Cross Correlation and Spin-Rotation Effects on Methyl Spin-Lattice Relaxation in Peptides: Tetragastrin

J. D. Cutnell[1] and Jay A. Glasel*

Contribution from the Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032. *Received March 8, 1976*

Abstract: ^1H and ^{13}C relaxation data relating to the methionine methyl group in the peptide tetragastrin are reported. An analysis of methyl spin-lattice relaxation times and the heteronuclear Overhauser effect is presented. This analysis illustrates an approach to the determination of motional information when dipolar and spin-rotation interactions dominate the relaxation mechanism and when the effect of cross correlations must be considered. The importance of these effects in studies of peptides in solution by NMR relaxation is emphasized.

We have recently reported the existence of nonexponential relaxation behavior for the protons of the methyl group of tetragastrin[2] (I) and have attributed this behavior to cross cor-

H-Trp-Met-Asp-Phe-NH_2

I

relations between the relaxing spin pairs. In addition, we have presented a detailed account of the relaxation of ^1H and ^{13}C nuclei in other groups of this molecule and have emphasized the conformation information implicit in the proper use of both ^1H and ^{13}C relaxation times and the ^1H-^{13}C heteronuclear Overhauser effect.[3]

This paper is an amplification of our original report,[2] and its importance stems from theoretical and experimental work[4-9] which indicates that accurate measurements of cross-correlation effects may be an aid in determining the nature of overall anisotropic molecular tumbling in molecules of the size with which we are dealing. Moreover, we recently have found nonexponential spin-lattice relaxation for the methionyl methyl protons of another peptide, the pharmacologically active pentapeptide, "methionine enkephalin".[10,11] Thus, nonexponential methyl proton relaxation and observable cross-correlation effects may be a general feature of methionine containing peptides. Also, specific ^{13}C enrichment of S-CH_3 sites has recently been reported,[12] and thus the increased use of methyl carbons as NMR probes of protein conformation seems likely. Accurate utilization of data gathered from biophysical application of ^{13}C methyl probes will potentially require consideration of auto- and cross-correlation effects in both the dipolar and spin-rotation interactions, since spin-rotation frequently plays a role in methyl relaxation.[13] In such a situation analysis of the relaxation is not straightforward, and in the present publication we illustrate an attack on the problem utilizing both ^1H and ^{13}C relaxation data.

The approach taken here utilizes analysis of the two different spin systems $^{12}CH_3$ and $^{13}CH_3$, the former relating to the proton spin system and the latter relating to the proton decoupled ^{13}C system. An alternative method involves analyzing the ^1H and ^{13}C relaxation directly in the fully coupled $^{13}CH_3$ species,[5] which has the advantage of potentially providing a complete description of the relaxation kinetics and thereby providing additional information from which motional parameters may be obtained. However, full utilization of this alternative method requires that ^{13}C enriched species be available and that facilities exist with which various kinds of spin system preparations may be used to establish the initial state away from which relaxation is monitored.

Experimental Section

Materials. Tetragastrin (as the trifluoroacetate) was synthesized according to published procedures[14] and was determined to be homogeneous to >95% by thin layer chromatography in two solvent systems before and after use as NMR samples.

Sample Preparation. The gastrin tetrapeptide amide trifluoroacetate was dissolved in 100% [$^2H_6$]dimethyl sulfoxide (DMSO-d_6) to yield a 0.3 M solution. This operation, as well as all sample transfers, was performed under dry nitrogen to minimize the amount of H_2O contaminating the solvent. Dissolved oxygen was removed via four freeze-pump-thaw cycles. The samples were transferred to 5 and 10 mm o.d. tubes for ^1H and ^{13}C studies respectively and sealed with pressure caps. For proton studies 0.015 and 0.03 M samples were prepared in a similar manner.[2]

NMR General. All spectra used in this study were obtained with a JEOL-PFT-100 Fourier transform spectrometer using a disk storage system, employing an internal deuterium lock, and operating at 100.0 and 25.15 MHz for ^1H and ^{13}C, respectively. Temperature was controlled to ±1 °C via the standard JEOL temperature controller. For all measurements of spin-lattice relaxation the standard 180°-τ-90°-t pulse sequence was used with the phase of the 90° pulse changed by 180° on every scan.[15] Except as noted previously[2] this pulse sequence was under computer control. Magnetization was taken to be proportional to peak heights which were read manually from recorded spectra. Spectral assignments were made as previously discussed.[3]

NMR ^{13}C. For ^{13}C spin-lattice relaxation measurements a bandwidth of 6.25 kHz was employed with 8192 data points. ^1H noise decoupling was used with a noise bandwidth of 2.5 kHz. The number of scans used per spectrum varied between 600 and 2284.

