Observation of Long-Range $^1H-^1H$ Distances in Solution by Dipolar Coupling Interactions

Mark R. Hansen,[†] Mark Rance,[‡] and Arthur Pardi*,[†]

Department of Chemistry and Biochemistry
University of Colorado
Boulder, Colorado 80309-0215
Department of Molecular Genetics
Biochemistry and Microbiology
University of Cincinnati College of Medicine
231 Bethesda Avenue, Cincinnati, Ohio 45267-0524

Received August 18, 1998
Revised Manuscript Received September 25, 1998

Dipolar couplings are a potentially extremely valuable source of distance and angular structural data for NMR studies of macromolecules; however, molecular tumbling averages these interactions to zero in isotropic solution.[1−3] Recently, Bax and co-workers demonstrated that partial alignment of proteins and nucleic acids could be achieved by dissolving macromolecules in a solution containing magnetically aligned bicelles and that residual one bond $^1H-^{13}C$ and $^1H-^{15}N$ dipolar couplings could be used to obtain angular constraints for structure refinement.[4,5] Here we introduce 2D dipolar coupling spectroscopy (DCOSY), which employs a TOCSY-type pulse sequence[6,7] to observe long-range through-space $^1H-^1H$ dipolar coupling interactions (DPIs) in partially aligned macromolecules. These $^1H-^1H$ DPIs are a function of r^{-3}, as opposed to r^{-6} for the nuclear Overhauser effect (where r is the distance between the protons) allowing observation of much longer $^1H-^1H$ distances than was previously possible by solution NMR techniques. This long-range distance data combined with the angular information contained in the $^1H-^1H$ DPIs make the DCOSY experiment a powerful tool for structural studies of macromolecules in solution.

We have developed a new method for alignment of biomacromolecules in which magnetically aligned Pf1 filamentous bacteriophage[8] forms an anisotropic medium that imparts partial alignment of macromolecular cosolutes.[9] The macromolecule is aligned by steric interactions with the magnetically aligned phage; thus the degree of alignment of the macromolecule is easily tuned by varying the phage concentration. This technique has been used to align multiple RNA, DNA, and protein systems[9] and in the current study is used to observe $^1H-^1H$ DPIs in several DNA duplexes. For an axially symmetric system, such as a DNA or RNA duplex, the $^1H-^1H$ dipolar coupling has the form[1,2,4]

$$D_{HH} = \frac{-\mu_0 \gamma_H^2 h S A_a}{4\pi^2 r^3}(3\cos^2\theta - 1) \qquad (1)$$

Where θ is the angle between the proton−proton internuclear vector and the molecular alignment axis, A_a, is the axial component of the molecular alignment tensor, and the other constants are as previously defined.[4] Thus, the dipolar couplings yield both angle and distance information for the inter-proton interactions.

The ability to observe long-range distances in the DCOSY spectrum is shown in Figure 1A and B where inter- and intrastrand adenine H2−H2 through-space interactions are compared in 1D slices of NOESY and DCOSY spectra. Interactions in the DNA 10mer duplex between the $A_6H2\cdots A_7H2$ (3.8 Å) and $A_6H2\cdots$

