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Journal of Magnetic Resonance 178 (2006) 72–76
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Effective rotational correlation times of proteins from NMR relaxation interference

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Received 19 May 2005; revised 21 August 2005
Available online 26 September 2005

Abstract

Knowledge of the effective rotational correlation times, τ_c, for the modulation of anisotropic spin–spin interactions in macromolecules subject to Brownian motion in solution is of key interest for the practice of NMR spectroscopy in structural biology. The value of τ_c enables an estimate of the NMR spin relaxation rates, and indicates possible aggregation of the macromolecular species. This paper reports a novel NMR pulse scheme, [^{15}N,^{1}H]-TRACT, which is based on transverse relaxation-optimized spectroscopy and permits to determine τ_c for ^{15}N–^{1}H bonds without interference from dipole–dipole coupling of the amide proton with remote protons. [^{15}N,^{1}H]-TRACT is highly efficient since only a series of one-dimensional NMR spectra need to be recorded. Its use is suggested for a quick estimate of the rotational correlation time, to monitor sample quality and to determine optimal parameters for complex multidimensional NMR experiments. Practical applications are illustrated with the 110 kDa 7,8-dihydroneopterin aldolase from *Staphylococcus aureus*, the uniformly ^{15}N-labeled *Escherichia coli* outer membrane protein X (OmpX) in 60 kDa mixed OmpX/DHPC micelles with approximately 90 molecules of unlabeled 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), and the 16 kDa pheromone-binding protein from *Bombyx mori*, which cover a wide range of correlation times.

Keywords: NMR; Cross-correlated relaxation; TROSY; Brownian motion; Effective rotational correlation time

1. Introduction

The effective rotational correlation time, τ_c, in a molecule is a key parameter for nuclear magnetic resonance (NMR) spectroscopy in solution [1,2]. In folded proteins, τ_c usually correlates to a good approximation with the molecular weight, and can, for example, indicate if aggregates are formed under the chosen conditions [3,4]. Knowing τ_c permits to optimize the NMR experiments, and to estimate spin relaxation rates and magnetization transfer properties of given experimental schemes. For proteins with molecular weights up to about 30 kDa, τ_c is commonly estimated from the ratios of transverse and longitudinal ^{15}N relaxation rates [5–8]. For larger proteins these approaches become unreliable due to the effect of the intramolecular motion on the longitudinal relaxation [8]. This paper presents a novel experiment for the determination of τ_c, [^{15}N,^{1}H]-TRACT (TROSY for rotational correlation times). Based on the TROSY principle, [^{15}N,^{1}H]-TRACT suppresses the influence of dipole–dipole (DD) relaxation by remote protons in backbone ^{15}N–^{1}H moieties as well as relaxation contributions from chemical exchange, and thus largely eliminates the influence of highly effective relaxation mechanisms that tend to deteriorate measurements of τ_c in large molecules. Since only one-dimensional spectra need to be recorded, the data can be rapidly analysed. The use of [^{15}N,^{1}H]-TRACT is suggested for rapid estimates of τ_c, and as a basis for the set-up of complex multidimensional NMR experiments.

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doi:10.1016/j.jmr.2005.08.014

2. Materials and methods

The [^{15}N,^{1}H]-TRACT experiment makes use of cross-correlation between CSA and DD relaxation in the amide groups of the protein backbone to estimate the rotational correlation time τ_c [9–11]. The two transitions of a ^{15}N nucleus in an amide moiety have different transverse relaxation rates [12]. Assuming an axially symmetric ^{15}N chemical shift tensor oriented with an angle θ between its unique axis and the N–H bond, the relaxation rates for the α- and β-spin states are given by R_α and R_β, respectively:

$$R_\alpha = \lambda - \eta_{xy} + R_H + R_{CS}, \quad (1)$$

$$R_\beta = \lambda + \eta_{xy} + R_H + R_{CS}, \quad (2)$$

λ is the auto-relaxation rate, η_{xy} stands for the transverse cross-correlated relaxation rate, R_{CS} is the relaxation contributed from chemical exchange, and R_H describes the transverse relaxation due to dipole–dipole (DD) coupling with remote protons [9,12,13]. η_{xy} can then be calculated as the difference of Eqs. (1) and (2) [9,12]

