References and Notes

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On the Interpretation of ^{13}C Spin–Lattice Relaxation Resulting from Ring Puckering in Proline

Robert E. London

Contribution from the Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545.

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Abstract: A calculation of ^{13}C relaxation parameters for a system undergoing overall isotropic motion as well as internal conformational fluctuation between two stable states is presented. Dependence of T_1 on the lifetimes of the two conformations, the angle defined by the particular C–H vector and the effective axis about which it rotates, and the range through which it rotates are considered. Relaxation data for proline and proline-containing peptides have been analyzed on the basis of several models for proline puckering: interconversion between two envelope forms with C^γ puckered, interconversion between two C^β–C^γ half-chair forms, and an intermediate case. Consideration of the ^{13}C T_1 values for each proline carbon indicates that for most cases the two stable conformational states correspond to approximate C^β–C^γ half-chairs with C^γ exhibiting a somewhat greater angular displacement than C^β. Reasonable values for the NT_1^γ/NT_1^α ratio can be obtained by requiring roughly equal lifetimes $\sim 10^{-11}$–10^{-12} s for the two states and a typical range of motion for the C^γ–H vectors ~ 50–$70°$. These results appear to be consistent with available theoretical calculations, x-ray data, and conclusions based on ^1H–^1H scalar coupling studies.

Introduction

^{13}C NMR offers a uniquely powerful tool for studying molecular dynamics due to the dominance of the spin relaxation by the dipolar interaction with the directly bonded protons.[1] The measured relaxation times can, with the aid of an appropriate model, be directly used to obtain information about the motion of individual C–H bonds. This situation differs from that encountered for most other nuclei; in particular, in ^1H NMR studies it is necessary to separate intra- and intermolecular contributions as well as to sort out all of the intramolecular interactions which may be significant.

The relaxation behavior of proline, both as the free amino acid and incorporated into various peptides, has stimulated considerable interest due to the marked differences in the T_1 values for the carbons in the pyrrolidine ring.[2-20] Such differences can be interpreted to reflect anisotropic motion of a basically rigid structure, internal motion such as would arise from a rapid interconversion of puckered conformations, or a combination of effects. The generality of these differences in relaxation time makes an explanation based primarily on anisotropic motion unlikely since the anisotropy exhibited by different peptides is likely to be very different. A recent quantitative evaluation of the relaxation effects of motional anisotropy based on crystallographic data substantiates this conclusion.[21] Of course, differences in motional anisotropy of the peptide backbone may contribute to the differences observed in various peptides. Attempts to describe the relaxation behavior in terms of internal motion have been limited to use of a free internal rotation model[13] and to an approximation in which the different relaxation times reflect a different effective correlation time for each carbon in the ring.[5,6] The interpretation of internal diffusion coefficients or correlation times deduced from the application of such models is, however, ambiguous. For example, if the ring alternates between only two conformational states, the lifetime of each conformation will be the same for all carbons in the ring, but T_1 differences can still be predicted due to differences in the angular factors involved, as is shown in the present calculation.

The approach considered here is based on a bistable system able to alternate between two different conformations. Such an approach leads to an evaluation of the observed relaxation times in terms of the overall diffusion rate, the lifetimes of the two states, the angle between the particular C–H vector, and the effective axis about which it rotates due to the jump, and the range through which the C–H vector jumps. This calculation probably represents an oversimplification for the proline ring system which may be able to adopt many puckered conformations. It can be justified on several grounds: (1) Studies of ^1H–^1H coupling constants indicate the existence of a conformational equilibrium between two equally populated conformations.[22-25] Roughly equivalent stabilities are also required based on the present relaxation calculations using the two-state model. (2) Theoretical energy calculations indicate the existence of two energy minima corresponding roughly to the states considered in the present calculations.[26-28] (3) The range through which the various C–H vectors must move to produce the observed relaxation rates is consistent with the degree of puckering observed in crystallographic studies.[28,29] (4) Development of the present model makes possible an evaluation of the relaxation effect of several models for internal

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motion consistent with the constraints of the ring and a determination of the motion most likely to significantly affect the relaxation rate. Finally, it is noted that the present calculation should adequately describe relaxation effects in a number of different but analogous systems. For example, the five-membered ring formed by ethylenediamine-metal ion chelation undergoes a rapid conformational equilibrium closely analogous to one of the models discussed here for proline.[30] In addition, a relaxation time pattern somewhat similar to that observed in proline has been reported for the five-membered carbon ring in prostaglandin $PGF_{2\alpha}$.[31] Presumably, similar internal puckering can occur in this case.

Theory

The model developed in this section is based on the assumption that (1) the overall tumbling of the molecule is isotropic; (2) the ^{13}C nuclear relaxation is dominated by the ^{13}C-^{1}H dipolar interaction with the directly bonded protons; (3) the molecule jumps internally between two stable states, A and B, with lifetimes τ_A and τ_B, respectively; (4) the jumps occur instantaneously so that at any given instant the molecule will either be in state A or state B. We first consider the orientational autocorrelation function for a C-H vector in the molecule which makes an angle β with the jump axis. Following Wallach,[32] this autocorrelation function is given by:

$$G(t) = \sum_a e^{-6D_0 t} |d_{a0}(\beta)|^2 \langle e^{ia[\gamma(0)-\gamma(t)]} \rangle \quad (1)$$

In the above expression, D_0 is the isotropic rotational diffusion coefficient, the $d_{a0}(\beta)$ are the reduced Wigner rotation matrices,[33] and the summations run from -2 to $+2$. β is the angle defined by the relaxation vector and the symmetry axis of the jump; it is analogous to the β which appears in the unrestricted internal rotation calculation.[32] The factor $\exp(ia\gamma)$ is defined using the usual Euler angle γ; it represents a rotation about the initial z axis (the internal jump axis) such that a subsequent rotation about the y axis will leave z' along the appropriate C-H vector; i.e., γ is chosen so that y is perpendicular to z and z'.[34] In obtaining the autocorrelation function, $\exp[ia\gamma(0)]$ represents the initial transformation to the C—H vector and $\exp[ia\gamma(t)]$ represents the transformation at time t. Subsequently we make the abbreviations $\gamma(0) = \gamma_0$ and $\gamma(t) = \gamma$. The above expression can readily be generalized to the case in which the overall motion is anisotropic,[35] or to include the effects of several additional internal rotations.[34,35] Since the purpose of the present calculation is to evaluate the effects of internal jumping only, these complicating factors have not been included.

