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A Simple Approach to Analyzing Protein Side-Chain Dynamics from ^{13}C NMR Relaxation Data

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A simple approach to deriving motional dynamics information of protein and peptide side chains by using ^{13}C NMR relaxation data is presented. By using linear approximation of internal rotational correlation functions, simple equations for relating side-chain conformation, bond rotational amplitudes, and rotational correlation coefficients with different NMR relaxation parameters have been obtained. Auto- and cross-correlation spectral densities are considered, and it is shown that proton-coupled ^{13}C NMR relaxation measurements allow detailed motional information to be obtained.

The goal of the present study is to develop a simple approach to deriving motional dynamics information on protein side chains from ^{13}C NMR relaxation data. There are many motional models and model-free approaches (*1*) available for such analyses; however, most are either too complicated, containing numerous parameters which all too often cannot be determined from NMR experiments with any reasonable accuracy, while others contain only a few simple parameters but provide no physically meaningful picture of internal motions. The approach presented herein retains the simplicity of a model-free approach while allowing a detailed understanding of side-chain internal rotations in terms of conformational parameters, particularly dihedral angles. Equations for calculating any auto- or cross-correlation spectral density $J_{\mathbf{ab}}(\omega)$ [**a** and **b** are motional vectors, usually CH bonds for ^{13}C NMR relaxation] are derived for use in obtaining internal rotational correlation times, rotational restrictions, correlated bond rotations, and peptide bond and side-chain geometry.

For this analysis, two reasonable assumptions have initially been made: (1) protein side-chain motions of a particular residue are determined primarily by that residue's χ_i ($i = 1, 2, \ldots, n$) and ϕ, ψ bond rotations, and (2) positions further out on a longer side chain have effectively no influence on the motions of CH bonds closer to the backbone. This latter assumption would mean, for example, that rotations of the $C_\beta H_2$ group in phenylalanine are not influenced by rotations of the phenyl ring. This is not strictly correct since $\chi_1(t)$ and $\chi_2(t)$ rotations can be correlated; however, due to recoil effects (*1*), this influence will be small. Likewise, side-chain rotations other than χ_1 will have only a small influence at best on backbone motions. Moreover, it should be emphasized that these assumptions concern only side-chain bond *rotational motions.* The average *conformation* of a side chain, on the other hand, can have a striking effect on backbone motions since moments of inertia, solvent accessibility, and intramolecular interactions depend upon the geometry of groups involved.

Since the following analysis considers only restricted internal rotations as opposed to completely free rotations, an average local conformation determined by average dihedral angles $\phi^0 = \langle\phi(t)\rangle$, $\psi^0 = \langle\psi(t)\rangle$, and $\chi_i^0 = \langle\chi_i(t)\rangle$ can be used. The symbols $\Delta\phi$, $\Delta\psi$, and $\Delta\chi_i$ indicate angular deviations from their equilibrium positions such that $\Delta\phi = \phi(t) - \phi^0$ and the average values of $\Delta\phi$, $\Delta\psi$, and $\Delta\chi_i$ are equal to zero. Equations are also expressed solely for isotropic tumbling of a ''rigid'' backbone triangle formed by the atoms N–C_α–C and tumbling with overall correlation time τ_0. The N–C_α–C triangle for a given residue, however, is allowed to fluctuate within the molecular frame due to ϕ, ψ bond rotations. τ_0 describes changes from the N–C_α–C equilibrium orientation (averaged over $\phi(t)$ and $\psi(t)$ rotations), e.g., $\Delta\phi = \Delta\psi = 0$. The assumption of isotropy is not crucial to this analysis, and a more general case for anisotropic tumbling may be developed using the appropriate equations (*1, 2*).

In general, the spectral density $J_{\mathbf{ab}}(\omega)$ can be written as

$$J_{\mathbf{ab}}(\omega) = \int C_{\mathbf{ab}}(t)\cos(\omega t)dt, \qquad [1]$$

where the correlation function $C_{\mathbf{ab}}(t)$ has the form (*1*)

$$C_{\mathbf{ab}}(t) = \frac{4\pi}{5}\exp(-t/\tau_0) \times \sum_m \langle Y_{2m}[\theta_{\mathbf{a}}^{\mathrm{M}}(t), \varphi_{\mathbf{a}}^{\mathrm{M}}(t)]Y^*_{2m}[\theta_{\mathbf{b}}^{\mathrm{M}}(0), \varphi_{\mathbf{b}}^{\mathrm{M}}(0)]\rangle. \qquad [2]$$

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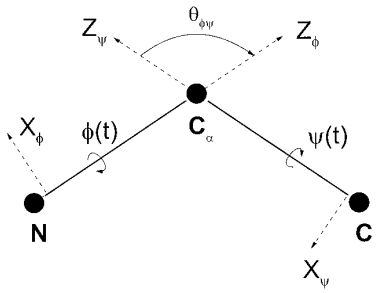

FIG. 1. The molecular frame used in calculations described in the text is shown with protein backbone N–C_α–C atoms.

