Internal motional amplitudes and correlated bond rotations in an α -helical peptide derived from ^{13}C and ^{15}N NMR relaxation

DJAUDAT IDIYATULLIN,¹ ALEXEI KRUSHELNITSKY,¹ IRINA NESMELOVA,¹
FRANCESCO BLANCO,² VLADIMIR A. DARAGAN,¹ LUIS SERRANO,²
AND KEVIN H. MAYO¹

¹Department of Biochemistry, Molecular Biology & Biophysics and the Biomedical Engineering Center,
University of Minnesota Health Science Center, 321 Church Street, Minneapolis, Minnesota 55455

²European Molecular Biology Laboratory, Structures and Biocomputing Programme, Meyerhofstraße 1,
69012 Heidelberg, Germany

(RECEIVED March 20, 2000; FINAL REVISION September 8, 2000; ACCEPTED September 8, 2000)

Abstract

Peptide GFSKAELAKARAAKRGGY folds in an α -helical conformation that is stabilized by formation of a hydrophobic staple motif and an N-terminal capping box (Munoz V, Blanco FJ, Serrano L, 1995, *Struct Biol* 2:380–385). To investigate backbone and side-chain internal motions within the helix and hydrophobic staple, residues F2, A5, L7, A8, and A10 were selectively ^{13}C - and ^{15}N -enriched and NMR relaxation experiments were performed in water and in water/trifluoroethanol (TFE) solution at four Larmor frequencies (62.5, 125, 150, and 200 MHz for ^{13}C). Relaxation data were analyzed using the model free approach and an anisotropic diffusion model. In water, angular variances of motional vectors range from 10 to 20° and backbone ϕ, ψ bond rotations for helix residues A5, L7, A8, and A10 are correlated indicating the presence of $\text{C}_\alpha\text{-H}$, $\text{C}_\alpha\text{-C}_\beta$, and N-H rocking-type motions along the helix dipole axis. L7 side-chain C_βH_2 and C_γH motions are also correlated and as motionally restricted as backbone C_αH , suggesting considerable steric hindrance with neighboring groups. In TFE which stabilizes the fold, internal motional amplitudes are attenuated and rotational correlations are increased. For the side chain of hydrophobic staple residue F2, wobbling-in-a-cone type motions dominate in water, whereas in TFE, the $\text{C}_\beta\text{-C}_\gamma$ bond and phenyl ring fluctuate more simply about the $\text{C}_\alpha\text{-C}_\beta$ bond. These data support the Daragan–Mayo model of correlated bond rotations (Daragan VA, Mayo KH, 1996, *J Phys Chem* 100:8378–8388) and contribute to a general understanding of internal motions in peptides and proteins.

Keywords: α -helix; anisotropic tumbling; ^{13}C and ^{15}N relaxation; correlated motions; molecular dynamics; NMR; peptide; trifluoroethanol

Peptide GFSKAELAKARAAKRGGY folds in an α -helix conformation that is stabilized by formation of a hydrophobic staple motif and an N-terminal capping box (Munoz et al., 1995). In water at 5 °C, numerous conformationally constraining, long-range ^1H nuclear Overhauser effects (NOEs) have been observed and distance geometry calculations demonstrate formation of α -helix conformation from K4 through A13 and an N-capping box motif between S3 and E6. Residues F2 and L7 comprise the structurally stabilizing hydrophobic staple. NOEs observed between F2 ring protons and side chains of L7 and A10, position A10 adjacent to this motif. The structure of the peptide from F2 to R11 is shown in

Figure 1. The C-terminal portion of the peptide is less structured and appears to form nascent helix. Within residues 2 through 10, the number of nonsequential NOEs, the large H_α chemical shift differences from random coil positions and the small $^3J_{\text{HN}\alpha}$ coupling constants are similar to those observed in proteins. Based on analysis of circular dichroism (CD) measurements, the peptide in water is estimated to be about 48% folded at 5 °C and about 20% folded at 30 °C (Munoz et al., 1995). In the presence of 40% (v/v) trifluoroethanol (TFE), which is known to stabilize folded structure, the peptide is about 90% folded at 5 °C and about 50% folded at 30 °C. Overall, this peptide is a good model system with which to investigate internal motional dynamics in a partially folded, α -helix-forming peptide using NMR relaxation techniques.

Structurally characterizing partially folded states has often proved problematic given their highly transient nature. Heteronuclear NMR relaxation, however, is well suited to the task. ^{13}C - and ^{15}N -NMR

Reprint requests to: Kevin H. Mayo, Department of Biochemistry, Molecular Biology & Biophysics and the Biomedical Engineering Center, University of Minnesota Health Science Center, 321 Church Street, Minneapolis, Minnesota 55455; e-mail: mayox001@maroon.tc.umn.edu.

Fig. 1. Structure of the hydrophobic staple, α -helical peptide. The five residues being investigated in the present study, F2, A5, L7, A8, and A10, are labeled. This figure is a modified version of the one in Munoz et al. (1995).

relaxation provides information on internal motional amplitudes of various bond vectors and their preferred axes of motion, i.e., motional geometry (Daragan et al., 1997). Such relaxation studies offer a unique approach to discerning the limits of conformational space accessible to a given residue via analysis of auto- and cross-correlation spectral densities using various motional models (Daragan & Mayo, 1997). To date, most studies on peptide and protein dynamics have been limited to analysis of ^{15}N NMR relaxation data, and therefore, to information from a single motional vector within the backbone. In addition, such data have usually been analyzed with the Lipari–Szabo model free approach, which assumes isotropic molecular motion. While this qualitative analysis of peptide dynamics may be sufficient to assess motional restrictions in the context of structure elucidation, it falls short of providing a comprehensive account of internal motions within a peptide. For this, inclusion of additional motional vectors from the backbone, as well as from side chains, is required.

$^{13}\text{C}/^{15}\text{N}$ -NMR relaxation investigations of internal motions of residues ‘X’ in short GXG-based tripeptides (Mikhailov et al., 1999) set the stage and provide a point of reference for interpretation of internal motions in larger peptides, like this α -helix-forming peptide. GXG peptides present the case where the backbone and side chain of residue ‘X’ are as fully solvent exposed and their motions are as unrestricted as possible in the context of a peptide. Even in these tripeptides, however, internal motions of side chains, particularly of β -branched and aromatic residues, are as restricted as those of backbone C_αH . Comparison of internal motional parameters from GXG peptides and from partially folded peptides (Daragan et al., 1997; Ramirez-Alvarado et al., 1998) or peptides

