J|A|C|S COMMUNICATIONS

Published on Web 06/18/2009

Chemical Shift Anisotropy of Imino ^{15}N Nuclei in Watson−Crick Base Pairs from Magic Angle Spinning Liquid Crystal NMR and Nuclear Spin Relaxation

Alexander Grishaev,[†] Lishan Yao,[†] Jinfa Ying,[†] Arthur Pardi,[‡] and Ad Bax*,[†]

Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, Maryland 20892-0520, and Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

Received April 22, 2009; E-mail: bax@nih.gov

Imino ^{15}N resonances contain a wealth of information on both the structure and dynamics of nucleic acids. In contrast to the very crowded spectral region of the (deoxy-)ribose resonances, the imino ^{1}H−^{15}N correlation spectrum is often remarkably well resolved, even for larger systems. Imino−imino NOEs provide important connectivity information between adjacent base pairs; $^{2h}J_{NN}$ couplings across hydrogen bonds can identify the hydrogen bonded partner and yield information on H-bond strength;[1,2] the ^{15}N relaxation rates provide information on both the rotational diffusion tensor and the amplitude and time scale of internal dynamics;[3−5] the ^{15}N−^{1}H residual dipolar coupling (RDC) provides information on the orientation of the N−H bond in weakly aligned systems; and the residual chemical shift anisotropy (RCSA)[6−8] can provide a second independent measurement for the orientation of the nucleotide relative to the alignment tensor. RCSA information is most accurately obtained from TROSY-based experiments. For it to be independent of that contained in RDCs, it is required that the ^{15}N−^{1}H dipolar and ^{15}N CSA tensors are not linearly correlated.[9,10] Both the interpretation of relaxation rates in terms of dynamics and the use of RCSA as a structural restraint require accurate knowledge of the imino ^{15}N CSA tensor. Quantum chemical computational work indicates that the CSA tensor is strongly affected by hydrogen bonding;[11,12] however, the only experimental data on these CSA tensors were obtained from solid state NMR studies on free nucleosides.[12,13]

The present study aims to define experimentally the ^{15}N CSA tensors for G−N_1 and U−N_3 nuclei in Watson−Crick base-paired nucleotides, under conditions commonly used for analysis of solution NMR data. Analogous to prior work in proteins,[14] we derive the ^{15}N CSA tensors from the RCSA observed for different nucleotides in the same molecule, $tRNA^{Val}$, upon weak alignment in a dilute liquid crystalline phase of Pf1.[15] With the structure of $tRNA^{Val}$ accurately known (PDB entry 1K4C),[16] its alignment relative to the magnetic field is defined by one-bond ^{15}N−^{1}H residual dipolar couplings (RDCs). The chemical shift change, $\Delta\delta$, between the aligned and isotropic phase is given by[14]

$$\Delta\delta = \sum_{i=x,y,z} \sum_{j=x,y,z} A_{jj} \cos^2 \theta_{ij} \delta_{ii} \qquad (1)$$

where θ_{ij} is the angle between the δ_{ii} principal axis of the traceless CSA tensor and the A_{jj} principal axis of the diagonalized traceless molecular alignment tensor. Because $\Delta\delta$ values are quite small, in the parts per billion (ppb) range, exceptional care needs to be taken that this difference is not impacted by small changes in sample conditions (pH, concentration, ionic strength). For this reason, measurements were carried out on the same solution, with one aliquot measured under static, aligned conditions in a Shigemi microcell, and measurements of isotropic chemical shifts under slow magic angle spinning conditions (∼630 Hz, to avoid precipitation of Pf1 phage), using a Bruker 4-mm triple resonance MAS probehead. The temperature of the MAS sample was adjusted to be the same (28 °C) as that of the static sample by ensuring an identical difference in chemical shift between the water resonance and internal trimethylsilylpropionic acid and verified by ensuring that the difference between static and MAS imino ^{1}H and ^{15}N chemical shifts is not correlated with their temperature coefficients (Supporting Information, SI).

