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ARTICLE

Sensitivity of hydrogen bonds of DNA and RNA to hydration, as gauged by $^1J_{NH}$ measurements in ethanol–water mixtures

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Abstract Hydrogen-bond lengths of nucleic acids are (1) longer in DNA than in RNA, and (2) sequence dependent. The physicochemical basis for these variations in hydrogen-bond lengths is unknown, however. Here, the notion that hydration plays a significant role in nucleic acid hydrogen-bond lengths is tested. Watson–Crick N1...N3 hydrogen-bond lengths of several DNA and RNA duplexes are gauged using imino $^1J_{NH}$ measurements, and ethanol is used as a cosolvent to lower water activity. We find that $^1J_{NH}$ values of DNA and RNA become less negative with added ethanol, which suggests that mild dehydration reduces hydrogen-bond lengths even as the overall thermal stabilities of these duplexes decrease. The $^1J_{NH}$ of DNA are increased in 8 mol% ethanol to those of RNA in water, which suggests that the greater hydration of DNA plays a significant role in its longer hydrogen bonds. The data also suggest that ethanol-induced dehydration is greater for the more hydrated G:C base pairs and thereby results in greater hydrogen-bond shortening than for the less hydrated A:T/U base pairs of DNA and RNA.

Keywords DNA · Ethanol · Hydration · Hydrogen-bond length · NMR · RNA

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Introduction

Hydrogen bonds are central to the structure and function of DNA and RNA (Saenger 1984). Recently, evidence was presented that suggests that hydrogen-bond lengths (1) of DNA are longer than those of RNA (Vakonakis and LiWang 2004), and (2) are sequence dependent (Manalo et al. 2005; Kim et al. 2006). However, the physicochemical basis for these observations is unclear. Our hypothesis is that hydration plays an important role in hydrogen-bond lengths of nucleic acids. We propose that the hydrogen-bond lengths of DNA are longer than those of RNA largely because it is more hydrated. Furthermore, we think that sequence dependent hydration plays an important role in modulating the hydrogen-bond lengths of DNA and RNA.

Hydrogen-bond strengths are sensitive to their environment (Perrin and Nielson 1997) and have been shown to increase in nonaqueous solvents relative to water for small molecules (Shan and Herschlag 1996). It is well recognized that water plays a significant role in the structure and stability of DNA and RNA (Saenger 1984; Westhof 1988; Jeffrey and Saenger 1991). RNA has been shown to have more structured water molecules in the major and minor grooves and around the ribose O2′ (Egli et al. 1996), although DNA is more hydrated overall (Chalikian et al. 1999; Kankia and Marky 1999). In addition, hydration of DNA is sequence dependent. Volumetric measurements (Chalikian et al. 1999) and computational studies (Feig and Pettitt 1999) found that GC-rich stretches are more hydrated than AT-rich tracts. Computational investigations also predict that electrostatic hydration free energies of DNA generally decrease as

poly(A) > poly(AT) > GC-rich (Elcock and McCammon 1995). Furthermore, the tendency of DNA duplexes to adopt the A-form conformation under low water activity is sequence dependent and goes as poly(G) > poly(AT) > poly(A) (Peticolas et al. 1988). We think that these sequence-dependent hydration patterns promote hydrogen-bond shortening in the same order. Indeed, an analysis of high-resolution X-ray crystal DNA and RNA structures has revealed that G:C hydrogen bonds are longer than those of A:T/U (Dingley and Grzesiek 1998; Dingley et al. 1999; Barfield et al. 2001): N1...N3 distances for G:C and A:T/U base pairs are 2.92 ± 0.05 and 2.81 ± 0.05 Å for DNA and 2.91 ± 0.08 and 2.83± 0.06 Å for RNA, respectively.