in low dielectric solvents (Mikhailov et al., 1995) may provide insight into the environment in which a particular residue resides. For example, residues in a more hydrophobic environment usually demonstrate increased motional amplitudes accompanied by lower bond rotational activation energies. This type of information has provided a better understanding of the ‘‘unfolded’’ state as being a collapsed state of the peptide (Daragan et al., 1997; Ramirez-Alvarado et al., 1998). Internal motions in peptides and proteins are also correlated, and correlated motions can contribute to NMR relaxation-derived auto- and cross-correlation spectral densities and values of motional order parameters (Daragan & Mayo, 1996a). Daragan and Mayo (1996a) developed a motional model to assess the influence of internally restricted correlated bond rotations on NMR relaxation-derived motional order parameters. In this model, backbone bond rotations, for example, are represented by vectors whose motions are correlated by a correlation coefficient, $c_{\phi\psi}$. Using molecular dynamics simulations on model peptides in α -helix and β -sheet structures, it was observed that the magnitude and sign of $c_{\phi\psi}$ are dependent upon ϕ, ψ angles within a structural element: in an α -helix, $c_{\phi\psi}$ is negative and in a β -sheet, $c_{\phi\psi}$ is positive. As initial verification of the model, $c_{\phi\psi}$ was found to be positive in two partially-folded β -sheet/hairpin-forming peptides, indicating that C_αH internal motions primarily occur along the sheet as ‘‘twisting’’ type motions (Daragan et al., 1997; Ramirez-Alvarado et al., 1998).

In the present study, ^{13}C and ^{15}N NMR relaxation experiments were performed on the hydrophobic staple-helix peptide in water and in water/TFE solution from 5 to 30 °C. Five hydrophobic residues, F2, A5, L7, A8, and A10, were uniformly $^{13}\text{C}/^{15}\text{N}$ -enriched, and proton-coupled and -decoupled NMR relaxation measurements were acquired at four Larmor frequencies (62.5, 125, 150, and 200 MHz for ^{13}C). From these relaxation data, auto- and cross-correlation spectral densities were determined and analyzed by using the model free approach (Lipari & Szabo, 1982a, 1982b) and an anisotropic motional model (Daragan & Mayo, 1997). Aside from providing internal motional amplitudes of backbone and side-chain C–H and N–H bonds in the peptide as the population of folded structure is varied, this study was designed to further validate the Daragan–Mayo model of correlated bond rotations (Daragan & Mayo, 1996a). If the model is correct, rotational correlation coefficients for residues within the helix region of this peptide should become more negative when the α -helix population is increased by lowering the temperature and/or by addition of TFE.

Results and discussion

For the helix-forming peptide GFSKAELAKARAARKGGY, backbone and side-chain ^{13}C and ^{15}N spin–lattice (T_1) and spin–spin (T_2) relaxation rates and heteronuclear NOEs were measured for isotopically-enriched residues F2, A5, L7, A8, and A10. For additional information on methylene and methyl group motions, ^{13}C multiplet spin–lattice relaxation rates were determined, allowing both auto-, $J_{\text{CH}}^*(\omega) = \frac{1}{10} [J(\omega_{\text{C}} - \omega_{\text{H}}) + 3J(\omega_{\text{C}}) + 6J(\omega_{\text{C}} + \omega_{\text{H}})]$, and cross-correlation, $J_{\text{HCH}}(\omega_{\text{C}})$, spectral densities to be derived. Relaxation data were acquired with the peptide in water at four magnetic field strengths (5.9, 11.8, 14.1, and 18.8 Tesla) and at temperatures from 5 °C where the peptide is about 48% folded (Munoz et al., 1995) to 30 °C where the peptide is only about 20% folded. To better assess ^{13}C -H and ^{15}N -H bond motions in a more folded state, relaxation measurements were also made with the

peptide in a 40/60 (v/v) mixture of TFE/water where the peptide is about 90% folded at 5°C and about 50% folded at 30°C. Relaxation data are exemplified in Figure 2, which shows the Larmor frequency dependence of W_1 ($= 1/T_1$) for $^{13}\text{C}_\alpha\text{H}$ (Fig. 2B) and W_2 ($= 1/T_2$) for ^{15}NH (Fig. 2A) of A5, L7, A8, and A10 in the presence and absence of TFE.

Helix residues A5, L7, A8, and A10

Using the Lipari–Szabo model free approach and Monte Carlo minimization, overall and internal correlation times, τ_o and τ_i , respectively, and order parameters, S^2 , were determined for backbone $^{13}\text{C}_\alpha\text{H}$ and ^{15}NH . In the presence and absence of TFE, the temperature dependence of τ_o and S^2 is presented in Figure 3 for $^{13}\text{C}_\alpha\text{H}$ and in Figure 4 for ^{15}NH .

In water, the overall correlation time for molecular tumbling τ_o increases with decreasing temperature as expected. At any given temperature, addition of TFE to the water solution increases τ_o due to increased viscosity of the solution. The dotted lines plotted in these figures illustrate the temperature dependence of the inverse of translational diffusion coefficients D for this peptide. In TFE, the slope is steeper, which reflects an increase in the energy of activation for molecular motion due to increased viscosity. Assuming that translational and rotational motions of the peptide are hydrodynamically mediated at low peptide concentrations as those

Fig. 2. Frequency dependence of (B) spin-lattice relaxation rates W_1 for $^{13}\text{C}_\alpha$ and (A) spin-spin relaxation rates W_2 for ^{15}N backbone nuclei of residues in the hydrophobic staple, α -helical peptide. Data are shown for the peptide in water in the absence and presence of TFE. Experimental errors are indicated by bars through data points; the absence of a bar indicates that the error is within the size of the symbol.

Fig. 3. The temperature dependence of overall correlation times and order parameters for backbone C_αH bonds using the Lipari–Szabo model-free approach. Data are shown for the peptide in water in the absence and presence of TFE.

used here, the temperature dependencies of τ_o and $1/D$ should be the same if the peptide were spherical and its conformation remained constant over this temperature range. For the most part, these temperature dependencies are not the same, with the temperature dependence of τ_o being somewhat less, i.e., it has a shallower slope, than that of $1/D$. This indicates that the shape of the peptide is not spherical and/or that the conformational distribution of the peptide changes with temperature. In the absence of TFE, the conformational distribution of this peptide does change significantly with increasing temperature and the shape of the structured peptide is more cylindrical than spherical (Munoz et al., 1995). Using the modified Kirkwood–Steele–Huntress theory (Daragan & Mayo, 1997), the ratio of the two main components of the rotational diffusion tensor, $D_{\parallel}$ and $D_{\perp}$, for the structured peptide was calculated to be 2.9 and not 1 as it would be for a spherical molecule. The ensemble-averaged shape of the peptide affects the rotational correlation time τ_o more than it does the translational diffusion coefficient D . Of particular note is that variations among τ_o values for helix residues A5, L7, A8, and A10 are relatively small at any temperature even though the helix content estimated by CD changes significantly with temperature. This may suggest that basic elements of the helix are preserved over this temperature range and that temperature-dependent changes in CD ellipticities are more a reflection of increased fluctuations of the helix at higher temperature rather than of a drastic change in the conformational ensemble average.

