Supporting information

Structural insights from ^{15}N relaxation data for an anisotropic collagen peptide

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Calculation of principal components of inertia tensor

Triple helical collagen model peptides are composed of three left-handed polyproline II-like helices supercoiled around a common axis with a one-residue stagger (Figure S1). The relative ratio of the principal components of the inertia tensor calculated from the x-ray structure of peptide T3-785 (PDB ID: 1BKV) is 1:1:0.03, indicating that the peptide can be modeled as an axially symmetric rotor.[1,2] The unique axis of the inertia tensor is observed to be aligned with the symmetric axis of the peptide. As the rotational properties for rigid molecules are largely characterized by its inertia tensor, the unique axis of the diffusion tensor is also expected to be approximately parallel to the symmetric axis of the peptide.[3]

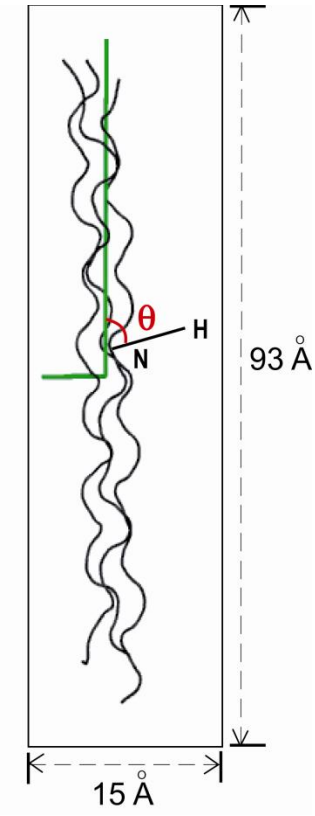

Figure S1. The structure of T3-785 positioned in the diffusion frame (in green). The θ angle between a representative N-H vector and the principal axis of the diffusion tensor is illustrated. The triple helical structure can be modeled as a cylinder with dimensions obtained from its X-ray structure.

Derivation of diffusion tensor from experimental R_2/R_1 values

As an axially symmetric molecule, the relaxation parameters R_1, R_2 and NOE for peptide T3-785 are defined by the spectral density function:[4-6]

$$J(\omega) = \frac{2}{5}\left\{S^2\left[\frac{A\tau_1}{1+(\omega\tau_1)^2} + \frac{B\tau_2}{1+(\omega\tau_2)^2} + \frac{C\tau_3}{1+(\omega\tau_3)^2}\right] + (1 - S^2)\tau_e\right\} \qquad (1)$$

where

$$1/\tau_1 = 6D_\perp,\ 1/\tau_2 = D_\parallel + 5D_\perp \text{ and } 1/\tau_3 = 4D_\parallel + 2D_\perp;$$

τ_1, τ_2 and τ_3 are the three correlation times; $D_\parallel$ and $D_\perp$ are the parallel and perpendicular components of the diffusion tensor; τ_e is the effective correlation time for the internal motion; S^2 is the generalized order parameter.

and

$$A = \frac{1}{4}(1 - 3\cos^2\theta)^2,$$

$$B = 3\sin^2\theta\cos^2\theta,$$

$$C = \frac{3}{4}\sin^4\theta;$$

A, B and C are coefficients dependent on the θ angle, which is the angle between the N-H vector and the principal axis of diffusion tensor as illustrated in Figure S1.

In the absence of significant amplitude internal motion ($\tau_e = 0$) and conformational exchange ($R_{ex} = 0$), the R_1, R_2 and NOE values for peptide T3-785 depend on the diffusion tensor constants $D_\parallel$ and $D_\perp$, the θ angle, and S^2 (Equation 1). However, the R_2/R_1 ratio depends only on the diffusion tensor constants and the θ angle, as S^2 is proportionally included in both R_1 and R_2, and thus can be crossed out in the R_2/R_1 ratio. Based on the experimental R_2/R_1 values and the known structure, the diffusion tensor can be derived using a fitting program R_2R_1_diffusion.[1] The program contains two

models: isotropic model and axially-symmetric motional model, and F-statistics are calculated to select the appropriate model. For the axially symmetric model, the structure of the molecule is rotated to the diffusion tensor frame. Basically, the program rotates the structure of the molecule in a diffusion tensor frame, in order to find a position where the differences in the calculated values of R_2/R_1 and the experimental values are minimized. Therefore, the diffusion tensor, defined by two diffusion constants $D_{\parallel}$ and $D_{\perp}$, and two Euler angles relative to the default molecular frame, theta and phi, can be derived from the fitting of experimental R_2/R_1 values to the structure. The θ angle of the N-H vectors can then be derived in the obtained diffusion tensor frame.

Table S1. The ^{15}N longitudinal and transverse relaxation rates R_1 and R_2 for labeled residues in T3-785 at 20°C at 500 MHz.