Empirical and computational $^{2h}J_{NN}$, $^{1}J_{NH}$, and δ_H measurements also indicated that N1...N3 hydrogen-bond lengths of Watson–Crick G:C base pairs are longer than those of A:T/U base pairs in both DNA and RNA (Dingley and Grzesiek 1998; Dingley et al. 1999; Barfield et al. 2001). Empirically, $^{2h}J_{NN}$ and $^{1}J_{NH}$ are strongly correlated with the isotropic chemical shift of the imino proton, δ_H, regardless of the type of base pair (Dingley et al. 1999). This correlation is reproduced by density functional calculations, and is the result of a strong dependence of $^{2h}J_{NN}$, $^{1}J_{NH}$, and δ_H on hydrogen-bond length and not on the chemical differences between the different base pair types (Barfield et al. 2001).

An advantage of $^{1}J_{NH}$ over $^{2h}J_{NN}$ is that measurements of several synthetic DNA and RNA duplexes can be achieved without ^{15}N isotopic enrichment. We recently developed a new one-dimensional NMR experiment optimized for the measurement of $^{1}J_{NH}$ of imino groups of DNA and RNA at ^{15}N natural abundance (Manalo et al. 2005). The $^{1}J_{NH}$ values are measured from the separation of the imino proton doublet components, which are partitioned into separate subspectra using the IPAP approach (Ottiger et al. 1998).

The addition of organic solvents reduces water activity and thereby the available water for hydration. Ethanol is widely used as a cosolvent to lower water activity (Hallsworth and Nomura 1999) and is known to dehydrate DNA (Ivanov et al. 1974; Umehara et al. 1990; Jeffrey and Saenger 1991). Also, molecular dynamics simulations predict that ethanol displaces water molecules from the primary hydration layer of DNA (Cheatham et al. 1997; Sprous et al. 1998). Thus, to test the notion that hydration plays an important role in hydrogen-bond lengths of nucleic acids, we compare $^{1}J_{NH}$ values of imino groups of canonical Watson–Crick A:T/U and G:C base pairs of DNA and RNA (Fig. 1) measured in water and dilute ethanol–water mixtures. $^{1}J_{NH}$ data of imino groups

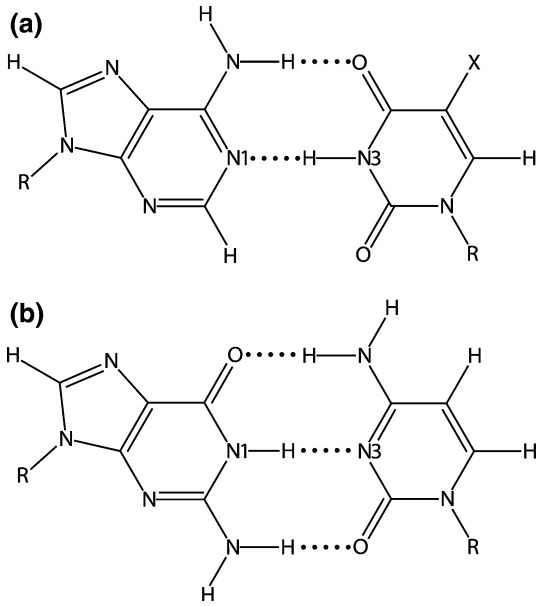

Fig. 1 Canonical Watson–Crick (**a**) A:T (X = CH_3), A:U (X = H) and (**b**) G:C base pairs

were collected for the following five isosequential pairs of self-complementary DNA and RNA dodecamers in a buffer with ethanol at a concentration of 8 mol% (~22% v/v at 25°C): $d(CGCGAATTCGCG)_2$ and $r(CGCGAAUUCGCG)_2$, $d(CGTTTTAAAACG)_2$ and $r(CGUUUUAAAACG)_2$, $d(CGAAAATTTTCG)_2$ and $r(CGAAAAUUUUCG)_2$, $d(CGTATATATACG)_2$ and $r(CGUAUAUAUACG)_2$, and $d(CGCGTATACGCG)_2$ and $r(CGCGUAUACGCG)_2$, which will be, respectively, referred to as **d1** and **r1**, **d2** and **r2**, **d3** and **r3**, **d4** and **r4**, and **d5** and **r5**.

Materials and methods

The DNA samples were purchased from Integrated DNA Technologies (Coralville, IA) and the RNA samples were purchased from Dharmacon (Lafayette, CO). All NMR samples contained 125 mM NaCl, 50 mM NaH_2PO_4, 0.75 mM EDTA, 0.2 mM DSS, 0.02% NaN_3, pH 7. DNA and RNA concentrations for NMR ranged from 1.7–4.3 and 2.1–3.7 mM duplex, respectively.