Repeated 90° pulses were used to obtain the equilibrium spectra from which NOe factors were calculated. Such spectra were determined with a spectral bandwidth of 6.25 kHz and 8192 data points in the presence and absence of ^1H noise decoupling power (2.5 kHz noise bandwidth). That there was sufficient decoupling power was evident from studies we have carried out with much broader resonances.[16] In the presence of noise decoupling power the time between 90° pulses was at least 3.4 times the longest T_1 characterizing the resonances. This factor was increased to 4.8 in the absence of decoupling power in order to account for the nonexponential approach to equilibrium of the coupled ^1H-^{13}C spin system.[17] The number of transients accumulated in determining these spectra was 8234. NOE factors were calculated from integrated intensities, these being determined by cutting out and weighing tracings (including noise) of the recorded spectra. This integration procedure was followed with at least two horizontal magnifications for each region of the spectrum and the results were averaged after being weighted by the appropriate factor. This procedure was used to ensure that intensity was not being neglected in the wings of the resonances. Except for one deviation of ±11% this procedure yielded areas agreeing within ±9%. Since not all resonances are completely resolved even in the decoupled spectrum, individual resonances could not be separately integrated in the fully coupled spectrum. Only five separate regions of the spectrum could be separately integrated. These regions ranged from the most downfield (carbonyl) to the most upfield (methyl) resonances and gave five separate values of the nonenhanced integrated intensity per carbon atom. These values agreed to within ±6%, which indicates that our 90° pulse was sufficiently uniform throughout the spectrum to justify using an average of these five values as the nonenhanced intensity per carbon in NOE calculations. Where NOE values correspond to the

Table I. Relaxation Parameters for the Methionine Residue of Tetragastrin at 30 °C in DMSO-d_6

	^{13}C		^{1}H	
	0.3 M T_1, s[a]	0.3 M NOE	0.3 M T_1, s	0.03 M T_1, s
α	0.0764 ± 0.0076	2.4[b]		
β	0.0591 ± 0.0072	2.6[c]		
γ	0.0562 ± 0.0077	*d*		
CH_3	1.37 ± 0.09	2.0	0.741[e]	0.873[e]

[a] ± figures denote approximate 95% confidence limits (≈ two standard deviations). [b] Resonances integrated as a unit with Asp α and Phe α. [c] Resonances integrated as a unit with Trp β and Asp β. [d] Integration not possible due to proximity of solvent resonances. [e] Average relaxation time determined from results of four-parameter regression analysis. Approximate 95% confidence limits available for the four separate parameters only.

average determined from the integrated intensity for a specific region of the enhanced spectrum, it is so indicated in our results, and reported values are accurate to ±10%.

NMR ^{1}H. For ^{1}H spin-lattice relaxation measurements a bandwidth of 2.00 kHz was employed with 8192 data points. The number of transients accumulated per spectrum varied between 2 and 40. The value of t in the 180°-τ-90°-t pulse sequence was 17 s. Such a large value is necessary for the methyl protons of tetragastrin since the relaxation is characterized by a nonexponential mechanism.[2]

Results

^{13}C Relaxation Times and NOE Factors. The T_1's determined for each methionine resonance are summarized in Table I. A single T_1 was found to be sufficient for characterizing the relaxation in each case and was determined via two parameter nonlinear regression analysis using an exponential decay function (not its logarithm) to describe the approach of the magnetization to equilibrium. The ± values shown in Table I are the approximate 95% confidence limits (corresponding to approximately two standard deviations) as determined by computer. The T_1's given reflect the results of nonlinear regression analysis using superimposed data from three different runs at the same temperature.

The NOE factor for each methionine resonance is also given in Table I, where we have been careful to indicate which NOE's were determined from integrating a particular region of the enhanced spectrum (containing several resonances). Attention should therefore be given to the footnotes appended to this table. In the case of Met γ an NOE value is not given in Table I because the proximity of this resonance to the solvent resonance prohibited accurate integration.

^{1}H Methyl Relaxation Times. For the methyl resonance it was necessary to employ a weighted sum of two exponentials to characterize the relaxation in 0.015 and 0.03 M solutions in DMSO-d_6.[2] Such was also the case for the 0.3 M DMSO-d_6 solution, further confirming our earlier results. The methyl T_1's shown in Table I are the average T_1's given by the initial slopes of the relaxation decay plots. These averages were calculated using the results of a four parameter nonlinear regression analysis according to $(T_1^{-1})_{av} = (aT_{1a}^{-1} + bT_{1b}^{-1})/(a + b)$, where a and b are the pre-exponential weighting factors and T_{1a} and T_{1b} are the corresponding time constants characterizing each exponential. Separate values for a, T_{1a}, b, and T_{1b} have been given previously for a 0.03 M solution.[2] It should be noted that the results in Table I for the 0.03 M solution do not reflect contributions from intermolecular interactions. Table I does indicate that such interactions come into play for concentrations between 0.03 and 0.3 M, but intermolecular interactions are not important at 0.03 M since the results for a 0.015 and 0.03 M solution were identical for the aromatic and -S-CH_3 T_1's.[2,3]