Fig. 4. The temperature dependence of overall correlation times and order parameters for backbone NH bonds using the Lipari–Szabo model-free approach. Data are shown for the peptide in water in the absence and presence of TFE.

The observation that τ_o for ^{15}NH is generally larger than it is for $^{13}\text{C}_\alpha\text{H}$ is related to the fact that the peptide is not spherical and tumbles anisotropically in solution. Molecular anisotropy is supported by $^{13}\text{C}_\alpha\text{H}$ and ^{15}NH spectral densities, which at zero frequency $J(0)$ (data not shown) do not have the same value as they would if the molecule were spherical. This is especially true at lower temperature and in the presence of TFE where the peptide is more structured. The NH spectral density at zero frequency is larger because in the helix conformation the NH bond is directed almost along the long axis of the helix (11° compared to 58° for the C_αH bond) where the average correlation time should be larger than that of the C_αH bond.

To take into account the more cylindrical shape of the molecule, relaxation data were also analyzed using an anisotropic motional model parameterized with rotational diffusion coefficients $D_\parallel$ and $D_\perp$ and two internal motional restriction parameters $\sigma_\theta^2 = \langle \Delta\theta^2 \rangle$ and $\sigma_\phi^2 = \langle \Delta\phi^2 \rangle$. For anisotropic overall tumbling, a single order parameter is not sufficient to describe the experimental data (see Materials and methods). At 5°C in water, the ratio $D_\parallel/D_\perp$ averaged over residues in the helix is 1.9. Deviation from the value of 2.9 calculated using the Kirkwood–Steele–Huntress theory results from (1) averaging of folded, partially folded and unfolded populations of the peptide conformational ensemble and (2) not taking into account the hydration shell around the peptide. Neglecting hydration effects and assuming a two state equilibrium of folded and unfolded peptide, the experimental value of 1.9 for $D_\parallel/D_\perp$ suggests

that approximately 50% of the ensemble is folded with a cylindrical shape at any given time. In TFE at 5°C , $D_\parallel/D_\perp$ averaged over residues in the helix is 2.6, indicating that about 90% of the ensemble is folded at any given time. These structural percentages are consistent with estimates made using CD and NMR chemical shift data.

Considering the anisotropic diffusion model, Figure 5 illustrates relationships between polar angles θ , ϕ , and angular variances σ_θ , σ_ϕ with respect to the Z_D axis and also shows average NH and C_αH bond orientations in an α -helix with respect to the same Z_D axis that runs parallel to the long axis of the helix. For motional vectors in this helical peptide, values for σ_θ and σ_ϕ derived using this model are plotted in Figure 6 and are given in Table 1 as the average of two low temperature values (5 and 10°C) and two high temperature values (25 and 30°C). In water, σ_ϕ for both C_αH and NH are, for the most part, larger than σ_θ values and remain fairly constant over this temperature range, whereas σ_θ values decrease with increasing temperature. The observation of smaller σ_θ values at higher temperature is consistent with the presence of correlated bond rotations as discussed below. Note also, that σ_ϕ and σ_θ values are larger for NH than for C_αH . Motions of the C_αH vector are probably more restricted than those of NH due to the presence of the C_βH_n side-chain group. Addition of TFE, which stabilizes the helix conformation of the peptide, also tends to restrict internal motions of both C_αH and NH, with the greater effect being observed in σ_ϕ . At higher temperature in TFE, however, σ_θ for C_αH and NH behaves differently by increasing, not decreasing, in value. Overall, angular variances are apparently less temperature dependent in water/TFE than in water alone, suggesting increased structural stability even at 30°C as indicated by τ_o values discussed above. One should note that if angular fluctuations follow a Gaussian distribution (as is the case for rotational fluctuations within a potential well), there is a 0.7 probability of finding a given motional vector within $\pm\sigma$ of the average orientation. If, on the other hand, angular fluctuations do not follow a Gaussian distribution, i.e., jumps, then $\sigma_\theta^2 = \langle (\theta - \theta_{ave})^2 \rangle$. In any event, a value of 2σ represents a lower limit in terms of the actual range in angular variation.

Angular variances σ calculated from S^2 values (Figs. 3, 4) are comparable to those derived using the anisotropic diffusion model. Average angular variances for ϕ, ψ backbone bond rotations have been estimated using order parameters for C_αH bonds. This angular variance is referred to as σ_α . The relationship between S^2 and σ_α is (Daragan & Mayo, 1996a):

$$S^2 = 1 - \frac{16}{3} \sigma_\alpha^2. \quad (1)$$

Fig. 5. Illustrations relating helix NH, C_αH , and C_βH bond orientations to polar angles θ and ϕ for angular variances as discussed in the text.

Fig. 6. Temperature dependence of restriction parameters σ_θ and σ_ψ for internal motions of backbone bonds and an effective order parameter S_{eff}^2 for residues in the α -helical part of the peptide. These values were calculated using the anisotropic diffusion model as discussed in the text.

Relative to residues in GXG-based tripeptides (Mikhailov et al., 1999), σ_α values for A5, L7, A8, and A10 in this peptide are generally smaller. For example, σ_α for L7 C α H is 13°, whereas for leucine C α H in the tripeptide GLG, σ_α is closer to 20°. This is consistent with the fact that this hydrophobic staple peptide has

Table 1. Average amplitudes of internal motions of C α H and NH backbone bonds for residues in the α -helical region of the peptide

	C α H		NH	
	5–10 °C	25–30 °C	5–10 °C	25–30 °C
Polar coordinates				
σ_ψ	15 ± 3	18 ± 3	23 ± 4	22 ± 3
+ TFE	13 ± 3	7 ± 3	17 ± 2	17 ± 3
σ_θ	11 ± 2	7 ± 2	16 ± 2	10 ± 3
+ TFE	9 ± 2	10 ± 2	15 ± 1	17 ± 3
Cartesian coordinates				
σ_z	10 ± 2	7 ± 3	14 ± 3	9 ± 2.5
+ TFE	8 ± 2	10 ± 2	13 ± 2	15 ± 2
$\sigma_x = \sigma_y^a$	10 ± 2	9 ± 2	14 ± 3	12 ± 2
+ TFE	8 ± 2	6 ± 2	11 ± 2	12 ± 2

^aValues assume that $\sigma_x = \sigma_y$ in Equation 14.

some defined structure and the tripeptide does not. In addition, σ_α values for these helix residues in the hydrophobic staple peptide tend to be relatively close together at any given temperature and to follow the same trend with temperature, supporting the idea that at least this segment of the peptide behaves as a single structural unit.