Y_{20} is the second-ranked spherical harmonics, and $\theta_{\mathbf{a}}^{M}(t)$ and $\varphi_{\mathbf{a}}^{M}(t)$ are polar angles defining the motional vector **a** in the molecular frame, i.e., N–C_α–C triangle for $\Delta\phi = \Delta\psi = 0$. As mentioned above, this molecular frame is allowed to vary its orientation due to overall tumbling and to conformational changes in the protein or peptide. Since the magnitude of these effects can be different for different residues, τ_0 should be calculated for each residue in any protein dynamics analysis.

For C_αH bond motions, only $\phi(t)$ and $\psi(t)$ backbone rotations need to be considered. When rotational amplitudes are small, the time dependence of the spherical harmonics [2] can be removed by performing the rotational transformation $\Delta\psi$, $-\theta_{\phi\psi}$, $\Delta\phi$ (Fig. 1). As a starting point for this transformation, the equilibrium conformation was chosen such that $\Delta\phi = \Delta\psi = 0$ and with the Z axis running through backbone C and C_α atoms. The corresponding Wigner matrix, D, has the form

$$D_{pq}^{(2)}(\Delta\psi, -\theta_{\phi\psi}, \Delta\phi) = \exp(-iq\Delta\psi)d_{pq}^{(2)}(-\theta_{\phi\psi})\exp(-ip\Delta\phi), \quad [3]$$

where $d^{(2)}$ is the second-order reduced D matrix. This transformation can also be performed by using the N–C_α bond as the Z axis in the molecular frame. For small $\Delta\psi$ and $\Delta\phi$ angles, the resulting expression will be similar. Following transformation, the correlation function can be written as

$$C_{\mathbf{ab}}(t) = \frac{4\pi}{5}\exp(-t/\tau_0)\sum_{p,q,q'} d_{pq}^{(2)}(-\theta_{\phi\psi}) \times d_{pq'}^{(2)}(-\theta_{\phi\psi})\Phi_{\phi\psi}(t)Y_{2q}(\theta_{\mathbf{a}}^{\phi}, \varphi_{\mathbf{a}}^{\phi}) \times Y_{2q'}^{*}(\theta_{\mathbf{b}}^{\phi}, \varphi_{\mathbf{b}}^{\phi}). \quad [4]$$

$\theta_{\mathbf{a}}^{\phi}$ and $\varphi_{\mathbf{a}}^{\phi}$ are polar angles for motional vector **a** (C_αH bond in this case) in the molecular frame $\{X_\phi, Y_\phi, Z_\phi\}$, where the N–$C_\alpha$ bond is the Z_ϕ axis and the X_ϕ axis lies in the N–C_α–C plane (see Fig. 1). The summation is performed over p, q, q' from -2 to 2, and the correlation function, $\Phi_{\phi\psi}(t)$, can be written as

$$\Phi_{\phi\psi}(t) = \langle\exp[ip(\Delta\psi(t) - \Delta\psi(0)) + i(q\Delta\phi(t) - q'\Delta\phi(0))]\rangle. \quad [5]$$

For small-amplitude (restricted) $\Delta\phi(t)$ and $\Delta\psi(t)$ rotations, Eq. [5] can be rewritten as

$$\Phi_{\phi\psi}(t) = 1 - \tfrac{1}{2}(2p^2\sigma_\psi^2 + (q^2 + q'^2)\sigma_\phi^2) - p(q + q')c_{\phi\psi}\sigma_\phi\sigma_\psi + p^2\langle\Delta\psi(t)\Delta\psi(0)\rangle + qq'\langle\Delta\phi(t)\Delta\phi(0)\rangle + pq'\langle\Delta\phi(t)\Delta\psi(0)\rangle + pq\langle\Delta\psi(t)\Delta\phi(0)\rangle, \quad [6]$$

where σ_ϕ and σ_ψ are the angular variances in $\Delta\phi(t)$ and $\Delta\psi(t)$, $\sigma_\psi^2 = \langle\Delta\psi^2(t)\rangle$ and $\sigma_\phi^2 = \langle\Delta\phi^2(t)\rangle$, and the rotational correlation coefficient, $c_{\phi\psi}$, is defined as

$$c_{\phi\psi} = \langle\Delta\phi(t)\Delta\psi(t)\rangle/(\sigma_\phi\sigma_\psi). \quad [7]$$

Note that this definition is identical to that given for $c_{\phi\psi}$ by Daragan and Mayo (*8*). Moreover, at infinite t, the limiting value for any correlation function defined by Eq. [6] is equal to zero (*3*):

$$\lim_{t\to\infty}\langle\Delta\psi(t)\Delta\phi(0)\rangle = \langle\Delta\psi(t)\rangle\langle\Delta\phi(t)\rangle = 0. \quad [8]$$