Discussion

A. Theory. For convenience the required dipolar relaxation formalism is summarized below. Equations 1-3 describe the relaxation parameters when the internuclear vector between two nuclei, the one relaxing the other, exhibits two kinds of motion. This vector reorients about an internal axis which in turn reorients by isotropic overall tumbling.[18-20] The internal reorientation is assumed to be rapid relative to the overall tumbling, which is assumed to be in the extreme narrowing limit. For ^{13}C:

$$T_1^{-1} = \left(\frac{\gamma_H^2\gamma_C^2\hbar^2 N}{4r^6}\right)(3\cos^2\theta - 1)^2\tau_R \quad (1)$$

$$\text{NOE} = 1 + \left(\frac{\gamma_H}{2\gamma_C}\right)f_{dd} \quad (2)$$

For ^{1}H:

$$T_1^{-1} = \left(\frac{3\gamma_H^4\hbar^2 N}{8r^6}\right)(3\cos^2\theta - 1)^2\tau_R \quad (3)$$

In eq 1-3 the symbols appearing are defined as follows: T_1 = spin-lattice relaxation time; NOE = ^{13}C nuclear Overhauser enhancement factor; θ = angle between the internuclear vector and the axis of internal reorientation = 109.5 and 90° for ^{13}C-^{1}H and ^{1}H-^{1}H interactions, respectively; τ_R = correlation time describing isotropic overall tumbling; γ_H, γ_C = magnetogyric ratios of ^{1}H and ^{13}C, respectively; h = Planck's constant divided by 2π; r = internuclear separation = 1.09 and 1.78 Å for ^{13}C-^{1}H and ^{1}H-^{1}H interactions, respectively; N = number of nuclei interacting with and situated at distance r from the nucleus whose relaxation rate is given by eq 1 or 3; f_{dd} = fraction of the ^{13}C spin-lattice relaxation caused by dipolar interactions.

Equations 1-3 were derived under the simplifying assumption that only autocorrelation functions need be considered. These functions describe the average correlation between the orientation of an internuclear vector at time $t = 0$ and the orientation of the same vector at a later time. Consideration of only autocorrelations generally suffices for most relaxation mechanisms. However, it is known to be insufficient under some circumstances, most notably when methyl reorientation is involved.[2] In this case the various internuclear vectors reorient as a unit, and thus cross correlations must be considered. These terms describe the average correlation between the orientation of one internuclear vector at an earlier time and the orientation of a different internuclear vector at a later time. The effects of cross correlations in nuclear spin relaxation have been considered by various workers.[4-9,21,26] While these effects are substantial in multiplet spectra[5] they may also be observed in singlet resonances.[2] In general the influence of cross correlations is always to retard the relaxation while leaving unaffected the relaxation rate determined from the initial slope of the decay plot.[23] Furthermore, the complexities introduced by cross correlations are sufficiently extensive that the relaxation can no longer be described by a single relaxation time as implied in eq 1-3. Multiple exponentials are required to characterize the approach of the magnetization to equilibrium. Unfortunately the mathematical description of this multiple exponential behavior is extremely complicated and hence it is not given here. We have written and will later use a computer program to calculate methyl ^{1}H spin-lattice relaxation decay plots based on the exact formulation of the problem.[4]

A formulation of the cross-correlation problem in ^{13}C methyl relaxation is also available.[6,24,25] Of this, only the effect on the NOE factor is needed here. To account for the influence of cross correlations[25] eq 2 must be modified as indicated:

$$\mathrm{NOE} = 1 + \left(\frac{\gamma_H}{2\gamma_C}\right)\left(\frac{f_{dd} - \Omega}{1 - \Omega}\right)$$

$$\Omega = \left(\frac{\gamma_H^2\gamma_C^2\hbar^2}{2r^6}\right)^2\left(\frac{(3\cos^2\theta - 1)^4\tau_R^2}{T_{1tC}^{-1}(T_{1tC}^{-1} + 2X)}\right) \qquad (4)$$

Equation 4 is given in the limit of extreme narrowing and rapid internal methyl reorientation. The term X in eq 4 is related to the effects of random field interactions in the proton frame.[25-27] It includes, for example, a contribution from both auto and cross correlations in the spin-rotation mechanism.[28] T_{1tC}^{-1} refers to the measured total methyl ^{13}C relaxation rate, including any contribution from spin-rotation interaction.

