medium decreases.[22] Since the effective dielectric constant of the micellar surface is lower than that of water,[1-3] this could be a contributing factor for the moderate increase in the apparent reactivity of micelle-bound thiosulfate.

In conclusion, we have demonstrated that the combined studies of thiosulfate binding to CTAB micelles and of the effect of CTA_2T micelles on the kinetics of reaction 1 can lead to the prediction of the effect of CTAB on the kinetics of this reaction. This implies that the reactivity of thiosulfate is identical in CTA_2T and CTAB micelles. We also have shown that the ion-exchange formalism is adequate to describe quantitatively the effect of detergents on reactions of divalent ions.

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Registry No. $S_2O_3^{2-}$, 14383-50-7; CTAB, 57-09-0; CTA_2T, 82209-37-8; butyl iodide, 542-69-8.

Model-Free Approach to the Interpretation of Nuclear Magnetic Resonance Relaxation in Macromolecules. 1. Theory and Range of Validity

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Abstract: A new approach to the interpretation of nuclear magnetic resonance relaxation experiments on macromolecules in solution is presented. This paper deals with the theoretical foundations and establishes the range of validity of this approach, and the accompanying paper demonstrates how a wide variety of experimental relaxation data can be successfully analyzed by using this approach. For both isotropic and anisotropic overall motion, it is shown that the unique information on fast internal motions contained in relaxation experiments can be completely specified by two model-independent quantities: (1) a generalized order parameter, $\mathcal{S}$, which is a measure of the spatial restriction of the motion, and (2) an effective correlation time, τ_e, which is a measure of the rate of motion. A simple expression for the spectral density involving these two parameters is derived and is shown to be exact when the internal (but not overall) motions are in the extreme narrowing limit. The model-free approach (so called because $\mathcal{S}^2$ and τ_e have model-independent significance) consists of using the above spectral density to least-squares fit relaxation data by treating $\mathcal{S}^2$ and τ_e as adjustable parameters. The range of validity of this approach is illustrated by analyzing error-free relaxation data generated by using sophisticated dynamical models. Empirical rules are presented that allow one to estimate the accuracy of $\mathcal{S}^2$ and τ_e extracted by using the model-free approach by considering their numerical values, the resonance frequencies, and the parameters for the overall motion. For fast internal motions, it is unnecessary to use approaches based on complicated spectral densities derived within the framework of a model because all models that can give the correct value of $\mathcal{S}^2$ work equally well. The unique dynamic information ($\mathcal{S}$ and τ_e) can be easily extracted by using the model-free approach. Moreover, if one desires a physical picture of the motion, the numerical values of $\mathcal{S}^2$ and τ_e can be readily interpreted within a physically reasonable model.

I. Introduction

Nuclear magnetic relaxation data on macromolecules in solution contain information concerning the nature of internal motions that occur in these systems. The usual approach[1] to extracting such information involves the use of dynamical models that are based on physical intuition and/or the ease of formulation. While such analyses can be useful, there is the danger of overinterpretation of limited data and the possibility that the resulting physical picture is not unique. Models cannot be proven; they can only be eliminated.

In this paper we seek to answer the questions: (1) what is the unique information content of a given set of relaxation data and (2) how can one extract that information? In order to clarify the nature of the problem, let us consider a hypothetical example of a ^{13}C NMR relaxation study of a lysine side chain in an isotropically reorienting protein. The relaxation of each carbon nucleus is determined by the fluctuations of the $^{13}C-H$ vectors with respect to the external magnetic field. The observed quantities are determined by the Fourier transform (the spectral density) of an appropriate time-correlation function evaluated at certain frequencies whose values depend on the external field strength. To obtain the time dependence of the correlation function, which contains all the potentially available dynamic information, one would, in principle, need to perform experiments at an infinite number of magnetic field strengths. Even then the dynamic information would be limited because of the nature of the correlation function. With current NMR technology, a typical data set consists of a few numbers (say, T_1's and NOE's at two magnetic fields). As a result of steric constraints and concerted motions, the dynamics of a side chain are extremely complicated, and one cannot expect to construct a detailed picture of the dynamics from a few experimentally accessible numbers that, as we shall see, may contain redundant information.

The simplest possible description of the internal dynamics of the side chain involves specifying (1) the rate (time scale) and (2) the spatial restriction of the motion of each carbon in the chain. Suppose we sit in a frame rigidly attached to the macromolecule

† Deceased June 19, 1982.

(1) For a review, see: London, R. E. In "Magnetic Resonance in Biology"; Cohen, J. S., Ed.; Wiley: New York, 1980; Vol. I, p 1.

and observe the reorientation of the $^{13}C-H$ vector associated with, say, the C_γ carbon. Because of intrachain and interchain steric interactions, this vector will not assume all possible orientations. That is, its motion is not isotropic but is restricted. The motion of other carbons in the chain may be more or less restricted. The time course of the trajectory of a $^{13}C-H$ vector as it explores the positions accessible to it is complicated. The rate of motion of this $^{13}C-H$ vector cannot be described by a single parameter (i.e., the correlation function is not a single exponential). However, different vectors in general do move at different "speeds" (i.e., the time dependence of their correlation functions differ), and it is desirable to define a correlation time that describes the rate of motion in an effective way.

In this paper we shall show that the information on fast internal motions (i.e., faster than about 0.3 ns) contained in an NMR relaxation experiment at currently available fields can be completely described by (1) a *generalized order parameter*, $\mathcal{S}$, which is the measure of the degree of spatial restriction of the motion, and (2) an effective correlation time, τ_e, which is a measure of the rate of the motion. The generalized order parameter and the effective correlation time will explicitly be defined in a model-independent way. For the special case that the overall motion can be described by a single correlation time τ_M, our approach to extracting the unique information (i.e., $\mathcal{S}$ and τ_e) is based on the following simple expression for the spectral density:

$$J(\omega) = \frac{2}{5}\left(\frac{\mathcal{S}^2\tau_M}{1 + (\tau_M\omega)^2} + \frac{(1 - \mathcal{S}^2)\tau}{1 + (\tau\omega)^2} \right) \quad (1)$$

with

$$\tau^{-1} = \tau_M^{-1} + \tau_e^{-1} \quad (2)$$

which we shall derive. Specifically, we propose to least-squares fit the experimental relaxation parameters to those calculated by using the above expression, treating $\mathcal{S}^2$ and τ_e as the only adjustable parameters. We refer to this approach as "model-free" because eq 1 is derived without invoking a specific model for internal motions and because $\mathcal{S}$ and τ_e are defined in a model-independent way. Once numerical values of $\mathcal{S}$ and τ_e are extracted from the data, then one can consider their interpretation within the framework of a particular model. This is to be contrasted with the usual approach in which the spectral density is evaluated within the framework of a model.

The above procedure is clearly very simple to apply. The question is whether it works. The answer involves two separate issues. First, can the above two-parameter expressions reproduce the data, and second, are the resulting parameters meaningful (i.e., do $\mathcal{S}$ and τ_e obtained by least-squares fitting the relaxation data agree with their exact values)? The strategy we have adopted in order to answer these questions is based on the analysis of "experimental" data that were generated by using a variety of sophisticated models of the internal dynamics (i.e., by using complicated spectral densities). The data generated in this way are error free, and $\mathcal{S}$ and τ_e are known exactly. Thus, both of the questions raised above can be answered unambiguously. By analyzing a large number of sets of simulated data, we have established the range of validity and the accuracy of our approach. Basically, we have found that the data can always be reproduced. $\mathcal{S}$ and τ_e are virtually exact when the internal motions (but not the overall motion) are close to the extreme narrowing limit; they are reasonably accurate ($\sim$25%) as long as $\omega\tau_e < 0.5$ and $\mathcal{S}^2 > 0.01$. More precise criteria will be presented later in this paper. In the accompanying paper, we shall consider the application of our approach to actual experimental results.

The spectral density in eq 1 has the same *functional form* as an approximate spectral density derived by several authors[2-6] within the framework of the diffusion in a cone model. In this model, the interaction vector is assumed to diffuse freely, with a wobbling diffusion constant D_w in a cone of semiangle θ_0. A major point of this paper is that eq 1 is more general than the diffusion in a cone model and is applicable to physical situations where this model is not reasonable. Stated another way, our model-free approach to analyzing experimental data (in the special case that the overall motion is isotropic) is *operationally* the same as using the diffusion in the cone model. However, the parameters $\mathcal{S}$ and τ_e that are extracted from the data have a more general significance and need not be interpreted within the framework of this model. Our approach is also formally similar to the work of Jardetzky and co-workers[7-9] (i.e., the spectral density is represented by a few Lorentzians with adjustable parameters). However, as will become apparent, the theoretical justification for using such an approach and, more importantly, the interpretation of the resulting parameters are different.

The outline of this paper is as follows. In section II we describe the theoretical basis of our model-free approach to analyzing relaxation data. We first consider the case that the overall motion is isotropic; the anisotropic case is treated subsequently. We consider the general expression for the correlation function for internal motions ($C_I(t)$) that describes dipolar relaxation and also quadrupolar and chemical shift anisotropy relaxation in the special case that the relevant tensors are axially symmetric. We analyze the behavior of this correlation function at short and long times. We express the infinite-time limit in terms of the generalized order parameter, $\mathcal{S}$, which is a model-independent measure of the degree of spatial restriction of the motion. We introduce a single-exponential approximation to $C_I(t)$ that is exact at $t = 0$ and $t = \infty$ and has the exact area. We then show that the spectral density obtained from the total correlation function constructed by using this approximation for $C_I(t)$ is in fact *exact* when the internal motions are sufficiently fast (i.e., they are in the extreme narrowing limit). We show how the development can be generalized to incorporate fluctuations in the internuclear distance. Finally, we consider anisotropic overall motion and conclude this section by making contact with approaches that are operationally similar to ours. In section III, we establish the range of validity of our model-free approach by analyzing a large number of simulated "experimental" data sets that were generated by using a variety of sophisticated dynamical models. Empirical rules are presented that can be used to determine whether the generalized order parameters and effective correlation times extracted from the data are meaningful.

II. Theory

The relaxation due to dipole–dipole interaction between two nuclei can be described by the correlation function[10,11]

$$C(t) = \langle D_{qo}^{(2)*}(\Omega_{LF}(0)) D_{qo}^{(2)}(\Omega_{LF}(t)) \rangle \quad (3)$$

where $D_{mn}^{(2)}(\Omega)$ is a Wigner rotation matrix element[12] and the Euler angles, Ω_{LF}, specify the orientation of the unit vector, $\hat{\mu}_{LF}$, connecting the two nuclei in the laboratory coordinate system. This correlation function also describes quadrupolar and chemical shift anisotropy relaxation in the special case where the relevant tensors are axially symmetric. For a system in solution, the correlation function does not depend on the index q and can be rewritten by using the addition theorem for spherical harmonics[12] as

$$C(t) = \tfrac{1}{5}\langle P_2(\hat{\mu}_{LF}(0)\cdot\hat{\mu}_{LF}(t)) \rangle \quad (4)$$

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where $P_2(x)$ is the second Legendre polynomial,

$$P_2(x) = \tfrac{1}{2}(3x^2 - 1) \tag{5}$$

The spectral density, which determines the relaxation parameters, is given by

$$J(\omega) = 2\int_0^\infty (\cos \omega t)C(t)\, dt \tag{6}$$

For ^{13}C NMR of protonated carbons, where at currently available fields the relaxation is primarily dipolar, the relaxation times and the nuclear Overhauser enhancement (NOE) are given by[13]

$$T_1^{-1} = \frac{\hbar^2\gamma_C^2\gamma_H^2}{4r_{CH}^6}(J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_C + \omega_H)) \tag{7a}$$

$$T_2^{-1} = \frac{\hbar^2\gamma_C^2\gamma_H^2}{8r_{CH}^6}(4J(0) + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)) \tag{7b}$$

$$\mathrm{NOE} = 1 + \frac{\gamma_H(6J(\omega_C + \omega_H) - J(\omega_H - \omega_C))}{\gamma_C(J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_C + \omega_H))} \tag{7c}$$

where ω_I, I = C, H, are the Larmor frequencies. For ^2H NMR of deuterated carbons, the quadrupolar tensor is axially symmetric about the C–^{2}H bond, and the relaxation times are given by[13]

$$T_1^{-1} = \frac{3}{16}\left(\frac{e^2qQ}{\hbar}\right)^2(J(\omega_D) + 4J(2\omega_D)) \tag{8a}$$

$$T_2^{-1} = \frac{1}{32}\left(\frac{e^2qQ}{\hbar}\right)^2(9J(0) + 15J(\omega_D) + 6J(2\omega_D)) \tag{8b}$$

Note that the correlation function has the same form in both cases: $\hat{\mu}_{LF}$ points along the ^{13}C–H bond in ^{13}C NMR, while it points along the C–^{2}H bond in ^2H NMR. For chemical shift anisotropy relaxation with an axially symmetric shift tensor, one has[13]

$$T_1^{-1} = (\omega_I(\delta_\parallel - \delta_\perp))^2 J(\omega_I) \tag{9a}$$

$$T_2^{-1} = \tfrac{1}{6}(\omega_I(\delta_\parallel - \delta_\perp))^2(4J(0) + 3J(\omega_I)) \tag{9b}$$

A. Isotropic Overall Motion We consider a macromolecule whose overall motion can be described by a single correlation time. In subsection B, we shall consider anisotropic overall motions. For the isotropic case, when it is assumed that the overall and internal motions are independent, the total correlation function can be rigorously factored as

$$C(t) = C_O(t)C_I(t) \tag{10}$$

where the correlation function for overall motion is

$$C_O(t) = \tfrac{1}{5}e^{-6D_M t} = \tfrac{1}{5}e^{-t/\tau_M} \tag{11}$$

where D_M and τ_M are the rotational diffusion constant and correlation time of the macromolecule, respectively. The correlation function for internal motions is

$$C_I(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t))\rangle \tag{12}$$

where the unit vector $\hat{\mu}$ describes the orientation of the interaction vector in a reference frame that is *rigidly attached* to the macromolecule.

