

Locally Anisotropic Internal Polypeptide Backbone Dynamics by NMR Relaxation

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The importance of the characterization of dynamical properties of biomolecules for understanding their function is widely recognized. Accordingly, NMR relaxation measurements providing information on intramolecular motions with high resolution in space and time have attracted significant attention.^{1,2} Most studies of peptide bond dynamics focus on ¹⁵N backbone T_1 , T_2 , and NOE relaxation data,³ and more recent work includes also backbone carbonyl ¹³C' relaxation measurements.⁴ Although ¹³C' and ¹⁵N atoms belonging to the same peptide plane are strongly correlated in their reorientational motion, so far, model-free analysis and analytical models for relaxation data interpretation treated each nucleus individually without taking advantage of such correlations.

We present here a comprehensive analytical description of anisotropic peptide-plane motion in terms of 3D harmonic local reorientational fluctuations that is consistent with molecular dynamics (MD) computer simulation. It is the NMR analogue to anisotropic temperature factors in X-ray crystallography.⁵

Selection of a suitable motional model is the most crucial step in the process of NMR relaxation interpretation. In a recent paper⁶ we introduced a general protocol that combines computational approaches based on molecular force fields with analytical treatments. Briefly, by analyzing an extended MD trajectory of the system, the basic motional processes affecting spin relaxation are determined, and analytical expressions are derived describing their effect on experimentally observable relaxation parameters. In a subsequent step, fitting of the model parameters is tested on its robustness using relaxation data calculated directly from the trajectory. This protocol is applied here to polypeptide backbone dynamics of the cyclic decapeptide antamanide [-V1-P2-P3-A4-F5-F6-P7-P8-F9-F10-] (Figure 1).

A 12 ns Langevin dynamics (LD) simulation at 400 K was performed with CHARMM⁸ on antamanide using the same protocol as described previously.⁶ Except for SHAKE,⁹ no constraints were applied. The coordinates were sampled every 0.5 ps leading to 24 000 snapshots. Overall motion was eliminated by aligning the molecular inertia tensors for every snapshot.

Backbone ¹³C' and ¹⁵N chemical shielding anisotropy (CSA) relaxation and dipolar ¹⁵N–¹H relaxation reflect both overall tumbling motion and intramolecular *angular* motion with respect to the tumbling frame.¹ As is well-known, the peptide planes

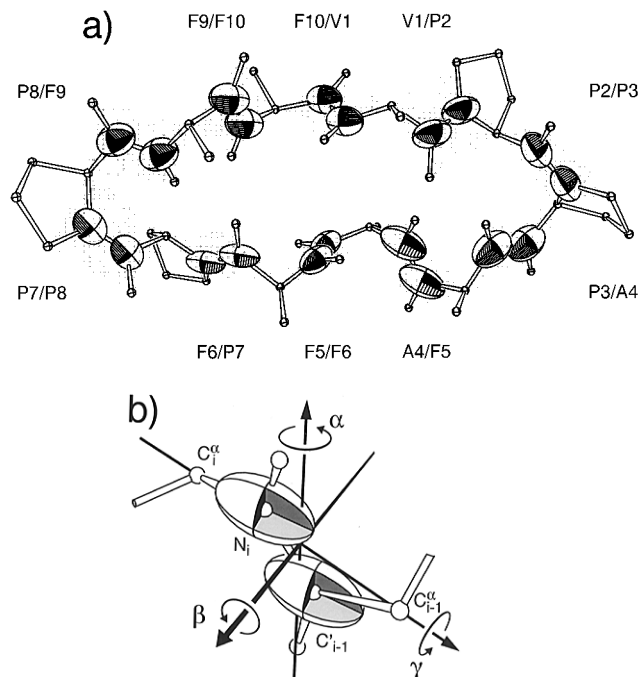


Figure 1. (a) Backbone structure of the cyclic decapeptide antamanide. The *axial fluctuation ellipsoids* determining NMR relaxation calculated from the LD trajectory are superimposed on the ¹³C' and ¹⁵N atoms of the peptide planes. The ellipsoid axes are parallel to the three orthogonal principal axes of *reorientational fluctuation* and the axis lengths are proportional to the fluctuation amplitudes σ_i . (b) Enlarged view of the A4/F5 peptide plane with the principal fluctuation axes α , β , γ indicated by straight arrows. The graphics were generated using ORTEP-III.⁷