The superimposed imino ^{1}H−^{15}N HSQC spectra, recorded under aligned and isotropic conditions, reveal small but readily measured chemical shift differences (Figure 1 and SI). Repeated measurements indicate that the reproducibility of the measured $\Delta\delta(^{15}N)$ values is *ca.* 5 ppb, yielding a 3 ppb random error in their averaged values, with the entire $\Delta\delta$ range spanning 170 ppb. Optimization of the fit between measured and predicted RCSA values by adjusting the ^{15}N CSA tensor is quite sensitive to uncertainty in the atomic coordinates (structural noise), and for this reason we also measured ^{15}N relaxation rates to further restrain the CSA tensor. By fitting the ratio of the transverse relaxation rate, R_2, and the CSA/N−H dipolar cross-correlated relaxation rate, Γ, the results become insensitive to small imperfections in the dynamic and structural model used to generate this ratio.[5] However, because the approximation of an axially symmetric CSA tensor collinear to the dipolar interaction is less appropriate for imino ^{15}N sites than for peptide amide ^{15}N, a reasonably accurate diffusion tensor is required for analysis of the R_2/Γ ratios, which was derived from R_1, R_2, and ^{15}N−{^{1}H} NOE values. Rates were fit to the axially symmetric diffusion model, using the recently RDC-refined structural

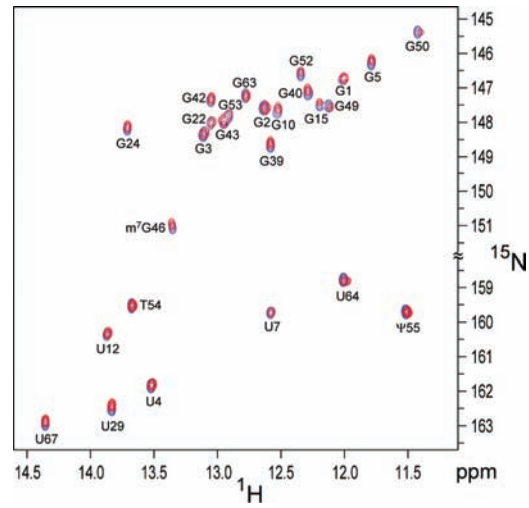

Figure 1. Superposition of the imino region of the 600 MHz $tRNA^{Val}$ HSQC spectra (0.7 mM in 90% H_2O, pH 7, 80 mM NaCl, 5 mM $MgCl_2$, 28 °C) in 10.5 mg/mL Pf1, recorded either on a static sample in a 300 μL Shigemi microcell (red) or in a 40 μL magic angle rotor, spinning at 630 Hz (blue).

[†] NIDDK.
[‡] University of Colorado.

10.1021/ja903244s CCC: $40.75

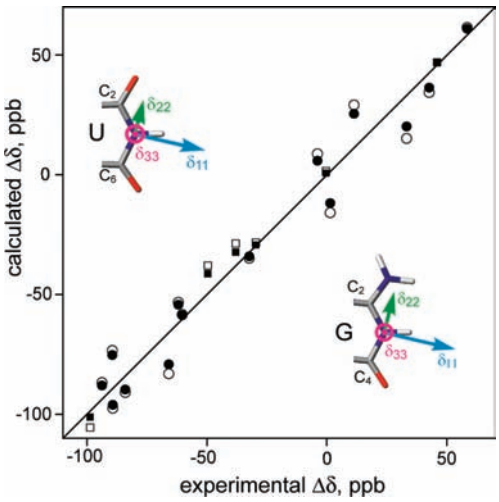

Figure 2. Correlations between the experimental and predicted $\Delta\delta(^{15}N)$. Filled symbols correspond to $\Delta\delta$ values for a CSA tensor optimized with all nucleotides of a given type (circles for G:N_1; squares for U:N_3) included in the CSA fit. Open symbols represent jack-knifed cross-validation results, where each (predicted/observed) pair is shown for a CSA tensor obtained without using the experimental data of that nucleotide.

