MAGNETIC RELAXATION ANALYSIS OF DYNAMIC PROCESSES IN MACROMOLECULES IN THE PICO- TO MICROSECOND RANGE

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ABSTRACT A formalism based on the theory of Markov processes and suitable for the analysis of multiple internal motions in macromolecules is presented. Computer calculations of specific motional models for ^{13}C nuclear magnetic resonance (NMR) relaxation, treated as special cases of the proposed formalism, demonstrate the potential of this approach for discriminating between different motional models on the basis of NMR relaxation data.

INTRODUCTION

It has been known for some time (1, 2) that nuclear magnetic resonance (NMR) relaxation data on macromolecular systems contain, at least in principle, a wealth of information on dynamic processes, including internal motions, association and dissociation rates for complexes, rates of energy transfer, etc. In recent years, experimental relaxation measurements on various macromolecules, using various nuclei (^{1}H, ^{13}C, ^{31}P, ^{9}F), have begun appearing in the literature in ever increasing numbers (3–10). In all cases reported so far the measurements were interpreted by assuming a specific relaxation model, a correlation time for the overall motion of the macromolecule, and one degree of internal motional freedom. The most widely used model is that calculated by Woessner (11, 12) for anisotropic diffusion or a methyl group rotation on a rigid sphere and the extension of this model to treat sequential rotations in a hydrocarbon chain (specifically that of a membrane lipid) developed by Levine et al. (13, 14). Other models dealing with correlated motions and a limited degree of internal rotation have also been treated (15–18). Interpretation of relaxation data in terms of specific models is undoubtedly instructive if a suitable model can be found and if the required calculation is tractable. The procedure is, however, not without its dangers. In the absence of a general theory which permits the comparison of different models, the inapplicability of an available model may not be easily recognized. Nor can the possibility be tested that the data may be compatible with more than one model. Yet no general theoretical effort has been made to deal with these problems.

We have recently pointed out that a general formalism for the analysis of NMR relaxation measurements on systems with multiple degrees of freedom can be based on

the theory of Markov processes and have derived a general equation for the analysis of data on systems undergoing an arbitrary number of motions of an arbitrary nature (19). In this communication we report a comparison between different relaxation models calculated within the framework of this formalism.

THEORETICAL

The calculation of relaxation parameters is in essence a calculation of spectral density functions $J_{F_j}(\omega)$ defined as

$$F_{F_j}(\omega) = \int_{-\infty}^{\infty} e^{i\omega\tau} \; \overline{F_j(t)F_j^*(t + \tau)}d\tau \tag{1}$$

for a dynamic variable F_j, with an autocorrelation function $\overline{F_j(t)F_j^*(t + \tau)}$ (1, 2). F_j may be, as is usual, taken to be the time-dependent part of an appropriate interaction Hamiltonian (1) or used to denote the total Hamiltonian for a given relaxation mechanism.

As already pointed out (19), the sole additional assumption necessary to develop a general formalism for the analysis of relaxation phenomena in systems with multiple degrees of freedom is that any motion contributing to relaxation is a Markov process. The simple case of rotational diffusion or exchange as a Markov process contributing to magnetic relaxation has been discussed by Kubo (20) and others (21, 22). Our formulation may be regarded as a generalization of these earlier treatments. It should be emphasized that the assumption of a general Markov process is far less restrictive than the common use of Markov statistics describing specific models. The assumption in its general form implies no more than that a probability can be assigned to each step in the process and that the system has no memory. The basic relationships that can be derived from it cover a wide range of specific models, and provide a general framework for systematic comparisons among them. As previously shown (19) for a system undergoing N independent motions, $J_{F_j}(\omega)$ is given by:

$$J_{F_j}(\omega) = \sum_{l_1 \dots l_N} \frac{2| <F_j, \prod_{k=1}^{N} {}^k\phi_{lk}^* >|^2 \; \sum_{k=1}^{N} \lambda_{lk}^k}{\left\{ \sum_{k=1}^{N} \lambda_{lk}^k \right\}^2 + \omega^2}, \tag{2}$$

where λ_n, ϕ_n are eigenvalues and ϕ_n, ϕ_n^* the eigenfunctions of the transition operator Ω so that

$$\Omega\phi_n = \lambda_n\phi_n, \tag{3}$$

and similarly for Ω^*, with $\phi_n, \phi_n^* = 1$. The general properties of the transition operator Ω are discussed elsewhere (19, 23). Of more immediate importance is

the physical significance of λ and ϕ. If F_j is a dynamic variable, ϕ_n are the amplitude parameters and λ_n the rate parameters of the motion, $\lambda_n = 1/\tau_n$, where τ_n is the correlation time for the nth individual motion. Eq. 2 thus allows us to calculate amplitudes and frequencies of any combination of internal motions or other processes affecting relaxation from a given set of relaxation data or, conversely, to predict a set of relaxation parameters from any assumed combination of processes. Although the equation strictly applies to independent processes, the condition of independence is not as restrictive as it may appear at first. If two or more processes are weakly coupled, it is possible to take this into account using standard perturbation theory, assuming

$$\Omega = \Omega_1 + \Omega_2 + \epsilon\Omega_{12}, \text{ where } \epsilon \text{ is small,} \tag{4}$$

and obtaining the solution in terms of Bohr expansions for eigenfunctions and eigenvectors (24).