Since the present model assumes that the molecule is in either conformation A or B at all times, the calculated correlation function and hence the relaxation times are independent of the particular path through which the C-H vector moves in going between conformations. For this reason, the choice of axes and hence the angular factors β and γ are not uniquely defined. Results are most readily interpreted by defining β in terms of a fixed molecular axis about which the C-H vector would rotate in going from conformation A to B, in analogy with the free internal rotation model. Equivalent relaxation times are obtained for equivalent conformational transitions regardless of the choice of axis. This point is considered in greater detail in the section dealing with particular dynamic models for proline.

The quantity $\langle \exp[ia(\gamma_0 - \gamma)] \rangle \equiv M(a)$ may be considered a five-component vector and can be evaluated by solving the time-dependent differential equations for the probability of the A and B states as done for a gauche $\rightleftarrows$ trans isomerism.[36] Letting A and B represent the normalized fraction of molecules in states A and B, the time-dependent probabilities that a molecule is in either state are described by the relations:

$$\frac{dA}{dt} = -\frac{A}{\tau_A} + \frac{B}{\tau_B} \quad (2)$$

$$\frac{dB}{dt} = \frac{A}{\tau_A} - \frac{B}{\tau_B} \quad (3)$$

which have the solutions:

$$A(t) = \tau_c \left[\frac{1}{\tau_B} + \frac{K}{\tau_B} e^{-t/\tau_c} \right] \quad (4)$$

$$B(t) = \tau_c \left[\frac{1}{\tau_A} - \frac{K}{\tau_B} e^{-t/\tau_c} \right] \quad (5)$$

where

$$\frac{1}{\tau_c} = \frac{1}{\tau_A} + \frac{1}{\tau_B} \quad (6)$$

and K is an arbitrary constant determined by the boundary conditions. Using the above results, the four conditional probabilities can be found:[36]

$$p(A,t|A,0) = \frac{\tau_A + \tau_B e^{-t/\tau_c}}{\tau_A + \tau_B}$$

$$p(B,t|A,0) = \frac{\tau_B(1 - e^{-t/\tau_c})}{\tau_A + \tau_B}$$

$$p(A,t|B,0) = \frac{\tau_A(1 - e^{-t/\tau_c})}{\tau_A + \tau_B}$$

$$p(B,t|B,0) = \frac{\tau_B + \tau_A e^{-t/\tau_c}}{\tau_A + \tau_B} \quad (7)$$

These probabilities can then be used to evaluate the averaged quantity in eq 1:

$$M(a) = \langle e^{ia(\gamma_0 - \gamma)} \rangle = \sum_{\gamma_0 = \pm\theta} \sum_{\gamma = \pm\theta} e^{ia(\gamma_0 - \gamma)} P(\gamma_0) P(\gamma,t|\gamma_0,0) \quad (8)$$

giving

$$M(2) = M(-2) = \frac{1}{(\tau_A + \tau_B)^2} [\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B \cos 4\theta + 2\tau_A\tau_B(1 - \cos 4\theta)e^{-t/\tau_c}] \quad (9)$$

$$M(1) = M(-1) = \frac{1}{(\tau_A + \tau_B)^2} [\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B \cos 2\theta + 2\tau_A\tau_B(1 - \cos 2\theta)e^{-t/\tau_c}]$$

$$M(0) = 1$$

where in the above expression the jump is assumed to change γ from $+(-)\theta$ to $-(+)\theta$. In the unrestricted internal rotation calculation the vector $M(a) = e^{-a^2 D_i t}$ where D_i is the internal diffusion coefficient. The vector $M(a)$ can be divided into two parts, $M(a) = M1(a) + M2(a)e^{-t/\tau_c}$. It can be noted that for $\theta = 0$, $M = 1$, the unit vector. Combining the above result with eq 1, it is apparent that the autocorrelation function decays as a sum of two exponentials:

$$G(t) = C1e^{-6D_0 t} + C2e^{-(6D_0 + 1/\tau_c)t} \quad (10)$$

where

$$C1 = \sum_a |d_{a0}(\beta)|^2 M1(a) = \frac{1}{(\tau_A + \tau_B)^2} \left[\frac{3}{4} \sin^4\beta(\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B \cos 4\theta) + 3 \sin^2\beta \cos^2\beta(\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B \cos 2\theta) + \left(\frac{3\cos^2\beta - 1}{2} \right)^2 (\tau_A + \tau_B)^2 \right] \quad (11)$$

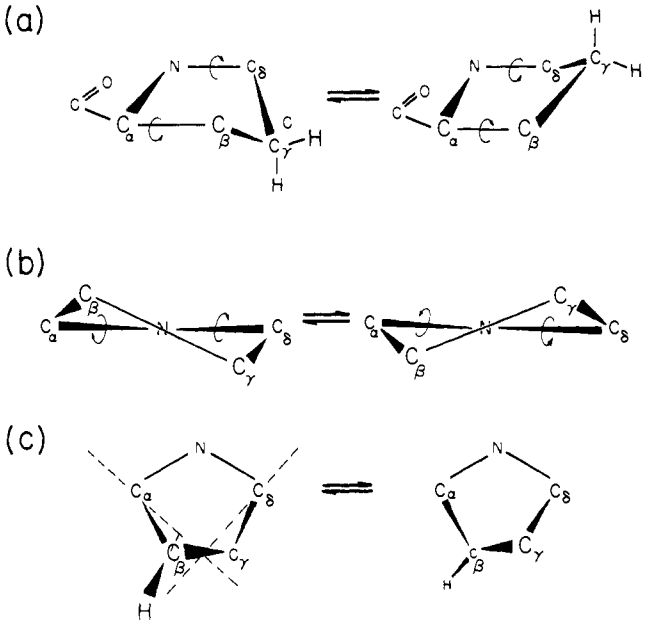

Figure 1. Dynamic models for ring puckering in proline: (a) exo ⇄ endo interconversion of C_γ with $\beta = 70.5°$ for C_β, C_δ, $\beta = 90°$ for C_γ. (b) Interconversion between two antisymmetric C_2 forms, $\beta = 70.5°$ for C_α, C_δ, $\beta = 75.4°$ for C_β, C_γ. The latter value was calculated assuming a C-C-C bond angle of 104°, C-C bond length of 1.53 Å, and an effective rotation axis as pictured in 1c. (c) Same as b viewed perpendicular to the C_δ-N-C_α plane. The two effective internal jump axes are indicated by dotted lines.

