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

Giovanni Lipari† and Attila Szabo*

Contribution from the Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205.
Received November 9, 1981

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.

periment) in terms of $\mathcal{S}$ and τ_e, without invoking a specific model for internal motions. In order to extract $\mathcal{S}$ and τ_e from experimental data, we treat these two quantities as adjustable parameters in the expression for the spectral density and vary them so as to least-squares fit the data. We then investigated the range of validity of our approach by analyzing a variety of relaxation data that were generated by using sophisticated dynamical models. Briefly, we found that the relaxation data can always be reproduced, and τ_e are virtually exact when the internal motions (not the overall motion) are in the extreme narrowing limit; they are fairly accurate ($\sim$25%) as long as $\omega\tau_e < 0.5$ and $\mathcal{S}^2 > 0.01$.

In this paper we consider the application of our model-free approach to the analysis of experimental data. This paper is largely self-contained, and references to paper 1 are kept to a minimum. In section II we tackle in general the problem of the interpretation of experimental data, discussing in detail how one can extract $\mathcal{S}$ and τ_e from a set of measurements and under what conditions one can expect the values of the generalized order parameter and the effective correlation time to be reliable. In section III we consider the interpretation of the generalized order parameter and of the effective correlation time. After the numerical values of $\mathcal{S}^2$ and τ_e have been determined without invoking any specific model for internal motions, one can use a variety of models to interpret the order parameter and the effective correlation time. We will see that the order parameter plays a crucial role in determining what models are going to be able to fit the data; models without sufficient flexibility to reproduce the numerical value of $\mathcal{S}^2$ extracted from the experiment are eliminated. Conversely, any model that can give the value of the order parameter will work. The rate of the internal motion does not place any restriction on possible models of the internal motions since any dynamical model contains at least an adjustable parameter related to the rate of the motion.

Section IV discusses the application of the model-free approach to the analysis of a wide variety of experimental data. We treat both isotropic and anisotropic overall motion. The generalized order parameter and the effective correlation time are extracted either by least-squares fitting the experimental data or, in favorable cases, by using simple analytical formulas. By using the numerically determined values of $\mathcal{S}^2$ and τ_e, we discuss librations, methyl rotations, restricted rotations of various kinds, segmental motions in long side chains, and the temperature dependence of internal motions. We show that for anisotropic overall reorientation a double exponential correlation function is adequate, even in cases where complicated distributions of correlation times have been invoked.

II. Extraction of the Generalized Order Parameter and the Effective Correlation Time from Experimental Data

The observable quantities in the NMR relaxation experiment are determined by certain linear combinations of the spectral density, $J(\omega)$, evaluated at different frequencies. $J(\omega)$ is given by

$$J(\omega) = 2\int_0^\infty C(t) \cos \omega t \, dt \tag{1}$$

where $C(t)$ is the appropriate correlation function. Heteronuclear dipolar relaxation expressions for T_1, T_2, and NOE in terms of $J(\omega)$ were given in paper 1, eq 7a–c. In the model-free approach we set

$$C(t) = C_O(t)C_I(t) \tag{2}$$

where $C_O(t)$ describes the overall motion and $C_I(t)$ is the correlation function for internal motion, given by

$$C_I(t) = \mathcal{S}^2 + (1 - \mathcal{S}^2)e^{-t/\tau_e} \tag{3}$$

where $\mathcal{S}^2$ ($0 \le \mathcal{S}^2 \le 1$) is the generalized order parameter and τ_e is an effective correlation time. These quantities are defined as follows:

$$\mathcal{S}^2 = \lim_{t\to\infty} C_I(t) \tag{4}$$

and

$$\tau_e = (1 - \mathcal{S}^2)^{-1}\int_0^\infty (C_I(t) - \mathcal{S}^2)\, dt \tag{5}$$

For overall isotropic motion, one has rigorously

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

where τ_M is the macromolecular correlation time. If the overall motion is anisotropic, we set

$$C_O(t) = \tfrac{1}{5}[Ae^{-t/\tau_1} + (1 - A)e^{-t/\tau_2}] \tag{7}$$

where A ($0 \le A \le 1$), τ_1, and τ_2 are adjustable parameters that can be determined from the relaxation data for a nucleus attached to the macromolecular backbone. Using eq 1, 2, 3, and 6, we readily obtain an expression for the spectral density in the case of overall isotropic motion

$$J(\omega) = \frac{2}{5}\left[\frac{\mathcal{S}^2\tau_M}{1 + (\omega\tau_M)^2} + \frac{(1 - \mathcal{S}^2)\tau}{1 + (\omega\tau)^2}\right] \tag{8}$$

where

$$\tau^{-1} = \tau_e^{-1} + \tau_M^{-1} \tag{9}$$

For overall anisotropic motion, from eq 1, 2, 3, and 7, one has for the spectral density

$$J(\omega) = \frac{2}{5}\left[\frac{A\mathcal{S}^2\tau_1}{1 + (\omega\tau_1)^2} + \frac{(1 - A)\mathcal{S}^2\tau_2}{1 + (\omega\tau_2)^2} + \frac{A(1 - \mathcal{S}^2)\tau'}{1 + (\omega\tau')^2} + \frac{(1 - A)(1 - \mathcal{S}^2)\tau''}{1 + (\omega\tau'')^2}\right] \tag{10}$$

where

$$(\tau')^{-1} = \tau_e^{-1} + \tau_1^{-1} \tag{11a}$$

$$(\tau'')^{-1} = \tau_e^{-1} + \tau_2^{-1} \tag{11b}$$

For a nucleus rigidly attached to an anisotropically reorienting macromolecular backbone, $\mathcal{S}^2 = 1$, and the expression for the spectral density given by eq 10 simplifies to

$$J(\omega) = \frac{2}{5}\left[\frac{A\tau_1}{1 + (\omega\tau_1)^2} + \frac{(1 - A)\tau_2}{1 + (\omega\tau_2)^2}\right] \tag{12}$$

When $\tau_1 = \tau_2$ or $A = 1$ in eq 12, one recovers $J(\omega)$ for isotropic overall motion if τ_1 is identified with τ_M.

The first step in the analysis of the experimental data is to establish the nature of the overall motion. For isotropic macromolecular motions, τ_M can be determined from hydrodynamic or light-scattering experiments or extracted from relaxation data for nuclei rigidly attached to the macromolecule. For anisotropic macromolecules or random-coil polymers, A, τ_1, and τ_2 have to be determined from relaxation data for a nucleus attached to the backbone. Once the parameters for the overall motion have been fixed, one can set out to extract the information on the internal motions. The procedure is operationally very simple. The values of $\mathcal{S}^2$ and τ_e uniquely determine T_1, T_2, and NOE via the spectral density given by eq 8 (for isotropic overall motion) or eq 10 (for anisotropic overall motion). One then only has to vary $\mathcal{S}^2$ and τ_e in such a way as to minimize the sum of the squared differences between the theoretical values and the experimental values. The formal definitions of $\mathcal{S}^2$ (eq 4) and τ_e (eq 5) play no role whatsoever at this stage of the analysis. $\mathcal{S}^2$ and τ_e are simply treated as adjustable parameters, the only constraint being that $\mathcal{S}^2$ can assume values between 0 and 1 and τ_e has to be positive. This procedure is easy to implement, since a number of efficient, user-oriented nonlinear least-squares fitting programs are readily available.[2] Once the numerical values of $\mathcal{S}^2$ and τ_e have been

(2) For the analysis presented in this chapter we used the subroutine STEPIT, by J. P. Chandler. The code can be obtained from QCPE, Chemistry Department, Indiana University, Bloomington, IN 47405 (Program No. 307).

extracted, one has to ascertain whether these quantities are meaningful; i.e., do the numerical values extracted from the fitting procedure agree with their actual values? In paper 1 we generated error-free "experimental" data using models for which the exact order parameter and effective correlation time are known. We then fitted these relaxation data using the model-free approach and compared the extracted $\mathcal{S}^2$ and τ_e to their exact values. Extensive numerical investigations allowed us to construct a set of empirical rules that, in principle, allow one to estimate the accuracy of $\mathcal{S}^2$ and τ_e determined from the fitting procedure by considering their numerical values, the resonance frequencies, and the parameters for overall motion. One should bear in mind that these rules are strictly valid in the limit of zero experimental error. Some aspects of this problem in the analysis of experimental data will be discussed in section IV.

The rules for isotropic motions can be briefly summarized as follows (in (i)–(iii) below, ω denotes the highest frequency that determines the observables, e.g., $\omega_C + \omega_H$ for ^{13}C dipolar relaxation):

(i) If $\tau_M \gg \tau_e$ ($\tau_e/\tau_M \leq 0.01$), the values of τ_e and $\mathcal{S}^2$ extracted from a set of two measurements (e.g., T_1 and NOE at one field or T_1's at two fields) are accurate to within a few percent as long as $\omega\tau_e \leq 0.1$ (e.g., for a ^{13}C frequency of 90 MHz, as long as $\tau_e \leq 50$ ps).

(ii) If $\tau_e/\tau_M \leq 0.1$, the values of $\mathcal{S}^2$ and τ_e extracted from a set of measurements at two fields (e.g., T_1 and NOE at two resonance frequencies) are fairly accurate (to within ~25%) as long as $\mathcal{S}^2 > 0.01$ and $\omega\tau_e \leq 0.5$ (e.g., for ^{13}C frequency of 90 MHz, as long as $\tau_e \leq 200$ ps).

(iii) If $\mathcal{S}^2 \geq 0.3$, the values of $\mathcal{S}^2$ and τ_e extracted from a set of measurements at two fields are fairly accurate (to within ~ 15%) with basically no restriction on the magnitude of τ_e, τ_M, or resonance frequency.

According to the above rules, if the value of τ_e extracted from the experimental data is sufficiently fast, then the corresponding order parameter is accurate. It should be emphasized that this conclusion was based on the analysis of data generated with the assumption that no internal motions are too far removed from the extreme narrowing limit. These rules are not applicable when some of the internal motions are neither in the extreme narrowing limit nor much slower than τ_M. For example, we have seen in paper 1, under certain circumstances τ_e can be short while the corresponding value of $\mathcal{S}^2$ is too large; i.e., the slower motions are only partially reflected in the value of the order parameter.

The rules for anisotropic motions are essentially identical with the above if τ_M is replaced by τ_2 (note that $\tau_1 > \tau_2$). A detailed discussion of these rules was given in paper 1, sections III and IV. Several examples of the use of these rules in applications are given later in this paper.

It is important to note that when rule i applies, the analysis of experimental data is particularly simple: $\mathcal{S}^2$ and τ_e can be extracted directly from any two relaxation parameters if the corresponding parameters for overall motion are known (see paper 1, eq 36 and 37). In particular, for T_1's at two fields (T_1 and $\tilde{T}_1$), one has

$$\mathcal{S}^2 = \frac{T_1^{-1} - \tilde{T}_1^{-1}}{(T_1^{-1})_O - (\tilde{T}_1^{-1})_O} \tag{13a}$$

$$\frac{\hbar^2\gamma_C^2\gamma_H^2}{r_{CH}^6}\tau_e = \frac{T_1^{-1}(\tilde{T}_1^{-1})_O - \tilde{T}_1^{-1}(T_1^{-1})_O}{T_1^{-1} - \tilde{T}_1^{-1} - (T_1^{-1})_O + (\tilde{T}_1^{-1})_O} \tag{13b}$$

For NOE and T_1 at one field, one has

$$\mathcal{S}^2 = \frac{(T_1)_O(2.988 - \mathrm{NOE})}{T_1(2.988 - \mathrm{NOE}_O)} \tag{14a}$$

$$\frac{\hbar^2\gamma_C^2\gamma_H^2}{r_{CH}^6}\tau_e = \frac{\mathrm{NOE} - \mathrm{NOE}_O}{T_1(2.988 - \mathrm{NOE}_O) - (T_1)_O(2.988 - \mathrm{NOE})} \tag{14b}$$

where $(T_1)_O$ and NOE_O are the relaxation parameters describing the overall isotropic or anisotropic motion. We shall present several applications of these simple expressions that show their utility and validity.

