Proline Ring Conformations Corresponding to a Bistable Jump Model from ^{13}C Spin-Lattice Relaxation Times

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Synopsis

A formalism for extracting the conformations of a proline ring based on the bistable jump model of R. E. London [(1978) *J. Am. Chem. Soc.* **100**, 2678–2685] from ^{13}C spin-lattice relaxation times (T_1) is given. The method is such that the relaxation data are only partially used to generate the conformations; these conformations are constrained to satisfy the rest of the relaxation data and to yield acceptable ring geometry. An alternate equation for T_1 of ^{13}C nuclei to that of London is given. The formalism is illustrated through an example.

INTRODUCTION

It is well known that ^{13}C relaxation times (T_1) reflect molecular dynamics, including internal motion, and are thus helpful in suggesting dynamic models for molecular motion.[1] One such example is the "jump" model for the pyrrolidine ring in proline-containing peptides.[2] In this model, the jump occurs between two conformations of the proline ring and hence the C–H dipole-dipole (d-d) vector jumps between two orientations corresponding to the two conformations. The jump of a C–H vector in the above model is characterized by two parameters: (1) β, the angle between the C–H vecto and the effective axis of rotation about which the C–H vector is assumed to rotate in going from one orientation to another; and (2) θ, half the range of angular motion involved in the reorientation of the C–H vector about the effective axis of rotation. Since this is a jump model, the parameters β and θ are not unique, and in principle, an infinite set of them exist that corresponds to the same two orientations of the C–H vector.[2]

Further, β and θ specify a pathway for the motion of the C–H vector, which is unrealistic in a jump model. In addition, in the work carried out using the above model,[2] β and θ are fitted to the T_1's of corresponding carbons individually, without considering the conformational and configurational implications fully and explicitly; whether the fitting maintains an acceptable proline ring geometry and if the conformational fitting of T_1's is self-consistent, have not been considered. As will be shown later, these are the actual constraints built into the present formalism.

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In this paper we suggest the following:

1. A fully equivalent, alternate equation for T_1 to that given by London[2] for a bistable jump model. The new equation (a) explicitly incorporates the direction cosines of the C–H vector in the two states, in an appropriate molecular frame of reference, which facilitates comprehension of the conformational implications of the fitting of T_1's; and (b) is free of parameters β and θ, which suggests a pathway for the motion of C–H vector during a jump and thus overcomes this conceptual difficulty.

2. A method of working out the conformations of the two states of the bistable jump model, wherein (a) the experimental T_1's of only two appropriate ring carbons are fitted to the necessary torsion angles in the two states. These completely specify the conformations in both states, which, in turn, determine the remaining two T_1's. The fit must be consistent with the experimental values of these remaining T_1's. (b) Prior to the fit, four bond lengths, three bond angles, and two torsion angles of the ring are required. In some cases, it may be necessary to invoke extra torsions, but these are ultimately absorbed into the ring torsions, as will be illustrated. The fitting procedure generates the values of the missing parameters, which should be in acceptable ranges. Thus, with the help of the constraints expressed in points (2a) and (2b), this method attempts to determine the conformations of the two states of the dynamic model. We illustrate the method using the model peptide system, *N*-acetyl-L-prolyl-D-alanyl-*N*-methylamide (APAMA).[3]

EXPERIMENTAL

APAMA was synthesized at our laboratory. The purified sample was dissolved in DMSO (d6) which had been dried over molecular sieves. Residual dissolved oxygen was removed by freezing and thawing the sample a few times and by bubbling nitrogen gas for more than ½ h. The sample was sealed under nitrogen.

The ^{13}C T_1's were measured on a Bruker WH-270 FT-NMR spectrometer operating at 67.89 MHz for ^{13}C by the inversion recovery method and using $(180°\text{-}t\text{-}90°\text{-}T\text{-})_n$ pulse sequence. The value of T was at least five times the value of T_1. The concentration of the sample was $0.15M$ (36 mg/mL). We can exclude the possibility of self-association of the peptide molecules at this concentration on the basis of proton T_1 data measured at different concentrations. (For example, the proton T_1 of alanine α-CH in APAMA was 1.50 and 1.48 s at concentrations of 0.15 and $0.075M$, respectively.) Each ^{13}C relaxation curve was the result of 12 points with 1500 accumulations per point. The experimentally obtained T_1 values for the proline ring carbons of APAMA are given in Table I.

