Journal of Biomolecular NMR, **15:** 335–338, 1999.
KLUWER/ESCOM

Line narrowing in spectra of proteins dissolved in a dilute liquid crystalline phase by band-selective adiabatic decoupling: Application to $^{1}H^{N}$-^{15}N residual dipolar coupling measurements

Craig W. Vander Kooi[a], Ēriks Kupče[d], Erik R.P. Zuiderweg[a,b,c,*] & Maurizio Pellecchia[c,*,**]
[a]*Department of Chemistry,* [b]*Department of Biological Chemistry, and* [c]*Biophysics Research Division, The University of Michigan, 930 North University Avenue, Ann Arbor, MI 48109, U.S.A.* [d]*Varian NMR Instruments, Application Laboratories, Oxford, U.K.*

Received 22 September 1999; Accepted 25 October 1999

Key words: adiabatic decoupling, bicelles, homonuclear decoupling, protein alignment, residual dipolar coupling

Abstract

Residual heteronuclear dipolar couplings obtained from partially oriented protein samples can provide unique NMR constraints for protein structure determination. However, partial orientation of protein samples also causes severe ^{1}H line broadening resulting from residual ^{1}H-^{1}H dipolar couplings. In this communication we show that band-selective ^{1}H homonuclear decoupling during data acquisition is an efficient way to suppress residual ^{1}H-^{1}H dipolar couplings, resulting in spectra that are still amenable to solution NMR analysis, even with high degrees of alignment. As an example, we present a novel experiment with improved sensitivity for the measurement of one-bond $^{1}H^{N}$-^{15}N residual dipolar couplings in a protein sample dissolved in magnetically aligned liquid crystalline bicelles.

Residual dipolar couplings are a new and increasingly valuable source of structural information for solution NMR studies of macromolecules (reviewed in Prestegard, 1998). Residual one- and two-bond heteronuclear dipolar couplings in high-resolution solution NMR have provided unique constraints in protein structure determination (Tolman et al., 1995; Tjandra et al., 1996a; Tjandra and Bax, 1997; Bewley et al., 1998; Drohat et al., 1999). Residual dipolar couplings are obtained from samples in which the macromolecule is partially aligned. Examples of effective media which induce tunable alignment of macromolecules in a magnetic field include liquid crystalline bicelles (Sander and Schwonek, 1992; Ottiger and Bax, 1998a), magnetically aligned filamentous bacteriophages (Clore et al., 1998a; Hansen et al., 1998a), and purple membrane fragments (Koenig et al., 1999; Sass et al., 1999). The magnitude of the residual dipolar couplings is determined by the degree of alignment of the macromolecule. Accordingly, it is desirable to work with samples with a high degree of alignment, allowing more couplings to be measured more accurately. However, in practice the use of such samples is limited by the broadening of the ^{1}H resonances due largely to ^{1}H-^{1}H residual dipolar couplings. This paper shows that the use of band-selective adiabatic homo-nuclear decoupling in the NMR spectra of partially oriented molecules represents an efficient way to suppress large aliphatic to amide-proton residual dipolar couplings, resulting in spectra that on the one hand contain useful dipolar information but on the other are still amenable to NMR analysis.

We illustrate the method using a 21 kDa fragment of the chaperone protein DnaK, dissolved in an aqueous solution containing 7.5% DMPC/DHPC/CTAB at 3.2:1:0.1 ratio (Losonczi and Prestegard, 1998). At 30 °C the bicelles are aligned and induce a considerable degree of protein alignment, which in turn

*To whom correspondence should be addressed. E-mail: zuiderwe@umich.edu
**Present address: TRIAD Biotechnology, 9390 Towne Centre Drive, San Diego, CA 92121, U.S.A. E-mail: mpellecchia@triadbiotechnology.com