It is important to bear in mind that eq 13 and 14 above were derived under the assumption of the same ^{13}C–H distance in the expression for T_1 and $(T_1)_O$. If r_{CH} is the proton–carbon internuclear distance for the observed carbon and r_O is the internuclear distance for the backbone (α) carbon, eq 13 and 14 can be generalized to the case of different ^{13}C–H bond lengths by replacing $(T_1^{-1})_O$ with $(T_1^{-1})_O(r_O/r_{CH})^6$. For example, neutron diffraction studies[3a] on alanine give C_α–H = 1.091 Å and an average C_β–H distance of 1.096 Å. These values, which were corrected for rigid-body motion, correspond to $(r_O/r_{CH})^6 = 1.03$; i.e., the true value of $\mathcal{S}^2$ is 1.03 times the value found with eq 13a or 14a. Similar differences in C–H bond lengths were found in neutron diffraction experiments on serine.[3b]

Finally, we point out that in the above development and in the subsequent analysis of experimental data, we ignore cross-correlation effects that in general lead to a nonexponential decay of the magnetization. For ^{13}C relaxation of a methyl group, when both the motions of and about the symmetry axis are in the extreme narrowing limit, it has been shown[4] that neglect of cross-correlation can lead to T_1's that are underestimated by almost 20%. However, the case (which is relevant to the systems considered in this paper) where the overall motion is not in the extreme narrowing limit has apparently not been studied in the literature. If it is assumed that in this case the T_1's are also underestimated by 20% independent of field, then it follows from eq 13a that the $\mathcal{S}$'s obtained by ignoring cross-correlation effects are 20% smaller than their true values.

III. Interpretation of the Generalized Order Parameter and the Effective Correlation Time

Once numerical values of $\mathcal{S}^2$ and τ_e have been extracted from experimental data, one can consider their interpretation within the framework of various models for internal motions. We shall see that these quantities cannot be uniquely interpreted, and their values are, in principle, consistent with an infinite number of physical pictures of the motion. However, the value of $\mathcal{S}^2$ can be used to disprove certain models.

As we have shown in paper 1, the generalized order parameter can be expressed in terms of the equilibrium orientational distribution ($p_{eq}(\Omega)$) of the interaction vector $\hat{\mu}$ as

$$\mathcal{S}^2 = \int\int d\Omega_1 d\Omega_2 p_{eq}(\Omega_1) P_2(\cos\theta_{12}) p_{eq}(\Omega_2) \tag{15}$$

where $P_2(x)$ is the second Legendre polynomial, $\Omega_i = (\theta_i, \phi_i)$ describes the orientation of $\hat{\mu}_i$ and θ_{12} is the angle between $\hat{\mu}_1$ and $\hat{\mu}_2$ (i.e., $\hat{\mu}_1\cdot\hat{\mu}_2 = \cos\theta_{12}$). $\mathcal{S}$ is a model independent measure of the degree of restriction of the motion in the sense that $\mathcal{S}^2 = 1$ if there is no motion (i.e., $p_{eq}(\Omega) = \delta(\Omega - \Omega_0)$) and $\mathcal{S}^2 = 0$ when the motion is isotropic (i.e., $p_{eq}(\Omega)$ is independent of Ω). To describe completely the amplitude or degree of restriction of the motion, one would like to know $p_{eq}(\Omega)$. Clearly, the numerical value of $\mathcal{S}^2$ does not uniquely determine $p_{eq}(\Omega)$. Nevertheless, one may be interested in "modeling" $\mathcal{S}^2$ to obtain a simple (but not unique) picture of the motion. This amounts to assuming some functional form for $p_{eq}(\Omega)$ that may contain an adjustable parameter chosen to reproduce the numerical value of $\mathcal{S}^2$ obtained from experiment. We shall now present several examples of this approach.

A simple model for the motion of $\hat{\mu}$ is to assume that it diffuses within a cone of semiangle θ_0.[5,6] For this model,

$$\mathcal{S}_{cone} = \tfrac{1}{2}(\cos\theta_0)(1 + \cos\theta_0) \tag{16a}$$

(3) (a) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. *J. Am. Chem. Soc.* **1972**, *94*, 2657. (b) Frey, M. N.; Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. *Acta Crystallogr., Sect. B* **1973**, *B29*, 876.
(4) Werbelow, L. G.; Grant, D. M. *Adv. Magn. Reson.* **1977**, *9*, 189.
(5) Lipari, G.; Szabo, A. *Biophys. J.* **1980**, *30*, 489. Lipari, G.; Szabo, A. *J. Chem. Phys.* **1981**, *75*, 2971.
(6) (a) Kinosita, K.; Kawato, S.; Ikegami, A. *Biophys. J.* **1977**, *20*, 289. (b) Howarth, O. W. *J. Chem. Soc., Faraday Trans. 2* **1979**, *75*, 863. (c) Wang, C. C.; Pecora, R. *J. Chem. Phys.* **1980**, *72*, 5333.

It is clear that one can always choose a cone angle θ_0 so as to reproduce the order parameter. Specifically,

$$\theta_0 = \cos^{-1}\left[\tfrac{1}{2}((1 + 8\mathcal{S}_{\text{cone}})^{1/2} - 1)\right] \tag{16b}$$

if one assumes $0 \leq \theta_0 \leq 90°$. Thus, one can always model the motion as diffusion in a cone, irrespective of whether it makes sense physically. For example, the diffusion-in-the-cone model is not unreasonable in describing the librational motion of an α carbon. However, one would not use it for the motion of the ^{13}C–H vector in a methyl group.

The simplest model for the motion of a methyl group is to assume that the ^{13}C–H vector diffuses freely or jumps among three equivalent sites about a symmetry axis.[7] If the angle between $\hat{\mu}$ and the symmetry axis is fixed at a value β, the order parameter for *both* models is the same and is given by

$$\mathcal{S}_{\text{Woessner}} = P_2(\cos\beta) \tag{17}$$

For a methyl group, assuming an ideal tetrahedral geometry, $\beta = 70.5°$ and so $\mathcal{S}^2$ is fixed at a value of 0.111. Small deviations from this ideal geometry have been observed; for example, neutron diffraction experiments on alanine[3b] gave $\beta = 69.8°$. The corresponding value of $\mathcal{S}^2$ is 0.103; i.e., a difference of $\sim 1\%$ in the bond angle brings about a change of 7% in the order parameter, indicating that $\mathcal{S}^2$ is a rather sensitive function of β. If the numerical value of $\mathcal{S}^2$ extracted from experiment is close to 0.111, then the motion is axially symmetric (free, three-equivalent-site jump or, more generally, motion in a potential with C_3 symmetry) about a fixed axis. If $\mathcal{S}^2$ is significantly different from 0.111, then the Woessner model can be eliminated.

The generalized order parameter for a methyl group whose rotational motion is axially symmetric is given by

$$\mathcal{S}^2 = \mathcal{S}_{\text{axis}}^2(P_2(\cos\beta))^2 = 0.111\mathcal{S}_{\text{axis}}^2 \tag{18}$$

where $\mathcal{S}_{\text{axis}}$ is the generalized order parameter for the motion of the symmetry axis of the methyl group (e.g., the C_α–C_β bond of alanine). In deriving eq 18, it has been assumed that the rotation of the methyl group and the motion of the axis are not coupled. Since $0 \leq \mathcal{S}_{\text{axis}}^2 \leq 1$, eq 18 predicts that $\mathcal{S}^2$ for an axially symmetric rotating methyl group must be ≤ 0.111. If the value $\mathcal{S}^2$ extracted from experiment is greater than 0.111, then the rotations of the methyl group must be slow on the NMR time scale and/or some relaxation mechanism other than dipolar must be operative. If $\mathcal{S}^2 < 0.111$, then $\mathcal{S}_{\text{axis}}^2$ can be calculated by using eq 18 and then modeled in various ways. For example, within the diffusion-in-the-cone model, the value of $\mathcal{S}_{\text{axis}}^2$ can be converted into a cone angle θ_0 via eq 16b.

An alternate model of the motion of the symmetry axis is the restricted-diffusion model.[8,9] This model is reasonable for the motion of the C_{δ_1} methyl group of an isoleucine residue: the C_{δ_1}–C_{γ_1} axis reorients because of restricted rotation about the C_β–C_{γ_1} bond. This model can be described more precisely as follows: the angle between the symmetry axis of the methyl group and a director $\hat{d}$ is fixed at a value β', and the rotation about the director is restricted to the angular range $\pm\gamma_0$. The order parameter for the symmetry axis of the methyl group (i.e., $\mathcal{S}_{\text{axis}}^2$) within this model is given by

$$\mathcal{S}_{\text{axis}}^2 = \mathcal{S}_{\text{rest}}^2 = (P_2(\cos\beta'))^2 + \frac{3\sin^2\beta'\sin^2\gamma_0}{\gamma_0^2}\left(\cos^2\beta' + \frac{1}{4}\sin^2\beta'\cos^2\gamma_0\right) \tag{19}$$

If $\mathcal{S}_{\text{axis}}^2$ is determined from the numerical value of $\mathcal{S}^2$ extracted from the data by using eq 18, γ_0 can be obtained by using eq 19 if the value of β' is fixed by the geometry of the residue (e.g., $\beta' = 70.5°$ for isoleucine and $\beta' = 80°$ for methionine). It is interesting to note that the minimum value of $\mathcal{S}_{\text{rest}}^2$ is $(P_2(\cos\beta'))^2$. This occurs when $\gamma_0 = \pi$ and corresponds to free rotation. Thus, if $\mathcal{S}^2 < (P_2(\cos\beta'))^2(P_2(\cos\beta))^2$, then the model cannot be used to interpret the data. That is, the motion of the symmetry axis of the methyl group cannot be described as free rotation about a symmetry axis d.

The interpretation of the generalized order parameters of various carbons in a long side chain such as that of lysine is more complicated. Consider the nth carbon in an aliphatic side chain (the α carbon corresponds to $n = 0$). If it is assumed that the reorientation about the successive internal rotation axes are free and independent (i.e., Wallach's generalization[10] of Woessner's model[7]), then

$$\mathcal{S}_n^2 = (P_2(\cos\beta))^{2n} \tag{20}$$

where β is the angle between successive rotation axes. Equation 20 predicts that $\mathcal{S}^2$ decreases monotonically as one goes out the side chain. More specifically, for an aliphatic side chain where $\beta = 70.5°$, eq 18 shows that $\mathcal{S}^2$ successively decreases by a factor of 0.111. If the values of $\mathcal{S}^2$ extracted from experiment do not follow this pattern, then the Wallach model can be ruled out and one can conclude that steric interactions are important and/or motions about different bonds are not independent. It is particularly interesting to note that $\mathcal{S}^2$ need not decrease monotonically as one goes out the side chain. Such behavior is predicted by the molecular dynamics calculations of Levy et al.[11] on a pseudoaliphatic side chain and also by the concerted jump model for a lysine side chain formulated by Wittebort and Szabo[8] (see paper 1).