The Internal Correlation Function. We now consider the properties of the correlation function that describes internal motions. Let us consider the relaxation of a carbon in some side chain in a protein (see Figure 1). The motion of the ^{13}C–H vector of this carbon in the macromolecular frame is, in general, complicated. If the motion is Markovian (e.g., diffusive or jumplike), then $C_I(t)$ can be expressed as a series of exponentials

$$C_I(t) = \sum_{i\geq 0} a_i e^{-t/\tau_i} \tag{13}$$

(13) See, for example: Table I of ref 11.

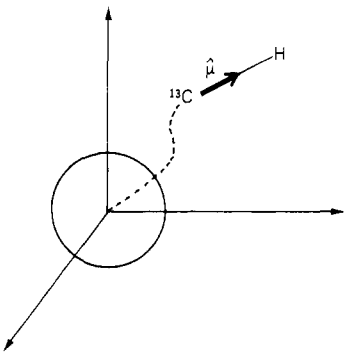

Figure 1. Orientation of the ^{13}C–H vector associated with a nucleus in a protein side chain relative to a macromolecule-fixed frame.

where $\tau_0 = \infty$, $\tau_1 > \tau_2 > \tau_3$ ..., and $0 \leq a_i \leq 1$ for all i. The length of this expansion and the magnitudes of the amplitudes, a_i, and the correlation times, τ_i, depend on the precise nature of the motion. For example, if $\hat{\mu}$ diffuses freely about an axis (the Woessner model[14]), the above expansion contains three terms. If the motion of $\hat{\mu}$ is restricted about this axis, then the sum contains an infinite number of terms.[11,15] If $\hat{\mu}$ can jump between N discrete sites, the expansion contains N terms.

Although the precise form of eq 13 depends on a model, we can obtain a number of properties of the internal correlation function independent of a particular model. The simplest is its value at $t = 0$,

$$C_I(0) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(0))\rangle = 1 \tag{14}$$

Now let us consider the value of $C_I(t)$ at long times. We will see that $C_I(\infty)$ is a rigorous model-independent measure of the degree of spatial restriction of the internal motion. By using the property of correlation functions that

$$\lim_{t\to\infty}\langle A(0)B(t)\rangle = \langle A\rangle\langle B\rangle \tag{15}$$

and the addition theorem for spherical harmonics

$$P_2(\hat{\mu}_1\cdot\hat{\mu}_2) = \sum_{m=-2}^{2} C_{2m}(\Omega_1)C_{2m}^*(\Omega_2) \tag{16}$$

where C_{2m} are the modified spherical harmonics of Brink and Satchler[12] ($C_{lm} = ((2l + 1)/4\pi)^{-1/2}Y_{lm}$) and $\Omega_i = (\theta_i,\phi_i)$ are the polar angles of $\hat{\mu}_i$, we have

$$C_I(\infty) = \sum_{m=-2}^{2}|\langle C_{2m}(\Omega)\rangle|^2 = \int\int d\Omega_1 d\Omega_2 p_{eq}(\Omega_1)P_2(\cos\theta_{12})p_{eq}(\Omega_2) \tag{17}$$

where θ_{12} is the angle between $\hat{\mu}_1$ and $\hat{\mu}_2$ and $p_{eq}(\Omega)$ is the normalized orientational distribution function of $\hat{\mu}$. The equilibrium average is defined as

$$\langle(\ldots)\rangle = \int d\Omega p_{eq}(\Omega)(\ldots) = \int_0^{2\pi} d\phi \int_0^{\pi} \sin\theta d\theta p_{eq}(\theta,\phi)(\ldots) \tag{18}$$

We equate $C_I(\infty)$ to the square of the *generalized order parameter* $\mathcal{S}$,

$$C_I(\infty) = \mathcal{S}^2 \tag{19}$$

We call $\mathcal{S}$ a *generalized* order parameter because it reduces to the usual order parameter (i.e., $\langle P_2(\cos\theta)\rangle$) when the motion is axially symmetric (see below). The usual order parameter plays an important role in the analysis of ^2H NMR[16] and fluorescence depolarization[4,17] of probes in membranes. Its relevance to NMR

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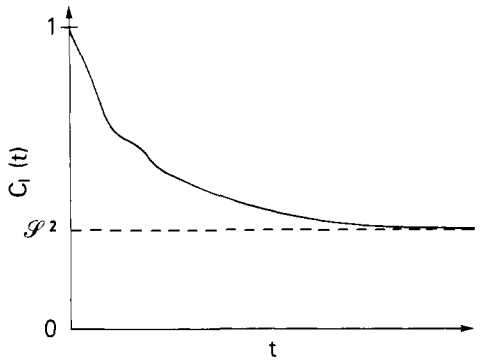

Figure 2. Idealized description of the time course of the correlation function for internal motions.

relaxation in solution has been recently recognized[6,18] in certain contexts. In this paper we show that the generalized order parameter plays a central role in the interpretation of NMR relaxation data for side chains in macromolecules.

$\mathcal{S}$ is a model-independent measure of the degree of spatial restriction of the motion. It satisfies the inequalities $0 \leq \mathcal{S}^2 \leq 1$. If the internal motion is isotropic (i.e., all orientations of $\hat{\mu}$ are equally probable, $p_{eq}(\Omega) = (4\pi)^{-1}$), then

$$\mathcal{S} = 0 \tag{20}$$

However, the converse is not true (see below). On the other hand, if the motion is completely restricted (i.e., $p_{eq}(\Omega) = \delta(\Omega - \Omega_0)$), then

$$\mathcal{S} = 1 \tag{21}$$

In the special case that the motion is azimuthally symmetric about an axis (i.e., $p_{eq}(\Omega) = p_{eq}(\theta)$, independent of ϕ, where θ is the angle between $\hat{\mu}$ and the symmetry axis), then

$$\mathcal{S} = \langle P_2(\cos\theta)\rangle = S \tag{22}$$

i.e., the generalized order parameter simply becomes the usual order parameter.

The order parameter S (and hence $\mathcal{S}$) can vanish even if the motion is not isotropic. For example, consider Woessner's model, which describes the free diffusion of $\hat{\mu}$ about a fixed axis. If β is the angle between $\hat{\mu}$ and this symmetry axis, then

$$S_{\text{Woessner}} = P_2(\cos\beta) \tag{23}$$

since $p_{eq}(\Omega) = (2\pi \sin\theta)^{-1}\delta(\theta - \beta)$. The order parameter for a model in which $\hat{\mu}$ jumps among three discrete equivalent sites about the symmetry axis is also given by eq 23. We note that the order parameter vanishes when β is at the "magic" angle (54.7°). A similar situation arises for the diffusion in the cone model where the order parameter (see eq A3) vanishes not only when $\theta_0 = \pi$ but also when $\theta_0 = \pi/2$ (i.e., when the interaction vector is restricted to move in a hemisphere). The generalized order parameter can easily be evaluated for a variety of models (see the Appendix for some examples).

We are now in a position to propose an approximation to the internal correlation function that plays a central role in our development. Figure 2 shows schematically the behavior of $C_I(t)$ as a function of time. The simplest approximation to $C_I(t)$ which is exact at $t = 0$ and at $t = \infty$ has the form

$$C_I^A(t) = \mathcal{S}^2 + (1 - \mathcal{S}^2)e^{-t/\tau_e} \tag{24}$$

where τ_e is an effective correlation time. To determine τ_e, one must place an additional requirement on the approximate correlation function. There exist several possibilities.[17] For the diffusion in the cone model, in which $C_I(t)$ is exactly given by an infinite sum of exponentials, it is known[4,19] that, as long as $\mathcal{S}^2$ (S_{cone}^2) is not too small, the single exponential approximation is very good if τ_e is determined by requiring the area of $C_I^A(t)$ to be exact. Because of this and for reasons that will become apparent later, we choose τ_e so that

$$\int_0^\infty (C_I^A(t) - \mathcal{S}^2)\,dt = \int_0^\infty (C_I(t) - \mathcal{S}^2)\,dt \tag{25}$$

or

$$\tau_e(1 - \mathcal{S}^2) = \int_0^\infty (C_I(t) - \mathcal{S}^2)\,dt \tag{26}$$

In the special case that $C_I(t) = e^{-t/\tau}$ ($\mathcal{S} = 0$), it follows from eq 26 that $\tau = \tau_e$. Thus, eq 26 can be regarded as the *definition* of an effective correlation time for an arbitrary correlation function. For future reference, we note that if expansion 13 is used for $C_I(t)$ ($a_0 = \mathcal{S}^2$ since $\tau_0 = \infty$), the effective correlation time becomes

$$\tau_e(1 - \mathcal{S}^2) = \sum_{i=1} a_i\tau_i \tag{27}$$

We have seen that the generalized order parameter can be expressed as an equilibrium average and contains no information about the time scale of dynamics; it is solely a measure of the spatial restriction of the motion. The effective correlation time, on the other hand, depends both on the microscopic diffusion or jump constants *and* the spatial nature of the motion. For example, for the Woessner model[14] in which the interaction vector diffuses freely about a symmetry axis with diffusion coefficient D,

$$\tau_e(1 - P_2(\cos\beta)^2) = D^{-1}\sum_{\substack{m=-2\\ m\neq 0}}^{2} (d_{m0}^{(2)}(\beta))^2 m^{-2} = 3D^{-1}(\sin^2\beta)(\cos^2\beta + \tfrac{1}{16}(\sin^2\beta)) \tag{28}$$

where β is the angle between $\hat{\mu}$ and the symmetry axis. This property of τ_e considerably complicates the interpretation of τ_e. In particular, while $\mathcal{S}$ has a model-independent significance, τ_e can be related to microscopic rate (diffusion) constants only within the framework of a particular model.

It is important to note that the above approximation for $C_I(t)$ is not equivalent to truncating the exact series expansion (eq 13) after two terms, i.e.,

$$C_I^A(t) \neq a_0 + a_1e^{-t/\tau_1} \tag{29}$$

Rather, the above approximation is the simplest form of a time-dependent Padé approximant. The area of the correlation function can be regarded as a *moment* of this function, and using the theory of moments, one can construct successively better approximations. For example, if in addition to $C_I(0)$, $C_I(\infty)$, and the area one also knows the behavior of $C_I(t)$ at short times (i.e., $(dC_I(t)/dt)_{t=0}$) and $\int_0^\infty dt\, t(C_I(t) - \mathcal{S}^2)$, one can construct the two-exponential approximation of the form

$$C_I^A(t) = A + Be^{-Ct} + De^{-Et} \tag{30}$$

where the coefficients are solutions to the equations

$$A = \mathcal{S}^2 \tag{31a}$$

$$A + B + D = C_I(0) = 1 \tag{31b}$$

$$BC + DE = -(dC_I(t)/dt)_0 = \sum_{i=1} a_i\tau_i^{-1} \tag{31c}$$

$$BC^{-1} + DE^{-1} = \int_0^\infty dt(C_I(t) - \mathcal{S}^2) = \sum_{i=1} a_i\tau_i \tag{31d}$$

$$BC^{-2} + DE^{-2} = \int_0^\infty dt\, t(C_I(t) - \mathcal{S}^2) = \sum_{i=1} a_i\tau_i^2 \tag{31e}$$

We shall not pursue such an extension here since, as we shall see, the simplest version of this scheme (i.e., eq 24) is adequate for our purposes.

The Spectral Density. A combination of eq 11 and 24 gives the total correlation function as

$$C(t) = \tfrac{1}{5}\mathcal{S}^2e^{-t/\tau_M} + \tfrac{1}{5}(1 - \mathcal{S}^2)e^{-t/\tau} \tag{32}$$

with

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(19) Kinoshita, K.; Kawato, S.; Ikegami, A. *Biophys. J.* **1977**, *20*, 289.

$$\tau^{-1} = \tau_M^{-1} + \tau_e^{-1} \tag{33}$$

and the corresponding spectral density as

$$J(\omega) = \frac{2}{5}\left(\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} + \frac{(1 - \mathcal{S}^2)\tau}{1 + (\omega\tau)^2} \right) \tag{34}$$

As discussed in the Introduction, eq 34 plays a central role in our approach to analyzing experimental data.