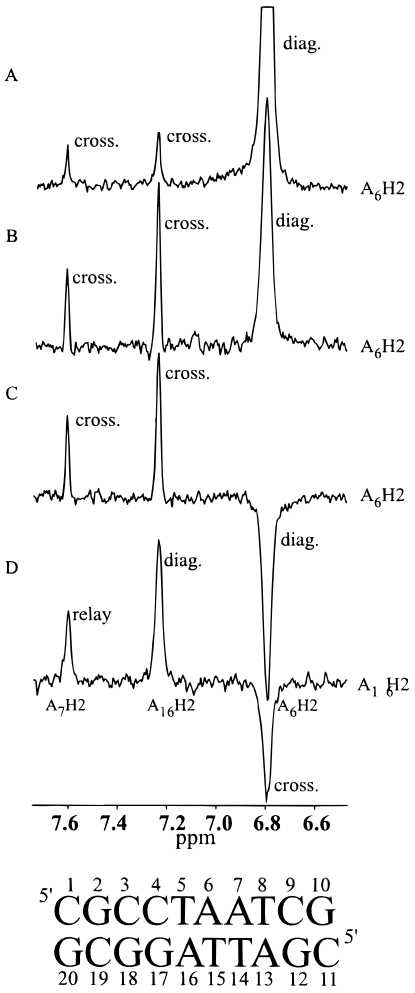

Figure 1. (A) Cross section corresponding to the A_6H2 of a 200 ms NOESY spectrum on the 1.0 mM DNA 10mer duplex without phage. For comparison, DCOSY spectra were collected on a 0.8 mM DNA 10mer dissolved in 46 mg/mL Pf1 phage. (B) 1D cross section corresponding to the A_6H2 resonance of an in-phase 80 ms (8.2 kHz) WALTZ-16[12] DCOSY experiment (see Figure S2A). (C) 1D cross section corresponding to the A_6H2 resonance of an orthogonal 80 ms (8.2 kHz) WALTZ-16 DCOSY experiment (see Figure S2B). The cross-peaks are plotted positive for comparison. (D) 1D cross section corresponding to the $A_{16}H2$ proton of a longer mixing time (155 ms) orthogonal (8.2 kHz) DIPSI-2[13] DCOSY experiment (see Figure S2B) to show effects of spin diffusion. The diagonal peaks (*diag.*), the DPI or NOE cross-peaks (*cross.*), and the cross-peak resulting from spin diffusion relayed between A_6H2 to A_{16}-H2 by the intervening A_7H2 (*relay*) are labeled. The sequence of the DNA 10mer duplex is shown. Each experiment was collected under identical conditions at 500 MHz and 25 °C in 25 h. Sample conditions are 10 mM Tris (d_{11}), pH 8.0, 0.005% NaN_3 in 99.9% D_2O. The Pf1 phage were prepared as previously described, and their concentration was determined using an extinction coefficient at 270 nm of 2.25 mL mg^{-1} cm^{-1}.[16,17]

The ability to observe long-range distances in the DCOSY spectrum is shown in Figure 1A and B where inter- and intrastrand adenine H2−H2 through-space interactions are compared in 1D slices of NOESY and DCOSY spectra. Interactions in the DNA 10mer duplex between the $A_6H2\cdots A_7H2$ (3.8 Å) and $A_6H2\cdots$

* To whom correspondence should be addressed.
[†] University of Colorado.
[‡] University of Cincinnati.

(1) Gayathri, C.; Bothnerby, A. A.; Vanzijl, P. C. M.; Maclean, C. *Chem. Phys. Lett.* **1982**, *87*, 192−196.
(2) Bastiaan, E. W.; Huis, L.; Maclean, C. *Mol. Phys.* **1989**, *67*, 615−632.
(3) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279−9283.
(4) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111−1114.
(5) Bax, A.; Tjandra, N. *J. Biomol. NMR* **1997**, *10*, 289−292.
(6) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521−528.
(7) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355−360.
(8) Torbet, J.; Maret, G. *J. Mol. Biol.* **1979**, *134*, 843−845.
(9) Hansen, M. R.; Mueller, L.; Pardi, A., submitted.

10.1021/ja9829665 CCC: $15.00
Published on Web 10/20/1998

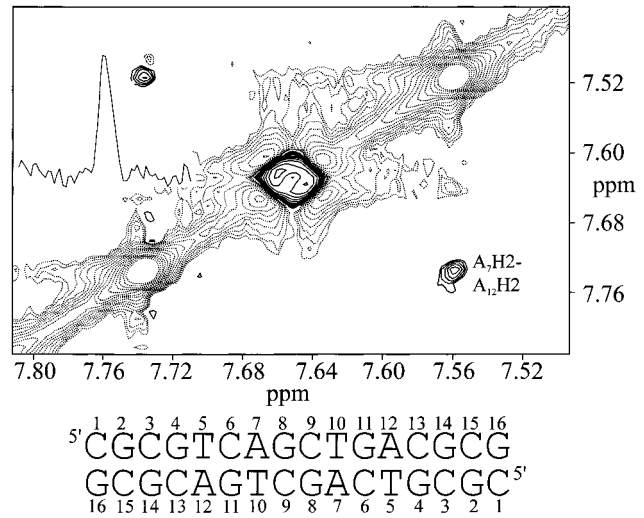

Figure 2. Part of the aromatic proton region of a DCOSY experiment showing a ~7.4 Å connectivity between the A_7H2 and the $A_{12}H2$ protons, which are separated by one base pair in the DNA 16mer duplex. Positive peaks are plotted as dotted lines and negative peaks as solid lines. A 1D cross section through the $A_7H2-A_{12}H2$ cross-peak is shown as an inset. The sequence of the DNA is shown. The experiment was collected at 600 MHz at 25 °C in 13 h using a 155 ms (8.2 kHz) orthogonal WALTZ-16 isotropic mixing sequence (Figure S2B). The sample conditions are 1.9 mM 16mer duplex, 39 mg/ml Pf1 phage, 40 mM NaH_2PO_4, 0.1 mM EDTA, 0.005% NaN_3, pH 8.0 in 99.9% D_2O.

$A_{16}H2$ (4.1 Å) proton pairs (distances in B-form DNA)[10] are of significantly greater intensity in the DCOSY than in the NOESY spectrum acquired for an equivalent time. This is a result of the r^{-3} distance dependence for the dipolar coupling as compared to the r^{-6} dependence of the nuclear Overhauser effect. Through-space DPIs at distances greater than 5 Å are easily detected. For example, Figure 2 shows a DCOSY cross-peak between the A_7H2 and the $A_{12}H2$ protons in the DNA 16mer duplex. These protons are two base pairs apart, separated by ~7.4 Å in B-form DNA. This distance is well beyond the range of what can be seen by an NOE but is readily observed in the DCOSY spectrum.