The effective correlation time τ_e is determined by the area under the correlation function for internal motion (see eq 5). In this sense, it is a model-independent quantity. It is not possible, however, to relate τ_e to some physical aspects of the internal motion in a model-independent way. The connection between τ_e and the microscopic diffusion constants or rates can only be established within the framework of a particular model; moreover, the value of τ_e depends on both the microscopic time constants and the spatial nature of the motion. For example, in Woessner's model,[7] for $\beta = 70.5°$, $\tau_e = (2.0 \times D)^{-1}$, where D is the diffusion coefficient for rotation of the interaction vector about the symmetry axis. For the diffusion in a cone model, for a cone semiangle $\theta_0 = 37°$, $\tau_e = (9.5 \times D_w)^{-1}$, where D_w is the wobbling diffusion constant. For $\theta_0 = 78°$, however, $\tau_e = (3.9\ D_w)^{-1}$.

In general, any dynamical model contains at least one adjustable rate parameter that is intrinsically flexible, since geometric constraints such as the ones on the order parameter in certain models do not limit the possible values of the rate. Thus, τ_e can be reproduced in any model. In models with more than one adjustable rate parameter, τ_e can be reproduced in an infinite number of ways.

Bearing these considerations in mind, in certain simple cases one can set out to interpret the numerical value of τ_e within some model. For example, Woessner's model could be used to describe the rotation of a methyl group whose symmetry axis is fixed relative to the macromolecule; the value of the diffusion coefficient D could be extracted from τ_e by using eq 28 of paper 1, which expresses τ_e on terms of the parameters of Woessner's model (D and $\beta = 70.5°$ for a methyl group). Similarly, one could use the diffusion in a cone model to describe the librational motion of an α carbon in a polypeptide. After having determined the cone semiangle θ_0 from $\mathcal{S}^2$ via eq 16b, one could calculate the value of the diffusion coefficient for the wobbling motion, D_w, by using eq A4 of paper 1.

Let us consider a more complicated case. A simple model for the motion of a methyl group of an alanine residue in a globular protein assumes that the methyl group undergoes free rotation about the C_α–C_β bond superimposed on wobbling in a cone motion of the methyl symmetry axis. An approximate expression for the

(7) Woessner, D. E. *J. Chem. Phys.* **1962**, *36*, 1.
(8) Wittebort, R. J.; Szabo, A. *J. Chem. Phys.* **1978**, *69*, 1722.
(9) London, R. E.; Avitabile, J. *J. Am. Chem. Soc.* **1978**, *100*, 7159.
(10) Wallach, D. *J. Chem. Phys.* **1967**, *47*, 5258.
(11) Levy, R. M.; Karplus, M.; Wolynes, P. G. *J. Am. Chem. Soc.* **1981**, *103*, 5998.

Table I. Model-Free Analysis of ^{13}C NMR Relaxation Data for Methyl Carbons in Basic Pancreatic Trypsin Inhibitor (τ_M = 4 ns)

residue	ω, MHz	NT_1,[a] s	NOE[a] $(1+\eta)$	$\mathcal{S}^2$	τ_e, ps	θ_0,[b] deg
Ala-16,40	25.1	0.480 (0.480)	2.27	0.039	55	46 (30)
	90.5	0.780 (0.780)	2.71			
Ala-25	25.1	0.456 (0.465)	1.98 (1.90)	0.058	38	37 (30)
	90.5	0.979 (0.960)	2.50			
Ala-27	25.1	0.438 (0.450)	2.40 (2.30)	0.035	70	48 (50)
	90.5	0.646 (0.630)	2.76			
Ala-48	25.1	0.449 (0.450)	2.01 (2.00)	0.057	40	37 (30)
	90.5	0.932 (0.930)	2.53			
Ala-58	25.1	0.900 (0.900)	2.52	0.013	37	62 (60)
	90.5	1.200 (1.200)	2.84			
Ile-18γ	25.1	0.610 (0.645)	2.29 (2.10)	0.030	43	51 (50)
	90.5	0.976 (0.930)	2.73			
Ile-19γ	25.1	0.621 (0.645)	1.94 (1.80)	0.044	25	43 (50)
	90.5	1.397 (1.350)	2.46			
Ile-18δ	25.1	1.130 (1.155)	2.19 (2.10)	0.018	20	58 (60)
	90.5	1.960 (1.920)	2.67			
Ile-19δ	25.1	0.900 (0.870)	2.02 (2.20)	0.028	20	52 (50)
	90.5	1.850 (1.920)	2.54			
Leu-6	25.1	0.944 (0.930)	2.03 (2.10)	0.026	19	53 (50)
	90.5	1.918 (1.950)	2.55			
Met-52	25.1	0.960 (0.975)	2.16 (2.10)	0.022	23	55 (60)
	90.5	1.704 (1.680)	2.66			

[a] Experimental data from Richarz et al.[13] fitted by using the model-free approach. Experimental values are in parentheses. [b] Cone semiangle (θ_0) describing the wobbling of the methyl-group symmetry axis calculated from $\mathcal{S}^2$ by using eq 18 and 16b. The θ_0's in parentheses were obtained by Richarz et al.[13] by using an approximate spectral density derived for a model in which free methyl rotation is superimposed on wobbling in a cone to fit the data.

correlation function for this model was given by Brainard and Szabo.[12] The value of τ_e in this model is

$$\tau_e = (1 - \mathcal{S}^2)^{-1}\{(1 - \mathcal{S}_{cone}^2)\tau_\perp + 2\sum_{b=1}^{2}[\mathcal{S}_{cone}^2 6\tau_\parallel/b^2 + (1 - \mathcal{S}_{cone}^2)(\tau_\perp/(1 + (b^2/6)(\tau_\perp/\tau_\parallel - 1)))]f_b(\beta)\} \quad (21)$$

where $\tau_\parallel$ is the correlation time for the methyl rotation, $\tau_\perp$ is the correlation time for the wobbling of the methyl axis, $f_1(\beta) = {}^3/_2 \sin^2\beta\cos^2\beta$, and $f_2(\beta) = {}^3/_8 \sin^4\beta$ ($\beta = 70.5°$ for a methyl group). From the structure of eq 21, it is obvious that infinite pairs of $\tau_\parallel$ and $\tau_\perp$ could give the same value of τ_e. Since only τ_e (and $\mathcal{S}^2$) essentially determine the relaxation data, if one used the complete dynamical picture of the model to extract $\tau_\parallel$ and $\tau_\perp$, these would not be uniquely determined, since any pair $(\tau_\parallel,\tau_\perp)$ that gives the correct value of τ_e would fit the data. For example, if $\tau_\parallel = \tau_\perp$, one has

$$\tau_\perp = \tau_e(1 - \mathcal{S}_{cone}^2/9)/(1.67\mathcal{S}_{cone}^2 + 1) \quad (22)$$

In section IV, we show that the experimental relaxation data of Richarz et al.[13] can be fitted by virtually any combination of $\tau_\parallel$ and $\tau_\perp$.

IV. Analysis of Experimental Data

In this section we analyze a number of experimental relaxation data on proteins and random-coil polymers. We discuss, first, results for systems where the overall reorientation is isotropic. The anisotropic case will be treated subsequently.

A. Isotropic Overall Motion. Richarz et al.[13] measured ^{13}C NMR relaxation data for a number of side-chain methyl groups in basic pancreatic trypsin inhibitor (BPTI). T_1 and NOE data at 25 MHz and T_1 data at 90 MHz were reported. Table I shows the results obtained by fitting the experimental data, using the model-free approach. In this fitting procedure, we assumed, following Richarz et al.,[13] that the overall motion of the protein can be described by overall isotropic reorientation with a correlation time τ_M = 4 ns. This value was estimated from the relaxation data for the α-carbon envelope. Later, we will investigate the effect of different descriptions of the overall motion on the physical picture of internal motions.

The time scale of internal motion is in the range 19–70 ps. For motions of this kind, measurements at two fields are likely to yield fairly accurate values of the order parameter as discussed in section II, rules i and ii. The order parameters for all resonances are substantially smaller (by almost an order of magnitude) than the values expected for free methyl rotation ($\mathcal{S}^2 = [P_2(\cos 70.5°)]^2 = 0.111$), thus showing the presence of additional motional flexibility. A simple model of these motions assumes that the axis about which free methyl rotation occurs can wobble within a cone of semiangle θ_0. This model is quite reasonable for alanine residues: for these side chains, the wobbling axis coincides with the C_α–C_β axis. Since we assumed that, on a first approximation, the overall motion of the protein could be described as isotropic reorientation, the extent of motion of the C_α–C_β axis is a measure of the local flexibility of the protein backbone. The cone angle for the wobbling motion can be extracted from $\mathcal{S}$ by using eq 18 and 16b, with $\beta = 70.5°$. The values of θ_0 determined in this way are shown in Table I. We find that the terminal Ala-58 residue has a larger cone angle (62°) than any other alanine residue (37°–48°) and the backbone region near Ala-27 seems to have a somewhat larger mobility. A fairly substantial amount of flexibility near the C-terminal group Ala-58 and for the protein fragment 25–28 was predicted from molecular dynamics calculations.[14] Table I also shows the values of the cone angle for the wobbling motion of the methyl rotation axis determined by Richarz et al.[13] by using an approximate formulation of the dynamics of the free methyl rotation superimposed on the wobbling in a cone of the methyl rotation axis. These values are in very good agreement with those obtained simply from the order parameter.

We now investigate the influence of alternative descriptions of the overall motion of the protein on the values of $\mathcal{S}^2$ and τ_e determined by using the model-free approach. If we extract the value of τ_M from the relaxation data for the α-carbon envelope by using only T_1 and NOE data at both fields (line widths but no T_2's were measured), and assuming that the backbone of the protein is rigid and diffuses isotropically, we obtain τ_M = 5.2 ns, i.e., a value 30% larger than the one used in the fitting procedure whose results are shown in Table I. Fitting the data using this new value for τ_M we obtain results for τ_e and $\mathcal{S}^2$ that are essentially identical. This indicates that the parameters describing the internal motions are fairly insensitive to the value used for τ_M.