If, on the other hand, the processes are strongly coupled, Ω for the combined process will not be diagonalizable in the separate components Ω_1 and Ω_2. Strongly coupled processes will therefore appear in this formalism as a single process, which may nevertheless be a reasonable approximation of physical reality. It is worth noting that Eq. 2 may be applied in one of two ways. Taking internal motions as an example, if the character of each motion is precisely known, the eigenvector ϕ_n can be derived from the geometric constraints of the motion, leaving λ_n as the sole unknown. In this form, Eq. 2 is a straightforward generalization of existing relaxation theory. Alternatively, the formalism defined by Eq. 3 and contained in Eq. 2 allows both the eigenvectors ϕ_n and eigenvalues λ_n to be treated as unknowns. Given a sufficient number of experimental measurements, Eq. 2 can thus be solved for both the amplitudes and the frequencies of each motion. If n motions are involved, $2^n + n - 1$ measurements will be necessary to obtain a self-consistent solution of Eq. 2. Considering that a single set of ϕ_n and λ_n should account for the measured values of all relaxation parameters (T_1, T_2, $T_{1\rho}$, NOE) at all frequencies and for all nuclei, the problem is of manageable size, especially in systems in which cross-correlation and spin-diffusion effects may be neglected or accounted for. The calculation of eigenfunctions and eigenvalues to interpret relaxation data will be a practical necessity in many cases of interest in macromolecules. In proteins where one may encounter overall rotation (diffusion), domain rotation, segmental flexibility, side chain motions, and relaxation by ligand binding, one is not likely to be able to rigorously specify the range of each motion for each observable group. This use of Eq. 2 will be discussed separately. The discussion in this paper is confined to the simpler problem of comparing different motional models, given that the relaxation mechanism is known.

MODEL CALCULATIONS

A crucial question for deciding whether an analysis of the proposed type can lead to physically interesting insight is whether it is possible to distinguish between different

kinds of motions (and combinations of motions), given a set of relaxation measurements. To answer this question we have carried out a series of computer calculations of relaxation rates according to Eq. 2, assuming a specific relaxation mechanism and varying (a) the amplitude, (b) the frequency of each motion, and (c) the number of motions contributing to relaxation. Our initial choice of motional models was determined by what we believe to be the simplest reasonable approximations to the actual internal motions occurring in proteins. T_1, T_2 and NOE for $^{13}C - {^1H}$ and $^1H - {^1H}$ dipolar interactions (at 100 and 360 MHz ^1H frequency) have been calculated from standard expressions (1). Calculations for $^{13}C - {^1H}$ relaxation will serve as an illustration here. Application to proton relaxation will be covered elsewhere.[1]

For $^{13}C - {^1H}$ relaxation:

$$\frac{1}{T_1} = K_1 R^{-6}\{J(\omega_H - \omega_C) + 3J(\omega_c) + 6J(\omega_H + \omega_C)\}$$

$$\frac{1}{\pi T_2} = K_2 R^{-6}\{4J(0) + 3J(\omega_C) + J(\omega_H - \omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)\}$$

$$NOE = 1 + \frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)} \cdot \frac{\omega_H}{\omega_C}$$

where $R = 1.09$ Å is the $^{13}C - {^1H}$ distance, K_1 and K_2 are proportionality constants, and $J(\omega)$ the spectral density functions.

The internal motions assumed in the calculation of $J(\omega)$ have been classified as follows and used singly or in combination. (i) Three-state Woessner rotation: This well-known model is typical of a methyl group in a side chain, jumping between its three low energy states. (ii) Two-state wobble: A jump between two different sites of varying amplitude, typical of a tetrahedral side-chain carbon in which two of the three low-energy states have a high-energy barrier due to steric hindrance, a cis-trans isomerization of proline, or a segment of a polypeptide chain constrained on a hinge. (iii) Three-state wobble: A restricted jump between three sites of varying amplitude, as may occur where segmental flexibility is allowed.