B. Motional Characteristics. General. Reference to Table I reveals that the $-S-CH_3$ ^{13}C relaxation time exceeds that of Met α, β, and γ by a factor of at least 18. Comparing NT_1's, the methyl carbon relaxation time should be shorter than those of the other carbons by factors of 3, 3/2, and 3/2, respectively, if all were rigidly fixed. In addition the measured NOE of 2.0 for $-S-CH_3$ indicates the presence of a nondipolar mechanism for relaxation, since the NOE is reduced relative to the theoretically allowed maximum of 3.0 for dipolar interactions (see eq 2). Thus, the factor by which the methyl dipolar T_1 exceeds the Met α, β, or γ T_1 (which reflect solely dipolar relaxation[3]) is larger than 18. On the other hand rapid internal reorientation can lengthen the methyl carbon T_1 by at most a factor of three relative to that for a methine carbon when both experience the same isotropic overall tumbling.[20] These considerations imply a substantial degree of motional freedom of the $-S-CH_3$ bond, in excess of that characterized by a single correlation time calculated from either the T_1 of Met α, β, or γ.

As a point of departure we assume that a single correlation time τ_R characterizes the motion of the methyl threefold symmetry axis and is in the extreme narrowing limit. Furthermore, we assume that internal reorientation about the threefold axis, described by a correlation time τ_G, is rapid compared to τ_R. In view of the NOE and the magnitude of the measured methyl ^{13}C dipolar T_1 (>1.37 s) there is little doubt that τ_R is in the extreme narrowing limit. The assumption that $\tau_G \ll \tau_R$ remains for the moment an a priori assumption. However, its tenability will be confirmed later by the successful prediction of the details of the methyl ^1H relaxation. It would be possible to eliminate the assumption that the overall tumbling of the threefold axis is isotropic since the effect of anisotropic overall tumbling has been treated theoretically.[29] It is doubtful, however, that the multiple correlation times which describe anisotropic overall tumbling offer an appreciable advantage over the single effective τ_R employed here. Our analysis will be limited in any case by complications in the formalism which treats the effect of spin-rotation interaction in the presence of dipolar cross correlations.[26,30,31]

C. Calculation of Correlation Time. If the effects of cross correlations were negligible, the approach from this point would be to use the measured NOE in eq 2 to calculate f_{dd}, with which the measured T_1 may be corrected to give T_{1ddC}, the ^{13}C dipolar T_1. T_{1ddC} would then provide a value of τ_R via eq 1. However, in view of the presence of nonexponential methyl ^1H relaxation and the resultant likelihood that cross-correlation effects are important, this approach cannot be used. The reason is that the presence of cross correlations vitiates the use of eq 2 to relate the measured NOE and f_{dd}. Therefore the alternative route via eq 4 must be taken. On the one hand T_{1tC}, the total ^{13}C T_1 (involving both dipolar and spin-rotation contributions), appears in eq 4 and is a known experimental quantity. On the other hand f_{dd} may be written as $T_{1ddC}^{-1}/T_{1tC}^{-1}$, and T_{1ddC}^{-1} is proportional to τ_R according to eq 1. However, a solution of eq 4 for τ_R requires an approximation for X.

As a first approximation[25,32] we may assume $X \approx 0$. The resulting quadratic in τ_R yields two solutions, one of which may be discarded immediately since it implies that $f_{dd} > 1$. The solution for τ_R, then, is $\tau_R = 5.95 \times 10^{-11}$ s, which is 15% larger than that obtained from eq 2 in the absence of cross correlations. Another possible approximation[25,28,33] is that X, in the limit of extreme narrowing and rapid internal reorientation, equals the contribution (from autocorrelations) of spin-rotation (sr) to T_{1tH}^{-1}, the total ^1H spin-lattice relaxation rate. If T_{1ddH}^{-1} is the dipolar contribution to T_{1tH}^{-1}, then $T_{1tH}^{-1} = 0.873^{-1} \approx X + T_{1ddH}^{-1}$ (see Table I). Noting that T_{1ddH}^{-1} is proportional to τ_R (see eq 3), we now have the requisite second equation with which eq 4 may be solved for τ_R. Thus, by using the methyl ^1H relaxation data eq 4 may be reduced again to a quadratic with two solutions for τ_R. Since one of these solutions predicts that the ^{13}C relaxation mechanism is essentially dipolar ($f_{dd} = 0.98$) it may be excluded. The remaining solution is that $\tau_R = 5.50 \times 10^{-11}$ s, a value which is 6% larger than that obtained by employing eq 2 and ignoring the effects of cross correlations. The validity of the assumptions made concerning X in the above analysis is unknown since literally no experimental data exist which relate to this point.[25] Nonetheless, it is important to note that the neglect of cross correlations leads to an overestimation of the dipolar correlation time by an amount which can be larger than the experimental error of carefully performed ^{13}C T_1 measurements.

