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A novel experiment for the quantitative measurement of CSA(^1H_N)/CSA(^{15}N) cross-correlated relaxation in ^{15}N-labeled proteins*

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Abstract

An experiment is presented which allows for the quantitative measurement of the relaxation interference between the ^1H_N CSA and ^{15}N CSA interactions in ^{15}N labeled proteins. A constant-time buildup scheme is used to measure the differential relaxation rate, η, between double-quantum (DQ) and zero-quantum (ZQ) ^1H_N-^{15}N coherences. The CSA/CSA experiment was recorded at three different B_o field strengths. The CSA(^1H_N)/CSA(^{15}N) cross-correlation rate was obtained from the linear fit of the measured rate, η, versus B_o^2 for 77 residues of the EH2 domain from mouse Eps15.

The measurement of cross-correlated relaxation rates has recently attracted a great deal of interest because of the high content of structural and dynamical information related to these rates (Reif et al., 1997; Kroenke et al., 1998). For instance, the H_N chemical shift anisotropy (CSA) derived from the measurement of CSA/dipolar (CSA/DD) relaxation interference in ^{15}N labeled proteins has proved to be a very good indicator for hydrogen bonding (Tessari et al., 1997a; Tjandra and Bax, 1997). Recent solid-state NMR data on crystalline hydrates have also shown that the orientation and asymmetry of the ^1H CSA tensor are highly correlated with hydrogen bonding geometry (Wu et al., 1998). For biomolecules, such information would also be of major importance because of the crucial role of the hydrogen bond in molecular structure and recognition.

Here, we present a novel experiment which allows for the quantitative measurement of the relaxation interference between the ^1H_N CSA and ^{15}N CSA interactions in ^{15}N labeled proteins. The combination of this experiment and previously reported CSA(^1H_N)/DD(^1H_N-^{15}N) and CSA(^{15}N)/DD(^1H_N-^{15}N) experiments (Tjandra et al., 1996; Tessari et al., 1997a,b; Tjandra and Bax, 1997) will provide a more complete picture of the ^1H_N CSA tensor.

The experiment is based on the measurement of the difference between the relaxation rates of double-quantum (Γ^{DQ}) and zero-quantum (Γ^{ZQ}) ^1H_N-^{15}N coherences. For a multi-spin system the differential rate, η, can be written as (Konrat and Sterk, 1993; Kumar and Kumar, 1996; Norwood et al., 1999):

$$\eta = \frac{\Gamma^{DQ} - \Gamma^{ZQ}}{2} = \eta^{cc} + \sigma^{dd} + \eta^{dd} + \eta^{exch} \quad (1)$$

where η^{cc}, σ^{dd}, and η^{dd} indicate the CSA(^1H_N)/CSA(^{15}N) cross-correlation rate, the ^1H_N-^{15}N cross-relaxation rate, and the sum of the cross-correlation rates between dipolar interactions involving other spins, *i*, close to the ^1H_N-^{15}N pair, respectively. The last term, η^{exch}, represents the cross-correlation rate between two rank-1 relaxation mechanisms, namely ^{15}N and ^1H chemical exchange. These rates are given by the following expressions:

$$\eta^{cc} = \frac{8}{45}\gamma_H\gamma_N\Delta\sigma_N\Delta\sigma_H B_o^2 J_{HN}(0) \quad (2a)$$