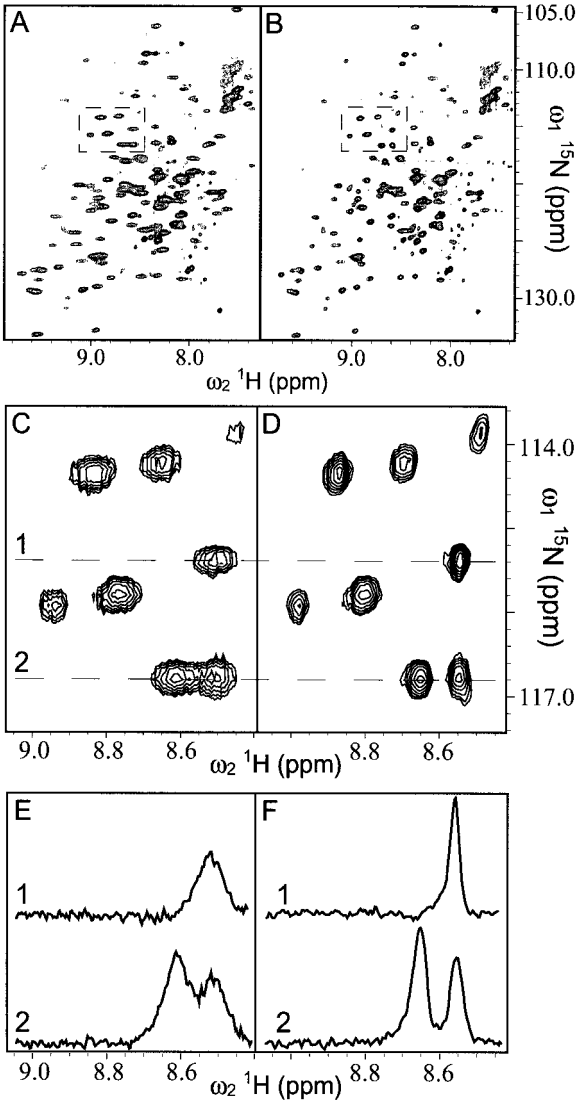

Figure 1. [^{15}N, ^{1}H] correlation spectra displaying only the slowly relaxing ^{15}N doublet component. The selection was achieved with the pulse scheme of Figure 2. The spectra were measured without (A) and with (B) WURST-4 homonuclear ^{1}H decoupling during acquisition. The measurements were done at 303 K with a ∼0.7 mM sample of ^{15}N labeled DnaK substrate binding unit (residues 386–561), dissolved in an aqueous solution containing 7.5% DMPC/DHPC/CTAB at 3.2:1:0.1 ratio, and 50 mM potassium phosphate buffer, pH = 7.2. (C) and (D): Zoomed regions taken from the spectra in (A) and (B), respectively. (E) and (F): Traces along ω_2 taken from the spectra in (C) and (D), respectively. A 1.6 ms WURST-4 adiabatic decoupling pulse was applied at 2.5 ppm during t_2 evolution which had a band width of 4000 Hz and employed a TPG supercycle used in a four-step MLEV-4 cycling (Levitt and Freeman, 1981). The acquisition time was 164 ms with a 5% duty cycle. The spectra were recorded on a Varian INOVA800 spectrometer operating at 800 MHz ^{1}H frequency equipped with a triple resonance probe with a shielded *z* gradient coil. The measuring time for the two experiments was ∼4 h each.

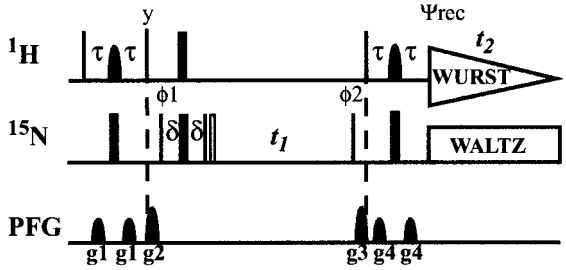

Figure 2. Pulse sequence to measure the $^{1}\Delta_{NH}$ coupling constants. Narrow and thin bars represent 90° and 180° rf pulses, respectively. Unless specified otherwise, pulse phases are along the *x*-axis. The ^{1}H carrier position was set to 9.2 ppm throughout the experiment and then shifted to 4.8 ppm just prior to acquisition. The 180° proton pulses in the middle of the INEPT steps are REBURP pulses (Geen and Freeman, 1991) of 1 ms duration and 6.04 kHz peak amplitude. Both pulses are designed not to excite water magnetization that is consequently suppressed by the pulsed field gradients. The pulsed field gradients have a duration of 1 ms and strengths of $g_1 = 20$ G/cm, $g_2 = 35$ G/cm, $g_3 = -35$ G/cm, $g_4 = 10$ G/cm. The delays are: $\tau = 2.60$ ms, $\delta = 1.34$ ms. States-TPPI quadrature detection is achieved by incrementing $\phi 2$. ^{15}N decoupling during t_2 was achieved with a 2.0 kHz WALTZ-16 decoupling (Shaka et al., 1983) sequence. WURST adiabatic homonuclear decoupling was applied during the acquisition (see text and legend to Figure 1). Phase cycle: $\phi 1 = \pi/4,5\pi/4$; $\phi 2 = x,x,-x,-x$; $\Psi_{rec} = x,-x,-x,x$. For the separation of the two doublet components a second experiment is recorded with the phase of the 90° nitrogen pulse (open box) prior to the t_1 evolution period set to $-x$.