The data for the α-carbon envelope cannot be fitted exactly by using a single correlation time. However, the T_1 and NOE data can be fitted by using the model-free approach, giving τ_M = 4.53 ns, τ_e = 20.1 ps, and $\mathcal{S}_L^2 = 0.874$. The subscript L indicates that the order parameter reflects the average librational motion of the backbone. We then fitted the data for the methyl carbons by using a correlation function of the form

$$C(t) = {}^1/_5 e^{-t/\tau_M}(\mathcal{S}_L^2 + (1 - \mathcal{S}_L^2)e^{-t/\tau_e})C_1(t) \quad (23)$$

where $C_1(t)$ has the usual model-free form and $\mathcal{S}_L^2$, τ_e, and τ_M are fixed at the values given above. The use of a correlation function of the form given by eq 23 amounts to describing the internal motions superimposed on an overall isotropic reorientation *and* an average librational motion of the backbone. Moreover, eq 23 assumes that all the motions are independent. Note that a correlation function of the form given by eq 23 is equivalent to the correlation function used for anisotropic overall motions (see eq 2 and 7) if one identifies $\mathcal{S}_L^2$ with A, τ_M with τ_1, and $(\tau_M^{-1} + \tau_e^{-1})^{-1}$ with τ_2. The values of the order parameter for internal motion, $\mathcal{S}_{int}^2$, extracted by using the correlation function of the form of eq 23, are, to within a few percent, identical with the values of $\mathcal{S}^2$ (extracted by using a single overall correlation time) multiplied by $1/\mathcal{S}_L^2$. This shows that, as expected, the inclusion

(12) Brainard, J. R.; Szabo, A. *Biochemistry* **1981**, *20*, 4618.
(13) Richarz, R.; Nagayama, K.; Wüthrich, K. *Biochemistry* **1980**, *19*, 5189.
(14) (a) McCammon, J. A.; Gelin, B. R.; Karplus, M. *Nature (London)* **1977**, *267*, 585. (b) Karplus, M.; McCammon, J. A. *Ibid.* **1979**, *277*, 578.

Table II. Comparison of $\mathcal{S}^2$ and τ_e Extracted from the Data of Richarz et al.[13] in Various Ways

	$\mathcal{S}^2$					τ_e, ps				
residue	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*
Ala-16,40	0.039	0.039		0.044		55	53		54	
Ala-25	0.058	0.055	0.061	0.060	0.066	38	39	31	40	34
Ala-27	0.035	0.031	0.040	0.035	0.043	70	69	60	70	61
Ala-48	0.057	0.056	0.056	0.062	0.062	40	40	39	42	42
Ala-58	0.013	0.014		0.015		37	36		37	
Ile-18γ	0.030	0.023	0.036	0.026	0.039	43	46	32	47	33
Ile-19γ	0.044	0.040	0.048	0.044	0.052	25	27	17	28	19
Ile-18δ }	0.018	0.017	0.020	0.019	0.022	20	21	17	21	18
Ile-19δ }	0.028	0.031	0.023	0.034	0.026	20	18	27	19	28
Leu-6	0.026	0.028	0.025	0.031	0.027	19	18	22	19	23
Met-52	0.022	0.021	0.024	0.023	0.026	23	24	21	24	22

[a] Least-squares fitting all the data with $\tau_M = 4$ ns as in Table I. [b] From the T_1's alone using eq 13. $(T_1)_O$'s were calculated by assuming $\tau_M = 4$ ns (39.9 and 210 ms at 25.1 and 90.5 MHz, respectively). [c] From the T_1 and NOE at 25.1 MHz using eq 14. $(T_1)_O$ and NOE_O were calculated by assuming $\tau_M = 4$ ns (39.9 ms and 1.45, respectively). [d] As in *b*, but with the experimental T_1's for the α carbons (45 and 260 ms at 25.1 and 90.5 MHz, respectively). [e] As in *c*, but with the experimental T_1 and NOE for the α carbons at 25.1 MHz (45 ms and 1.40, respectively).

Table III. Analysis of the Data of Richarz et al.[13] within the Framework of a Model in which Free Methyl Rotation (with Correlation Time $\tau_\parallel$) Is Superimposed on Wobbling (with Correlation Time $\tau_\perp$) in a Cone of Semiangle θ_0[a]

residue	ω, MHz	NOE[c] $(1+\eta)$	NT_1,[c] ms	$\tau_\parallel$,[c] ps	$\tau_\perp$,[c] ps	$\mathcal{S}^2$ [b]
Met-52	25.1	2.17 (2.1)	0.958 (0.975)	17.2	17.2	0.0223
	90.5	2.65	1.71 (1.68)			(0.0223)
	25.1	2.17 (2.1)	0.959 (0.975)	7.51	75.1	0.0224
	90.5	2.65	1.71 (1.68)			(0.0223)
	25.1	2.20 (2.1)	0.944 (0.975)	37.0	3.7	0.0217
	90.5	2.56	1.73 (1.68)			(0.0223)
Ala-48	25.1	2.02 (2.0)	0.447 (0.450)	21.5	21.5	0.0562
	90.5	2.50	0.935 (0.930)			(0.0566)
	25.1	2.02 (2.0)	0.448 (0.450)	13.6	136	0.0564
	90.5	2.51	0.934 (0.930)			(0.0566)
	25.1	2.04 (2.0)	0.445 (0.450)	29.0	2.9	0.0556
	90.5	2.46	0.941 (0.930)			(0.0566)

[a] This table shows that the data does not uniquely determine both $\tau_\parallel$ and $\tau_\perp$. [b] $\mathcal{S}^2$ calculated from θ_0 as described in text. The values of $\mathcal{S}^2$ obtained by using the model-free approach in Table I are in parentheses. [c] The experimental data (in parentheses) were least-squares fitted by using the correlation function of Brainard and Szabo[12] with $\tau_M = 4$ ns, $\beta = 70.5°$, and θ_0, $\tau_\perp$, and $\tau_\parallel$ varied, keeping $\tau_\parallel/\tau_\perp$ fixed at 1, 0.1, and 10, respectively.

of a librational motion term in the correlation function simply corresponds to a "change-of-reference frame" for the internal motions: the internal motions are viewed as superimposed on the overall rotation of the molecule *and* on the "average" librational motion of the backbone rather than on the overall isotropic motion only. Since the librational motion and the internal motion were considered independent as a first approximation, the order parameter for internal motions in a macromolecule-fixed frame is the product of the order parameter $\mathcal{S}_L^2$ times the order parameter for internal motions viewed as superimposed on the average librational motion of the backbone. Motions of small amplitude, however, seem to have the dominant effect on the "average" librational motion: the C_α–C_β axis of the alanine residues wobbles considerably, even relative to the backbone. This follows from the fact that the values of $\mathcal{S}_{int}^2$ for the alanines are between 39% and 85% smaller than the value (0.103) calculated for methyl rotation alone by using neutron diffraction geometry.[3a]

So far in the analysis of the relaxation data on BPTI, we have used all the experimental information available to extract the order parameter and the effective relaxation time using a least-squares method. According to rule i in section II, however, motions on this time scale are extremely fast, and, in principle, the values of $\mathcal{S}^2$ and τ_e could be extracted from eq 13a and 13b by using the T_1 values at both fields or from eq 14a and 14b by using T_1 and NOE values at low field. It is interesting to investigate the validity of this rule for a case where experimental error is present. Table II shows the results of this analysis and compares the values of $\mathcal{S}^2$ and τ_e obtained analytically to those extracted from the fitting procedure. The agreement is very good for all the resonances. The fact that the values of $\mathcal{S}^2$ and τ_e obtained by using only the T_1's and only the T_1 and NOE values at 25 MHz are so close clearly shows that the information content of the NMR experiment concerning fast internal motions can indeed be completely specified by $\mathcal{S}^2$ and τ_e and that the simple analytical formulas for extracting this information work remarkably well. The use of a larger experimental data set, however, does decrease the influence of experimental error. It is interesting to note that the values of $\mathcal{S}^2$ obtained by using the experimental relation data for the α-carbon envelope are about 10% larger than the values obtained by using $\tau_M = 4$ ns for the overall motion. This has a simple physical interpretation. Since the α carbons themselves move with respect to a frame rigidly attached to the macromolecule, the methyl groups are more restricted (i.e., larger $\mathcal{S}^2$) when viewed against the average librational motion of the α carbons. This agrees rather well with the picture of internal motions obtained by using a correlation function of the form of eq 23 (i.e., we found values of the order parameter ~13% larger than those extracted for internal motions superimposed on isotropic reorientation).

We finally investigated the amount of information that could by obtained on the rate of internal motions in BPTI by using a dynamical model in which the methyl group rotates freely about its axis, which, in turn, diffuses in a cone of semiangle θ_0. Specifically, we wished to establish whether the data could be uniquely interpreted to yield correlation times for both the methyl rotation and the "wobbling" of the axis. We used the approximate formulation of the above model due to Brainard and Szabo[12] (which has some advantages[12] over the formulation of the same model given by Richarz et al.[13]) to fit the experimental data. The required correlation function is obtained by specializing eq 15 of their paper[12] to the cone model. This model has two rate parameters: $\tau_\parallel$ for the free methyl rotation and $\tau_\perp$ for the wobbling in a cone of the methyl symmetry axis. Table III shows the results for two residues. The ratio $\tau_\parallel/\tau_\perp$ was held fixed at the values of 1, 0.1, and 10. In all cases we have been able to reproduce the experimental data. Note that the order parameters for the best fit for this model, calculated from the values of the semiangle ($\mathcal{S}^2 = 0.111 \times 0.25 \times (\cos^2\theta_0)(1 + \cos\theta_0)^2$) are virtually identical with the ones extracted by using the model-free approach, whereas the $\tau_\parallel$ and $\tau_\perp$ values vary widely. When $\tau_\parallel = \tau_\perp$, one can calculate these parameters via eq 22 using τ_e and $\mathcal{S}^2$ given in Table I ($\mathcal{S}_{cone}^2 = 9\mathcal{S}^2$). For Met-52 we find $\tau_\parallel = \tau_\perp = 16.9$ ps, and for Ala-48 we obtain $\tau_\parallel = \tau_\perp = 20.6$ ps, in excellent agreement with the values in Table III. The above clearly shows that $\mathcal{S}^2$ and τ_e do indeed completely specify the unique information on fast internal motions contained in the NMR experiment.

In summary, the detailed application discussed above illustrates the advantages of the model-free approach extremely well. The approach is simple to use: one does not have to bother with often rather complicated spectral densities derived in the framework of a model. In favorable cases, one can even extract $\mathcal{S}^2$ and τ_e directly from the data by using analytical formulas (i.e., eq 13 and 14). Once one obtains numerical values of $\mathcal{S}^2$ and τ_e, one can, if one wishes to, interpret them within the framework of a particular model. It is considerably simpler to evaluate $\mathcal{S}^2$ in a model than to derive the entire correlation function (or spectral density). In this way, one can get the same physical picture as that obtained by directly least-squares fitting the data using

Table IV. ^{13}C NMR Relaxation of C_{δ_1} Carbons of Isoleucine Residues in Sperm Whale Myoglobin at 67.9 MHz (τ_M = 16 ns)

chemical shift, ppm	NT_1,[a] s	NOE[a] $(1+\eta)$	$\mathcal{S}^2$	τ_e, ps	γ_0,[b] deg	θ_0,[b] deg
14.16	3.05	1.32	0.12	1.6		
13.17	1.95	1.82	0.13	10		
12.85	2.46	2.27	0.066	12	45 (50)	33
12.51	2.34	2.23	0.073	13	40 (50)	30
11.75	2.18	1.31	0.17	2.1		
11.40	2.13	2.39	0.063	16	46 (50)	34
9.80	2.06	2.16	0.091	14	27 (30)	21
9.20	2.13	2.19	0.084	13	32 (30)	24

[a] Data from Wittebort et al.[16] The $\mathcal{S}^2$ and τ_e values were determined from eq 14a and 14b. [b] Angular range of restricted diffusion extracted from $\mathcal{S}^2$ by using eq 18 and 19 with $\beta = \beta' = 70.5°$. The values obtained by Wittebort et al.[16] by using the complete dynamical formulation of the model are in parentheses. [c] Value of cone semiangle extracted from $\mathcal{S}^2$ by using eq 18 and 16b with $\beta = 70.5°$.

complicated spectral densities. However, one does not lose sight of the fact that this picture is not unique and is prevented from overinterpreting the data (e.g., obtaining two different rate parameters that are meaningless).