σ_θ and σ_ψ , derived using the anisotropic diffusion model, are related to Cartesian coordinates in the molecular diffusive frame by

$$\sigma_x^2 + \sigma_y^2 = \cos^2 \theta \sigma_\theta^2 + \sin^2 \theta \sigma_\psi^2 \quad (2A)$$

and

$$\sigma_z^2 = \sin^2 \theta \sigma_\theta^2 \quad (2B)$$

where σ_x^2 , σ_y^2 , and σ_z^2 are variances of the projections of a unit motional vector on to the respective axis indicated. For C α H and NH, θ is the angle that C-H and N-H bonds make with respect to the long axis of the helix, i.e., 58 and 11°, respectively. Values for σ_z and $\sigma_x = \sigma_y$, given in Table 1, indicate that at lower temperature and in water alone, both C α H and NH bond vectors fluctuate parallel to the dipole axis of the helix (z -axis) to the same extent as in the plane perpendicular to that axis. Such C α H and NH bond motions are effectively fluctuating within a nearly symmetric cone originating at C α or N. At higher temperature, motional amplitudes in the x, y plane are greater, making the cone more elliptical in shape. In TFE, both C α H and NH motional amplitudes are attenuated along all axes at lower temperature and increase along the z -axis while decreasing in the x, y plane at higher temperature. The most likely explanation for bond vector fluctuations tracing out a cone shape is that ϕ, ψ bond rotations, which primarily dictate C α -H and N-H bond motions, are correlated.

The presence of correlated ϕ, ψ bond rotations is also evident in the temperature dependence of S^2 , derived either from the Lipari–Szabo model (Figs. 3, 4) or from the anisotropic motional model, which yields an effective order parameter S_{eff}^2 (Fig. 6). S_{eff}^2 is derived from the value of the correlation function at $t \ll 1/D_\perp, 1/D_\parallel$ and is equal to $[1 - 3(\langle \Delta\theta^2 \rangle + \sin^2 \theta \langle \Delta\phi^2 \rangle)]$ (Daragan & Mayo, 1999). S_{eff}^2 values for C α H bonds are nearly the same as S^2 obtained using the Lipari–Szabo model. Notice that both S_{eff}^2 and S^2 increase slightly with increasing temperature. This is more convincing for the peptide in the presence of TFE. Although this appears to run contrary to the fact that the peptide is more structured at lower temperature where motional order parameters should be larger and not smaller as observed, this trend can be explained by considering that ϕ, ψ bond rotations are correlated (Daragan & Mayo, 1996a). Since internal motions of any C α H bond are dictated primarily by ϕ, ψ bond rotations, the order parameter for C α H bond motion can be expressed (Daragan & Mayo, 1998) as

$$S_{\text{CH}}^2 = 1 - \frac{8}{3} (\sigma_\phi^2 + \sigma_\psi^2) + \frac{8}{3} \sigma_\phi \sigma_\psi c_{\phi\psi} \quad (3)$$

where σ_ϕ^2 and σ_ψ^2 are angular variances of ϕ and ψ bond rotations, and $c_{\phi\psi}$ is the rotational correlation coefficient ($-1 < c_{\phi\psi} < 1$). The value of $c_{\phi\psi}$ is dependent upon the ϕ and ψ angles within a structural element. Molecular dynamics studies on short peptides demonstrated that $c_{\phi\psi}$ should be negative for residues in an α -helix (Daragan & Mayo, 1996a, 1997). At low temperature (5 °C) where helical structure for this peptide is most stable, $c_{\phi\psi}$ for A5, L7, A8,

and A10, therefore, should be negative according to the Daragan–Mayo model of correlated ϕ, ψ bond rotations. Assuming that σ_ϕ and σ_ψ are equal and using a reasonable value of 15° as presented above, $c_{\phi\psi}$ is estimated to be -0.5 . As the temperature is increased and helical structure is reduced, $c_{\phi\psi}$ should become more positive and S_{CH}^2 should increase according to Equation 3. The alternative would be to consider that σ_ϕ and σ_ψ decrease significantly as the temperature is increased. However, it has been already been observed that σ_ϕ and σ_ψ for residues in this peptide as well as for other peptides (see above), vary little, if at all, with temperature.

One test for the Daragan–Mayo model of correlated ϕ, ψ bond rotations is to compare the temperature dependence of S^2 for residues in the peptide with low helix content to that when the helix content is increased. When helix content is increased, $c_{\phi\psi}$ should become more negative and S^2 should demonstrate an even greater “inverse” temperature dependence. Here, this has been accomplished by the addition of TFE which is known to increase conformational stability of a peptide (Buck, 1998). In Figure 3, notice that in the presence of TFE, $S_{\text{C}\alpha\text{H}}^2$ for helical residues A5, L7, A8, and A10, becomes larger at higher temperature. For the peptide in water, S^2 for NH groups displays a more normal temperature dependence (Fig. 4), decreasing with increasing temperature as the structured population is reduced. In the presence of TFE, however, the temperature dependence of S_{NH}^2 also becomes more like that of $S_{\text{C}\alpha\text{H}}^2$. For the NH group, this indicates correlated rotations of $\psi(i, i-1)$ and $\phi(i, i)$ backbone bonds. For α -helix structure, S_{NH}^2 can be expressed as (Daragan & Mayo, 1996a)

$$S_{\text{NH}}^2 = 1 - \sigma^2(3.7 - 2.0c_{\psi'\phi} - 0.1c_{\phi\psi}) \quad (4)$$

where $c_{\psi'\phi}$ is the correlation coefficient for $\psi(i, i-1)$ and $\phi(i, i)$ backbone bonds. From Equation 4, it is evident that the influence of the rotational correlation coefficient $c_{\phi, \psi}$ is small because of the small value of its coefficient. The primary contribution to S_{NH}^2 arises from the coefficient $c_{\psi'\phi}$, which, due to the relative rigidity of the $\text{NH}=\text{CO}$ plane, is usually negative (Daragan & Mayo, 1996a). The $c_{\psi'\phi}$ term has a greater negative contribution to S_{NH}^2 as the peptide fold is stabilized, in this case by addition of TFE. This helps explain why S_{NH}^2 increases as the temperature is increased. A decrease in S_{NH}^2 as the temperature is lowered may indicate that these rotational correlation coefficients contribute more to the or-

der parameter and is consistent with the presence of correlated ϕ, ψ bond rotations and motions occurring along the helix dipole axis.

For the L7 side chain, correlated bond rotations can also be deduced from analysis of side-chain order parameters. S^2 values for L7 C_βH_2 and C_γH groups (Table 2) were derived by using the Lipari–Szabo model and a value for τ_o taken as the average τ_o of C_αH s within the helix. At 5°C in the absence or presence of TFE, the L7 C_βH_2 group is nearly as motionally restricted as its backbone C_αH . The motional restriction map (Daragan & Mayo, 1995, 1997) shown in Figure 7 plots S_{CH}^2 vs. S_{HCH}^2 for L7 C_βH_2 . On this map, relationships between S_{CH}^2 and S_{HCH}^2 for three different motional models are plotted as solid lines. Even though internal motions of L7 C_βH_2 are dominated by rotations about the $\text{C}_\alpha\text{--C}_\beta$ bond, correlated bond rotations do contribute to the motional ensemble. At 5°C where the peptide is more structured, off-bond-axis motions contribute to this ensemble. These motions arise when backbone and side-chain bond rotations are negatively correlated and result from $\text{C}_\alpha\text{--C}_\beta$ bond fluctuations occurring parallel to the helix dipole axis as described above for the L7 $\text{C}_\alpha\text{--H}$ bond (see Fig. 6). At 30°C , wobbling-in-a-cone type motions contribute to the ensemble. The same types of correlated motions are found to occur in the presence of TFE.