Following the approach used by Lipari and Szabo (*3*), let us assume that all correlation functions in Eq. [6] can be described by an exponential decay with a single time constant, the correlation time τ_α. Since NMR relaxation data are normally insensitive to the internal motional correlation time, this approximation is sufficient for our analysis. In this case, Eq. [6] can be simplified to

$$\Phi_{\phi\psi}(t) = 1 - p^2\sigma_\psi^2[1 - \exp(-t/\tau_\alpha)] - \sigma_\phi^2(q^2 + q'^2)/2 + qq'\sigma_\phi^2\exp(-t/\tau_\alpha) - p(q + q')c_{\phi\psi}\sigma_\phi\sigma_\psi[1 - \exp(-t/\tau_\alpha)]. \quad [9]$$

Using the addition theorem for spherical harmonics and the properties of $d^{(2)}$ matrices,

$$\sum_p d_{pq}^{(2)}d_{pq'}^{(2)} = \delta_{qq'}, \quad [10]$$

Eq. [4] can be transformed to

$$C_{\mathbf{ab}}(t) = \exp(-t/\tau_0)[S^2_{\mathbf{ab}} + A^{\mathbf{ab}}_{\phi}\exp(-t/\tau_\alpha)\sigma^2_\phi + A^{\mathbf{ab}}_{\psi}\exp(-t/\tau_\alpha)\sigma^2_\psi + B^{\mathbf{ab}}_{\phi\psi}\exp(-t/\tau_\alpha)\sigma_\phi\sigma_\psi c_{\phi\psi}] \quad [11a]$$

$$S^2_{\mathbf{ab}} = P_2(\cos\theta_{\mathbf{ab}}) - A^{\mathbf{ab}}_{\phi}\sigma^2_\phi - A^{\mathbf{ab}}_{\psi}\sigma^2_\psi - B^{\mathbf{ab}}_{\phi\psi}\sigma_\phi\sigma_\psi c_{\phi\psi}. \quad [11b]$$

$\theta_{\mathbf{ab}}$ is the angle between motional vectors **a** and **b**, and $P_2(x) = (3x^2 - 1)/2$ is the second-order Legendre polynomial. The squared order parameter $S^2_{\mathbf{ab}}$ (*3–6*) is defined as the limiting value of the internal rotational correlation function. This has been calculated from Eqs. [6] and [8]. The coefficients $A^{\mathbf{ab}}_{\phi}$ and $A^{\mathbf{ab}}_{\psi}$ can be expressed as

$$A^{\mathbf{ab}}_k = 3\cos\theta^k_{\mathbf{a}}\cos\theta^k_{\mathbf{b}}\sin\theta^k_{\mathbf{a}}\sin\theta^k_{\mathbf{b}}\cos(\varphi^k_{\mathbf{a}} - \varphi^k_{\mathbf{b}}) + 3\sin^2\theta^k_{\mathbf{a}}\sin^2\theta^k_{\mathbf{b}}\cos(2\varphi^k_{\mathbf{a}} - 2\varphi^k_{\mathbf{b}}), \quad [12]$$

where $k = \phi, \psi$; and $\theta^k_{\mathbf{a}}$ and $\varphi^k_{\mathbf{a}}$ are polar angles for vector **a** in the molecular frame in which the Z axis is coincident with the direction of the kth rotation. Since $(\varphi^k_{\mathbf{a}} - \varphi^k_{\mathbf{b}})$ is the only difference term in Eq. [12], the direction of the X axis is irrelevant for calculating $A^{\mathbf{ab}}_k$ coefficients which describe the influence of kth bond rotations on the motions of bond vectors **a** and **b**. For the autocorrelation function, Eq. [12] can be significantly simplified:

$$A^{\mathbf{aa}}_k = A^{\mathbf{a}}_k = 3\sin^2\theta^k_{\mathbf{a}}. \quad [13]$$

For rotations about a single axis, this equation has already been derived for the autocorrelation function by Brüschweiler and Wright (*7*), and similar equations have been obtained by Daragan and Mayo (*8*) for multiple bond rotations by using vector algebra.

Coefficients $B^{\mathbf{ab}}_{\phi\psi}$, which describe the influence of $\phi(t)$ and $\psi(t)$ rotational correlations on the motions of bond vectors **a** and **b**, can be written in the form

$$B^{\mathbf{ab}}_{\phi\psi} = \frac{4\pi}{5}\sum_{p,q} pq d^{(2)}_{pq}(-\theta_{\phi\psi})[Y_{2q}(\theta^{\phi}_{\mathbf{a}}, \varphi^{\phi}_{\mathbf{a}})Y^*_{2p}(\theta^{\psi}_{\mathbf{b}}, \varphi^{\psi}_{\mathbf{b}}) + Y^*_{2q}(\theta^{\phi}_{\mathbf{b}}, \varphi^{\phi}_{\mathbf{b}})Y_{2p}(\theta^{\psi}_{\mathbf{a}}, \varphi^{\psi}_{\mathbf{a}})]. \quad [14]$$