$$C2 = \sum_a |d_{a0}(\beta)|^2 M2(a)$$
$$= \frac{1}{(\tau_A + \tau_B)^2}\left[\frac{3}{2}\sin^4\beta\tau_A\tau_B(1 - \cos 4\theta) + 6\sin^2\beta\cos^2\beta\tau_A\tau_B(1 - \cos 2\theta)\right]$$

Finally, the relevant spectral densities will be given by:

$$J(\omega) = C1\frac{(6D_0)^{-1}}{1 + [\omega/(6D_0)]^2} + C2\frac{(6D_0 + 1/\tau_c)^{-1}}{1 + [\omega/(6D_0 + 1/\tau_c)]^2} \quad (12)$$

It should be noted that the spectral densities correspond to only half the value obtained by Fourier transforming $G(t)$ from $-\infty$ to $+\infty$. The extra factor of 2 can be absorbed into the coefficients used to calculate the relaxation times.

The relation obtained above for the spectral density in the two-state model is closely analogous to the corresponding expression for the unrestricted internal rotation case:[37-39]

$$J(\omega) = \left(\frac{3\cos^2\beta - 1}{2}\right)^2\frac{(6D_0)^{-1}}{1 + [\omega/6D_0]^2} + 3\sin^2\beta\cos^2\beta\frac{(6D_0 + D_i)^{-1}}{1 + [\omega/(6D_0 + D_i)]^2} + \frac{3}{4}\sin^4\beta\frac{(6D_0 + 4D_i)^{-1}}{1 + [\omega/(6D_0 + 4D_i)]^2}$$

In comparing the two results, we note that the coefficient $C1$ is somewhat less sensitive to the value of β than the corresponding coefficient of the first term in the above expression. In particular, $C1$ does not vanish for values of β close to 54.7°. Both coefficients equal 1.0 for $\beta = 0$ and are both equal to $\frac{1}{4}$ for $\beta = 90°$ if, in the two-state model, the conditions $\tau_A = \tau_B$ and $\theta = 45°$ are also fulfilled. Analogous behavior is obtained in the limit $1/\tau_c \to 0$ or $D_i \to \infty$; if all motions are in the extreme narrowing limit and the conditions $1/\tau_c \gg D_0$ or $D_i \gg D_0$ are fulfilled, only the first terms in either spectral density equation will contribute. Alternatively, if the extreme narrowing condition is not fulfilled for D_0, this approximation is not necessarily valid and a quantitative analysis of the various spectral density terms is required.

T_1, T_2, and the nuclear Overhauser enhancement (NOE) are given by:

$$\frac{1}{T_1} = \frac{\gamma_C^2\gamma_H^2\hbar^2}{10r_{CH}^6}[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (13)$$

$$\frac{1}{T_2} = \frac{\gamma_C^2\gamma_H^2\hbar^2}{20r_{CH}^6}[4J(0) + 6J(\omega_H) + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_C + \omega_H)] \quad (14)$$

$$\eta = \frac{\gamma_H}{\gamma_C}\left[\frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)}\right] \quad (15)$$

As shown recently by Werbelow and Grant,[40,41] the existence of cross correlations in the motions of the different C-H vectors for a particular carbon will lead to nonexponential relaxation with the initial rate given by eq 13 above. The cross-correlation function analgous to (1) will be given by:

$$G^{CC}(t) = \sum_a e^{ia(\phi-\phi')}e^{-6D_0t}|d_{a0}(\beta)|^2\langle e^{ia(\gamma_0-\gamma)}\rangle \quad (16)$$

In determining both the auto- and cross-correlation functions, the Wigner rotation matrices represent a time-dependent transformation from a system with the z axis parallel to the effective internal rotation axis to a system with the z' axis along the C-H vector. The additional factor $\exp[ia(\phi-\phi')]$ in the cross-correlation function arises from having one transformation to the C-H vector and one to the C-H′ vector. The angle $\phi-\phi'$ represents a rotation about the initial z axis so that the x axis of the coordinate system rotates from an orientation perpendicular to the z-z' plane to an orientation perpendicular to the z-z'' plane where z' and z'' represent vectors along the C-H and C-H′ bonds, respectively. To illustrate, we consider one of the models for exo ⇄ endo interconversion used in the present calculations and pictured in Figure 1a. For carbons C^δ and C^β, $\phi-\phi' = 120°$; for carbon C^γ, the effective rotation axis is perpendicular to both C-H vectors so that $\phi-\phi'$ is the H-C-H′ bond angle. Neglecting deviations of the angles from a perfectly tetrahedral geometry, it is therefore 109.5°. Because of the summation over a in eq 16, the additional term adds a factor $2\cos[a(\phi-\phi')]$ so that the matrix elements analogous to $C1$ and $C2$ for the cross-correlation function are given by:

$$C1^{cc} = \frac{1}{(\tau_A + \tau_B)^2} \times \left[\frac{3}{4}\sin^4\beta\cos[2(\phi-\phi')](\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B\cos 4\theta) + 3\sin^2\beta\cos^2\beta\cos(\phi-\phi')(\tau_A^2 + \tau_B^2 + 2\tau_A\tau_B\cos 2\theta) + \left(\frac{3\cos^2\beta - 1}{2}\right)^2(\tau_A + \tau_B)^2\right] \quad (17)$$

$$C2^{cc} = \frac{1}{(\tau_A + \tau_B)^2}\left[\frac{3}{2}\sin^4\beta\cos[2(\phi-\phi')]\tau_A\tau_B(1 - \cos 4\theta) + 6\sin^2\beta\cos^2\beta\cos(\phi-\phi')\tau_A\tau_B(1 - \cos 2\theta)\right]$$

The corresponding spectral densities can be obtained analogous to the autocorrelation function case substituting $C1^{cc}$ and $C2^{cc}$ for $C1$ and $C2$.