L7 $\text{C}_\beta\text{--C}_\gamma$ bond rotations are more restricted than those of the $\text{C}_\alpha\text{--C}_\beta$ bond. At 5°C , angular variances for χ_1 ($\text{C}_\alpha\text{--C}_\beta$) bond rotations, σ_{χ_1} , and χ_2 ($\text{C}_\beta\text{--C}_\gamma$) bond rotations σ_{χ_2} , (Table 3) [calculated as described by Ramirez-Alvarado et al., 1998], are about 30 and 14° , respectively, in water, and about 20 and 13° , respectively, in TFE. Similar trends are also found at 30°C in the presence or absence of TFE. C_βH_2 motional amplitudes are mostly contributed to by rotations about χ_1 and χ_2 bonds, whereas C_γH motional amplitudes are dominated by rotations about the χ_2 bond. However, C_β is bonded to two hydrogens that are less bulky than the two methyls bonded to C_γ . This may account for differences in the motional amplitudes of these groups, even though the C_γ group is further along the side chain.

N-terminal residue F2

Using the Lipari–Szabo model, τ_o and S^2 for F2 $^{13}\text{C}_\alpha\text{H}$ and ^{15}NH (Figs. 3, 4) are found, for the most part, to be smaller than they are for helical residues A5, L7, A8, and A10. This is consistent with F2

Table 2. Order parameters for L7 and F2 side-chain CH bonds

Temperature ($^\circ\text{C}$)	Residue/ bond	S_{CH}^2 H_2O	S_{HCH}^2 H_2O	S_{CH}^2 $\text{H}_2\text{O} + \text{TFE}$	S_{HCH}^2 $\text{H}_2\text{O} + \text{TFE}$
5	Leu7 C_βH	0.60 ± 0.04	-0.10 ± 0.03	0.58 ± 0.04	-0.09 ± 0.03
30	Leu7 C_βH	0.36 ± 0.04	-0.05 ± 0.02	0.43 ± 0.04	-0.07 ± 0.03
5	Leu7 C_γH	0.49 ± 0.05	—	0.51 ± 0.04	—
30	Leu7 C_γH	0.31 ± 0.03	—	0.37 ± 0.03	—
5	Phe2 C_βH	0.50 ± 0.04	-0.13 ± 0.03	0.53 ± 0.04	-0.10 ± 0.03
30	Phe2 C_βH	0.21 ± 0.03	-0.01 ± 0.02	0.51 ± 0.04	-0.10 ± 0.03
5	Phe2 $\text{C}_{\epsilon,\delta}\text{H}$	0.74 ± 0.05	—	0.80 ± 0.05	—
30	Phe2 $\text{C}_{\epsilon,\delta}\text{H}$	0.73 ± 0.05	—	0.52 ± 0.05	—
5	Phe2 $\text{C}_\epsilon\text{H}$	0.77 ± 0.05	—	0.80 ± 0.05	—
30	Phe2 $\text{C}_\epsilon\text{H}$	0.38 ± 0.04	—	0.61 ± 0.04	—

Fig. 7. Motional restriction map for $C_{\beta}H_2$ methylene groups of L7 and F2. The solid lines drawn on this motional restriction map are where both order parameters would lie for three different models: wobbling-in-a-cone, restricted rotation about an axis ($\theta = 70.5^\circ$, tetrahedral angle), which coincides with any one bond originating from that carbon, and restricted rotation about an axis ($\theta = 90^\circ$, tetrahedral angle), which bisects the angle between the $C_{\alpha}H$ and $C_{\alpha}C_{\beta}$ bonds. The wobbling-in-a-cone type motion arises from rotations about multiple bonds, whereas the latter type of motion is best stated as up-down motions occurring about an axis that runs through the α -carbon and orthogonal to the $H-C_{\alpha}-C_{\beta}$ plane.

being at the N-terminus of the peptide where motions are usually less restricted than those within a structured region like the helix domain. Furthermore, the F2 $C_{\alpha}H$ angular variance σ_{α} is 15° at 5°C and 17° at 30°C , which is nearly the same as σ_{α} for phenylalanine in the tripeptide GFG. In the presence of TFE, however, S^2 values for F2 NH and $C_{\alpha}H$ groups at lower temperature are nearly the same as for residues within the helix. This increase in F2 motional restriction reflects the increased structural stability of the hydrophobic staple at lower temperature and in the presence of TFE.

Internal motions of F2 side-chain $C_{\beta}H_2$ and ring CH groups are relatively restricted at 5°C in the absence or presence of TFE (see order parameters in Table 2). In fact, the F2 phenyl ring is considerably more motionally restricted than its backbone atoms. The motional restriction map (Fig. 7) indicates that motions of the F2 $C_{\beta}H_2$ group are highly influenced by the presence of TFE. In

water, both wobbling-in-a-cone and rotations about the $C_{\alpha}-C_{\beta}$ bond contribute to motions of the F2 $C_{\beta}H_2$ group. Wobbling-in-a-cone motions are also indicative of correlated bond rotations as was the case with the L7 $C_{\beta}H_2$ group. However, addition of TFE abrogates contributions from correlated bond rotations. Irrespective of temperature, TFE shifts the motional ensemble such that single axis rotations about the $C_{\alpha}-C_{\beta}$ bond clearly dominate.

Using F2 $C_{\beta}H_2$ auto- and cross-correlation terms, the angular variance about the χ_1 bond, σ_{χ_1} (Table 3), was calculated as described by Ramirez-Alvarado et al. (1998). For this calculation, the most energetically favored rotamer state, i.e., $\chi_1^0 = -60^\circ$ [dihedral angle defined by $NC_{\alpha}C_{\beta}C_{\gamma}$] was used and rotational correlations were initially neglected. In the absence of TFE, σ_{χ_1} is $18^\circ \pm 2^\circ$ at 5°C and $23^\circ \pm 3^\circ$ at 30°C . Similar values were obtained using the second most energetically favored rotamer state, $\chi_1 = 180^\circ$. For the tripeptide GFG in water, σ_{χ_1} is 16° (Mikhailov et al., 1999). In TFE, σ_{χ_1} is about the same at 5°C , whereas it is significantly smaller at 30°C suggesting that the phenylalanine side chain may be involved in a collapsed state of the peptide as suggested above.