The coefficients A and B can be readily calculated, yielding equations for autocorrelation order parameters as well as for cross-correlation order parameters like $J_{\mathrm{CH,CH'}}(\omega)$ and $J_{\mathrm{CH,HH'}}(\omega)$ which can be derived from ^{13}C proton-coupled relaxation experiments (*1*) on, for example, the glycine $C_\alpha H_2$ group. The primary order parameters $S_{\mathrm{CH}} = S_{\mathrm{CH,CH}}$, $S_{\mathrm{HCH}} = S_{\mathrm{CH,CH'}}$, and $S_{\mathrm{CHH}} = S_{\mathrm{CH,HH'}}$ can be expressed as

$$S^2_{\mathrm{CH}} = 1 - \tfrac{8}{3}(\sigma^2_\phi + \sigma^2_\psi) + \tfrac{8}{3}\sigma_\phi\sigma_\psi c_{\psi\phi} \quad [15a]$$

$$S^2_{\mathrm{HCH}} = -\tfrac{1}{3} + \tfrac{4}{3}(\sigma^2_\phi + \sigma^2_\psi) - \tfrac{32}{9}\sigma_\phi\sigma_\psi c_{\psi\phi} \quad [15b]$$

$$S^2_{\mathrm{CHH}} = \tfrac{1}{2} - \tfrac{4}{3}(\sigma^2_\phi + \sigma^2_\psi). \quad [15c]$$

These equations are analogous to those derived using a different approach (*8*). Equation [15a] defines the autocorrelation order parameter, e.g., for the $C_\alpha H$ methine group, while S^2_{HCH} and S^2_{CHH} define cross-correlation order parameters for CH_2 methylene groups. S^2_{CHH}, obtainable from heteronuclear NMR relaxation experiments (*9*), has a unique property—the absence of any influence from $\phi(t)$ and $\psi(t)$ rotational correlations. Despite having three Eqs. [15] and three motional parameters, it is impossible to obtain σ_ϕ, σ_ψ, and $c_{\psi\phi}$ independently, and only two combinations $\sigma^2_\phi + \sigma^2_\psi$ and $\sigma_\phi\sigma_\psi c_{\psi\phi}$ can be determined from ^{13}C NMR relaxation data. However, assuming that $\sigma_\phi = \sigma_\psi$, $c_{\psi\phi}$ and $\sigma_\phi = \sigma_\psi$ can be estimated. As will be shown later, using ^{13}C relaxation data on C_β carbons can provide additional information to help resolve this problem.

Note that Eq. [11a] can also be expressed as

$$C_{\mathbf{ab}}(t) = \exp(-t/\tau_0)\{S^2_{\mathbf{ab}} + [P_2(\cos\theta_{\mathbf{ab}}) - S^2_{\mathbf{ab}}\exp(-t/\tau_\alpha)]\}. \quad [16]$$

When $\mathbf{a} = \mathbf{b}$ and $P_2(\cos\theta_{\mathbf{ab}}) = 1$, this equation is identical to the ''classical'' Lipari–Szabo equation (3) for the autocorrelation function. For uncorrelated $\phi(t)$ and $\psi(t)$ bond rotations, the equation for the correlation function for $C_\alpha H$ bond motions looks like

$$C_\alpha(t) = \exp(-t/\tau_0) \times [1 - \tfrac{16}{3}(1 - \exp(-t/\tau_\alpha))\sigma^2_\alpha], \quad [17]$$

where

$$\sigma^2_\alpha = (\sigma^2_\phi + \sigma^2_\psi)/2. \quad [18]$$

These expressions can be used to estimate angular variances, for example, in relatively unstructured parts of proteins where rotational correlations are small. In this case, only three experimental parameters are required to derive σ_α, τ_α, and τ_0.

For protein side-chain CH bond motions, Eqs. [11] can be generalized as

$$C_{\mathbf{ab}}(t) = \exp(-t/\tau_0)[S^2_{\mathbf{ab}} + \sum_k A^{\mathbf{ab}}_k\exp(-t/\tau_k)\sigma^2_k + \sum_{i\neq k} B^{\mathbf{ab}}_{ik}\exp(-t/\tau_{ik})\sigma_i\sigma_k c_{ik}] \quad [19a]$$

$$S^2_{\mathbf{ab}} = P_2(\cos\theta_{\mathbf{ab}}) - \sum_k A^{\mathbf{ab}}_k\sigma^2_k - \sum_{i\neq k} B^{\mathbf{ab}}_{ik}\sigma_i\sigma_k c_{ik}, \quad [19b]$$

where $k = \phi, \psi, \chi_1, \chi_2, \ldots$. Equation [12] can be used to calculate coefficients A, and coefficients B can be written in a general form:

$$B_{ik}^{\mathbf{ab}} = \tfrac{4\pi}{5} \sum_{m_1 m_2} m_1 m_2 d^{(2)}_{m_1 m_2}(\beta_{ik})[Y_{2m_2}(\theta^i_{\mathbf{a}}, \varphi^i_{\mathbf{a}}) Y^*_{2m_1}(\theta^k_{\mathbf{b}}, \varphi^k_{\mathbf{b}}) + Y^*_{2m_2}(\theta^i_{\mathbf{b}}, \varphi^i_{\mathbf{b}}) Y_{2m_1}(\theta^k_{\mathbf{a}}, \varphi^k_{\mathbf{a}})]. \quad [20]$$