The approach described above may be altered to include the situation where X is neither zero nor equal solely to the contribution of spin-rotation (from auto correlations) to the total ^1H relaxation mechanism. In the limit of extreme narrowing and rapid internal reorientation $X = 2(k^{11} - k^{12})$ where k^{11} and k^{12} are respectively the auto- and cross-correlation spectral densities for the ^1H spin-rotation interaction.[25,33] It is well known that temperature studies of the ^1H relaxation may be used to separate the spin-rotation ($2k^{11}$) from the dipolar contribution to the ^1H relaxation mechanism.[34] With this added piece of information concerning $2k^{11}$ our approach utilizing ^{13}C data has the potential to provide estimates of $2(k^{11} - k^{12})$. Alternatively we note that k^{11} and k^{12} also appear in the formalism which describes the kinetics of the ^1H relaxation.[26,30,31] Thus, a complete solution to eq 4 requires that nonlinear regression techniques be applied jointly to eq 4 and the expressions which describe the ^1H relaxation. We do not pursue such an approach here for reasons discussed in section D.

It may be objected that in the above analysis the total ^{13}C and ^1H spin-lattice relaxation rates were implicitly, and potentially erroneously, assumed to be the sum of $T_{1dd}^{-1} + T_{1sr}^{-1}$. However, it is known[23] that the initial slope of the ^1H dipolar relaxation decay plot is independent of cross correlations. Furthermore, it may be shown from the results of Buchner[24] that cross correlations in the dipolar or the spin-rotation mechanism do not contribute to the average T_1 determined from the initial slope of the ^{13}C decay plot. Since exponential methyl ^{13}C relaxation is observed and since the ^1H T_1 given in Table I is indeed determined from the initial slope of the decay plot, the above analysis is valid within the limits of the stated approximations, assuming that intermolecular dipolar interactions and nondipolar mechanisms other than spin-rotation may be ignored. Intermolecular dipolar interactions may be discounted on the basis of our dilution experiments (see Results). Chemical shift anisotropy[35] is a possible additional source for nondipolar relaxation. However, the nondipolar contribution implied by the measured NOE of 2.0 and eq 2 would correspond[35] to a chemical shift anisotropy of 1400 ppm. Such a value is larger than known values in analogous systems[35-37] by at least an order of magnitude, especially at the relatively low field strength used in this study. Another possible source of nondipolar relaxation is modulated scalar coupling, but such a mechanism is not at issue here in

view of the absence of other spins which are subject to rapid relaxation (e.g., by quadrupolar interactions) or which are chemically exchanging.[26] We discount the possible effects of paramagnetic impurities since dissolved oxygen has been removed from our samples (see Experimental Section) and since calculations utilizing the dipolar formalism to predict ^{1}H and quaternary ^{13}C relaxation rates from T_1 data on protonated carbons reveal no systematic errors attributable to such effects.[3] In view of the above considerations and the well known ability of internally mobile methyl groups to exhibit appreciable spin-rotation interaction[38] our analysis is appropriate.

With an estimate of τ_R now available it is possible to proceed with the analysis of the details of the ^{1}H relaxation. Equation 3 may be used with $\tau_R = 5.50 \times 10^{-11}$ s (chosen to give maximum sr interaction) to show that $T_{1ddH} = 1.35$ s. Since $T_{1tH}^{-1} = T_{1ddH}^{-1} + T_{1srH}^{-1}$ and $T_{1tH} = 0.873$ s, it is apparent that $T_{1srH} = 2.47$ s and that spin-rotation interaction provides 35% of the ^{1}H spin-lattice relaxation rate. We note that this separation of the dipolar and spin-rotation contributions to the ^{1}H relaxation rate was achieved without the usual temperature studies. The possibility of using ^{13}C T_1 and NOE measurements to effect this separation is a welcome one, especially in cases where temperature studies cannot be undertaken profitably. However, in such situations the effect discussed above (see eq 4), which is caused by the influence on the NOE of the proton auto- and cross-correlation spin-rotation spectral densities, should be kept in mind.

The 35% contribution of spin-rotation to the ^{1}H relaxation is to be compared with the corresponding contribution to the ^{13}C relaxation. Equation 1 may be used with $\tau_R = 5.50 \times 10^{-11}$ s to show that $T_{1ddC} = 2.55$ s. The total ^{13}C T_1 of 1.37 s may now be corrected for the dipolar contribution to show that $T_{1srC} = 2.96$ s, corresponding to a 46% contribution from spin-rotation. Thus, the contribution of spin-rotation is not greatly different in the ^{13}C and ^{1}H relaxation mechanisms, a result similar to that found for acetonitrile.[34,39]

