Journal of Biomolecular NMR **29:** 65–72, 2004.

Trans-hydrogen bond deuterium isotope effects of A:T base pairs in DNA

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Received 21 October 2003; Accepted 25 November 2003

Key words: Hydrogen bond, Watson-Crick, DNA, deuterium isotope effect, vibrational potential well, chemical shift

Abstract

The chemical shifts of ^{13}C2 of adenosine residues of DNA were observed to experience a through-space or trans-hydrogen bond isotope effect as a result of deuterium substitution at the imino hydrogen site of base-paired thymidine residues. NMR measurements of several self-complementary DNA duplexes at natural abundance ^{13}C in 50% H_2O, 50% D_2O solvent mixtures yielded an average trans-hydrogen bond isotope effect, $^{2h}\Delta^{13}$C2, of -47 ppb. The data suggest that stronger hydrogen bonds have more negative $^{2h}\Delta^{13}$C2 values, which means that A:T N1$\cdots$H3 hydrogen bonds increase the anharmonicity of the effective vibrational potential of H3. However, $^{2h}\Delta^{13}$C2 values do not correlate with intra-residue $^{2}\Delta^{13}$C4 values of thymidine observed here and earlier (Vakonakis et al., 2003), which suggests that $^{2h}\Delta^{13}$C2 is not determined entirely by hydrogen bond strength. Instead, the variations observed in $^{2h}\Delta^{13}$C2 values suggest that they may also be sensitive to base pair geometry.

Introduction

Hydrogen bonds are central to the structure and function of nucleic acids (Saenger, 1984; Jeffrey and Saenger, 1991). Characterization of biomolecular hydrogen bonds using NMR, once limited to mostly chemical shift analysis (Wagner et al., 1983) and measurement of hydrogen/deuterium exchange (Hvidt and Nielson, 1966; Roder et al., 1985), has advanced significantly in recent years. New experiments include measurements of trans-hydrogen bond scalar couplings (Dingley and Grzesiek, 1998; Pervushin et al., 1998; Cordier et al., 1999; Dingley et al., 1999), chemical shift anisotropies (Tjandra and Bax, 1997; Czernek et al., 2000), deuterium quadrupole couplings (Boyd et al., 1997; LiWang and Bax, 1997), and equilibrium deuterium/protium fractionation factors (LiWang and Bax, 1996; Coman and Russu, 2003; Vakonakis et al., 2003). However, the information provided by the different approaches is not completely redundant. For instance, $^{1}H^{N}$ CSA values describe the distortion of the shielding surface as a function of hydrogen bond length, and fractionation factors report on vibrational aspects of hydrogen-bonded groups. Trans-hydrogen bond scalar couplings reflect the extent of electron delocalization across the hydrogen bond, whereas deuterium quadrupole couplings gauge changes in electric field gradients due to hydrogen bonds.

Deuterium isotope effects (DIE) on the chemical shifts of ^{13}C nuclei two bonds removed from the site of isotopic substitution, defined as $^{2}\Delta^{13}C = \delta^{13}C(H) - \delta^{13}C(D)$, in inter- and intramolecularly hydrogen-bonded small molecules have been shown to have linear correlations with the isotropic chemical shift of the hydrogen-bonded proton in a variety of small compounds (Reuben, 1986; Dziembowska et al., 1997). It is now well established that $^{2}\Delta^{13}$C can be sensitive indicators of hydrogen bond strength in small molecules (Reuben, 1987; Tüchsen and Hansen, 1991; Hansen et al., 1992; Jameson, 1996; Perrin and Nielson, 1997; Abildgaard et al., 1998; Ruud et al., 2001). Recently, we reported intramolecular two-bond DIE on the chemical shifts of the thymidine ^{13}C2 and ^{13}C4 nuclei, $^{2}\Delta^{13}$C2 and $^{2}\Delta^{13}$C4, of Watson-Crick base paired DNA (Vakonakis et al., 2003). It was observed

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that increasing values of $^{2}\Delta^{13}$C2 and $^{2}\Delta^{13}$C4 correlate with increasing values of δ_{H3}, which indicates that $^{2}\Delta^{13}$C2 and $^{2}\Delta^{13}$C4 depend largely on the N1$\cdots$H3 hydrogen bond strength. It was also observed that equilibrium deuterium/protium fractionation factors, Φ, of the imino site decrease with increasing δ_{H3}. Together, the data indicated that as an A:T N1$\cdots$H3 hydrogen bond becomes stronger, the effective vibrational potential well of the imino hydrogen widens with a corresponding increase in anharmonicity.

