Effective rotational correlation times of proteins from NMR relaxation interference

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Abstract

Knowledge of the effective rotational correlation times, \( \tau_c \), 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 \( \tau_c \) 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, \([^{15}\text{N},^{1}\text{H}]-\text{TRACT}\), which is based on transverse relaxation-optimized spectroscopy and permits to determine \( \tau_c \) for \(^{15}\text{N}–^{1}\text{H} \) bonds without interference from dipole–dipole coupling of the amide proton with remote protons. \([^{15}\text{N},^{1}\text{H}]-\text{TRACT}\) 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 \( \text{Staphylococcus aureus} \), the uniformly \(^{15}\text{N}\)-labeled \( \text{Escherichia coli} \) 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 \( \text{Bombyx mori} \), which cover a wide range of correlation times.

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1. Introduction

The effective rotational correlation time, \( \tau_c \), in a molecule is a key parameter for nuclear magnetic resonance (NMR) spectroscopy in solution [1,2]. In folded proteins, \( \tau_c \) 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 \( \tau_c \) 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, \( \tau_c \) is commonly estimated from the ratios of transverse and longitudinal \(^{15}\text{N} \) 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 \( \tau_c \), \([^{15}\text{N},^{1}\text{H}]-\text{TRACT}\) (TROSY for rotational correlation times). Based on the TROSY principle, \([^{15}\text{N},^{1}\text{H}]-\text{TRACT}\) suppresses the influence of dipole–dipole (DD) relaxation by remote protons in backbone \(^{15}\text{N}–^{1}\text{H} \) 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 \( \tau_c \) in large molecules. Since only one-dimensional spectra need to be recorded, the data can be rapidly analysed. The use of \([^{15}\text{N},^{1}\text{H}]-\text{TRACT}\) is suggested for rapid estimates of \( \tau_c \), and as a basis for the set-up of complex multidimensional NMR experiments.

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2. Materials and methods

The \[^{15}\text{N},^{1}\text{H}\]-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 \(\tau_c\) [9–11]. The two transitions of a \(^{15}\text{N}\) nucleus in an amide moiety have different transverse relaxation rates [12]. Assuming an axially symmetric \(^{15}\text{N}\) chemical shift tensor oriented with an angle \(\theta\) between its unique axis and the N–H bond, the relaxation rates for the \(\alpha\)- and \(\beta\)-spin states are given by \(R_{\alpha}\) and \(R_{\beta}\), respectively:

\[
R_{\alpha} = \lambda - \eta_{xy} + R_{\text{H}} + R_{\text{CS}},
\]

\[
R_{\beta} = \lambda + \eta_{xy} + R_{\text{H}} + R_{\text{CS}},
\]

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

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

where \(p\) is the DD coupling between \(^1\text{H}\) and \(^{15}\text{N}\) of the \(^{15}\text{N},^{1}\text{H}\) moiety

\[
p = \mu_0\gamma_{\text{H}}\gamma_{\text{N}}h/(16\pi^2\sqrt{2}r_{\text{HN}}),
\]

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

\[
\delta_N = \gamma_{\text{N}}B_0\Delta\delta_N/(3\sqrt{2}).
\]

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

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

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

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

\[
\sigma(a) = S_{\alpha} + S_{\beta}.
\]

Fig. 1. 1D \[^{15}\text{N},^{1}\text{H}\]-TRACT pulse scheme for measuring the effective rotational correlation time, \(\tau_c\), in macromolecules (TRACT = TROSY for rotational correlation times). A \(\tau_c\)-determination is based on a series of 1D \[^{15}\text{N},^{1}\text{H}\]-TRACT recordings with variable \(\Lambda\) values, and with phase cycle-selection of the \(\alpha\)- and \(\beta\)-spin states of \(^{15}\text{N}\). In the \(^1\text{H}\) and \(^{15}\text{N}\) radiofrequency (rf) channels, narrow and wide black bars stand for nonselective 90° and 180° rf-pulses, respectively. Phases \(x\), unless indicated otherwise above the pulse. The \(^1\text{H}\) selective pulses (curved shapes) are used to maintain the water magnetization along the positive z-axis. The \(^1\text{H}\) and \(^{15}\text{N}\) carrier frequencies are set at the water resonance, and at 118 ppm, respectively. \(\tau_c = 1/4\ ,\ 1/8\ ,\ \ldots\ ,\ 1/16\) ms. In a special case, \(\tau_c\) is known from measurements of \(R_{\alpha}\) and \(R_{\beta}\) (Eqs. (1) and (2)).