We now examine some limiting cases of the above spectral density. If the overall motion is considerably slower than the internal motions ($\tau_e \ll \tau_M$), eq 34 simplifies to

$$J(\omega) = \frac{2}{5}\left(\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} + \frac{(1 - \mathcal{S}^2)\tau_e}{1 + (\omega\tau_e)^2} \right) \tag{35}$$

Furthermore, if τ_e is sufficiently fast and the internal motion is in the extreme narrowing limit ($(\tau_e\omega)^2 \ll 1$, where ω is the largest frequency at which the spectral density must be evaluated, i.e., $\omega_C + \omega_H$ for ^{13}C NMR and $2\omega_D$ for ^2H NMR), we have

$$J(\omega) = \frac{2}{5}\left(\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} + (1 - \mathcal{S}^2)\tau_e \right) \tag{36}$$

Note that we have not assumed that the overall motion is in the extreme narrowing limit. By using the spectral density 36 in eq 7a and 7b, we find that T_1 and T_2 for dipolar relaxation are

$$T_i^{-1} = \mathcal{S}^2(T_i^{-1})_O + \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}(1 - \mathcal{S}^2)\tau_e \tag{37}$$

where $(T_i)_O$, $i = 1, 2$, are the relaxation times for an isotropically reorienting (with correlation time τ_M) macromolecule. The analogous expression for the NOE is

$$2T_1^{-1}(\mathrm{NOE} - 1)\gamma_C\gamma_H^{-1} = 2\mathcal{S}^2(T_1^{-1})_O(\mathrm{NOE}_O - 1)\gamma_C\gamma_H^{-1} + \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}(1 - \mathcal{S}^2)\tau_e \tag{38}$$

The second term in eq 37 and 38 arises exclusively from internal motions. However, the contribution due to the overall motion is reduced by the square of the generalized order parameter resulting from internal motions.

Although we have derived the spectral density in eq 36 by using our single exponential approximation to $C_I(t)$ (eq 24), we shall now show that this expression for the spectral density, and hence eq 37 and 38 for the relaxation times and the NOE, are actually *exact* when (1) the overall motion is isotropic, (2) internal motions are much faster than the overall motion and lie in the extreme narrowing limit, and (3) τ_e is defined as the area of the correlation function (eq 26). This is an important result. It explains why our model-free approach to analyzing relaxation data works so well in many cases of interest.

To show this, we begin by noting that eq 13 for the internal correlation function can be rewritten as (since $\mathcal{S}^2 = a_0$)

$$C_I(t) = \mathcal{S}^2 + \sum_{i=1} a_i e^{-t/\tau_i} \tag{39}$$

The spectral density (including the overall motion) then becomes

$$J(\omega) = \frac{2}{5}\int_0^\infty dt\ (\cos\omega t)(\mathcal{S}^2 e^{-t/\tau_M} + \sum_{i=1} a_i e^{-t(\tau_M^{-1} + \tau_i^{-1})^{-1}}) \tag{40}$$

Assuming that $\tau_M \gg \tau_i$ and evaluating the integral, we have

$$J(\omega) = \frac{2}{5}\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} + \frac{2}{5}\sum_{i=1}\frac{a_i\tau_i}{1 + (\omega\tau_i)^2} \tag{41}$$

Finally, assuming that the internal motions are in the extreme narrowing limit ($(\tau_i\omega)^2 \ll 1$), we have

$$J(\omega) = \frac{2}{5}\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_H)^2} + \frac{2}{5}\sum_{i=1} a_i\tau_i \tag{42}$$

which is indeed identical with eq 36 when τ_e is defined via the area criterion (see eq 27). The above result is actually more general than this derivation might suggest. A proof can be easily constructed for an arbitrary internal correlation function (not necessarily of the form given in eq 39) as long as the three conditions stated above hold.

In the above development, we have assumed that all the internal motions are faster than the overall motion (i.e., $\tau_i \ll \tau_M$ for all i). What happens if some component of the internal correlation function decays more slowly than the overall motion? Suppose, for example, that in eq 39 $\tau_1 \gg \tau_M$ but $\tau_i \ll \tau_M$ for $i = 2, 3, \ldots$. Then, proceeding as in eq 40–42, it can be seen that eq 42 should be replaced by

$$J(\omega) = \frac{2}{5}\frac{\mathcal{S}'^2\tau_M}{1 + (\omega\tau_M)^2} + \frac{2}{5}\sum_{i=2} a_i\tau_i \tag{42'}$$

where $\mathcal{S}'^2 = \mathcal{S}^2 + a_1$. $\mathcal{S}'^2$ is an order parameter that is a measure of the degree of restriction on a time scale determined by the overall motion; that is, $\mathcal{S}'^2$ is the limiting value of the internal correlation function for times less than τ_M. The internal correlation function can of course decay further for longer times, but the NMR experiment contains no information about such behavior. The situation when one of the components of the internal correlation function is neither in the extreme narrowing limit nor much slower than τ_M is more complicated, and the model-free approach based on eq 1 is not strictly applicable. However, by using the spectral density in eq 1 in such a case, we expected to obtain an effective order parameter with a value somewhere between $\mathcal{S}^2$ and $\mathcal{S}'^2$.

Equation 42′ shows that the model-free approach is also exact when the internal motions fall into two distinct time scales: (1) some much slower than the overall motion and (2) the remaining so much faster that they are in the extreme narrowing limit. When such separation of time scales occurs, the generalized order parameter extracted from the relaxation data is a measure of the restriction of the internal motion on a time scale faster than the overall motion. Since eq 42 and 42′ are formally identical, one cannot distinguish between $\mathcal{S}^2$ and $\mathcal{S}'^2$, and the prime can be dropped if it is remembered that the generalized order parameters extracted from NMR relaxation data do not contain any information about motions slower than the overall motion.

In summary, when the internal motions are sufficiently fast, the NMR experiment only measures the asymptote (i.e., $\mathcal{S}^2$) of the internal correlation function for times shorter than the overall motion and the corresponding area (i.e., τ_e) and contains no information about the details of its time dependence. Since the unique information content of an NMR experiment concerning fast internal motions is contained in $\mathcal{S}^2$ and τ_e, one can eliminate all physical models that cannot reproduce the numerical values of these parameters (e.g., in certain models such as those of Woessner[14] and Wallach[10] the order parameter is fixed by the geometry of the side chain). Conversely, all dynamical models that have the flexibility to reproduce the numerical values of $\mathcal{S}^2$ and τ_e can describe the relaxation data equally well even though the physical pictures of the motion inherent in such models differ. For example, we have shown[18] that multinuclear NMR relaxation experiments on nucleic acid fragments could be described equally well by using different (two-site jump and diffusion in a cone) models of the internal motion. These models correspond to different physical pictures, but their parameters that reproduce the data yield the same numerical value of the order parameter.

The Effective ^{13}C–H Internuclear Distance. Before generalizing the development to treat anisotropic overall motions, we consider the limit that the internal motions are infinitely fast ($\tau_e \to 0$). We will show that the influence of the generalized order parameter in the expressions for ^{13}C NMR dipolar relaxation times can be mimicked by using an effective carbon–hydrogen internuclear distance. In the limit that $\tau_e \to 0$, the spectral density (eq 34) becomes

$$J(\omega) = \frac{2}{5}\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} \tag{43}$$

and the corresponding dipolar relaxation times (eq 37) become

$$T_i^{-1} = \mathcal{S}^2(T_i^{-1})_0 \qquad i = 1, 2 \tag{44}$$

where $(T_i)_0$ is the result for an isotropically reorienting sphere. We note that this equation can formally be rewritten as

$$T_i = (T_i')_0 \tag{45}$$

where $(T_i')_0$ is the sphere result calculated by using an effective internuclear distance, r_{CH}', given by eq 46. Since $0 \leq |\mathcal{S}| \leq 1$,

$$r_{CH}' = r_{CH}|\mathcal{S}|^{-1/3} \tag{46}$$

the effective internuclear distance, r_{CH}', is always *larger* than r_{CH}. For example, if r_{CH} is 1.09 Å and $\mathcal{S}$ is estimated by using the diffusion in the cone model (eq A3) with a cone angle (θ_0) equal to 16°, then $r_{CH}' = 1.11$ Å. It is important to note that bending vibrations (librations) lead to values of $\mathcal{S}$ smaller than unity and, hence, *increase* the effective internuclear distance. Stretching vibrations in the *harmonic approximation* result in a *decrease* in the effective internuclear distance. Thus, the observation of Dill and Allerhand[20] that the NMR data at two magnetic fields for the α carbons of lysozyme can be fitted with a single correlation time when $r_{CH}' = 1.11$ Å is likely to be an indication of the presence of librational motions (bending vibrations or some other fast motions that reorient the ^{13}C–H vectors).

For the sake of completeness, we show how the theory of this paper can be formulated when fluctuations not only in the orientation but also in the internuclear separation are considered. The generalization of eq 12 is

$$\bar{C}_I(t) = \left\langle \frac{P_2(\hat{\mu}(0)\cdot\hat{\mu}(t))}{r^3(0)r^3(t)} \right\rangle \tag{47}$$

The generalized order parameter becomes

$$\bar{\mathcal{S}}^2 = \sum_{m=-2}^{2} |\langle C_{2m}(\Omega)/r^3 \rangle|^2 \tag{48}$$

The analogue of eq 24 is

$$\bar{C}_I(t) = \bar{\mathcal{S}}^2 + (\langle r^{-6} \rangle - \bar{\mathcal{S}}^2)e^{-t/\tau_e} \tag{49}$$

and finally, the generalization of eq 37 is

$$T_i^{-1} = \bar{\mathcal{S}}^2(r_0)^6(\bar{T}_i^{-1})_0 + \hbar^2\gamma_C^2\gamma_H^2(\langle r^{-6} \rangle - \bar{\mathcal{S}}^2)\tau_e \tag{50}$$

where $(T_i)_0$ is the relaxation time calculated for an isotropically reorienting sphere when r_{CH} is set equal to r_0.

B. Anisotropic Overall Motion. We now consider the generalization of the development to the situation where the overall motion cannot be described by a single correlation time (e.g., a cylindrical macromolecule or a random-coil polymer). When the overall motion is anisotropic, the first complication that arises is that the total correlation function *cannot* be rigorously factored into a product of contributions due to overall and internal motions even when it is assumed that these motions are independent (i.e., eq 10 is no longer rigorous). Nevertheless, we approximate the total correlation function as a product. Numerical evidence for the validity of this decoupling approximation will be presented later in this paper.

We shall use the same functional form for the internal correlation function as in the isotropic case (i.e., eq 24), so we are left with the problem of describing the correlation function for overall motion. For random-coil polymers, the usual approach to describing the highly anisotropic nature of the motion of the backbone involves the use of a distribution of correlation times[21,22] (or equivalently diffusion coefficients). Specifically, if $p(\tau)$ is the normalized probability that τ is between τ and $\tau + d\tau$, then the overall correlation function is represented as

$$C_O(t) = \tfrac{1}{5}\int p(\tau)e^{-t/\tau}\, d\tau \tag{51}$$

A number of different ad hoc expressions have been used in the literature.[21,22] One does not expect the resulting expression for $C_O(t)$ to be very sensitive to the particular form of the distribution. The situation is analogous to the theory of heat capacities of solids where the Einstein, Debye, and exact normal-mode frequency distributions are very different, yet the temperature dependence of the heat capacities are rather similar (except of course at very low temperatures). In this paper we set

$$p(\tau) = A\delta(\tau - \tau_1) + (1 - A)\delta(\tau - \tau_2) \tag{52}$$

and hence

$$C_O(t) = \tfrac{1}{5}Ae^{-t/\tau_1} + \tfrac{1}{5}(1 - A)e^{-t/\tau_2} \tag{53}$$

where A, τ_1, and τ_2 are adjustable parameters that can be determined by fitting the relaxation data of a nucleus that is attached to the macromolecular backbone. When $A = 1$, we recover the isotropic result (eq 11). It is interesting to note that eq 53 for the overall correlation function describing anisotropic motions has *formally* the same functional form as the total correlation function (overall and internal) for the case when the overall motion is isotropic (i.e., eq 32, $A \rightarrow \mathcal{S}^2$, $\tau_M \rightarrow \tau_1$, and $\tau \rightarrow \tau_2$). In summary, our expression for the total correlation function when the overall motion is anisotropic is

$$C(t) = \tfrac{1}{5}(Ae^{-t/\tau_1} + (1 - A)e^{-t/\tau_2})(\mathcal{S}^2 + (1 - \mathcal{S}^2)e^{-t/\tau_e}) \tag{54}$$

The generalized order parameter $\mathcal{S}$, which describes the spatial restriction of the internal motions, is now defined with respect to a frame attached to a backbone nucleus whose anisotropic motion is described by A, τ_1, and τ_2.

