by +0.4 ± 0.4 Hz corresponds to a hydrogen bond distance change of approximately −0.02 ∓ 0.02 Å at an N1···N3 distance of 2.80 Å. A plot of $^{2h}\Delta^{13}$C2 versus $^1J_{NH}$ is shown in Figure 2, where it is observed that less negative $^1J_{NH}$ values coincide with more negative $^{2h}\Delta^{13}$C2 values. Stronger hydrogen bonds are predicted to respectively increase and decrease $^1J_{NH}$ and $^{2h}\Delta^{13}$C2,[14] and the trend

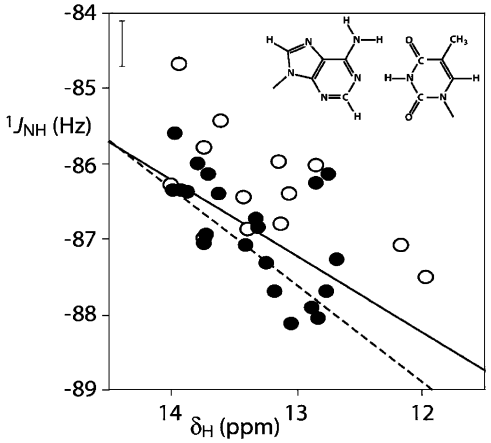

Figure 1. Plot of the isotropic chemical shift of the imino proton, δ_H, versus $^1J_{NH}$ of RNA (open circles) and DNA (solid circles). The solid black line is a linear fit to the 21 DNA data points, which yields a correlation coefficient of $r = 0.60$ and a probability of $P < 0.005$. The dashed line is a linear fit determined by Dingley et al. to data on a DNA triplex.[8] (Upper left corner): Average uncertainty in the RNA and DNA $^1J_{NH}$ values. (Upper right corner): Drawing of an A:T base pair. A correlation plot of RNA versus DNA $^1J_{NH}$ values is given in the Supporting Information.

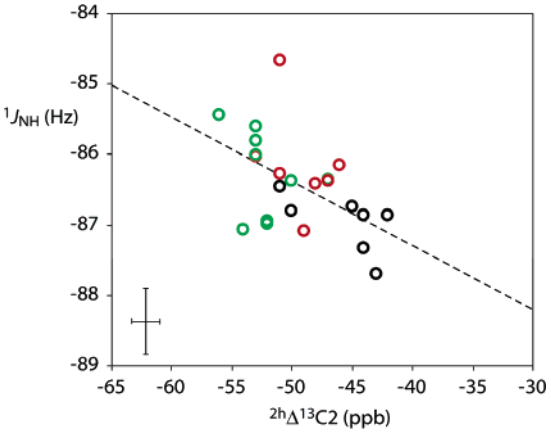

Figure 2. Plot of $^{2h}\Delta^{13}$C2 versus $^1J_{NH}$ of RNA and DNA. The dashed line is the best linear fit of the data and yields a linear correlation coefficient of $r = 0.53$ and a probability of $P < 0.02$. Black symbols denote uracil and thymine bases that have intrastrand 5′ and 3′ purine nearest neighbors. Green symbols are used for pyrimidine nearest neighbors. Red symbols are used for purine−pyrimidine and pyrimidine−purine nearest neighbors. $^{2h}\Delta^{13}$C2 values are taken from Kim et al.[19] (Lower left corner): Average uncertainty. Note that $^1J_{NH}$ is measured at the hydrogen bond donor, whereas $^{2h}\Delta^{13}$C2 is measured at the hydrogen bond acceptor.

between these two independent NMR observables further supports the notion that $^1J_{NH}$ and $^{2h}\Delta^{13}$C2 are sensitive to hydrogen-bond strengths in RNA and DNA.