model of $tRNA^{Val}$ (PDB entry 1K4C).[16] As expected for these R_2/Γ ratios, which are relatively insensitive to details of the dynamic model employed, they cluster in narrow ranges (1.43 ± 0.11 for U−N_3 and 1.26 ± 0.08 for G−N_1 at 500 MHz, 1.86 ± 0.15 and 1.55 ± 0.09 at 800 MHz) and provide strong constraints on the magnitude and orientation of the ^{15}N CSA tensor. We note, however, that optimizing the fit between experimental and predicted values of both $\Delta\delta$ and R_2/Γ values by iterative adjustment of the ^{15}N CSA tensor is sensitive to the N−H bond length used to calculate the dipolar interaction. The strength of the $^{15}N-^{1}H$ dipolar interaction relates inversely to the magnitude of the RDC-derived alignment tensor, and the optimized CSA tensor therefore scales with $\langle r_{NH}^{-3}\rangle$. High resolution crystal structures, hydrogen bond $^{2h}J_{NN}$ couplings, and quantum chemical calculations point to a shorter $N_1...N_3$ hydrogen bond length for U−A than for G−C Watson Crick basepairs.[2,17] Equilibrium N−H bond lengths of $r_{NH} = 1.036$ and 1.050 Å, computed for such Watson−Crick base-paired U and G imino groups, respectively,[2] need to be adjusted for zero-point stretching and bending motions to derive an effective bond length, r_{NH}^{eff}, which determines the observed RDCs and NMR relaxation rates.[18,19] Assuming that the zero-point motion corrections to the $^{15}N-^{1}H$ dipolar interactions are similar for peptide and imino N−H groups yields r_{NH}^{eff} values of 1.043 (G) and 1.057 (U) Å.[19] It is worth noting that if the same r_{NH} bond lengths (1.04 Å) were used for U and G, generalized order parameters (S^2) for U (0.91 ± 0.05) fall significantly below those of G (0.97 ± 0.02), whereas higher and very similar values are obtained for $r_{NH}^{eff} = 1.043$ Å for G ($S^2 = 0.99 \pm 0.03$) and $r_{NH}^{eff} = 1.057$ Å for U ($S^2 = 0.97 \pm 0.06$). Heteronuclear $^{15}N-\{^{1}H\}$ NOE values (0.76 ± 0.11), very close to the theoretical limit, also point to the absence of significant root-mean-squared amplitudes of the internal dynamics for the bases in the tRNA Watson−Crick base pairs.

Under the assumption that the ^{15}N CSA tensor has the same value for all Watson−Crick base-paired G−N_1 sites, and similarly for all U−N_3, the CSA tensor values are obtained from an iterative nonlinear fitting procedure that restrains the δ_{33} component of the CSA tensor to be orthogonal to the base plane. Excellent agreement is observed between measured and predicted $\Delta\delta$ values, not only when these $\Delta\delta$ values are included in the CSA optimization procedure (filled symbols in Figure 2) but also when evaluating the agreement in a cross-validated manner (open symbols). CSA tensor parameters summarized in Table 1 show good agreement with solid state data regarding the magnitudes of the tensors but differ considerably in asymmetry (SI Table S3).[12,13] As predicted by quantum chemical calculations, for a traceless shift tensor, hydrogen bonding strongly increases the difference between δ_{22} and δ_{33}.[11] For the shorter U−A hydrogen bonds, $|\delta_{33}|$ becomes larger than $|\delta_{11}|$. The sign of the angle between δ_{22} and the G:N_1−H bond is the same as previously calculated,[11] but its magnitude is *ca.* 6° smaller. The quality of the fit between observed and predicted $\Delta\delta$ values is limited by both structural noise and the assumption that all tensors are the same for a given nucleotide type. In this respect it is interesting to note that if the analysis is carried out using a high quality homology model for $tRNA^{Val}$ (used as the starting model for RDC refinement),[20] the prediction becomes 35% worse for G and 83% worse for U. If the experimental $\Delta\delta$ values and the $tRNA^{Val}$ model are assumed to be error free, scatter seen in Figure 2 can be attributed to random variations between nucleotides, $\sigma(\delta)$, in the CSA tensor. Variations in H-bond length are expected to impact δ_{22} and δ_{33} in opposite directions, with a correlated small change in the orientation of these two principal axes and little effect on δ_{11}.[11] Using these assumptions, we require $\sigma(\delta_{33}) = 8$ ppm for U, 14 ppm for G, to reproduce the scatter seen in Figure 2. These $\sigma(\delta_{33})$ values correspond to upper limits for the rms variations in H-bond length of ±0.13 Å for U−A and ±0.29 Å for G−C.[11]