Eight specific models have been compared thus far. (a) Isotropic diffusion; (b) semi-anisotropic diffusion (ellipsoid of revolution), which is formally equivalent to (c); (c) isotropic diffusion with internal continuous Woessner rotation; (d) isotropic diffusion with internal three-state Woessner rotation; (e) isotropic diffusion with internal two-state wobble; (f) isotropic diffusion with internal three-state wobble on a cone; (g) isotropic diffusion with an internal four-state (or n-state) wobble; (h) isotropic diffusion with internal three-state Woessner rotation plus a two-state wobble. To illustrate the calculation we use case f. Calculations for cases a–c have been published in detail by previous workers and are included here only for purposes of comparison. Calculations for cases d and e are equally simple. For case f we have

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[1] King, R., and O. Jardetzky. Manuscript in preparation.

$$J(\omega) = \frac{2}{5} \sum_{n=-2}^{2} \left\{ \frac{\frac{1}{\tau_0} | <\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_1^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0}\right)^2} \right.$$

$$+ \frac{\left(\frac{1}{\tau_0} + 2\mu\right)| <\tilde{Y}_n | \phi_2^{(w)} \cdot \phi_1^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0} + 2\mu\right)^2}$$

$$+ \frac{\left(\frac{1}{\tau_0} + 3\eta\right) | <\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_2^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0} + 3\eta\right)^2}$$

$$+ \frac{\left(\frac{1}{\tau_0} + 2\mu + 3\eta\right)| <\tilde{Y}_n | \phi_2^{(w)} \cdot \phi_2^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0} + 2\mu + 3\eta\right)^2}$$

$$+ \frac{\left(\frac{1}{\tau_0} + 3\eta\right) | <\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_3^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0} + 3\eta\right)^2}$$

$$\left. + \frac{\left(\frac{1}{\tau_0} + 2\mu + 3\eta\right) | <\tilde{Y}_n | \phi_2^{(w)} \cdot \phi_3^{(j)} > |^2}{\omega^2 + \left(\frac{1}{\tau_0} + 2\mu + 3\eta\right)^2} \right\}$$

with the inner products:

$$<\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_1^{(j)} > = \frac{1}{6} \sum_{i=1}^{2} \sum_{j=1}^{3} \tilde{Y}_n(\theta_{ij}, \varphi_{ij})$$

$$<\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_2^{(j)} > = \frac{1}{6} \sum_{i=1}^{2} \frac{3}{2} \{\tilde{Y}_n(\theta_{i2}, \varphi_{i2}) - \tilde{Y}_n(\theta_{i3}, \varphi_{i3}')\}$$

$$<\tilde{Y}_n | \phi_1^{(w)} \cdot \phi_3^{(j)} > = \frac{1}{6} \sum_{i=1}^{2} \frac{1}{2} \{\tilde{Y}_n(\theta_{i2}, \varphi_{i2}) + \tilde{Y}_n(\theta_{i3}, \varphi_{i3}) - \tilde{Y}_n(\theta_{i1}, \varphi_{i1})\}$$

$$<\tilde{Y}_n | \phi_2^{(w)} \cdot \phi_1^{(j)} > = \frac{1}{6} \sum_{j=1}^{3} \{\tilde{Y}_n(\theta_{1j}, \varphi_{1j}) - \tilde{Y}_n(\theta_{2j}, \varphi_{2j})\}$$

$$<\tilde{Y}_n | \phi_2^{(w)} \cdot \phi_2^{(j)} > = \frac{1}{6} \cdot \frac{3}{2} \{\tilde{Y}_n(\theta_{12}, \varphi_{12}) - \tilde{Y}_n(\theta_{13}, \varphi_{13})$$

$$- \tilde{Y}_n(\theta_{22}, \varphi_{22}) + \tilde{Y}_n(\theta_{23}, \varphi_{23})\}$$

$$\langle \tilde{\mathbf{Y}}_n \mid \phi_2^{(w)} \cdot \phi_3^{(j)} \rangle = \frac{1}{6} \cdot \frac{1}{2} \{ -2\tilde{Y}_n(\theta_{11}, \varphi_{11}) + \tilde{Y}_n(\theta_{12}, \varphi_{12})$$

$$+ \tilde{Y}_n(\theta_{13}, \varphi_{13}) + 2\tilde{Y}_n(\theta_{21}, \varphi_{21}) - \tilde{Y}_n(\theta_{22}, \varphi_{22}) - \tilde{Y}_n(\theta_{23}, \varphi_{23}) \}$$

where $\lambda_0 = 1/\tau_0$ is the rate of isotropic diffusion, μ the wobble (w) rate, η the jump (j) rate, and $\tilde{Y}_n$ (for a C − H vector) the nth second-order normalized spherical harmonic in the coordinate system $\tilde{Y}_n = \tilde{Y}_n \exp(\text{in } \varphi)$ describing overall rotational diffusion

$$\tilde{Y}_0 = \left(\frac{5}{4}\right)^{1/2} (1 - 3\cos^2\theta),$$

$$\tilde{Y}_{1,-1} = \left(\frac{15}{2}\right)^{1/2} \cos\theta \sin\theta,$$

$$\tilde{Y}_{2,-2} = -\left(\frac{15}{8}\right)^{1/2} \sin^2\theta.$$

$\tilde{\mathbf{Y}}_n$ is the vector obtained by computing $\tilde{Y}_n$ for the C − H vector at each of the wobble and jump states and $\langle\ \rangle$ is the inner product weighted by the equilibrium probabilities of the states in the Markov processes.

