Model-Independent and Model-Dependent Analysis of the Global and Internal Dynamics of Cyclosporin A

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Abstract: The global and internal dynamics of the cyclic peptide cyclosporin A are analyzed according to the model-free theory of Lipari and Szabo (Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546–4559). This theory allows the unique information of nuclear magnetic resonance (NMR) relaxation experiments to be represented by two model-independent parameters describing the internal motions. The formulation also distinguishes between isotropic and anisotropic global molecular reorientation and determines the corresponding correlation time(s). Using only the ^{13}C NMR spin–lattice relaxation times and nuclear Overhauser enhancements measured at 75 and 125 MHz, parameters describing the motions at 39 carbons and the overall reorientation are obtained. The results indicate that the molecular reorientation of cyclosporin A in chloroform is isotropic with a correlation time of 0.475 ns/rad. The generalized order parameters provide a measure of the amplitudes of the internal motions. Physical insight into the nature of these motions is gained by comparison with order parameters derived from specific motional models. Comparisons of this kind indicate that motions of certain side chains are severely restricted while others experience less restrictions at carbons furthest from the main chain. The degree of torsion of the planar peptide bonds as measured by the dynamics of *N*-methyls correlates with the involvement of the associated carbonyl in hydrogen bonds. The dynamic information obtained for the carbons of the unsaturated side chain of residue 1 confirms the geometry of its trans configuration. In certain cases, the effective correlation times obtained are related to physically meaningful diffusion constants.

The internal motions of proteins occur over a wide range of time scales from femtosecond to picosecond vibrations and torsional oscillations through to larger segmental motions on the microsecond time scale.[1,2] Some of these motions are relevant to protein function.[3,4] Accordingly, a wide variety of experimental techniques have been used to characterize the internal motions of biopolymers over a large range of relevant time scales. The use of nuclear magnetic resonance (NMR)[5] spectroscopy, although a potentially rich source of dynamic information in the subnanosecond time scale, has historically been hindered principally by the difficulty in obtaining extensive resonance assignments and by the complicated origin of NMR relaxation phenomena. The former problem has recently been overcome by the advent of multidimensional NMR techniques[6,7] and the development of isotopic enrichment methodologies.[8-10] The extraction of meaningful and unbiased parameters describing relaxation processes has been somewhat simplified by the development of a model-independent formulation by Lipari and Szabo.[11,12] This approach has been used in several instances[13,14] but has not previously been comprehensively applied to a polypeptide system. Here we use the model-independent approach to provide a comprehensive description of both the global and the internal motions of the immunosuppressive cyclic peptide cyclosporin A (Figure 1). It is shown that the overall molecular reorientation is isotropic rather than anisotropic, with a correlation time of approximately 0.5 ns/rad. Generalized order parameters and effective correlation times are found for internal motions occurring on the picosecond time scale. The generalized order parameters have a model-independent significance which allows one not only to characterize the internal motions, but also to draw conclusions concerning the nature of both structural dynamics and static geometry. In general, these kinds of information are necessary to provide an insight into the impact of internal dynamics upon biological function, to provide further experimental contact with, for example, the results of molecular dynamics calculations, crystallographic temperature factor analyses, and hydrogen exhange studies, as well as to potentially provide a means to reconcile the effects of internal dynamics upon solution structure determinations.

Materials and Experimental Procedures

Cyclosporin A, a gift of Sandoz Research Institute (East Hanover, NJ), was used without further purification. A 183 mM sample in 0.6 mL of $CDCl_3$ was purged with argon gas to remove oxygen and sealed in a 5-mm NMR tube.

The published assignments of Kessler et al.[15] for the ^{13}C resonance signals were used. Spectra were recorded at 125.76 MHz on a Bruker AM-500 spectrometer and at 75.46 MHz on a Bruker AM-300 spectrometer. All measurements were performed on the same sample at a temperature of 298 K. One-dimensional ^{13}C T_1 and NOE spectra were acquired at 75.46 MHz with a sweepwidth of 15625 Hz, 8192 complex points, 256 scans, 3-Hz linebroadening, and a 20-s relaxation delay. Similar spectra were acquired at 125.76 MHz with a sweepwidth of 26316 Hz, 4096 complex points zero-filled to 8192 complex points, 256 scans, 5-Hz linebroadening, and a 10-s relaxation delay. T_1 determinations used a $180°-\tau-90°$ inversion recovery scheme with saturation of the proton resonances via composite pulse decoupling at all times. The steady-state NOE enhancement was found by measuring peak intensities of the carbon signals recorded with decoupling of protons before and during acquisition, and comparing to the intensities recorded with decoupling of protons only during acquisition.

Most of the carbons with bound protons were well-resolved at both fields used. Certain resonances exhibited degeneracies, such as the α carbons of residues 4, 5, and 6; therefore, those carbons were excluded from the analysis since the necessary data could not be unambiguously obtained. All data transformations and preliminary analyses were per-

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(5) Abbreviations: CSA, chemical shift anisotropy; $\Delta\sigma$, chemical shift difference; τ_e, effective correlation time; *S*, generalized shift anisotropy; $\Delta\sigma$, chemical shift difference; τ_e, effective correlation time; *S*, generalized order parameter; ω, Larmor frequency; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; τ_m, overall molecular correlation time; NT_1, spin–lattice relaxation time.
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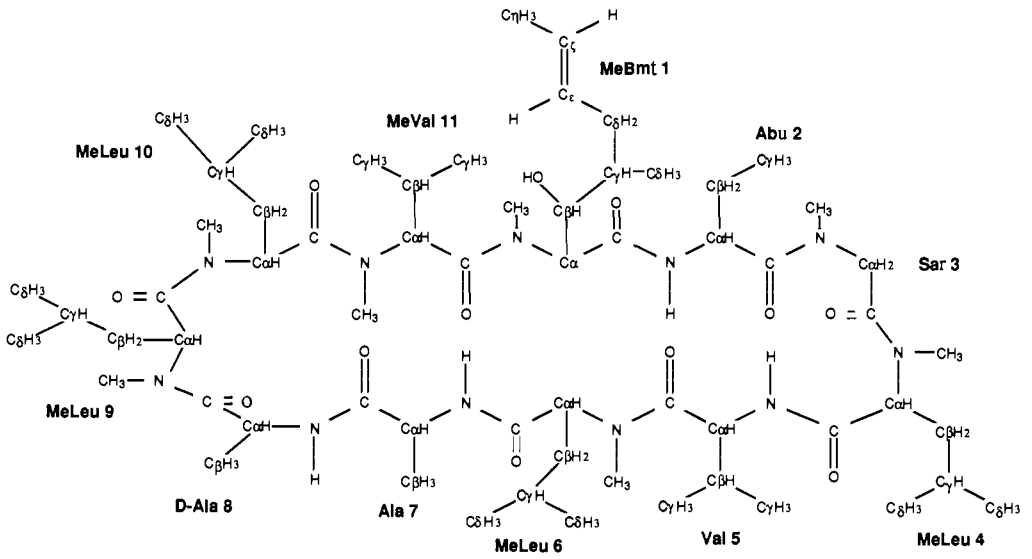

Figure 1. Cyclosporin A, molecular weight 1202.5. MeBmt is (4*R*)-4((*E*)-2-butenyl)-4,*N*-dimethylthreonine. Abu is α-aminobutyric acid. Sar (= sarcosine) is *N*-methylglycine.

formed using the data analysis software of the spectrometers. Subsequent numerical analyses (see Results) were carried out using standard IMSL routines on a Vax 8800. Graphical analyses were done with an Evans and Sutherland PS390 and the program FTNMR (Hare Research, Woodinville, WA).