As in the case of isotropic overall motion, it can be shown that eq 37 (where now $(T_i)_0$ are the relaxation times for anisotropic motion) is exact when (1) the internal motions are in the extreme narrowing limit and the internal correlation function decays much faster than the overall correlation function (i.e., $\int_0^\infty C_O(t)(\cos \omega t)(C_I(t) - \mathcal{S}^2)\, dt = \int_0^\infty (C_I(t) - \mathcal{S}^2)\, dt$), (2) the decoupling approximation is valid, and (3) τ_e is defined as the area of the internal correlation function. It is important to note that in proving the above, one does not require the overall correlation function to have the form given in eq 53 and, thus, the result holds more generally.

C. Relation to Previous Work. We shall now discuss the relation of our model-free approach to analyzing NMR relaxation data to previous work. As mentioned in the introduction, the functional form for the internal correlation function (eq 24), on which our approach is based, is identical with an *approximation* to the correlation function within the diffusion in a cone model[2-6] (in this model $\hat{\mu}$ is restricted to diffuse in a cone of semiangle θ_0). Specifically, if the generalized order parameter is evaluated within this model (i.e., $\mathcal{S} = S_{cone}$), then the expressions are identical. It should be emphasized that the analysis of experimental data based on eq 24 is meaningful independent of whether the cone model is physically reasonable. In this regard, we mention the interesting work of Howarth,[3] who analyzed a variety of experimental relaxation data by using the cone model. He showed that the data could be well reproduced even in cases where the cone model is not reasonable (e.g., for the carbons of an aliphatic side chain of a random-coil polymer). Our work explains why this is so: the cone angles he extracted may have no physical significance; however, the corresponding order parameters are physically meaningful.

Jardetzky and co-workers[7-9] have previously considered "how much definite information on internal mobility in proteins can be deduced from NMR relaxation measurements" and proposed an approach to "mapping internal motions in proteins". Although their approach is operationally similar to ours in that the spectral density is represented as a sum of Lorentzians containing adjustable parameters, the motivation for using such expressions and interpretation of the parameters is quite different. For example, their work contains no reference to the concept of an order parameter. Moreover, they suggest that more and more experimental information (T_1, T_2, and NOE's at a large number of fields) will allow one to learn more and more about internal motions. We, on the other hand, emphasize that the information content of such data over a large range of frequencies on fast internal motions

(20) Dill, K.; Allerhand, A. *J. Am. Chem. Soc.* **1979**, *101*, 4376.
(21) Schaefer, J. A. *Macromolecules* **1973**, *6*, 882.
(22) Wittebort, R. J.; Szabo, A.; Gurd, F. R. N. *J. Am. Chem. Soc.* **1980**, *102*, 5723.

is redundant and, in fact, can be specified by just two quantities (i.e., $\mathcal{S}^2$ and τ_e).

Since the papers of Jardetzky and co-workers[7-9] use the technical jargon of the mathematical theory of Markov processes, it is perhaps useful to summarize our understanding of their work. In their first paper,[7] they show *formally* how the spectral density can be expressed in terms of the eigenfunctions and eigenvalues of a transition operator. They obtain a correlation function of the form

$$C(t) = \sum_n \alpha_n e^{-\lambda_n t} \tag{55}$$

and a corresponding spectral density

$$J(\omega) = 2\sum_n \frac{\alpha_n \lambda_n}{\lambda_n^2 + \omega^2} \tag{56}$$

where $-\lambda_n$ are the eigenvalues of the transition operator. They then proceed to generalize the above expression to "N independent motions" and obtain a spectral density of the same general form as above. The entirely formal nature of this paper can be contrasted with the work of Wittebort and Szabo,[11] who explicitly show how to construct the spectral density for a general jump model (i.e., a general discrete Markov process) and illustrate the formalism by considering the *concerted* motions of a lysine side chain. Jardetzky and co-workers[7-9] refer to λ_n^{-1} in eq 55 and 56 as the correlation time for the "nth individual motion". However, it is well-known that simple models containing only a few types of motions lead to correlation functions containing a large number of λ_n's. For example, even in the Woessner model[14] (a single free internal rotation superimposed on isotropic overall motion) one has *three* λ's (i.e., $\lambda_0 = 6D_M$, $\lambda_1 = 6D_M + D$, and $\lambda_2 = 6D_M + 4D$, where D_M and D are the overall and internal diffusion coefficients). Moreover, the exact correlation function for the diffusion in the cone model[4,19] (see eq A2) has an infinite number of components. In any case, they propose to use sufficiently extensive sets of relaxation data to determine all amplitudes α_n and relaxation times λ_n^{-1}. This is, of course, not even possible in principle because the NMR experiment contains information about the individual values of only those α_n and λ_n's for which λ_n^{-1} is sufficiently slow so as not to be in the extreme narrowing limit.

In a more recent paper,[9] Jardetzky and co-workers indicate how to implement their approach in practice. They write the spectral density as

$$J(\omega) = 2\sum_{i=1}^{M} \frac{\bar{\alpha}_i \bar{\lambda}_i}{\bar{\lambda}_i^2 + \omega^2} \tag{57}$$

including as many terms as required to fit the experimental data. They refer to M as the "number of independent motions", and $\bar{\alpha}_i$ and $\bar{\lambda}_i$ are the effective amplitude and rate for the ith physical motion. As discussed above, we find this terminology misleading. They say that $\bar{\alpha}_i$ does not have a simple physical interpretation. They normalize $\bar{\alpha}_i$ such that $\sum_{i=1}^{M} \bar{\alpha}_i = 1$ and call $100\bar{\alpha}_i$ the percent contribution of motion i to the relaxation. It is clear that all this is quite different from our terminology.

One of the disadvantages of eq 57 is that the contribution to the spectral density of the overall motion of the macromolecule is not clearly shown. If there is an independent overall motion common to all parts of the system, there cannot be a component in the correlation function that decays more slowly than the overall motion. In our notation this can be seen immediately by using eq 11 and 13 in eq 10. Thus, within their approach, if one wanted to analyze NMR data on a protein by using the information on the overall motion extracted from the rotational diffusion coefficient measured independently, this would imply two constraints: to fix one of the eigenvalues, say $\bar{\lambda}_1$, and $\bar{\lambda}_i \geq \bar{\lambda}_1$ for all $i = 2, \ldots, M$. Since Jardetzky and co-workers do not impose restrictions on the relative magnitudes of the $\bar{\lambda}$'s, they can arrive at physical conclusions that are artifacts. This occurs in their analysis of relaxation data for various residues in bovine pancreatic trypsin inhibitor. To interpret the relaxation parameters, they set $\bar{\lambda}_1 = 6 \times 10^8\ s^{-1}$, which is characteristic of the overall motion, and then find a component with $\bar{\lambda}_2 \approx 10^7\ s^{-1}$, which they attribute to a general low-frequency distortion of the backbone. However, since the internal motion is superimposed on the overall motion, internal motions (slow or otherwise) only result in components that decay faster than $\bar{\lambda}_1$ (e.g., $\bar{\lambda}_2 = \bar{\lambda}_1 + \bar{\lambda}_I$, where $\bar{\lambda}_I$ is the rate of internal motion). The slow component could, in principle, arise from anisotropic overall motion, but this seems unlikely considering the shape of the molecule.

Finally, we point out that the simplest version of their approach ($M = 2$) is in fact *formally* identical with the simplest version of ours. Setting $M = 2$ in eq 57 and using $\bar{\alpha}_1 + \bar{\alpha}_2 = 1$, we have

$$J(\omega) = \frac{2\bar{\alpha}_1\bar{\lambda}_1}{\bar{\lambda}_1^2 + \omega^2} + \frac{2(1 - \bar{\alpha}_1)\bar{\lambda}_2}{\bar{\lambda}_2^2 + \omega^2} \tag{58}$$

which apart from the factor 5^{-1} (which is incorporated into the expressions for the relaxation parameters) is identical with our eq 34 when $\bar{\lambda}_1 = \tau_M^{-1}$, $\bar{\lambda}_2 = \tau^{-1}$, and $\bar{\alpha}_1$ is identified with the generalized order parameter ($\bar{\alpha}_1 = \mathcal{S}^2$). However, *operationally*, eq 58 and 34 are identical only if the constraint $\bar{\lambda}_1 \leq \bar{\lambda}_2$ is imposed, since $\tau_M^{-1} \leq \tau^{-1}$ (see eq 33).

III. Range of Validity

In this section we investigate the range of validity of the model-free approach by analyzing NMR relaxation data that were generated by using sophisticated dynamical models for which the values of $\mathcal{S}^2$ and τ_e are known exactly. Using the model-free expressions for the spectral density (e.g., eq 34 for isotropic overall motion) and treating $\mathcal{S}^2$ and τ_e as the only adjustable parameters, we least-squares fit the relaxation data and then compare the resulting numerical values of $\mathcal{S}^2$ and τ_e with their exact values. The rather complicated spectral densities that were used in generating the data are described in the Appendix. We have seen in the Theory section that the model-free approach is exact when the internal motions are in the extreme narrowing limit. Here we wish to quantify this result and determine how well the approach works for slower internal motions. We will give a set of empirical rules that allow one to determine the accuracy of the extracted values of τ_e and $\mathcal{S}^2$ by considering only their numerical values, the parameters for overall motion, and the resonance frequencies. This is important in the analysis of experimental data (see accompanying paper), where, of course, the exact values of the order parameter and the effective correlation times are not known. We have analyzed a large number of "simulated" data sets, and we present below the results of only a representative sample. We consider both isotropic (subsection A) and anisotropic (subsection B) overall motions.

A. Isotropic Overall Motion. We consider systems whose overall motion can be described by a single correlation time, τ_M. Before presenting the results of a systematic study based on relaxation data that we have generated using the models described in the appendix, we show how well the model-free approach works for the relaxation data calculated by Levy et al.[23] for a pseudo side chain in a macromolecule using molecular dynamics. Briefly, Levy et al.[23] obtained trajectories describing the motion of a heptane molecule by using the diffusive Langevin equation. Then they mimicked a pseudo side chain of a macromolecule by isotropically reorienting the C_1–C_2 bond (i.e., C_2 is an "α carbon" whose relaxation is described by τ_M). Finally, they calculated T_1, T_2, and NOE for carbons C_3, C_4, C_5, and C_6 at two fields with τ_M = 1, 10, and 100 ns. The generalized order parameters defined in this paper can be related to "configuration averages of Wigner functions", which they tabulate. Specifically, $\mathcal{S}^2$ for the carbons 3–6 is simply 4π times the entries in their Table X. It is interesting to note that $\mathcal{S}^{-2}$ is identical with the "motional averaging scale factor" Levy and co-workers define in another paper.[24]

In Table I we present the results of the analysis of the simulated data of Levy et al.[23] using the model-free approach. We fixed

(23) Levy, R. M.; Karplus, M.; Wolynes, P. G. *J. Am. Chem. Soc.* **1981**, *103*, 5998.

(24) Levy, R. M.; Karplus, M.; McCammon, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 994.

Table I. Model-Free Analysis of the Relaxation Data Calculated by Levy et al.[23] for a Pseudoaliphatic Side Chain by Using Molecular Dynamics[a]

	ω, MHz	NOE	NT_1, ms	NT_2, ms	$\mathcal{S}^2$	τ_e, ps
C3	15	1.62 (1.63)	120 (120)	80 (80)	0.16 (0.16)	90
	68	2.46 (2.44)	460 (460)	114 (114)		
C4	15	2.72 (2.74)	488 (486)	444 (446)	0.0078 (0.0073)	81
	68	2.93 (2.91)	581 (584)	488 (494)		
C5	15	2.52 (2.50)	733 (736)	625 (626)	0.0090 (0.0093)	46
	68	2.92 (2.95)	988 (984)	722 (716)		
C6	15	2.59 (2.60)	1039 (1038)	907 (908)	0.0054 (0.0053)	34
	68	2.94 (2.92)	1328 (1330)	1025 (1030)		

[a] The C2 carbon moves isotropically with $\tau_M = 10$ ns. Only T_1 and NOE's are fitted. The T_2's are predicted. The exact results are in parentheses.

τ_M at 10 ns and least-squares fit their T_1 and NOE values (but not T_2's) at the two fields simultaneously to extract numerical values of $\mathcal{S}^2$ and τ_e. We then predicted the T_2 values at both fields. It can be seen that the model-free approach can reproduce the data extremely well. Moreover, the predicted T_2's are in excellent agreement with the exact results. Finally, the generalized order parameters extracted from the relaxation data agree remarkably well with their exact values. Note, in particular, that the nonmonotonic behavior of $\mathcal{S}^2$ as one goes out the chain, which appears to be a hallmark of concerted motions, is reproduced. A similar comparison for τ_e is not possible since Levy et al.[23] did not present exact values of this quantity. We have performed similar analyses of their data for $\tau_M = 1$ and 100 ns. The results are almost as impressive ($\mathcal{S}^2$ for C_4 is somewhat overestimated when $\tau_M = 1$ ns, and T_2 information is required when $\tau_M = 100$ ns to get highly accurate values of $\mathcal{S}^2$). In fact, we were able to find a misprint in a preprint of their paper (we correctly predicted that the NOE at 15 MHz for C_4 with $\tau_M = 100$ ns should be 2.93 instead of 2.83).