The transition matrices, the equilibrium probability vector ρ, and the eigenvalues and eigenvectors for the internal motions are given by:

Two-state wobble

	1	2	ρ	λ	φ	
1	$-\mu_{12}$	μ_{12}	$\mu_{21}/\mu_{12} + \mu_{21}$	0	$\sqrt{\dfrac{\mu_{12}}{\mu_{21}}}$	$-\sqrt{\dfrac{\mu_{21}}{\mu_{12}}}$
2	μ_{21}	$-\mu_{21}$	$\mu_{12}/\mu_{12} + \mu_{21}$	$\mu_{12} + \mu_{21}$		

θ and φ arbitrary

Three-state Woessner

	1	2	3	ρ	λ	φ		
1	-2η	η	η	$1/3$	0	1	1	1
2	η	-2η	η	$1/3$	3η	0	$\sqrt{3}/2$	$-\sqrt{3}/2$
3	η	η	-2η	$1/3$	3η	$-\sqrt{2}$	$(\sqrt{2})^{-1}$	$(\sqrt{2})^{-1}$

	1	2	3	
	$71°$	$71°$	$71°$	θ
	0	$120°$	$240°$	φ

with θ and φ obtainable by straightforward combination. The results of calculations for different motional models can be compared on plots of pairs of predicted relaxation
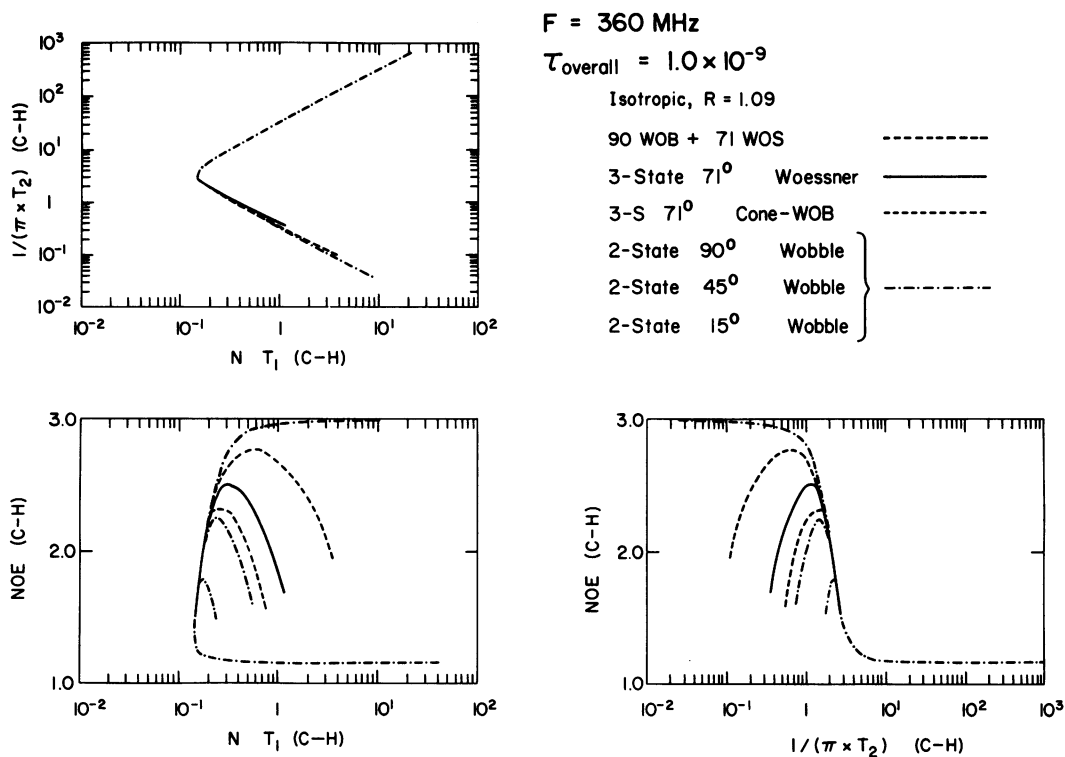
FIGURE 1 ^{13}C NMR relaxation phase diagrams for different models of internal motion in a macromolecule with a rotational diffusion constant $\tau = 10^{-9}$.

parameters, i.e., T_1 vs. $1/\pi\ T_2$, T_1 vs. NOE, NOE vs. $1/\pi\ T_2$, etc. In each plot as shown in Figs. 1–3, τ_0 is constant, τ_I and τ_{II} for internal motion are variable. We find this presentation particularly useful and refer to it as a "relaxation phase diagram." It clearly defines the extent to which different combinations of assumed motions predict different combinations of observed relaxation parameters. This is important, because a unique interpretation of the data is not possible if different models account for them equally well. Furthermore, the phase diagrams emphasize that for a meaningful interpretation several relaxation parameters at several spectrometer frequencies must be accounted for by the same model. Inconsistencies between predicted and observed values for even one of the relaxation measurements should be taken as prima facie evidence that the chosen model does not apply.