are quite rigid entities,¹⁰ which is reflected in $\pm 5^\circ$ fluctuation amplitudes of the intraplane dihedral angles (C_{i-1}^α , C_{i-1}^β , N_i , C_i^α) observed during the LD simulation. Thus, the peptide planes exhibit rigid-body motions that depend on the adjacent backbone dihedral angles ψ_{i-1} , φ_i whose fluctuations are significantly anticorrelated¹¹ (see Table 1). However, the quite large deviations of the correlation coefficients $\rho_{\psi_{i-1}\varphi_i}$ from -1 demonstrate that the relaxation-active interactions of spins belonging to the peptide plane ($i-1$, i) do not only experience fluctuations about ψ_{i-1} and φ_i ; but are affected by other motion too,¹² such as fluctuations of the adjacent planes ($i-2$, $i-1$) and (i , $i+1$).

In several studies,¹³ probability distributions of backbone N–H vector orientations were determined from MD simulations. For peptide planes that do not undergo ψ_{i-1} , φ_i flips, distributions of N–H vectors were found to typically lie between Gaussian axial fluctuations about a single axis¹⁴ and distributions that are rotationally symmetric about the N–H equilibrium direction.^{15,16} The same behavior is exhibited by antamanide in the present LD simulation. In search of a general analytical description we find that for the LD trajectory the angular motions of all peptide planes, which are predominantly harmonic, can be adequately described by independent Gaussian axial fluctuations about three orthogonal axes α , β , γ . They

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Table 1. Langevin Dynamics (LD) and Fit Results of the 3D GAF Model for Antamanide

$(i-1, i)^a$	LD results (deg)							fit values (deg) ^e	
	$\sigma_{\psi_{i-1}^b}$	$\sigma_{\varphi_i^b}$	$\rho_{\psi_{i-1}, \varphi_i^b}$	σ_α^c	σ_β^c	σ_γ^c	χ^d	$\sigma_{\alpha\beta}^{\text{fit}}$	$\sigma_\gamma^{\text{fit}}$
F10-V1	16	17	-0.5	4.9	6.6	14.0	8	7.6	15.9
P3-A4	23	17	-0.5	8.8	7.4	18.4	11	13.0	17.5
A4-F5	22	20	-0.8	5.7	10.4	23.2	12	8.9	27.1
F5-F6	20	17	-0.5	4.6	6.0	14.7	8	7.9	16.4
P8-F9	18	14	-0.6	7.7	6.3	13.7	7	9.6	14.4
F9-F10	19	17	-0.8	5.5	6.5	18.6	10	8.8	21.6