Why does the model-free approach work so well? Basically, because the internal motions are sufficiently fast so as to be close to the extreme narrowing limit. In this limit, the information on internal motions contained in the NMR relaxation experiment is rigorously completely specified by $\mathcal{S}^2$ and τ_e. Therefore, one should be able to extract $\mathcal{S}^2$ and τ_e from just two experimental relaxation parameters when these parameters are known for the overall motion. Suppose we know T_1's at two fields in the presence and absence of internal motion (i.e., T_1, $\bar{T}_1$ and $(T_1)_0$, $(\bar{T}_1)_0$). In the extreme narrowing limit, these quantities are related by eq 37. Since we have two equations (one for each field) in two unknowns ($\mathcal{S}^2$ and τ_e), we immediately have

$$\mathcal{S}^2 = \frac{T_1^{-1} - \bar{T}_1^{-1}}{(T_1^{-1})_0 - (\bar{T}_1^{-1})_0} \tag{59a}$$

and

$$\frac{\hbar^2\gamma_C^2\gamma_H^2}{r_{CH}^6}\tau_e = \frac{T_1^{-1}(\bar{T}_1^{-1})_0 - \bar{T}_1^{-1}(T_1^{-1})_0}{T_1^{-1} - \bar{T}_1^{-1} - (T_1^{-1})_0 + (\bar{T}_1^{-1})_0} \tag{59b}$$

Identical equations hold for T_2. If one knows T_1 and NOE at one field, then, using eq 38, we have

$$\mathcal{S}^2 = \frac{(T_1)_0(2.988 - \text{NOE})}{T_1(2.988 - \text{NOE}_0)} \tag{60a}$$

$$\frac{\hbar^2\gamma_C^2\gamma_H^2}{r_{CH}^6}\tau_e = \frac{\text{NOE} - \text{NOE}_0}{T_1(2.988 - \text{NOE}_0) - (T_1)_0(2.988 - \text{NOE})} \tag{60b}$$

where $(T_1)_0$ and NOE_0 are the relaxation parameters describing the overall motion. In favorable cases (e.g., sufficiently fast internal motions and accurate data), these equations allow one to obtain $\mathcal{S}^2$ and τ_e *directly* from experimental data. We will make extensive use of these equations below and in part 2 of this series, where we will show that they work remarkably well for real systems.

In Table II we compare $\mathcal{S}^2$ calculated via eq 59a and 60a from the data of Levy et al.[23] using (1) only the T_1's at both fields, (2) T_1's and NOE's at 15 MHz, and (3) T_1's and NOE's at 68 MHz, with the exact results and the values extracted from T_1 and NOE data at both fields using a least-squares method (see Table I). The $(NT_1)_0$ and NOE_0 are respectively 23.8 ms and 1.30 at 15 MHz and 266 ms and 1.16 at 68 MHz. Considering the simplicity of the analysis, the general agreement among the values is remarkable. The $\mathcal{S}^2$'s obtained by using only the T_1's are excellent, except for C_4, where $\mathcal{S}^2$ is slightly overestimated. The $\mathcal{S}^2$'s obtained from the T_1's and NOE's at low field are virtually exact. This is to be expected since at this frequency the internal motions are very close to the extreme narrowing limit. The $\mathcal{S}^2$'s obtained using only T_1's and NOE's at high field are quite poor except for C_3. This is a consequence of the relative slowness of the internal motions (i.e., at high field the internal motions are further from the extreme narrowing limit) and the fact that the exact values of the order parameters are very small. As we shall see below, T_1 and NOE data at 68 MHz determine $\mathcal{S}^2$ and τ_e better when $\mathcal{S}^2$ is larger (i.e., $\mathcal{S}^2 > 0.01$) and/or τ_e is faster.

Table II. Comparison of the Generalized Order Parameter Extracted From the Simulated Data of Levy et al.[23] in Various Ways ($\tau_M = 10$ ns)

	$\mathcal{S}^2$				
	exact	T_1 and NOE at 15 and 68 MHz[a]	T_1 at 15 and 68 MHz[b]	T_1 and NOE at 15 MHz[c]	T_1 and NOE at 68 MHz[d]
C3	0.16	0.16	0.16	0.16	0.17
C4	0.0073	0.0078	0.0090	0.0072	0.019
C5	0.0093	0.0090	0.0090	0.0093	0.0056
C6	0.0053	0.0054	0.0055	0.0053	0.0074

[a] Least-square fitting T_1 and NOE at both fields as in Table I. [b] Using eq 59a with $(NT_1)_0 = 23.8$ and 266 ms at 15 and 68 MHz, respectively. [c] Using eq 60a with $(NT_1)_0 = 23.8$ ms and $\text{NOE}_0 = 1.30$. [d] Using eq 60a with $(NT_1)_0 = 266$ ms and $\text{NOE}_0 = 1.16$.

We now present the results of a systematic investigation of the range of validity of the model-free approach. The conclusions reached by exploring a large number of simulated data will be illustrated with numerous examples.

We consider first the case where the internal motions are in the extreme narrowing limit. In this limit, the spectral density is given exactly by eq 36; i.e., one has exactly

$$\frac{\tau}{1 + (\omega\tau)^2} = \tau_e \tag{61}$$

where $\tau^{-1} = \tau_M^{-1} + \tau_e^{-1}$. We call *fast* internal motions those for which eq 61 holds to within 10% for the largest frequency determining the relaxation parameters, e.g., $\omega_C + \omega_H$ for ^{13}C dipolar relaxation. We found that for fast internal motions, virtually exact values of $\mathcal{S}^2$ and τ_e can be obtained from a pair of relaxation parameters (e.g., NOE and T_1) at two fields. For example, for a ^{13}C NMR frequency of ~90 MHz, if $\tau_M \sim 10$ ns, motions such that $\tau_e \lesssim 100$ ps may be considered fast. At a given magnetic field and fixed τ_M, as τ_e decreases the agreement between the two sides of eq 61 gets better and, of course, if τ_e is fast enough, eq 61 becomes an identity. For example, for ^{13}C–H dipolar relaxation at a Larmor frequency of 90.5 MHz, if $\tau_M = 10$ ns and $\tau_e = 25$

Table III. Model Free Analysis of ^{13}C Dipolar Relaxation Data Generated by Using the Woessner Model[14] with $\tau_M = 10$ ns, $1/6D = 10.6$ ps, and $\beta = 70.5°$ [a]

ω, MHz	NOE $(1 + \eta)$	T_1, ms	T_2, ms	$\mathcal{S}^2$	τ_e, ps
25	1.56 (1.57)	332 (335)	146 (149)	0.113 (0.111)	31.4 (31.9)
90	2.45 (2.45)	1194 (1194)	179 (182)		

[a] Only T_1's and NOE's at high field were fitted. The T_2's at high field and all the relaxation parameters at low field are predicted. The exact results are in parentheses.

ps, $(\omega\tau_e)^2 = 5 \times 10^{-3}$, $\tau_e/\tau_M = 2.5 \times 10^{-3}$, and eq 61 errs by only ~0.7%. If the contribution to the spectral density of the τ_e term is really frequency independent, one should be able to extract the exact values of $\mathcal{S}^2$ and τ_e and predict very accurately the relaxation data at any other field (such that eq 61 still holds) only from, say, the measurement of two relaxation data at one field. On the basis of extensive numerical experimentation, we conjecture that if eq 61 holds to within ~2%, this is indeed the case. Motions of this nature will be called *extremely fast* internal motions. In the case of extremely fast motions, eq 61 is virtually exact and $\mathcal{S}^2$ and τ_e can be determined very accurately by using the analytical expressions 59a and 59b for T_1 data at two fields or eq 60a and 60b for T_1 and NOE data at one field. For example, for ^{13}C–^1H dipolar relaxation with $\tau_M = 10^{-8}$, at a ^{13}C resonance frequency of ~90.5 MHz, internal motions such that $\tau_e \lesssim 50$ ps fall in this category. Table III illustrates an example of extremely fast internal motions for Woessner's diffusional model. Exact data were generated for $\tau_M = 10^{-8}$ s, $\beta = 70.5°$, and $1/6D = 10.6$ ps. Fitting only T_1 and NOE at high field, we can extract $\mathcal{S}^2$ and τ_e almost exactly and predict very accurately the other relaxation data. For these fast internal motions, one can use simple analytical formulas to extract $\mathcal{S}^2$ and τ_e from a set of two relaxation data, e.g., T_1 and NOE at 90.5 MHz. Using eq 60a and 60b with $(T_1)_0 = 461$ ms, $NOE_0 = 1.16$, one obtains $\mathcal{S}^2 = 0.114$, $\tau_e = 31.1$ ps (with an error of less than 1%); using eq 37 with $(T_2)_0 = 22.6$ ms, one predicts $T_2 = 178$ ms at 90.5 MHz (exact value 182 ms). For the relaxation parameters at low field, using eq 37 for T_1 and T_2 and eq 38 for the NOE with $(T_1)_0 = 46.6$ ms, $(T_2)_0 = 18.1$ ms, and $NOE_0 = 1.21$, one predicts $T_1 = 332$ ms (exact value 335 ms), $T_2 = 146$ ms (exact value 149 ms), and NOE = 1.55 (exact value 1.51).

It is of some interest that, in the limit of extremely fast internal motions, the spectral density at nonzero frequencies is fairly insensitive to large percent variations of $\mathcal{S}^2$ when $\mathcal{S}^2$ is very small, say $\lesssim 0.005$, whereas the zero-frequency term depends strongly on $\mathcal{S}^2$, especially if τ_M is relatively slow. For example, if $\tau_M = 10^{-7}$ s, $\tau_e = 19$ ps, and $\mathcal{S}^2 = 3 \times 10^{-3}$, a change in $\mathcal{S}^2$ of 53% brings about a variation in T_1 and NOE of less than 0.1%, whereas the T_2 values change by 29%. However, given the physical interpretation of the generalized order parameter, it is uninteresting to determine $\mathcal{S}^2$ very accurately when it is that small. In general, for large amplitude motions, small changes in the allowed spatial range bring about a large *percent* change in the value of the generalized order parameter. For example, in the diffusion in a cone model, an order parameter of 3.0×10^{-3} corresponds to a cone angle of ~84°; $\mathcal{S}^2 = 4.5 \times 10^{-3}$ corresponds to a cone angle of ~83°. Thus, a variation of ~50% in the order parameter involves a change of only ~1% in the cone angle. Even for extremely fast motions, if $\mathcal{S}^2 \lesssim 10^{-3}$, usually T_2 information is required to extract the exact value of the order parameter. Thus, if one *uses* $\mathcal{S}^2$ values extracted only from T_1 and NOE data to predict T_2 values when $\mathcal{S}^2$ is very small, the T_2 values are likely to be inaccurate.

We now present some examples of the model-free analysis of relaxation data when some of the internal motions are not fast while others are in the extreme narrowing limit. To illustrate this point, let us consider the model of Brainard and Szabo[6] for the motion of a carbon nonrigidly attached to a spherical macromolecule in such a way that the C–H vector can rotate freely about an axis d, which in turn is allowed to wobble within a cone of semiangle θ_0 about a director $\vec{d}$ attached to the macromolecule. The angle between the C–H vector and $\vec{d}$ is fixed at a value β. The correlation time for the motion about $\vec{d}$ is $\tau_{\parallel}$, and $\tau_{\perp}$ is the correlation time for the wobbling of the d axis. Exact NMR data were generated for $\beta = 70.5°$, $\theta_0 = 30°$, $\tau_M = 10$ ns, $\tau_{\perp} = 100$ ns, and $\tau_{\parallel} = 10$ ps by using eq 15 of ref 6. The order parameter for this model is 0.072. For no motion of the d axis, $\mathcal{S}^2 = 0.11$. Fitting T_1 and NOE values at 25 and 90 MHz by using the model-free approach, we obtained $\tau_e = 30$ ps and $\mathcal{S}^2 = 0.11$, which correspond to no motion of $\vec{d}$. Addition of T_2 information gives virtually identical results. In this case, for one internal motion, the slow internal motion is "invisible". We then considered the Brainard–Szabo model with all the parameters as in the above example except that $\tau_{\perp}$ was set at a value of 1 ns. Fitting T_1 and NOE values at both fields, we obtained $\mathcal{S}^2 = 0.089$ and $\tau_e = 40$ ps. The addition of T_2 information gives $\mathcal{S}^2 = 0.079$ and $\tau_e = 41$ ps. The effective correlation time appears to be fast, but the order parameter has a value that is intermediate between 0.11 (corresponding to rotation about the d axis only) and 0.072 (corresponding to rotation and wobbling).

We now turn to the case where all the internal motions are not close to the extreme narrowing limit. In this case, both terms in the model-free expression for the spectral density depend on frequency. It is important to realize that, in general, the correlation function for internal motions is not accurately described by a single exponential. The consequences of the one-exponential approximation are particularly severe for motions that are not very restricted (small values of $\mathcal{S}^2$) or, irrespective of the spatial extent of the motions, if the time scale of the motions is slow. For relatively slow motions, we observed that, with very few exceptions, the trend is to predict values of $\mathcal{S}^2$ that are too large and values of τ_e that are too small.