The first measurement of a trans-hydrogen bond DIE in DNA was on a self-complementary hexamer specifically ^{15}N labeled at a single position on the adenosine residue, d[CGT(^{15}N1)ACG] (Wang et al., 1991). The ^{15}N1 resonance shifts downfield by $\sim$0.34 ppm as a result of deuterium substitution at the thymidine H3. In H_2O/D_2O solvent mixtures, additional shifts from the isotopomers of the adenosine amino group result in an overall quintet splitting pattern of ^{15}N1. No other trans-hydrogen bond DIE have been reported in DNA or RNA to our knowledge. As there are no theoretical treatments of the effect, our understanding of the physical basis is incomplete. It was not clear *a priori* whether trans-hydrogen bond DIE would be dominated by hydrogen bond strength, or also by other factors such as base pair geometry, which necessitated an empirical approach.

Here, we report N1$\cdots$H3 trans-hydrogen bond imino DIE on the ^{13}C2 chemical shifts of Watson-Crick base paired adenosine residues, $^{2h}\Delta^{13}$C2 ($= \delta_{C2}\{^{1}H3\} - \delta_{C2}\{^{2}H3\}$), of five DNA dodecamers at natural abundance ^{13}C. It was hoped that observation of DIE at ^{13}C2 would result in simpler line shapes than those of ^{15}N1 and remove the necessity for isotope enrichment. Well-studied, self-complementary dodecameric DNA duplexes were used for this study: $[d(CGCGAATTCGCG)]_2$, $[d(CGTTTTAAAACG)]_2$, $[d(CGAAAATTTTCG)]_2$, $[d(CGTATATATACG)]_2$ and $[d(CGCGTATACGCG)]_2$ which will be referred to as **1**, **2**, **3**, **4** and **5**, respectively. The sequences were chosen based upon their structural and dynamic differences at the A:T base pairs. Studies have shown that the A:T base pairs of poly-A tracts have longer base pair lifetimes (Patel et al., 1983; Leijon and Gräslund, 1992; Nuutero et al., 1994) and should possess a higher propeller twist relative to those at TpA steps (Yoon et al., 1988; Yanagi et al., 1991; Shui et al., 1998), leading to a variety of base pair geometries available for study in our samples.

Materials and methods

All experiments described here were performed on non-isotopically enriched samples of **1**, **2**, **3**, **4** and **5** equilibrated in a solvent mixture of 50% H_2O and 50% D_2O. Samples of deoxy-adenosine 5′-monophosphate (dAMP) in 100% H_2O or 100% D_2O were also used. Proton resonance assignments were based on WATERGATE NOESY spectra of the dodecamers and on earlier work (Gupta et al., 1988; Sarma et al., 1988; Sklenár et al., 1993). Assignments of ^{13}C aromatic chemical shifts of thymidine and adenosine residues were determined from a combination of one-bond and long-range ^{1}H, ^{13}C heteronuclear single-quantum correlation (HSQC) spectra of samples in D_2O (Bax et al., 1996). All chemical shifts were referenced to internal DSS (Markley et al., 1998).

Measurement of $^{2}\Delta^{13}$C4 was performed as described earlier (Vakonakis et al., 2003). To measure $^{2h}\Delta^{13}$C2, we acquired ^{1}H, ^{13}C gradient enhanced TROSY-HSQC spectra correlating adenosine ^{1}H2 with ^{13}C2 (Pervushin et al., 1997; Brutscher et al., 1998). Correlations of ^{1}H2, ^{13}C2 in identically acquired one-bond ^{1}H, ^{13}C HMQC spectra were significantly broader and weaker with respect to those in TROSY-HSQC spectra (Figure 1). This is in contrast to pyrimidine ^{1}H6, ^{13}C6 correlations, which were weak (Figure 1B). In addition, partial overlap of the in-phase 'doublet' components in the HMQC spectra leads to a systematic underestimation of the true splittings by approximately 8 ppb, as determined from simple lineshape simulations. In contrast, lineshape simulations of the TROSY-HSQC spectra indicate that over the range of splittings observed here there is no systematic deviation between true splittings and $^{2h}\Delta^{13}$C2 values determined by 3 point interpolation.