At the time point \(b\) after the relaxation period \(\Lambda\), the transverse magnetization is given by

\[
\sigma(b) = S_{\alpha}\exp[-R_{\alpha}\Lambda] + S_{\beta}\exp[-R_{\beta}\Lambda].
\]

During the time period from \(b\) to \(c\) (Fig. 1), either \(S_{\alpha}\) or \(S_{\beta}\) is transferred to the slowly relaxing \(^1\text{H}\) 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)

\[
\sigma(c) \propto \exp[-R_{\alpha}\Lambda]
\]

or by Eq. (10)

\[
\sigma(c) \propto \exp[-R_{\beta}\Lambda].
\]

Using the values for \(R_{\alpha}\) and \(R_{\beta}\) obtained from Eqs. (9) and (10), \(\tau_c\) 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, $R_s$ and $R_B$ 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 $\tau_c$, 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 $\tau_c$ 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 $\tau_c$ 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 $\tau_c$-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 $R_s$ and $R_B$ as the experimental observables has the advantage of canceling the influence of relaxation caused by remote protons (Fig. 2). For both $\alpha$-helical and $\beta$-sheet secondary structure, the effect of remote protons on the longitudinal $^{15}$N relaxation time depends strongly on $\tau_c$, while the corresponding effect on the transverse $^{15}$N relaxation time in the time range of interest nearly independent of $\tau_c$ (Fig. 2). Therefore, when calculating $\tau_c$ 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-15N]-OmpX/DHPC and 2 mM [u-15N,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 $\Delta R$. The relaxation rates $R_s$ and $R_B$ 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 $\alpha$- and $\beta$-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 $\tau_c$ 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 $\alpha$-helix (thin line) and a $\beta$-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: $r_{11nm} = 1.02$ Å [27], $\Delta \delta_m = 160$ ppm, and the following distances from remote protons: $\alpha$-helix, $^1$H$^\beta$(i-2), $^1$H$^\alpha$(i-1), $^1$H$^\alpha$(i+1), $^1$H$^\alpha$(i+2), $^1$H$^\beta$(i), $^1$H$^\beta$(i+1), $^1$H$^\beta$(i+2), $^1$H$^\alpha$(i-4), $^1$H$^\alpha$(i-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 2.5 Å, respectively, and in $\beta$-sheet, $^1$H$^\alpha$(i-1), $^1$H$^\alpha$(i+1), $^1$H$^\beta$(j), $^1$H$^\alpha$(i), $^1$H$^\alpha$(j), $^1$H$^\alpha$(i-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].]
good agreement with previous studies of OmpX/DHPC using different techniques, where $\tau_c$ 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% 2H,u-15N]-OmpX/DHPC; 4 h for 2 mM [u-15N,10% 13C]-OmpX/DHPC). For routine measurements, the number of data points can be reduced about 5-fold without noticeable effects on the precision of the $\tau_c$-measurement.

In addition to the experiments with OmpX/DHPC, we applied 1D [15N,1H]-TRACT with the 110 kDa 7,8-dihydropyrimidinopterin 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 $\tau_c$-values obtained previously with different experiments [23] (F. Damberger, ETH Zurich, personal communication).

4. Conclusion

The presently introduced 1D [15N,1H]-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 15N-labeled proteins in structures with molecular weights up to approximately 200 kDa. It relies on cross-correlated relaxation in 15N–1H moieties and is not affected by dipole–dipole relaxation with remote protons or by chemical exchange. Therefore, 1D [15N,1H]-TRACT can be used either with or without deuterium labelling of the protein. Efficient measurement of $\tau_c$ 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.

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References