An attempt was made to estimate σ_{χ_2} by using order parameters for phenyl ring CH bonds. However, since these order parameters are quite large, suggesting considerable motional restriction, only very small or even negative values for σ_{χ_2} resulted. There are two possible explanations for this. First, if χ_2 rotational amplitudes are large, as would occur with ring flip motions, angular variances cannot be calculated using this approach since these equations were derived under the assumption of small rotational amplitudes of less than one radian. The second explanation is that χ_1 and χ_2 rotations are correlated. Large amplitude rotations about the $C_{\beta}-C_{\gamma}$ bond (χ_2) define F2 ring flip motions that have been observed to occur on the millisecond to microsecond timescale (Wüthrich & Wagner, 1978). Since chemical shifts for F2 2,6 and 3,5 proton resonances are averaged, ring flip rates must be greater than 10^3 to 10^4 s^{-1} . ^{13}C relaxation data are not sensitive to motions on this timescale, and σ_{χ_2} values, therefore, refer to χ_2 rotational amplitudes when the F2 ring is in one or the other flipped orientation. This being the case, the second explanation of correlated bond rotations is the more likely.

Conclusions

The Daragan–Mayo model of correlated internal motions has been validated. Internal motions of α -helix backbone $C_{\alpha}-H$ and $N-H$ bonds arise from negatively correlated ϕ, ψ bond rotations. In terms of internal motional amplitudes, $N-H$, $C_{\alpha}-H$, and $C_{\alpha}-C_{\beta}$ bonds show angular variances of about 15° in water and slightly less in TFE. Relative to the molecular frame of the helix, these bond vectors fluctuate both parallel and perpendicular to the helix dipole axis, consistent with the idea of helix “breathing” motions.

Materials and methods

Peptides having the amino acid sequence GFSKAELAKARAARKGGY were synthesized using standard Fmoc solid-phase methodology on either a Milligen/Millipore Excell automatic peptide synthesizer or on an Applied Biosystems 431A Peptide Synthesizer (Atherton & Sheppard, 1989) and were purified by high-performance liquid chromatography (HPLC) using a linear acetonitrile/water gradient. Three such peptides were made, each incorporating one or two $^{13}\text{C}/^{15}\text{N}$ -enriched amino acids (CIL, Cambridge, UK). The residues enriched were F2, A5, L7, A8, and A10. Peptide purity

Table 3. Angular variances for L7 and F2 side-chain χ_1 and χ_2 bond fluctuations

Temperature ($^\circ\text{C}$)	Solvent	Residue	σ_{χ_1} (deg)	σ_{χ_2} (deg)
5	H ₂ O	L7	30 ± 4	14 ± 1
30	H ₂ O	L7	24 ± 2	13 ± 2
5	H ₂ O + TFE	L7	20 ± 2	13 ± 1
30	H ₂ O + TFE	L7	24 ± 2	15 ± 2
5	H ₂ O	F2	18 ± 2	
30	H ₂ O	F2	23 ± 3	
5	H ₂ O + TFE	F2	19 ± 2	
30	H ₂ O + TFE	F2	13 ± 2	

was checked by MALDI-TOF mass spectrometry and analytical HPLC on a C18 Bondclone (Phenomenex) column.

For NMR measurements, freeze-dried samples were dissolved in D₂O for ¹³C relaxation measurements or in a H₂O/D₂O (90/10) mixture for ¹⁵N relaxation measurements. Peptide concentration, determined from the dry weight of freeze-dried samples, was 15 mg/mL. The pH was adjusted to pH 6 by adding microliter quantities of NaOD or DCl. NMR relaxation experiments were performed on Varian Inova-500, 600, 800 NMR spectrometers equipped with triple-resonance probes and on a Bruker AM-250 NMR spectrometer. The temperature was varied from 5 to 30 °C. Temperature calibration was performed by using chemical shifts of resonances from methanol.

Pulse field gradient (PFG) self-diffusion measurements were performed on a Varian Unity INOVA-500 spectrometer operating at the ¹H frequency of 500 MHz and capable of generating magnetic field gradients along the *z*-axis. Diffusion coefficients D_s were evaluated from the diffusion decays of absorption line amplitudes $A(g)$ in proton spectra that were obtained by using the PFGSE pulse sequence (Stejskal & Tanner, 1965) at different amplitudes of magnetic field gradient pulses g . This is defined by the equation $A(g) = \exp(\gamma^2 \delta^2 t_d D_s g^2)$, where γ is magnetogyric ratio for protons, t_d is diffusion time, and δ is duration of the field gradient pulses. Since variances in D_s over the peptide concentration range studied (1 to 15 mg/mL) could be ascribed solely to changes in viscosity, it was concluded that peptide aggregation was not occurring.

¹³C spin-lattice relaxation rates were determined by using the direct homonuclear inversion-recovery method with a composite 180° pulse (90°_x-180°_y-90°_x). In all experiments, broad band ¹H decoupling GARP (Shaka et al., 1985) was used. The number of acquisitions was chosen to have a signal-to-noise ratio greater than 10. Therefore, the number of transients varied from 200 to 1000. Ten to 15 time incremented (partially relaxed) spectra were routinely acquired for each relaxation measurement. Relaxation rates were determined by using methods described by Daragan et al. (1993). In short, ¹³C-¹H NOE coefficients were measured by using the standard gated-decoupling technique, and cross-correlation spectral densities $J_{\text{HCH}}(\omega_C)$ were obtained from ¹³C proton-coupled inversion-recovery experiments. For methylene and methyl groups, $J_{\text{HCH}}(\omega_C)$ was determined from the difference of initial slopes of relaxation curves for the outer W_o and inner W_i lines of ¹³C-multiplet spectra as described by Ramirez-Alvarado et al. (1998).

Steady-state {¹H-¹⁵N} NOEs were determined from spectra recorded in the presence and absence of proton saturation (Farrow et al., 1994). Saturation was achieved by application of 120° ¹H pulses every 5 ms (Markley et al., 1971) during repetition times of 5 s. When proton saturation was absent, a net relaxation delay of 10 s was used. Five pairs of these spectra were recorded in the same way, and data reported here are the average of these five pairs of spectra.

¹⁵N spin-lattice and spin-spin relaxation rates were measured by using the heteronuclear single quantum coherence (HSQC) sequence reported by Farrow et al. (1994). This pulse sequence employs pulsed field gradients for the coherence transfer pathway, whereby magnetization passes from ¹H to ¹⁵N and back again to ¹H for observation. The water flip-back method was used to minimize water saturation during the pulse sequence. During the relaxation time, ¹³C broad band decoupling WALTZ-16 (Shaka et al., 1983) was used. The delay in the CPMG train was 0.9 ms. Spin-lattice and spin-spin relaxation decays for all residues follow single exponential decays, except for F2, which shows two distinguish-

able exponents. After carrying out additional experiments with different concentrations of D₂O in H₂O, it was determined that the initial fast decay is related to proton detection and proton-deuterium exchange. Therefore, to more accurately determine relaxation parameters for F2, the slower decaying component was used.