All experiments were conducted at 14.1 T static field strength (600 MHz ^{1}H frequency) and a sample temperature of 25 °C. Chemical shift evolution for the oligonucleotides was 50 ms along t_2 (300*, n* represents n complex data points) and 181 ms along t_1. Spectral widths along t_1 varied between 528 Hz (96*) and 600 Hz (110*). An exponential 1 Hz line narrowing function was applied along the t_1 dimension during processing. Final digital resolutions were 5.9 and 1.8 Hz along $F2$ and $F1$, respectively. Each spectrum was collected in approximately 20 h, used an interscan delay of 2.5 s, and a delay of 3.3 ms for each INEPT dephasing period. The chemical shifts of dAMP in 100% H_2O were measured using a concentric NMR tube in which the solution of dAMP

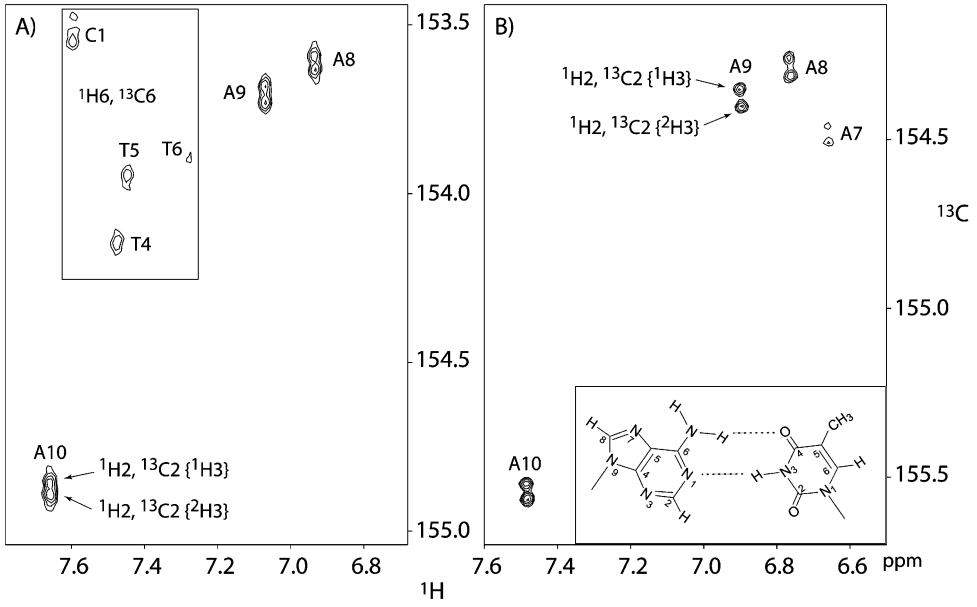

Figure 1. Region of (A) the ^{1}H, ^{13}C HMQC spectrum and (B) the ^{1}H, ^{13}C TROSY-HSQC spectrum of **2** collected at 14.1 *T* and 25 °C. The spectra are plotted at the same signal-to-noise ratio and contouring interval. The ^{1}H, ^{13}C HMQC spectrum was collected in approximately 96 h while the ^{1}H, ^{13}C TROSY-HSQC spectrum was acquired in less than 20 h. 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. Residue A7 is at the TpA step and is much weaker, probably due to conformational exchange. The inset is a diagram of an A:T base pair.

was placed in the outer chamber and D_2O for field-frequency locking was placed in the inner chamber. Data of dAMP were acquired using ^{1}H, ^{13}C HMQC experiment as two-dimensional matrices of 600* × 120* points along t_2 (100 ms) and t_1 (398 ms), respectively. Sample concentrations were 100 mM for dAMP and varied between 2.5–3.0 mM duplex for the oligonucleotides. Buffer conditions were 125 mM NaCl, 50 mM NaH_2PO_4, 0.75 mM EDTA, 0.2 mM DSS, 0.02% NaN_3, pH 7. Peak positions were determined using polynomial interpolation with the program PIPP (Garrett et al., 1991).

