Direct Determination of ^{15}N- and ^{19}F-NMR Correlation Times from Spin−Lattice and Spin−Spin Relaxation Times

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The NMR rotational correlation equations for dipolar relaxation between ^{1}H and the nuclei ^{15}N and ^{19}F have been solved for viscous solutions using the R_2/R_1 dipolar ratio. The rotational correlation times have been determined over the dipolar R_2/R_1 range 1.1−1200 at field strengths of 4.7, 6.35, 7.05, 9.4, 11.75, and 14.1 T. The calculated correlation times at each field strength have been fitted to pairs of polynomials that reproduce the correlation times from R_2/R_1 values at a given temperature. These polynomials are used to determine correlation times in two studies where molecular rotation is slow ($\omega\tau > 1$). The studies include (1) where the ^{15}N−^{1}H correlation-time polynomial equations are used to determine the correlation times of the enzyme 4-oxalocrotonate tautomerase and the backbone correlation time of the intestinal fatty acid binding protein and (2) where the ^{19}F−^{1}H polynomial equations are used to characterize the rotational mobility of 5-fluorouracil-substituted *Escherichia coli* $tRNA_1^{Val}$ and to establish the existence of scalar relaxation in the case of an isomer of the peptide complex [Co(benzyloxycarbonyl-cys-pro-leu-cys-gly-NHC$_6$H$_4$-*m*-F)$_2$]$^{2-}$.

Introduction

The use of NMR relaxation methods often provides extensive information about the dynamics and structure of chemical systems in both liquid and solid phases. This information includes rotational correlation times, internuclear distances, and, when appropriate, quadrupolar coupling constants. One of the most useful parameters is the rotational correlation time that can be used to indicate the degree of binding in complex molecular systems such as enzyme−inhibitor and enzyme−substrate complexes.

The determination of rotational correlation times outside the region of extreme narrowing ($\omega\tau < 1$) has often been limited to low-temperature studies ($\omega\tau > 1$) where the correlation equation passes through a minimum and can be solved directly.[1,2] Although this is clearly an accurate method, numerous chemical systems of major interest do not lend themselves to such an approach. Unlike dipolar nuclei, quadrupolar nuclei can be studied in viscous media where liquid-state correlation times and nuclear quadrupole coupling constants can be determined.[3−6] This method is simplified by the fact that the ratio of the relaxation rates for a quadrupolar nucleus, $R_1(=1/T_1)/R_2(=1/T_2)$, can be represented by a quartic equation that reduces to a simple quadratic.[3−6]

In a method similar to that used for quadrupole relaxation, the determination of rotational correlation times outside the region of extreme narrowing ($\omega\tau < 1$) has recently been simplified[7] for dipolar relaxation between ^{1}H, ^{13}C, ^{31}P, ^{113}Cd and neighboring protons at six magnetic field strengths (4.70, 6.35, 7.05, 9.4, 11.75, and 14.1 T). This method[7] is appropriate when either the relaxation process is predominantly dipolar or the fraction of dipolar contribution to the spin−lattice and spin−spin relaxation mechanisms is essentially the same. Under either of these conditions, a ratio of spin−spin to the spin−lattice relaxation times can be used in conjunction with a polynomial to calculate the rotational correlation time for the nucleus of interest (^{1}H, ^{13}C, ^{31}P, or ^{113}Cd).[7]

In this study, the same method[7] is extended to include ^{15}N and ^{19}F nuclei interacting with neighboring protons. The ratio of dipolar relaxation equations is solved for R_1/R_2, and rotational correlation times are obtained for ^{15}N−^{1}H and ^{19}F−^{1}H dipolar relaxation. The values for the dipolar correlation times are then fitted to a series of polynomial equations that can be used by an investigator, once the ratio of $(R_2/R_1 = T_1/T_2)_{dipolar}$ is known. The range of values for the $(R_2/R_1)_{dipolar}$ ratio varies from 1.1 to 1200 using two sets of polynomials that accurately calculate the rotational correlation times (c.c. = correlation coefficient $\geq$ 0.999 for all the polynomials included herein).