Several general features emerge from the examination of diagrams such as those shown in Figs. 1–3.

(a) If the overall rotational correlation time is relatively low, discrimination between different models is best when the correlation times for internal motions are short. It becomes progressively more difficult as the correlation times for internal motions increase.
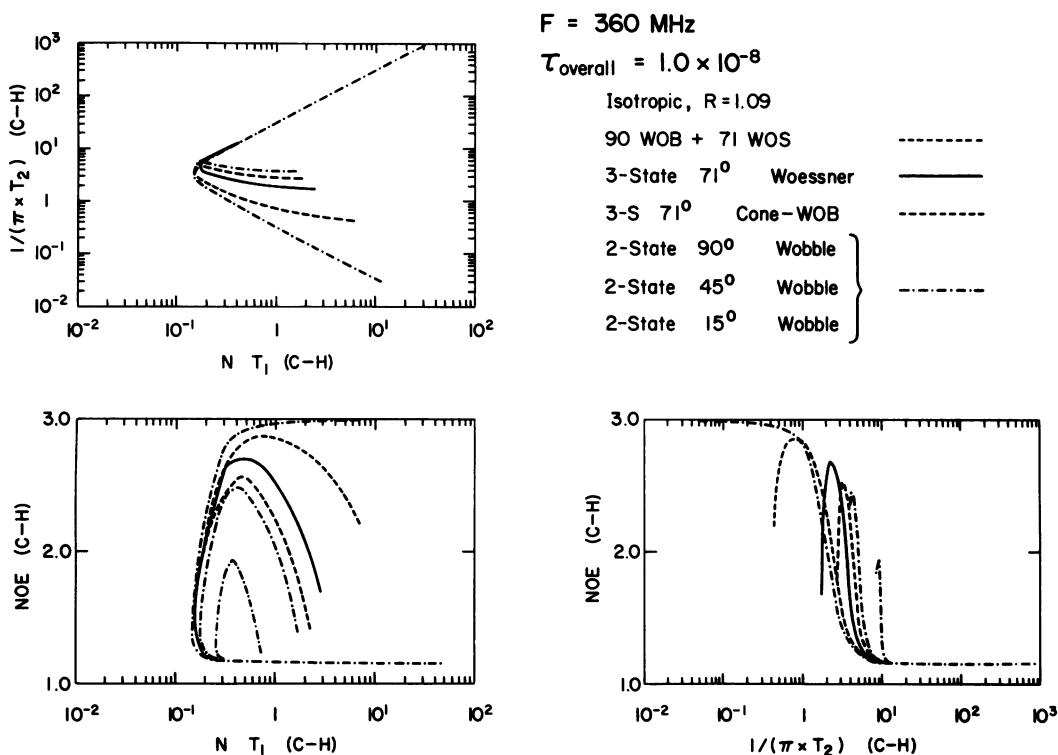
FIGURE 2 ^{13}C NMR relaxation phase diagrams for different models of internal motion in a macromolecule with a rotational diffusion constant $\tau = 10^{-8}$.

(*b*) For a given internal correlation time, motions of different amplitude can be distinguished if the amplitudes are relatively small. At large amplitudes, different motions become relatively indistinguishable.

(*c*) Discrimination between the effects of different motions is possible when either their frequencies or their amplitudes are very different. If a slower motion is superimposed in a rapid motion, discrimination from a model with a single correlation time intermediate between the two is generally possible. If two rapid motions of large amplitude are superimposed, no distinction can be made from a model with a single short correlation time. In the extreme narrowing limit, some discrimination between different motional models is still possible by considering the NOE, but not from T_1 and T_2 alone.

(*d*) If the overall rotational correlation is short, contributions of internal motions become undetectable or indistinguishable from each other.

(*e*) Motions of low amplitude at a given τ are readily discriminated by their T_2 values, but their frequencies can only be determined by a comparison of NOE and T_1 or NOE and T_2 data.