Table IV shows the results obtained by using the model-free approach to analyze ^{13}C relaxation data generated by using Woessner's model[14] for $\tau_M = 10$ ns, $1/6D = 99.3$ ps, and $\beta = 70.5°$. For these relatively slow internal motions, it is still possible to extract fairly accurate values of $\mathcal{S}^2$ and τ_e (in error by 25% and 15%, respectively) from a set of T_1 and NOE measurements at two fields. The predicted values of T_2 are in good agreement with the exact values at both fields (with an error of 11% at high field and of 7% at low field).

Table V shows the results obtained by fitting NMR data for ^{13}C–^1H dipolar relaxation at 25.1 and 90.5 MHz for the diffusion in a cone model with $\tau_M = 10^{-8}$ s, $1/6D_w = 7.03$ ns and 0.703 ns, and two cone angles, $\theta_0 = 36.9°$ and 66.4°. In both cases, by fitting T_1 and NOE at both fields, one can reproduce very accurately the exact data. The values of $\mathcal{S}^2$ and τ_e extracted improve as the time scale of the motion gets faster. $\mathcal{S}^2$ is about 9% larger than its true value, $1/6D_w = 7.03$ ns. One predicts a value in error by only 1.7% by including T_2 information at both fields. Notice that τ_e extracted by fitting T_1 and NOE values at both fields ($1/6D_w = 7.03$ ns) is 3.80 ns. Inclusion of T_2 information at both fields in the fitting procedure for $1/6D_w = 7.03$ ns gives $\tau_e = 4.20$ ns, which is closer to the true value of τ_e. For faster internal motions, however, ($1/6D_w = 0.703$ ns) τ_e predicted by using T_1 and NOE information at both fields is 0.437 ns, i.e., only 1.6% shorter than its exact value. Inclusion of T_2 information has little or no effect on the extracted value of τ_e. For a cone angle of $\theta_0 = 66.4°$, the exact data can be reproduced, but the predicted values of $\mathcal{S}^2$ and τ_e are not accurate for $\tau_e = 9.9$ ns. $\mathcal{S}^2$ errs by a factor of 2, and inclusion of T_2 information does not improve this value. Using T_1 and NOE at both fields, one obtains $\tau_e = 6.80$ ns. The situation is better for the faster motion ($\tau_e = 0.99$ ns). In this case, the addition of T_2 information improves substantially the predicted value of $\mathcal{S}^2$ (the error is 16% vs. 73% for the prediction based on the T_1 and NOE information at two fields only). For

Table IV. Model-Free Analysis of ^{13}C Relaxation Data Generated by Using the Woessner Model with $\tau_M = 10$ ns, $1/6D = 99.3$ ps, and $\beta = 70.5°$

ω, MHz	NOE $(1 + \eta)$	T_1, ms	T_2, ms	$\mathcal{S}^2$	τ_e, ps
25	2.25 (2.34)	135 (135)	82.6 (88.8)	0.138 (0.111)	252 (298)
68	2.57 (2.39)	226 (231)	97.5 (110)		

[a] T_1 and NOE values at both fields were fitted; T_2's at both fields are predicted. Exact values are given in parentheses.

Table V. Model-Free Analysis of ^{13}C Relaxation Data from the Diffusion in a Cone Model with $\tau_M = 10$ ns (Exact Values Are in Parentheses)

θ_0	$1/6D_w$, ns	ω, MHz	NOE $(1 + \eta)$	T_1,[a] ms	T_2,[a] ms	$\mathcal{S}^2$ [a]	$\mathcal{S}^2$ [b]	τ_e,[a] ns	τ_e,[b] ns
36.9°	7.03	25	1.43 (1.42)	44.9 (44.7)	23.0 (23.5)	0.566 (0.518)	0.527 (0.518)	3.80 (4.44)	4.20 (4.44)
		90	1.19 (1.21)	262 (262)	31.6 (36.6)				
	0.703	25	1.64 (1.65)	65.2 (66.0)	30.0 (30.6)	0.530 (0.518)	0.522 (0.518)	0.437 (0.444)	0.435 (0.444)
		90	1.88 (1.86)	271 (274)	38.2 (39.0)				
66.4°	7.03	25	1.41 (1.42)	40.9 (41.2)	26.4 (27.0)	0.224 (0.0784)	0.175 (0.0784)	6.38 (9.93)	6.77 (9.93)
		90	1.18 (1.19)	235 (236)	40.0 (41.4)				
	0.703	25	2.40 (2.37)	68.8 (69.3)	51.1 (56.1)	0.135 (0.0784)	0.0911 (0.0784)	0.819 (0.993)	0.832 (0.993)
		90	1.67 (1.67)	170 (168)	79.0 (92.0)				

[a] Obtained by fitting NOE and T_1 at both fields. T_2 values are predicted. [b] Obtained by fitting all the relaxation data at both fields.

$1/6D_w = 0.703$ ns, the value of τ_e extracted by fitting T_1 and NOE at both fields is 0.819 ns, i.e., closer to its exact value (0.993 ns).

Tables IV and V show certain trends that extensive numerical investigations have confirmed for all the dynamical models studied. In general, one can fit NMR data from *any* model at all fields and arbitrary time scale and overall motion. The accuracy of the predicted values of $\mathcal{S}^2$ and τ_e, however, depends crucially on the spatial restriction and time scale of the motion. As the order parameter gets smaller, the accuracy of the prediction of the value of $\mathcal{S}^2$ gets worse. For a given order parameter, the accuracy of the prediction improves as the time scale of the motion gets faster. For motions that are not fast, we conjecture that up to values of τ_e such that $(\omega\tau_e)^2 \sim 1$, where ω is the largest relevant frequency determining the relaxation data, the order parameter and τ_e can be extracted from a set of measurements of T_1 and NOE at two fields with an error no larger than ~25%. For a ^{13}C Larmor frequency of ~90.5 MHz, one has $(\omega\tau_e)^2 \sim 1$ for $\tau_e \sim 350$ ps. For example, for the diffusion in a cone model, for $\tau_M = 10^{-8}$ s and $1/6D_w = 167$ ps, one predicts, using NOE and T_1 at 25.1 and 90.5 MHz, $\mathcal{S}^2 = 0.0898$ (exact value 0.0784) and $\tau_e = 218$ ps (exact value 236 ps).

In applications, one is sometimes interested in internal motions that are slow on the NMR time scale, i.e., $(\omega\tau_e)^2 > 1$. There seems to be no simple criterion to determine accurately the magnitude of the error involved. We estimate, however, that order parameters larger than ~0.3 can be predicted with an accuracy of ~30%. For order parameters larger than 0.5, the accuracy is likely to be better, probably ~15%.

It is also interesting to consider the question whether measurements at several different fields can provide more information on the dynamics of the relaxation process. Table VI shows the results obtained for the jump model of Wittebort and Szabo for the lysine side chain attached to a spherical macromolecule. For $\tau_M = 10^{-8}$ s, data were generated at eight different magnetic fields, corresponding to Larmor frequencies ranging from 15 to 100 MHz. The table shows the results obtained by fitting T_1 and NOE at two fields and the results obtained by fitting T_1 and NOE at eight fields. The exact values of τ_e range from 278 to 31.5 ps, and $\mathcal{S}^2$ from 0.33 to 0.0046. Remarkably, the values of τ_e and $\mathcal{S}^2$ extracted by fitting T_1 and NOE at 25.1 and 67.9 MHz and by fitting T_1 and NOE at eight fields are almost identical. The fitted values of T_1 and NOE and the predicted T_2's are also very close. For the β carbon, $\tau_e = 266$ ns and $\mathcal{S}^2 = 0.333$. The extracted values of $\mathcal{S}^2$ and τ_e are good, since $\mathcal{S}^2$ is large. For the γ carbon, however, the exact value of $\mathcal{S}^2$ is 0.00926 and the extracted value of $\mathcal{S}^2$ is about twice as big. The δ and ϵ carbons are in the fast-motion regime, and one can extract the values of τ_e and $\mathcal{S}^2$ accurately. This example indicates that the addition of information of data measured at a number of different frequencies does not change the values of $\mathcal{S}^2$ and τ_e predicted. Some of the motions in the above example are rather fast. However, considering motions 1 order of magnitude slower, i.e., the jump model of Wittebort and Szabo with $k_1 = k_2 = k_3$, $k_1^{-1} = 10^{-9}$ s, $\tau_M = 10^{-8}$ s, we obtained similar results.

B. Anisotropic Overall Motion. In subsection IIB we considered the application of the model-free approach to cases where the overall motion cannot be described by a single correlation time. The correlation function is factored in two terms, one describing the overall motion (eq 53) and the other for internal motions relative to the macromolecule, which has the usual model-free expression given by eq 32. In order to justify this approximation, we show first with an example that for anisotropic motions such "decoupling" of internal and overall motion is accurate. The expression for the correlation function for the diffusion in a cone model superimposed on anisotropic overall motion is given in the Appendix, eq A11. We now consider a correlation function of the form

$$C_{\text{decoupled}}(t) = \frac{1}{5}\sum_{b=-2}^{2} \exp\{-[6D_x + b^2(D_z - D_x)]t\} \times (d_{b0}^{(2)}(\beta_{MD}))^2[S_{\text{cone}}^2 + (1 - S_{\text{cone}}^2)e^{-t/\tau_e}] \quad (62)$$

which factors out the internal motion contribution. Equation 62 has a form similar to the isotropic case, except for the fact that the correlation function for the macromolecular motion has a more complicated structure. Table VII shows the results obtained by fitting ^{13}C NMR relaxation data generated at 25.1 and 90.5 MHz from eq A11 with a correlation function given by eq 62. D_x, D_z, and β_{MD} were held fixed while $\mathcal{S}$ and τ_e were allowed to vary. Exact data correspond to $1/6D_x = 10$ μs, $1/6D_z = 0.5$ μs, $1/6D_w = 2.16$ ns, $\theta_0 = 66.4°$ ($\beta_{MD} = 15°$) and $1/6D_w = 2.60$ ns, $\theta_0 = 36.9°$ ($\beta_{MD} = 55°$). Values of $1/6D_x$ in the range 1–20 μs and of $1/6D_z$ in the range 0.1–0.05 μs are typical of DNA fragments ~10^2 base pairs long.[18] The relaxation data can be reproduced well, and $\mathcal{S}^2$ and τ_e are predicted accurately in both cases. Notice that the two cases considered here are rather unfavorable, since the motions are relatively slow. For $\beta_{MD} = 15°$, the amplitude of the motion is rather large, and for $\beta_{MD} = 55°$, the order parameter for the total correlation function (i.e., the limit of the total correlation function as $t \to \infty$) given by eq A13 is extremely small (3.33×10^{-6}), since β_{MD} is close to the "magic angle". This numerical example shows that it is reasonable to separate the correlation function into a term describing the macromolecular motion and a term describing the internal motion. In applications, one has to determine the parameters for the overall motion from relaxation data for a nucleus attached to the macromolecular backbone. Such measurements may be actually available (e.g., data for α carbons in proteins in a helical conformation) or may

Table VI. Model-Free Analysis of ^{13}C Relaxation Data Generated by Using the Jump Model of Wittebort and Szabo[11] for a Lysine Side Chain with $\tau_M = 10$ ns, $k_1 = k_2 = k_3$, and $k_t^{-1} = 100$ ps (Exact Results Are in Parentheses)

carbon	ω, MHz	NOE[a] $(1+\eta)$	NOE[b] $(1+\eta)$	T_1,[a] ms	T_1,[b] ms	T_2,[a] ms	T_2,[b] ms	$\mathcal{S}^{2\,a}$	$\mathcal{S}^{2\,b}$	τ_e,[a] ps	τ_e,[b] ps
β	25	1.78 (1.80)	1.77	91.9 (92.1)	91.1	45.5 (45.1)	44.0	0.342 (0.333)	0.347 (0.333)	266 (278)	266 (278)
	68	2.27 (2.23)	2.27	228 (229)	228	53.7 (54.7)	53.1				
γ	25	2.83 (2.87)	2.84	214 (213)	216	191 (199)	193.4	0.0167 (0.00926)	0.0157 (0.00926)	212 (223)	210 (223)
	68	2.79 (2.72)	2.80	251 (254)	252	210 (225)	213				
δ	25	2.46 (2.53)	2.45	420 (416)	424	286 (299)	287	0.0328 (0.0278)	0.0334 (0.0334)	81.6 (89.8)	80 (80)
	68	2.83 (2.73)	2.83	564 (570)	574	319 (341)	320				
ϵ	25	2.75 (2.72)	2.75	1316 (1310)	1323	1060 (1086)	1070	0.00526 (0.00463)	0.0527 (0.00463)	30.4 (31.5)	30.2 (31.5)
	68	2.89 (2.93)	2.89	1502 (1509)	1512	1132 (1165)	1137				

[a] From fitting procedure using T_1 and NOE values at 25 and 68 MHz. T_2 values are predicted. [b] From fitting procedure using T_1 and NOE values at 15, 25, 38, 50, 68, 75, 90, and 100 MHz. T_2 values are predicted.

be simulated if the parameters for the motion of the macromolecule are known from other experiments (e.g., hydrodynamic or light-scattering measurements). One has to determine A, τ_1, and τ_2 in this fashion; subsequently, when data are interpreted for nuclei that have internal motions, the parameters for internal motions will be allowed to vary while A, τ_1, and τ_2 are held fixed.