Theoretical Section

Relaxation Mechanisms. The spin−lattice relaxation rate ($R_1 = 1/T_1$) typically provides useful information concerning molecular dynamics in solution.[2,7−10] The relaxation mechanisms that can contribute to the spin−lattice relaxation rates (R_1) include dipole−dipole, chemical-shift anisotropy, spin rotation, scalar relaxation, chemical exchange, and paramagnetic relaxation. In particular, dipolar relaxation can be directly related to rotational motion with the use of spherical harmonic functions.[9,10] Solution of the resulting autocorrelation functions produces spectral density functions, assuming exponential decay.[9,10] The resulting spectral density equations[9] used herein are correlation-time-dependent at a set frequency.[9,10]

Heteronuclear Dipolar Relaxation. The same dipolar relaxation mechanism that occurs between ^{1}H and ^{13}C, ^{31}P, or ^{113}Cd can also be applied to the ^{15}N−^{1}H and ^{19}F−^{1}H systems. An essential requirement for relaxation is that interactions causing fluctuations at or near the Larmor frequency will be the most effective in causing relaxation. Therefore, the correlation-time equation for dipolar spin−lattice relaxation includes terms that are within the range of resonance frequencies. The intramolecular dipole−dipole (rotational-motion) contribu-

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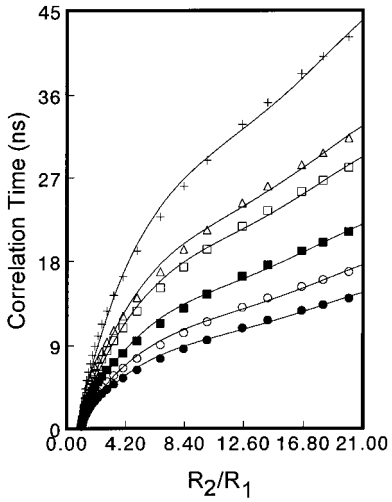

Figure 1. NMR ^{15}N correlation times (ns) at 14.1 (●), 11.75 (○), 9.4 (■), 7.05 (□), 6.35 (△), and 4.70 (+) T vs R_2/R_1 from 0 to 20.

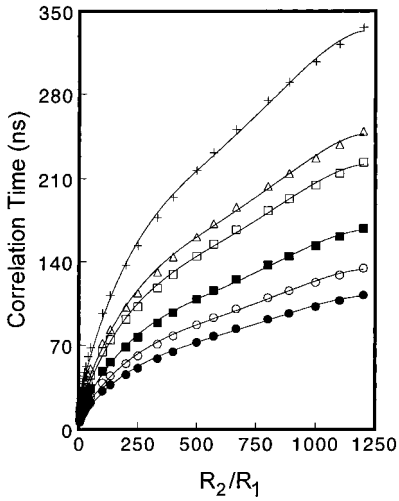

Figure 2. NMR ^{15}N correlation times (ns) at 14.1 (●), 11.75 (○), 9.4 (■), 7.05 (□), 6.35 (△), and 4.70 (+) T vs R_2/R_1 from 20 to 1200.

tion to spin−lattice relaxation for unlike nuclei of spin $^1/_2$ such as ^{15}N, or ^{19}F being relaxed by neighboring hydrogens, is as follows:[9]

$$R_1 = [N_H\gamma_X{}^2\gamma_H{}^2\hbar^2/(10r_{XH}{}^6)][\tau_c/(1+\omega_-{}^2\tau_c{}^2) + 3\tau_c/(1+\omega_X{}^2\tau_c{}^2) + 6\tau_c/(1+\omega_+{}^2\tau_c{}^2)] \quad (1)$$

where N_H is the number of hydrogen atoms attached to (or interacting with) X (^{15}N or ^{19}F), γ_X is the magnetogyric ratio of ^{15}N or ^{19}F, γ_H is the magnetogyric ratio of ^{1}H, $\omega_H = 2\pi\nu_H$, $\omega_X = 2\pi\nu_X$, $\omega_- = \omega_H - \omega_X$, $\omega_+ = \omega_H + \omega_X$, r_{XH} is the distance between either ^{15}N or ^{19}F and a neighboring ^{1}H, and τ_c is the effective correlation time that usually varies exponentially with temperature