β_{ik} is the Euler angle for rotation of the ith bond (defined by a molecular frame where the Z axis is coincident with the direction of the ith rotation) to the kth bond (defined by a molecular frame where the Z axis is coincident with the direction of the kth rotation). Directions of the X axes can be chosen arbitrarily; however, the choice of these axes should be taken into account when calculating polar angles $\theta_{\mathbf{a}}$, $\varphi_{\mathbf{a}}$, $\theta_{\mathbf{b}}$, and $\varphi_{\mathbf{b}}$. Therefore, in order to calculate ϕ and ψ rotational correlations, the angle β_{ik} is equal to $\beta_{ik} = -\theta_{\phi\psi}$ when the molecular frames are as shown in Fig. 1 [12]. To calculate, for example, the influence of ϕ and ψ rotational correlations on motions of the $C_\gamma H$ bond, additional transformations of the spherical harmonics are required. Equation [19a] is different from the Lipari–Szabo equation (3) because it contains multiexponential decay terms to describe the correlation function for internal bond rotations.

Coefficients A and B depend on the conformation of the side chain, i.e., on equilibrium values of the dihedral angles $\chi_i^0 = \langle \chi_i(t) \rangle$. For example, consider rotations of $C_\beta H$ and $C_\beta H_2$ groups in a side chain. For the $C_\beta H$ group, χ_1^0 is defined by atoms N–C_α–C_β–H_β, and the coefficients A and B for $\phi(t)$, $\psi(t)$, and $\chi_1(t)$ bond rotations are given in Table 1. For the $C_\beta H_2$ group, χ_1^0 must be defined by the atoms N–C_α–C_β–C_γ with averaging being done over the two $C_\beta H$ bonds (see Tables 1 and 2). If $\sigma_\phi = \sigma_\psi = \sigma_\alpha$, the coefficients A_α can be calculated (Tables 1 and 2) with $A_\alpha = A_\phi + A_\psi$ and with summing being performed over $k = \alpha, \chi_1, \chi_2, \cdots$ in Eq. [14].

TABLE 1

The Coefficients A and B for Autocorrelation Functions of $C_\beta H$ and $C_\beta H_2$ Groups for Different Values of the χ_1^0 Dihedral Angles

χ_1^0	180°	−60°	60°
A_ϕ^{CH} ($C_\beta H$)	0	8/3	8/3
A_ψ^{CH} ($C_\beta H$)	8/3	0	8/3
A_1^{CH} ($C_\beta H$)	8/3	8/3	8/3
A_ϕ^{CH} ($C_\beta H_2$)	8/3	4/3	4/3
A_ψ^{CH} ($C_\beta H_2$)	4/3	8/3	4/3
A_1^{CH} ($C_\beta H_2$)	8/3	8/3	8/3
A_α^{CH} ($C_\beta H$)	8/3	8/3	16/3
A_α^{CH} ($C_\beta H_2$)	4	4	8/3
$B_{\phi\psi}^{CH}$ ($C_\beta H$)	0	0	−8/3
$B_{\phi\psi}^{CH}$ ($C_\beta H_2$)	−4/3	−4/3	0
$B_{\phi 1}^{CH}$ ($C_\beta H$)	0	8/3	8/3
$B_{\phi 1}^{CH}$ ($C_\beta H_2$)	8/3	4/3	4/3
$B_{\psi 1}^{CH}$ ($C_\beta H$)	8/3	0	8/3
$B_{\psi 1}^{CH}$ ($C_\beta H_2$)	4/3	8/3	4/3

Note. Coefficients A_α^{CH} were calculated under the assumption that $\sigma_\phi = \sigma_\psi = \sigma_\alpha$ and $\tau_\phi = \tau_\psi = \tau_\alpha$. A and B for CH_2 groups were calculated by averaging over two CH bonds. The dihedral angle χ_1^0 is defined by N–C_α–C_β–C_γ atoms for the $C_\beta H_2$ group and by N–C_α–C_β–H_β atoms for the $C_\beta H$ group.

TABLE 2

The Coefficients A and B for Cross-Correlation Functions of $C_\beta H_2$ Groups for Different Values of χ_1^0 Dihedral Angles

χ_1^0	180°	−60°	60°
A_ϕ^{HCH} ($C_\beta H_2$)	−4/3	0	0
A_ψ^{HCH} ($C_\beta H_2$)	0	−4/3	0
A_1^{HCH} ($C_\beta H_2$)	−4/3	−4/3	−4/3
A_ϕ^{CHH} ($C_\beta H_2$)	4/3	2/3	2/3
A_ψ^{CHH} ($C_\beta H_2$)	2/3	4/3	2/3
A_1^{CHH} ($C_\beta H_2$)	4/3	4/3	4/3
A_α^{HCH} ($C_\beta H_2$)	−4/3	−4/3	0
A_α^{CHH} ($C_\beta H_2$)	2	2	4/3
$B_{\phi\psi}^{HCH}$ ($C_\beta H_2$)	−4/9	−4/9	8/9
$B_{\phi\psi}^{CHH}$ ($C_\beta H_2$)	−4/3	−4/3	4/3
$B_{\phi 1}^{HCH}$ ($C_\beta H_2$)	−32/9	4/9	4/9
$B_{\phi 1}^{CHH}$ ($C_\beta H_2$)	0	4/3	4/3
$B_{\psi 1}^{HCH}$ ($C_\beta H_2$)	4/9	−32/9	4/9
$B_{\psi 1}^{CHH}$ ($C_\beta H_2$)	4/3	0	4/3