$$R_\beta - R_\alpha = 2\eta_{xy} = 2p\delta_N(4J(0) + 3J(\omega_N))(3\cos^2\theta - 1), \quad (3)$$

where p is the DD coupling between ^{1}H and ^{15}N of the ^{15}N–^{1}H moiety

$$p = \mu_0\gamma_H\gamma_N h/(16\pi^2\sqrt{2}r_{HN}^3), \quad (4)$$

and δ_N is the chemical shift anisotropy (CSA) of the ^{15}N nucleus,

$$\delta_N = \gamma_N B_0 \Delta\delta_N/(3\sqrt{2}). \quad (5)$$

γ_H and γ_N are the gyromagnetic ratios of ^{1}H and ^{15}N, respectively. h is the Planck constant, r_{HN} is the ^{15}N–^{1}H internuclear distance, $\Delta\delta_N$ is the difference of the two principal components of the axially symmetric ^{15}N chemical shift tensor, and $J(\omega)$ represents the spectral density function at the frequency ω [13]

$$J(\omega) = 0.4\tau_c/[1 + (\tau_c\omega)^2]. \quad (6)$$

In Eq. (3), the effective rotational correlation time, τ_c, is contained only in the spectral density functions (Eq. (6)), and τ_c can thus be calculated if η_{xy} is known from measurements of R_α and R_β (Eqs. (1) and (2)). The value thus obtained for τ_c represents a lower limit for the overall rotational correlation time, due to the rigid body assumption used.

The transverse cross-correlated relaxation rate, η_{xy}, can efficiently be measured using the new pulse scheme [^{15}N,^{1}H]-TRACT (Fig. 1). Thereby, a series of experiments with different relaxation periods Δ_i is recorded, where each experiment consists of two measurements with different phase cycles that select the α- and β-spin states, respectively. The ^{1}H magnetization transfer to ^{15}N at time point a (Fig. 1) is represented by the single-transition operators S_α and S_β [9]