causes significant line broadening due to the presence of large ^{1}H-^{1}H residual dipolar couplings, with an average $^{1}H^{N}$ line width of about 65 Hz (Figure 1A). This situation is dramatically improved by introducing aliphatic homonuclear decoupling during acquisition, which results in an average $^{1}H^{N}$ line width of about 35 Hz (Figure 1B). Although there is in principle an intrinsic loss in sensitivity associated with the use of homonuclear decoupling, (primarily associated with the duty cycle, *dc*, with a loss of signal equal to $[1 - \sqrt{(1 - dc)}]$, where $dc = 5\%$), and potentially also due to the dephasing of water magnetization, we observe substantial resonance line narrowing that largely compensates for these small losses. The overall improvement can be clearly observed throughout the spectra (Figure 1).

The spectra were obtained using a novel efficient experiment that was designed to determine residual $^{1}H^{N}$-^{15}N one-bond dipolar couplings ($^{1}D_{NH}$) in partially oriented biomolecules. Our experiment (Figure 2) exploits the recently introduced $S^{3}E$ method for ^{15}N doublet component separation (Meissner et al., 1997, 1998; Sørensen et al., 1997), selecting single transitions in the indirect dimension for the sake

of spectral simplification. Two [^{15}N, ^{1}H] correlation spectra are recorded where the only difference between the two is that the phase of the 90° nitrogen pulse just prior to the nitrogen evolution (indicated with an open box in Figure 2) is inverted. After Fourier transformation, the sum of the two spectra leads to a sub-spectrum containing the upfield components of the ^{15}N doublet in the indirect dimension. The difference between the two spectra, followed by a 90° phase shift in the indirect dimension, yields a sub-spectrum containing only the downfield components. The differences in ^{15}N resonance positions between the two sub-spectra give the $^{1}\Delta_{NH}$ couplings ($^{1}J_{NH}+{}^{1}D_{NH}$). The residual $^{1}D_{NH}$ dipolar couplings are obtained by comparison with similar spectra recorded in isotropic solution, that yield values for $^{1}J_{NH}$ only.

As recently pointed out by Ottiger and Bax (1998b) and also by Lerche et al. (1999), the correct timing for the S^3E element is more sensitive to variations of the coupling constants when compared with other methods for the measurements of one-bond ^{15}N-^{1}H couplings (Ottiger and Bax, 1998b; Lerche et al., 1999). However, when compared with these methods our experiment presents a reduced length of the delays needed to select individual components, which makes it particularly advantageous when working with large molecules at high degrees of alignment, which exhibit rapid transverse magnetization dephasing due to fast relaxation and residual dipolar couplings. In practice, even with medium size molecules and a modest degree of protein alignment, a reduced number of delays outweigh the losses due to variations of the coupling constants. Furthermore, in the two experiments used for S^3E selection, the doublet components follow identical relaxation pathways, leading to better selection. Due to the large ^{15}N CSA/^{1}H-^{15}N dipole–dipole cross-correlation the two components will exhibit a very large differential line broadening (Gueron et al., 1983; Tjandra et al., 1996b). This has been constructively exploited in the TROSY type of experiments (Pervushin et al., 1997). Therefore, at high magnetic field it is preferable to select only the slowly relaxing component and to suppress the fast relaxing component. This is easily achieved by eliminating the hollow 90° nitrogen pulse just prior to t_1 (Pervushin et al., 1998). In this case, the $^{1}D_{NH}$ can be measured by comparison with a regular decoupled [^{1}H, ^{15}N] HSQC spectrum recorded in the same medium. Depending on whether both components or only the slowly relaxing component is to be selected, Boltzmann magnetization can be used either to enhance the weak or the strong component, respectively (Pervushin et al., 1997, 1998; Cordier et al., 1999). With our method, this can be easily achieved by appropriate selection of the phase of the first 90° nitrogen pulse.

