Published in final edited form as:

J Am Chem Soc. 2009 April 22; 131(15): 5380–5381. doi:10.1021/ja900771b.

Facilitated Assignment of Adenine H2 Resonances in Oligonucleotides using Homonuclear Long-range Couplings

Jin Zhang, Alexander Spring, and Markus W. Germann*

Departments of Chemistry and Biology, Georgia State University, Atlanta, Georgia.

NMR structural studies of nucleic acids require a near complete and unambiguous assignment of the proton resonances. This includes the adenine H2 protons which, especially in B type DNA, are generally far from other protons rendering their assignment cumbersome. In both A and B type nucleic acid helices, the H2 protons are located in the minor grooves where they serve as useful monitors for binding [1]. The conventional assignment method, which relies on NOE's recorded in D₂O solution, generally does not allow complete assignment of H2 resonances. In practice, these resonances are often assigned from their NOE to imino protons of A•U or A•T base pairs [2,3]. This requires that experiments are carried out in H₂O and the presence of detectable imino protons whose exchange with the solvent is sufficiently slow. For adenine residues in unpaired regions, other strategies are needed. Oligonucleotides that are ¹³C labeled allow magnetization transfer between H2 and H8 protons via common couplings to intervening ¹³C nuclei [4,5]. However, this approach is hampered by fast relaxing carbons. Subsequently, triple resonance experiments using [¹³C, ¹⁵N] labeled oligonucleotides were developed to correlate H2 and H8 protons (H-¹⁵N->¹³C->H) [6,7]. Such schemes feature increased sensitivity but require labeled oligonucleotides and that experiments are recorded in H₂O. The development of higher sensitivity NMR probes and the use of low artifact gradient enhanced experiments enabled the assignments of H2 and H8 protons at natural abundance using HMBC experiments in D₂O [8]. This experiment relies on a common heteronuclear long range coupling of both H2 and H8 to C4. However, the inherently low sensitivity requires very long acquisition times and/or high sample concentrations. In D₂O solution the adenine H2 resonances are readily identified since they appear as narrow singlets with long longitudinal and transverse relaxation times. The long T₁ value is often a nuisance because it limits the repetition rate in many NMR experiments. On the other hand long T₂ values permit the use of pulse sequences with longer durations, which aid detection of even small couplings. Long-range 6 bond homonuclear couplings have been previously reported for polycyclic aromatic systems. [22]. Therefore, we explored if there is a homonuclear coupling between H2 and H8 of adenine that could be exploited.

Small coupling constants, particularly when they are on the order of the linewidths, are difficult to measure [18,19,20]. Inspection of adenosine NMR spectra does not provide any obvious support for long-range H2-H8 couplings. Therefore, we used quantum mechanical calculations to predict a long range coupling of -0.18 Hz [15]. However, these calculation also forecast several other non-vanished couplings of H2 to H3', H5' and H5'' which are suspect (Figure S1 A). In view of these results, experimental verification is clearly needed. The H2-H8 coupling was assessed using a long-range optimized COSY experiment of adenosine 5'-monophosphate [14]. The coupling became apparent for long range evolution delays larger than 0.10 s. We

*mwg@gsu.edu .

Supporting Information Available: Long range optimized COSY and XLOC spectra of 5'-AMP; H2-H8 TOCSY cross peak intensity of a DNA decamer, 200 ms NOESY spectrum of the DNA hairpin. Complete Ref. 15. This material is available free of charge via the Internet at <http://pubs.acs.org>

also noted a long-range COSY correlation for H8 and H1' protons (Figure S1). To estimate the H2-H8 coupling constant, the long-range evolution delay was varied (Figure 1).

The maximum cross peak intensity was obtained for a delay of 0.75 s (Figure 1). NMR-SIM simulations of this experiment, accounting for relaxation, are consistent with a coupling of approximately 0.5 Hz. Sørensen et al introduced an innovative experiment, named XLOC, which allows a direct measurement of small homonuclear couplings [9,10]. This approach disentangles small homonuclear J_{HH} couplings (F2) by the large one-bond $^1J_{CH}$ coupling in the F1 dimension. Using XLOC on a 5'-AMP sample, a coupling constant of 0.5 Hz $\pm$ 0.2 Hz is obtained, consistent with the previous result (Figure S2). The coupling between H2 and H8 protons also resulted in a cross peak in the TOCSY experiment at long mixing times (0.20 s to 1.25 s). Such durations are possible because of the slow relaxation behavior of the A H2 proton.

A selective TOCSY experiment using an E-Burp pulse for excitation of the base region was applied to several DNA samples in D₂O. The selective pulse limits the appearance of unwanted signals and permits the use of large receiver gains. For a 10-mer DNA duplex at mixing times larger than 0.25 s a clear correlation of all H2 and H8 protons was observed (Figure 2). The cross peak intensity remains nearly constant for mixing times between 0.40 and 1.0 s (Figure S3).

Unambiguous assignment was also obtained for a 0.75 mM 18-mer DNA hairpin using a mixing time of 0.30 s in just 45 minutes (Figure 3).

Depending on sequence and structural peculiarities H2 protons can be assigned from regular NOESY experiments, particularly for RNA [21]. Often however, the relevant cross peaks are missing, weak, ambiguous or in overlapped regions (Figure S4). Although not observed for the nucleic acids shown here (Figure 2, 3), a ROE artifact could potentially also arise if H2 is close to another proton. However a direct ROE has the opposite sign compared to a TOCSY cross peak and a through space correlation is also readily recognized from a companion NOESY experiment.

