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COMMUNICATIONS

Improved Estimation of Protein Rotational Correlation Times from ^{15}N Relaxation Measurements

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In the study of protein backbone dynamics by ^{15}N relaxation measurements, an initial estimation of the isotropic global correlation time, τ_m, is usually obtained from the average T_1/T_2 ratio of nuclear spins that do not exhibit slow internal motion and with T_2 values not significantly shortened by chemical or conformational exchange processes. Different methods have been used for identification of the rates of internal motion. However, the number of nuclear spins included in the τ_m estimation is often larger than the number that ultimately can be fitted to a single-order parameter, S^2, implying that some nuclear spins involved in the initial τ_m estimation actually have an effective internal correlation time, τ_e, not as fast as assumed. As a consequence, τ_m is underestimated, since internal motion reduces the T_1/T_2 ratio. This situation becomes more obvious if the molecule has a large τ_m value because the reduction in T_1/T_2 ratio arising from internal motion is more significant than for molecules with smaller τ_m and the same degree of internal motion. This Communication describes a more reliable method for identifying nuclear spins which should be excluded from the τ_m estimation because of insufficiently rapid internal motion. This results in an improved τ_m value, giving a much better agreement between the number of nuclear spins fitted successfully to a single-order parameter, S^2, and those used in the τ_m estimation.

Key Words: **^{15}N relaxation measurements; global correlation time; protein dynamics; rate of internal motion; NOE.**

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^{15}N relaxation measurements have been used widely to gain information on the backbone dynamics of proteins in solution (*1–8*). Although most molecules exhibit some degree of anisotropy of their global motion, and fully anisotropic analyses of the ^{15}N relaxation data have been reported recently (*9–13*), ^{15}N relaxation data (^{15}N T_1, T_2 and $\{^1H\}$–^{15}N NOE) are often interpreted with the approximation of isotropic global motion because of the significant simplification of data analysis afforded. Under this isotropic approximation, the motional parameters are usually obtained by using the model-free formalism (*14*), with the isotropic global correlation time of the molecule being determined from the T_1/T_2 ratio (*1, 2*).

Nuclear spins with an effective internal correlation time, τ_e, much faster than the global correlation time of the molecule have a spectral density function, as adopted by the model-free formalism (*14*), of

$$J(\omega) = S^2\tau_m/[1 + (\omega\tau_m)^2], \qquad [1]$$

where S^2 is the order parameter, reflecting the extent of spatial restriction of the NH bond, and τ_m is the global correlation time of the molecule. Using Eq. [1], τ_m can be determined from the average T_1/T_2 ratio, provided that nuclear spins with slow internal motion or with T_2 values that are significantly shortened due to chemical or conformational exchange are excluded (*1*). As internal motion results in a reduction in both the T_1/T_2 ratio and the NOE (see below), whereas a significant contribution from exchange processes leads to an increase in the T_1/T_2 ratio, alternative methods have been used to identify nuclear spins for the estimation of τ_m values. Thus, nuclear spins with slow internal motion can be identified by their small NOE values (for example, a lower limit in the range 0.6–0.7 is frequently adopted), whereas nuclear spins having a significantly shortened T_2 due to chemical or conformational exchange are identified by a T_1/T_2 ratio greater than the average (over the nuclear spins with NOE larger than the preselected value) plus one standard deviation (*1*). Alternatively, nuclear spins exhibiting either slow internal motion or chemical or conformational exchange can be identified exclusively from the T_1/T_2 ratio (for example, only nuclear spins with a T_1/T_2 ratio within one standard deviation of the averaged value are used for the τ_m estimation (*2*), or, equivalently, a trimmed weighted average value of the T_1/T_2 ratio can be used (*6*)). These methods are used widely for identification of the character of internal motion of nuclear spins before making an initial estimation of the isotropic global correlation time, although an estimation of τ_m obtained by making

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TABLE 1
Comparison of the Number of Nuclear Spins Used in the τ_m Estimation with Those Fitted to a Single S^2 Value for Several Proteins from the Literature where Both Numbers Were Reported

Protein	No. of spins used in τ_m estimation	Estimated τ_m (ns)	Critical value[a]	No. of spins fitted to a single S^2	Reference
Interleukin-1β	113	8.3 ± 0.05	$T_1/T_2 = \langle T_1/T_2 \rangle$ − SD	54	*2*
SH2 domain:					*5*
Free	67	9.3 ± 0.4	$T_1/T_2 = \langle T_1/T_2 \rangle$ − SD	4	
Complexed	63	6.7 ± 0.2	$T_1/T_2 = \langle T_1/T_2 \rangle$ − SD	29	
Interleukin-3	55	6.50 ± 0.04	NOE = 0.65	30	*7*

[a] Nuclear spins with T_1/T_2 ratios or NOE values smaller than the critical values are considered to have insufficiently rapid internal motion.

use of structural information (for example, from the T_1/T_2 ratios of nuclear spins located in regions that are well defined in the solution structure) has also been reported (*15*).