Results

As exchange with solvent was slow for the iminos of the A:T base pairs, under the experimental conditions used here, $^{2h}\Delta^{13}$C2 were observed as splittings of the ^{1}H2, ^{13}C2 correlations in the ^{13}C dimension by −47 ppb on average (Table 1). The upfield and downfield 'doublet' components were assigned to the protonated and deuterated states of the imino site of thymidine, respectively, by acquiring similar spectra in 100% H_2O and 100% D_2O (data not shown). The downfield shift of the ^{13}C2 resonance upon deuteration of the H3 site of the base paired thymidine residue is in agreement with previous studies (Wang et al., 1991). Shown in Figure 1B is a small region of the ^{1}H, ^{13}C TROSY-HSQC spectrum of **2** in 50% H_2O, 50% D_2O.

The ^{13}C2 DIE for dAMP is +17 ppb, as measured from samples in 100% H_2O and 100% D_2O (Table 2). Likely, this positive DIE is the result of both isotopic substitution at the exchangeable amino group and solvent effects on vibrational amplitudes. Similar isotope effects were also observed for the chemical shifts of ^{15}N3, ^{15}N7 and ^{15}N9 (Table 2) and agree with the chemical shift changes measured in ions in H_2O/D_2O solvents (Loewenstein et al., 1968; Radley and Reeves, 1971; Gustavsson et al., 1978). We expect solvation effects to be different between free dAMP and DNA, and thus no solvent-effect correction was applied to our DNA $^{2h}\Delta^{13}$C2 values. Further, isotopic fractionation at the amino site of adenosine in DNA can be assumed to be independent of that at the imino site of the base-paired thymidine. In the equimolar mixture of 50% H_2O, 50% D_2O the different isotopomers of the amino group would simply broaden the 'doublet' components of the ^{1}H2, ^{13}C2 correlations in the DNA spectra. Note that the sign of the ^{13}C2 DIE of dAMP is positive, opposite to that observed for base-