^a Backbone peptide plane connecting amino acids $i-1$ and i . ^b RMSD backbone dihedral angle fluctuations $\sigma_{\psi_{i-1}}$, σ_{φ_i} and correlation coefficient $\rho_{\psi_{i-1}, \varphi_i} = \langle (\psi_{i-1} \varphi_i) - \langle \psi_{i-1} \rangle \langle \varphi_i \rangle \rangle / (\sigma_{\psi_{i-1}} \sigma_{\varphi_i})$. ^c Reorientational fluctuation amplitudes about principal axes α , β , γ according to eqs 1 and 2. ^d Angle between γ axis and $C_{i-1}^\alpha - C_i^\alpha$ vector. ^e Best fit results using eq 3 applied to $^{13}\text{C}' T_1$ and $^{15}\text{N} T_1$ and NOE values for $B_0 = 400, 600, 800$ MHz calculated from LD trajectory. The $^{13}\text{C}'$ and ^{15}N CSA tensors were taken from ref 21. The γ axis was kept fixed parallel to the $C_{i-1}^\alpha - C_i^\alpha$ axis. Since $\sigma_\alpha, \sigma_\beta$ are much smaller than σ_γ , they were combined to a single parameter $\sigma_{\alpha\beta} = \sigma_\alpha = \sigma_\beta$. The fitting errors of $\sigma_{\alpha\beta}^{\text{fit}}$ and $\sigma_\gamma^{\text{fit}}$ are $\pm 1^\circ$ and $\pm 2^\circ$, respectively. Dipolar relaxation of the $^{13}\text{C}_i$ spins by remote protons was not explicitly taken into account.

can be visualized as ellipsoids (Figure 1) with principal axes parallel to α , β , γ and lengths that are proportional to the fluctuation amplitudes $\sigma_\alpha, \sigma_\beta, \sigma_\gamma$. The characteristic time scales of these fluctuations are below 10 ps.

The axis directions α, β, γ and amplitudes $\sigma_\alpha, \sigma_\beta, \sigma_\gamma$ are obtained from the LD trajectory by evaluation of the motion of three orthogonal unit vectors $e_1(t), e_2(t), e_3(t)$ that are rigidly attached to the peptide plane with (orthonormal) equilibrium orientations e_1^0, e_2^0, e_3^0 , e.g., two vectors lying in the plane and one orthogonal to it. Linear averaging of $e_1(t), e_2(t), e_3(t)$ over the whole trajectory yields $\langle e_1 \rangle, \langle e_2 \rangle, \langle e_3 \rangle$, which generally are neither normalized nor orthogonal. They determine the symmetric 3×3 matrix \mathbf{M} with elements

$$M_{ij} = \langle e_i \rangle^T \cdot \langle e_j \rangle \quad (i, j = 1, 2, 3) \quad (1)$$

\mathbf{M} is diagonalized by the transformation $\mathbf{R}^T \mathbf{M} \mathbf{R}$, where the columns of \mathbf{R} are the normalized eigenvectors of \mathbf{M} belonging to eigenvalues $\lambda_1, \lambda_2, \lambda_3$. The principal axes α, β, γ are then given by $\mathbf{R}e_1^0, \mathbf{R}e_2^0, \mathbf{R}e_3^0$ with axial variances

$$\sigma_\alpha^2 = \frac{1}{2} \left[\log \frac{\lambda_1}{\lambda_2 \lambda_3} \right], \quad \sigma_\beta^2 = \frac{1}{2} \left[\log \frac{\lambda_2}{\lambda_1 \lambda_3} \right], \quad \sigma_\gamma^2 = \frac{1}{2} \left[\log \frac{\lambda_3}{\lambda_1 \lambda_2} \right] \quad (2)$$

The corresponding LD results for antamanide are summarized in Table 1. The axial fluctuation ellipsoids are almost axially symmetric ($\sigma_\alpha \approx \sigma_\beta$) with the principal axis γ about which the largest fluctuations occur always nearly colinear to the $C_{i-1}^\alpha - C_i^\alpha$ vector (see Table 1 and Figure 1).

The effect of internal motion on $^{13}\text{C}'$ and ^{15}N spin relaxation can be described by NMR order parameters $S_{\mu\nu}^2$,¹⁷ where μ and ν indicate two axially symmetric rank 2 tensor interactions. Using a procedure similar to the one outlined in the Appendix of ref 6, the effect of Gaussian axial fluctuations about the three orthogonal axes α, β, γ on $S_{\mu\nu}^2$ is given by