*This paper is dedicated to the memory of Gino Tessari.
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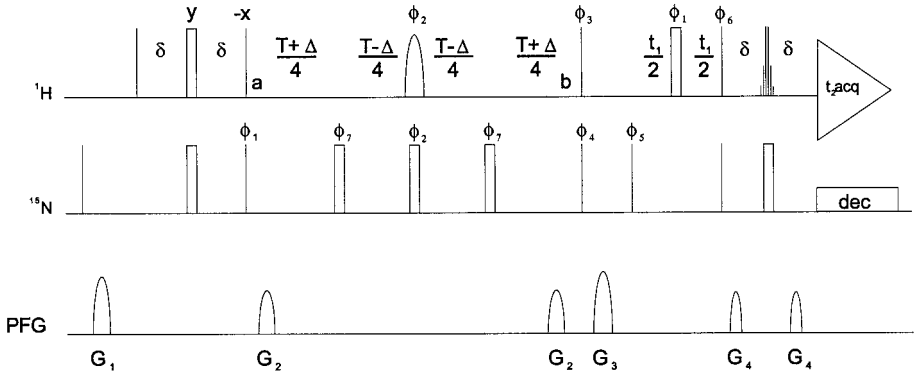

Figure 1. Pulse scheme for the quantitative measurement of relaxation interference effects between ^1H_N CSA and ^{15}N CSA. Narrow and wide pulses correspond to flip angles of 90° and 180°, respectively. Scheme A is used to record the magnetization terms arising from CSA(^1H_N)/CSA(^{15}N) cross correlation in the period Δ, while scheme B is used for the reference experiment. The phase of all pulses is assumed x, unless indicated. Phase cycling: ϕ_1 = y,−y; ϕ_2 = 8(x),8(y),8(−x),8(−y); ϕ_3 = 4(x),4(−x) (scheme A) or ϕ_3 = 4(y),4(−y) (scheme B); ϕ_4 = 2(x),2(−x) (scheme A) or ϕ_4 = 2(y),2(−y) (scheme B); ϕ_5 = x; ϕ_6 = 16(x),16(−x); ϕ_7 = 32(x),32(y),32(−x),32(−y); receiver = R, R̲, R, R̲, R̲, R, R̲, R, where R = x,−x,−x,x and R̲ = −x,x,x,−x. Quadrature detection in the t_1 dimension is accomplished by incrementing ϕ_5 in the States-TPPI manner. Delay durations: T = 30 ms; δ = 2.75 ms; Δ = 20, 30 ms at 11.7 T; 12.8, 25.6 ms at 14.1 T; 15, 25 ms at 17.6 T. All gradients are sine-bell shaped; $G_{1,2,3,4}$ = 1.0 ms, 20 G/cm; 1.0 ms, 17 G/cm; 1.5 ms, 27 G/cm; 1.0 ms, −7 G/cm. A REBURP pulse (Geen and Freeman, 1991) with the center of its excitation profile at 8.1 ppm and a width of 4.76 ms at 500 MHz is used to selectively refocus the signals in the amide region. In the reverse INEPT step a 3-9-19-19-9-3 WATERGATE refocusing pulse is used (Sklenar et al., 1993). Typically 256 scans were recorded for each hypercomplex increment, yielding a total experimental time of ∼20 h.

$$\sigma^{dd} = \frac{1}{10}\left(\frac{\mu_o}{4\pi}\right)^2\left(\frac{\gamma_H\gamma_N\hbar}{r_{HN}^3}\right)^2 [6J_{HNHN} \times(\omega_H + \omega_N) - J_{HNHN}(\omega_H - \omega_N)] \quad (2b)$$

$$\eta^{dd} = \frac{2}{5}\left(\frac{\mu_o}{4\pi}\right)^2\gamma_H\gamma_N\hbar^2\sum_i\frac{\gamma_i^2}{r_{Ni}^3 r_{Hi}^3}\left[J_{HiNi}(0) + \frac{3}{4}J_{HiNi}(\omega_i)\right] \quad (2c)$$

$$\eta^{exch} = 2p_A p_B\delta\omega_N\delta\omega_H\tau_{exch} = 2p_A p_B\gamma_N\gamma_H\delta\sigma_N^{iso}\delta\sigma_H^{iso}B_o^2\tau_{exch} \quad (2d)$$