Recently Ribeiro et al.[15] also reported T_1's, T_2's, and NOE's, obtained at two magnetic fields, for a large number of carbon resonances of BPTI. In contrast to the data of Richarz et al.,[13] we have not been able to fit all their data for the two frequencies simultaneously using the model-free approach. The difficulty can be traced to their reported values of the T_2's. If we consider only T_1 and NOE data at both frequencies, we obtain good fits of the experimental data, and the values of $\mathcal{S}^2$ and τ_e are similar to the values extracted from the data of Richarz et al.[13] The T_2's that are predicted in this way agree with the values reported by Ribeiro et al.[15] to within a factor of 2. Ribeiro et al.[15] fit their data by using a total correlation function that is a sum of three exponentials with normalized coefficients (eq 57 of paper 1 for $M = 3$ gives the corresponding spectral density). One of the exponents reflecting the overall motion was held fixed at a value determined independently from light-scattering experiments. The remaining adjustable parameters turn out to be slightly frequency dependent. For most resonances, in order to reproduce the T_1, NOE, and T_2 values simultaneously at a single frequency, Ribeiro et al. are forced to use a very slow component (approximately 60 times slower than the overall motion) in the total correlation function. The very long correlation time is crucial in reproducing the T_2 values that are essentially determined by slow motions. Anisotropic overall motion could give rise to a slow component that would be the same for all the resonances. In view of the structure of the BPTI molecule (axial ratio $\sim$ 3), we estimate that the slow and fast diffusion coefficients for the overall motion should differ by a factor of $\sim$3. As discussed in paper 1, it is impossible to have in the total correlation function a component that decays more slowly than the slowest component resulting from the overall motion, as long as the internal and overall motions are independent, as is explicitly assumed in the formalism of Ribeiro et al. This has important consequences: first, the attribution of the slow-motional component to a slow-warping motion of the protein backbone is simply an artifact of their analysis; second, no correlation function represented as a sum of exponentials (even an infinite number) could reproduce the T_1, NOE, and T_2 data simultaneously if the proper constraints (i.e., no component can be slower than the isotropic overall) are used, since such constraints would rule out the possibility of having the rather small exponent required to reproduce the T_2 values correctly.

Wittebort et al.[16] presented natural-abundance ^{13}C NMR relaxation data for eight isoleucine residues in sperm whale myoglobin. Spin–lattice relaxation times and NOE's were measured at 68 MHz for eight well-resolved resonances between 9 and 15 ppm assigned to C_{δ_1} of isoleucine residues. Table IV presents the results of the model-free analysis of these data. We used eq 14a and 14b to extract $\mathcal{S}^2$ and τ_e. The overall motion was assumed to be isotropic, with τ_M = 16 ns (i.e., $(T_1)_O$ = 412 ms and NOE_O = 1.16). Identical results are obtained if the data are least-squares fitted, using $\mathcal{S}^2$ and τ_e as adjustable parameters. The effective correlation times are in the range 1.5–15.7 ps. Since internal motions on this time scale monitored at a Larmor frequency of 68 MHz can be considered extremely fast (rule i, section II), the values of $\mathcal{S}^2$ and τ_e should be meaningful (if the experimental error is small and no relaxation mechanisms other than dipolar are operative (see below)).

In order to make contact with the analysis of Wittebort et al., we now model the value of the order parameters using a physically plausible description of the motion of the $^{13}C_{\delta_1}$–H bond. In myoglobin, the hydrophobic isoleucine side chains are substantially completely buried in the protein interior, and the packing density around these chains is high. This circumstance is likely to limit rather severely the configuration space spanned by the side chains. Rapid methyl rotations about the C_{γ_1}–C_{δ_1} bond, however, can occur without modifying the chain configuration and are not likely to be hindered. In addition, in myoglobin, eight of the nine isoleucine residues are in helical regions; rotations about the C_α–C_β and C_β–C_{γ_1} bonds are restricted by the helical backbone. The allowed amplitude of rotation about the C_α–C_β bond has to be very small (less than 30°) to prevent the collision of the C_{γ_2} methyl group with the backbone. A reasonable model, then, allows for free methyl rotation about the C_{γ_1}–C_{δ_1} bond, while rotation about C_β–C_{γ_1} is restricted. The rotation about the C_α–C_β bond is strongly hindered; therefore, it is not likely to have a sizable effect on the NMR relaxation and can be neglected on a first approximation. If the motions are independent, then from eq 18 one can determine the order parameter for the motion of the C_{γ_1}–C_{δ_1} axis by using the value of $\mathcal{S}^2$ extracted from the experimental data. The value of $\mathcal{S}_{axis}^2$ can in turn be interpreted by using a model for the restricted motion of the axis. Using the restricted diffusion model ($\mathcal{S}^2$ given by eq 19), one can extract the values of the allowed angular range $\pm\gamma_0$. Table IV shows the numerical values of γ_0 obtained in this way. The model of free methyl rotation superimposed on restricted diffusion was used by Wittebort et al.[16] to interpret their relaxation data. The values determined by these authors using the complicated spectral density[8,9] obtained within this model are given in parentheses in Table IV. These values were obtained from an approximate estimate rather than from a least-squares procedure (they only considered γ_0 = 20°, 30°, 50°, or 80°). The agreement is good. For the two resonances at 14.16 at 13.7 ppm, we obtained $\mathcal{S}^2$ = 0.123 and 0.135, respectively. These values are just slightly higher than the order parameter predicted for free methyl rotation and completely hindered rotation about the C_β–C_{γ_1} bond (γ_0 = 0°). This might be due to a small error in the experimentally determined T_1's and NOE's and/or might be an indication of the presence of another relaxation mechanism. The value of $\mathcal{S}^2$ extracted for the resonance at 11.75 ppm seems to negate the model proposed above, as found by Wittebort et al.[16] These authors suggest that in addition to dipolar relaxation, paramagnetic relaxation might be operative. It is interesting to note that within the model-free approach, it is only after modeling the order parameter that we recognize that dipolar relaxation alone may not be adequate to interpret the data in a physically reasonable way. For the other resonances, we obtained values of γ_0 roughly between 27° and 45° and values of 12.6–15.7 ps for τ_e. There is no unique way to relate the single effective correlation time for internal motions to the rate of the two motions considered in the above model. Wittebort et al.[16] obtained correlation times in the range 4–6.5 ps for the rotation of the C_{δ_1} methyl group and 12–20 ps for the motion about C_β–C_{γ_1}, but these pairs of values are certainly not unique. There exist an infinite combination of rates that yield the same value of τ_e.

The order parameter could also be interpreted in the framework of the wobbling-in-a-cone model by using eq 18 and 16b, which

(15) Ribeiro, A. A.; King, R.; Restivo, C.; Jardetzky, O. *J. Am. Chem. Soc.* **1980**, *102*, 4040.

(16) Wittebort, R. J.; Rothgeb, T. M.; Szabo, A.; Gurd, F. R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1059.

Table V. ^{13}C NMR Relaxation of C_ϵ Carbons of Methionine Residues in Bacterial Dihydrofolate Reductase at 25 MHz (τ_M = 20 ns)

chemical shift, ppm	NT_1,[a] s	NOE[a] $(1+\eta)$	$1/\pi T_2$,[a] Hz	$\mathcal{S}^2$ [a]	τ_e,[a] ps	γ_0,[b] deg	θ_0,[c] deg
15.37	2.16	2.2	1.6 (2.4)	0.015	12		60
15.24	1.20	1.5	4.7 (3.0)	0.051	7.4	53	40
14.95	1.23	1.5	4.6 (3.2)	0.050	7.2	54	40
14.74	0.999	1.5	5.7 (4.4)	0.062	9.0	45	35
14.57	1.38	1.5	4.1 (3.2)	0.045	6.4	58	43
14.18	1.11	1.6	4.8 (5.0)	0.052	10	53	40

[a] Data for enzyme-methotrexate complex from Blakley et al.[17] Experimental and theoretical values of NOE and T_1 obtained by using the model-free approach are identical to within the given figures. Only T_1 and NOE were included in the fitting procedure, since the estimate of T_2 values on the basis of line-width measurements alone is not likely to be accurate. The theoretical values of the line widths we report are predicted by using $\mathcal{S}^2$ and τ_e extracted from the fitting procedure. Measured line widths are enclosed in parentheses. [b] Angular range of restricted diffusion extracted from $\mathcal{S}^2$ by using eq 18 and 19 with $\beta' = 80°$ and $\beta = 70.5°$. [c] Cone semiangle extracted from $\mathcal{S}^2$ by using eq 18 and 16b.

gives cone angles in the range 21–34° for the angular range of the motion of the symmetry axis of the methyl group.

It is interesting to compare the internal motion of isoleucine residues in myoglobin (Table IV) and in BPTI (Table I). With the exception of the resonance at 12.51 ppm, the order parameters for isoleucine C_{δ_1} carbons in myoglobin are larger than the ones in BPTI, indicating that motions are more restricted in the heme protein. The values of τ_e are smaller in myoglobin, suggesting a greater relative importance of fast methyl rotations. Although it is intrinsically difficult to compare experiments performed under different conditions of temperature and concentrations, the difference in internal flexibility in the two proteins might be related to the difference in the secondary structure of the environments of isoleucine residues in BPTI and myoglobin. In the heme protein, eight out of the nine isoleucine residues are located in helical regions, whereas Ile-18 and -19 in BPTI are in the twisted-β-sheet region.