It has often been found, however, that the number of nuclear spins included in the τ_m determination is larger than the number finally fitted to a single, internal motion-related parameter, S^2 (*2, 5, 7*), as shown in Table 1. In other words, if the original model-free formalism is followed (fitting to S^2 and τ_e), the number of nuclear spins exhibiting extremely fast effective internal correlation times in the final results is usually less than that used in the τ_m determination. This implies that these methods may have failed to identify some nuclear spins for which effective internal motion correlation times are not as fast as assumed and for which Eq. [1] is therefore not strictly valid. As a consequence, the global correlation time of the molecule is underestimated (see below). This is significant because the initial value of τ_m might dominate the final fitting results for particular residues. For example, an underestimated τ_m value will result in the data for some nuclear spins being satisfactorily fitted only with the addition of an explicit exchange term, R_{ex}. Furthermore, a final optimization of the τ_m value, after the best combination of parameters related to internal motion has been determined for each individual nuclear spin, usually gives only a very limited improvement in the τ_m value itself. Thus, the value of the global correlation time is important in the analysis of ^{15}N relaxation data, and an improvement in its estimation is desirable.

An improved τ_m value can be obtained if nuclear spins for which internal motions are not sufficiently rapid (i.e., where Eq. [1] becomes invalid) can be identified and then excluded from the estimation of τ_m. Figures 1A and 1B compare the theoretical predictions of the T_1/T_2 ratio and $\{^1H\} - ^{15}N$ NOE for an ^{15}N spin as a function of τ_m for different rates and amplitudes of internal motion, with the spectral density function taking the form used in the model-free formalism (*14*). This illustrates that: (i) internal motion gives rise to a reduction in both the T_1/T_2 ratio and NOE, but the extent of reduction is much more significant for the NOE, (ii) for the same magnitude of internal motion this effect is more pronounced for molecules with larger global correlation times, and (iii) the NOE value is much less sensitive to τ_m than the T_1/T_2 ratio. From Figs. 1A and 1B, it is clear, first, that inclusion of nuclear spins with internal effective correlation times that are not sufficiently rapid will result in an underestimated τ_m because internal motion causes a decrease in the T_1/T_2 ratio. Second, since the ^{15}N NOE exhibits a much higher sensitivity to internal motion, the NOE should serve as a better identifier of internal motion than the T_1/T_2 ratio; i.e., nuclear spins with relatively slow internal motion may not be obvious from their T_1/T_2 ratio, but should be detectable from their NOE value. Finally, even though the ^{15}N NOE is also independent of S^2 when the spectral density function takes the form of Eq. [1], its high sensitivity to internal motions and insensitivity to τ_m makes it less favorable for an independent determination of the global correlation time.

When the NOE is used for identification of the rates of internal motion, it is obvious that the outcome of the τ_m estimation will depend strongly on the selection of the critical NOE value, NOE_c (nuclear spins with an NOE value larger than this value are considered to have relatively rapid internal motion and thus the use of Eq. [1] is valid), and use of a higher NOE_c value will improve the performance for the estimation of τ_m. In practice, however, the T_1/T_2 ratios of those few nuclear spins with the largest NOE values are not suitable for the τ_m estimation due to experimental error in the NOE measurements, even though in theory a larger NOE value implies less effect from internal motion. In addition, NOE_c is molecule- and field-dependent. A uniform value does not exist.