Extensive numerical investigations showed that even in the case of the anisotropic motions, the internal motions can be adequately described by using the model-free approach. The accuracy of the predicted values of $\mathcal{S}^2$ and τ_e depends on the time scale of the motion. However, the procedure used in determining the parameters for the overall motion introduces an additional error in the approximate correlation functions, and the inclusion of T_2 information may improve slightly the accuracy of the predicted values of $\mathcal{S}^2$ and τ_e. We will briefly summarize the results of our numerical investigations and then present some illustrative examples.

The first case of interest is the one of extremely fast motions, i.e., motions such that eq 61 is satisfied to within ~2%, where now τ is defined as $\tau^{-1} = \tau_2^{-1} + \tau_e^{-1}$ (recall that we assume that τ_1 and τ_2 are defined in such a way that $\tau_1 > \tau_2$). In this case, a set of measurements (e.g., NOE and T_1) at one field can give accurate values of $\mathcal{S}^2$ and τ_e, and relaxation data at all other fields such that (4) is satisfied to within ~2% can be predicted accurately. For anisotropic overall motion, however, the inclusion of information at another field (e.g., T_1 and NOE) does slightly improve the extracted values of $\mathcal{S}^2$ and τ_e, probably due to the method employed in determining the parameters for the macromolecular motion. A simple general criterion to determine the accuracy of the predicted values of $\mathcal{S}^2$ for arbitrary values of τ_1 and τ_2 does not seem to exist; however, we estimate that as long as the overall motion is not too slow ($\tau_1 < 0.1$ μs), data at one field should give $\mathcal{S}^2$ values accurate to within ~5%, with the accuracy improving for larger order parameters. For fast internal motions, i.e., motions such that eq 61 is satisfied to within ~10%, one can extract accurate values of $\mathcal{S}^2$ and τ_e from a set of measurements (e.g., T_1 and NOE) at two fields. Again, the situation is slightly worse than in the isotropic case. We estimate that as long as $\tau_2 < 0.1$ μs, one can predict $\mathcal{S}^2$ with an error no larger than ~20%, with the error being substantially smaller for larger order parameters. By including T_2 information, one obtains virtually exact answers.

Slower motions follow a pattern similar to the one observed in the isotropic case. For motions such that $(\omega\tau_e)^2 \lesssim 1$, one can obtain fairly accurate values of $\mathcal{S}^2$ from a set of measurements at both fields including T_2 information. For large order parameters ($\mathcal{S}^2 \gtrsim 0.4$), one can obtain accurate values of $\mathcal{S}^2$ and τ_e for motions on all time scales.

Table VIII describes results obtained for the diffusion in a cone model superimposed to an overall anisotropic motion with $1/6D_x = 1$ μs, $1/6D_z = 0.05$ μs, and $\beta_{MD} = 15°$. Exact results were generated for ^{13}C–^1H dipolar relaxation at 25.1 and 90.5 MHz: for $\theta_0 = 66.4°$, $1/6D_w = 10.9$, 56.4 and 97.6 ps. For the fastest motion ($1/6D_w = 10.9$ ps), we obtain an accurate prediction of both $\mathcal{S}^2$ and τ_e just by fitting T_1 and NOE at high field. The extracted $\mathcal{S}^2$ value is slightly improved by fitting NOE and T_1 at both fields. For $1/6D_w = 56.4$ ps, using T_1 and NOE at both fields, one obtains $\mathcal{S}^2 = 0.116$ (the exact value is 0.0784), $\tau_e = 81.9$ ps (the exact value is 79.8 ps). By including T_2 information, one obtains $\mathcal{S}^2 = 0.0783$ and $\tau_e = 78.9$ ps. For the slowest motion ($1/6D_w = 97.6$ ps, $\tau_e = 137$ ps), one obtains $\mathcal{S}^2 = 0.2041$ and $\tau_e = 155$ ps by fitting just NOE and T_1 at both fields; i.e., without including T_2 information, the predicted value of the order parameter is about three times bigger than the exact value. The inclusion of T_2 information, however, allows a very accurate prediction of both $\mathcal{S}^2$ and τ_e that differs from their exact values by 0.1% and 1.5%, respectively.

We now show that the model-free approach for overall anisotropic motions is accurate even in cases where a distribution of correlation times has been invoked (e.g., for random-coil polymers). Table IX describes ^{13}C–^1H NMR relaxation data generated for the jump model of Wittebort and Szabo for a lysine side chain

Table VII. ^{13}C Relaxation Data Generated by Using the Diffusion in a Cone Model Superimposed on Anisotropic Overall Reorientation[a]

β_{MD}	ω, MHz	NOE $(1+\eta)$	T_1, ms	T_2, ms	$\mathcal{S}^2$	τ_e, ns
15°	25	1.67 (1.71)	46.1 (48.6)	0.34 (0.34)	0.0788 (0.0784)	2.83 (3.06)
	90	1.21 (1.27)	184 (195)	0.35 (0.35)		
55°	25	2.15 (2.10)	106 (107)	0.24 (0.24)	0.529 (0.518)	1.60 (1.64)
	90	1.31 (1.36)	307 (302)	0.24 (0.24)		

[a] Data determined by using $1/6D_x = 10$ μs, $1/6D_z = 0.5$ μs, $1/6D_w = 2.16$ ns, $\theta_0 = 66.4°$ (for $\beta_{MD} = 15°$), and $\theta_0 = 36.9°$ (for $\beta_{MD} = 55°$), fitted by using the "decoupled" correlation function of eq 62 where the only adjustable parameters were $\mathcal{S}^2$ and τ_e. T_2 values are predicted. The exact results are in parentheses.

Table VIII. Model-Free Analysis of ^{13}C Relaxation Data Generated by Using the Diffusion in a Cone Model Superimposed on Anisotropic Overall Reorientation[a]

$1/6D_w$, ps	ω, MHz	NOE $(1+\eta)$	T_1, s	T_2, ms	τ_e, ps	$\mathcal{S}^2$
10.9	25	2.78[d] (2.79)	2.90[d] (2.91)	3.25 (3.50)	15.6,[d] 15.5[b] (15.5)	0.0835,[d] 0.0787 (0.0784)
	90	2.97[d] (2.98)	3.24[d] (3.24)	3.25 (3.50)		
56.4	25	2.92[b] (2.94)	0.623[b] (0.621)	2.33[b] (3.46)	81.9,[b] 78.9[c] (79.8)	0.116,[b] 0.0783[c] (0.0784)
	90	2.93[b] (2.91)	0.663[b] (0.663)	2.33[b] (3.46)		
97.6	25	2.95[c] (2.95)	0.372[c] (0.366)	3.44[c] (3.44)	155,[b] 135[c] (137)	0.204,[b] 0.0783[c] (0.0784)
	90	2.86 (2.79)	0.409[c] (0.419)	3.44[c] (3.44)		

[a] Data determined by using $1/6D_x = 1$ μs, $1/6D_z = 0.05$ μs, $\beta_{MD} = 15°$, and $\theta_0 = 66.4°$. Exact results are in parentheses. The parameters for overall motion are $\tau_1 = 0.960$ μs, $\tau_2 = 0.183$ μs, and $A = 0.865$. [b] Results obtained by fitting NOE and T_1 at both fields. [c] Results obtained by fitting all relaxation data at both fields. [d] Results obtained by fitting NOE and T_1 at high field.

Table IX. Model-Free Analysis of ^{13}C Relaxation Data Generated by Using the Jump Model of Wittebort and Szabo for a Lysine Side Chain Attached to a Macromolecule Whose Overall Motion is Described by a Distribution of Correlation Times, with $k_1 = k_2 = k_3$, $k_1^{-1} = 100$ ps, $D_L = 2.5 \times 10^6$ s^{-1}, and $D_U = 5.0 \times 10^8$ s^{-1} [a]

carbon	ω, MHz	NOE $(1+\eta)$	T_1, ms	T_2, ms	$\mathcal{S}^2$	τ_e, ps
α	15.0	1.77 (1.75)	36.8 (37.0)	16.3 (14.1)	A = 0.509	τ_1 = 18.8 ns
	67.9	1.53 (1.52)	185 (184)	21.3 (17.8)		τ_2 = 1.11 ns
β	15.0	2.09 (2.09)	79.8 (80.3)	41.5 (33.9)	0.341 (0.333)	256 (278)
	67.9	2.29 (2.25)	213 (213)	52.9 (47.6)		
γ	15.0	2.86 (2.91)	226 (225)	202 (203)	0.0163 (0.00926)	206 (223)
	67.9	2.81 (2.75)	269 (271)	224 (227)		
δ	15.0	2.55 (2.61)	407 (406)	281 (257)	0.0325 (0.0278)	78.4 (89.8)
	67.9	2.82 (2.73)	580 (582)	325 (320)		
ε	15.0	2.77 (2.79)	1296 (1294)	1055 (990)	0.00520 (0.00496)	29.9 (31.5)
	67.9	2.92 (2.89)	1523 (1526)	1146 (1127)		

[a] NOE's and T_1's were fitted at both fields, T_2's are predicted. Exact values are enclosed in parentheses.

attached to a macromolecule undergoing an overall motion associated with a distribution of diffusion coefficients given by eq A14 with $D_L = 2.5 \times 10^6$ s^{-1} and $D_U = 5.0 \times 10^8$ s^{-1}. The motion of the α carbon in the laboratory frame is identical with the overall motion of the macromolecule. T_1 and NOE for the α carbon at two fields were fitted to a correlation function of the form of eq 32. The parameters for the overall motion were held fixed in the fitting procedure for carbons β through ε. Figure 3 shows the exact and approximate spectral densities for the overall motion plotted vs. the frequency ω. The two curves are very similar in shape but tend to coincide only at the frequencies that determine the relaxation data. The inset shows the exact and approximate distributions of diffusion coefficients. $p(D)$ is plotted vs. log D, where D is defined by $\tau = 1/6D$. The "double-δ-function" distribution is represented by two spikes whose height is proportional to the normalized probability of each value of D. The contrast between the similarity of the shape of the spectral density curves and the drastically different distributions is striking. It is also very interesting that the parameters for internal motion (predicted by using a simple double-δ-function distribution of diffusion coefficients for the overall motion) are excellent in the sense that the accuracy is very similar to that achieved for internal motions on the same time scale when the overall motion is isotropic.

Table X shows a comparison of the values of $\mathcal{S}^2$ and τ_e obtained in various ways from the relaxation parameters in Table IX. The exact $\mathcal{S}^2$ and τ_e, their values extracted from a fitting procedure,

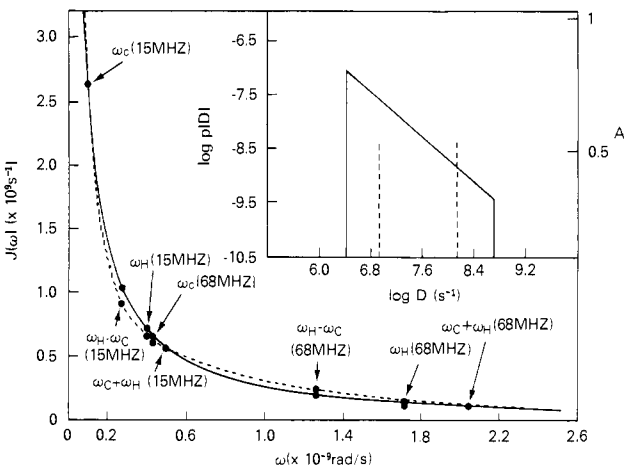

Figure 3. Comparison of the spectral density of eq A16 calculated by using a distribution of diffusion coefficients (eq A14) (solid line) and the spectral density corresponding to the double-exponential correlation function of eq 53 (dashed line) whose parameters (A, $\tau_1 = 1/6D_1$, and $\tau_2 = 1/6D_2$) were determined by fitting the relaxation data generated with eq A16. The inset shows the corresponding probability distribution, $p(D)$, plotted vs. log D. The double-δ-function distribution is represented by two spikes whose heights are proportional to the normalized probability of each value of D.