Theory

The ^{13}C relaxation data have been analyzed according to the model-independent approach set forth by Lipari and Szabo.[11,12] This approach allows the data to be fit by a minimum of parameters, without making specific assumptions about the motions of the ^{13}C–^{1}H vectors involved. The spin–lattice relaxation time (T_1) and nuclear Overhauser enhancement (NOE) for a protonated carbon where the relaxation is primarily dipolar are[11,16]

$$\left(\frac{1}{T_1}\right)^{DD} = \frac{\hbar^2\gamma_H^2\gamma_C^2}{4r_{CH}^6}[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (1)$$

$$\mathrm{NOE} = 1 + \frac{\gamma_H[6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)]}{\gamma_C[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)]} \quad (2)$$

where r_{CH} is taken to be 1.1 Å, and ω is the Larmor frequency in rad·s^{-1}; $\hbar$ is given in units of erg·s·rad^{-1} (equivalent to G^2·cm^3·s·rad^{-1}) and γ is in units of rad·s^{-1}·G^{-1}. Both T_1 and correlation times (see below) are in s·rad^{-1}. When more than one proton is attached to the carbon, the measured T_1 must be multiplied by the number of protons, N, assuming that they have identical bond lengths r_{CH}. Note that the expression for T_1 corresponds to our experimental conditions, where relaxation of the ^{13}C resonances occurs in the presence of broadband decoupling of protons. This ensures that the inversion recovery curve is a single exponential, ignoring cross-correlation effects.[16,17]

Within the framework of the model-free approach, a simple form for the spectral density function $J(\omega)$ has been proposed. For a ^{13}C–^{1}H vector attached to an isotropically reorienting molecule, the spectral density function is given by[11,12]

$$J(\omega) = \frac{2}{5}\left[\frac{S^2\tau_m}{1 + \omega^2\tau_m^2} + \frac{(1 - S^2)\tau}{1 + \omega^2\tau^2}\right] \quad (3)$$

where

$$\frac{1}{\tau} = \frac{1}{\tau_m} + \frac{1}{\tau_e}$$

Here, τ_m is the correlation time describing the overall isotropic motion of the molecule, S is a generalized order parameter, and τ_e is an effective correlation time for reorientation of the ^{13}C–^{1}H vector of interest.

The generalized order parameter S describes the spatial restriction of the internal motion. When $S^2 = 1$, the motion at that carbon is completely restricted and the spectral density reduces to the case where no internal motion is present (i.e., rigid rotor approximation). In the limit of completely unrestricted motions, S^2 would equal zero, although the converse is not necessarily true.[12] The effective correlation time τ_e provides an upper limit for the time scale of the internal motions and is formally defined as the area of the internal correlation function. Accordingly, it is not possible to ascribe a physical interpretation to the derived value of τ_e, unless it is in the context of a specific motional model.[11,12]

In cases where the overall motion cannot be described by a single correlation time, an approximation is made using two correlation times. This formulation conveniently describes anisotropic overall motions even in cases where these motions involve a distribution of correlation times.[12] This anisotropic form of $J(\omega)$ is given by[11,12]

$$J(\omega) = \frac{2}{5}\left[S^2\left\{\frac{A\tau_1}{1 + \omega^2\tau_1^2} + \frac{(1 - A)\tau_2}{1 + \omega^2\tau_2^2}\right\} + (1 - S^2)\left\{\frac{A\tau_{1e}}{1 + \omega^2\tau_{1e}^2} + \frac{(1 - A)\tau_{2e}}{1 + \omega^2\tau_{2e}^2}\right\}\right] \quad (4)$$

$$\frac{1}{\tau_{1e}} = \frac{1}{\tau_1} + \frac{1}{\tau_e}$$

$$\frac{1}{\tau_{2e}} = \frac{1}{\tau_2} + \frac{1}{\tau_e}$$

where τ_1 and τ_2 are the two correlation times describing the overall reorientation of the molecule, and A is a mixing coefficient ranging from 1 to 0. When $A = 1$ or 0 (or equivalently, when $\tau_1 = \tau_2$), then the isotropic form of the spectral density function is recovered. It is usual to define $\tau_1 > \tau_2$.

Equations 3 and 4 assume only that the intermal motions of the C–H vectors are completely independent of the overall reorientation of the molecule. In the limit where internal motions are much more rapid than the overall motion, making the assumption that $\tau_e \ll \tau_m$ or $\tau_e \ll \tau_1, \tau_2$ allows a simplification of the formula for $J(\omega)$, by making the substitution $\tau = \tau_e$. These simplifications would be expected to be justified for cases involving large proteins where overall motion is slow. An additional simplification is possible if one assumes extreme narrowing of the

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internal motions (i.e., $\omega^2\tau_e^2 \ll 1$, where $\omega = \omega_H + \omega_C$). At current spectrometer field strengths and for the range of τ_m one would expect to encounter for small peptides (τ_m of the order of nanoseconds), assuming extreme narrowing of internal motions is tantamount to assuming that $\tau_e \ll \tau_m$. For extreme narrowing to hold within 5% (i.e., for $\omega^2\tau_e^2 \leq 0.05$) at $\omega_C = 125$ MHz, τ_e must be 57 ps/rad or less; for $\tau \approx \tau_e$ to also hold within 5% requires that $\tau_e/\tau_m \leq 0.05$, a more stringent requirement when, for example, $\tau_m = 1$ ns/rad or less. We have chosen *not to make either assumption* in analyzing the observed relaxation, since the assumption of extreme narrowing is not required by the theory and may be unwarranted at higher field strengths.

Results

Numerical Analysis. Using the anisotropic form of the spectral density function (eq 4), the relaxation data from N carbons should be describable by three global parameters A, τ_1, and τ_2, and N sets of parameters $\{S^2, \tau_e^2\}_i$, where $i = 1...N$, for a total of $2N + 3$ parameters. Measurement of T_1 and NOE relaxation data at two fields yields $4N$ experimental observations. Clearly, it should be possible to fit parameters to the data and determine all parameters for $N = 2$ or greater. When $N = 2$, the data may be fit uniquely; however, the effect of experimental error in the data would lead to inaccurate determinations. When N is large, it is expected that the errors in the data would average out in the determination of the global parameters, allowing a confident determination of the anisotropic correlation times τ_1 and τ_2.