Note. Coefficients A_α^{HCH} and A_α^{CHH} were calculated under the assumption that $\sigma_\phi = \sigma_\psi = \sigma_\alpha$ and $\tau_\phi = \tau_\psi = \tau_\alpha$. The dihedral angle χ_1^0 is defined by N–C_α–C_β–C_γ atoms.

Consider the simple case of uncorrelated $\phi(t)$, $\psi(t)$, and $\chi_1(t)$ rotations. Using Eq. [11b] and Table 1, a different order parameter for the $C_\beta H$ group, $S^2_{C_\beta H}$, is obtained for different values of χ_1^0:

$$\chi_1^0 = 180°, \quad S^2_{C_\beta H} = 1 - \tfrac{8}{3}(\sigma^2_\psi + \sigma^2_{\chi 1}) \quad [21a]$$

$$\chi_1^0 = -60°, \quad S^2_{C_\beta H} = 1 - \tfrac{8}{3}(\sigma^2_\phi + \sigma^2_{\chi 1}) \quad [21b]$$

$$\chi_1^0 = 60°, \quad S^2_{C_\beta H} = 1 - \tfrac{8}{3}(\sigma^2_\phi + \sigma^2_\psi + \sigma^2_{\chi 1}). \quad [21c]$$

When the $C_\beta H$ group is symmetric with respect to the N–C_α–C plane ($\chi_1^0 = 60°$), $S^2_{C\beta H}$ is at a minimum and is always less than the $C_\alpha H$ order parameter, S^2_α, which for uncorrelated $\phi(t)$ and $\psi(t)$ rotations is equal to $1 - \frac{8}{3}(\sigma^2_\phi + \sigma^2_\psi)$. If $\chi_1^0 = -60°$ and $\sigma_{\chi 1} < \sigma_\phi$ or if $\chi_1^0 = 180°$ and $\sigma_{\chi 1} < \sigma_\psi$, $S^2_\alpha < S^2_{C_\beta H}$. In other words, for highly restricted $\chi_1(t)$ rotations, $C_\beta H$ bond motions are more restricted than $C_\alpha H$ bond motions. This has been observed for residues in a short,

partially folded β-sheet peptide 20mer (*10*). In another example with a partially folded β-hairpin peptide 12mer (*11*), different values for order parameters were found for different χ_1^0 dihedral angles. In this case, order parameters for $C_\beta H$, $C_\beta C_{\gamma 1}$, and $C_\beta C_{\gamma 2}$ bonds in a valine residue were determined from analysis of ^{13}C NMR relaxation data on $C_\beta H$ and $C_\gamma H_3$ groups (*11*). These examples show how ^{13}C NMR relaxation data from side-chain carbons can yield unique information on backbone motions.

From proton-coupled ^{13}C NMR multiplet relaxation of the $C_\beta H_2$ group, three motional order parameters, S_{CH}, S_{HCH}, and S_{CHH}, can be obtained to describe restrictions of CH and HH motional vectors. For simplicity, let us see what occurs when angular variances for $\phi(t)$ and $\psi(t)$ rotations are equal. With $\chi_1^0 = 180°, -60°$ (the label β is omitted to simplify equations),

$$S^2_{CH} = 1 - 4\sigma^2_\alpha - \tfrac{8}{3}\sigma^2_{\chi 1} + \tfrac{4}{3}c_{\phi\psi}\sigma^2_\alpha \qquad [22a]$$

$$S^2_{HCH} = -\tfrac{1}{3} + \tfrac{4}{3}\sigma^2_\alpha + \tfrac{4}{3}\sigma^2_{\chi 1} + \tfrac{4}{9}c_{\phi\psi}\sigma^2_\alpha \qquad [22b]$$

$$S^2_{CHH} = \tfrac{1}{2} - 2\sigma^2_\alpha - \tfrac{4}{3}\sigma^2_{\chi 1} + \tfrac{4}{3}c_{\phi\psi}\sigma^2_\alpha \qquad [22c]$$

and with $\chi_1^0 = 60°$,

$$S^2_{CH} = 1 - \tfrac{8}{3}\sigma^2_2 - \tfrac{8}{3}\sigma^2_{\chi_1} \qquad [23a]$$

$$S^2_{HCH} = -\tfrac{1}{3} + \tfrac{4}{3}\sigma^2_{\chi 1} - \tfrac{8}{9}c_{\phi\psi}\sigma^2_\alpha \qquad [23b]$$

$$S^2_{CHH} = \tfrac{1}{2} - \tfrac{4}{3}\sigma^2_\alpha - \tfrac{4}{3}\sigma^2_{\chi 1} - \tfrac{4}{3}c_{\phi\psi}\sigma^2_\alpha. \qquad [23c]$$

