Journal of Biomolecular NMR, **19:** 63–67, 2001.
KLUWER/ESCOM

Simple multidimensional NMR experiments to obtain different types of one-bond dipolar couplings simultaneously

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Received 31 July 2000; Accepted 17 October 2000

Key words: Bax, dipolar couplings, liquid crystal, protein structure

Abstract

In order to measure residual dipolar couplings, the molecule under study has to be partially oriented in the presence of the magnetic field. It has been observed that some protein samples are not stable under the conditions imposed by the orienting media. If different types of dipolar couplings are measured sequentially, their values will not agree with a unique alignment tensor that is changing slowly over time. This could bias the structure calculation. It would be more appropriate to obtain different types of dipolar couplings simultaneously, such that all the data correspond to one effective alignment tensor. We describe here a general NMR strategy designed to do so, that can be adapted to various existing pulse sequences.

Residual dipolar coupling has proven to be a very valuable parameter in the structure calculation of biopolymers (Tolman et al., 1995; Tjandra and Bax, 1997a). It provides long-range information that, in contrast to the nuclear Overhauser effect, can relate structural elements that are not in proximity. In proteins, the most common measured dipolar couplings are the one-bond amide NH, $C_\alpha H_\alpha$, $C'N$ and $C_\alpha C'$. The residual dipolar coupling (D) appears as an addition to the scalar coupling (J) under anisotropic conditions, and can be calculated by subtraction of the J value present under isotropic conditions. In order to obtain most of the dipolar couplings, 2D or pseudo-2D NMR experiments are used (Ottiger et al., 1998a, b). However, the analysis of 2D experiments can be difficult when studying large proteins, especially when these are mainly α-helical, since spectral overlap can be severe. In this case it will be more advantageous to use three-dimensional (3D) NMR experiments to measure dipolar couplings. Additionally, it would be preferable to measure as many types of J+D values as possible within the committed time of the 3D experiment, especially when the protein is unstable under the orienting media conditions. In this way, if the magnitude and/or orientation of the alignment tensor are changing slowly over time due to the instability of the sample, the different sets of dipolar couplings will be consistent with a unique effective alignment tensor. On the contrary, if the J+D values corresponding to different types of bond vectors are measured sequentially and the sample conditions are changing slowly over time, the alignment tensor will be systematically different for each type. This will bias the protein structure calculation process.

In order to illustrate this idea we have modified the 3D NMR experiments HNCO (Kay et al., 1994) and (HA)CA(CO)NH (Tjandra and Bax, 1997b). The modification consists of allowing one or more J couplings to evolve during the chemical shift evolution periods of the indirect dimensions. In addition, the IPAP strategy (Ottiger et al., 1998a) is employed and it serves to reduce spectral overlap by acquiring the in-phase and anti-phase components resulting from J coupling in an interleaved manner. The in-phase and anti-phase components are processed separately. The data are added and subtracted giving rise to two spec-

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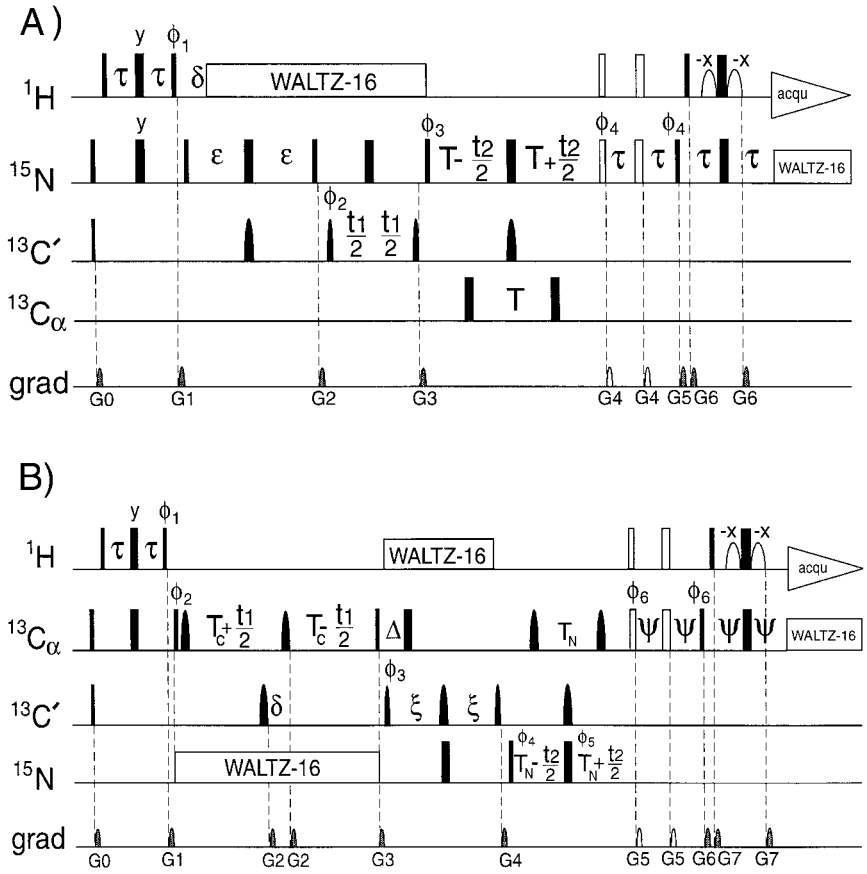