The complete set of parameters which best reproduce the data corresponds to a global minimum in the error. The target function used here was defined as the sum of the squares of the fractional differences between calculated and observed values of T_1 and NOE at both fields,

$$\text{error} = \sum_{\omega_1,\omega_2 i=1}^{N}\sum \left(\frac{T_1^{\text{obs}} - T_1^{\text{calc}}}{T_1^{\text{obs}}} \right)_i^2 + \left\{ \frac{(\text{NOE}/T_1)^{\text{obs}} - (\text{NOE}/T_1)^{\text{calc}}}{(\text{NOE}/T_1)^{\text{obs}}} \right\}_i^2 \quad (5)$$

For a perfect fit, the target function would equal zero. A fractional error was used to obtain equal weighting of the observed T_1 and NOE data. Parameters were fit by searching a grid formed by the three global parameters τ_1, τ_2, and A. For a given point in the grid, the global parameters were held constant and the best pair of parameters $\{S^2,\tau_e\}_i$ for the ith carbon were found by regression. The regression thus involves only two parameters determined by four observables. Initial estimates for each pair $\{S^2,\tau_e\}_i$ were calculated directly from the data using the simplified formulas given by Lipari and Szabo.[11] The point of lowest error in the grid determines the best values for the global parameters τ_1, τ_2, and A. The target function was displayed as a three-dimensional grid which allowed visual assessment of whether a unique minimum had been obtained.

Accurate determination of each S^2 and τ_e will depend not only on the accurate determination of the global parameters, but also on the number of individual observations for each carbon. Insofar as the global parameters are well-determined for large N, the parameters S^2 and τ_e are over-determined within each regression. Thus, when determining the local parameters, S^2 and τ_e, at the best values for the global parameters τ_1, τ_2, and A, experimental errors will tend to average out. Semiempirical rules for evaluating the error in S^2 and τ_e have been set forth by Lipari and Szabo.[11] However, these rules were based on the asssumption of extreme narrowing of internal motions. In the present analysis this assumption is not made, as discussed above. It is expected therefore that the current analysis will be valid over the same or greater range of values of the parameters.

Table I is a summary of the T_1 and NOE data obtained at two fields for 39 carbons as well as the model-free parameters obtained. The standard deviations of all the T_1 determinations were roughly ±0.03 s/rad before multiplication by N. The precision of the NOE measurements was roughly 10%. The parameters obtained reproduced the data very well, with the highest relative error between an observation and the calculated value being about 15% and most deviations being less than 10%. The T_1 for the N-methyl carbons of MeLeu-4 and MeVal-11 at 125 MHz are smaller than the T_1 at 75 MHz, indicating that relaxation mechanisms other than dipolar may be active (see Discussion). The fitted parameters for MeLeu-4 result in an anomalously high error in reproducing the T_1 at the higher field, and the NOE at both fields. The fitted parameters for MeVal-11 reproduce the observed data to within 10%. However, in both cases, the resultant calculated T_1 is greater at the high field compared to the low field, as required by the theory. Excluding these data from the fit did not influence the determination of the remaining parameters.

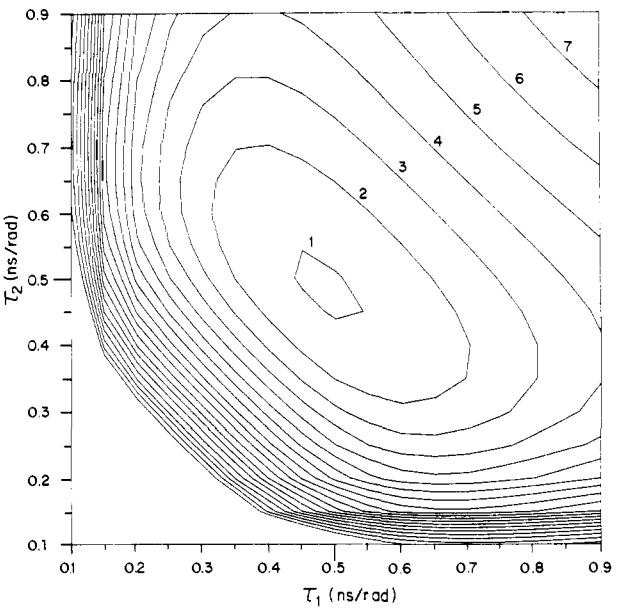

Figure 2. Contour plot of the error as a function of τ_1 and τ_2 for $A = 0.5$. Changes in τ_1 and τ_2 were made in steps of 0.05 ns/rad. The minimum occurs at $\tau_1 = \tau_2 = 0.475$ ns/rad. The error function is given by eq 5 for the fitting procedure described in the text; the error levels for each contour are as follows: (1) 0.637, (2) 0.682, (3) 0.730, (4) 0.781, (5) 0.835, (6) 0.894, (7) 0.956.

Molecular Correlation Times. The results of fitting data for relaxation of 39 carbons indicate that the overall motion of cyclosporin A can be described isotropically with one correlation time $\tau_m = 0.475$ ns/rad with a precision of ±0.025 ns/rad. The three-dimensional error grid can be dissected into planes showing contours of error vs τ_1 and τ_2 at constant values of A. One such contour plot is shown in Figure 2, for $A = 0.5$. Only one distinct minimum was found. Note that for cyclosporin, it was found that $\tau_1 = \tau_2$, so that the value of A is meaningless. This is manifested in the error volume as a minimum at $\tau_1 = \tau_2$ for all values of A.

The value of τ_m is very reasonable given the size and shape of the molecule. The dimensions of cyclosporin estimated from the crystal structure correspond roughly to a prolate ellipsoid with major and minor axes of 23 and 10 Å, respectively. The radius of a sphere of equivalent volume is about 13 Å. This can be compared to a radius derived using the simple formula[18]

$$\tau_m = 4\pi r^3\eta/3kT \quad (6)$$

where η = viscosity. For a viscosity, η, of 5.42×10^{-3} g/cm·s for chloroform at 298 K,[19] the value of r obtained is 10 Å. This approximation is in reasonable agreement with that predicted from the crystal model. It is clearly possible to determine an accurate molecular correlation time or times based on the model-free analysis of NMR data, without making assumptions about solvent viscosity or molecular shape.