D. Nonexponential Methyl ^{1}H Spin–Lattice Relaxation. We turn now to the question of the nonexponentiality of the ^{1}H relaxation, an effect which has been attributed to cross correlations between relaxing spin pairs.[2] Our reasons for rejecting other explanations of nonexponentiality have been given previously.[2] The experimental decay plot is reproduced in Figure 1 (see curve labeled exptl) from the decay function $(M_0 - M)/2M_0 = (0.817 \pm 0.162)\exp[-t/(0.762 \pm 0.126)] + (0.155 \pm 0.168)\exp[-t/(3.77 \pm 4.65)]$, which was determined by a four-parameter nonlinear regression analysis of the raw data.[3,40]

A problem arises because of the presence of the spin-rotation contribution to the ^{1}H relaxation. The experimental ^{1}H relaxation decay plot in Figure 1 is seen to be distinctly nonexponential. Correcting this experimental curve for the effect of spin-rotation is *not* a straightforward matter, however, since the spin-rotation interaction does not affect each portion of the nonexponential curve to the same extent.[24,26,30,31] The presence of spin-rotation, while influencing the decay curve nonequivalently in different regions, apparently does not significantly affect the preexponential factors which weight the two separately decaying exponentials needed to characterize the relaxation. This conclusion is evident from a comparison of the measured preexponential values of 0.840 and 0.160 (normalized to unity) with those given by Hubbard,[22] who considers three spin-one-half nuclei at the corners of an equilateral triangle. This triangle undergoes hindered reorientation with respect to a molecule which exhibits rotational Brownian motion, and in the limit of extreme narrowing and rapid internal motion the predicted preexponential constants are 0.833 and 0.167 for the rapidly and more slowly decaying exponentials, respectively. The agreement with our measured values

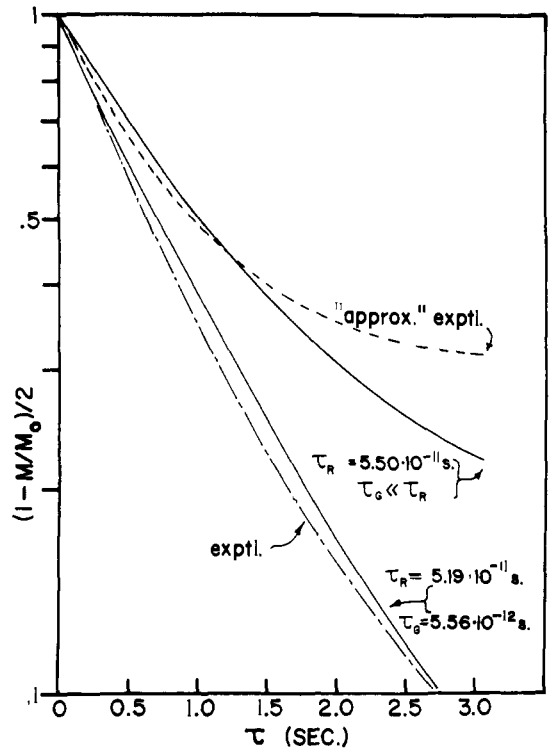

Figure 1. ^{1}H relaxation for the methyl protons of the Met residue in tetragastrin. Solid curves denote results calculated from formalism of Werbelow and Marshall.[4] Solid curve labeled $\tau_R = 5.19 \times 10^{-11}$ s and $\tau_G = 5.56 \times 10^{-12}$ s indicates that erroneous fits of the raw experimental data may be obtained if spin-rotation interaction is not accounted for. With reference to combined results at 30 °C for a 0.03 M DMSO-d_6 solution[2] the dashed curves denote raw experimental data (– – –) and the results after approximately separating the effects of spin-rotation (- - -).

is striking and supports the tenability of our initial assumption that internal methyl reorientation is rapid.

Further analysis of the nonexponentiality of the ^{1}H relaxation requires that the raw experimental data be fit by nonlinear regression techniques to the appropriate decay function. Exact formulations of the problem exist[26,30,31] and calculations indicate the possibility of interesting effects as a result of cross correlations in both the dipolar and spin-rotation mechanisms, which effects are absent if either mechanism is present alone.[41] However, a priori knowledge of the spin-rotation interaction parameters is required if the application of these complicated formulations is to be meaningful. Such information is presently unavailable for tetragastrin, and hence we proceed[42] according to the approximation implied by eq 5. The left side of eq 5 is the usual form for the decay function in a 180–90° experiment, where M_0 and M refer respectively to the equilibrium magnetization and the magnetization at a 180–90° pulse separation of τ. The right side of eq 5 reflects the assumption that the total decay function is the product of two separate decay functions. One corresponds to the spin-rotation contribution and includes the ^{1}H spin-rotation relaxation time of 2.47 s determined earlier in section C. The other corresponds to the dipolar contribution and is given in eq 5 as the sum of two exponentials, each with its own characteristic decay time (T_{1a} or T_{1b}) and corresponding preexponential weighting factor (a or b). It is precisely such factorization of decay functions which is normally used when multiple relaxation pathways contribute to the overall mechanism and the total relaxation rate is assumed to be the sum of the individually contributing rates. Such an approach is strictly valid here only when dealing with the initial slopes of the relaxation curves, which are unaffected by cross correlations.[23] Thus, the parameters a, T_{1a}, b, and T_{1b} have no significance other than as a parameterization of the data.