where $\Delta\sigma_H$ and $\Delta\sigma_N$ indicate the anisotropy of the chemical shift ($\sigma_{||} - \sigma_\perp$) for the ^1H_N and ^{15}N nuclei, respectively. The symbols B_o, γ_H, γ_N, γ_i, r_{HN}, r_{Hi}, and r_{Ni} have their usual meanings, where i is defined as above. $J_{HN}(\omega)$, $J_{HiNi}(\omega)$ and $J_{HNHN}(\omega)$ denote the spectral density for the CSA-CSA cross-correlation, the dipolar cross-correlation, and the dipolar auto-correlation, respectively. Expression 2d holds in the case of fast conformational exchange between two sites with populations p_A and p_B and an exchange correlation time τ_{exch}. The symbols $\delta\sigma_N^{iso}$ and $\delta\sigma_H^{iso}$ denote the difference in ^{15}N and ^1H isotropic chemical shift, respectively.

In the simple case of isotropic motion of a rigid body, the following equalities hold:

$$J_{HN}(\omega) = J(\omega)\left(\frac{3\cos^2\theta_{HN} - 1}{2}\right) = \frac{\tau_c}{1+\omega^2\tau_c^2}\left(\frac{3\cos^2\theta_{HN} - 1}{2}\right) \quad (3a)$$

$$J_{HiNi}(\omega) = J(\omega)\left(\frac{3\cos^2\theta_{HiNi} - 1}{2}\right) = \frac{\tau_c}{1+\omega^2\tau_c^2}\left(\frac{3\cos^2\theta_{HiNi} - 1}{2}\right) \quad (3b)$$

$$J_{HNHN}(\omega) = J(\omega) = \frac{\tau_c}{1+\omega^2\tau_c^2} \quad (3c)$$

where τ_c indicates the rotational correlation time and θ_{HN} and θ_{HiNi} denote the angle between the axes of the two CSA tensors, assumed here as being axially symmetric (Gerald et al., 1993), or the two dipolar interactions, respectively.

Figure 1 shows the pulse scheme used for the quantitative measurement of the CSA(^1H_N)/CSA(^{15}N) relaxation interference. Rather than measuring Γ^{DQ} and Γ^{ZQ} separately, as recently proposed by Norwood et al. (1999), our experiment allows the direct observation of the terms generated by the

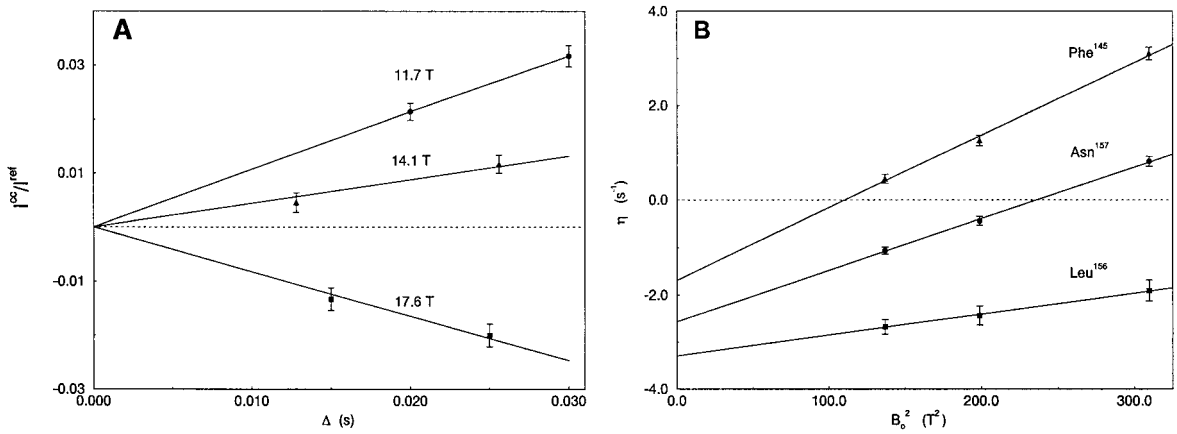

Figure 2. (A) Buildups of the ratio I^{cc}/I^{ref} for residue Asn^{157} at 11.7 T (circle), 14.1 T (triangle) and 17.6 T (square). The curves obtained from the linear fits of the ratio I^{cc}/I^{ref} versus the time interval Δ, according to Equation 4, are shown. (B) Linear fit of the measured η rate versus the square of the field strength, B_o, for residues Phe^{145} (triangle), Leu^{156} (square) and Asn^{157} (circle) in mEH2.