^{13}C NMR data for bacterial dihydrofolate reductase labeled by incorporation of [*methyl*-^{13}C]methionine have been obtained by Blakley et al.[17] T_1 and NOE ^{13}C NMR relaxation data were measured at a Larmor frequency of 25.1 MHz for the carbons of methionine residues for the enzyme alone and the complexes enzyme–methotrexate and enzyme–methotrexate–NADPH. Table V presents the results of the fitting procedure using the model-free approach for the enzyme–methotrexate binary complex. The relaxation behavior of the other two systems studied by Blakley et al.[17] is very similar, and our analysis of these data showed that the basic picture of the internal motions is the same in all three systems. Only T_1 and NOE values were included in the fitting procedure since no direct T_2 measurements were performed and T_2 values estimated from line widths are not likely to be accurate. The line widths given in Table V were predicted from the values of $\mathcal{S}^2$ and τ_e extracted from NOE and T_1 data alone ($w = (\pi T_2)^{-1}$). The overall motion of the enzyme was described as isotropic overall reorientation, with a correlation time τ_M = 20 ns given by Blakley et al.[17] The fitting procedure yields values of τ_e in the range 6.5–12 ps. Motions on this time scale at a resonance frequency of 25 MHz may be considered extremely fast according to rule i of section II, and one can extract accurate values of $\mathcal{S}^2$ and τ_e from a set of measurements of T_1 and NOE at one field. Indeed, the values of $\mathcal{S}^2$ and τ_e calculated by using eq 14a and 14b are identical. Blakley et al.[17] pointed out that a model involving only free methyl rotation could not explain the data. However, even a model that allows free rotation about the preceding CH_2–S bond is inadequate; therefore, Blakley et al.[17] considered a motional model where the free methyl rotation is superimposed on restricted rotation about the CH_2–S bond.[8,9] In Table V we present the values of γ_0, the allowed angular range for restricted diffusion, obtained from $\mathcal{S}^2$ by using eq 18 and 19. β' in eq 19 was taken to be 80°, since from the crystal structure of methionine, one derives a C_γ–S–C_ϵ angle of 100°.[17] The values of γ_0 are in the range 45–58° except for the resonance at 15.37 ppm. The order parameter for this resonance (0.015) is smaller than the value predicted for free methyl rotation superimposed on free diffusion about the C_γ–S bond (0.022). Thus, the motion of the C_ϵ–S bond must be less restricted than that one would expect from free rotation about the C_γ–S bond. An alternative model assumes wobbling in a cone of the C_ϵ–S bond; using eq 18 and 16b, we extract from $\mathcal{S}^2$ a value of $\theta_0 = 60°$. By modeling the order parameter in the same way for the other resonances, we obtain values of the cone semiangle ranging between 35° and 43°. London and Avitabile,[9] using the restricted diffusion model, reached similar conclusions. They did not analyze each individual reasonance in detail but presented illustrative values in a table. In one instance one can use their table to accurately fit the data; results for the resonance at 14.18 ppm can be reproduced for $1/6D_1$ = 8.3 ps, $1/6D_2$ = 4.2 ps, and $\gamma_0 = 55°$, where D_1 and D_2 are diffusion coefficients for rotations about the C_γ–S bond and methyl rotation, respectively. We obtained τ_e = 10.5 ps and $\mathcal{S}^2 = 0.052$, which corresponds to $\gamma_0 = 53°$. The agreement between the γ_0 obtained by interpreting the value of $\mathcal{S}^2$ and the γ_0 obtained by using the complicated spectral density within the restricted diffusion model is excellent. As discussed in some detail previously, the τ_e cannot be decomposed into correlation times for methyl rotations and restricted rotations about the C_γ–S bond. Thus, the above values of D_1 and D_2 are not unique.

Jones et al.[18] measured relaxation data for the C_ϵ resonance of two selectively enriched methionines (residues 55 and 131) in sperm whale myoglobin. NOE and T_1 values at 15 and 25 MHz and T_1 values at 68 MHz were reported. The overall motion of the protein was described as rigid isotropic reorientation. Table VI shows the results obtained by using the model-free approach. The results for the two residues are very similar. The T_2's evaluated from the measured line widths were not included in the fitting procedure. Compared to methionines in dihydrofolate reductase (see Table V), internal motions of methionine in myoglobin are more restricted and slightly slower (see the values of γ_0 obtained from $\mathcal{S}^2$ in Tables V and VI). A possible explanation is that myoglobin has a more densely packed structure. If we use for τ_M the value of 12 ns proposed by Gilman,[19] we obtain values of $\mathcal{S}^2$ that are smaller (~25%) and slightly (6%) faster τ_e's compared to the computations for τ_M = 20 ns. This shows that a large change in the value of τ_M does not substantially affect the picture of internal motions. Thus, a very accurate determination of τ_M is not crucial for the extraction of $\mathcal{S}^2$ and τ_e.

Gilman[19] measured ^{13}C NMR relaxation data at a resonance frequency of 25 MHz for ethyl isocyanide bound to myoglobin and hemoglobin. The ligand was ^{13}C labeled in the ethyl side chain. Table VII shows the results for carbon 2 (the methyl carbon of the side chain). The NMR relaxation data were fitted by using the model-free approach. T_1 and NOE at 25 MHz were included in the fitting procedure. The results obtained by using eq 14a and 14b are identical. The overall motion of the macromolecule was described by isotropic diffusion with τ_M = 12 ns for sperm whale mylglobin (SWM) and harbor seal myoglobin (HSM) and τ_M = 41 ns for human hemoglobin (HHb) at a concentration of 8.7 mM. The τ_M values were extracted by Gilman[19] from the α-carbon envelope. The segmental motion of the ethyl side chain is likely to be hindered due to collision of the side chain with groups present in the heme pocket. Rotations of the methyl carbon (carbon 2) of the side chain can take place freely, since they do not change the orientation of the ethyl side chain; rotations of the methylene carbon of the side chain, however, are likely to be

(17) Blakley, R. L.; Cocco, L.; London, R. E.; Walker, T. E.; Matwiyoff, N. A. *Biochemistry* **1978**, *17*, 2284.

(18) Jones, W. C.; Rothgeb, T. M.; Gurd, F. R. N. *J. Biol. Chem.* **1976**, *251*, 7452.

(19) Gilman, J. C. *Biochemistry* **1979**, *18*, 2273.

Table VI. ^{13}C NMR Relaxation of C_ϵ Carbons of Methionine Residues in Sperm Whale Myoglobin (τ_M = 20 ns)

residue	ω, MHz	NOE[a] $(1+\eta)$	NT_1,[a] s	line width,[a] Hz	$\mathcal{S}^2$ [b]	τ_e,[b] ps	γ_0,[c] deg
55	15	1.39 (1.5)	0.346 (0.474)	8.2 (8.0)			
	25	1.58 (1.7)	0.730 (0.588)	7.4 (6.5)	0.079 (0.059)	16 (13)	59 (74)
	68	2.38	2.14 (2.42)	9.3 (6.9)			
131	15	1.37 (1.5)	0.343 (0.513)	8.4 (6.8)			
	25	1.55 (1.7)	0.733 (0.579)	7.5 (5.9)	0.081 (0.059)	14 (12)	58 (74)
	68	2.33	2.25 (2.59)	7.0 (9.0)			

[a] Data from Jones et al.[18] Experimental values of the relaxation parameters are enclosed in parentheses. The values of $\mathcal{S}^2$ and τ_e obtained for τ_M = 12 ns are in parentheses. [b] Obtained by using the model-free approach. The values of $\mathcal{S}^2$ and τ_e obtained for τ_M = 12 ns are in parentheses. [c] Value of γ_0 in the restricted-diffusion model, calculated from $\mathcal{S}^2$ by using eq 18 and 19. Values corresponding to $\mathcal{S}^2$ extracted for τ_M = 12 ns are in parentheses.

Table VII. ^{13}C NMR Relaxation Data at 25 MHz for the Methyl Group of Ethyl Isocyanide Bound to Myoglobin and Hemoglobin

protein[a]	chemical shift, ppm	NT_1,[b] s	NOE[b] $(1+\eta)$	line width,[c] Hz	$\mathcal{S}^2$ [b]	τ_e,[b] ps	γ_0,[d] deg
SWM	15.9	0.918	1.7	2.7 (5)	0.040	15	65
	15.9	0.975	1.5	2.8 (5)	0.044	8.6	62
HSM	15.7	0.900	1.3	3.3 (5)	0.054	3.3	53
	15.9	0.996	1.3	3.0 (5)	0.049	3.0	57
HHb	16.1	1.839	1.5	11.1 (11)	0.064	5.0	46

[a] Abbreviations used: SWM, sperm whale myoglobin; HSM, harbor seal myoglobin; and HHb, human hemoglobin. Each entry represents an experiment on an independently prepared sample. The overall motion of the protein was described as overall isotropic reorientation with τ_M = 12 ns for SWM and HSM and τ_M = 41 ns for HHb. [b] Data from Gilman[19] fitted by using the model-free approach. Only T_1 and NOE were used in the fitting procedures. Experimental and theoretical values are identical to within the given figures. [c] The line widths are calculated from the T_2 values predicted by $\mathcal{S}^2$ and τ_e extracted from the fitting procedure. Experimental values are in parentheses. [d] Value of γ_0 in the restricted diffusion model predicted from $\mathcal{S}^2$ by using eq 18 and 19 with β = 70.5°.

restricted, since they can substantially modify the chain configuration. A simple model that takes into account these excluded volume effects pictures the motion of carbon 2 as free methyl rotation superimposed on restricted diffusion in the angular range $\pm\gamma_0$ of carbon 1 about an axis along the bond that connects it to the macromolecule.[8,9] The values of $\mathcal{S}^2$ and τ_e extracted from T_1 and NOE data at 25 MHz are probably reliable since by inspection of the model-free parameters (τ_e in the range 3–15 ps) the parameters are such that the internal motion can be considered extremely fast (rule i of section II). The angle γ_0 can be extracted from $\mathcal{S}^2$ by using eq 18 and 19 and is shown in Table VI for the proteins studied. The values of γ_0 are in the range of 46–65°, in good agreement with the results obtained by Gilman[19] with the restricted diffusion dynamical model.[8,9] The similarity between the values of γ_0 obtained for the ligand bound to the two myoglobins and to human hemoglobin seems to indicate that the ethyl isocyanide environment is fairly similar to the three proteins. T_1 values and line widths were measured for carbon C_1 of the ethyl side chain; the results obtained by using the model-free approach are not reliable since the amount of data is too limited. It is interesting, however, that the spin–lattice relaxation times can be reproduced by using a value of $\mathcal{S}_{axis}^2$ extracted from the C_2 data; even the line widths predicted are in fairly good agreement with the experimental data.

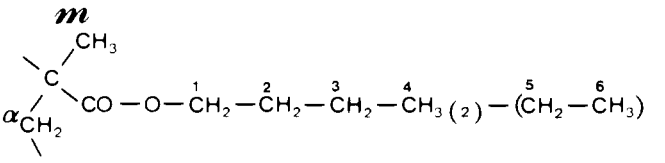
B. Anisotropic Overall Motion. In this section we use the model-free approach to analyze experimental data on a number of systems where the overall macromolecular motion is not adequately described by a single correlation time. Such a situation arises for macromolecules whose shape deviates considerably from a sphere or systems where the very notion of "macromolecular shape" loses meaning because the polymers are in the random-coil conformation. As discussed in paper 1 and in section II of this paper, the model-free approach to the description of anisotropic motions simply amounts to using a double-exponential correlation function for the overall motion multiplied by the usual model-free expression for the internal motion. We will show that a double-exponential correlation function for the overall motion can adequately reproduce the experimental data even in cases where distributions of correlation times of various complexity have been invoked.

Table VIII. Parameters for Overall Motion of Poly(*n*-butyl methacrylate) as a Function of Temperature[a]

T, °C	A	τ_1, ns	τ_2, ps
0	0.80	21	185
20	0.54	9.6	80
40	0.57	6.2	66
60	0.58	3.7	78
80	0.57	3.0	75
100	0.42	3.7	136

[a] Data from Levy et al.[20] analyzed by least-squares fitting T_1 and NOE at 23 and 68 MHz for the backbone carbon by using a double-exponential correlation function.