$$\sigma(a) = S_\alpha + S_\beta. \quad (7)$$

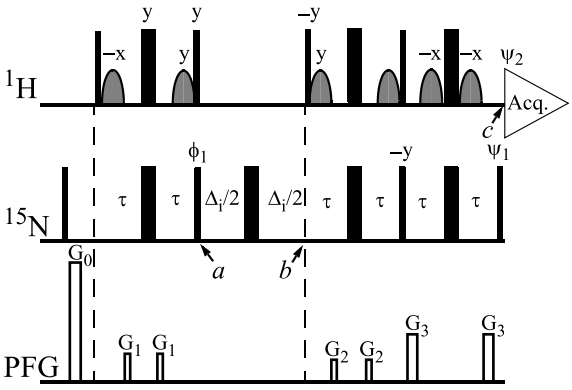

Fig. 1. 1D [^{15}N,^{1}H]-TRACT pulse scheme for measuring the effective rotational correlation time, τ_c, in macromolecules (TRACT = TROSY for rotational correlation times). A τ_c-determination is based on a series of 1D [^{15}N,^{1}H]-TRACT recordings with variable Δ_i values, and with phase cycle-selection of the α- and β-spin states of ^{15}N. In the ^{1}H and ^{15}N radio-frequency (rf) channels, narrow and wide black bars stand for nonselective 90° and 180° rf-pulses, respectively. Phases are x, unless indicated otherwise above the pulse. The ^{1}H selective pulses (curved shapes) are used to maintain the water magnetization along the positive z-axis. The ^{1}H and ^{15}N carrier frequencies are set at the water resonance, and at 118 ppm, respectively. $\tau = 1/(4\ {}^{1}J_{NH}) = 2.7$ ms. The α-spin state is selected with $\phi_1 = \{y,-y,-x,x\}$, $\psi_1 = \{-x\}$, ψ_2 (receiver) $= \{y,-y,-x,x\}$, and the β-spin state with $\phi_1 = \{y,-y,x,-x\}$, $\psi_1 = \{x\}$, ψ_2 (receiver) $= \{y,-y,-x,x\}$. For proteins smaller than about 10 kDa it may be preferable to suppress cross relaxation by setting $\Delta_i = n_i/{}^{1}J_{NH}$, where n_i is an arbitrary integer [12,24]; in our measurements this modification of the scheme had no detectable influence. A 2D version of [^{15}N,^{1}H]-TRACT is obtained by replacing the two $\Delta_i/2$-periods with $(\Delta_i/2 - t_1/2)$ and $(\Delta_i/2 + t_1/2)$, respectively. The evolution period, t_1, is thus incremented independently of the value chosen for Δ_i. To obtain phase-sensitive recordings along the t_1 dimension, a second FID for each t_1 value is recorded with the following phase cycling: for the α-spin state of ^{15}N, $\phi_1 = \{y,-y,x,-x\}$, $\psi_1 = \{x\}$ and ψ_2 (receiver) $= \{-x,x,y,-y\}$, and for the β-spin state, $\phi_1 = \{y,-y,x,-x\}$, $\psi_1 = \{-x\}$ and ψ_2 (receiver) $= \{-x,x,-y,y\}$. The data is processed using the method described in [25]. The pulsed magnetic field gradients (PFG) along the z-axis are: G_0, amplitude = 80 G/cm, duration = 1 ms; G_1, 19 G/cm, 0.5 ms; G_2, 15 G/cm, 0.5 ms; G_3, 32 G/cm, 0.9 ms.

At the time point b after the relaxation period Δ_i, the transverse magnetization is given by

$$\sigma(b) = S_\alpha \exp[-R_\alpha\Delta_i] + S_\beta \exp[-R_\beta\Delta_i]. \quad (8)$$

During the time period from b to c (Fig. 1), either S_α or S_β is transferred to the slowly relaxing ^{1}H transition in the single transition-to-single transition polarization transfer step (ST2-PT) [14,15]. The phase cycles have been optimized in order to minimize baseline distortions due to the solvent signal (see caption to Fig. 1). The proton signal at time point c (Fig. 1) is given either by Eq. (9)

$$\sigma(c) \propto \exp[-R_\alpha\Delta_i] \quad (9)$$

or by Eq. (10)

$$\sigma(c) \propto \exp[-R_\beta\Delta_i]. \quad (10)$$

Using the values for R_α and R_β obtained from Eqs. (9) and (10), τ_c is determined with Eqs. (3)–(6), whereby no corrections for contributions from DD relaxation by remote protons or chemical exchange are needed.

In our basic approach for well-structured proteins, R_α and R_β are determined with the use of Eqs. (9) and (10) by fitting the integrals over the entire amide proton chemical shift region with a single exponential. Due to possible internal motions in the protein, the value of τ_c resulting from this treatment of the experimental data with integration over all backbone amide proton signals will in general be an average of different effective correlation times for the individual amide protons, which represents a lower limit for the overall rotational correlation time. For globular proteins that are well structured over the entire polypeptide chain, experience shows that this lower limit closely approximates the actual overall rotational correlation time. If the protein under investigation contains extensive flexible polypeptide segments, this simple approach may yield a τ_c-value that is significantly shorter than the overall rotational correlation time for an equivalent sphere representing the protein studied (possible effects from deviations from spherical shape are discussed, for example, in [5,8,16]). The impact of the flexible residues on the measured τ_c value can be reduced by limiting the integration to a spectral region between approximately 8.0 and 10.0 ppm, where mostly amide proton resonances from well-structured regions are located [1]. Alternatively, for a more precise but more time-consuming determination of the correlation time, a 2D version of [^{15}N,^{1}H]-TRACT can be used (see caption to Fig. 1), with selective integration of cross-peaks assigned to structured regions of the protein. In summary, with these different possible strategies for data collection and analysis, [^{15}N,^{1}H]-TRACT will always yield a τ_c-value that is either a lower limit to or the actual value of the overall rotational correlation time.