This parameterization is a useful one, however, in that it gives an approximate indication of the manner in which the dipolar relaxation decay function approaches its equilibrium value. The results are given in Figure 1 by the dashed curve marked "approx." exptl.

$$\frac{M_0 - M}{2M_0} = e^{-\tau/2.47}(ae^{-\tau/T_{1a}} + be^{-\tau/T_{1b}}) \qquad (5)$$

Comparison between experiment and cross-correlation theory may now be made. Using the value of $\tau_R = 5.50 \times 10^{-11}$ s determined in section C for isotropic overall tumbling and the assumption of rapid internal motion, our computer solution of the Werbelow and Marshall formalism[4] yields the result shown in Figure 1 by the appropriately labeled solid curve. Comparison between the theoretical curve and the curve labeled "approx." exptl. indicates that we have been able to utilize methyl ^{13}C relaxation data to predict the methyl ^1H relaxation behavior, even to the extent of the presence of observable cross-correlation effects. The lack of complete agreement, particularly at later stages of the relaxation, is no doubt due to our method of separating the dipole–dipole from the spin-rotation contribution. It should be emphasized here that the agreement shown in Figure 1 between experiment and theory confirms the tenability of our initial (and a priori) assumption that internal methyl reorientation is rapid compared to τ_R. The confirmation derives not from the early stages of the relaxation where $\tau \rightarrow 0$ in Figure 1, since the correspondence in this region of the curves was predetermined by our use of the initial slopes of the measured relaxation decay plots. Rather, the confirmation derives from the agreement in the later stages of the relaxation where $\tau > 0$ and cross-correlation effects are important. Even the approximate agreement shown in Figure 1 at $\tau > 0$ need not have been if our assumption that $\tau_G \ll \tau_R$ were seriously in error. Furthermore, the approximate agreement emphasizes that cross-correlation effects play a major role in the relaxation of the *S*-methyl protons in this peptide.[2]

Also shown in Figure 1 is a computer generated curve showing the influence of cross correlations when $\tau_R = 5.19 \times 10^{-11}$ s and $\tau_G = 5.56 \times 10^{-12}$ s. It is included to stress the importance, when analyzing cross-correlation effects, of correctly accounting for nondipolar effects in the ^1H relaxation mechanism. Had spin-rotation been ignored, it would have been obviously possible (see Figure 1) to reproduce the raw ^1H relaxation data using fallacious values for τ_R and τ_G in the cross-correlation formalism. This possibility arises because the effect of a spin-rotation mechanism for relaxation appears nonequivalently in different regions of the overall decay plot. The latter stages of the relaxation, where the inefficiency induced by dipolar cross correlations becomes increasingly apparent, are more affected by the additional relaxation pathway provided by spin-rotation than the early stages of the relaxation, where the dipolar mechanism is independent of cross correlations.[43] The net effect of the spin rotation is to linearize the overall decay plot and this linearization can easily be confused with the effect of varying the dipolar correlation times describing overall tumbling and internal reorientation.

The question may be raised as to why the influence of cross correlations is readily apparent in the ^1H but not in the ^{13}C spin–lattice relaxation decay curve. To answer this question the value of $\tau_R = 5.50 \times 10^{-11}$ s (see section C) may be used in Buchner's formalism for ^{13}C relaxation (including the effects of cross correlations on dipolar and spin-rotation interactions).[24] In the limit of extreme narrowing and rapid internal reorientation this formalism shows that the departure from exponential relaxation is within the experimental error and hence unobservable. This occurs for ^{13}C and not for ^1H because of the difference in the angle made by the H–H internuclear vectors (90°) and the threefold symmetry axis, as compared to that made by the C–H internuclear vectors (109.5°).

The previous analysis provides the groundwork whereby cross-correlation effects, when observed in singlet resonances, may be used to provide information concerning molecular motion. In complex situations the overall molecular tumbling may be anisotropic and thus not describable by a single τ_R. Such a situation might be expected to arise in studies which employ ^{13}C enriched methyl carbons as NMR relaxation probes in proteins.[12] The analytical approach used above may be expanded in such a case since anisotropic overall tumbling is readily included in the formalism of Werbelow and Marshall.[4] In this case the last step in the analysis would be the generation of a series of curves as in Figure 1, only now as a function of one of the additional correlation times required to describe the anisotropic overall tumbling. By comparison between the curvature in the experimental and theoretical ^1H relaxation decay plots an estimate of one additional correlation time may be obtained. If such an analytical approach is to be profitably applied, however, the ^1H relaxation formalism[26,30,31] which quantitatively accounts for the contribution of spin-rotation in the presence of dipolar cross correlations must be used and details of the spin-rotation interaction known in advance.