¹³C spin-spin relaxation rates were measured by using the proton-detected HSQC pulse sequence as described above for the ¹⁵N CPMG experiment (Farrow et al., 1994) with some modification. Since each ¹³C nucleus in this peptide has at least one ¹³C-bonded carbon, *J*-coupling induces oscillations between in-phase and anti-phase coherence that modulates the spin-echo decay (Tokuhiro & Fraenkel, 1968) and thus precludes measuring T_2 relaxation times with the CPMG pulse sequence. To eliminate effects from *J*-coupling, soft rectangular pulses at the ¹³C resonance of interest, were used. The duration d of the 180° pulse was 0.4 to 0.7 ms, and the time delay t in the CPMG pulse train was 0.9 ms. During the relaxation time, ¹⁵N and ¹H broadband WALTZ-16 decoupling (Shaka et al., 1983) was used. For ¹³C _{α} relaxation, soft ¹³C pulses applied at the resonance of interest are selective; therefore, with each 180° pulse, the sign of the antiphase coherence changes and the contribution is eliminated. This is not the case with pulses applied at side-chain carbon resonances, and 180° pulses are not sufficiently selective. However, *J*-coupling effects could be eliminated by reducing the difference between d and t . When the ratio d/t is approximately equal to one, antiphase coherence is abolished as occurs in the spin-lock experiment (Peng & Wagner, 1994). The value of d was increased until effects from *J*-coupling were eliminated. The smoothness in the T_2 relaxation decay curves indicates that effects from *J*-coupling have been essentially eliminated. Using this method, T_2 values are in good agreement with T_2 values measured by using the spin-locking experiment with small rf fields. This approach to measuring T_2 relaxation curves that are devoid of *J*-coupling effects is, in effect, a combination of CPMG and spin-lock experiments.

Motional parameters were determined by using a Monte Carlo minimization protocol (Daragan et al., 1993). The function to be minimized is

$$\chi^2 = \sum_i [(W_{1\text{exp}}^i - W_{1\text{theor}}^i)/W_{1\text{exp}}^i]^2 + [(W_{2\text{exp}}^i - W_{2\text{theor}}^i)/W_{2\text{exp}}^i]^2 + [(\text{NOE}_{\text{exp}}^i - \text{NOE}_{\text{theor}}^i)/\text{NOE}_{\text{exp}}^i]^2 \quad (5)$$

where $W_{1\text{exp}}^i$, $W_{2\text{exp}}^i$, and $\text{NOE}_{\text{exp}}^i$ are experimental values and $W_{1\text{theor}}^i$, $W_{2\text{theor}}^i$, and $\text{NOE}_{\text{theor}}^i$ are calculated values of NMR relaxation parameters are expressed as spectral densities of molecular motions:

$$W_1 = \frac{1}{10} k_{dd} [J(\omega_C - \omega_H) + 3J(\omega_C) + 6J(\omega_C + \omega_H)] + \frac{2}{15} \Delta\sigma^2 \omega_C^2 J(\omega_C) \quad (6A)$$

$$W_2 = \frac{1}{20} k_{dd} [J(\omega_C - \omega_H) + 3J(\omega_C) + 6J(\omega_C + \omega_H) + 4J(0) + 6J(\omega_H)] + \frac{1}{45} \Delta\sigma^2 \omega_C^2 [4J(0) + 3J(\omega_C)] + W_{ex} \quad (6B)$$

$$\text{NOE} = \frac{1}{10} \frac{\gamma_H}{\gamma_C} \frac{k_{dd} [6J(\omega_C + \omega_H) - J(\omega_C - \omega_H)]}{W_1} \quad (6C)$$

where $k_{dd} = n\omega_C^2\gamma_H^2\hbar^2/r_{CH}^6$; γ_C and γ_H are magnetogyric ratios for ^{13}C and ^1H nuclei; $\hbar$ is Plank's constant; r_{CH} is the length of CH bond, and n is the number of bonded protons. For CH_2 and CH_3 groups, equations for the NOE are more complicated (Daragan & Mayo, 1997; Werbelow & Grant, 1977). During minimization, the contribution due to chemical exchange W_{ex} was taken as being proportional to the Larmor frequency squared. The summation in Equation 4 was performed over all NMR frequencies for which data were acquired. A similar approach was used for analysis of ^{15}N relaxation data.

Analysis of NMR relaxation data

Spectral densities of molecular motion $J(\omega)$ can be written as the sum of Lorentzians:

$$J(\omega) = \sum_{i=1}^N \frac{c_i \tau_i}{1 + (\omega\tau_i)^2} \quad (7)$$

where c_i are weighting coefficients.

$$\sum_{i=1}^N c_i = 1. \quad (8)$$

For isotropic overall tumbling, $J(\omega)$ for two motional vectors $\mathbf{a}$ and $\mathbf{b}$ can be approximated using two terms (Lipari & Szabo, 1982a, 1982b; Daragan & Mayo, 1997):

$$J_{ab}(\omega) = \frac{S_{ab}^2 \tau_o}{1 + (\omega\tau_o)^2} + \frac{(P_2(\cos \theta_{ab}) - S_{ab}^2) \tau}{1 + (\omega\tau_o)^2} \quad (9)$$

where $P_2(x) = 0.5(1 - 3x^2)$, and S_{ab}^2 is the well-known order parameter. τ_o is the overall correlation time, and $\tau = \tau_o\tau_i/(\tau_o + \tau_i)$ where τ_i is the correlation time for internal motion. For a single motional vector ($\mathbf{a} = \mathbf{b}$), $P_2(\cos \theta_{ab}) = 1$ and Equation 9 becomes the classical Lipari–Szabo equation.

For anisotropic molecular tumbling, two restriction parameters $\langle\Delta\theta^2\rangle$ and $\langle\Delta\varphi^2\rangle$ should be used where

$$\Delta\theta = \theta - \theta_o \quad (10A)$$

and

$$\Delta\varphi = \varphi - \varphi_o \quad (10B)$$

where θ_o and φ_o are average values of angles that characterize the orientation of the motional vector in the molecular frame (a symmetric top is the assumed shape). The autocorrelation spectral density for this type of motion can be expressed (Daragan & Mayo, 1999) as

$$J_a(\omega) = \sum_{m=0}^2 \left[(\lambda_{m1} + \langle\Delta\theta^2\rangle\lambda_{m2} + \langle\Delta\varphi^2\rangle\lambda_{m3}) \frac{D_m}{D_m^2 + \omega^2} + (\langle\Delta\theta^2\rangle\lambda_{m4} + \langle\Delta\varphi^2\rangle\lambda_{m5}) \frac{\tau_m}{1 + (\omega\tau_m)^2} \right] \quad (11)$$