TABLE I
Experimental ^{13}C T_1's of Proline Ring Carbons of APAMA and T_1's from Fitting Procedure

Carbon	T_1 from Present Formalism (s)	Experimental[a] $NT_1 = N \times$ Experimental T_1 (s)	Absolute Percentage Deviation (s)
C^α	0.73	0.76 ± 0.01	3.64
C^β	1.25[b]	1.21 ± 0.01	3.29
C^γ	1.63[b]	1.62 ± 0.02	0.60
C^δ	0.96	1.00 ± 0.02	4.53

[a] Standard deviations given are from least-squares fitting.
[b] Values obtained from the fit.

RESULTS AND DISCUSSION

Theory

The equation for T_1 for the proton decoupled ^{13}C spectra in the case of relaxation by dipole–dipole mechanism is

$$\frac{1}{T_1^C} = \frac{(\gamma_C \gamma_H \hbar)^2}{r_{CH}^6} \frac{1}{2} (1/2 + 1)[1/12 J_0(\omega_C - \omega_H) + 3/2 J_1(\omega_C) + 3/4 J_2(\omega_C - \omega_H)] \quad (1)$$

where $J_h(\omega)$ are the spectral densities, γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H nuclei, respectively, and r_{CH} is the C–H internuclear distance. The spectral densities are the Fourier transforms of the orientational correlation function, $G_h(\tau)$, pertaining to reorientation of the C–H (d-d) vector given by[4]

$$J_h(\omega) = \int_{-\infty}^{\infty} G_h(\tau) e^{-i\omega\tau} \, d\tau \quad (2)$$

Woessner[5] has treated the case of an ellipsoidal molecule undergoing rotational Brownian motion in which the d-d vector independently undergoes internal motion. When the overall motion of the molecule is isotropic (i.e., when all the three rotational diffusion constants[5] are equal), the correlation function given by Woessner[5] simplifies to

$$G_h(\tau) = 1/2 K_h e^{-|\tau|/\tau_0} \langle 3(l'l'' + m'm'' + n'n'')^2 - 1 \rangle_{av} \quad (3)$$

where

$$K_0 = 4/5, \quad K_1 = 2/15, \quad K_2 = 8/15 \quad (4)$$

and $\langle \ \rangle_{av}$ denotes averaging over internal motion and τ_0 is the isotropic correlation time defined by Woessner[5]; l', m', and n' are the direction cosines of the d-d vector at a time t; and l'', m'', and n'' are those at a time $t + \tau$, with respect to a molecular reference frame undergoing isotropic rotational Brownian motion.

In the bistable jump model,[2] any d-d vector has only two orientations, corresponding to states A and B. Let l_A, m_A, and n_A be the direction cosines of a d-d vector in state A, and l_B, m_B, and n_B be the direction cosines of the same d-d vector in state B.

Let $P(A,B;\tau)$ denote the probability that the system (the proline ring) is in state A at time t and state B at time $t + \tau$. Similarly, we have three more probabilities: $P(B,A;\tau)$, $P(A,A;\tau)$, and $P(B,B;\tau)$.

Using these definitions, Eq. (3) can be put into the form

$$G_h(\tau) = \tfrac{1}{2}K_h e^{-|\tau|/\tau_0}\,[\{3(l_A l_B + m_A m_B + n_A n_B)^2 - 1\} \times \{P(A,B;\tau) + P(B,A;\tau)\} + \{3(l_A^2 + m_A^2 + n_A^2)^2 - 1\} \times P(A,A;\tau) + \{3(l_B^2 + m_B^2 + n_B^2)^2 - 1\}\,P(B,B;\tau)] \quad (5)$$

with

$$Z = 3(l_A l_B + m_A m_B + n_A n_B)^2 - 1 \quad (6)$$

Equation (5) now simplifies to

$$G_h(\tau) = \tfrac{1}{2}K_h e^{-|\tau|/\tau_0}[Z\{P(A,B;\tau) + P(B,A;\tau)\} + 2\{P(A,A;\tau) + P(B,B;\tau)\}] \quad (7)$$