Both spin−lattice and spin−spin relaxation are affected by rotational and diffusional motion in liquids. As in the case of dipolar spin−lattice relaxation, dipolar spin−spin relaxation can be directly related to rotational motion with the use of spherical harmonic functions. In addition to the rotational and diffusional motions that affect spin−lattice relaxation, random forces that modulate the spin energy levels at very low frequencies contribute to spin−spin lattice relaxation without inducing transitions. Consequently, the dipolar spin−spin relaxation correlation-time equation contains a zero-frequency spectral density term that is not present in eq 1. The intramolecular dipole−dipole (rotational-motion) contribution to spin−spin relaxation for unlike nuclei of spin $^1/_2$ such as ^{15}N or ^{19}F being relaxed by neighboring hydrogens is[9]

$$R_2 = [N_H\gamma_X{}^2\gamma_H{}^2\hbar^2/(20r_{XH}{}^6)][4\tau_c + \tau_c/(1+\omega_-{}^2\tau_c{}^2) + 3\tau_c/(1+\omega_X{}^2\tau_c{}^2) + 6\tau_c/(1+\omega_+{}^2\tau_c{}^2) + 6\tau_c/(1+\omega_H{}^2\tau_c{}^2)] \quad (2)$$

where N_H is the number of hydrogen atoms attached to (or interacting with) X (^{15}N or ^{19}F), γ_X is the magnetogyric ratio of ^{15}N or ^{19}F, γ_H is the magnetogyric ratio of ^{1}H, $\omega_H = 2\pi\nu_H$, $\omega_X = 2\pi\nu_X$, $\omega_- = \omega_H - \omega_X$, $\omega_+ = \omega_H + \omega_X$, r_{XH} is the distance between either ^{15}N or ^{19}F and a neighboring ^{1}H, and τ_c is the effective correlation time that usually varies exponentially with temperature.

Dipolar Relaxation Ratios. The exact solution of eqs 1 and 2 often can only be obtained at a T_1 or T_2 minimum via a low-temperature study. This requirement often eliminates relaxation studies as a source of information. In this study, we provide an alternative method that provides rotational correlation times for ^{15}N and ^{19}F in viscous media, subject to certain requirements.

A ratio of spin−spin to spin−lattice relaxation times approach can be used to determine rotational correlation times[7] in cases where (a) dipolar relaxation is the major contributing relaxation mechanism, as is often the case with ^{1}H−^{1}H [11−14] and ^{13}C−^{1}H (where the carbon is directly bonded to a neighboring hydrogen) relaxation[15] or (b) the fraction of dipolar contribution to the spin−lattice and spin−spin relaxation mechanisms is essentially the same for each mechanism. Condition b would appear to be a reasonable approximation for cases such as those referred to in both this and a previous study.[7] As in the case of quadrupolar nuclei,[3−6] the ratio of R_1/R_2 (eq 1/eq 2) eliminates a number of terms contained in eqs 1 and 2. The resulting eq 3 (=eq 1/eq 2) can be solved by iterative methods, assuming a range of values for R_1/R_2. Once the iterative solutions of eq 3 are known for each field strength, it is then possible to represent these solutions by a separate polynomial for each field strength. Knowledge of this polynomial then allows the investigator to determine rotational correlation times at a particular field strength from a limited number of measurements:

$$R_1/R_2 = T_2/T_1 = [2/(1+\omega_-{}^2\tau_c{}^2) + 6/(1+\omega_X{}^2\tau_c{}^2) + 12/(1+\omega_+{}^2\tau_c{}^2)]/[4 + 1/(1+\omega_-{}^2\tau_c{}^2) + 3/(1+\omega_X{}^2\tau_c{}^2) + 6/(1+\omega_+{}^2\tau_c{}^2) + 6/(1+\omega_H{}^2\tau_c{}^2)] \quad (3)$$

Results

Solutions of Eq 3. The solution of eq 3 (^{15}N and ^{19}F relaxed by ^{1}H) at field strengths of 4.70, 6.35, 7.05, 9.4, 11.75, and 14.1 T can be accurately (c.c. = 0.999) represented by two series of polynomial equations for R_2/R_1 values from 1.10 to 20 and 20 to 1000. Typical plots of the polynomials (c.c. = 0.999) that cover the two ranges of R_2/R_1 are shown in Figures 1 and 2 for ^{15}N relaxed by ^{1}H over the entire range of field strengths (4.70, 6.35, 7.05, 9.4, 11.75, and 14.1 T).