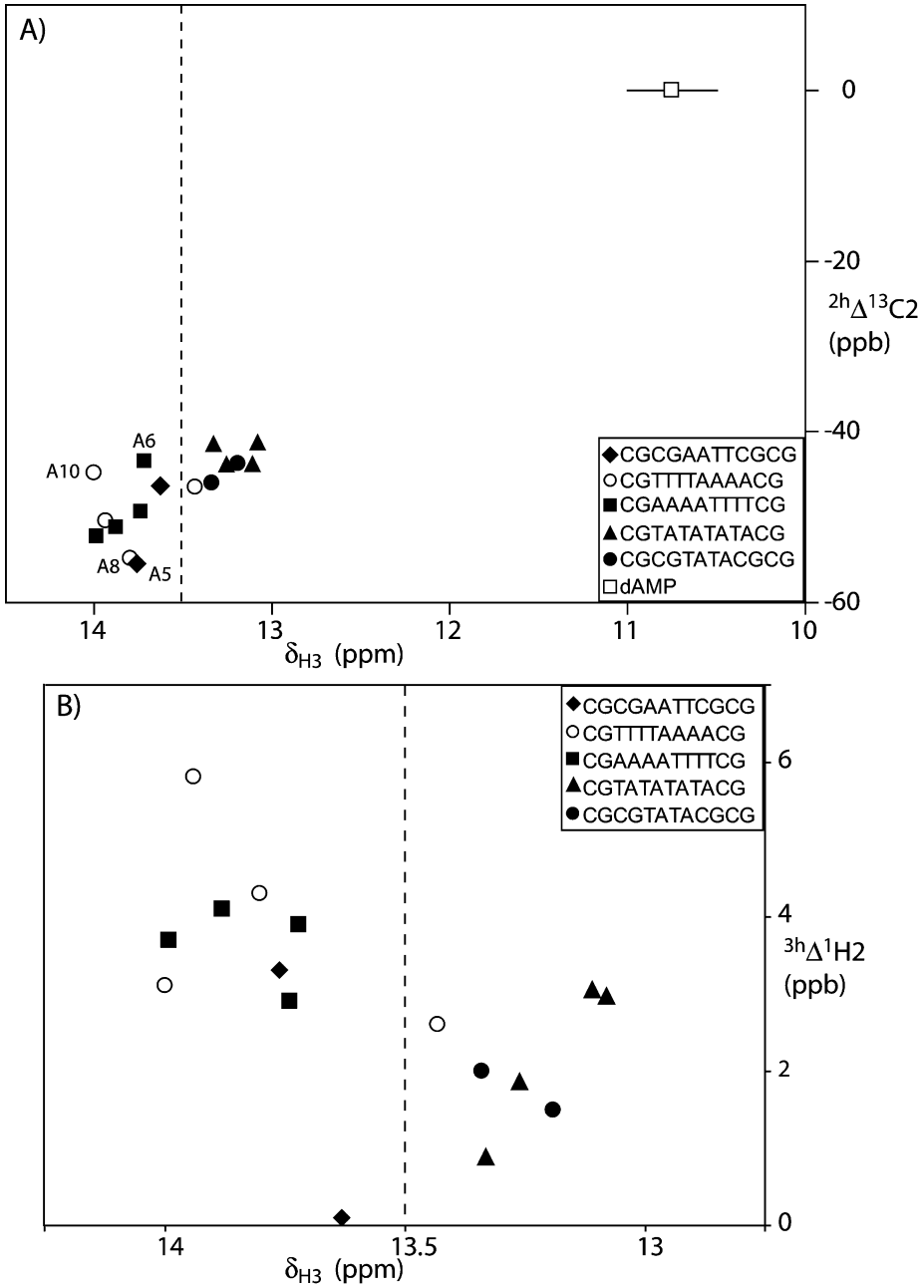

Figure 2. (A) Values of $^{2h}\Delta^{13}$C2 of adenosine residues are plotted here versus δ_{H3} of the base-paired thymidine residues. The values and uncertainties are reported in Table 1. Outliers are labeled. The vertical dashed line is placed at $\delta_{H3} = 13.5$ ppm. (B) Values of $^{3h}\Delta^{1}$H2 are plotted versus δ_{H3} of the base-paired thymidine residues. The vertical dashed line is placed at $\delta_{H3} = 13.5$ ppm.

Table 1. Spectral parameters and DIE measurements on DNA dodecamers and dAMP[a]

	$^{2h}\Delta^{13}C2$[b]	$^{2}\Delta^{13}C4$[c]	$^{3h}\Delta^{1}H2$[b]	δ_{H3}
1, A5:T8	-55 ± 1.1	168 ± 2[d]	3.3 ± 0.8	13.76[d]
1, A6:T7	-46 ± 1.1	168 ± 1[d]	0.1 ± 0.8	13.63[d]
2, T3:A10	-45 ± 1.1	173 ± 3	3.1 ± 0.8	14.00
2, T4:A9	-50 ± 1.1	170 ± 3	5.8 ± 0.8	13.94
2, T5:A8	-44 ± 1.1	169 ± 3	4.3 ± 0.8	13.80
2, T6:A7	-47 ± 1.1	166 ± 3	2.6 ± 0.8	13.43
3, A3:T10	-49 ± 1.1	163 ± 3	2.9 ± 0.8	13.74
3, A4:T9	-51 ± 1.1	176 ± 3	4.1 ± 0.8	13.88
3, A5:T8	-52 ± 1.1	175 ± 3	3.7 ± 0.8	13.99
3, A6:T7	-43 ± 1.1	180 ± 3	3.9 ± 0.8	13.72
4, T3:A10	-44 ± 1.2[e]	N.A	3.1 ± 1.9[e]	13.11
4, A4:T9	-42 ± 0.8[e]	164 ± 3	3.0 ± 0.9[e]	13.08
4, T5:A8	-44 ± 0.4[e]	N.A	1.9 ± 0.1[e]	13.26
4, A6:T7	-42 ± 0.9[e]	162 ± 3	0.9 ± 0.2[e]	13.33
5, T5:A8	-46 ± 1.1	164 ± 1	2.0 ± 0.8	13.34
5, A6:T7	-44 ± 1.1	162 ± 2	1.5 ± 0.8	13.19
dAMP / dTMP	17 ± 0.2[e]	76 ± 1[d]	9.2 ± 0.2[e]	11.0–10.5[f]