On this basis it is possible to define the general requirements for the interpretation of
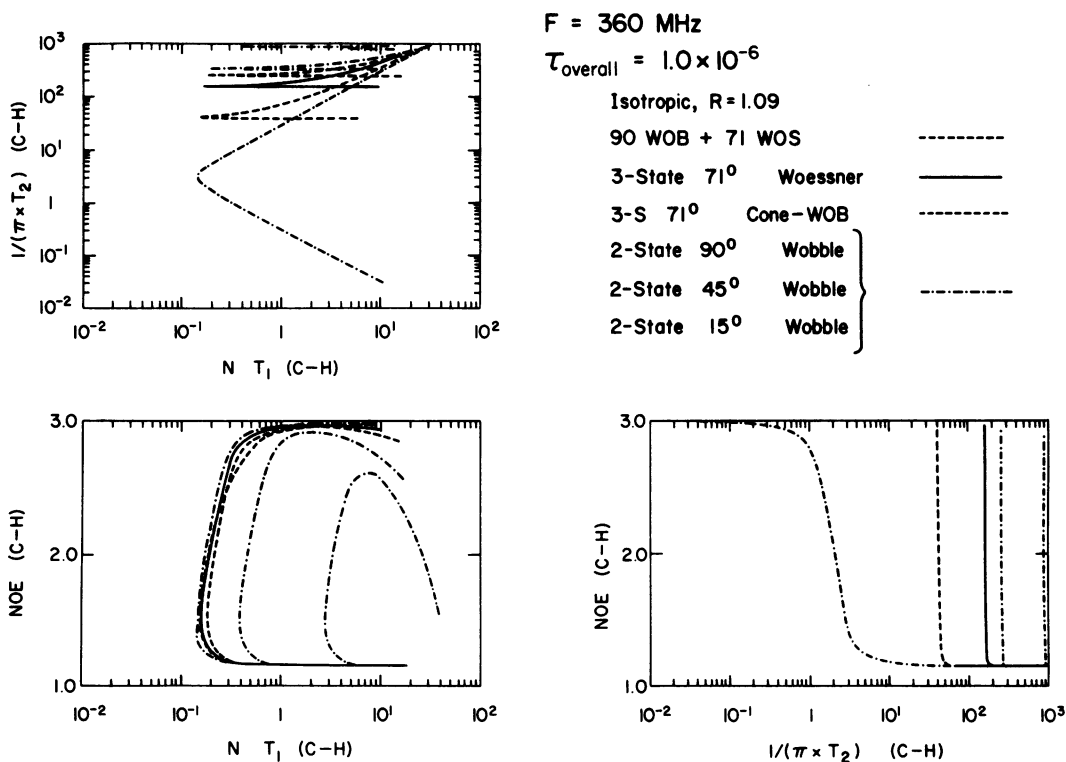
110

FIGURE 3 ^{13}C NMR relaxation phase diagrams for different models of internal motion in a macromolecule with a rotational diffusion constant $\tau = 10^{-6}$.

relaxation data on systems with multiple motions: (*a*) Data for all three, T_1, T_2, and NOE, measurements are necessary to provide a check of internal consistency; (*b*) the dominant relaxation mechanism must be established; (*c*) if the system has an overall motion corresponding to the rotation of a coordinate system originating in the center of mass, the frequency, amplitude, and symmetry of this motion should be determined by an independent method. (*d*) The experimental values for T_1, T_2, and NOE can be checked against a variety of motional models in the diagrams plotted for the appropriate parameters of the overall motion. (*e*) For existing models the values of τ_0 and τ_1 can be read off a conventional T_1 vs. τ plot for the chosen model. Fig. 4 is a previously unpublished sample plot for our case (*f*).

In some cases it will be found that the measurements correspond to a single specific model. In others, several models will fit the data. It might be noted that the probability of finding some model that fits all the data is very high. The probability of finding more than one model is high for those cases where the motional amplitudes are large and the overall τ is short. This is clearly a case in which a unique interpretation of relaxation data is impossible in principle. This is not to say, however, that the data provide no information about the system. It should be possible to define a class of
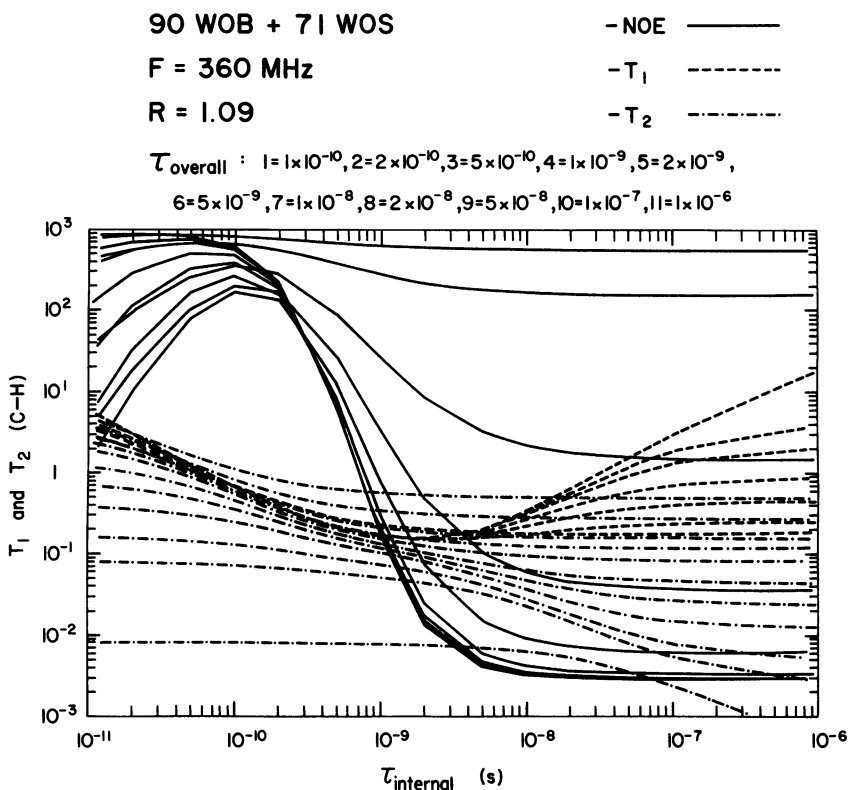
90 WOB + 71 WOS   −NOE ————