Using the probability functions evaluated by London,[2] Eq. (7) assumes the form

$$G_h(\tau) = \frac{K_h}{2}\frac{1}{(\tau_A + \tau_B)^2}\,[(\tau_A^2 + \tau_B^2)(Z + 2)e^{-|\tau|/\tau_0} + \{4\tau_A\tau_B - Z(\tau_A^2 + \tau_B^2)\}e^{-|\tau|/\tau_{eff}}] \quad (8)$$

where $\tau_{eff}^{-1} = \tau_C^{-1} + \tau_0^{-1}$ and $\tau_C^{-1} = \tau_A^{-1} + \tau_B^{-1}$.

Substituting Eq. (8) in Eq. (2), we get

$$J_h(\omega) = K_h J(\omega) \quad (9)$$

where

$$J(\omega) = \frac{1}{(\tau_A + \tau_B)^2}\left[(\tau_A^2 + \tau_B^2)(Z + 2)\frac{\tau_0}{1 + \omega^2\tau_0^2} + \{4\tau_A\tau_B - Z(\tau_A^2 + \tau_B^2)\}\frac{\tau_{eff}}{1 + \omega^2\tau_{eff}^2}\right] \quad (10)$$

Substituting Eqs. (4) and (10) in Eq. (1), we get

$$\frac{1}{T_1^C} = \frac{(\gamma_C\gamma_H\hbar)^2}{r_{CH}^6}\frac{1}{20}\,[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (11)$$

Under extreme narrowing conditions ($\omega\tau_0 \ll 1$), the $J(\omega)$ becomes frequency independent:

$$J(\omega) = J = \frac{1}{(\tau_A + \tau_B)^2}\,[(\tau_A^2 + \tau_B^2)(Z + 2)\tau_0 + \{4\tau_A\tau_B - Z(\tau_A^2 + \tau_B^2)\}\,\tau_{eff}]$$

$$\frac{1}{T_1^C} = \frac{(\gamma_C\gamma_H\hbar)^2}{r_{CH}^6}\frac{J}{2}$$

when

$$\tau_A = \tau_B$$

$$\frac{1}{T_1^C} = \frac{(\gamma_C \gamma_H \hbar)^2}{r_{CH}^6} \frac{1}{4} [(2 + Z)\tau_0 + (2 - Z)\tau_{eff}] \tag{12}$$

Prediction of Conformations of the Two States of the Jump Model

We now illustrate the scheme of fitting T_1 that would yield the conformations of states A and B of the proline ring, as mentioned in the Introduction, for a model system such as APAMA. We assume (a) isotropic rotational Brownian motion for overall motion of the molecule[2] and (b) equal lifetimes for states A and B. (London has pointed out that the most appropriate values for lifetimes of the states of the bistable jump model proposed by him are of the order of 10^{-12} s.) This seems reasonable since the theoretical conformational energy calculations[6-8] indicate that in most cases, the proline ring is essentially bistable, with nearly equal energies. Since we have assumed isotropic motion for the overall motion of the molecule, τ_0 can be derived from the T_1 of a backbone carbon,[2] which, in our case, is C^α of the alanine residue. Using the equation

$$\frac{1}{(T_1)_{\text{Ala } C^\alpha}} = \frac{(\gamma_C \gamma_H \hbar)^2}{r_{CH}^6} \tau_0$$

the value of τ_0 obtained (0.68×10^{-11} s) satisfies the condition for an extreme narrowing limit; hence the use of Eq. (12) is justified.