Circular dichroism experiments

Circular dichroism (CD) spectra of DNA in water and 8 mol% ethanol were acquired on an AVIV 62 DS spectrometer. CD spectra of RNA in water and 8 mol% ethanol were acquired on an AVIV 202 SF spectrometer. The concentration of all samples was 5 μM duplex. The temperature was set at 25°C and the

bandwidth was 1.0 nm. The wavelength was scanned from 320 to 220 nm with a step resolution of 0.5 nm. The averaging time and settling time were both set to 1 s. Two scans were collected and averaged for each sample. The CD spectrum of the buffer was subtracted from the CD spectra of the samples to obtain the response of the DNA or RNA alone.

T_m experiments

The pre-annealed DNA and RNA samples were diluted into the appropriate buffer to 2.5 μM duplex concentration and loaded into capped cuvettes tightly sealed with Teflon tape. The samples were then subjected to thermal denaturation monitored on a Cary 1 UV/VIS spectrophotometer equipped with a temperature controller. Up-melts were performed using a temperature ramp rate of 0.5°C/min from 5 to 80°C. The resulting melting profiles ($\partial A/\partial T$ versus T) acquired at 260 and 280 nm were simultaneously fit to a multiple sequential interacting transition unfolding model using the ''t-melt'' fitting program (Theimer et al. 1998) to obtain T_m values.

NMR spectroscopy

All NMR experiments were carried out at 11.7 T and 25°C sample temperature. All spectra were referenced relative to internal DSS (Wishart et al. 1995). Spectra were processed using nmrPipe and analyzed using nmrDraw (Delaglio et al. 1995). $^1J_{NH}$ splittings were measured at ^{15}N natural abundance using an ^{15}N-filtered, ^{1}H-detected, one-dimensional, in-phase/anti-phase (IPAP) pulse sequence (Manalo et al. 2005). This IPAP experiment allows the separate recovery of individual ^{15}N-coupled proton doublet components in the directly detected dimension by simple addition and subtraction of the IP and AP spectra (IP ± AP). Thus, the spectral complexity of each IPAP spectrum is identical to that of a ^{15}N-decoupled spectrum. Gradient coherence selection is used to suppress the 2×269-times larger ^{14}N-attached proton signals.

Carrier positions for ^{1}H and ^{15}N were 4.7 and 155 ppm, respectively. Spectra were collected using an acquisition time of 200 ms and a recovery delay of 1.35 s. The size of free induction decay was 2,000 complex points. The final digital resolution was 1.2 Hz/pt. In-phase and anti-phase free-induction decays were interleaved every 512 scans with 53,248 scans each for a total of 46.7 h per data set. Between three and 16 data sets were collected for each sample. Shown in Fig. 2 are IPAP spectra of **d2** and **r2** collected in 8 mol% ethanol.

Results and discussion

CD spectra of A- and B-form duplexes are distinct (Ivanov et al. 1973; Gray et al. 1992; Wang et al. 1992) as a result of significantly different base–base interactions (Johnson and Tinoco 1969). B-type duplexes display both positive and negative lobes of approximately equal intensity centered at ~260 nm with the positive lobe at longer wavelengths. CD spectra of A-type duplexes, on the other hand, are dominated by a large positive lobe. CD is also sensitive to small variations in secondary structure of nucleic acids. Shown in Fig. 3 are CD spectra of DNA and RNA in water and in 8 mol% ethanol. The CD spectra reveal that in 8 mol% ethanol the DNA and RNA duplexes maintain their respective B- and A-form conformations. Furthermore, imino proton spectra for the samples in 8 mol% ethanol are similar to those in water (Supplemental Figures 1 and 4). Thus we can gauge changes in the Watson–Crick hydrogen bonds due to this ethanol perturbation in the absence of significant structural changes.