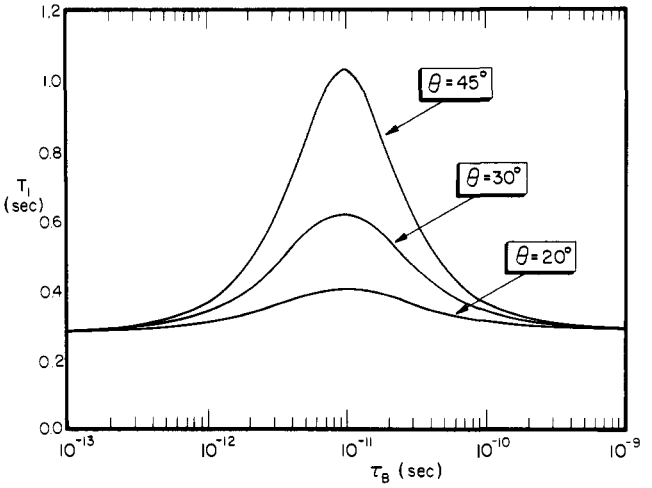

Figure 2. Calculated ^{13}C spin-lattice relaxation time for a system undergoing internal jumps characterized by $\beta = 90°$, $D_0 = 10^9\ s^{-1}$, $\tau_A = 10^{-11}$ s, 10^{-13} s $< \tau_B < 10^{-9}$ s, and corresponding to the values of θ indicated.

Numerical Calculations

Before proceeding to evaluate the present model in terms of experimental proline relaxation data, we consider the general dependence of the calculated T_1 values on the various parameters. In the absence of data suggesting significantly nonexponential decays, cross-correlation effects have been neglected in these calculations. It is apparent from eq 9 that in the limit $\tau_A \ll \tau_B$ or $\tau_A \gg \tau_B$, $M \rightarrow 1$ so that eq 1 becomes:

$$G(t) \rightarrow \sum_a e^{-6D_0 t}|d_{a0}(\beta)|^2 \equiv e^{-6D_0 t} \qquad (18)$$

Thus the internal jump motion will produce no effect. Physically, this result indicates that if the molecule spends an overwhelming portion of time in either the A or B conformation, the internal jump mechanism fails to affect the relaxation rate. A plot of the calculated T_1 corresponding to $D_0 = 10^9\ s^{-1}$, $\tau_A = 10^{-11}$ s, $\beta = 90°$, and 10^{-13} s $< \tau_B < 10^{-9}$ s is given in Figure 2 for several values of θ. For τ_B a factor of 2 larger or smaller than τ_A, the T_1 increase is only 75% that corresponding to $\tau_A = \tau_B$. It is thus apparent that to have a significant effect on the relaxation time we require $\tau_A \sim \tau_B$.

The dependence of T_1 on the relative overall and internal correlation times is straightforward and is illustrated for the case of $\beta = 2\theta = 90°$ (Figure 3). The plot of T_1 ($\theta = 45°$)/T_1 ($\theta = 0°$) as a function of $\tau_A = \tau_B$ corresponding to various values of D_0 illustrates the fractional change in T_1 due to jumping with a range (2θ) of 90° compared with the no-jump case. For $\tau_A \gg \tau_0 = \frac{1}{6}D_0$, the internal motion does not contribute to the relaxation process so that the ratio $T_1(\theta = 45°)/T_1(\theta = 0°) \equiv R = 1.0$. In the limit $\tau_A = \tau_B \ll \tau_0 = \frac{1}{6}D_0$ only the first term in the spectral density eq 12 is significant. For the case of $\beta = 2\theta = 90°$, $C1 = \frac{1}{4}$ so that the relaxation time will be a factor of 4 greater than that corresponding to the absence of internal jumping. In the intermediate region T_1 will increase monotonically if all motions are in the extreme narrowing limit but can decrease if the overall motion is in the slow tumbling range since in this case the second term in eq 12 will contribute significantly. Analogous behavior occurs in the free internal rotation calculation for T_1.[38-40] For values of θ other than 45°, similar results are obtained with the T_1 ratio R being closer to 1.0 so that the dip in the T_1 curves corresponding to $D_0 = 10^6$ and $10^7\ s^{-1}$ is less pronounced and the asymptote for $\tau_A = 10^{-12}$ s is lower than 4.0.

We next consider the dependence of T_1 on the two angular factors which enter the calculation. In Figure 4 we have plotted

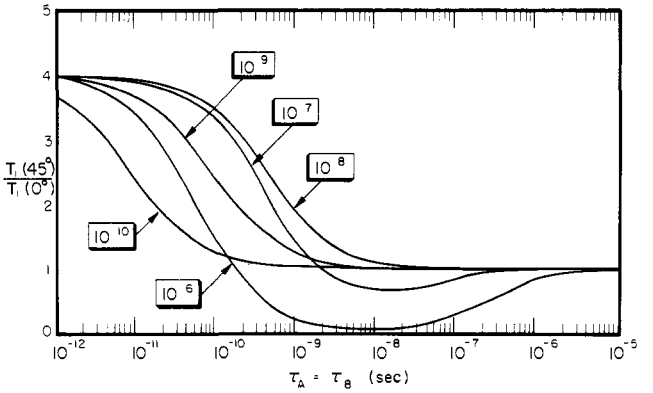

Figure 3. Calculated ^{13}C spin-lattice relaxation time ratio, $T_1(\theta = 45°)/T_1(\theta = 0°)$ corresponding to $\beta = 2\theta = 90°$ plotted as a function of $\tau_A = \tau_B$ for $D_0 = 10^6 - 10^{10}\ s^{-1}$ are indicated for each curve.