The present use of the difference between R_α and R_β as the experimental observable has the advantage of canceling the influence of relaxation caused by remote protons (Fig. 2). For both α-helical and β-sheet secondary structure, the effect of remote protons on the longitudinal ^{15}N relaxation time depends strongly on τ_c, while the corresponding effect on the transverse ^{15}N relaxation time is in the time range of interest nearly independent of τ_c (Fig. 2). Therefore, when calculating τ_c from the ratio of the ^{15}N longitudinal and transverse relaxation rates, contributions from remote couplings have to be taken into account, especially when large, incompletely deuterated molecules are studied. In contrast, 1D [^{15}N,^{1}H]-TRACT measurements can be made on samples with arbitrary deuteration levels without the need for additional corrections.

3. Results and discussion

As an initial practical application, the 1D [^{15}N,^{1}H]-TRACT experiment was applied for the measurement of the rotational correlation time of the outer membrane protein X (OmpX) from *Escherichia coli* reconstituted in mixed micelles with 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) (OmpX/DHPC). Two uniformly ^{15}N-labeled protein preparations with and without deuterium labelling of the protein were used, i.e., 0.5 mM [u-70% ^{2}H,u-^{15}N]-OmpX/DHPC and 2 mM [u-^{15}N,10% ^{13}C]-OmpX/DHPC (the 10% ^{13}C-labeled OmpX was used because it was available from earlier studies [17]; the ^{13}C-labeling had no impact on the work described here). Fig. 3 presents stacked plots of a series of 1D [^{15}N,^{1}H]-TRACT spectra of the 70% deuterated OmpX/DHPC recorded with different values for the relaxation period Δ_i. The relaxation rates R_α and R_β were extracted by fitting a single exponential to the integrals over the backbone amide proton region from 6.5 to 10 ppm (Fig. 4). The data from the 70% deuterated OmpX/DHPC micelles yielded ^{15}N relaxation rates of the α- and β-spin states of 13 and 64 Hz, respectively (Fig. 4A), whereas for the non-deuterated OmpX/DHPC we obtained 22 and 80 Hz, respectively (Fig. 4B). Based on these rates, Eqs. (3)–(6) yielded effective rotational correlation times of 21 ns and 24 ns, respectively, for the samples with 0.5 mM 70% deuterated OmpX and 2 mM fully protonated OmpX in mixed micelles with DHPC at natural isotope distribution. The difference between the two values is mainly due to the slightly different lipid concentrations in the two samples. The agreement between the two experiments confirms the prediction from Eq. (3) that remote protons do not significantly affect τ_c measurements with [^{15}N,^{1}H]-TRACT. The results obtained here are also in