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Table I. Primary Data and Model-Free Analysis of the ^{13}C Dipolar Relaxation of Cyclosporin A[a]

residue	carbon	75 MHz NT_1 (s/rad)	75 MHz NOE	125 MHz NT_1 (s/rad)	125 MHz NOE	S^2	t_e (ps/rad)
MeBmt-1	$C_{N\text{-}Me}$	2.48 (2.41)	2.53 (2.46)	2.90 (3.05)	2.06 (2.21)	0.0508	5.84
	C_α	0.19 (0.18)	2.33 (2.26)	0.25 (0.26)	1.68 (1.80)	0.906	~0
	C_β	0.17 (0.19)	2.19 (2.26)	0.30 (0.27)	1.94 (1.80)	0.884	~0
	$C_{\delta\text{-}Me}$	1.50 (1.46)	2.79 (2.68)	1.61 (1.66)	2.43 (2.57)	0.0492	21.2
	$C_{\delta H2}$	0.38 (0.39)	2.38 (2.64)	0.44 (0.46)	2.42 (2.48)	0.189	110
	C_ϵ	0.53 (0.49)	2.65 (2.36)	0.62 (0.66)	1.84 (2.01)	0.295	19.5
	C_ζ	0.54 (0.51)	2.47 (2.49)	0.56 (0.63)	1.92 (2.26)	0.224	43.8
	C_η	3.17 (3.12)	2.36 (2.38)	3.89 (4.11)	1.93 (2.06)	0.0446	2.75
Abu-2	C_α	0.23 (0.23)	2.35 (2.60)	0.27 (0.29)	2.25 (2.28)	0.241	400
	C_γ	2.30 (2.21)	2.95 (2.46)	2.99 (2.80)	2.33 (2.21)	0.055	6.43
Sar-3	$C_{N\text{-}Me}$	2.57 (2.42)	2.56 (2.53)	2.59 (2.95)	1.98 (2.35)	0.0431	8.13
	C_α	0.19 (0.20)	2.21 (2.26)	0.31 (0.28)	2.09 (1.80)	0.852	~0
MeLeu-4	$C_{N\text{-}Me}$	2.44 (2.42)	2.27 (2.75)	2.12 (2.67)	2.05 (2.69)	0.0221	14.5
	$C_{\delta 2}$	1.45 (1.42)	2.99 (2.82)	1.59 (1.53)	2.77 (2.77)	0.0272	29.0
Val-5	C_β	0.27 (0.23)	2.80 (2.42)	0.27 (0.31)	1.76 (2.09)	0.549	149
	$C_{\gamma 1}$	1.50 (1.38)	2.71 (2.30)	1.86 (1.91)	1.78 (1.88)	0.115	1.84
	$C_{\gamma 2}$	0.89 (0.84)	2.89 (2.43)	1.08 (1.07)	2.13 (2.16)	0.153	16.9
MeLeu-6	$C_{N\text{-}Me}$	2.32 (2.22)	2.32 (2.34)	2.69 (2.99)	1.74 (1.98)	0.0667	2.69
	C_β	0.30 (0.28)	2.62 (2.48)	0.34 (0.36)	2.03 (2.21)	0.406	120
	C_γ	0.37 (0.38)	2.29 (2.59)	0.43 (0.46)	2.30 (2.40)	0.230	98.8
	$C_{\delta 1}$	1.37 (1.31)	2.82 (2.55)	1.56 (1.59)	2.28 (2.37)	0.077	16.6
Ala-7	C_α	0.21 (0.20)	2.61 (2.30)	0.29 (0.28)	1.98 (1.88)	0.792	71.9
	C_β	0.98 (0.96)	2.81 (2.45)	1.34 (1.22)	2.40 (2.19)	0.129	15.7
D-Ala-8	C_α	0.22 (0.22)	2.71 (2.40)	0.30 (0.29)	2.09 (2.06)	0.597	187
	C_β	1.28 (1.21)	2.50 (2.42)	1.41 (1.56)	1.87 (2.14)	0.108	10.3
MeLeu-9	$C_{N\text{-}Me}$	2.72 (2.61)	2.45 (2.36)	3.28 (3.49)	1.85 (2.01)	0.0558	2.57
	C_α	0.18 (0.18)	2.33 (2.30)	0.27 (0.25)	2.03 (1.87)	0.879	190
	C_β	0.26 (0.27)	2.33 (2.26)	0.40 (0.38)	1.79 (1.80)	0.617	~0
	C_γ	0.33 (0.32)	2.65 (2.30)	0.49 (0.45)	2.10 (1.89)	0.488	16.8
MeLeu-10	$C_{N\text{-}Me}$	2.45 (2.51)	2.14 (2.42)	2.97 (3.24)	1.96 (2.14)	0.0519	4.59
	C_α	0.20 (0.20)	2.64 (2.44)	0.26 (0.26)	2.13 (2.05)	0.518	500
	C_β	0.29 (0.29)	2.38 (2.51)	0.35 (0.36)	2.16 (2.26)	0.360	131
	C_γ	0.34 (0.33)	2.53 (2.47)	0.40 (0.41)	2.12 (2.20)	0.363	82.4
	$C_{\delta 1}$	1.24 (1.22)	2.79 (2.69)	1.37 (1.38)	2.55 (2.60)	0.0550	27.1
MeVal-11	$C_{N\text{-}Me}$	3.18 (3.03)	2.89 (2.79)	3.03 (3.29)	2.43 (2.74)	0.0149	12.3
	C_α	0.21 (0.20)	2.44 (2.36)	0.27 (0.27)	1.95 (1.99)	0.693	178
	C_β	0.24 (0.24)	2.17 (2.54)	0.29 (0.31)	2.24 (2.25)	0.365	244
	$C_{\gamma 1}$	0.76 (0.76)	2.45 (2.26)	1.13 (1.07)	1.87 (1.80)	0.220	~0
	$C_{\gamma 2}$	1.49 (1.35)	2.99 (2.42)	1.70 (1.75)	2.00 (2.13)	0.0977	8.69

[a] Model-free parameters were determined globally using τ_m = 0.475 ns/rad as described in the text. Values in parentheses were calculated using these parameters. The experimental observations for MeLeu-4 $C_{N\text{-}Me}$ and MeVal-11 $C_{N\text{-}Me}$ were included in the fit. T_1 values were determined from the exponential inversion recovery profile by regression on at least 13 points. The fitted T_1 values had standard deviations of about $\pm 0.03N$ s/rad; this is generally less than 10% of the determined T_1. The precision of the NOE measurements was estimated to be about 10%. The T_1 and NOE values were experimentally reproducible to within 10%. To avoid arbitrary roundoff effects, numerical calculations involving T_1 and NOE values were carried to three significant digits.

Comparison of Model-Free Parameters to Specific Models of Internal Motion. One can relate the generalized order parameter derived from the data to the order parameters dictated by specific models for the internal motions. In this way one may attempt to exclude certain models which are not consistent with the model-independent result. Outlined below are several models which have been applied to the analysis of the model-free parameters.