Among the several possible band-selective broadband decoupling schemes (Zuiderweg and Fesik, 1991; McCoy and Mueller, 1993) we found that WURST adiabatic decoupling (Kupče and Freeman, 1995; Kupče and Wagner, 1995) represents the best choice as it is simple to implement and enjoys a very high figure of merit in terms of the effective bandwidth for a given rf power. The best choice for the length of the WURST pulse depends on the sum of the residual proton–proton dipolar couplings that need to be suppressed. In practice, we found that a WURST pulse length greater than $1/(5*\Delta LW)$, where ΔLW represents the difference between the observed average amide proton line widths in an isotropic solution and in the liquid crystalline phase, gives the best results. The band width (BW) to be decoupled should be large enough to cover the entire aliphatic region, without perturbing the amide region, but also has to satisfy the condition $(BW/5*\Delta LW) \geq 5$ to preserve the advantages of adiabatic decoupling. The ratio $(BW/5*\Delta LW)$ also determines the WURST-*n* index. A high *n* index yields a decoupling region with only small transitional regions and also helps to reduce sidebands. An *n* value $0.5(BW/5*\Delta LW)$ is recommended, although it is not critical (Kupče and Freeman, 1995; Kupče and Wagner, 1995). In addition to WURST aliphatic proton decoupling during the acquisition, our scheme also reduces the losses of magnetization due to amide to aliphatic protons residual dipolar couplings during the INEPT steps by using amide-proton selective REBURP refocusing pulses (Geen and Freeman, 1991).

Using this novel experiment allowed the measurement of relatively large residual heteronuclear dipolar couplings. Although the homonuclear decoupling introduces a slight Bloch–Siegert shift in the direct dimension, this does not affect the determination of the $^{1}D_{NH}$ residual dipolar couplings, as they are measured in the indirect dimension (see also Figure 1 C,D). Based on the distribution of the $^{1}D_{NH}$ residual dipolar couplings (Figure 3) we estimated an axial component of the alignment tensor (D_a^{NH}) of about -40 Hz (Clore et al., 1998), a value that is two- to fivefold larger than those commonly reported for use in structure refinement (Bewley et al., 1998; Cai et al., 1998; Drohat et al., 1999).

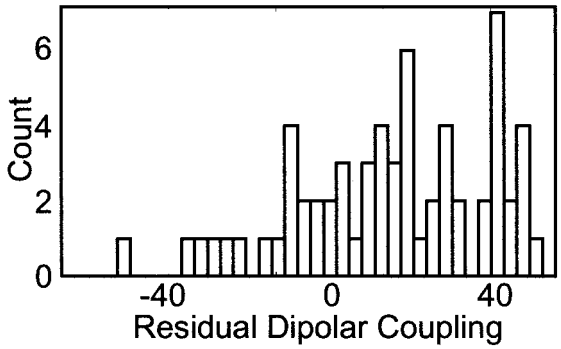

Figure 3. Distribution of the $^{1}D_{NH}$ residual dipolar couplings measured with the scheme of Figure 2 with a ~0.7 mM sample of ^{15}N labeled DnaK substrate binding unit (residues 386–561), dissolved in an aqueous solution containing 7.5% DMPC/DHPC/CTAB at 3.2:1:0.1 ratio. At 30 °C this solution exhibits a residual quadrupolar ^{2}H coupling of 9 Hz.

In conclusion, we demonstrate that selective homonuclear proton decoupling can be effectively employed to obtain spectra that on the one hand contain large residual heteronuclear dipolar coupling information (the $^{1}D_{NH}$ couplings in the example reported in this paper) but on the other hand are still amenable to solution NMR analysis. This observation also suggests the possibility of further increasing the degree of alignment in NMR samples without requiring perdeuteration, opening the way to the measurement of heteronuclear residual dipolar couplings which are currently inaccessible, as well as long-range dipolar ^{1}H-^{1}H interactions (Bolon and Prestegard, 1998; Hansen et al., 1998a,b), that may provide useful structural information.

Acknowledgements

This work was supported by a University of Michigan Regent's Fellowship to C.W.V.K. and by the National Institutes of Health Grant GM 52421.

References

Bewley, C.A., Gustafson, K.R., Boyd, M.R., Covell, D.G., Bax, A., Clore, G.M. and Gronenborn, A.M. (1998) *Nat. Struct. Biol.*, **5**, 571–578.