TABLE 3

Coefficients $\langle A\rangle$ and $\langle B\rangle$ for Autocorrelation Functions of $C_\gamma H$ and $C_{\delta,\epsilon}H$ Groups (Phenyl Ring) for Different Values of the χ_1^0 Dihedral Angles

χ_1^0	180°	−60°	60°
A_ϕ^{CH} ($C_\gamma H$)	8/3	16/9	16/9
A_ψ^{CH} ($C_\gamma H$)	16/9	8/3	16/9
A_1^{CH} ($C_\gamma H$)	16/9	16/9	16/9
A_2^{CH} ($C_\gamma H$)	8/3	8/3	8/3
A_ϕ^{CH} ($C_{\delta,\epsilon}H$)	2.25	1.917	1.917
A_ψ^{CH} ($C_{\delta,\epsilon}H$)	1.917	2.25	1.917
A_1^{CH} ($C_{\delta,\epsilon}H$)	1.917	1.917	1.917
A_2^{CH} ($C_{\delta,\epsilon}H$)	2.25	2.25	2.25
$B_{\phi\psi}^{CH}$ ($C_\gamma H$)	−16/9	−16/9	−8/9
$B_{\phi 1}^{CH}$ ($C_\gamma H$)	16/9	8/9	8/9
$B_{\psi 1}^{CH}$ ($C_\gamma H$)	8/9	16/9	8/9
$B_{\phi 2}^{CH}$ ($C_\gamma H$)	16/3	−16/9	−16/9
$B_{\psi 2}^{CH}$ ($C_\gamma H$)	−16/9	16/3	−16/9
B_{12}^{CH} ($C_\gamma H$)	16/9	16/9	16/9
$B_{\phi\psi}^{CH}$ ($C_{\delta,\epsilon}H$)	−1.5	−1.5	−1.167
$B_{\phi 1}^{CH}$ ($C_{\delta,\epsilon}H$)	1.5	1.167	1.167
$B_{\psi 1}^{CH}$ ($C_{\delta,\epsilon}H$)	1.167	1.5	1.167
$B_{\phi 2}^{CH}$ ($C_{\delta,\epsilon}H$)	4.5	−1.5	−1.5
$B_{\psi 2}^{CH}$ ($C_{\delta,\epsilon}H$)	−1.5	4.5	−1.5
B_{12}^{CH} ($C_{\delta,\epsilon}H$)	1.5	1.5	1.5

Note. coefficients A and B for $C_{\delta,\epsilon}H$ groups were calculated by averaging over $C_\delta H$ and $C_\epsilon H$ bonds of the phenyl ring. The dehedral angle χ_1^0 is defined by $N-C_\alpha-C_\beta-C_\gamma$ atoms.

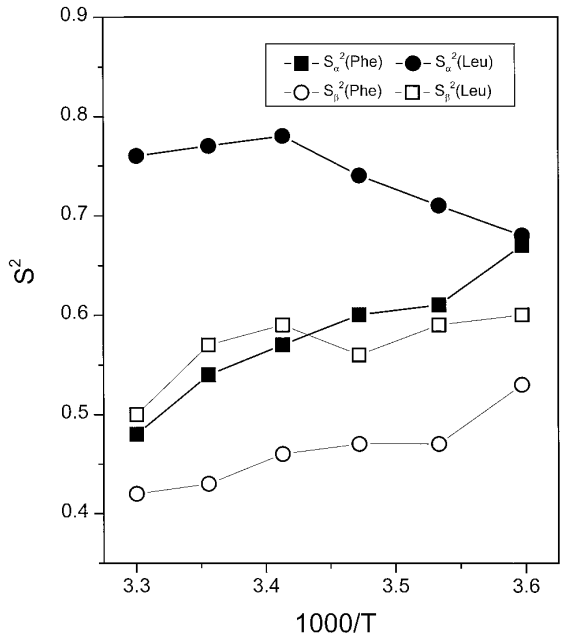

FIG. 2. The temperature dependence of order parameters for $C_\alpha H$ and $C_\beta H$ bonds of F2 and L7 in the peptide GFSKAELAKARAAKRGGY which forms relatively stable populations of α-helix structure at low temperature.

These equations allow angular variances, rotational correlation coefficients, and the average value of the χ_1 dihedral angle (defined by $N-C_\alpha-C_\beta-C_\gamma$ atoms) to be calculated. For phenylalanine, for example, relaxation measurements on the $^{13}C_\zeta H$ group (*para*-position) yield the order parameter S^2_{CC} for the $C_\beta-C_\gamma$ bond. Using the same definition for χ_1, equations for these order parameters can be written for $\chi_1^0 = 60°$,

$$S^2_{CC} = 1 - \tfrac{16}{3}\sigma^2_\alpha - \tfrac{8}{3}\sigma^2_{\chi 1} + \tfrac{8}{3}c_{\phi\psi}\sigma^2_\alpha \qquad [24a]$$

and for $\chi_1^0 = 180°, -60°$,

$$S^2_{CC} = 1 - \tfrac{8}{3}\sigma^2_\alpha - \tfrac{8}{3}\sigma^2_{\chi 1}. \qquad [24b]$$