It is also interesting to note that there may be a dependence of $^1J_{NH}$ and $^{2h}\Delta^{13}$C2 on context, although it is weaker for $^1J_{NH}$. When

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the uracil or thymine base is flanked by pyrimidine nearest neighbors, $^1J_{NH}$ and $^{2h}\Delta^{13}C2$ are less and more negative, respectively (Figure 2). On the other hand, when the nearest neighbors are both purines, $^1J_{NH}$ and $^{2h}\Delta^{13}C2$ are respectively more and less negative. It appears as though hydrogen-bond strength may be sequence dependent, with the strongest N1···N3 hydrogen bonds occurring in polypyrimidine:polypurine tracts. Previously, hydrogen-bond free energies have been shown to depend on context in an RNA hairpin,[15] and sequence-dependent pK_a values have been observed for single-stranded RNA and DNA.[16] The context-dependent $^1J_{NH}$ and $^{2h}\Delta^{13}C2$ values suggest that hydrogen-bonding and base-stacking interactions are coupled, which may have implications for cooperativity and long-range structure and stability. Recent calculations have suggested that $\pi-\pi$ interactions between aromatic heterocycles play a significant role in the hydrogen-bonding potential of an aromatic nitrogen base.[17] Coupling between base stacking and hydrogen bonding may contribute to the difference between N1···N3 hydrogen bond strengths of RNA (A-form stacking) and DNA (B-form stacking). A recent calculation of isolated base pairs, but with geometries found in the crystal structures of RNA and DNA duplexes, revealed no differences in the hydrogen-bond strengths of A:U and A:T dimers.[18] However, ab initio calculations of $^1J_{NH}$ and $^{2h}\Delta^{13}C2$ as a function of context will be an important test of whether they reflect a sequence dependence of hydrogen-bond strengths.

Previously, the $^1J_{NH}$ value of a deoxyribose A:U base pair in double-stranded *DNA* was found to be ~0.4 Hz less negative than that of a DNA A:T base pair,[20] which is similar to the difference we find here between RNA and DNA (Figure 1). It was suggested that the lower pK_a of uracil, as determined from monomeric 2′-deoxyuridine (pK_a = 9.3) and thymidine (pK_a = 9.8) nucleosides, results in a stronger N1···N3 hydrogen bond for A:U relative to that for A:T. However, as the authors point out,[20] pK_a values of nucleobases free in solution vis-à-vis those of nucleobases embedded within a double helix probably differ. Furthermore, it is unknown how the pK_a differs between A- and B-form duplexes. Thus, it may be premature to attribute the difference between RNA and DNA $^1J_{NH}$ values to C7 methyl group-induced pK_a shifts. The contribution of the chemical difference between uracil and thymine to the RNA and DNA $^1J_{NH}$ values reported here can be resolved either through further measurements on chemically modified RNA and DNA or ab initio calculations.

It should be noted that relaxation interference between the $^{15}N-^1H$ dipolar and ^{15}N and ^1H chemical shift anisotropy (CSA) interactions will contribute to the measured doublet splittings through the imaginary components of cross-correlation spectral density functions.[21,22] Different N1···N3 hydrogen-bond lengths will change N−H covalent bond lengths, which will change the dipolar and CSA interactions and thereby result in different contributions of the interference terms to the measured doublet splittings. Calculations have estimated that as the N1···N3 distance decreases from 2.95 to 2.65 Å, the $^{15}N3$ and $^1H3$ CSA values respectively decrease and increase from −115 to −136 ppm and 21 to 30 ppm.[23] Also, Barfield et al.[9] have calculated for a Watson−Crick A:T base pair of a T•A:T base triple that, as the N1···N3 distance decreases from 3.0 to 2.6 Å, the N−H covalent bond length increases from 1.04 to 1.08 Å. We estimate that the increase in the doublet splitting due to decreasing ^{15}N CSA is largely offset by the concomitant increase in ^1H CSA and decrease in $^{15}N-^1H$ dipolar interactions (Supporting Information). Thus, interference effects are estimated to contribute <0.1 Hz to the measured splittings.

Finally, it should be noted that the N6···O4 hydrogen bond of A:U/T base pairs was not observed in our experiments and any conclusions regarding differences in overall hydrogen-bond strengths requires consideration of both hydrogen bonds. However, a recent study has suggested that cooperativity between the N1···N3 and N6···O4 hydrogen bonds of an A:T base pair contribute 31% to the overall stability,[24] which raises the possibility that N6···O4 hydrogen bonds in RNA are stronger than those of DNA as well.

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Supporting Information Available: Table of $^1J_{NH}$ values, 1D IPAP pulse sequence with parameters, IP and AP spectra, relaxation interference calculations, and a correlation plot between isosequential pairs of $^1J_{NH}$ values of RNA and DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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