Here we describe a method for the determination of NOE_c solely from the relaxation data. An initial τ_m is obtained from the T_1/T_2 ratio of all nuclear spins with an NOE larger than a preselected value, say $NOE_c = 0.6$ (excluding, as usual, nuclear spins with T_2 values shortened significantly by chemical or conformational exchange). A corresponding estimate of the global correlation time, τ'_m, is then derived from the measured NOEs of the same nuclear spins in a similar manner. As τ'_m will be smaller than τ_m, the procedure

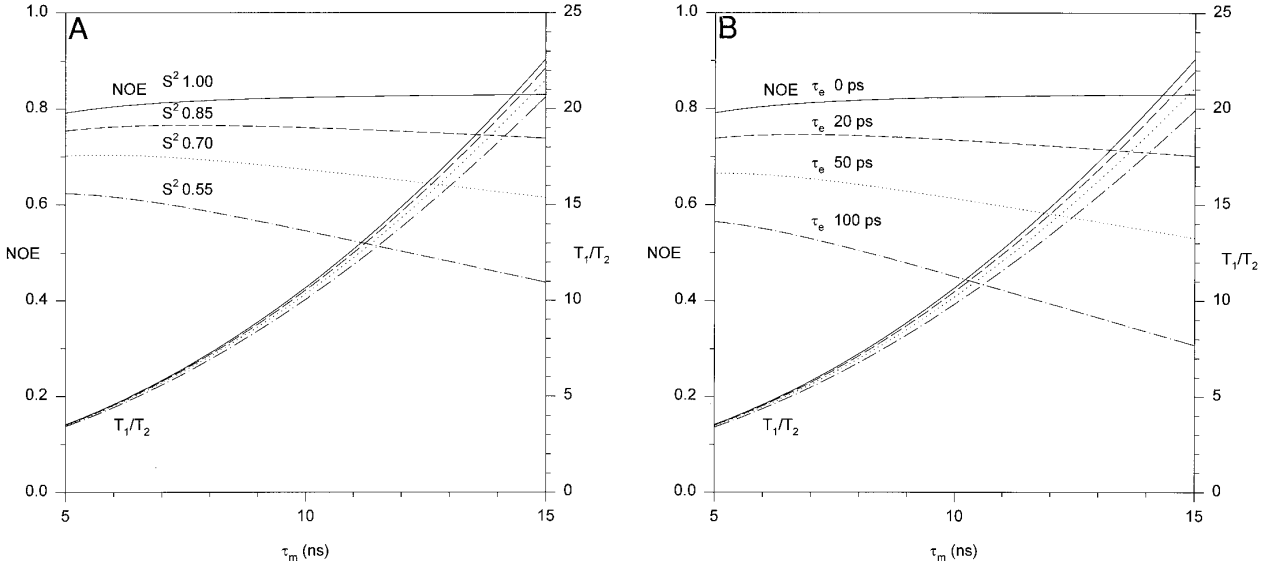

FIG. 1. Theoretical plots of the T_1/T_2 ratio and $\{^1H\}-^{15}N$ NOE value for an ^{15}N spin versus global correlation time, in a typical range of τ_m values, 5.0–15.0 ns, for a number of S^2 and τ_e values. The spectral density function takes the form adopted by the model-free formalism, and the ^{15}N resonance frequency is −60.81 MHz. (A) For a constant effective internal correlation time, τ_e, of 20 ps, from top to bottom with $S^2 = 1.0$ (solid line), 0.85 (dashed line), 0.7 (dotted line), and 0.55 (single dotted and dashed line), respectively. (B) For a constant order parameter, S^2, of 0.8, from top to bottom with $\tau_e = 0$ ps (solid line), 20 ps (dashed line), 50 ps (dotted line), and 100 ps (single dotted and dashed line), respectively.

is repeated with NOE_c increased by a small step, e.g., 0.02. The whole process is then continued in an iterative manner until a minimum difference between τ_m and τ'_m is obtained or τ'_m becomes greater than τ_m.

The variation of τ_m and τ'_m as a function of NOE_c for relaxation data from the 20-kDa cytokine leukaemia inhibitory factor (LIF), as measured at an ^{15}N frequency of −60.81 MHz, is shown in Fig. 2. It is clear that the global correlation times derived from the ratio of T_1/T_2 and the NOE value approach the same value as NOE_c increases, i.e., as more nuclear spins with relatively slow internal motion (where Eq. [1] is not strictly valid) are excluded. As a result, τ_m increased from 9.47 ± 0.62 to 9.75 ± 0.46 ns. The global correlation time of 9.47 ± 0.62 ns was derived from the average T_1/T_2 ratio of 113 nuclear spins (from a total of 143 measured nuclear spins) using an $NOE_c = 0.6$, but subsequently only 70 spins were fitted satisfactorily to a single-order parameter, S^2 (indicating they had sufficiently rapid internal motions). In comparison, when the global correlation time of 9.75 ± 0.46 ns was used, 49 nuclear spins from the 50 involved in the estimation of τ_m were fitted satisfactory to a single S^2 value. It is obvious that even though the value of τ_m changed by only a small amount (within the range of the error), use of the new τ_m value gave a much better agreement between the number of nuclear spins fitted successfully to a single-order parameter, S^2, and those used in the estimation of τ_m.