A simple model which may be reasonable for the motion of an α carbon assumes that the C–H vector diffuses freely in a cone of semiangle θ_0; in this model, the generalized order parameter is equated with the usual order parameter, S_{cone}. Noting that it is always possible to choose θ_0 in such a way as to reproduce the order parameter, the semiangle is given by[12,20]

$$\theta_0 = \cos^{-1}\left[\tfrac{1}{2}((1 + 8S_{cone})^{1/2} - 1)\right] \quad (7)$$

$$0° \le \theta_0 \le 90°$$

Table II lists angles derived for the eight α carbons for which generalized order parameters were obtained. For most, the derived angles fall in the range of about 15° to 35°, indicating that the motions of these carbons are restricted. This is as expected for a vector which is attached to the backbone of the peptide chain. Two interesting cases are worth noting. The angle derived for the Abu-2 C_α is large relative to the other α carbons. However, the value of τ_e for this carbon is nearly as large as the overall correlation time. Similarly, the effective correlation time for the MeLeu-10 C_α is actually equal to the overall correlation time. In such cases the value of S^2 is ill-defined mathematically; thus the level of confidence in angles derived from these two results is low. It is important to note that for the MeLeu-10 C_α, the value of S^2 obtained was equal to zero when the assumption that $\tau_e \ll \tau_m$ was used; such a result is clearly physically unrealistic as it would indicate completely unrestricted motion at the α carbon.

Table II. Interpretation of the Model-Free Parameters for α Carbons Using the Diffusion in a Cone Model

C_α	S^2	θ_0[a] (deg)	τ_e (ps/rad)	$1/(6D_w)$[b] (ns/rad)
MeBmt-1	0.906	14.7	~0	~0
Abu-2[c]	0.241	52.4	400	0.37
Sar-3	0.852	18.6	~0	~0
Ala-7	0.792	22.4	71.9	0.28
D-Ala-8	0.597	32.9	187	0.36
MeLeu-9	0.879	16.7	190	1.31
MeLeu-10[c]	0.518	36.9	500	0.79
MeVal-11	0.693	27.9	178	0.46

[a] Calculated using eq 7. [b] Calculated using eq 8. [c] The τ_e values for Abu-2 and MeLeu-10 are close to the value of τ_m; therefore the errors in the parameters may be large. Accordingly, values for θ_0 and $1/(6D_w)$ should be interpreted with caution.

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Table III. Derived Order Parameter for Motion of a Methyl Symmetry Axis from the Model-Free Order Parameter[a]

	methyl	S^2	S^2_{axis}[b]
C_β	Ala-7	0.129	~1
	D-Ala-8	0.108	~1
$C_{N\text{-}Me}$	MeBmt-1	0.0508	0.458
	Sar-3	0.0431	0.388
	MeLeu-4	0.0221	0.199
	MeLeu-6	0.0667	0.601
	MeLeu-9	0.0558	0.503
	MeLeu-10	0.0519	0.468
	MeVal-11	0.0149	0.134
C_γ	Abu-2	0.0553	0.498
	Val-5-γ_1	0.115	~1
	Val-5-γ_2	0.153	
	MeVal-11-γ_1	0.220	
	MeVal-11-γ_2	0.0977	0.880
C_δ	MeBmt-1	0.0492	0.443
	MeLeu-4-δ_1	0.0272	0.245
	MeLeu-6-δ_1	0.0772	0.695
	MeLeu-10-δ_1	0.0550	0.495
C_η	MeBmt-1	0.0446	0.402

[a] Values for S^2_{axis} are calculated using eq 10. [b] Where no value is given, the generalized order parameter is too large to be considered within the Woessner model. Values indicated as ~1 have generalized order parameters approximately equal to that predicted for the simple Woessner model (eq 9).

This emphasizes the difficulties associated with making the assumption $\tau_e \ll \tau_m$, which only provides a modest simplification in any case. The value for S^2 at these carbons should be considered approximately 1.0, indicating no internal motion which is distinguishable from the overall molecular reorientation.

In the case of isotropic overall motion, the effective correlation time can be related to the diffusion coefficient D_w for diffusion of the internal vector according to the formula

$$D_w(1 - S^2_{cone})\tau_e = \chi_0^2(1 + \chi_0)^2\{\log[(1 + \chi_0)/2] + (1 - \chi_0)/2\}/[2(\chi_0 - 1)] + (1 - \chi_0)(6 + 8\chi_0 - \chi_0^2 - 12\chi_0^3 - 7\chi_0^4)/24 \quad (8)$$

where $\chi_0 = \cos\theta_0$.[11,20] Values for $1/(6D_w)$ calculated using the cone angles derived for the α carbons are given in Table II, including those for Abu-2 and MeLeu-10 for which the model-free parameters may be inaccurate. The value of τ_e cannot be uniquely related to a physically meaningful parameter or parameters in models which contain more than one adjustable time parameter. Thus no attempt has been made to interpret τ_e in the models discussed below.

The simplest model for motion of a methyl group is the Woessner model, which predicts an order parameter $S^2_{Woessner}$ based on the angle β between the C–H bond and the methyl symmetry axis. In this model, reorientation occurs by either free diffusion about the symmetry axis or a jump-rotation among three equivalent sites[12,21]

$$S^2_{Woessner} = (P_2(\cos\beta))^2 \quad (9)$$

For aliphatic carbons with tetrahedral geometry, $\beta = 70.5°$, corresponding to an order parameter $S^2 = 0.111$. Significant deviations of the generalized order parameter from this value indicate that the Woessner model is inappropriate. When S^2 is much less than 0.111, one may attempt to include the motion of the symmetry axis of the methyl group as well. The generalized order parameter in this case is predicted to be[12]

$$S^2 = (P_2(\cos\beta))^2 S^2_{axis} = 0.111 S^2_{axis} \quad (10)$$

Table III shows values of S^2_{axis} obtained in this way for all of the methyls of cyclosporin A. In some cases, one cannot reasonably extract such a value because S^2 is significantly greater than 0.111. In this case one must conclude that either methyl rotation is not free or that some other relaxation mechanism is contributing (e.g., chemical shift anisotropy). Because of the consideration of the possible contribution of CSA (see Discussion), the former possibility seems likely.

Table IV. Interpretation of Derived S^2_{axis} for *N*-Methyl Carbons Using the Diffusion in a Cone Model

$C_{N\text{-}methyl}$	S^2_{axis}	θ_0[a] (deg)	$\theta_0(C_\alpha)$ (deg)
MeBmt-1	0.458	40	15
Sar-3	0.388	44	19
MeLeu-4	0.199	55	*b*
MeLeu-6	0.601	33	*b*
MeLeu-9	0.503	38	17
MeLeu-10	0.468	39	37
MeVal-11	0.134	61	28

[a] Calculated using eq 7 with $S_{cone} \equiv S_{axis}$. Note that τ_e from the *N*-methyl can no longer be uniquely related to a diffusion coefficient since at least two motions are occurring: a methyl rotation superimposed on diffusion in a cone. [b] Data for this C_α not resolvable.

The generalized order parameters for the C_γ methyls of the two valine residues, Val-5 and MeVal-11, are inconsistent with free rotation of the methyl groups. For Val-5, the order parameter for γ_1 is reasonably close to 0.111, thus predicting an S^2_{axis} of ~1.0. The γ_2 methyl has an order parameter too large for this model to be considered. The generalized order parameter of the C_β carbon for comparison is approximately 0.5, again implying that S^2_{axis} should be significantly less than 1.0. In the case of MeVal-11, the order parameter found for γ_1 is also larger than 0.111. On the other hand, the γ_2 methyl has an order parameter much less than 0.111, and a value of $S^2_{axis} = 0.882$ can be obtained. The generalized order parameter for the C_β carbon is 0.365 for comparison. Such differing results for both valines suggest that one methyl is hindered and unable to rotate as freely as the other methyl (see Discussion).