Residue	R_1	Error	R_2	Error	R_2/R_1	Error
1G15	1.99	0.03	11.4	0.2	5.7	0.11
2G15	2.02	0.02	11.8	0.1	5.87	0.12
3G15	1.99	0.02	11.8	0.2	5.95	0.12
1L16	1.9	0.02	11.5	0.2	6.06	0.12
2L16	1.96	0.03	11.4	0.2	5.78	0.12
3L16	1.93	0.02	11.5	0.2	5.95	0.12
1A17	1.82	0.03	11.8	0.3	6.49	0.13
2A17	1.79	0.02	11.7	0.2	6.5	0.13
3A17	1.81	0.02	12	0.2	6.64	0.13
1G18	2.02	0.03	11.6	0.3	5.72	0.11
2G18	2.02	0.03	11.3	0.2	5.6	0.11
3G18	2.04	0.03	11.9	0.1	5.86	0.12
G24	2.04	0.03	11.5	0.2	5.68	0.11

Hydrodynamic models for triple helical peptides

For anisotropic molecules, the rotational properties can be estimated by modeling the molecule as either a cylinder or a prolate ellipsoid.[3] Diffusion tensors for peptide T3-785 are predicted from both models and are compared to the experimentally derived diffusion tensor.

1. Cylinder model

The diffusion tensor constants for a cylinder model are given as follows:[6]

$D_{\parallel} = k_B T / \pi \eta b^2 L$

$D_{\perp} = 3k_B T / \pi \eta L^3 \{\mathrm{Ln}(2L/b) - 1.57 + 7[1/\mathrm{Ln}(2L/b) - 0.28]^2\}$

where L and b are the length and width of the cylinder, T is the temperature and η is the viscosity. The overall correlation time τ_c is calculated using the equation $1/\tau_c = 2D_{\parallel} + 4D_{\perp}$.

The triple helical structure of peptide T3-785 can be approximated as a cylinder (Figure S1) with length of ~93 Å and width of ~ 15 Å, which are obtained from the X-ray structure of T3-785 (PDB ID: 1BKV) considering the hydration layer and one extra residue Tyr at the C-terminal. By using the above equations, the ratio of the principal values of the diffusion tensor $D_{\parallel}/D_{\perp}$ is 12.3, and the overall correlation time τ_c is 6.98ns.

2. Prolate ellipsoid model

For a prolate ellipsoid model, the ratio of the principal components of the diffusion tensor $D_{\parallel}/D_{\perp}$ can be approximated using the equation $D_{\parallel}/D_{\perp} \approx (I_{\perp}/I_{\parallel})$, where $I_{\perp}$ and $I_{\parallel}$ are the principal components of the inertia tensor.[7] The relative ratio of the principal components of the inertia tensor calculated from the x-ray structure of T3-785 is 1:1:0.03, indicating that the peptide can be modeled as an axially symmetric rotor.[1] Using a simple prolate ellipsoid model, the ratio of the principal values of the diffusion tensor $D_{\parallel}/D_{\perp}$ is 11.9.

By fitting the experimental data of Leu-16-Ala17, the ratio of the principal values of the diffusion tensor $D_{\parallel}/D_{\perp}$ is 13.1, and the overall correlation time τ_c is 6.92ns. These

values are comparable to those obtained from the cylinder model ($D_{\parallel}/ D_{\perp}$=12.3 and τ_c=6.98ns) and the prolate ellipsoid model ($D_{\parallel}/ D_{\perp}$=11.9).

Chemical shift anisotropy (CSA) contribution to R_2/R_1

Two factors, the dipolar interaction between ^{15}N-^{1}H and ^{15}N chemical shift anisotropy (CSA), contribute to R_1 and R_2. The dipolar terms of the spectral density function depend on the θ angle between the NH vector and the principal axis of the diffusion tensor, while the CSA terms of the spectral density function depend on the angle θ' between the principal axis of the ^{15}N shielding tensor and the principal axis of the diffusion tensor:[8]

$$J(\omega)_{CSA} = \frac{2}{5} S^2 \left[\frac{A'\tau_1}{1 + (\omega\tau_1)^2} + \frac{B'\tau_2}{1 + (\omega\tau_2)^2} + \frac{C'\tau_3}{1 + (\omega\tau_3)^2} \right] \qquad (2)$$

$$A' = \frac{1}{4}(1 - 3\cos^2\theta')^2,$$

$$B' = 3\sin^2\theta'\cos^2\theta',$$

$$C' = \frac{3}{4}\sin^4\theta';$$

θ' will be equal to θ if the principal axis of the ^{15}N shielding tensor is collinear with the ^{15}N-H bond vector. Studies have indicated that the principal axis of the shielding tensor can be inclined away from the NH bond by an angle α ~ 20°.[8] The θ angles for Gly15 and Gly18 are about 85° and therefore the θ' angle for the two Gly could be as small as 65°. R_2/R_1 values can then be calculated at a given θ and θ' by using equations 1 and 2 assuming diffusion constants obtained from fitting. Re-calculating the R_2/R_1 values of Gly15 and Gly18 assuming an angle of θ=85° and θ'=65° results in a value of ~6.38, still far from the experimental R_2/R_1value of ~5.78, suggesting that non-collinearity of the principal axis of the shielding tensor with the NH bond does not account for the inability to fit the Gly residues. As the CSA term contributes only ~18% to the R_1 and R_2 values, we expect that the adjusted θ' value would not change the values of R_2/R_1 very significantly and we see this through the calculation described above.

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