Conclusion

Our analysis illustrates that details of ^1H spin-rotation interaction may be determined by joint ^1H and ^{13}C measurements and that cross-correlation effects in methyl ^1H singlet resonances may be analyzed with the aid of ^{13}C data. Correct analysis of the nonexponentiality induced in the relaxation of methyl ^1H singlet resonances by dipolar cross correlations is severely complicated by spin rotation, which is generally a likely competing mechanism for relaxation. The need for information about the spin-rotation interaction is a requirement which is present even in the approach to the problem utilizing the complete relaxation behavior of the fully coupled $^{13}CH_3$ spin system.[5] It is possible that the additional information inherent in the relaxation behavior of the fully coupled $^{13}CH_3$ spin system may be used to provide the requisite information about spin-rotation. However, if such proves to be the case, it is likely to be at the expense of information about the details of the motional processes inherent in the dipolar mechanism. In any event the complications introduced by spin-rotation in any analysis of dipolar cross-correlation effects are formidable.

Our results also indicate that the effects of dipolar cross correlation must be considered in the ^{13}C frame even though nonexponential ^{13}C relaxation may not be observable. The influence of dipolar cross correlations enters via the NOE, in the presence of spin rotation, with the result that errors of 6–15% can be made in correlation time determinations. We emphasize that if methyl ^{13}C T_1 probes are used to monitor molecular motion, such errors can exceed the accuracy of carefully performed T_1 measurements. For example, methyl groups in a peptide may be used to monitor overall tumbling via $^{13}CH_3$ relaxation, and although the use of ^{13}C enriched methyl carbon probes offers exciting possibilities for studies of biophysically interesting macromolecules, our results show that the power of such studies will be enhanced considerably if collateral methyl ^1H data are available. The observation of a reduced ^{13}C methyl NOE and nonexponential methyl ^1H spin–lattice relaxation provide the clues which indicate that the analysis of the raw ^{13}C data may not be straightforward. Depending upon the contribution of dipolar cross correlation (as determined by methyl rotational correlation times, anisotropy of overall tumbling, etc.) and spin-rotation, the derived reorientation times may be substantially in error.

Note Added in Proof. After this work was completed and in press, a particularly attractive group theoretical treatment of methyl relaxation has appeared (G. B. Matsen, *J. Chem. Phys.*, in press). This treatment leads to spectral density functions identified with symmetry labels rather than with auto- and cross-correlation. The advantage is to provide a physical interpretation for the pre- and postexponential terms in methyl relaxation behavior characterized by multiple exponentials. Subsequent work from this laboratory will use this development.

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Proton Magnetic Resonance and Conformational Energy Calculations of Repeat Peptides of Tropoelastin: the Tetrapeptide

Md. Abu Khaled, V. Renugopalakrishnan, and Dan W. Urry*

Contribution from the Laboratory of Molecular Biophysics and the Cardiovascular Research and Training Center, University of Alabama Medical Center, Birmingham, Alabama 35294. *Received April 8, 1976*

Abstract: The detailed conformation of a tetrapeptide of tropoelastin, *t*-Boc-L-Val_1-L-Pro_2-Gly_3-Gly_4-OMe in $CDCl_3$, has been obtained from a combined analysis of ^1H NMR spectra and conformational energy calculations. The observations of Gly_3 and Gly_4 methylene protons as ABX spin systems indicate a fixed conformation similar to a cyclic peptide stabilized by hydrogen bond formation. Temperature dependence and solvent perturbation of NH protons and conformational energy calculations each showed the presence of a β-turn, a ten atom hydrogen-bonded ring involving the Gly_4 NH and Val_1 C=O, and a segment of an antiparallel β-pleated sheet stabilized by a hydrogen bond between the Val_1 NH and the Gly_4 C=O. Conformational angles obtained from the observed $^3J_{\alpha CH-NH}$ coupling constants and from conformational energy calculations were in good agreement. The secondary structure of this tetramer is shown to be the same as previously proposed for the high polymer of the tetramer in water at elevated temperature.

An understanding of the essential functional role of elastin in vascular wall and its molecular pathology in atherosclerosis requires an understanding of its conformation and dynamics. Structural features at the molecular level are largely responsible for the dynamic properties and interactions of elastin. Gray, Sandberg, and co-workers[1,2] have shown that soluble