The intercepts and polynomial coefficients that can be used for the calculation of correlation times for R_2/R_1 ratios are given in Tables 1 and 2. Equation 4 is the form of the polynomial used for the calculation of correlation times:

$$\tau_c(\text{ns}) = a_0 + a_1(R_1/R_2) + a_2(R_1/R_2)^2 + a_3(R_1/R_2)^3 + a_4(R_1/R_2)^4 \quad (4)$$

TABLE 1: ^{15}N and ^{19}F Coefficients for Eq 4 over the R_2/R_1 Range 1−20

	magnetic field (T)					
coefficient	14.1	11.75	9.4	7.05	6.35	4.70
			^{15}N−^{1}H R_2/R_1 Range of 1−20			
a_0	−1.628 222	−1.945 766	−2.438 107	−3.237 252	−3.606 243	−4.864 452
a_1	2.567 863	3.074 462	3.847 965	5.116 525	5.698 016	7.683 783
a_2	−0.233 774	−0.279 159	−0.350 314	−0.463 435	−0.517 854	−0.696 461
a_3	0.010 857	0.0129 42	0.016 289	0.021 429	0.024 025	0.032 217
a_4	−0.000 018	−0.000 215	−0.000 271	−0.000 355	−0.000 399	−0.000 533
			^{19}F−^{1}H R_2/R_1 Range of 1−20			
a_0	−2.072 640	−2.973 340	−3.107 300	−4.145 864	−4.619 417	−6.237 507
a_1	1.780 686	2.146 968	2.670 532	3.563 822	3.970 939	5.357 377
a_2	−0.092 934	−0.113 675	−0.139 706	−0.186 999	−0.209 537	−0.280 471
a_3	0.002 702	0.003 406	0.004 109	0.005 490	0.006 216	0.008 179
a_4	−0.000 029	−0.000 039	−0.000 046	−0.000 060	−0.000 069	−0.000 089

TABLE 2: ^{15}N and ^{19}F Coefficients for Eq 4 over the R_2/R_1 Range 20−1200

	magnetic field (T)					
coefficient	14.1	11.75	9.4	7.05	6.35	4.70
			^{15}N−^{1}H R_2/R_1 Range of 20−1200			
a_0	8.184 222	9.821 894	12.270 852	16.339 177	18.187 255	24.561 503
a_1	0.266 321	0.319 601	0.399 628	0.533 289	0.592 543	0.799 024
a_2	−0.000 456	−0.000 547	−0.000 686	−0.000 916	−0.001 016	−0.001 369
a_3 ($\times 10^7$)	4.364 055	5.234 258	6.573 424	8.769 680	9.732 072	13.102 010
a_4 ($\times 10^{10}$)	−1.512 002	−1.812 972	−2.280 019	−3.040 989	−3.374 968	−4.544 723
			^{19}F−^{1}H R_2/R_1 Range of 20−1200			
a_0	7.354 148	8.838 231	11.032 027	14.685 003	16.336 371	22.107 651
a_1	0.268 468	0.322 170	0.402 594	0.536 923	0.597 207	0.804 161
a_2	−0.000 467	−0.000 560	−0.000 701	−0.000 936	−0.001 041	−0.001 394
a_3 ($\times 10^7$)	4.499 276	5.369 408	6.744 833	9.014 260	10.022 340	13.374 540
a_4 ($\times 10^{10}$)	−1.564 684	−1.860 361	−2.344 293	−3.135 111	−3.484 832	−4.638 227

^{15}N-NMR Correlation Times. The free and inhibitor-bound protein, 4-oxalocrotonate tautomerase, was the subject of a ^{15}N-NMR relaxation study[16] at 14.1 and 11.75 T. The mean R_1 values for the free protein are 1.15 ± 0.12 and 0.93 ± 0.12 s^{-1} at 11.75 and 14.1 T. The mean R_2 values for the free protein are 17.52 and 18.22 s^{-1} at 11.75 and 14.1 T. Correlation times of 14.3 and 14.1 ns for the free protein are calculated using R_2/R_1 ratios of 15.23 and 19.59, and eq 4 with the coefficients from Table 1. These values compare favorably with an average value of 14.2 ± 0.4 ns reported by the investigators.