Shown in Fig. 4 is a correlation plot of $^1J_{NH}$ between corresponding residues of isosequential pairs

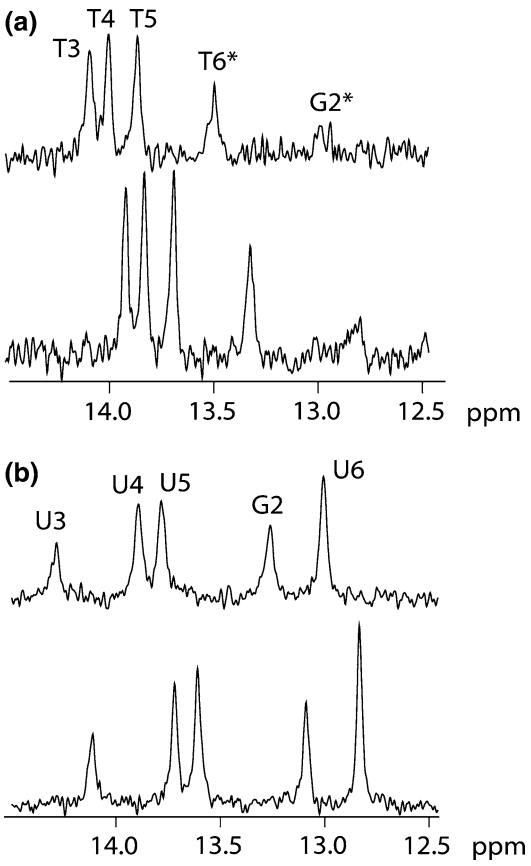

Fig. 2 One-dimensional ^{15}N-coupled ^{1}H IPAP spectra of the imino region of (**a**) **d2** and (**b**) **r2** in 8 mol% ethanol. Residues for which splittings were not measured are labeled with asterisks

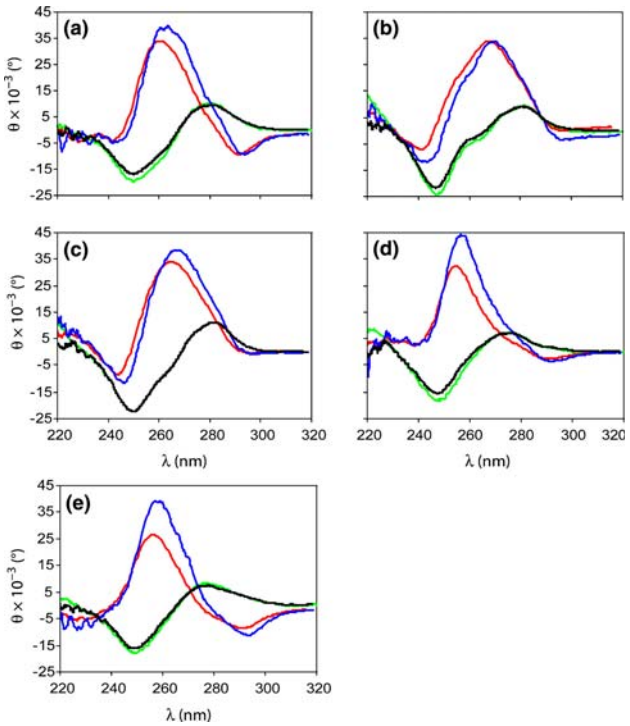

Fig. 3 Circular dichroism spectra of DNA and RNA in water and 8 mol% ethanol. The panels correspond to (**a**) **1**, (**b**) **2**, (**c**) **3**, (**d**) **4**, and (**e**) **5**. Black, green, red, and blue colors are used for DNA in water, DNA in 8 mol% ethanol, RNA in water, and RNA in 8 mol% ethanol