Figure 1. Schematic representation of the pulse programs used for the modified version of the HNCO (A) and the (HA)CA(CO)NH (B) experiments. In Figure 1A, $\tau = 2.65$ ms, $\delta = 5.4$ ms, $\varepsilon = 12.5$ ms, $2T = 27.2$ ms. All pulses for which there is no specification of phase are applied in x. The phase cycling of the rest of the pulses is: $\phi1 = y, -y$; $\phi2 = 4(x), 4(-x)$; $\phi3 = x$; $\phi4 = 2(-x), 2(x)$; Rec. = x, 2(−x), x, −x, 2(x), −x. Quadrature detection in the ^{13}C and ^{15}N dimensions is achieved by States-TPPI on $\phi2$ and $\phi3$, respectively. All gradients are sine-bell shaped with a strength of 25 G/cm when applied on x or y and 35 G/cm on z. The durations of the gradients are : $G_{0,1,2,3,4,5,6} = 2.5$, 1.1, 0.7, 1.3, 1.0, 2.7, 0.4 ms, with respective gradient axes: yz, x, yz, xz, xy, yz, z. In Figure 1B, $\tau = 1.5$ ms, $\delta = 8.6$ ms, $T_c = 12.6$ ms, $\Delta = 4.5$ ms, $\xi = 13$ ms, $2T_N = 29.6$ ms, $\psi = 2.65$ ms. The phase cycling is: $\phi1 = y, -y$; $\phi2 = x$; $\phi3 = 2(x), 2(-x)$; $\phi4 = x$; $\phi5 = 4(x), 4(y)$; $\phi6 = 4(x), 4(-x)$; Rec. = x, 2(−x), 2(x), 2(−x), x. Quadrature detection in the ^{13}C and ^{15}N dimensions is achieved by States-TPPI on $\phi2$ and $\phi4$, respectively. The lengths of the gradients are: $G_{0,1,2,3,4,5,6,7} = 2.5$, 1.0, 0.7, 1.3, 1.2, 1.0, 2.5, 1.0 ms, and their respective axes are: yz, x, xyz, xz, y, xy, yz, z. For both figures, narrow rectangular bars represent 90° pulses, while wide bars represent 180° pulses. Shaped pulses are represented by non-rectangular bars and are (sinx)/x in shape, except the second and third 180° pulses in C_α of Figure 1B, whose shape is hyperbolic-secant type with a squareness level of 3. The white bars represent the extra pulses that are applied in an interleaved manner to collect the in-phase ^{15}N magnetization. The anti-phase ^{15}N magnetization is collected with the application of the white pulses.

tra, each containing one of the components of the multiplet.

In the modified HNCO (Figure 1A), the $C_\alpha C'$ coupling is active during C' chemical shift evolution. Therefore, it allows the measurement of the $C_\alpha C'$ dipolar coupling. The IPAP strategy (Ottiger et al., 1998a) is incorporated after the ^{15}N constant time evolution, during which the ^{1}H-^{15}N coupling is active. This allows one to obtain the amide J coupling. In the IPAP strategy the anti-phase magnetization N_yH_z present after the ^{15}N constant time is transferred to anti-phase proton magnetization with a regular INEPT step symbolized by the last ^{15}N and ^{1}H 90° pulses of Figure 1A. Subsequently, ^{1}H in-phase is generated and then recorded. After the constant time evolution, the N_x in-phase magnetization is transformed into ^{15}N anti-phase magnetization during the time 2τ, in the middle of which two 180° pulses, one in ^{1}H and the other in ^{15}N, are applied simultaneously (white pulses in Figure 1A) to allow ^{1}H-^{15}N J coupling evolution. The two 90° pulses represented as white bars in Figure 1A do no affect the N_x in-phase magnetization,

while they serve as purge pulses. The in-phase and anti-phase components are collected separately in an interleaved manner. The pulses represented by white bars in Figure 1 are applied only when collecting the in-phase magnetization. Pulses symbolized as black bars (Figure 1) are applied when collecting both the in-phase and the anti-phase magnetization. Once the two experiments are processed, the ^{15}N in-phase and anti-phase multiplets of the three-dimensional spectra are added and subtracted such that spectral overlap is reduced. This same idea has also been applied to an (HA)CA(CO)NH type experiment (Figure 1B). In this example, $C_\alpha H_\alpha$ dipolar coupling is measured during C_α chemical shift evolution and the IPAP (Ottiger et al., 1998a) strategy is applied after the ^{15}N constant time evolution. In this case amide NH dipolar couplings can also be obtained since ^{1}H-^{15}N J coupling is active during the constant time.