Table 1. Average Chemical Shift Tensor Values for Imino ^{15}N in Watson−Crick Base-Paired Nucleotides[a]

	β, deg	δ_{11}, ppm	δ_{22}, ppm	δ_{33}, ppm
G:N_1	13.1 ± 1.4	77.9 ± 1.7	−12.4 ± 2.2	−65.5 ± 2.5
U:N_3	11.4 ± 2.4	68.5 ± 1.6	2.3 ± 2.9	−70.8 ± 3.2

[a] Using $r_{NH}^{eff} = 1.043$ Å for G and $r_{NH}^{eff} = 1.057$ Å for U.

Acknowledgment. This work was supported in part by the Intramural Research Program of the NIDDK, NIH and NIH Grant AI33098 (A.P.). We thank Werner Maas and Jochem Struppe (Bruker Instruments) for help with the MAS measurements.

Supporting Information Available: One table with measured $\Delta\delta$ values and relaxation rates; one table with temperature coefficients. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dingley, A. J.; Grzesiek, S. *J. Am. Chem. Soc.* **1998**, *120*, 8293–8297.
(2) Barfield, M.; Dingley, A. J.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **2001**, *123*, 4014–4022.
(3) Tjandra, N.; Feller, S. E.; Pastor, R. W.; Bax, A. *J. Am. Chem. Soc.* **1995**, *117*, 12562–12566.
(4) Dayie, K. T.; Wagner, G.; Lefevre, J. F. *Annu. Rev. Phys. Chem.* **1996**, *47*, 243–282.
(5) Fushman, D.; Tjandra, N.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, *120*, 10947–10952.
(6) Sanders, C. R.; Hare, B. J.; Howard, K. P.; Prestegard, J. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 421–444.
(7) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836–6837.
(8) Hansen, A. L.; Al-Hashimi, H. M. *J. Magn. Reson.* **2006**, *179*, 299–307.
(9) Tate, S.; Shimahara, H.; Utsunomiya-Tate, N. *J. Magn. Reson.* **2004**, *171*, 284–292.
(10) Grishaev, A.; Ying, J. F.; Bax, A. *J. Am. Chem. Soc.* **2006**, *128*, 10010–10011.
(11) Czernek, J.; Fiala, R.; Sklenar, V. *J. Magn. Reson.* **2000**, *145*, 142–146.
(12) Stueber, D.; Grant, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 10539–10551.
(13) Hu, J. Z.; Facelli, J. C.; Alderman, D. W.; Pugmire, R. J.; Grant, D. M. *J. Am. Chem. Soc.* **1998**, *120*, 9863–9869.
(14) Cornilescu, G.; Bax, A. *J. Am. Chem. Soc.* **2000**, *122*, 10143–10154.
(15) Hansen, M. R.; Mueller, L.; Pardi, A. *Nat. Struct. Biol.* **1998**, *5*, 1065–1074.
(16) Grishaev, A.; Ying, J.; Canny, M. D.; Pardi, A.; Bax, A. *J. Biomol. NMR* **2008**, *42*, 99–109.
(17) Dingley, A. J.; Masse, J. E.; Peterson, R. D.; Barfield, M.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 6019–6027.
(18) Case, D. A. *J. Biomol. NMR* **1999**, *15*, 95–102.
(19) Yao, L.; Voegeli, B.; Ying, J. F.; Bax, A. *J. Am. Chem. Soc.* **2008**, *130*, 16518–16520.
(20) Grishaev, A.; Tugarinov, V.; Kay, L. E.; Trewhella, J.; Bax, A. *J. Biomol. NMR* **2008**, *40*, 95–106.

JA903244S