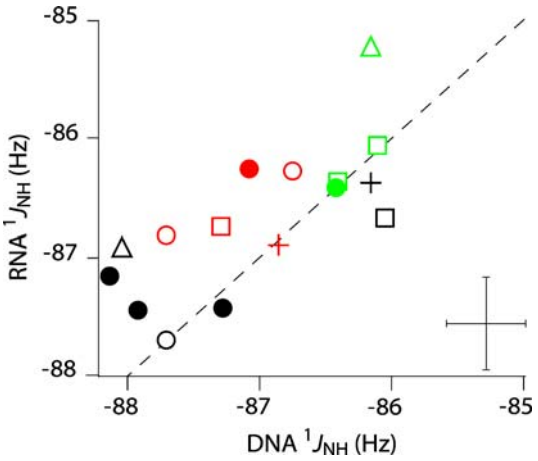

Fig. 4 Correlation plot of $^1J_{NH}$ values between corresponding residues of isosequential pairs of DNA and RNA duplexes in aqueous buffer. Solid circles, open squares, triangles, ''+'' symbols, and open circles are used for $^1J_{NH}$ values of **d1** and **r1**, **d2** and **r2**, **d3** and **r3**, **d4** and **r4**, and **d5** and **r5**, respectively. Green is used for $^1J_{NH}$ values of U or T with a U or T 3′ nearest neighbor. Red is used for $^1J_{NH}$ values of U or T with any other 3′ nearest neighbor. Black is used for $^1J_{NH}$ values of G residues. The mean pairwise difference is 0.3 ± 0.5 Hz and a paired Student's *t*-test yields a *P* value of 0.025. The average uncertainties for the DNA and RNA measurements are shown in the lower right corner of the plot. The dashed line is along the diagonal. Significantly more data sets were collected here relative to a similar plot published earlier (Manalo et al. 2005)

of DNA and RNA in aqueous buffer. The $^1J_{NH}$ values of RNA are 0.3 ± 0.5 Hz less negative than those of DNA, as given by the mean pairwise difference. A paired Student's *t*-test gives a value of 0.025, which suggests that this difference is statistically significant. The calculated dependence of $^1J_{NH}$ on the N1...N3 distance for A:T and G:C base pairs by Barfield et al. (2001) predicts that RNA A:U hydrogen bonds are shorter than those of DNA A:T by 0.02 ± 0.02 Å. Similarly, RNA G:C hydrogen bonds are shorter than those of DNA by 0.02 ± 0.04 Å. These values are consistent with the 0.04 ± 0.03 Å difference predicted from trans-hydrogen bond deuterium isotope shifts (Kim et al. 2006).

Consistent with earlier observations, there is a dependence of the $^1J_{NH}$ values on sequence (Dingley and Grzesiek 1998; Dingley et al. 1999). We find that $^1J_{NH}$ of Gs are more negative than those of Ts and Us of DNA and RNA: –87.4 ± 0.8 and –87.1 ± 0.5 Hz for Gs of DNA and RNA, and –86.6 ± 0.6 and –86.3 ± 0.5 Hz for Ts and Us of DNA and RNA, respectively (Supplemental Table 1). Using the calculations of Barfield et al. (2001), these differences in $^1J_{NH}$ values suggest that the N1...N3 hydrogen-bond lengths of DNA A:T and RNA A:U base pairs are, respectively, shorter than those of G:C by 0.06 ± 0.05 and 0.05 ± 0.03 Å. Crystallographically, these differences are estimated to be 0.11 ± 0.07 and 0.08 ± 0.10 Å for DNA and RNA (Dingley and Grzesiek 1998; Dingley et al. 1999; Barfield et al. 2001), respectively, and are consistent with our $^1J_{NH}$ measurements. The least negative $^1J_{NH}$ values are observed for Ts and Us with 3′ T and U nearest neighbors, which is similar to the sequence dependence observed for trans-hydrogen bond deuterium isotope shifts (Kim et al. 2006).

Shown in Fig. 5 are $^1J_{NH}$ values of DNA and RNA in H_2O plotted against the corresponding values measured in 8 mol% ethanol. On average, $^1J_{NH}$ values measured in 8 mol% ethanol are 0.2 ± 0.6 Hz less negative than those measured in water. A paired Student's *t*-test yields a *P* value of 0.029, which suggests that this change in $^1J_{NH}$ values is statistically significant. Using the calculations of Barfield et al. (2001), the 0.2 ± 0.6 Hz increase in $^1J_{NH}$ upon the addition of ethanol corresponds to an average decrease in the N1...N3 hydrogen-bond distance of 0.01 ± 0.03 Å. Such a hydrogen-bond shortening may be driven by ethanol-induced dehydration of the DNA and RNA duplexes.