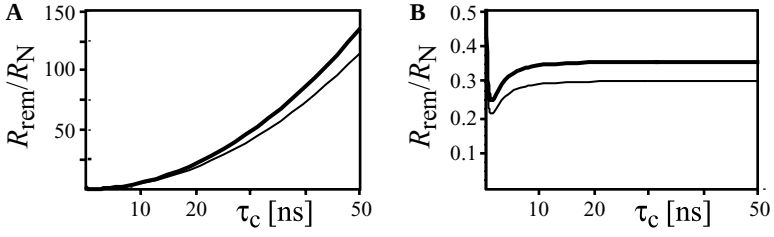

Fig. 2. Influence of remote protons on the ^{15}N relaxation rates in amide groups. (A) Longitudinal relaxation. (B) Transverse relaxation. Ratios of the amide ^{15}N relaxation rates from remote protons (R_{rem}) and from auto-relaxation (R_N) were calculated for an α-helix (thin line) and a β-sheet (thick line). R_{rem} was calculated with Eq. (5) in [9]; R_N was obtained using from Ref. [8] Eqs. (31) and (64) for longitudinal relaxation and Eqs. (34) and (66) for transverse relaxation. The parameters used were: $r_{HN} = 1.02$ Å [27], $\Delta\delta_N = 160$ ppm, and the following distances from remote protons: α-helix, $^{1}H^{N}(i\text{-}2)$, $^{1}H^{N}(i\text{-}1)$, $^{1}H^{N}(i{+}1)$, $^{1}H^{N}(i{+}2)$, $^{1}H^{\alpha}(i)$, $^{1}H^{\alpha}(i\text{-}2)$, $^{1}H^{\alpha}(i\text{-}3)$, $^{1}H^{\alpha}(i\text{-}4)$, $^{1}H^{\beta}(i\text{-}1)$, and $^{1}H^{\beta}(i)$ at distances of 4.2, 2.8, 2.8, 4.2, 3.5, 2.6, 4.4, 3.4, 4.2, 3.2, and 2.5Å, respectively, and in β-sheet, $^{1}H^{N}(i\text{-}1)$, $^{1}H^{N}(i{+}1)$, $^{1}H^{N}(j)$, $^{1}H^{\alpha}(i)$, $^{1}H^{\beta}(j)$, $^{1}H^{\beta}(j)$, $^{1}H^{\beta}(i\text{-}1)$, and $^{1}H^{\beta}(i{+}1)$ at distances of 4.3, 4.3, 3.3, 2.2, 3.2, 2.5, 3.6, and 3.6 Å, respectively [1].

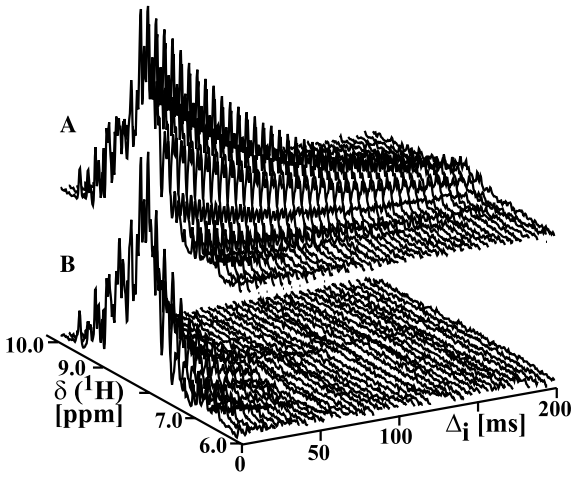

Fig. 3. Stacked plots of 750 MHz 1D [^{15}N,^{1}H]-TRACT spectra recorded with variable relaxation periods Δ_i on a Bruker DRX 750 spectrometer equipped with a triple resonance probe with a shielded z-gradient coil. (A) α-spin state of ^{15}N. (B) β-spin state. The spectra were recorded with 0.5 mM [u-70% ^{2}H,u-^{15}N]-OmpX/DHPC in 95%/5% $^{1}H_2O/^{2}H_2O$ containing 20 mM sodium phosphate at pH 6.5 and 100 mM NaCl, $T = 30$ °C (512 complex points, $t_{1,max} = 50$ ms, and $\Delta_i = 2, 4, 6, \ldots, 200$ ms, 256 transients per Δ_i value). A base line correction with a second degree polynomial function was applied after Fourier transformation.