These examples demonstrate that a simple semi selective TOCSY experiment optimized for very small couplings allows straight-forward and rapid assignment of A H2 protons in unlabeled oligonucleotides via a 6/7 bond homonuclear coupling. The through bonds correlations are unambiguous while NOE-based assignment can be problematic, especially in non-helical regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Christopher N. Johnson for the decamer assignments and assistance with NMR experiments. This work was supported by NIH Grant AI/GM47459, the Georgia Cancer Coalition and Molecular Basis of Disease (A.S.) and Brains & Behavior (J.Z.) fellowships from GSU.

REFERENCES

1. Halle B, Denisov VP. Biopolymers 1998;48:210–233. [PubMed: 10699841]
2. Varani G, Tinoco I Jr. Q. Rev. Biophys 1991;24:479–532. [PubMed: 1723809]
3. Wuthrich, K. NMR of Proteins and Nucleic Acids. John Wiley & Sons; New York: 1986.
4. Legault P, Farmer TB, Mueller L, Pardi A. J. Am. Chem. Soc 1994;116:2203–2204.

5. Marino JP, Prestegard JH, Crothers DM. *J. Am. Chem. Soc* 1994;116:2205–2206.
6. Simorre J, Zimmermann GR, Mueller L, Pardi A. *J. Am. Chem. Soc* 1996;118:5316–5317.
7. Simon B, Zanier K, Sattler M. *Journal of Biomolecular NMR* 2001;20:173–176. [PubMed: 11495248]
8. van Dongen MJP, Wijmenga SS, Eritja R, Azorín F, Hilbers CW. *J. Biomol. NMR* 1996;8:207–212. [PubMed: 8914275]
9. Thøgersen H, Sørensen OW. *J. Magn. Reson. Series A* 1994;110:118–120.
10. Meissner A, Sørensen OW. *Magn. Reson. Chem* 2001;39:49–52.
11. Vold LR, Waugh SJ, Klein PM, Phelps ED. *J. Chem. Phys* 1968;48:3831.
12. Kingsley BP. *Concepts Magn. Reson* 1999;11:243–276.
13. Meiboom S, Gill D. *Rev. Sci.Instrum* 1958;29:688.
14. Bax A, Freeman R. *J. Magn. Reson* 1981;44:542–561.
15. Frisch, MJ., et al. Gaussian 03, Revision C.02. Gaussian, Inc.; Wallingford CT: 2004.
16. Cammi R, Mennucci B, Tomasi J. *J. Phys. Chem. A* 1999;103:9100–9108.
17. Cammi R, Mennucci B, Tomasi J. *J. Phys. Chem. A* 2000;104:5631–5637.
18. Mahi L, Duplan JC. *Magn. Reson. Chem* 1997;35:379–383.
19. Meissner A, Schulte-Herbrüggen T, Sørensen OW. *J. Am. Chem. Soc* 1998;120(31):7989–7990.
20. Bertini I, Jiménez B, Piccioli M, Poggi L. *J. Am. Chem. Soc* 2005;127:12216–12217. [PubMed: 16131187]
21. Heus HA, Pardi A. *J. Am. Chem. Soc* 1991;113:4360.
22. Cho BP, Harvey RG. *J. Org. Chem* 1987;52:5679–5684.

Figure 1.

Dependence of the H2-H8 cross-peak height on the evolution delay in the long-range COSY experiment of 5'-AMP, 50 mM, in 99.9 % D₂O, 10 mM sodium phosphate, 0.5 mM EDTA, pH* 6.6 at 298 K and 500 MHz). (●), long range COSY magnitude mode experiment. From the maximal response of the experimental curve a coupling constant around 0.6 Hz is predicted using $J = 1/(2\tau)$. (.....) NMR SIM long-range COSY calculations for a coupling constant of 0.5 Hz. (- - -) simulation for a coupling constant of 0.3 Hz. T₁ and T₂ relaxation times of 5'-AMP were determined from inversion recovery [11,12] and CPMG [13] experiments. Simulation were carried out with NMR-SIM 3.1 (Bruker) using: H2 = 8.17 ppm T₁ = 6.0 s, T₂ = 1.6 s; H8 = 8.54 ppm T₁ = 2.1 s, T₂ = 1.1 s.

Figure 2. 600MHz selective TOCSY spectrum of a 0.5 mM decamer DNA duplex, recorded at 298 K (99.99 % D₂O, 10 mM sodium phosphate, 0.1 mM EDTA, 100 mM NaCl, pH* 6.6) with a mixing time of 500 ms and a relaxation delay of a 5.0 s. An E-Burp pulse of 4.5 ms was used for excitation at 7.7 ppm. A 1024×100 data matrix was acquired in a spectral window of 2.4×2.4 ppm resulting in an acquisition time of 5 h.

Figure 3. 500 MHz selective TOCSY spectrum of a 0.75 mM DNA hairpin, recorded at 298 K (99.99 % D₂O, 10 mM sodium phosphate, 0.1 mM EDTA, 50 mM NaCl, pH* 6.84) with a relaxation time of 3.5s and mixing time of 300 ms. A 1024×128 data point matrix was acquired using 4 scans per increment resulting in an experiment time of 45 minutes on a TXI cryogenic probe.