[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 for $^{2h}\Delta^{13}C2$ and $^{3h}\Delta^{1}H2$ of **1**, **2**, **3** and **5** are values from a single data set. Uncertainties were estimated from the average of the uncertainties of **4** multiplied by $\sqrt{2}$.
[c]Only one set for $^{2}\Delta^{13}C4$ was acquired for **2**, **3** and **4**. Uncertainties were roughly estimated from the average of the uncertainties of **1** and **5** multiplied by $\sqrt{5}$.
[d]Values and uncertainties are the results of five datasets and are taken from Vakonakis et al. (Vakonakis et al., 2003).
[e]Shown for $^{2h}\Delta^{13}C2$ and $^{3h}\Delta^{1}H2$ of **4** and dAMP are values from two separate data sets and the uncertainties in the average values ($= 1/2|x_1 - x_2|$). This dodecamer exhibits the lowest $|^{2h}\Delta^{13}C2|$ values and has weak $^{1}H2$, $^{13}C2$ correlations.
[f]Chemical shift from apparently non-hydrogen bonded imino protons of thymidine and uridine residues of DNA and RNA in aqueous solutions (Escaja et al., 2000; Dejong et al., 2002).

Table 2. Deuterium isotope effects on free dAMP[a]

$\Delta^{13}C2$	17 ± 0.2[b]
$\Delta^{13}C4$	-2 ± 0.3[b]
$\Delta^{13}C5$	74 ± 2[c]
$\Delta^{13}C6$	96 ± 2[c]
$\Delta^{13}C8$	16 ± 1[b]
$\Delta^{15}N1$	212 ± 2[c]
$\Delta^{15}N3$	97 ± 2[c]
$\Delta^{15}N7$	57 ± 2[c]
$\Delta^{15}N9$	55 ± 2[c]

[a]Units of $\Delta A = \delta_A(H_2O) - \delta_A(D_2O)$ are in ppb. ^{13}C chemical shifts were measured using ^{1}H, ^{13}C HMQC and HMBC experiments. ^{15}N chemical shifts were measured using long-range ^{1}H, ^{15}N HSQC experiments. Samples of non-isotopically enriched dAMP in 100% H_2O and 100% D_2O were used. Solution conditions were 100 mM dAMP, 125 mM NaCl, 50 mM NaH_2PO4, 0.75 M EDTA, 0.2 mM DSS, 0.02% NaN_3, pH 7. It is important to note that due to differences in the solvation state of adenosine in DNA and the free state, the DIE values reported here are not directly applicable for DNA.
[b]Uncertainties derived from multiple data sets.
[c]Uncertainties conservatively estimated from the digital error of the experiment to be ± 2 ppb.

paired adenosines in DNA, which indicates a different origin of the effect.

Values of $^{2h}\Delta^{13}C2$ range from -42 to -55 ppb (Table 1) while values of δ_{H3} span the narrow range of 13.08–14.00 ppm. In general, downfield-shifted $^{1}H3$ resonances are correlated with more negative $^{2h}\Delta^{13}C2$ values (Figure 2A). A linear fit to the data yielded $^{2h}\Delta^{13}C2 = -8.9\,\delta_{H3} + 74.1$ ppb with a correlation coefficient of $r = -0.67 (p < 0.005)$. All δ_{H3} values from adenosines located at TpA steps are upfield of 13.5 ppm and tend to have less negative $^{2h}\Delta^{13}C2$ values. TpA steps are known to have shorter base pair lifetimes (Leroy et al., 1988; Kennedy et al., 1993). Indeed, $^{1}H2$, $^{13}C2$ correlations of TpA steps in the TROSY-HSQC spectra had uniformly weaker intensities than those of other adenosine residues. Although the value of $^{2h}\Delta^{13}C2$ of dAMP for hydrogen-bonded water molecules could not be measured due to isotope effects at the amino group and solvent effects, we anticipate that it would be much smaller than those measured in DNA and assigned it an assumed value of zero (open square in Figure 2A). Trans-hydrogen bond isotope effects on the chemical shifts of $^{1}H2$, $^{3h}\Delta^{1}H2$, are <6 ppb but measureable. Shown in Figure 2B is a plot of $^{3h}\Delta^{1}H2$ values against corresponding δ_{H3} values. Note that $^{3h}\Delta^{1}H2$ tends to become more positive while $^{2h}\Delta^{13}C2$ becomes more negative with increasing δ_{H3}.