In the case of overall isotropic motion, the T_1's are independent of any molecular reference frame chosen with respect to which direction cosines of the C–H (d-d) vector are determined. This is because, for isotropic rotational Brownian motion, all three rotational diffusion coefficients are equal[5]; hence, any arbitrary reference frame, attached rigidly to the molecule, can be chosen in which the coordinates of the ring atoms can be calculated. The notation employed for torsional angles of the ring is illustrated in Fig. 1. The coordinates of hydrogens attached to the ring carbons can be calculated by fixing the torsions involving hydrogens at ±120° to

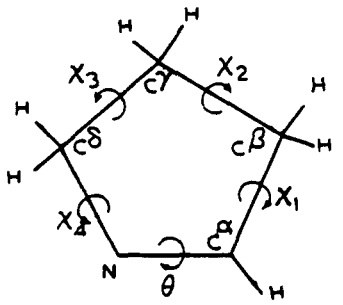

Fig. 1. Proline ring with the conventional ring torsions.

the appropriate ring torsion. For example, to fix the coordinates of one of the hydrogens of C^{γ} atom (see Fig. 1) in a given reference frame, for states A and B, we can use

$$\begin{aligned} \chi^{A}(C^{\alpha}\text{–}C^{\beta}\text{–}C^{\gamma}\text{–}H) &= \chi_2^{A} + 120 \\ \chi^{B}(C^{\alpha}\text{–}C^{\beta}\text{–}C^{\gamma}\text{–}H) &= \chi_2^{B} + 120 \end{aligned} \tag{13}$$

Here, the superscripts on the torsions correspond to the state. The two hydrogens, attached to each of the carbons C^{β}, C^{γ}, and C^{δ} are considered to be identical, hence it is sufficient to consider the reorientation of the d-d vector, involving any one of them, in determining the T_1 of the corresponding carbon. Referring to Fig. 1, the molecular frame of reference was chosen with N as the origin, N-C^{α} as the x-axis, and the direction found by rotating N-C^{α} by 90° (anticlockwise), in the plane of the paper (mean plane of the ring) as the y-axis. The z-axis is automatically fixed for the right-handed Cartesian system chosen.

Now we proceed to illustrate that by fitting the T_1's of two appropriate carbons, conformations of both states A and B can be obtained that are consistent with the other T_1's and the ring geometry.

The two carbon atoms chosen for this purpose were C^{α} and C^{δ}. From Eq. (12) and those similar to Eq. (13), it is clear that T_1 of C^{α} depends on θ^{A} and θ^{B}, while that of C^{δ} depends on χ_4^{A} and χ_4^{B}. We fix

$$\begin{aligned} \theta^{B} &= -\theta^{A} \\ \chi_4^{B} &= -\chi_4^{A} \end{aligned} \tag{14}$$

As already stated, we assume that two states, A and B, are of near-equal energy.[6–8] Wherever required, standard values of bond lengths and bond angles were used from the x-ray crystal structure studies of proline rings of proline-containing peptides.[6,9] Because θ and χ_4 are independent, the fitting of T_1's of C^{α} and C^{δ} [in conjunction with Eq. (14)] does not specify the sign relationship between θ and χ_4, in a particular state. It is then a matter of choice because once a particular relationship is chosen in one state [from Eq. (14)], it follows that the same sign relationship is preserved in the other state. These values of θ^{A}, θ^{B}, χ_4^{A}, and χ_4^{B}, chosen to fit the T_1's

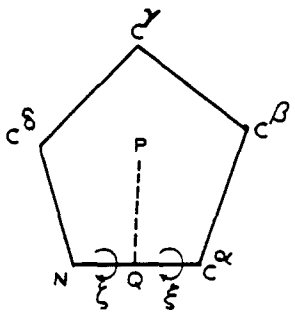

Fig. 2. Two torsions introduced in the fitting procedure.

TABLE II
Torsions of Proline Ring of APAMA in the Two Conformations Together with X-Ray Data

Torsion	From the Present Formalism		From X-Ray Structure (deg)
	Conformation A (deg)	Conformation B (deg)	
ξ	9	−9	—
ζ	−5	5	—
θ	4	−4	−2.5
χ_1	−26.6[a]	26.6[a]	22.0
χ_2	39.1	−39.1	−33.4
χ_3	−35.8[a]	35.8[a]	30.8
χ_4	21.0	−21.0	−18.3

[a] Values obtained from the fit.