Where a reasonable value for S^2_{axis} can be obtained, it can also be interpreted within the framework of specific models. Table IV shows the S^2_{axis} order parameters for carbons in the seven *N*-methyl groups present. This includes the parameters obtained for MeLeu-4 which, as mentioned, exhibits anomalous T_1 values. Analogous to the treatment of C_α carbons, the values of S^2_{axis} have been converted into cone angles. The cone angles describing the motions of the N–C axes are much larger than those of the associated α carbons, which are shown in the table for comparison. This seems to indicate a greater net flexibility of the planar peptide bonds. Torsions about the ψ_i and ϕ_{i+1} dihedral angles could allow significant angular displacements of the peptide bond and its attached methyl, while introducing only modest displacements of the neighboring α carbon. Such a negative correlation between changes in ψ_i and ϕ_{i+1} has been observed in a molecular dynamics simulation of bovine pancreatic trypsin inhibitor.[22] While the dispersion in the cone angle derived here is not great, there appear to be two classes. Five of the seven cone angles fall in the range 33°–44°; the remaining two angles are 55° and 61°. Three of the smaller angles involve peptide bonds which have hydrogen-bonded carbonyls (i.e., the carbonyls of Abu-2, Val-5, and MeVal-11). The cone angle for the *N*-methyl of MeLeu-9 includes the motion of the carbonyl of D-Ala-8, which may be involved in a hydrogen bond with its own amide NH.[23] The *N*-methyl of MeLeu-10 is part of a cis peptide bond; in this case, a major torsion of the peptide plane would significantly distort the surrounding backbone conformation. In contrast to these cases, the two larger angles involve residues whose peptide linkages are free of hydrogen-bonding interactions.

A model for the motion of the methyl symmetry axis which may be reasonable in the case of extended side chains of leucines and valines is the restricted-diffusion model.[24,25] The symmetry

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Table V. Interpretation of Side-Chain Dynamics Using the Restricted Diffusion Model

	S^2_{restr}	γ_0[a] (deg)
Abu-2-C_γ	0.498[b]	53
MeLeu-4-δ_1	0.245[b]	85
MeLeu-6-δ_1	0.695[b]	37
MeLeu-10-δ_1	0.495[b]	53
MeVal-11-γ_2	0.880[b]	22
MeBmt-1-C_ϵ	0.295[c]	81
MeBmt-1-C_ζ	0.224[c]	94

[a] Calculated using eq 11. [b] $S^2_{restr} \equiv S^2_{axis}$ with $\beta' = 70.5°$. [c] $S^2_{restr} \equiv S^2$ with $\beta' = 60°$.

axis is assumed to rotate about a director axis which is at a fixed angle β' to the methyl symmetry axis. Rotation about the director is restricted within some angular range $\pm\gamma_0$. In this model, the order parameter for the axis is given by[12]

$$S^2_{axis} = S^2_{restr} = (P_2(\cos\beta'))^2 + \frac{3\sin^2\beta'\sin^2\gamma_0}{\gamma_0^2}(\cos^2\beta' + \tfrac{1}{4}\sin^2\beta'\cos^2\gamma_0) \quad (11)$$

For aliphatic carbons, β' is fixed at 70.5° (tetrahedral geometry). The minimum value occurs when $\gamma_0 = \pi$, corresponding to free rotation of the symmetry axis about the director. In this case, $S^2_{axis} = (P_2(\cos\beta'))^2$. As long as the order parameter for the symmetry axis is in the range $1.0 \geq S^2_{axis} \geq (P_2(\cos\beta'))^2$, then a restriction angle γ_0 can always be found.

Some angles γ_0 derived from the S^2_{axis} for representative side-chain carbon–methyls are given in Table V. In all cases, the angles indicate fairly restricted motions, consistent with a compact globular structure. An interesting result is obtained in the case of the two ethylene carbons, C_ϵ and C_ζ, of MeBmt-1, for which the generalized order parameters can be directly equated to S^2_{restr}. If the carbon–carbon double bond is considered to be rigid, the C_ϵ–H axis is at an angle $\beta' = 60°$ with the director axis C_δ–C_ϵ; the C_ζ–H vector may also be at a 60° angle to the same director (trans conformation), or the angle may be 0° (i.e., in the cis conformation the bonds C_δ–C_ϵ and C_ζ–H are parallel in space). In the first case (trans), the order parameters $S_{restr}{}^2$ should be the same, neglecting any torsions of the planar bond. For a cis conformation, the order parameters should be very different. In fact, the generalized order parameters for these two carbons are nearly equal; this clearly indicates a trans conformation, which is confirmed by the crystal structure. Applying a similar analysis to the reorientation of the C_ζ–C_η bond predicts that diffusion about the director axis C_δ–C_ϵ cannot contribute to the observed S^2 since $\beta' = 0$ (i.e., the C_δ–C_ϵ and C_ζ–C_η bonds are parallel). Diffusion with respect to other director axes not constrained to be parallel (for example, C_γ–C_δ) would still contribute to the order parameter. Thus, the value $S^2_{axis} = 0.402$ for the C_ζ–C_η bond is not inconsistent with this interpretation.

In the limit where orientations about successive rotation axes in the aliphatic side chains are free and independent, then an order parameter describing motions about each axis would be predicted such that

$$S_n{}^2 = (P_2(\cos\beta))^{2n} \quad (12)$$

where n represents the nth rotation axis and β the angle between the successive axes.[12,26] This prediction highlights the general expectation that the order parameter should decrease as one moves to the extremities of the side chain. The generalized order parameters obtained for cyclosporin A side chains generally exhibit this progression, though certainly the limiting behavior expected for completely free rotations is not observed. The progression is not strictly maintained in the side chain of MeBmt-1, where the order parameters for the C_ϵ and C_ζ carbons show a slight increase over that of the $C_{\delta H2}$ carbon. This could indicate greater angular mobility of the C_δ relative to the end carbons of this extended chain. Since the value of τ_e is relatively large for the $C_{\delta H2}$ carbon, it is possible that the generalized order parameter is poorly determined in this case.