Table 4. λ_{mk} coefficients

	$m = 0$	$m = 1$	$m = 2$
$k = 1$	$\frac{1}{4}(3\cos^2\theta - 1)^2$	$3\cos^2\theta\sin^2\theta$	$\frac{3}{4}\sin^4\theta$
$k = 2$	$\frac{3}{4}(3\cos^2\theta - 1)\sin^2\theta$	$-3\cos^2\theta\sin^2\theta$	$\frac{3}{4}(1 + \cos^2\theta)\sin^2\theta$
$k = 3$	$\frac{3}{2}\cos^2\theta\sin^2\theta$	$\frac{1}{4}(2 + \cos 2\theta + \cos 4\theta)$	$\frac{1}{4}(3 + \cos 2\theta)\sin^2\theta$
$k = 4$	$\frac{9}{2}\cos^2\theta\sin^2\theta$	$-\frac{3}{2}(1 + 2\cos 2\theta)\sin^2\theta$	$-\frac{3}{2}\sin^4\theta$

where

$$D_m = 6D_{\perp} + (D_{\parallel} - D_{\perp})m^2 \quad (12)$$

and

$$1/\tau_m = 1/\tau_o + D_m \quad (13)$$

where $D_{\parallel}$ and $D_{\perp}$ are rotational diffusion coefficients about the molecular symmetry axis and about some axis perpendicular to that symmetry axis. The coefficients λ_{mk} are given in Table 4. For isotropic motion, Equation 11 is transformed into the Lipari–Szabo form with $S_a^2 = 1 - 3\langle\Delta\theta^2\rangle$ (Brueschweiler & Wright, 1994; Daragan & Mayo, 1997).

Acknowledgments

This work was supported by research grants from the National Science Foundation (NSF, MCB-9729539) and from the North Atlantic Treaty Organization (NATO, CRG 960694) and benefited from use of the high field NMR facility at the University of Minnesota.

References

- Atherton E, Sheppard RC. 1989. *Solid phase peptide synthesis: A practical approach*. Oxford, UK: IRL Press.
- Brueschweiler R, Wright PE. 1994. NMR order parameters of biomolecules: A new analytical representation and application to the Gaussian axial fluctuation model. *J Am Chem Soc* 116:8426–8427.
- Buck M. 1998. Trifluoroethanol and colleagues: Cosolvents come of age. Recent studies with peptides and proteins. *Quart Rev Biophys* 31:297–355.
- Daragan VA, Ilyina E, Fields CG, Fields GB, Mayo KH. 1997. Backbone and side chain dynamics of residues in a partially folded β -sheet peptide from PF4. *Protein Sci* 6:355–363.
- Daragan VA, Kloczewiak MA, Mayo KM. 1993. ^{13}C nuclear magnetic resonance relaxation-derived ϕ, ψ bond rotational energy barriers and rotational restrictions for glycine $^{13}\text{C}_\alpha$ methylenes in a GXX-repeat hexadecapeptide. *Biochemistry* 32:10580–10590.
- Daragan VA, Mayo KH. 1995. Using the model free approach to interpret ^{13}C NMR multiplet relaxation data from peptides and proteins. *J Magn Reson* 107:274–278.
- Daragan VA, Mayo KH. 1996a. Analysis of internally restricted correlated rotations in peptides and proteins using ^{13}C and ^{15}N NMR relaxation data. *J Phys Chem* 100:8378–8388.
- Daragan VA, Mayo KH. 1996b. A novel model-free analysis of ^{13}C NMR relaxation of alanine-methyl side chain motions in peptides. *J Magn Reson* 110:164–175.
- Daragan VA, Mayo KH. 1997. Motional model analyses of protein and peptide dynamics using ^{13}C and ^{15}N NMR relaxation. *Prog NMR Spectrom* 32:63–105.
- Daragan VA, Mayo KH. 1998. A simple approach to analyzing protein side chain dynamics from ^{13}C -NMR relaxation data. *J Magn Reson* 130:329–334.
- Daragan VA, Mayo KM. 1999. Using the model free approach to analyze NMR relaxation data in cases of anisotropic molecular diffusion. *J Phys Chem* 103:6829–6843.
- Farrow N, Muhandiram R, Singer A, Pascal S, Kay C, Gish G, Shoelson S, Pawson T, Forman-Kay J, Kay L. 1994. Backbone dynamics of a free and

- phosphopeptide-complexed Src homology 2 domain studied by ^{15}N NMR relaxation. *Biochemistry* 33:5984–6003.
- Lipari G, Szabo A. 1982a. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. I. Theory and range of validity. *J Am Chem Soc* 104:4546–4559.
- Lipari G, Szabo A. 1982b. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. II. Analysis of experimental results. *J Am Chem Soc* 104:4559–4570.
- Markley JL, Horsley WJ, Klein MP. 1971. Spin-lattice relaxation measurements in slowly relaxing complex spectra. *J Chem Phys* 55:3604–3605.
- Mikhailov D, Daragan VA, Mayo KH. 1995. ^{13}C -multiplet NMR relaxation-derived ring puckering and backbone dynamics in proline-containing glycine-based peptides. *Biophys J* 68:1540–1550.
- Mikhailov DV, Washington L, Voloshin AM, Daragan VA, Mayo KH. 1999. Angular variances for internal bond rotations of side chains in GXG-based tripeptides derived from ^{13}C -NMR relaxation measurements. *Biopolymers* 49:373–383.
- Munoz V, Blanco FJ, Serrano L. 1995. The hydrophobic-staple motif and a role for loop-residues in α -helix stability and protein folding. *Struct Biol* 2:380–385.
- Peng JW, Wagner G. 1994. Investigation of protein motions via relaxation measurements. *Methods Enzymol* 239:563–596.
- Ramirez-Alvarado M, Daragan VA, Serrano L, Mayo KH. 1998. Motional dynamics of residues in a β -hairpin peptide measured by ^{13}C -NMR relaxation. *Protein Sci* 7:720–729.
- Shaka AJ, Barker PB, Freeman R. 1985. Computer-optimized decoupling scheme for wideband applications and low-level operation. *J Magn Reson* 64:547–552.
- Shaka AJ, Keeler J, Frenkiel T, Freeman R. 1983. An improved sequence for broadband decoupling: WALTZ-16. *J Magn Reson* 52:335–338.
- Stejskal EO, Tanner JE. 1965. Spin diffusion measurements: Spin echoes in the presence of a time dependent field gradient. *J Chem Phys* 42:288–292.
- Tokuhiro T, Fraenkel G. 1968. Modulation of spin echoes in multi-half-spin systems. Closed formulas of Carr-Purcell spin echoes in several A_nBX_x systems. *J Chem Phys* 49:3998–4008.
- Werbelow LG, Grant DM. 1977. Intramolecular dipolar relaxation in multi-spin systems. *Adv Magn Reson* 9:189–299.
- Wüthrich K, Wagner G. 1978. Dynamics of aromatic amino acid residues in BPTI. *Trends Biochem Sci* 3:227–230.