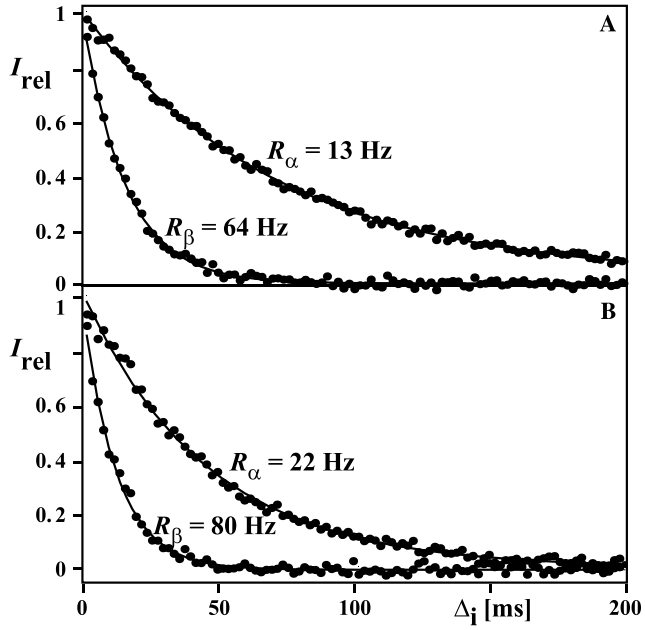

Fig. 4. Decay of the relative intensity of the ^{1}H NMR signal, I_{rel}, due to ^{15}N relaxation. I_{rel} was determined from integration of the 1D spectrum over the region 6.5–10.0 ppm (Fig. 3). (A) 0.5 mM [u-70% ^{2}H,u-^{15}N]-OmpX/DHPC. (B) 2 mM [u-^{15}N,10% ^{13}C]-OmpX/DHPC. In (A) and (B), the upper and lower curves correspond to the slowly relaxing α-spin state of ^{15}N and the more rapidly relaxing β-spin state, respectively. Exponential fits (solid lines) yielded R_α and R_β values as indicated. Using Eqs. (3)–(6) with the parameters $\theta = 17°$ [26], $r_{HN} = 1.02$ Å [27], and $\Delta\delta_N = 160$ ppm, values of $\tau_c = 21$ ns and $\tau_c = 24$ ns, respectively, were obtained from the data in (A) and (B). These values should be considered as a lower limit of τ_c due to a rigid body assumption (Eq. (6)).

good agreement with previous studies of OmpX/DHPC using different techniques, where τ_c values in the range from 21 to 25 ns were obtained [18–20].

The large number of points used for illustrative purposes in the data sets of Figs. 3 and 4 resulted in rather long experiments (15 h for 0.5 mM [u-70% ^{2}H,u-^{15}N]-OmpX/DHPC; 4 h for 2 mM [u-^{15}N,10% ^{13}C]-OmpX/DHPC). For routine measurements, the number of data points can be reduced about 5-fold without noticeable effects on the precision of the τ_c-measurement.

In addition to the experiments with OmpX/DHPC, we applied 1D [^{15}N,^{1}H]-TRACT with the 110 kDa 7,8-dihydroneopterin aldolase from *Staphylococcus aureus* (DHNA) [21], and the 16 kDa pheromone-binding protein from *Bombyx mori* (BmPBP) [22]. Effective rotational correlation times of 47 and 9 ns for DHNA and BmPBP were obtained, respectively (data not shown), which coincides closely with τ_c-values obtained previously with different experiments [23] (F. Damberger, ETH Zurich, personal communication).

4. Conclusion

The presently introduced 1D [^{15}N,^{1}H]-TRACT experiment enables highly efficient measurements of the effective rotational correlation times in biological macromolecules, when compared to other techniques [5–7]. The method is applicable for uniformly ^{15}N-labeled proteins in structures with molecular weights up to approximately 200 kDa. It relies on cross-correlated relaxation in ^{15}N–^{1}H moieties and is not affected by dipole–dipole relaxation with remote protons or by chemical exchange. Therefore, 1D [^{15}N,^{1}H]-TRACT can be used either with or without deuterium labelling of the protein. Efficient measurement of τ_c in large structures permits to characterize the solution conditions in the NMR sample, and is an attractive alternative to light scattering measurements for assessing possible aggregation. It further allows to estimate NMR relaxation rates, and on this basis to optimize the more complex multidimensional NMR techniques to be used with a given system.

Acknowledgments

Financial support was obtained from the Schweizerischer Nationalfonds and the ETH Zürich through the National Center for Competence in Research (NCCR) Structural Biology.

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