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Discussion

The results of fitting the data for cyclosporin A to the model-free parameters show that this method can indeed reliably account for the relaxation behavior of the molecule. Tests using simulated data sets indicate that the final algorithm is not very sensitive to random errors in the data. A small degree of random error distributed between +/−10% results in a very shallow but smooth target function with respect to the global parameters. Conventional regression routines were inefficient in locating a minimum because of this shallowness. The grid searching method outlined above (see Results) explicitly provides a range of discreet values for the global parameters, which reduces the computational burden. Consequently, it is much more efficient, regardless of the number of parameters being fitted. This is highly advantageous as the errors in the experimental observations will tend to average out in the determination of the global parameters. This was confirmed with simulations. These results indicate that the model-independent approach of Lipari and Szabo may be used to distinguish isotropic and anisotropic molecular reorientation and its time scale, as well as address the question of internal motions. In several instances, the obtained effective correlation times tend toward zero while the associated generalized order parameters are relatively larger (see Table I). The extremely fast motions underlying these parameters are highly restricted. This is a physically realistic situation. Furthermore, if $\tau_m \gg \tau_e$ and $(\omega_C + \omega_H)\tau_e \ll 1$, then the obtained S^2 is very accurately defined.[12] With few exceptions, the generalized order parameters and effective correlation times are estimated to accurately reflect the actual values to better than 10%. This assessment is based on the empirical rules set forth by Lipari and Szabo and does not include the effects of experimental error.[12]

It is difficult to make generalized statements on the effects of experimental error on the determination of S^2 and τ_e. However, if as Figure 2 suggests, the global parameter (τ_m) is well-defined, then for $\tau_e \ll \tau_m$ the error in obtained S^2 and τ_e parameters is proportional to the experimental errors of T_1 and NOE. This is borne out in the predicted T_1 and NOE values obtained for cases where the derived τ_e is much less than τ_m; i.e., for cases where the model-free theory is accurate[11,12] the deviation between observed and calculated T_1 and NOE values is well within the precision of the experimental measurements. This is as expected. Further, as pointed out previously,[12] the use of four observables for the determination of each S^2 and τ_e pair generally improves their accuracy. Thus, for the vast majority of carbon sites studied here, the accuracy of the obtained generalized order parameter and effective correlation time should be better than the estimated precision of the T_1 and NOE values used in their determination.

No attempt has been made here to include the effects of relaxation by chemical shift anisotropy. The contribution to the spin–lattice relaxation including this mechanism is given by[16,27,28]

$$1/T_1 = 1/T_1{}^{DD} + 1/T_1{}^{CSA} \quad (13)$$

$$1/T_1{}^{CSA} = (1/3)(\Delta\delta)^2(\omega_c)^2 J(\omega_c) \quad (14)$$

where the breadth of the chemical shift tensor, $\Delta\delta$, is given in parts per million (ppm). For an axially symmetric chemical shift tensor, $\Delta\delta = \delta_\parallel - \delta_\perp$; in general, it is given by[28]

$$\tfrac{2}{3}(\Delta\delta)^2 = (\delta_{11} - \bar{\delta})^2 + (\delta_{22} - \bar{\delta})^2 + (\delta_{33} - \bar{\delta})^2 \quad (15)$$

where δ_{11}, δ_{22}, and δ_{33} are the principal values of the chemical shift tensor, and

$$\bar{\delta} = \tfrac{1}{3}(\delta_{11} + \delta_{22} + \delta_{33}) \quad (16)$$

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In general, the breadth of the chemical shift tensor for aliphatic carbons is believed to be small, of the order of 20 ppm or less.[28] At the highest field used in this work, 11.3 T, and setting $\Delta\delta$ = 20 ppm and τ_m = 0.5 ns/rad, the contribution $1/T_1^{CSA}$ is approximately 0.014 rad·s^{-1} (ignoring internal motions, i.e., $S^2 \sim 1$). As the time scale is reduced, the value of $1/T_1^{CSA}$ decreases and becomes insignificant for correlation times on the order of picoseconds. Accordingly, the terms in $J(\omega)$ containing τ_e can usually be neglected, and including the generalized order parameter leads to a reduction in $1/T_1^{CSA}$ by a factor S^2 over the result given above. That reduction means that for cyclosporin A, the contribution of $1/T_1^{CSA}$ to the total T_1 is probably no more than 1%, well within experimental error. For the carbons studied in this work, relaxation due to chemical shift anisotropy can be disregarded. This also avoids the difficulty of assigning specific values to the elements of the chemical shift tensor. In a recent paper by Weaver et al.,[14] the relaxation data for the tryptophan $C_{\delta 1}$ ring carbon in a small peptide bound to a micelle was fit assuming isotropic overall motion. The effects of CSA were included in their analysis. However, the breadth of the chemical shift tensor was assumed to be 180 ppm, much larger than expected for aliphatic carbons. Such a large chemical shift difference would contribute significantly to the observed relaxation of the carbon.

The results of comparing the generalized order parameters to model-dependent order parameters is informative. Although the expected decrease in order parameters with side-chain length is observed, several order parameters are inconsistent with the models most likely to account for the motions of the carbon involved. This is true for some of the methyl carbons, such as in the valines and Ala-8. In some cases, derived values of S^2_{axis} are not consistent with the observed generalized order parameters for C–H bonds attached to the same vertex. These inconsistencies may be due to restricted motion in the methyl groups, which are assumed to freely rotate in this analysis. Such a situation is likely, for example, in the case of the methyls of MeVal-11. The γ_1 methyl (*pro-R*) has an associated S^2 value which is inconsistent with the Woessner interpretation of methyl rotation ($S^2 > 0.111$) while the motion of the γ_2 methyl (*pro-S*) appears consistent with the Woessner model. Examination of the crystal structure indicates that the γ_1 methyl may be eclipsed by the *N*-methyl of MeBmt-1. A similar trend is found for the γ-methyls of Val-5, where the γ_2 methyl (*pro-R*) has a generalized order parameter which cannot be interpreted within the Woessner model. The crystal structure shows this methyl to be in close proximity to the *N*-methyl of MeLeu-6. The cone angles associated with the *N*-methyls of MeBmt-1 and MeLeu-6 are relatively small. As discussed earlier (see Results), these *N*-methyls are associated with carbonyls involved in hydrogen-bonds. The small cone angles indicative of restricted motion are consistent with both this observation and the possibility of steric hindrance.

It is important to examine the validity of the assumption concerning the independence of overall and internal motions. In the foregoing analysis, it is assumed that the motions associated with internal fluctuations do not contribute to the bulk reorientation of the molecule. This allows the separation of the autocorrelation function into a product of two functions separately describing the two types of motions.[11] For very small molecules, it is entirely possible that this separation is not valid. For instance, rotation about the C–C bond in an ethanol molecule could provide a major contribution to the overall tumbling motion; the distinction between overall and internal motions may be physically unrealistic in such a case. However, for larger macromolecules such as proteins and nucleic acids, the physical distinction between internal and overall motions should be valid. This is particularly so in cases for which τ_e is very much smaller than τ_m; the corresponding internal motions would be complete within a time frame in which the overall motion is insignificant. When $\tau_e \sim \tau_m$, correlation between internal and overall motions may conceivably occur.

The best evidence for the validity of independent overall and internal motions occurs for cases where overall isotropic reorientation is observed. Clearly many of the internal motions are restricted and highly anisotropic. If these motions were significantly contributing to the overall motions, then the observation of isotropic rather than anisotropic molecular reorientation would be very unlikely. Yet for cyclosporin A, isotropic overall motion is, in fact, observed, and the validity of the assumption is fully upheld.