Recently Levy et al.[20] reported a very extensive series of ^{13}C NMR relaxation data on poly(*n*-alkyl methacrylates). T_1 and NOE values at two different resonance frequencies were measured over a range of temperatures of about 100 °C for poly(*n*-butyl methacrylate) (PBMA) and poly(*n*-hexyl methacrylate) (PHMA). This set of data is interesting since it allows the extraction of dynamic information as a function of temperature. The structure and numbering scheme of PBMA and PHMA is



As usual, one extracts information on the overall motion of the polymer from relaxation data for the backbone carbon. A single-exponential correlation function cannot reproduce the relaxation data at any temperature. With a double-exponential correlation function, however, the NMR data for the backbone carbon can be reproduced accurately at all temperatures. Levy et al.[20] used three different distributions of overall correlation times (or, equivalently, of diffusion coefficients) to describe the macromolecular motion. They showed that distributions of quite different shapes could reproduce the experimental data. The double-exponential correlation function results from using a distribution of correlation times of the form

$$p(\tau) = A\delta(\tau - \tau_1) + (1 - A)\delta(\tau - \tau_2) \qquad (24)$$

where $0 \le A \le 1$ and $\delta(x)$ is Dirac's delta function. In general, the parameters A, τ_1, and τ_2 do not have a simple physical meaning. It does not seem worthwhile, however, to try to use $p(\tau)$ of a more complicated form than in eq 24, since that simple form of the distribution appears to be adequate. As will be discussed

(20) Levy, G. C.; Axelson, D. E.; Schwartz, R.; Hochmann, J. *J. Am. Chem. Soc.* **1978**, *100*, 410.

Table IX. ^{13}C Data for Poly(*n*-butyl methacrylate) at 60 °C[a]

carbon	ω, MHz	NOE (1 + η)	NT_1, ms	$\mathcal{S}^2$	τ_e, ps
α[b]	23	1.62 (1.64)	61.1 (60.7)		
	68	1.44 (1.44)	188 (189)		
C_1	23	1.66 (1.77)	193 (176)	0.31	11
	68	1.57 (1.67)	559 (633)		
C_2	23	2.08 (2.74)	767 (711)	0.053	24
	68	2.38 (2.33)	1389 (1534)		
C_3	23	2.22	1860 (1860)	0.018	12
	68	2.53	3000 (3000)		
C_4	23	2.16 (2.37)	5480 (5292)	0.0068	3.4
	68	2.47 (2.43)	9291 (9660)		
m	23	2.01 (2.42)	178 (165)	0.24	140
	68	2.24 (2.27)	352 (391)		

[a] Data from Levy et al.[20] Experimental values are enclosed in parentheses. [b] Data for the α carbon were fitted by using a double-exponential correlation function with $\tau_1 = 3.66$ ns, $\tau_2 = 78.4$ ps, and $A = 0.580$. These parameters for the overall motion were held fixed while fitting the data for all the other carbons.

below, we have not been able to find a counter example in the NMR relaxation data published to date.

Table VIII shows the variation of the α-carbon parameters as a function of temperature, extracted by least-squares fitting the T_1 and NOE values at 23 and 68 MHz. The dependence of the τ_1, τ_2, and A values on temperature does not show a well-defined pattern, even though the effective correlation times tend to be substantially longer at very low temperatures and A is fairly constant (with the exception of the 0 and 100 °C values). The absence of a simple trend in the parameters of the correlation function for overall macromolecular motion is not surprising since the double exponential merely describes in an effective way the distribution of correlation times for overall motion and is not directly related to the physical properties of the system.

We then investigated systematically the internal motions of the other carbons of PBMA. At each temperature, the parameters for the overall motion extracted from the NMR data for the α carbon were held fixed and $\mathcal{S}^2$ and τ_e describing the internal motions were allowed to vary. For the side-chain carbons at all temperatures, the motions were found to be very fast ($\tau_e < 100$ ps). As an example of the goodness of the fit and of the orders of magnitude of $\mathcal{S}^2$ and τ_e, Table IX presents the results obtained for $T = 60$ °C. It is interesting to note that the order parameters for the butyl side chain decrease monotonically going out the chain. The methyl carbon of the side chain has a very small value of $\mathcal{S}^2$ (0.0068), indicating a large degree of mobility. The methyl carbon attached to the backbone (m), however, has an abnormally slow τ_e (140 ps) and a very large value of $\mathcal{S}^2$ (0.24). We note that the value of $\mathcal{S}^2$ is higher than would be expected for a simple methyl group rotation.

We then investigated the temperature dependence of the parameters for internal motion in PBMA. Figures 1 and 2 show $\mathcal{S}^2$ and τ_e, respectively, as a function of temperature for all carbons.

The effective correlation times decrease with increasing temperature; however, the order parameters (and hence the amplitudes of the motions) are reasonably constant over the whole temperature range (with the exception of the backbone methyl group). Similar results were obtained by Howarth,[6b] who analyzed the temperature dependence of the internal motions within the framework of the diffusion-in-a-cone model (he plotted the cone angle instead of the order parameter against T). It is tempting to extract an apparent "activation energy" from an Arrhenius plot of log τ_e vs. $1/T$. The linearity of these curves is fairly good: correlation coefficients are greater than 0.9 for all carbons. However, given the rather simple data-smoothing procedure used to obtain the relaxation parameters at the same temperature for both resonance frequencies, the values of the activation energy are not likely to be reliable. One obtains values ranging from ~8 kJ/mol for the C_4 carbon to ~19 kJ/mol for the side-chain methyl group.

We then investigated the effect of different side chains on the dynamics of the polymer. For a hexyl side chain (PHMA), the trends of $\mathcal{S}^2$ and τ_e along the chain and the rather unusual behavior

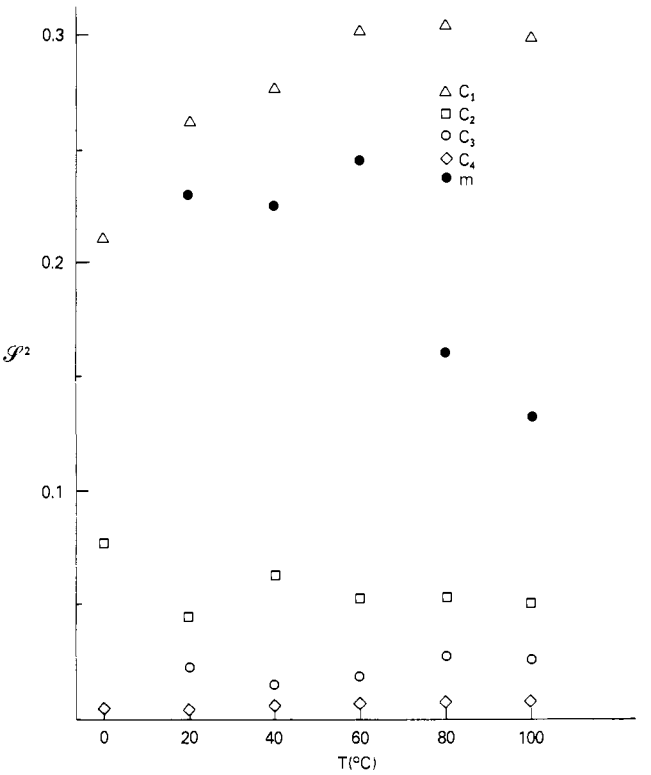


Figure 1. $\mathcal{S}^2$ as a function of temperature, extracted from the data on poly(*n*-butyl methacrylate) obtained by Levy et al.[20]

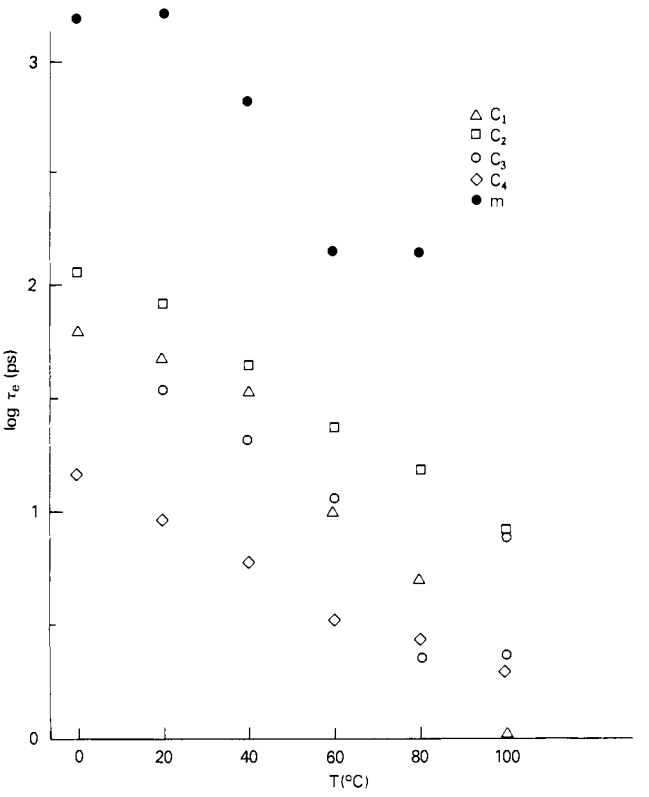


Figure 2. τ_e as a function of temperature, extracted from the data on poly(*n*-butyl methacrylate) obtained by Levy et al.[20]

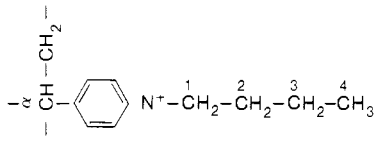
of the m carbon are similar to those of PBMA. Even for the longer chain, the values of $\mathcal{S}^2$ decrease monotonically as one goes from C_1 to C_6. The pattern of $\mathcal{S}^2$, however, is not $[P_2(\cos\beta)]^{2n}$, where n is the nucleus C_n, so that the rotations about the C–C bonds are not free and independent. This explains why Levy et al.[20] had difficulties in fitting their data using a model with multiple independent rotations.

Table X. ^{13}C Relaxation Data for Poly(4-vinyl-*N*-*n*-butylpyridinium bromide) in Methanol Solution at 37% Degree of Quaternization

carbon	ω, MHz	NOE[a] $(1+\eta)$	NT_1,[a] ms	$\mathcal{S}^2$ [b]	τ_e,[b] ps
α[c]	25	1.92 (1.9)	55.5 (54)		
	63	1.71 (1.8)	123 (120)		
C_1	25	1.97 (1.8)	119 (114)	0.44 [0.30]	35 [74]
	63	1.83 (2.0)	248 (260)		
C_2	25	2.35 (2.2)	435 (440)	0.076 [0.078]	49 [53]
	63	2.47 (2.6)	647 (640)		
C_3	25	2.51 (2.5)	1146 (1160)	0.023 [0.030]	24 [27]
	63	2.64 (2.6)	1517 (1500)		
C_4	25	2.59 (2.7)	3836 (3840)	0.0053 [0.0083]	7.8 [7.5]
	63	2.72 (2.6)	4804 (4800)		

[a] Data from Ghesquiere et al.[21] Experimental values of the relaxation parameters are enclosed in parentheses. [b] From fitting procedure using the model-free approach. The values of $\mathcal{S}^2$ and τ_e in brackets are the ones extracted for P4VPC4 at 100% degree of quaternization. [c] The overall motion is described by a double-exponential correlation function with $\tau_1 = 4.20$ ns, $\tau_2 = 649$ ps, and $A = 0.455$. For 100% degree of quaternization: $\tau_1 = 4.38$ ns, $\tau_2 = 188$ ps, and $A = 0.587$.