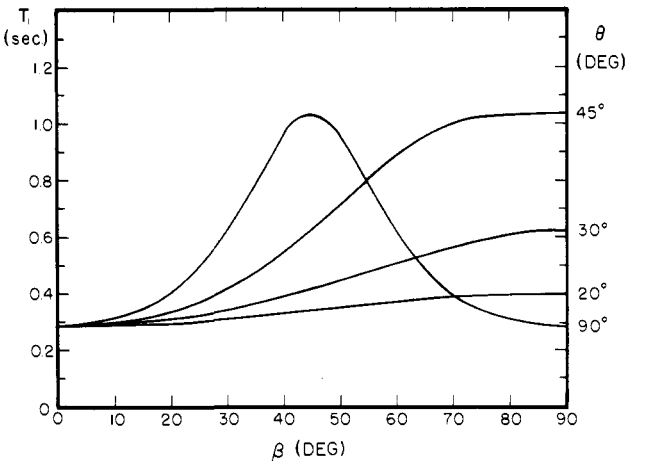

Figure 4. Calculated ^{13}C spin-lattice relaxation times corresponding to $D_0 = 10^9\ s^{-1}$; $\tau_A = \tau_B = 10^{-11}$ are plotted as a function of β for several values of θ.

T_1 as a function of β for several values of θ. It should be noted that since the C-H vector jumps between $+\theta$ and $-\theta$, the total range through which it moves is 2θ. This calculation reveals an important feature about the present model: for relatively small jump ranges, i.e., $\theta \leq 45°$, and all motion in the extreme narrowing limit, the relaxation time is a monotonically increasing function of both β and θ. Thus, as the range increases the relaxation time will increase. Further, as the C-H vector becomes more perpendicular relative to the effective jump axis, the relaxation time increases. This result enables different models for ring puckering to be evaluated in terms of how sensitive the various relaxation times will be to the internal motion.

In order to estimate the effect on T_1 of internal jumps in the absence of information on the jump range, a plot of T_1 vs. θ corresponding to various values of D_0 and τ_A and corresponding to $\beta = 90°$ is shown in Figures 5a-c. The symmetry of these plots about $\theta = 45°$ is evident from eq 11. If $\beta \neq 90°$, the curves become skewed so that the maximum occurs for a larger value of θ and the maximum increase in T_1 is reduced. Also significant is the reduction in the effectiveness of the internal jump process when $\tau_0 = \frac{1}{6}D_0$ becomes comparable to or shorter than $\tau_A = \tau_B$. This situation occurs for free hydroxyproline for which all of the NT_1 values are approximately equal.[42] It should be pointed out, however, that in this case the two states may have significantly different stabilities so that $\tau_A \gg \tau_B$ so that even if $\tau_A \ll \tau_0$ no relaxation effect will be observed.

A final result worth noting which is apparent in Figures 4

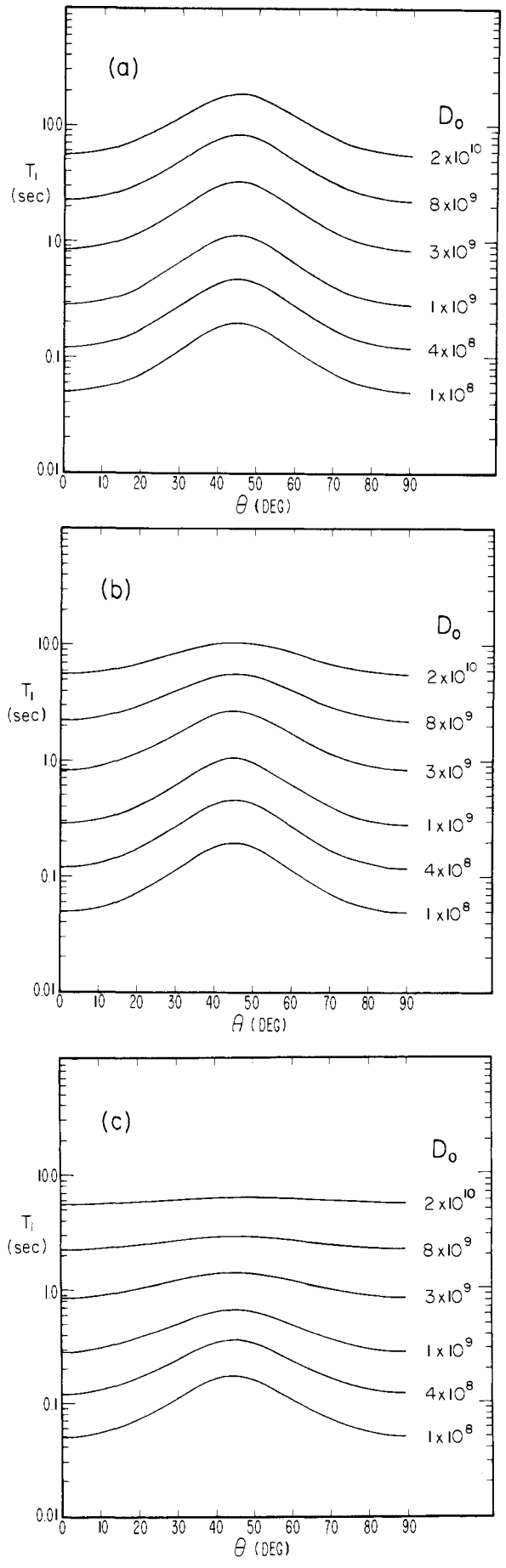

Figure 5. Calculated ^{13}C spin-lattice relaxation time plotted as a function of θ for $\beta = 90°$ and (a) $\tau_A = \tau_B = 10^{-12}$ s; (b) $\tau_A = \tau_B = 10^{-11}$ s; (c) $\tau_A = \tau_B = 10^{-10}$ s. Values of D_0 are indicated in the figure.

and 5 is the T_1 behavior corresponding to $\theta = \beta = 90°$. In this case, the internal motion corresponds to instantaneous jumps of the C-H vector between parallel and antiparallel orientations. Since the dipolar interaction is invariant to such a motion, the jump has no effect.

Calculations for T_2 have not been presented since for nearly all of the data available all motion falls into the extreme narrowing limit with $T_1 = T_2$. Similarly, for typical parameters, calculated NOE values are close to the theoretical maximum, consistent with measurements on peptides.[20] Reduction of the NOE does occur for sufficiently slow values of D_0 and the internal jump model predicts an increased NOE dependent on the rate and range of the internal motion. Use of the cross-correlation function derived in the previous section and the equation of Werbelow and Grant[40,41] indicates that for most cases of interest deviation of the spin-lattice relaxation behavior from exponentiality is minor. Two relevant parameters in describing these latter effects are the ratio of the two "relaxation times" and the ratio of the two preexponential factors. Nonexponential recovery will be most pronounced if the first ratio is either much greater or smaller than 1 and the second is close to 1. For C^γ of Figure 1a ($\beta = 90°$, ϕ-$\phi' = 109.5°$), significant deviations from exponential decay only occur for θ very close to 45°, a situation which is probably unusual. In the absence of any data indicating such behavior of the spin-lattice relaxation, detailed calculations are not included at present.