Residual dipolar couplings are measured by allowing J coupling evolution between a pair of nuclei while the chemical shift of one of them is recorded. The presence of J coupling implies that the signal to noise ratio will be reduced due to the splitting of the signal. If J+D values of different types of one-bond vectors are to be measured simultaneously in one NMR experiment, the signal will be halved the corresponding number of times. In the experiments proposed herein, the signal is halved twice in order to measure two different types of one-bond dipolar coupling. The signal to noise ratio will be the square-root of 2 smaller if the J+D values are measured simultaneously than if they are measured sequentially, using the same NMR experimental time. As it will be shown later, it is possible to obtain two values for each J+D. The use of the average value will compensate for the loss in sensitivity, such that the error in the measured dipolar couplings depending on the signal to noise ratio will be the same no matter if they are measured sequentially or simultaneously.

The modified experiments HNCO and (HA)CA (CO)NH have been used to measure dipolar couplings of the apoptotic inducing protein Bax (Oltvai et al., 1993). This protein has been selected as an example since it is all α-helical and it is a relatively large protein for NMR structure determination (∼22 kDa); thus, severe NMR signal overlap has been observed. In fact, using the conventional 2D experiments, the dipolar couplings of only ∼20% of the residues can be obtained. The use of the 3D NMR experiments described here greatly reduces spectral overlap. The protein is stable in the fd bacteriophages solution (Clore et al., 1998; Hansen et al., 1998) that served to create the anisotropic environment. Nevertheless, there is evidence that a number of proteins are unstable in orienting media such as bicelles (Clore et al., 1998). Additionally, it is known that certain phospholipids used to form bicells, which contain ester bonds, are hydrolyzed over time, thus the orienting properties of the solution are modified too (Ottiger and Bax, 1999). For these cases it may be convenient to measure dipolar couplings of different bond vectors simultaneously, and the utilization of the pulse programs presented herein allows one to do so.

Figure 2 shows selected portions of the modified versions of the HNCO and the (HA)CA(CO)NH spectra acquired for the protein Bax. As can be observed, two values of the dipolar coupling of each bond vector can be measured. The frequency difference between the two components of each multiplet can be used to obtain two values of the $C_\alpha C'$ (in the case of the HNCO) and $C_\alpha H_\alpha$ (in the case of the (HA)CA(CO)NH) dipolar couplings for a particular residue. In addition, the difference in frequency of the ^{15}N up-field and down-field components of each of the signals of the multiplet allows one to obtain two values of the amide NH dipolar coupling from each NMR experiment.

Figures 3A and B represent the amide NH and $C_\alpha H_\alpha$ J+D values obtained for the protein Bax using the proposed experiments, in which different J-couplings are measured simultaneously, versus the same type of dipolar couplings obtained using the HNCO and the (HA)CA(CO)NH experiments in which only one J-coupling is measured. While developing the pulse programs presented here, we have found systematic deviations between the two sets of data depending on the position in the sequence of the selection of the individual components of the doublet. The good correlation observed indicates that the modifications introduced in the pulse programs to obtain several J-couplings simultaneously do not affect the results in any way.

In conclusion, the proposed experiments are expected to facilitate the measurement of dipolar couplings in medium-sized proteins, since the incorporation of the IPAP strategy plus the use of three dimensions will reduce spectral overlap. Additionally, in the case of an unstable anisotropic sample, the values corresponding to different types of one-bond dipolar couplings are measured simultaneously, and this will ensure an agreement between the obtained data and a unique effective alignment tensor. The reduction in