F = 360 MHz   −T_1 --------

R = 1.09   −T_2 —·—·—·—

$\tau_{overall}$: $1 = 1 \times 10^{-10}, 2 = 2 \times 10^{-10}, 3 = 5 \times 10^{-10}, 4 = 1 \times 10^{-9}, 5 = 2 \times 10^{-9},$

$6 = 5 \times 10^{-9}, 7 = 1 \times 10^{-8}, 8 = 2 \times 10^{-8}, 9 = 5 \times 10^{-8}, 10 = 1 \times 10^{-7}, 11 = 1 \times 10^{-6}$

FIGURE 4   Dependence of ^{13}C NMR relaxation parameters on both the overall diffusional (τ_c) and the internal (τ_i) correlation times for the 90° Woessner model of internal motion (cf. text).

models compatible with the data and to set outer limits to the amplitudes and frequencies of these motions. Thus, for example, it might be possible to conclude that a given set of measurements cannot be accounted for by low amplitude motions of any frequency but is compatible with any number of high amplitude motions in a certain frequency range. Another set of measurements may not be compatible with any high amplitude motion, but requires a low amplitude motion in addition to the overall rotation for its explanation. Even if the amplitudes and frequencies can be specified only as allowed ranges, for some systems this provides considerably more insight into their internal dynamics than is possible by any other means.

If the system under study is a folded linear polymer, further discrimination between models in a given allowed class may be possible by (a) determining the overall motion of the macromolecule and its symmetry by light scattering or other techniques and using the parameters as knowns in Eq. 2, common to all observed groups; and (b) comparing allowed motions for neighboring segments. Most of the models will require that at least some of the motions of adjacent segments be correlated. Thus, if diffusional

parameters and the sequence of the polymer chain are known, it should be possible to narrow down the choice of allowed models to one or, at most, to very few.

REFERENCES

1. ABRAGAM, A. 1961. The Principles of Nuclear Magnetism, Oxford University Press, New York.
2. JARDETZKY, O. 1964. The study of specific molecular interactions by nuclear magnetic relaxation measurements. *Adv. Chem. Phys.* **7**:499.
3. OLDFIELD, E., R. S. NORTON, and A. ALLERHAND. 1975. Studies of individual carbon sites of proteins in solution natural abundance carbon 13 nuclear magnetic resonance spectroscopy. *J. Biol. Chem.* **250**:6368.
4. OPELLA, S. J., D. J. NELSON, and O. JARDETZKY. 1976. An approach to the quantitative study of internal motions in proteins by measurements of longitudinal relaxation times and nuclear Overhauser enhancements in proton decoupled carbon-13 NMR spectra. *Am. Chem. Soc. Symp. Ser.* **34**:397.
5. DESLAURIERS, R., E. RALSTON, and R. L. SOMORJAI. 1977. Conformational studies of angiotensin-II. Least-squares fit of carbon 13 nuclear magnetic resonance relaxation times to extended and folded conformations. *J. Mol. Biol.* **113**:697.
6. JONES, W. C., T. M. ROTHGEB, and F. R. N. GURD. 1976. Nuclear magnetic resonance studies of sperm whale myoglobin specifically enriched with ^{13}C in the methionine methyl groups. *J. Biol. Chem.* **251**:7452.
7. TORCHIA, D. A., and D. L. VANDERHART. 1976. ^{13}C magnetic resonance evidence for anisotropic molecular motion in collagin fibrils. *J. Mol. Biol.* **104**:315.
8. HULL, W. W., and B. D. SYKES. 1975. Fluorotyrosine alkalene phosphatase: internal mobility of individual tyrosines and the role of chemical shift anisotropy as a ^{19}F nuclear spin relaxation mechanism in proteins. *J. Mol. Biol.* **98**:121.
9. WUTHRICH, K. 1975. NMR in Biological Research: Peptides and Proteins. American Elsevier Publishing Co., Inc., New York.
10. JARDETZKY, O., K. AKASAKA, D. VOGEL, S. MORRIS, and K. C. HOLMES. 1978. Unusual segmental flexibility in a region of tobacco mosaic virus coat protein. *Nature (Lond.).* In press.
11. WOESSNER, D. E. 1962. Nuclear spin relaxation in ellipsoids undergoing rotational Brownian motion. *J. Chem. Phys.* **37**:647.
12. WOESSNER, D. E., B. S. SNOWENDEN, JR., and G. H. MEYER. 1969. Nuclear spin-lattice relaxation in axially symmetric ellipsoids with internal motion. *J. Chem. Phys.* **50**:719.
13. LEVINE, Y. K., P. PARTINGTON, and G. C. K. ROBERTS. 1973. Calculation of dipolar nuclear magnetic relaxation times in molecules with multiple internal rotations. I. Isotropic over-all motion of the molecule. *Mol. Phys.* **25**:497.
14. LEVINE, Y. K., N. J. M. BIRDSALL, A. G. LEE, J. C. METCALFE, P. PARTINGTON, and G. C. K. ROBERTS. 1974. Calculation of dipolar nuclear magnetic relaxation times in molecules with multiple internal rotations. II. Theoretical results for anisotropic overall motion of the molecule, and comparison with ^{13}C relaxation. *J. Chem. Phys.* **60**:2890.
15. HUNTRESS, W. T. 1970. The study of anisotropic rotation of molecules in liquids by NMR quadrupolar relaxation. *Adv. Magn. Res.* **4**:1.
16. WALLACH, D. 1967. Effect of internal rotation on angular correlation functions. *J. Chem. Phys.* **47**:5258.
17. VAN PUTTE, K. 1970. Spin-lattice relaxation in anhydrous sodium and lithium soaps between 23° and 180°C. *J. Magn. Res.* **2**:23.
18. NOACK, F. 1971. Nuclear magnetic relaxation spectroscopy. *In* NMR: Basic Principles and Progress. P. Diehl, et al., editors. **3**:83.