Table X. Comparison of the Generalized Order Parameters and Effective Correlation Times Extracted from ^{13}C NMR for the Jump Model of Wittebort and Szabo for a Lysine Side Chain Superimposed on Overall Reorientation Described by a Distribution of Correlation Times[a]

carbon	exact	from fitting procedure[d]	T_1 and NOE at 15 MHz[b]	T_1 and NOE at 68 MHz[b]	T_1's at 15 and 68 MHz[c]
			$\mathcal{S}^2$		
β	0.333	0.341	0.334	0.434	0.359
γ	0.00926	0.0163	0.0104	0.110	0.0349
δ	0.0278	0.0325	0.0278	0.0556	0.0345
ϵ	0.00496	0.00520	0.00457	0.00805	0.00544
			τ_e, ps		
β	278	256	240	193	200
γ	223	206	196	162	169
δ	89.8	78.4	82.1	70.0	74.0
ϵ	31.5	29.9	30.4	28.8	29.4

[a] The exact and fitted values of $\mathcal{S}^2$ and τ_e are compared to the values obtained from the data in Table XII by using the analytical formulas given in eq 59a, 59b, 60a, and 60b. [b] Evaluated by using eq 59a for $\mathcal{S}^2$ and 59b for τ_e. [c] Evaluated by using eq 60a for $\mathcal{S}^2$ and 60b for τ_e. [d] Obtained by fitting T_1 and NOE at 15 and 68 MHz as in Table IX.

and the results obtained by using the analytical formulas given in eq 59a, 59b, 60a, and 60b are presented. The values of $\mathcal{S}^2$ and τ_e extracted from T_1 and NOE at 15 MHz by using eq 60a and 60b, respectively, are excellent since at this frequency the motions of all the carbons are close to the extreme narrowing limit. The situation is different for the values of $\mathcal{S}^2$ and τ_e extracted from T_1 and NOE at 68 MHz; at this resonance frequency, only the motions for carbons δ and ϵ are not far from the extreme narrowing limit. The $\mathcal{S}^2$'s and τ_e's obtained from T_1's at both fields by using eq 59a and 59b, respectively, are better than the values obtained analytically from T_1 and NOE at high field, but not as accurate as the results from T_1 and NOE at low field. It is remarkable how good the general agreement between the values is, considering the simplicity of the analysis of this system where the motion is highly anisotropic. Of course the agreement between exact and calculated values would be even better for internal motions on a faster time scale. In summary, the trend in the accuracy of the extracted $\mathcal{S}^2$ and τ_e seems to be basically unaltered by the form of the correlation function for overall motion. The accuracy of $\mathcal{S}^2$ and τ_e depends essentially only on the magnitude of the order parameter and the time scale of internal motions. Even in the anisotropic case, for fast internal motions accurate values of $\mathcal{S}^2$ and τ_e can be extracted by using a straightforward fitting procedure or, for motions close to the extreme narrowing limit, from simple analytical formulas.

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Appendix

In this Appendix we describe the various dynamic models that we have used to generate relaxation data. We do this not only for the sake of completeness but also to contrast the complexity of the correlation functions or spectral densities of these models with the simplicity of eq 32 and 54, which are used in the model-free approach.

In Woessner's model,[14] a single free internal rotation is superimposed on isotropic reorientation. Specifically, the interaction vector $\hat{\mu}$ diffuses freely, with diffusion coefficient D, about a symmetry axis rigidly attached to the macromolecule. The angle β between $\hat{\mu}$ and the symmetry axis does not changes with time. The total correlation function is[14,10]

$$C(t) = \tfrac{1}{5}e^{-t/\tau_M}\sum_{b=-2}^{2}\exp(-b^2Dt)(d_{b0}^{(2)}(\beta))^2 \qquad (A1)$$

where $d_{b0}^{(2)}(\beta)$ is a reduced Wigner rotation matrix element.[12] The order parameter for this model is given by eq 23, and the effective correlation time is given by eq 28.

In the diffusion in the cone model, the unit vector $\hat{\mu}$, with orientation $\Omega = (\theta,\phi)$, diffuses freely in the angular region $0 \le \theta \le \theta_0$, $0 \le \phi \le 2\pi$ with diffusion coefficient D_w. The exact internal correlation function is

$$C_I(t) = S_{cone}^2 + \sum_{i=1}^{\infty}a_ie^{-b_iD_wt} \qquad (A2)$$

where

$$S_{cone} = \tfrac{1}{2}\cos\theta_0(1 + \cos\theta_0) \qquad (A3)$$

The coefficients a_i and b_i have been calculated numerically by Kinosita et al.[19] In our calculations we employed a truncated form of eq A2. For $\theta_0 = 66.4°$, the nonzero values of a_i and b_i are respectively 0.5523, 3.069; 0.2157, 8.827; 0.1295, 10.949; 0.0156, 21.559; 0.0014, 35.020; 0.0034, 36.648; and 0.0016, 54.62. For $\theta_0 = 36.9°$, the nonzero values of a_i and b_i are respectively 0.4224, 8.680; 0.339, 24.194; 0.0223, 35.463; 0.0015, 118.865; and 0.0007, 110.023. Although the coefficients a_i and b_i cannot be expressed as closed-form functions of θ_0, the effective correlation time, τ_e, has been shown[4] to equal

$$D_w(1 - S_{cone}^2)\tau_e = \sum_{i=1}a_ib_i^{-1} =$$
$$\chi_0^2(1 + \chi_0)^2\{\log[(1 + \chi_0)/2] + (1 - \chi_0)/2\}/[2(\chi_0 - 1)] +$$
$$(1 - \chi_0)(6 + 8\chi_0 - \chi_0^2 - 12\chi_0^3 - 7\chi_0^4)/24 \qquad (A4)$$

where $\chi_0 = \cos\theta_0$.

The jump model of Wittebort and Szabo[11] for the concerted motions of a lysine side chain can be described as follows. The possible configurations of the entire chain are limited to 24 discrete configurations on a diamond lattice as shown in Figure 6b of their paper. The rate matrix $\boldsymbol{R}$ describing the interconversion of their configurations by means of n-bond ($n = 1, 2, 3$) motions is represented in their Figure 8b. The orientations $\hat{\mu}_i$ (described by polar angles β_{NF}^i and α_{NF}^i) of the various ^{13}C–H vectors is contained in their Tables II, III, and IV.

By using their general jump formalism,[11] we can express the correlation function for the case in which the overall motion is isotopic in terms of the eigenvalues and eigenvectors of a *symmetrized* rate matrix, $\bar{R}$, i.e.,

$$\bar{R}\tilde{X} = -\lambda\tilde{X} \qquad (A5)$$

as

$$C(t) = \tfrac{1}{5}e^{-t/\tau_M}\sum_{n=1}^{24}e^{-\lambda_nt}\sum_{b=-2}^{2}|C_{bn}|^2 \qquad (A6)$$

with

$$C_{bn} = \sum_{l=1}^{24}d_{b0}^{(2)}(\beta_{NF}^l)\exp(ib\alpha_{NF}^l)\tilde{X}_l^{(0)}\tilde{X}_l^{(n)} \qquad (A7)$$

where $\tilde{X}^{(0)}$ is the eigenvector corresponding to $\lambda = 0$. Equation A6 can be shown to be equivalent to eq A8 by using the addition theorem for spherical harmonics.[12]

$$C(t) = \frac{1}{5}e^{-t/\tau_M}\sum_{n,k,l=1}^{24}e^{-\lambda_nt}\tilde{X}_l^{(0)}\tilde{X}_l^{(n)}\tilde{X}_k^{(0)}\tilde{X}_k^{(n)}P_2(\hat{\mu}_k\cdot\hat{\mu}_l) \qquad (A8)$$

The order parameter in this model is

$$\mathcal{S}^2 = \sum_{b=-2}^{2}|C_{b0}|^2 = \sum_{i,j=1}^{24}p_{eq}(i)P_2(\hat{\mu}_i\cdot\hat{\mu}_j)p_{eq}(j) \qquad (A9)$$

where $p_{eq}(i)$ is the probability that at equilibrium $\hat{\mu}$ assumes

position i. The effective correlation time in this model is

$$\tau_e(1 - \mathcal{S}^2) = \sum_{n=1}^{24} \lambda_n^{-1} \sum_{b=-2}^{2} |C_{bn}|^2 \quad \text{(A10)}$$

We now consider the situation where diffusion in a cone is superimposed on anisotropic overall motion. The macromolecule is assumed to be of cylindrical symmetry. D_x and D_z are the diffusion coefficients for reorientation of and about the C_∞ axis of the molecule, respectively. The unit vector $\hat{\mu}$ diffuses in a cone of semiangle θ_0 about a director, $\vec{d}$, which forms a fixed angle β_{MD} with the C_∞ axis of the cylinder. The correlation function for this model is[18]

$$C(t) = \frac{1}{5} \sum_{b=-2}^{2} \sum_{c=-2}^{2} \exp\{-[6D_x + b^2(D_z - D_x)]t\}(d_{bc}^{(2)}(\beta_{MD}))^2 G_c(t) \quad \text{(A11)}$$

with

$$G_c(t) = \langle D_{co}^{(2)*}(\Omega(0)) D_{co}^{(2)}(\Omega(t)) \rangle \quad \text{(A12)}$$

where Ω is the orientation of $\hat{\mu}$ relative to $\vec{d}$. In generating relaxation data by using this model, we have used the essentially exact expressions for $G_c(t)$ given by Lipari and Szabo.[25] The exact order parameter for this model is

$$\mathcal{S}^2 = S_{cone}^2 (P_2(\cos \beta_{MD}))^2 \quad \text{(A13)}$$

where S_{cone} is given by A3.

Finally, we consider the jump model of Wittebort and Szabo[11] for the motion of a lysine side chain for the situation where the overall motion is described by a distribution of correlation times (or equivalently, a distribution of diffusion coefficients since $\tau_M = (6D_M)^{-1}$). We use the normalized distribution function[22]

$$p(D_M) = [D_M \log (D_U/D_L)]^{-1} \quad D_L \le D_M \le D_U$$
$$= 0 \quad \text{otherwise} \quad \text{(A14)}$$

The spectral density is

$$J(\omega) = 2 \int_{D_L}^{D_U} p(D_M) \int_0^\infty C(t) (\cos \omega t)\, dt\, dD_M \quad \text{(A15)}$$

where $C(t)$ is given by eq A6.

Performing the integrations in eq A15 we obtain

$$J(\omega) = \frac{2}{5} \sum_{n=1}^{24} \sum_{b=-2}^{2} \left\{ |C_{bn}|^2 [\log (D_U/D_L) \times (\lambda_n^2 + \omega^2)]^{-1} \left[\omega \tan^{-1} \left(\frac{6(D_U - D_L)\omega}{\omega^2 + (6D_U + \lambda_n)(6D_L + \lambda_n)} \right) + \frac{\lambda_n}{2} \log \left(\frac{D_U^2(\omega^2 + (6D_L + \lambda_n)^2)}{D_L^2(\omega^2 + (6D_U + \lambda_n)^2)} \right) \right] \right\} \quad \text{(A16)}$$

The generalized order parameter is the same as in the case of isotropic overall motion (i.e., eq A9).

(25) Lipari, G.; Szabo, A. *J. Chem. Phys.* **1981**, *75*, 2971. Equation 2.7a of this paper should read

$$D_w \tau_0 = x_0^2(1 + x_0)^2\{...$$

Model-Free Approach to the Interpretation of Nuclear Magnetic Resonance Relaxation in Macromolecules. 2. Analysis of Experimental Results

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Abstract: In the preceding paper it has been shown that the unique dynamic information on fast internal motions in an NMR relaxation experiment on macromolecules in solution is specified by a generalized order parameter, $\mathcal{S}$, and an effective correlation time, τ_e. This paper deals with the extraction and interpretation of this information. The procedure used to obtain $\mathcal{S}^2$ and τ_e from experimental data by using a least-squares method and, in certain favorable circumstances, by using an analytical formula is described. A variety of experiments are then analyzed to yield information on the time scale and spatial restriction of internal motions of isoleucines in myoglobin, methionines in dihydrofolate reductase and myoglobin, a number of aliphatic residues in basic pancreatic trypsin inhibitor, and ethyl isocyanide bound to myoglobin, hemoglobin, and aliphatic side chains in three random-coil polymers. The numerical values of $\mathcal{S}^2$ and τ_e can be readily interpreted within the framework of a variety of models. In this way, one can obtain the same physical picture of internal motions as that obtained by using complicated spectral densities to fit the data. The numerical value of the order parameter, unlike the effective correlation time τ_e, plays a crucial role in determining what models can be used to describe the experiment; models in which the order parameter cannot be reproduced are eliminated. Conversely, any model that can yield the correct value of $\mathcal{S}$ works.

I. Introduction

In the preceding paper[1] (hereafter referred to as paper 1), we addressed the question of the information content of NMR relaxation data and of the extraction of this information. We presented a model-free approach to this problem, showing that the dynamic information on fast internal motions contained in an NMR experiment is essentially specified by two parameters: (1) a generalized order parameter, $\mathcal{S}$, which is a measure of the degree of spatial restriction of the motion, and (2) an effective correlation time, τ_e, which is a measure of the rate (time scale) of the motion. These two parameters were defined in a model-independent way. For both isotropic and anisotropic overall motion we derived expressions for the appropriate spectral density (which determines the observable quantities in the NMR relaxation ex-

† Deceased June 19, 1982.

(1) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.*, preceding paper in this issue.