of C^α and C^δ, determine the coordinates of C^β and C^γ carbons in both states. At this stage, coordinates of all the ring atoms are known in both states. Now, by using equations similar to Eq. (13) (to derive the direction cosines of the C^γ–H and C^β–H vectors in both states, by using χ_2 and χ_1, which are generated from the fit) and Eq. (12), we can determine the remaining T_1's, viz., that of C^γ and C^β. It may be noted that during the entire operation, C^α, N, and C^δ were not moved with respect to the mean plane of the ring. An equivalent procedure to the one above is to fit the T_1's of C^δ and C^γ, or those of C^α and C^β, instead of C^α and C^γ. However, in the procedure outlined above, where we started by fitting T_1's of C^α and C^δ, we need (a) all the ring bond lengths, except C^β–C^γ, and (b) the bond angles C^δ–N–C^α, N–C^α–C^β, and C^γ–C^δ–N. These, along with the two torsions, θ and χ_4, from the fit, determine the remaining ring parameters in both states.

When C^δ was held stationary, with respect to the mean plane, as mentioned earlier, for any sign relationship chosen between θ and χ_4, the values specified for θ^A, θ^B, χ_4^A, and χ_4^B to fit the T_1's of C^α and C^δ were inconsistent with the experimental values for the two remaining T_1's. But the values derived from the above trials indicated (a) C^δ should also be moved with respect to the mean plane of the ring, (b) a sign relationship, wherein θ and χ_4 have the same sign, and (c) that the puckering of C^β and C^δ would be in the same sense with respect to the mean plane in a state. This requires two

TABLE III
Bond Lengths and Angles of the Proline Ring of APAMA

Bond Lengths (Å)		Bond Angles (deg)	
N–C^α	1.472	C^δ–N–C^α	112.0
C^α–C^β	1.519	N–C^α–C^β	102.5
C^β–C^γ	1.555[a]	C^α–C^β–C^γ	104.7[a]
C^γ–C^δ	1.497	C^β–C^γ–C^δ	100.9[a]
C^δ–N	1.462	C^γ–C^δ–N	104.1

[a] Values obtained from the fit.

more torsions to be invoked, as shown in Fig. 2. Here we have chosen Q as the midpoint of the bond N–C^{α}, PQ $\perp$ NC, and PNC^{α} as the mean plane; the reference frame already chosen is retained. It is clear from Fig. 2 that the T_1 of C^{α} is determined by ξ^A and ξ^B; and T_1 of C^{δ} by ζ^A, ζ^B, χ_4^A, and χ_4^B. As we did previously with θ and χ_4, we fix

$$\begin{aligned} \xi^A &= -\xi^B \\ \zeta^A &= -\zeta^B \\ \chi_4^A &= -\chi_4^B \end{aligned} \tag{15}$$

It can be seen that ξ and ζ determine θ in a state. As already mentioned, C^{δ} and C^{β} should have the same sense of puckering (with respect to the mean plane), and hence, ξ and ζ were chosen to be of opposite sign in a state [the same relationship is preserved in the other state also by virtue of Eq. (15)]. χ_4 and ξ were chosen to be of the same sign. ξ^A, ζ^A, and χ_4^A were all iterated over appropriate ranges to get a fit corresponding to a maximum deviation of less than 5% from the experimental values of T_1. This also yielded acceptable values for the bond length and bond angles generated from the fit. The results are tabulated in Tables I–III. Another point illustrating the geometrical consistency of the method is that since the same values were given for respective bond lengths and bond angles in both the states, the fit remained the same in both conformations A and B; similarly, the respective torsions in states A and B derived from the fit were of equal magnitude and opposite sign.

We thus conclude that ^{13}C spin-lattice relaxation data can be used to yield conformations of the proline ring in the two states corresponding to a bistable jump model. The conformations thus generated can be compared with those that can be obtained from an analysis of ^1H-^1H coupling constant data and x-ray structure analysis. But in many proline-containing compounds the analysis of the proton nmr spectra are very complicated, and no useful information can be obtained regarding conformations. Even in such cases, the proton noise-decoupled ^{13}C spectra are usually quite simple, and hence our method based on ^{13}C T_1 data analysis might prove to be very useful in deriving conformational information of proline-containing peptides.

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