Another ^{15}N relaxation study[17] concerns the backbone mobility of the intestinal fatty acid binding protein. The T_1's at 11.75 T are 438.1 and 439.7 ms for the apo- and holoenzyme. The T_2's for the apo- and holoenzyme are 116.4 ± 10.3 and 124.9 ± 7.9 ms. The authors report correlation times of 6.7 and 6.2 ns for the apo- and holoenzyme. One obtains 6.3 and 6.0 ns for the apo- and holoenzyme forms using eq 4, the coefficients from Table 1, and the reported relaxation times.

^{19}F Correlation Times. ^{19}F is potentially a very useful correlation time probe because of its high sensitivity. An excellent example of this is an ^{19}F-NMR relaxation study[18] of 5-fluorouracil-substituted *Escherichia coli* tRNA$_1^{Val}$ in which ^{19}F NOE's, T_1's and T_2's were measured at several magnetic field strengths. The dipolar relaxation data is analyzed using two-state jump and diffusion in a cone model, and the correlation time for overall tRNA reorientation was set at 30 ns. R_2/R_1 ratios have been calculated using the T_1 and T_2 ^{19}F data at 7.05 T for 11 of the peaks in the ^{19}F spectrum of 5-fluorouracil-substituted *Escherichia coli* tRNA$_1^{Val}$. The labeled peaks and their R_2/R_1 ratios are the following: A, 74.2; B, 50.5; C, 43.2; D, 13.2; E/F, 12.0; H, 32.0; I, 66.1; J, 41.9; L, 50.6; M, 43.1; N, 34.9. The corresponding correlation times (in nanoseconds) calculated from eq 4 and the coefficients in Tables 1 and 2 are the following: A, 50; B, 40; C, 36; D, 22; E/F, 21; H, 31; I, 46; J, 36; L, 40; M, 36; N, 32. The average of these correlation times is 35 ± 8 ns, which agrees reasonably well with the assumed value of 30 ns. Peaks A and B are assigned to FUra 55 and 54 in the TYC loop, while peaks D−H correspond to FUra residues that are highly exposed to solvent and are located in loop regions of the tRNA.[18,19] These latter peaks (D−H) have the shortest correlation times (21−31 ns) as one would expect.

Another ^{19}F-NMR relaxation study[20] focuses on several mononuclear Co(II) complexes and reports T_1 and T_2's for [Co(benzyloxycarbonyl-cys-pro-leu-cys-gly-NHC$_6$H$_4$-*m*-F)$_2$]$^{2-}$ at 11.75 T. This complex exists as two isomers in solution with ^{19}F peaks at −98.0 and −103.5 ppm. The peak at −98.0 ppm is considerably broader (198 Hz) than the peak at −103.5 ppm (56 Hz), although the T_1's for these peaks are similar (22.9 and 23.1 ms). The authors offer the formation of a NH−S hydrogen bond as a possible explanation for the differences in line widths. The R_2/R_1 ratios for these peaks are 14.2 and 4.07, which yield correlation times of 13.2 and 4.6 ns from eq 4 and the coefficients in Table 1. These unrealistic correlation times, which should be similar in value, may be explained by the presence of scalar coupling, which would lengthen the T_2 value of the peak at −98.0 ppm without affecting this isomer's T_1.[9,21] This is consistent with one isomer having its ^{19}F nucleus closer to the paramagnetic Co(II) as indicated in this study.[20] This points out the hazard of using the R_2/R_1 ratio indiscriminately and particularly in cases where a paramagnetic species is near the nucleus under investigation.

Summary

The NMR dipolar correlation equations for the ratio of R_1 (=1/T_1) to R_2 (=1/T_2) are solved jointly for ^{15}N and ^{19}F interacting with ^{1}H in viscous solutions. Sets of polynomials have been generated that provide correlation times for these two nuclei undergoing dipolar relaxation with ^{1}H once the ratio

of $(R_2/R_1)_{\text{dipolar}}$ is known. These equations are applicable in the region where $\omega\tau > 1$ and $R_2 > R_1$.

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