Intraresidue deuterium isotope effects on the chemical shifts of $^{13}C4$ (and $^{13}C2$) of thymidine residues due to isotopic substitution at the imino hydrogen, $^{2}\Delta^{13}C4$, was shown earlier to be a reporter of hydrogen bond strength in two DNA molecules (Vakonakis et al., 2003). $^{2}\Delta^{13}C4$ values measured on the additional DNA duplexes studied here show a correlation with δ_{H3} that corroborate the earlier observations (Figure 3). A linear fit to the data yielded $^{2}\Delta^{13}C4 = 13.2\,\delta_{H3} - 11.7$ ppb with a correlation coefficient of $r = 0.71 (p < 0.005)$. The measured $^{13}C4$ DIE of free dTMP is likely influenced by solvation effects similar to $^{13}C2$ DIE of free dAMP, and, thus, was not used for the linear fit.

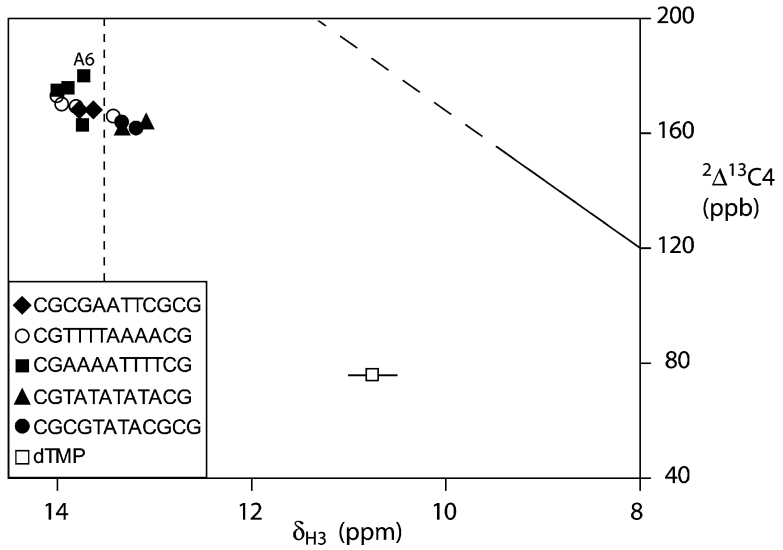

Figure 3. Values of $^2\Delta^{13}$C4 are plotted against δ_{H3} values. Values for d[(CGCGAATTCGCG)]$_2$, d[CGCGTATACGCG)]$_2$ and dTMP were taken from Vakonakis et al. (2003). The values and uncertainties are reported in Table 1. The outlier is labeled. The solid line is from a linear fit of $^2\Delta^{13}$C versus δ_{NH2} of intramolecular hydrogen bonded amino protons of a variety of aniline derivatives and is given by $^2\Delta = 23.6\,\delta_{NH2} - 68.4$ ppb (Reuben, 1987). The dashed line is an extrapolation of the fit of the aniline data beyond the original experimental data points. The horizontal offset between values of the aniline derivatives and those of DNA is likely due to the different hydrogen-bonding groups involved. The vertical dashed line is placed at $\delta_{H3} = 13.5$ ppm.