It appears as though the ethanol perturbation increases $^1J_{NH}$ values of Gs more than Ts and Us. Linear regression fits to the DNA and RNA data in Fig. 5

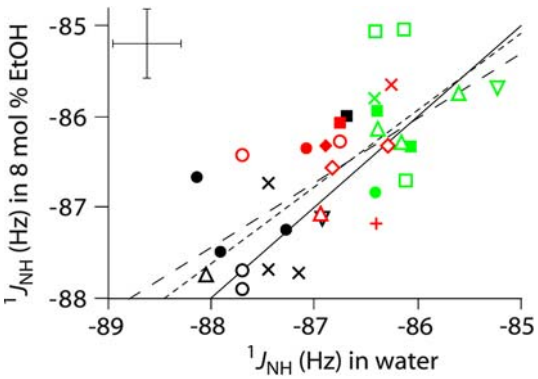

Fig. 5 Correlation plot of $^1J_{NH}$ values of DNA and RNA duplexes measured in water and 8 mol% ethanol. Solid circles, " × " symbols, open squares, solid squares, triangles, inverted triangles, "+" symbols, solid diamonds, open circles, and open diamonds are used for $^1J_{NH}$ values of **d1**, **r1**, **d2**, **r2**, **d3**, **r3**, **d4**, **r4**, **d5**, and **r5**, respectively. Green is used for $^1J_{NH}$ values of U or T with a U or T 3′ nearest neighbor. Red is used for $^1J_{NH}$ values of U or T with any other 3′ nearest neighbor. Black is used for $^1J_{NH}$ values of G residues. The long- and short-dashed lines are from linear fits to the DNA and RNA data, respectively. A solid line is drawn along the diagonal. The average uncertainty in each dimension is shown in the upper left-hand corner

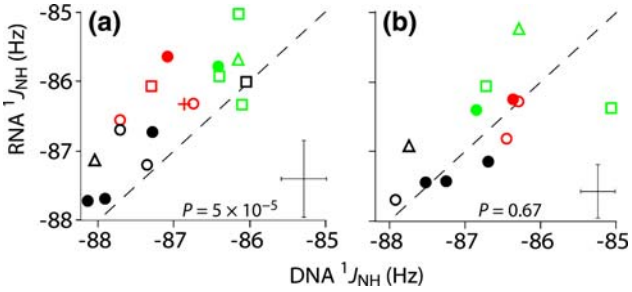

Fig. 6 Plots of $^1J_{NH}$ values between corresponding residues of isosequential pairs of DNA and RNA in water and 8 mol% ethanol. (**a**) DNA in water and RNA in 8 mol% ethanol, and (**b**) DNA in 8 mol% ethanol versus RNA in water. Symbols and colors are defined in the caption to Fig. 4. The dashed lines are along the diagonal. The average uncertainties in $^1J_{NH}$ values are shown in the lower right-hand corners. The *P* values from paired Student's *t*-tests of these data are given at the bottom of each plot

have slopes <1.0 with $r = 0.7$ and $p < 0.005$. This observation suggests that G:C base pairs, which have been shown to be more hydrated (Elcock and McCammon 1995; Chalikian et al. 1999; Feig and Pettitt 1999), undergo more ethanol-induced dehydration and thus hydrogen-bond shortening than A:T/U base pairs. The observation is consistent with the hypothesis that hydrogen-bond lengths are sensitive to local hydration.

The difference between the $^1J_{NH}$ values of DNA and RNA is enhanced upon the addition of ethanol to RNA (Fig. 6a). The data imply that dehydration of RNA leads to further hydrogen-bond shortening and thus a greater discrepancy with the hydrogen-bond lengths of DNA in water. In contrast, there is no statistical difference between $^1J_{NH}$ values of DNA in 8 mol% ethanol and those of RNA in water (Fig. 6b), which suggests that the hydrogen bonds of partially dehydrated DNA can be as short as those of A-form RNA while still maintaining the B-form secondary conformation. These observations support the notion that the higher degree of hydration of DNA relative to RNA contributes significantly to its longer hydrogen bonds.