Dynamic Models for Proline Puckering

Quantitative estimates for the T_1 values in proline require that the states A and B be defined. Some x-ray data[29] and ^1H-^1H coupling data[22-25] suggest that in a variety of proline derivatives the atoms C^β-C^α-N-C^δ are nearly coplanar, with C^γ occupying an endo or exo position relative to the proline carboxyl. The data provide one basis for evaluating the relaxation behavior with the states A and B pictured in Figure 1a. It is important to note that a separate calculation must be done for each carbon. The C^α-H vector is not affected by the jump process and thus, along with the C^α-N vector, is assumed to rotate isotropically. The angle β is 90° for C^γ and ~70.5° for C^β and C^δ. The latter value which is just the complement of the tetrahedral angle is obtained assuming that the jump involves rotations about the C^α-C^β and N-C^δ axes as pictured in Figure 1. There are also differences in the range through which the C-H vectors rotate. An approximate relationship between the ranges for C^γ-H and C^β-H and C^δ-H can be obtained for a regular pentagon based on the calculations of Abraham and McLauchlan in connection with studies of the ^1H-^1H coupling constants.[43] Clearly, these relationships are only a rough approximation of the true motion with time-dependent deviations of C^β-C^α-N-C^δ from planarity the most significant omission. Thus, from Table I of ref 41, our angle θ for C^γ corresponds to θ in Table I, and our θ for C^β,C^δ corresponds to ω_{23} in Table I. It is apparent that this definition of states A and B will lead to T_1 values which have the relation $NT_1^\gamma > NT_1^\beta = NT_1^\delta > NT_1^\alpha$ (N is the number of directly bonded protons). Examination of the available ^{13}C relaxation data for a variety of peptides, particularly peptides having N terminal proline residues, indicates that approximately this pattern is observed in some cases; however, more frequently there are significant differences between T_1^β and T_1^δ while $T_1^\beta \simeq T_1^\gamma$. In at least one case, $T_1^\beta > T_1^\gamma$.[6] This effect can be qualitatively interpreted by assuming that formation of the peptide bond involving the proline nitrogen effectively immobilizes the proline nitrogen to a greater degree than C^α. Of the possible puckering modes then available, the conversion between the two half-chair C_2 forms pictured in Figures 1b,c should be an effective mechanism for the relaxation of C^β and

C^γ since the C-H vectors make an angle of 75.4° with the effective internal rotation axes. This model, therefore, leads to the T_1 pattern $NT_1^\gamma = NT_1^\beta > NT_1^\delta = NT_1^\alpha$ which is approximately satisfied for a variety of proline containing peptides. Based on a recent survey of crystallographic data for 40 proline derivatives, DeTar and Luthra have found a range of C^β-C^γ half-chairs to be the most common conformational state.[28]

Most typically, neither of the above models is completely appropriate since the observed T_1 pattern corresponds to $NT_1^\gamma > NT_1^\beta > NT_1^\delta > NT_1^\alpha$. Such a pattern can be predicted by a model somewhere between the two extreme cases illustrated in Figure 1. Thus, the appropriate values of β will be 70.5° for C^δ, between 70.5 and 75.4° for C^β, and between 75.4 and 90° for C^γ. Although C^α may be subject to some internal motion, it is apparent from Figure 5 that for small displacements corresponding to a range $\lesssim 10°$ the internal jump will contribute very slightly to the T_1 calculated assuming no internal motion so that T_1^α provides a reasonable basis for calculating D_0. Evidence in support of this approximation has been obtained by Torchia and Lyerla.[5] This will not be the case if the overall motion is so slow that the internal motion makes the dominant contribution to the spectral density; however, for all peptides for which data are available all motion is in the extreme narrowing limit. Thus, it is apparent that by an appropriate choice of states A and B it is possible to predict any observed T_1 pattern without postulating differences in the correlation times characterizing the motion of the different C-H vectors. Such a prediction is based on the assumption that only two states are significantly populated in which case the differences in relaxation rate must reflect primarily differences in the angular factors β and θ for the different C-H vectors.

It must be emphasized at this point that although the internal jump is uniquely defined by a particular dynamic model, the choice of the angles β, θ which describe the conformational transition is not. In the preceding discussion β has been defined using the axis about which the various C-H vectors are assumed to rotate in making the conformational jump. The values of β and θ thus obtained are therefore most readily interpreted in terms of a molecular model. However, since the model derived here assumes that the molecule is always in conformation A or B, the relaxation parameters calculated are independent of the motional pathway between these states. In general, an infinite choice exists for the angles (β,θ) describing the transition. In addition to the choices discussed here, a convenient choice is frequently $\beta = 90°$, i.e., the jump axis perpendicular to the initial and final orientations of the C-H vector. If the jump is initially described using the angles β and θ, it can be described using the angles $\beta' = 90°$ and θ', where simple geometry gives:

$$1 - \cos 2\theta' = \sin^2 \beta(1 - \cos 2\theta)$$

Both sets of angles lead to equal relaxation times as can be checked for several cases using Figure 4. For example, (β,θ) values of (45°,45°) and (90°,30°) give the same T_1 value.

Comparison with Experimental Data

Estimates for the various parameters of the model can be made on the basis of comparison with available experimental data, although the problem is underdetermined. The simplest initial approximation is that the various peptides can be classed according to molecular weight with the rate of internal motion remaining relatively constant. If the lifetimes τ_A and τ_B are sufficiently slow so that for the lowest molecular weight samples $\tau_A, \tau_B \gtrsim \tau_0 = \frac{1}{6}D_0$, the effect of internal motion as measured, for example, by the ratio NT_1^γ/NT_1^α will decrease as illustrated in Figure 5c. Alternatively, if $\tau_A, \tau_B \ll \tau_0$ for all of the peptides, no such decrease will occur, as in Figure 5a. In general, however, this approximation appears to be too much of an oversimplification. For example, the ratio NT_1^γ/NT_1^α is 2.0 for proline and 2.2 for lysine vasopressin.[11] In addition, the steric factors present, for example, in small cyclic peptides also result in strong deviations from predictions based on the above assumptions.[6,11] For L-proline, NT_1 values close to those observed and corresponding to reasonable values of θ can be obtained for $\tau_A = \tau_B < 10^{-12}$ s. A particular example, based on the model of Figure 1a, is given in Table I.