Discussion

Hydrogen bonding to an electronegative atom shifts the hydrogen-bonded proton to higher frequencies (Becker, 1996). The chemical shifts of exchangeable protons in proteins (Wagner et al., 1983) and nucleic acids (Wüthrich, 1986) are sensitive to hydrogen bonding. However, chemical shifts are also modulated by other geometric and ring current effects (Giessner-Prettre and Pullman, 1987; Case, 1995), and it is difficult to use the isotropic chemical shift as a quantitative measure of the hydrogen bond strength of proteins and nucleic acids. Thus, there has been much interest in using other probes of hydrogen bond strength that are less susceptible to these effects. However, most of the new methods require isotopic enrichment of the sample, which is more technically demanding to accomplish for nucleic acids than for proteins (Batey et al., 1995; Smith et al., 1997). One attractiveness of $^n\Delta^{13}$C values as gauges of hydrogen bond strength of A:T base pairs is that even without isotopic enrichment they can be measured with significant precision and accuracy.

$^{2h}\Delta^{13}$C2 is a through-space isotope effect and originates from the anharmonicity of the effective vibrational potential of H3: Relative to ^{1}H3, the lower zero-point energy of ^{2}H3 allows a smaller average displacement from N3 towards N1, which results in a reduction in the nuclear shielding at ^{13}C2. Previously, we found that stronger A:T N1$\cdots$H3 hydrogen bonds have more anharmonic vibrational potentials (Vakonakis et al., 2003), which is in agreement with studies of the effect of molecular zero-point vibrational motions on electronic shielding of nuclei (Smirnov et al., 1996; Ruud et al., 2001). Larger values of $^2\Delta^{13}$C4 correspond with downfield-shifted δ_{H3} values, indicating that stronger A:T N1$\cdots$H3 hydrogen bonds result in more anharmonic vibrational potentials at H3.

The $^{2h}\Delta^{13}$C2 and $^{3h}\Delta^1$H2 data presented here (Figure 2) corroborate that finding but as measured from the hydrogen-bond acceptor group. Stronger A:T base pairs have more negative and positive values of $^{2h}\Delta^{13}$C2 and $^{3h}\Delta^1$H2, respectively. However, $^2\Delta^{13}$C4 does not show a significant correlation with $^{2h}\Delta^{13}$C2 (or $^{3h}\Delta^1$H2) (Table 1), which indicates that intramolecular and trans-hydrogen bond isotope effects are affected differentially by other factors. Indeed, it has been well established that isotope effects, especially over three bonds, are strongly modulated by geometrical factors (Yashiro et al., 1986; Aydin et al., 1988; Tüchsen and Hansen, 1988). Deuterium isotope effects over two bonds have also been shown to be dependent on conformation. For example, in proteins $^2\Delta^{13}C^\alpha$ was found to depend upon the backbone ϕ

and ψ angles (Ottiger and Bax, 1997). In DNA, different base pair conformations (propeller, buckle, shear, stagger) may modulate the transmission of the isotope effect across the hydrogen bond.

It should be noted that at present precise determination of base pair conformation is still difficult to obtain by NMR. Recently, however, an exceptionally large data set of residual dipolar couplings was used to determine base pair propeller with a precision of $\pm 1^\circ$ (Wu et al., 2003). If a quantitative relationship between $^{2h}\Delta^{13}$C2 (and $^{3h}\Delta^{1}$H2) and propeller, buckle, shear, and/or stagger is found, base pair conformation may be determinable with $^{2h}\Delta^{13}$C2 (and $^{3h}\Delta^{1}$H2) and fewer residual dipolar couplings values. *Ab initio* calculations of $^{2h}\Delta^{13}$C2 and $^{3h}\Delta^{1}$H2 of A:T base pairs could reveal such a quantitative relationship. The values of $^{2h}\Delta^{13}$C2, $^{3h}\Delta^{1}$H2 and $^{2}\Delta^{13}$C4 presented here are likely complementary and differentially gauge geometric and hydrogen bond strengths of A:T base pairs.

Acknowledgements

We are thankful for helpful discussions with Marlon Manalo and Xiaolian Gao and NMR assistance by Karl Koshlap. We are also grateful for the financial support provided by the Robert A. Welch Foundation Grant A-1471. The NMR instrumentation at Texas A&M University was supported by National Science Foundation Grant DBI-9970232 and by the Texas Agricultural Experiment Station.

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