An interesting feature of the DCOSY experiment is the change in sign of the DPIs depending upon the specific trajectory of the proton magnetization during the mixing period. As seen in Figure 1B and C, at short mixing times the cross-peak has the same sign as the diagonal peaks when the proton magnetization is spin-locked by an isotropic mixing sequence that is applied along the same axis as the proton magnetization. However, these cross-peaks are inverted relative to the diagonal peaks when the proton magnetization is orthogonal to the axis for the mixing sequence. Applying the Hausdorff formula[11] with the appropriate effective nuclear spin Hamiltonian, it can be shown that magnetization along the x axis, I_x, that evolves under a dipolar coupling interaction with spin S during an in-phase (x axis) WALTZ[12] or DIPSI[13] mixing sequence has the form

$$I_x \rightarrow {}^1/_2 I_x (1 + \cos \pi D_{IS} t) + {}^1/_2 S_x (1 - \cos \pi D_{IS} t) + (I_y S_z - I_z S_y) \sin \pi D_{IS} t \quad (2)$$

whereas, I_z, magnetization that begins orthogonal to the (x axis) mixing sequence, has the form

$$I_z \rightarrow I_z \{{}^1/_2 \cos({}^3/_2 \pi D_{IS} t) + {}^1/_2 \cos({}^1/_2 \pi D_{IS} t)\} + S_z \{{}^1/_2 \cos({}^3/_2 \pi D_{IS} t) - {}^1/_2 \cos({}^1/_2 \pi D_{IS} t)\} + (I_y S_x + I_x S_y) \sin({}^3/_2 \pi D_{IS} t) + (I_y S_x - I_x S_y) \sin({}^1/_2 \pi D_{IS} t) \quad (3)$$

where D_{IS} is the dipolar coupling between spins I and S, t is the isotropic mixing time, the I_x or I_z terms represent diagonal peaks, the S_x or S_z terms represent $^1H-{}^1H$ dipolar coupling cross-peaks, and $(I_y S_x - I_x S_y)$ and $(I_y S_x + I_x S_y)$ represent zero and double quantum coherences, respectively. A more detailed theoretical analysis of the DCOSY experiment will be presented elsewhere.[14]

Equation 3 predicts that the diagonal and cross-peaks have opposite signs in a DCOSY spectrum that employs an orthogonal mixing sequence, which means that the cross-peak for a three-spin interaction change will have a sign opposite to that of a cross-peak for a direct interaction (similar to three-spin interactions in ROESY).[15] This is illustrated in Figure 1D where, for a longer mixing time in the DCOSY spectrum, the $A_{16}H2$ shows a cross-peak to the A_7H2 that has a sign opposite to that of the cross-peak between the A_{16} H2 and the neighboring A_6 H2. The $A_{16}H2-A_7H2$ cross-peak arises from a three-spin interaction relayed through the intervening A_6H2 proton. The dependence of the signs of the cross-peaks as a function of the mixing time provides a valuable distinction between direct interactions and indirect three-spin interactions in the DCOSY experiment. This contrasts with the NOESY experiment where, for macromolecules, the direct and spin-diffusion cross-peaks cannot be distinguished on the basis of the sign of the cross-peak.[15]

The angular dependence of the dipolar couplings yields a fundamentally different type of structural data, which complement the NOESY-derived distance constraints. The DCOSY spectrum shows more variation in cross-peak intensity than the NOESY spectrum due to the distance and angular dependence of the DPI. This is illustrated in the aromatic to H1′ region of the DCOSY where a nearly complete sequential aromatic proton to H1′ walk can be performed (see Figure S1 in Supporting Information). However, except for relatively isolated spin systems such as the adenine H2 protons, complications due to $^1H-{}^1H$ J-couplings make it difficult to unambiguously interpret DCOSY cross-peaks strictly in terms of distance and angle. As demonstrated for the 7.5 Å $^1H-{}^1H$ interaction observed in the DNA 16mer, the DPIs in the DCOSY experiment provide through-space distance information at greater distances than is possible with standard NOESY experiments.

Acknowledgment. This work was supported by NIH Grants AI33098 (to A.P.), GM40089 (to M.R.), and a postdoctoral fellowship from the Leukemia Society of America 5562-98 (to M.R.H.). We thank Dr. Luciano Mueller for valuable discussions.

JA9829665

(10) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(11) Banwell, C. N.; Primas, H. *Mol. Phys.* **1963**, *6*, 225−256.
(12) Shaka, A. J.; Keeler, J.; Frenkiel, T.; Freeman, R. *J. Magn. Reson.* **1983**, *52*, 335−338.
(13) Shaka, A. J.; Lee, C. J.; Pines, A. *J. Magn. Reson.* **1988**, *77*, 274−293.
(14) Rance, M.; Hansen, M. R.; Pardi, A. manuscript in preparation.
(15) Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 1989.
(16) Cross, T. A.; Tsang, P.; Opella, S. J. *Biochemistry* **1983**, *22*, 721−726.
(17) Cross, T. A.; Opella, S. J. *Biochemistry* **1981**, *20*, 290−297.