An important parameter in the model is the range, 2θ, through which the various C-H vectors must move in order to produce the observed relaxation effects. The largest effect of puckering, as measured by a NT_1^γ/NT_1^α ratio of 3.93, has been reported for Pro-Leu-Gly-NH_2.[2] This is sufficiently close to the theoretical maximum (Figure 3) to require $\beta = 2\theta = 90°$ and $\tau_A = \tau_B \leq 10^{-12}$ s. This result is suspect, however, owing to the much lower ratio observed in Pro-Leu-Gly-$N(CH_3)_2$,[2] as well as in subsequent studies.[19,44] A more reasonable range would be 50-70° which is closer to the values obtained for more typical NT_1^γ/NT_1^α ratios ~2.

We next consider some specific examples. The pattern $NT_1^\gamma > NT_1^\beta \simeq NT_1^\delta > NT_1^\alpha$ which is predicted using the model of Figure 1a is approximately satisfied in a number of cases including free proline,[11] *cyclo*-$(Pro)_3$,[18] and Pro-Leu-Gly-NH_2.[2,19] The data for *cyclo*-$(Pro)_3$ have been fitted by first obtaining D_0 from the observed $C^\alpha T_1$ value assuming isotropic motion of the peptide (Table I). The values $\tau_A = \tau_B = 10^{-12}$ s were used based on the assumption that the internal puckering rate is similar to that in free proline and acetyl-Pro-NH_2. Using $\tau_A = \tau_B = 10^{-11}$ s results in a very minor perturbation increasing θ for C^α from 26 to 27°. Alternatively, further reducing τ_A and τ_B to 10^{-10} s renders even $\theta = 45°$ insufficient to fit the observed $C^\gamma T_1$. It appears that in general lifetimes in the range 10^{-11} to 10^{-12} s fit the data adequately whereas longer lifetimes are inconsistent with the data. This point is considered in greater detail below. It is worth noting here that given the differences in β, the θ values obtained for C^β, C^δ, and C^γ are very similar. This result contrasts with expectation based on the calculations of Abraham and McLauchlan[43] which predict a substantially larger range for the C^γ-H motion. Using the notation of ref 43, the ratio of C^γ-H to C^β-H range of motion is given by $\theta/\omega_{23} \simeq 0.60$. In none of the examples fitted using the current model has such a low ratio been obtained. This result suggests that a more realistic model would include additional motion of C^β and C^δ relative to the center of mass. For example, a slight perturbation of the model of Figure 1a would include motion of C^β and C^δ opposing the motion of C^γ. This would lead to relatively small changes in β and be consistent with more similar values of θ for the three ring carbons undergoing internal motion.

As noted in the previous section, nonterminal residues typically exhibit significant differences between T_1^β and T_1^δ and thus undergo ring puckering more like that illustrated in Figure 1b. Although in this model all of the pyrrolidine ring C-H vectors exhibit some internal motion, it has been assumed that the motion of C^α-H is of a sufficiently small amplitude to justify the use of T_1^α for calculating D_0. This is based, in part, on the results given in Figure 5 indicating that for $\theta \lesssim 10°$, the effect of internal jumping on T_1 is within experimental error. Results obtained for acetyl-Pro-NH_2 and for poly(Pro) are summarized in Table I and compared with data from the literature.[5,11] T_1 values for the former have been calculated using $\tau_A = \tau_B = 10^{-12}$ s as well as $\tau_A = 10^{-12}$ s, $\tau_B = 2 \times 10^{-12}$ s. Using the latter values results in small increases in the values of θ. The resulting range of motion is not unreasonable, and we conclude that differences of a factor of 2 between the lifetimes of the two states are possible. A maximal factor of 4 for τ_B/τ_A is possible in order to fit the data although the latter value corresponds to a range $2\theta \simeq 76°$ for C^γ; a factor of 5 is

Table I. Observed and Calculated ^{13}C Relaxation Times for Proline[a]

Peptide or amino acid	Carbon	D_0, s^{-1}	β, deg	θ, deg	NT_1, s Calcd	NT_1, s Obsd	Ref
L-Proline	C^α	1.5×10^{10}			4.2	4.3	11
(pH 6.4)	C^β		70.5	27	7.4	7.5	
	C^γ		90	29	8.7	8.6	
	C^δ		70.5	26	7.1	7.0	
cyclo-(Pro)$_3$	C^α	2.2×10^9			0.62	0.62	18
$CDCl_3$	C^β		70.5	25	1.02	1.01	
	C^γ		90	26	1.16	1.17	
	C^δ		70.5	26	1.07	1.09	
Acetyl-Pro-NH_2	C^α	7.0×10^9	70.5	0	1.96	1.9	11
	C^β		75.4	25	3.31	3.4	
	C^γ		75.4	24	3.20	3.2	
	C^δ		70.5	12	2.20	2.2	
Acetyl-Pro-NH_2[b]	C^α	7.0×10^9	70.5	0	1.96	1.9	11
	C^β		75.4	28	3.42	3.4	
	C^γ		75.4	26	3.18	3.2	
	C^δ		70.5	13	2.21	2.2	
Poly(Pro-Gly)	C^α	4.4×10^8	70.5	0	0.130	0.129	5
	C^β		75.4	25	0.223	0.222	
	C^γ		75.4	26	0.234	0.236	
	C^δ		70.5	16	0.163	0.164	
Bradykinin	C^α	6.0×10^8			0.173	0.176	42
(Pro^7)	C^β		73	24	0.282	0.286	
	C^γ		83	34	0.480	0.480	
	C^δ		70.5	15	0.208	0.208	

[a] In all calculations except acetyl-Pro-NH_2,[b] $\tau_A = \tau_B = 10^{-12}$ s. [b] For acetyl-Pro-NH_2,[b] $\tau_A = 10^{-12}$ s; $\tau_B = 2 \times 10^{-12}$ s.

inconsistent with the data.