CSA(^{1}H$_N$)/CSA(^{15}N) cross-correlated relaxation. A constant-time buildup scheme is used, as previously employed for measuring the CSA(^{1}H$_N$)/DD(^{1}H$_N$-^{15}N) and CSA(^{15}N)/DD(^{1}H$_N$-^{15}N) cross-correlation rates (Tessari et al., 1997a,b).

Between time points *a* and *b* of the pulse scheme, the $2N_xH_x$ term of the density operator, corresponding to the superposition of DQ and ZQ ^{1}H$_N$-^{15}N coherences, precesses in the transverse plane for a period of duration T. During the time interval Δ, as a consequence of the differential relaxation of DQ and ZQ coherences, $2N_xH_x$ coherence is partially converted into $2N_yH_y$ coherence. Neither scalar coupling nor chemical shift evolution contribute to the creation of the $2N_yH_y$ term, since they are both refocused at time point *b* of the sequence. Depending on the phases of the ^{15}N 90° and ^{1}H 90° pulses following time point *b*, the $2N_yH_y$ term (scheme A) or the $2N_xH_x$ term (scheme B) can be selected. After the purging gradient G$_3$, a HSQC-like sequence is employed for chemical shift labeling and acquisition.

Scheme B of the experiment is used to record the reference spectrum with $\Delta = 0$. Because of the interconversion of DQ and ZQ coherences, no net differential relaxation occurs, and no $2N_yH_y$ term is present at time point *b* of the sequence.

The ratio of the signal intensities measured with the two schemes, I^{cc} and I^{ref}, is given by:

$$\frac{I^{cc}}{I^{ref}} = \frac{C\exp(-\lambda T)\sinh(-\eta\Delta)}{C\exp(-\lambda T)} = \sinh(-\eta\Delta) \approx -\eta\Delta \qquad (4)$$

where λ denotes the DQ/ZQ auto-relaxation rate, η is defined as in Equation 1 and the proportionality factor *C* resulting from the experimental setup is the same for both schemes since the number of pulses and the duration of delays are identical.

A buildup curve of the ratio I^{cc}/I^{ref} can be obtained by recording several experiments at different values of the delay Δ (scheme A), and one reference spectrum (scheme B). A linear fit of the buildup curve provides the value of η. Assuming a typical value of −0.2 for the ^{15}N-{^{1}H$_N$} NOE and of 0.7 s for ^{15}N T_1 yields a cross relaxation rate $\sigma^{dd} \sim 0.015$ s^{-1}, well below the experimental error (vide infra). Therefore, the contribution of σ^{dd} to the value of η will not be considered in the following. For biomolecules, $J_{HiNi}(\omega_i)$ can be neglected from Equation 2c, in which case Equations 1 and 2 show that η depends linearly on the square of the field. By measuring η at different field strengths it is possible to separate the contribution of the field-independent term, η^{dd}, from $\eta^{cc} + \eta^{exch}$.