The model-free analysis yields several points of contact with other experimental and theoretical techniques. The correlation times describing the global reorientation of the molecule can be theoretically related to knowledge of the molecular shape, or compared with those correlation times obtained from light-scattering or fluorescence lifetime experiments. Anisotropic tumbling is readily distinguishable from isotropic motion. This information is obtained from data encompassing the whole molecule and so is less subject to bias arising from local motions examined in isolation.

In their study of cyclosporin A, Loosli et al.[23] concluded that there was no appreciable variation in the dynamics of the backbone. This conclusion was based on a comparison of NT_1 values. Here, the spin–lattice relaxation times for the eight α carbons that could be measured varied between 0.18 and 0.23 s/rad (Table II). In contrast, the corresponding order parameters ranged from 0.2 to 0.9. This confirms the expectation that NT_1 values are not a sensitive measure of internal dynamics.

Loosli et al. also compared NT_1 values to temperature factors derived from crystallographic studies but found no correlation.[23] The generalized order parameter may allow a more direct comparison to such temperature factors[29] since only the internal motion is being measured. For example, comparison of the generalized order parameters for α carbons of cyclosporin A (Table II) to their root mean squared (rms) positional fluctuations derived from crystallographic β-factors (Figure 3a, ref 30) shows a general correlation between these parameters. In contrast, comparison of rms fluctuations for side-chain methyls derived from a restrained molecular dynamics simulation of cyclosporin A in $CDCl_3$ (Figure 9, ref 30) shows little correlation to the generalized order parameter. However, it should be noted that the experimental generalized order parameters should be compared to the generalized order parameters calculated directly from the molecular dynamics trajectories.[31] The effective correlation times determined in this study are generally in the picosecond time range and may also provide a solid experimental basis for comparison to or evaluation of molecular dynamics trajectories. A recent paper by LeMaster et al.[32] has also proposed a correlation between temperature factors and order parameters derived from molecular dynamics simulations.

Summary

NMR relaxation studies are potentially powerful because many sites within the molecule may be probed simultaneously with one set of experiments. The results of this study show that the internal dynamic behavior can be directly mapped to the structural features giving rise to these motions. A capability for distinguishing discreet configurational features, such as cis or trans isomers, has also been shown. The use of intrinsically dynamic parameters to provide information on static structure (i.e., geometry) is not new and has been successfully used in this context in the solid state.[33,34] Here, however, the potential complications due to molecular reorientation are analytically rather than physically removed from the experimental data. The analysis presented here is equally applicable to dipolar relaxation of ^{15}N nuclei, which should be of special

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interest in studies of proteins. When applied to larger proteins of interest, comparison of backbone and side-chain dynamics in different kinds of secondary structure will be possible. Importantly, changes in dynamics accompanying transitions between functionally distinct states can be studied. This kind of information is required to characterize the internal dynamics of biopolymers and to ascertain whether or not these motions hinder, aid, or are irrelevant to function. Equally important is the possibility of using unbiased information on the internal dynamics of biopolymers during the generation and refinement of structures based upon the distance-geometry transform.[35]

The methodology presented here is experimentally simple and direct. In order to extend such work to proteins of significant size, isotopic enrichment can be used in conjunction with selective proton labeling of nitrogens by deuterium-hydrogen exchange. We have initiated such studies of human ubiquitin and cytochrome *c*.

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Structure of $(SiO)_2$: A Comparison between $(AlF)_2$, $(SiO)_2$, and $(PN)_2$. Matrix Infrared Investigation and ab Initio Calculation

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Abstract: The structure of dimeric SiO is reexamined by a study of the IR spectra of the matrix-isolated species. Spectra of isotopomers from experiments with ^{29}Si- and ^{18}O-enriched samples are discussed with the help of normal-coordinate analysis. The results are in line with data of the geometrical and electronic structure obtained by ab initio SCF calculations. The dimerization energy calculated by quantum chemical methods agrees well with experimental data, which have been recalculated. With the help of additional ab initio calculations on the isoelectronic species PN and AlF, interesting correlations between their tendency toward dimerization can be obtained. Structural data of SiO are compared with the analogous ones of similar molecules (e.g., BF).

1. Introduction

When quartz is heated above 1500 K monomeric SiO is by far the most favored species in the gas phase.[1] But during cocondensation of such a high-temperature molecule with an excess of argon on a helium-cooled surface, oligomers of SiO are formed. The composition of these oligomers strongly depends on the experimental conditions. By this method the molecules $(SiO)_2$ and $(SiO)_3$ have been detected with infrared spectroscopy some time ago.[2,3] As the results of these investigations are called in question in a recent study of dimeric SiO[4] and as there is an increased interest in the system Si/O in the last years, stimulated by surface reactions of silicon wafers, we took up this subject once more in the discussion presented here. New findings could be expected (1) by experiments with samples enriched in ^{29}Si, (2) by a detailed normal-coordinate analysis including the unsymmetrical species $^{28}Si_2{}^{16}O^{18}O$ and $^{29}Si^{28}Si^{16}O_2$, (3) by ab initio SCF calculations, (4) and by the recalculation of the dissociation energy of $(SiO)_2$. In order to understand structural data and bonding of SiO and its dimer, they are compared with those of similar molecules.

2. Technical Details

2.1. Starting Compounds, Matrix Isolation, and Spectroscopy. SiO is generated when O_2 is passed over heated Si (Wacker) at about 1500 K in an Al_2O_3 furnace. Experiments with ^{29}Si-enriched SiO have been performed in the following way: $^{29}SiO_2$ (Oak Ridge Laboratory) was first reduced with the help of Be in a Knudsen cell. Subsequently, ^{29}SiO was generated by passing O_2 over the heated mixture as described above. A mixture of Ar/SiO (with the composition 200:1) was condensed for about 2 h on a helium-cooled Cu surface. The setup with the flow cryostat has been described before.[5] The IR spectra were recorded using a Perkin-Elmer 225 spectrometer.

Normal-coordinate analysis was carried out using a modified version of the Shimanouchi program system.[6]

2.2. Details of Computation. Ab initio SCF computations were performed for the electronic ground states of BF, AlF, PN, SiO, $(SiO)_2$, $(AlF)_2$, and $(PN)_2$ by using the Karlsruhe version[7] of the Columbus system of programs.[8,9] A gradient program[10] was used for geometry optimization.

The following CGTO basis was used:[11] B, (9,5,1)/[5,3,1], η(d) = 0.5; O, (9,5,1)/[5,3,1], η(d) = 1.0; Al, (11,7,1)/[6,4,1], η(d) = 0.3; P,

(1) As the dissociation energy of $(SiO)_2$ is about 200 kJ/mol (298 K) (section 3.4), the ratio in partial pressures of $SiO/(SiO)_2$ is on the order of 10000 at the conditions of our experiments.
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