Table 2 compares the results obtained by fitting the relaxation data with the model selection approach using τ_m values

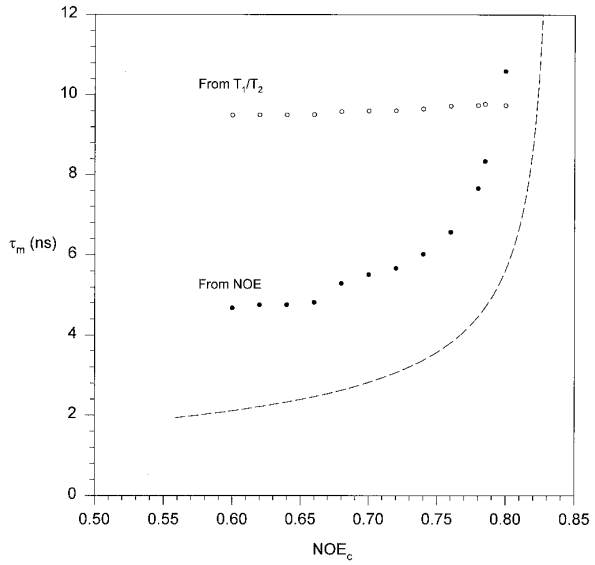

FIG. 2. Dependence of the isotropic global correlation time of leukaemia inhibitory factor (LIF) determined from the T_1/T_2 ratio (open circles) and NOE value (closed circles) as a function of different critical NOE values (see text). The dashed line represents the theoretical dependence of the $\{^1H\}-^{15}N$ NOE value on the global correlation time, τ_m, for a rigid molecule with the spectral density function taking the form of Eq. [1] and with an ^{15}N frequency of −60.81 MHz. Note that the offset between the curve traced by the solid dots and the theoretical dashed line reflects largely the contribution of possible internal motions, even though they are very rapid in comparison with τ_m.

TABLE 2

Distribution of the Number of Residues Fitted Satisfactorily to Each Combination of Model-Free Parameters for the 20-kDa Cytokine Leukaemia Inhibitory Factor (LIF)[a]

Parameters optimized	Residues fitted satisfactorily	
	τ_m 9.47 ns	τ_m 9.75 ns
S^2	71 (71/103)[b]	67 (49/50)
S^2, τ_f	12	14
S^2, R_{ex}	16	9
S^2, τ_f, R_{ex}	1	3
S_f^2, S_s^2, τ_s	35	42

[a] In the model selection approach (*6*), using the extended model-free formula (*16*) for the expression of the spectral density function, up to three parameters related to the internal motion are fitted, while the effects of the remaining parameters are assumed to be negligible. Previously described selection criteria were followed for the determination of model-free parameter combinations for each individual residue (*6*).

[b] The number of nuclear spins exhibiting sufficiently rapid internal motion in the final results vs that assumed initially to have rapid internal motion is shown in parentheses.

obtained with $NOE_c = 0.6$ and those obtained with the approach proposed here. The previously described selection criteria of Mandel *et al.* (*6*) were followed. It is clear that upon using the τ_m value obtained with the method described in the present paper, the number of residues requiring an explicit contribution of chemical or conformation exchange (Rex) for a satisfactory fit was reduced, as anticipated. Furthermore, more residues were fitted satisfactorily with relatively slower effective internal correlation times, as a consequence of the increased τ_m value.

In summary, we have shown that an underestimated τ_m value (due to the inclusion of nuclear spins with relatively slow internal motion in the τ_m estimation) is responsible for the discrepancy between the number of nuclear spins that can be fitted to a single S^2 value and the number used in the estimation of τ_m. Although the intrinsically high sensitivity of the ^{15}N NOE value to internal motion when compared with the T_1/T_2 ratio limits its utility as an independent determination of the global correlation time, it can be used to obtain a better identification of the magnitude of internal motion and thus to obtain an improved initial estimation of the global correlation time from the T_1/T_2 ratio in the study of protein dynamics by ^{15}N relaxation measurements. This improvement is readily achievable from existing ^{15}N relaxation data. It will be more significant for molecules with longer global correlation times since the same degree of internal motion will give a relatively larger reduction in the T_1/T_2 ratio. Therefore, if a molecule has a relatively long global correlation time and isotropic overall motion is assumed, the method described in this Communication should give a better estimate of the global correlation time.

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