To analyze motions of $C_\gamma H$ and $C_\gamma H_2$ groups, different values of χ_1^0 and χ_2^0 need to be considered. Usually $C_\gamma H$ groups are more mobile than $C_\beta H$ groups, and there is considerably greater angular variance in χ_2 than there is in χ_1. Therefore, averaging over χ_2 can be done when analyzing NMR relaxation data. Table 3 gives A and B coefficients for autocorrelation functions of $C_\gamma H$ bonds and phenyl ring CH bonds. For the phenyl ring, averaging was done over $C_\delta H$ and $C_\epsilon H$ groups. In all cases, the dihedral angle χ_1^0 was defined by the atoms $N-C_\alpha-C_\beta-C_\gamma$.

The number of unknowns can be significantly reduced by neglecting rotational correlations between nonadjacent bonds, e.g., ϕ and χ_2, and by initially assuming that all

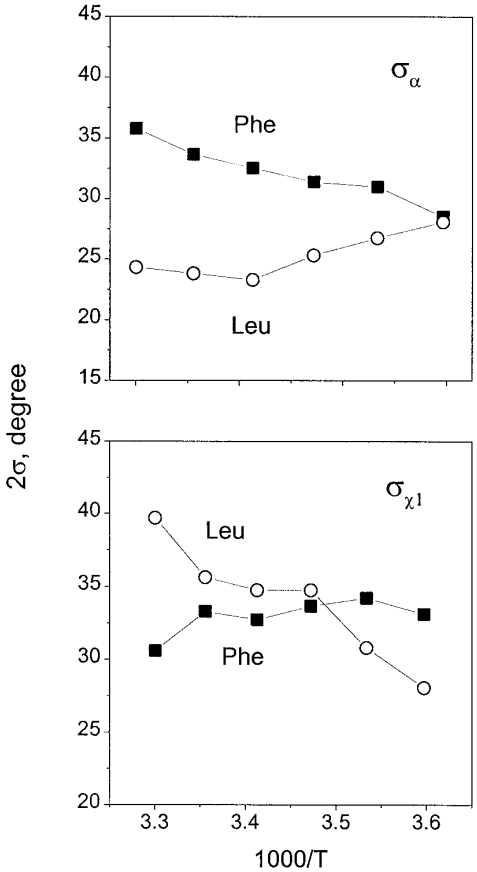

FIG. 3. The temperature dependence of angular variances for F2 and L7 in the peptide GFSKAELAKARAAKRGGY. Angular variances have been calculated under the assumption that internal rotations are uncorrelated.

rotational correlations are equal to zero. For unrealistic angular variances (e.g., those that decrease with increasing temperature) which result following the first round of calculations, fits can be improved by introducing rotational correlations as was done by Ramizez-Alvarado *et al.* (*11*). Another example is provided with the 18-residue peptide GFSKAELAKARAAKRGGY which, at low temperature, forms an α-helix structure that is stabilized by a hydrophobic staple motif (*12*). By measuring ^{13}C spin–lattice relaxation times and ^{13}C–{^{1}H} NOEs at two frequencies, S^2_{CH} values for CH bonds of several residues have been determined (*13*). These are exemplified in Fig. 2 for phenylalanine (F2) and leucine (L7). The equilibrium value of $\chi_1^0 = -60°$ was derived from bond rotation energy profiles calculated using the DISCOVER program (Version 2.3.5, Biosym Technologies). Assuming that internal rotations are uncorrelated, angular variances were calculated for different temperatures (Fig. 3). For L7, $\sigma^2_\alpha = (\sigma^2_\phi + \sigma^2_\psi)/2$ increases on going to lower temperature. This is physically unrealistic, particularly in view of the fact that the peptide is more folded at lower temperature. The only way to explain this apparent contradiction is to consider that $\phi(t)$ and $\psi(t)$ bond rotations are strongly negatively correlated. This is consistent with the proposal that in α-helices, $\phi(t)$ and $\psi(t)$ bond rotations are negatively correlated (*1*). For F2, more mobile and less correlated rotations may be the result of its proximity to the N-terminus. Examples of using cross-correlation spectral densities to derive information on bond rotational correlations and amplitudes of internal bond rotations can be found in other papers (*1, 8, 10, 11*). Rotational correlation coefficients can also be estimated by using molecular dynamics simulations or by using appropriate analytical methods (*8*). Measuring dipolar–CSA (chemical shift anisotropy) cross-correlation terms and ^{13}C proton-coupled relaxation for $C_\gamma H_2$ groups can provide further information to determine additional motional model parameters.

In conclusion, this simple approach can be used to derive information on protein and peptide side-chain motional dynamics via analysis of ^{13}C NMR relaxation data. Even though more detailed descriptions of internal mobilities in proteins are required to fully analyze all available experimental data, this approach represents a first approximation for estimating bond rotational amplitudes and correlation coefficients in proteins.

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