Shown in Supplemental Figure 2 are the melting temperatures (T_m) of the DNA and RNA duplexes as a function of ethanol concentration. As can be seen from the figure, the addition of ethanol lowers the melting temperatures of the DNA and RNA duplexes, which is due to a reduction of hydrophobic aromatic stacking between adjacent bases (Albergo and Turner 1981; Guckian et al., 2000). Thus, the addition of ethanol to solutions of DNA and RNA appears to have opposite effects on the two major (and largely orthogonal) weak interactions in DNA and RNA: reducing hydrophobic aromatic stacking while shortening base-pair hydrogen bonds.

A thermodynamic comparison of isosequential 12 base-pair duplexes found that RNA was more stable than deoxyuridine-substituted DNA by 3.8 kcal/mol and that 5-methyl cytosine substituted RNA was more stable than DNA by 4.7 kcal/mol, which gives an average of 0.4 kcal/mol more stabilization per base pair for RNA (Wang and Kool 1995). Density functional theory (DFT) calculations with basis set superposition error (BSSE) correction on isolated A:T and A:U base pairs give 3 cal/mol change in total energy for a 0.01 Å change from the fully optimized N1...N3 distance of 2.88 Å (Supplemental Table 4). This calculated energy change is smaller than that reported previously (Kim et al. 2006) because for these calculations we changed the hydrogen-bond distance by 0.01 Å instead of estimating the energy change from a coarser 0.1 Å step, as was done earlier. Thus, the contribution of the 0.02 Å-shorter RNA hydrogen bonds is roughly estimated to contribute only ~2% to the 0.4 kcal/mol of extra stabilization per RNA base pair.

It is unclear how 8 mol% ethanol perturbs the hydration layers of the DNA and RNA. Water molecules have been shown to hydrate the phosphates and functional groups of Watson–Crick base pairs along the major and minor grooves of DNA (Saenger 1984; Westhof 1988; Jeffrey and Saenger 1991). In addition to the primary layer, there is at least a second hydra-

tion layer. Studies show that the residence times of water molecules in the minor groove of B-form DNA are significantly longer than for those in the major groove (Phan et al. 1999), and suggest that the spine of hydration in the minor groove stabilizes the B-form conformation of DNA (Dickerson et al. 1982; Lan and McLaughlin 2001). As the native B-form conformation of the DNA is preserved in the 8 mol% ethanol–water mixture (Fig. 3), we anticipate that the stabilizing waters in the minor groove are not perturbed significantly by this concentration of ethanol. In addition, hydration of the phosphate groups are distinctly different for the A- and B-form conformations (Dickerson et al. 1982), which leads us to suspect that the primary layer of hydration of the phosphate groups is also not significantly perturbed in 8 mol% ethanol.

Other factors besides hydrogen bonding can affect $^1J_{NH}$ values. Solvent effects can be quite substantial (Barfield and Johnston 1973), and it has been shown that $^1J_{NH}$ of substituted pyrroles varied up to 1 Hz between various solvents (King et al. 1976). We do not know the solvent effect of the added ethanol on the $^1J_{NH}$ values measured here, but the NMR and CD spectra suggest that in 8 mol% ethanol the DNA and RNA structures are fairly similar to those in water. Therefore, we assume that electronic changes to the purine and pyrimidine bases due to direct interactions with ethanol are negligible, and that ethanol-induced shifts in $^1J_{NH}$ are predominantly due to changes in hydrogen-bond lengths.

The possibility that $^1J_{NH}$ depends on base pair type was another concern. However, $^1J_{NH}$ of imino groups of different base pair types of a DNA triplex was found to follow the same linear trend with respect to the isotropic chemical shift of the corresponding imino proton (Dingley et al. 1999). In addition, DFT calculations predicted that $^1J_{NH}$ of imino groups of A:T, A:U, and G:C base pairs have the same dependence on the N1...N3 hydrogen-bond length (Barfield et al. 2001). Finally, it cannot be excluded that differences in base stacking between DNA and RNA, and the reduced base-stacking propensity in the presence of ethanol, may affect the contribution of susceptibility-induced magnetic alignment to the measured splittings.

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