The CSA/CSA experiment was recorded on a 1.5 mM solution of uniformly ^{15}N labeled EH2 from mouse Eps15 (mEH2) in acetic buffer at pH 5.1, 100 mM NaCl, 95% ^{1}H$_2$O, 5% ^{2}H$_2$O, T = 288 K, using Varian Inova spectrometers at 11.7 and 17.6 T and a Bruker DRX spectrometer at 14.1 T. The buildup curve of the ratio I^{cc}/I^{ref} for residue Asn^{157} at three field strengths is shown in Figure 2A. A change in the sign of the slope is observed increasing the field strength from 11.7 T to 17.6 T, indicating that the rates ($\eta^{cc} + \eta^{exch}$) and η^{dd} have opposite signs. Figure 2B shows the fit of the rate η as a function of B_o^2 for residues Phe^{145}, Leu^{156}, and Asn^{157}. The two

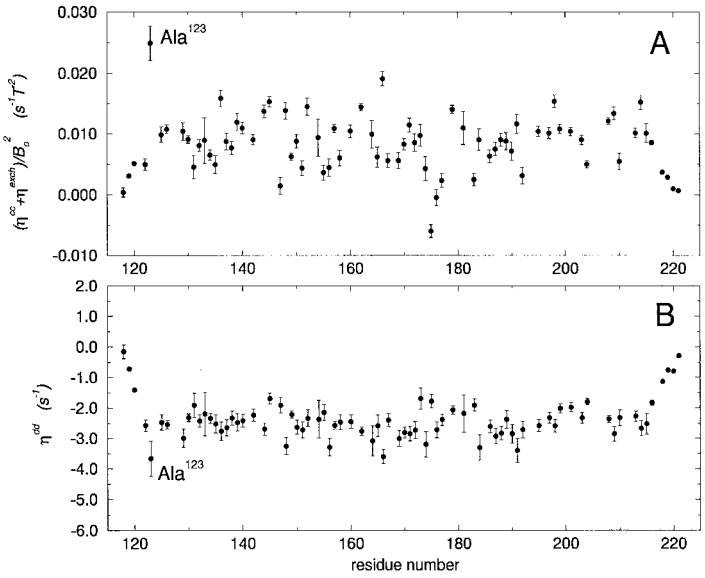

Figure 3. Values of $(\eta^{cc} + \eta^{exch})/B_0^2$ (A) and η^{dd} (B) as a function of residue number in mEH2. Residue Ala^{123} is indicated.

contributions $(\eta^{cc} + \eta^{exch})/B_o^2$ and η^{dd} correspond to the slope and intercept of these fits, respectively. For mEH2, data were obtained for 77 non-overlapping signals out of 95 non-proline residues. The values of $(\eta^{cc} + \eta^{exch})/B_o^2$ and η^{dd} are shown in Figure 3 as a function of the residue number. The magnitude of $(\eta^{cc} + \eta^{exch})/B_o^2$ for the residues located in the structured regions of mEH2 spans a range of 0.005–0.025 $s^{-1}T^{-2}$, with an average value of 0.008 $s^{-1}T^{-2}$. An extreme value of $(\eta^{cc} + \eta^{exch})/B_o^2$ is found for residue Ala^{123} (cf. Figure 3), probably as a consequence of the η^{exch} contribution. This observation is consistent with the presence of chemical exchange for this residue, as indicated by the analysis of ^{15}N relaxation data. No significant chemical exchange was detected for the other residues, for which we can safely neglect η^{exch}. We find that the rate η^{cc}/B_o^2 is positive for most residues, which is in agreement with the single observation by Norwood et al. (1999). Using ^{15}N CSA values obtained from ^{15}N relaxation studies, a rotational correlation time τ_c of ∼9 ns, and assuming $\Delta\sigma_H$ of 10 ppm, the calculated η^{cc}/B_o^2 rates fall in the 0.007–0.009 $s^{-1}T^{-2}$ range. A complete analysis of the results of the CSA(^1H_N)/CSA(^{15}N) experiment, in combination with the results of the CSA(^1H_N)/DD(^1H_N-^{15}N) and CSA(^{15}N)/DD(^1H_N-^{15}N) experiments, will yield a more detailed understanding of the CSA of individual ^{15}N and ^1H spins in a protein. This analysis is currently in progress, and will be reported elsewhere.

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