For most of the peptides which have been studied, the relaxation behavior is intermediate between that calculated for the two models considered above. Thus, $NT_1^\gamma > NT_1^\beta > NT_1^\delta > NT_1^\alpha$ is approximately valid for most cases. By allowing C^β to move out of the C^α-N-C^δ plane in the opposite sense of the C^γ motion and at a slightly reduced amplitude, the T_1 pattern indicated above can be predicted. Such motion corresponds to values for β intermediate to those of Figures 1a and b. As an approximation, we have used average values of β of 70.5° for C^δ, 73° for C^β, and 83° for C^γ. A fit of the Pro^7 relaxation data measured for bradykinin[45] corresponding to this model is included in Table I. The differences in β between C^γ and C^β are not sufficient to explain the observed T_1 differences so that a greater range for C^γ must be used to fit the data. In general, differences in β for any of the ring carbons corresponding to the models most likely to correspond to the actual motion are minor suggesting that, as a rough approximation, most of the T_1 differences correspond to differences in the range of motion, 2θ. Several alternative models which have been considered appear to be inconsistent with the observed data. Thus, if the motion of C^β and C^γ is in the same sense relative to the C^α-N-C^δ plane, the values for β are considerably smaller and require a much larger range to fit the observed data. In some cases, no fit can be obtained. Finally, we note that the model used for the bradykinin calculations is consistent with theoretical calculations indicating that the most stable proline conformations correspond to puckering of C^β and C^γ in opposite senses with the amplitude greater for C^γ.[28]

The lifetimes of 10^{-12} s used for the calculations in Table I are somewhat shorter than the correlation times for internal motion of the pyrrolidine ring obtained in ref 5 and 6. The correlation times τ_{ring} thus obtained are based on the difference between isotropic rotational correlation times calculated for C^α (τ^α) and for the ring carbons. Using this approach values for τ_{ring} of 4.0×10^{-10} s for poly(Pro-Gly) C^γ (5) and 6.7×10^{-11} s for linear (Gly-L-Pro) C^γ (6) are obtained. A similar calculation for free L-proline using the data of ref 11 gives $\tau_{ring} = 1.1 \times 10^{-11}$ s. Although it is reasonable to expect ring motion to become slower in the larger peptides due to additional steric interactions, the approximate treatment leads to changes in τ_{ring} similar to, and in some cases slightly larger than, the changes in overall motional correlation times. Thus, if NT_1^γ and NT_1^α differ by a factor of 2 as is typical, the value for $\tau_{ring} = \tau^\alpha$. It is more probable that changes in internal ring motion will be smaller than changes in overall correlation time. The data of Table I demonstrate that appropriate restriction of the internal motion is consistent with the observed NT_1 values for a wide range of molecular weight peptides assuming a constant rate of internal motion chosen to be sufficiently rapid to give reasonable NT_1 values for the lowest molecular weight species. The possibility that τ_A and τ_B become longer for the larger molecular weight peptides cannot be excluded; however, it is not required. In general, the shorter internal jump correlation times obtained using the present treatment reflect in part the fact that since the motion is more restricted it must be more rapid to produce an equivalent effect.

Conclusions

The present calculations demonstrate that the observed ^{13}C spin-lattice relaxation data for proline-containing peptides can be described using a model which assumes overall isotropic motion and internal equilibrium between two stable conformations. The stable conformations used correspond well with theoretical calculations as well as with available x-ray data, giving reasonable values for the range of motion of the various C-H vectors. In order to obtain NT_1^γ/NT_1^α ratios of the order of 2 or greater, it is further necessary to have approximate equality between the lifetimes of the two states, a result consistent with interpretations of ^1H-^1H coupling data. This conclusion has significant implications on the conformational interpretations of NMR results. In some peptides, particularly small cyclic proline-containing peptides[6] the observed NT_1^γ/NT_1^α ratio falls significantly below 2, suggesting that the stabilities of the two proline conformations may differ considerably.[6] This is also consistent with theoretical calculations indicating that only one of the two major conformations of the proline ring which represent nearly extreme positions of the γ carbon is appreciably populated in these peptides.[46,47] Alternatively, Haar et al. have interpreted three-bond ^{13}C-^{13}C

coupling constants to indicate a strongly favored endo conformation for proline in thyrotropin releasing factor.[48] Based on the observed ratio $NT_1^{\gamma}/NT_1^{\alpha} = 2.6$,[7] it can be concluded that, subject to the assumptions inherent in the present calculation, the lifetimes of the two conformations differ by substantially less than an order of magnitude, and probably a factor of 2-3 at most.

An interpretation of the internal motion which occurs in proline is useful both for obtaining a more detailed understanding of that motion and because it appears to be strongly coupled to peptide structure. For example, Deslauriers et al.[14] have interpreted differences in proline relaxation times between *cyclo*-(L-Pro-L-Leu) and *cyclo*-(L-Pro-D-Leu) in terms of differences in internal ring motion, noting that overall motional anisotropy differences cannot explain the data. Similarly, Prange et al.[49] have suggested that differences in the stability of cis and trans poly(Pro) are related to differences in steric interactions involving the pyrrolidine rings. The lower $NT_1^{\gamma}/NT_1^{\alpha}$ values for cis Gly-L-Pro compared with trans Gly-L-Pro (6) is consistent with a prediction of greater steric interactions in the former. As more data becomes available, greater correlations between proline ring puckering as monitored by ^{13}C NMR and peptide secondary structure may become apparent. Frequency dependent data should be particularly useful for determining τ_A and τ_B in larger peptides.

Finally, it should be noted that although it is possible to obtain an exact fit of the proline relaxation data using the parameters inherent in the two-state model, the relaxation data do not uniqueiy define a dynamic model. It is quite possible for more than the two states considered here to be significantly populated, thereby complicating the analysis considerably. The important point is that using a two-state model, reasonable values for the various parameters are obtained. If the $NT_1^{\gamma}/NT_1^{\alpha}$ ratio were $\gtrsim 4$, the above class of models would have to be discarded. It is also clear that certain dynamic models, such as rigid anisotropic rotation, can be effectively ruled out as shown by the calculations of Deslauriers et al.,[21,43,49] although such effects may explain some of the differences observed in various peptides.

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