Bolon, P.J. and Prestegard, J.H. (1998) *J. Am. Chem. Soc.*, **120**, 9366–9367.

Cai, M., Huang, Y., Zheng, R., Wei, S., Ghirlando, R., Lee, M.S., Craigie, R., Gronenborn, A.M. and Clore, G.M. (1998) *Nat. Struct. Biol.*, **5**, 903–909.

Clore, G.M., Starich, M.R. and Gronenborn, A.M. (1998a) *J. Am. Chem. Soc.*, **120**, 10571–10572.

Clore, G.M., Gronenborn, A.M. and Bax, A. (1998b) *J. Magn. Reson.*, **133**, 216–222.

Cordier, F., Dingley, A.J. and Grzesiek, S. (1999) *J. Biomol. NMR*, **13**, 175–180.

Drohat, A.C., Tjandra, N., Baldisseri, D.M. and Weber, D.J. (1999) *Protein Sci.*, **8**, 800–809.

Geen, H. and Freeman, R. (1991) *J. Magn. Reson.*, **93**, 93–141.

Gueron, M., Leroy, J.L. and Griffey, R.H. (1983) *J. Am. Chem. Soc.*, **105**, 7262–7266.

Hansen, M.R., Mueller, L. and Pardi, A. (1998a) *Nat. Struct. Biol.*, **5**, 1065–1074.

Hansen, M.R., Rance, M. and Pardi, A. (1998b) *J. Am Chem. Soc.*, **120**, 11210–11211.

Koenig, B.W., Hu, J., Ottiger, M., Bose, S., Hendler, R.W. and Bax, A. (1999) *J. Am. Chem. Soc.*, **121**, 1385–1386.

Kupče, Ē. and Freeman, R. (1995) *J. Magn. Reson.*, **A115**, 273–276.

Kupče, Ē. and Wagner, G. (1995) *J. Magn. Reson.*, **B109**, 329–333.

Lerche, M.H., Meissner, A., Poulsen, F.M. and Sørensen, O.W. (1999) *J. Magn. Reson.*, **140**, 259–263.

Levitt, M.H. and Freeman, R. (1981) *J. Magn. Reson.*, **43**, 502–507.

Losonczi, J.A. and Prestegard, J.H. (1998) *J. Biomol. NMR*, **12**, 447–451.

McCoy, M.A. and Mueller, L. (1993) *J. Magn. Reson.*, **A101**, 122–130.

Meissner, A., Duus, J.O. and Sørensen, O.W. (1997) *J. Biomol. NMR*, **10**, 89–94.

Meissner, A., Schulte-Herbruggen, T. and Sørensen, O.W. (1998) *J. Am. Chem. Soc.*, **120**, 3803–3804.

Ottiger, M. and Bax, A. (1998a) *J. Biomol. NMR*, **12**, 361–372.

Ottiger, M., Delaglio, F. and Bax, A. (1998b) *J. Magn. Reson.*, **131**, 373–378.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1998) *J. Am. Chem. Soc.*, **120**, 6394–6400.

Prestegard, J.H. (1998) *Nat. Struct. Biol.*, **5**, 517–522.

Sanders, C.R. and Schwonek, J.P. (1992) *Biochemistry*, **31**, 8898–8905.

Sass, J., Cordier, F., Hoffmann, A., Rogowski, M., Cousin, A., Omichinski, J.G., Löwen, H. and Grzesiek, S. (1999) *J. Am. Chem. Soc.*, **121**, 2047–2055.

Shaka, A.J., Keeler, J., Frenkiel, T. and Freeman, R. (1983) *J. Magn. Reson.*, **52**, 335–338.

Sørensen, M.D., Meissner, A. and Sørensen, O.W. (1997) *J. Biomol. NMR*, **10**, 181–186.

Tjandra, N., Grzesiek, S. and Bax, A. (1996a) *J. Am. Chem. Soc.*, **118**, 6264–6272.

Tjandra, N., Szabo, A. and Bax, A. (1996b) *J. Am. Chem. Soc.*, **118**, 6986–6991.

Tjandra, N. and Bax, A. (1997) *Science*, **278**, 1111–1114.

Tolman, J.R., Glanagan, J.M., Kennedy, M.A. and Prestegard, J.H. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 9279–9283.

Zuiderweg, E.R.P. and Fesik, S.W. (1991) *J. Magn. Reson.*, **93**, 653–658.