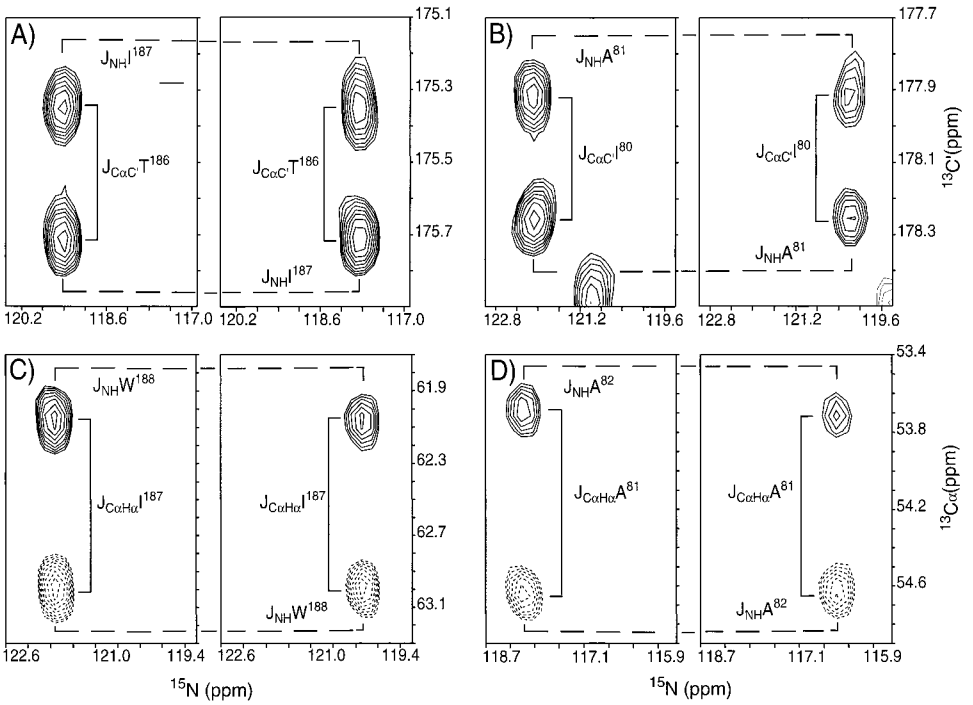

Figure 2. Selected regions of the HNCO-based (A and B) and the (HA)CA(CO)NH-based experiments (C and D). A and B show the two J+D values that can be measured for NH of residues I187 and A81 (dashed lines), as well as the two values for J+D of $C_\alpha C'$ of residues T186 and I80 (solid lines). J+D values of NH of residues W188 and A82 (dashed lines), together with J+D values of $C_\alpha H_\alpha$ of residues I187 and A81 (solid lines) are shown in parts C and D.

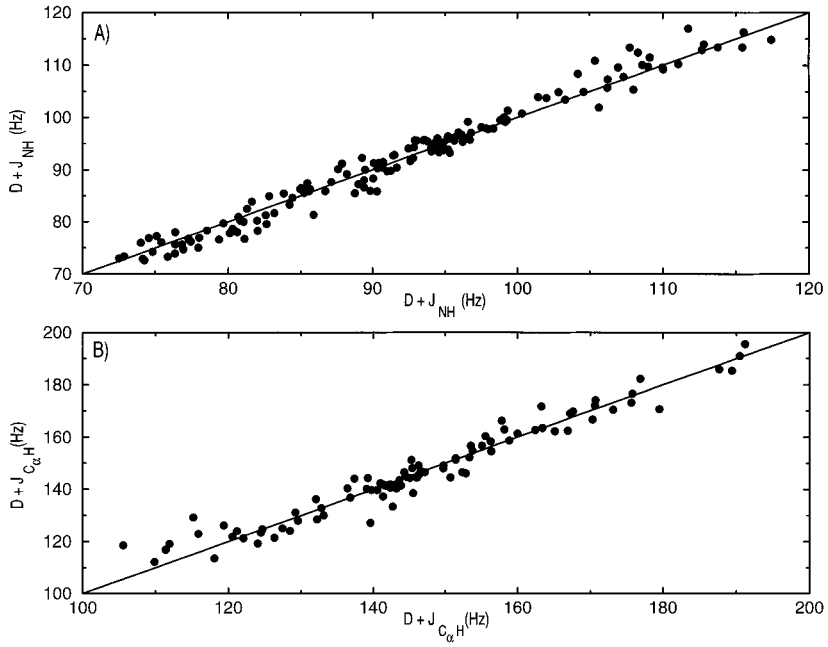

Figure 3. J+D values of NH (A) and $C_\alpha H_\alpha$ (B) of the protein Bax in bateriophages measured with the modified HNCO and (HA)CA(CO)NH experiments to measure various J-couplings simultaneously, versus the same type of data obtained with the original versions of these experiments.

signal to noise ratio is compensated in terms of error in the measurement by the use of the average of the two values obtained for each bond vector. More types of one-bond dipolar couplings can easily be measured if additional couplings are kept active during chemical shift evolution. For example, the D+J value of the C′N bond vector can be obtained if the coupling between both nuclei is active during the ^{15}N constant time. For medium-sized proteins (∼20 kDa) up to four different types of dipolar couplings can be measured simultaneously without generating severe overlap. The strategy presented is general and can easily be applied to various 3D experiments.

Acknowledgements

E.d.A. is recipient of a postdoctoral fellowship from the Human Frontier Science Program.

Note added in proof

During the writing of this manuscript the work reported by Permi et al. has been published in *J. Biomol. NMR* (vol. 17 (2000) 43–54) in which the IPAP strategy is incorporated into the HNCO-based experiment.

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