Ghesquiere et al.[21] measured T_1 and NOE ^{13}C relaxation data at two resonance frequencies (25 and 63 MHz) for various poly(4-vinyl-*N*-*n*-alkylpyridinium bromides) in methanol solutions. We analyzed the more extensive data on the butyl side chain (P4VPC4) for two different degrees of quaternization (37% and 100%). Table X shows the results obtained for P4VPC4 at 37% degree of quaternization. The structure and numbering scheme of P4VPC4 are



The parameters for overall motion were extracted from the relaxation data for the α carbon by using a double-exponential correlation function. The order parameter for the C_1 carbon is rather large (0.44), indicating a substantial motional restriction due to the presence of the aromatic ring; the values of the order parameters for the other side-chain carbons are quite small (<0.1) and show that the amplitude of their motion is fairly large. The terminal methyl group is virtually free ($\mathcal{S}^2 = 0.0053$) and moves very fast ($\tau_e = 7.8$ ps). The values of the order parameters for the side-chain carbons decrease monotonically going out the chain.

Table X also shows (in brackets) the values of $\mathcal{S}^2$ and τ_e extracted from the NMR relaxation data for P4VPC4 at 100% degree of quaternization. The values of $\mathcal{S}^2$ are fairly similar in both cases, and also the τ_e's are quite close, with the exception of the C_1 carbon. The description of the overall motion is also not very much affected by the degree of quaternization: one extracts from the relaxation data for the α carbon $\tau_1 = 4.4$ ns, $\tau_2 = 190$ ps, and $A = 0.59$ for 100% degree of quaternization; $\tau_1 = 4.2$ ns, $\tau_2 = 650$ ps, and $A = 0.46$ for 37% degree of quaternization. In summary, the degree of quaternization does not seem to have a large effect on either the overall or the internal motions.

The internal motions in P4VPC4 are rather fast ($\tau_e < 50$ ps for carbons that are the furthest from the backbone). In paper 1 and in the analysis of the NMR data reported by Richarz et al.,[13] we discussed how experimental information at two magnetic fields is redundant when the internal motions are close to the extreme narrowing limit and the data are very accurate. It is interesting to investigate this in a case where extensive experimental data are available for an anisotropic system. Table XI compares the values of $\mathcal{S}^2$ and τ_e for P4VPC4 (37% degree of quaternization) extracted by using different amounts of experimental information. Table XI shows $\mathcal{S}^2$ and τ_e obtained by using a fitting procedure that included T_1 and NOE data at both fields, the values determined from T_1 data at the two fields by using eq 13a and 13b and the values determined from T_1 and NOE data at one field (25 or 63 MHz) via eq 14a and 14b. The order parameters and effective correlation times obtained in these four different ways are remarkably similar, especially since the experimental data are not error free. Note that the agreement between experimental and theoretical relaxation parameters obtained by using a fitting procedure within the model-free approach is not perfect. If in eq 13a, 13b, 14a, and 14b we use the *calculated* T_1's and NOE's rather than their experimental values, the $\mathcal{S}^2$'s and τ_e's determined analytically in all possible ways and the ones extracted from the fitting procedure are virtually identical. Even with the experimental T_1's and NOE's, however, the relative ordering of $\mathcal{S}^2$ and τ_e values is preserved, and the numerical agreement is excellent, considering the smallness of the order parameters for carbons furthest removed from the backbone. These results show that there is redundance in NMR data at two resonance frequencies for fast internal motion. The experimental error, however, is likely to introduce an additional uncertainty in the extracted values of the generalized order parameter and the effective correlation time. Such uncertainty is likely to be reduced by the use of a larger amount of experimental data, due to the random nature of experimental errors.

Ghesquiere et al.[21] analyzed their data by using a number of models: (A) free diffusion; (B) three equivalent sites corresponding to t, g^+, and g^- conformers, and (C) the same as B but with t inequivalent to g^+ and g^-. They found that a satisfactory fit was obtained only with model C. This is to be expected since in models A and B the generalized order parameter is fixed by the geometry. It is straightforward to interpret the generalized order parameters obtained by using the model-free approach in terms of model C. For example, for the C_1 carbon, using the analogue of eq A9 of paper 1, we have

$$\mathcal{S}^2 = N_t^2 + \tfrac{4}{3}N_g(N_g - N_t) \qquad (25a)$$

where N_t is the equilibrium probability that the trans conformer is realized ($N_t + 2N_g = 1$). Solving eq 25a for N_t, we find

$$N_t = \tfrac{1}{6}(2 + (18\mathcal{S}^2 - 2)^{1/2}) \qquad (25b)$$

Using the value of $\mathcal{S}^2$ for C_1 in Table X for 37% degree of quaternization, we find $N_t = 0.74$. Using the rate constants given by Ghesquiere et al.[21] in their Table IX, we have $N_t = 0.71$. The corresponding values of N_t for 100% degree of quaternization are 0.64 and 0.63, respectively. The agreement is remarkably good.

Wittebort et al.[22] recently reported ^{13}C NMR data at two resonance frequencies for poly(L-lysine). T_1 and NOE values at 68 MHz and T_1 values at 15 MHz were measured at 28 °C for a material with a degree of polymerization of 129. Table XII shows the results obtained by using the model-free approach to fit the experimental data. The overall motion is described by a double exponential whose parameters are determined from the data for the α carbon. We obtained $\tau_1 = 4.50$ ns, $\tau_2 = 240$ ps, and $A = 0.654$. The values of τ_e extracted for the β (288 ps) and γ (191 ps) carbons are rather slow. The corresponding values of $\mathcal{S}^2$, however, are quite large (0.420 for the β carbon and 0.242 for the γ carbon). Thus, according to the operational criteria discussed in paper 1 and in section II of this paper, the values of $\mathcal{S}^2$ and τ_e are likely to be reliable. The remaining carbons have values of $\tau_e < 100$ ps, so $\mathcal{S}^2$ and τ_e for these carbons are also meaningful. The values of $\mathcal{S}^2$ for the β and γ carbons indicate that their internal motions have a fairly large degree of restriction. Such restriction is less severe for carbons further removed from the backbone. Any model used in interpreting these experimental

(21) Ghesquiere, D.; Chachaty, C.; Tsutsumi, A. *Macromolecules* **1979**, *12*, 775.

(22) Wittebort, R. J.; Szabo, A.; Gurd, F. R. N. *J. Am. Chem. Soc.* **1980**, *102*, 5723.

Table XI. Values of $\mathcal{S}^2$ and τ_e for Poly(4-vinyl-*N*-*n*-butylpyridinium bromide) (37% Degree of Quaternization) Extracted in Various Ways from the Data of Ghesquiere et al.[21]

carbon	from fitting procedure[a]	T_1 and NOE at 25 MHz[b]	T_1 and NOE at 63 MHz[b]	T_1's at 25 and 63 MHz[c]	from fitting procedure[a]	T_1 and NOE at 25 MHz[d]	T_1 and NOE at 63 MHz[d]	T_1's at 25 and 63 MHz[e]
C_1	0.44	0.52	0.38	0.48	35	*f*	49	*g*
C_2	0.076	0.089	0.061	0.070	49	32	52	49
C_3	0.023	0.021	0.026	0.019	24	23	22	24
C_4	0.0053	0.0037	0.0082	0.0051	7.8	9.0	6.6	7.8

[a] From a fitting procedure as in Table X. [b] From eq 13a; parameters for overall and internal motion as in Table X. [c] From eq 14a; parameters for overall and internal motion as in Table X. [d] From eq 13b; parameters for overall and internal motion as in Table X. [e] From eq 14b; parameters for overall and internal motion as in Table X. [f] One obtains a negative value of τ_e by using the experimental values of T_1 and NOE. With the calculated value of the NOE (1.97), one obtains $\tau_e = 47$ ps and $\mathcal{S}^2 = 0.44$. [g] One obtains a negative value of τ_e by using the experimental values of T_1. With the calculated values of T_1 at 25 MHz (119 ms) and 63 MHz (248 ms), one obtains $\tau_e = 37$ ps and $\mathcal{S}^2 = 0.43$.

Table XII. ^{13}C Relaxation Data on Poly(L-lysine)[a]

carbon	ω, MHz	NOE $(1 + \eta)$	T_1, ms	$\mathcal{S}^2$	τ_e, ps
α[b]	15		36 (36)		
	68	1.6 (1.6)	170 (170)		
β	15		69 (72)	0.42	288
	68	2.17 (2.0)	206 (202)		
γ	15		108 (108)	0.24	191
	68	2.40 (2.4)	264 (264)		
δ	15		201 (208)	0.12	93
	68	2.52 (2.3)	443 (430)		
ϵ	15		481 (484)	0.033	61
	68	2.78 (2.7)	738 (734)		

[a] Data from Wittebort et al.[22] The experimental values are enclosed in parentheses. [b] Overall motion described by a double-exponential correlation function whose parameters are determined from the data for the α carbon: $\tau_1 = 4.50$ ns, $\tau_2 = 240$ ps and $A = 0.654$.

data has to take this restriction into account, i.e., must have enough flexibility to reproduce the large order parameters for the β and γ carbons and the small values of $\mathcal{S}^2$ for the δ and ϵ carbons. We note that the rates of internal motions are rather slow compared to aliphatic side chains in organic polymers and proteins. This may be because the terminal group of the lysine (NH_3^+), which is hydrated, slows down the motion of the entire side chain.

It is also interesting that the values of $\mathcal{S}^2$ and τ_e can be extracted by using the analytical formulas of eq 13a, 13b, 14a, and 14b. For example, by using T_1 values at two fields, from eq 13a and 13b, one calculates $\mathcal{S}^2 = 0.41$, $\tau_e = 201$ ps for C_β; $\mathcal{S}^2 = 0.25$, $\tau_e = 144$ ps for C_γ; $\mathcal{S}^2 = 0.11$, $\tau_e = 87$ ps for C_δ; and $\mathcal{S}^2 = 0.032$, $\tau_e = 57$ ps for C_ϵ. These values are in excellent agreement with the ones obtained from a fitting procedure (shown in Table XII) using all the available experimental information. As expected, a calculation of $\mathcal{S}^2$ and τ_e from T_1 and NOE at 68 MHz yields poorer results, since at this resonance frequency the internal motions are farther from the extreme narrowing limit.

Finally, we point out that if we use the restricted diffusion model to interpret the value of $\mathcal{S}^2$ for the β carbon, we extract $\gamma_0 = 59°$ using eq 18. Wittebort et al.[22] obtained $\gamma_0 = 60°$ using the restricted diffusion model superimposed on an overall motion described by a distribution of diffusion coefficients, given by

$$p(D) = [\ln (D_U/D_L)D]^{-1} \qquad (26)$$

for $D_L \leq D \leq D_U$; $p(D) = 0$ otherwise. In this model the spectral density is extremely complicated and is given in terms of certain transcendental functions. The fact that the value of γ_0 can be extracted so simply with model-free approach is remarkable. It shows that our double-exponential approximation for $C_O(t)$ works very well: the parameters for internal motions are insensitive to the specific form of the correlation function for overall motion, even in the case of highly anisotropic motions. Dynamic information that is obtained after lengthy calculations within the framework of a complicated model can be extracted in a simple, straightforward way by using the model-free approach.

Acknowledgment. G.L. is partially supported by U.S. Public Health Service Grant HL-21483 awarded to Professor F. R. N. Gurd and thanks the Foundation Stiftelsen Blanceflor, Boncompagni-Ludovsisi, född Bildt, for a fellowship.