19. KING, R., and O. JARDETZKY. 1978. A general formulism for the analysis of NMR relaxation measurements on systems with multiple degrees of freedom. *Chem. Phys. Lett.* **55**:15.
20. KUBO, R. 1962. *In* Fluctuation, Relaxation and Resonance in Magnetic Systems, J. ter Haar, editor. Oliver and Boyd Ltd., Edinburgh. 23.
21. ANDERSON, P. W. 1954. A mathematical model for the naming of spectral lines by exchange or motion. *J. Phys. Soc. Jpn.* **9**:316.
22. JOHNSON, C. S. 1965. Chemical rate processes and magnetic resonance. *Adv. Magn. Res.* **1**:33.
23. BHARUCHA-REID, A. T. 1960. Elements of the Theory of Markov Processes and their Applications. McGraw Hill Book Company, New York.
24. MESSIAH, A. 1961. Quantum Mechanics. North Holland Publishing Co. Amsterdam.

DISCUSSION

NAGESWARA RAO: Could you please elaborate on some of the terms in your theory? For example, what criteria do you use to determine whether two or more motional processes are weakly or strongly coupled? This puzzles me, especially because you have invoked independent motions in writing Eq. 2 in your paper. It would be especially helpful if you could provide some illustrative examples of weakly coupled and strongly coupled motions within the framework of your independent motion model.

JARDETZKY: If you are using the method with prejudice, that is, using a specific model for every motion, you can use mathematical criteria for deciding whether they are weakly or strongly coupled. You can see whether you can best fit the data by one or another specific motional models with either weak or strong coupling on the transition operator. If you are using the method without prejudice, you have to do this by trial and error, and you have to see, for example, whether you can account for the data by, let us say, two motions, or whether you require a model of three motions, two of which are weakly coupled.

NAGESWARA RAO: You see, in your Eq. 2 the eigenvalues seem to be τ_n^{-1}. Now, if I just use my elementary knowledge of quantum mechanics in considering whether the two states are coupled, I would consider the magnitude of the off-diagonal element and compare it to the difference between the two diagonal elements. What is this off-diagonal element in your case?

JARDETZKY: If you are using the method without prejudice, I suppose you would call it a degree of coupling.

NAGESWARA RAO: Well, let's go back to the quantum mechanics that you invoked as the analogy. In quantum mechanics we talk of an interaction causing this off-diagonal element. Since you didn't pick an example, let me pick one. One could be an overall reorientation. The other could be a segmental motion. The two uncoupled motions may be represented by the correlation times for the two motions. What would you consider an off-diagonal interaction that would couple an overall motion to the segmental motion?

KING: If the overall motion were discrete and made transitions between several states, and if while that overall motion was changing among those states, the molecule made a segmental motion so that the motions were stochastically coupled and not statistically independent, then you would have an off-diagonal interaction term.

NAGESWARA RAO: I don't think you are in a position really to write down the quantum mechanical states of these molecules, especially for the kind of molecules you are thinking of.