$$S_{\mu\nu}^2 = \frac{4\pi}{5} \sum_{l,k,k',m,m'=-2}^2 (-i)^{k-k'} e^{-\sigma_\alpha^2 l^2 - \sigma_\beta^2 (k^2 + k'^2)/2} e^{-\sigma_\gamma^2 (m^2 + m'^2)/2} \times d_{kl}^{(2)} \left(\frac{\pi}{2} \right) d_{k'l}^{(2)} \left(\frac{\pi}{2} \right) d_{mk}^{(2)} \left(\frac{\pi}{2} \right) d_{m'k'}^{(2)} \left(\frac{\pi}{2} \right) Y_{2m}(\mathbf{e}_\mu^{pp}) Y_{2m'}^*(\mathbf{e}_\nu^{pp}) \quad (3)$$

where Y_{2m} are the second order spherical harmonics, $\mathbf{e}_\mu^{pp} = (\theta_\mu, \varphi_\mu)$ defines the direction of the symmetry axis of interaction μ in the α, β, γ frame rigidly attached to the peptide plane (pp) and $d_{kl}^{(2)}(\pi/2)$ are the reduced Wigner matrix elements.¹⁸ This 3D GAF model for anisotropic local motion reduces to 1D GAF¹⁴ if all but one σ_i disappear. If needed, lattice jump motion can be easily incorporated into eq 3.⁶ The model is not suitable

for highly flexible or unfolded parts of a polypeptide chain, where the peptide planes spend much time in nonharmonic energy regions. The X-ray crystallographic analogue to the 3D GAF model are the anisotropic temperature factors which also include translational fluctuations.⁵

The feasibility to extract the reorientational fluctuation tensors of the peptide planes from NMR relaxation data is tested by calculating from the LD trajectory $^{13}\text{C}' T_1, ^{15}\text{N} T_1$, and $\{^1\text{H}\}^{15}\text{N}$ NOE values at three different magnetic field strengths B_0 (400, 600, 800 MHz). The relaxation parameters were calculated according to standard equations⁶ with an overall tumbling correlation time $\tau_c = 150$ ps.¹⁹ The 3D GAF model of eq 3 was fitted for a symmetrized fluctuation tensor ($\sigma_\alpha = \sigma_\beta$) to the relaxation parameters of the six non-proline peptide planes and the robustness of the fitted model parameters was assessed by a Monte Carlo error analysis with $\pm 2\%$ Gaussian errors added to the relaxation parameters. The results, compiled in Table 1, show that these parameters can be extracted with good accuracy by the fitting procedure. Very similar fit results were obtained with $\tau_c = 800$ ps, 5 ns, and 15 ns, respectively. Thus, these model calculations suggest that the favorable relative orientations of the dipolar and CSA interaction tensors of backbone $^{13}\text{C}'$ and ^{15}N nuclei allow a reliable extraction of the 3D GAF parameters for a wide range of polypeptides.

Changes in fluctuation amplitudes $\{\sigma_\alpha^A, \sigma_\beta^A, \sigma_\gamma^A\} \rightarrow \{\sigma_\alpha^B, \sigma_\beta^B, \sigma_\gamma^B\}$ between two different polypeptide states A and B contribute to free energy changes^{16,20} according to

$$\Delta G_{3D\text{GAF}}^{A \rightarrow B} = -kT \sum_i \log \left(\frac{\sigma_{\alpha,i}^B \sigma_{\beta,i}^B \sigma_{\gamma,i}^B}{\sigma_{\alpha,i}^A \sigma_{\beta,i}^A \sigma_{\gamma,i}^A} \right) \quad (4)$$

where the sum includes all peptide planes.²²

In conclusion, the 3D GAF model provides a detailed but simple interpretation of anisotropic fast-time scale polypeptide backbone dynamics by combining spatially directed dipolar and CSA relaxation information of the $^{13}\text{C}'$, ^{15}N nuclei. The framework is applicable to other fragments of proteins and nucleic acids that exhibit isotropic or anisotropic 3D Gaussian internal reorientational motion.

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