NOTE

A two-dimensional spin-diffusion NMR study on the local structure of a water-soluble model peptide for *Nephila clavipes* dragline silk (MaSp1) before and after spinning

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INTRODUCTION

The dragline silk of the golden orb web spider *Nephila clavipes* has received significant attention because of its remarkable mechanical properties, which include toughness and high tensile strength.^{1,2} This silk contains two structural proteins designated as major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2).^{3,4} The dominant MaSp1 can be described as AB block-copolymers consisting of numerous alternating poly-Ala (A) and Gly-rich (B) blocks. The dragline silk fiber is produced from an aqueous spinning solution (dope) containing ~20% w/v spidroins at ambient pressure and temperature with substantial conformational change. Several factors have been thought to be important for controlling this conformational change, including mechanical strain, the concentration of fibroin, changes in pH, and the concentration of other ions.^{5,6} A pH change from 6.9 ± 0.1 to 6.3 ± 0.1 from the start of the silk gland duct to the spigot is crucial for gelling the fibroin, increasing its sensitivity to shear, and initiating the conformational transition.^{1,6,7}

We previously performed solid-state NMR structural analysis of ^{13}C selectively labeled versions of the water-soluble model peptide, (E)₈GGLGGQGAG(A)₆GGAGQGG-YGG, to gain insight about the local structure of the MaSp1 protein before and after spinning.⁸ Polyglutamic acid (E)₈ included at the amino terminus makes the peptide water soluble, leading to mimicking the predominance of amino acids with acidic side chains in this part of the sequence in spider fibroins and the induction of the conformational transition by lowering the pH.^{6,9} One-dimensional ^{13}C -cross polarization magic angle-spinning NMR spectra of the lyophilized and acid-treated peptide samples revealed that the conformational change induced by acidification primarily occurred in the poly-Ala, and to a lesser extent in the GAGA and GGA sequences. Moreover, the amount of β -sheet was the largest in the center of the poly-Ala domain compared with its periphery. These observations suggest that the peptide can be used as a model

for studying the effects of acidification on changes in the local conformation.⁸ However, further detailed structures, such as the torsion angles in the β -sheet fraction, are still unclear.

Two-dimensional (2D) proton-driven spin-diffusion solid-state NMR under off-magic-angle spinning (OMAS) coupled with ^{13}C isotope double labeling of specific residues has been used as a powerful method for determining the torsion angles of the backbone amino-acid residues for the silk model peptides.^{10–18} The combination with rotational-echo double-resonance measurements is a more quantitative approach, as previously reported,^{10,15,18} but it is not easy to quantitatively determine the structure when the structure is heterogeneous, such as a mixture of β -sheets and random coil conformations. In this case, it is better to first rigorously determine the fraction of the mixed conformations of individual residues. We should then try to determine the structure using spin-diffusion solid-state NMR by taking the fraction into account.

In this paper, we will propose an analytical method for estimating the detailed local structures in the conformational ensemble system using 2D spin-diffusion OMAS NMR experiments combined with information about the fractions of different conformations at specific sites obtained from the 1D spectra.⁸ Furthermore, we will also discuss the detailed structures of the acid-treated model peptide, such as the torsion angles in the β -sheet component.

EXPERIMENTAL PROCEDURE

Three types of ^{13}C selectively double-labeled carbonyl carbon sites of the water-soluble spider silk model peptides with the same structure were designed to obtain detailed information on the local conformation of the Ala residue, as summarized in Table 1. The labeled residues in the P1, P2 and P3 are located at Gly¹⁷-Ala¹⁸, Ala²⁰-Ala²¹, and Gly²⁵-Ala²⁶, respectively, and consequently, the torsion angles of the Ala¹⁸, Ala²¹ and Ala²⁶ residues can be determined using 2D spin diffusion analysis. These peptides were synthesized using the F-moc

Table 1 Synthesized ^{13}C double-labeled peptides used for the 2D spin-diffusion analysis

	Peptide	information
P1	(E) $\square$ GGLGGQGA[1- ^{13}C]G[1- ^{13}C]A AAAAGGAGQGQGYGG	Ala $^{18}(\phi, \psi)$
P2	(E) $\square$ GGLGGQGA[1- ^{13}C]A[1- ^{13}C]AAAGGAGQGQGYGG	Ala $^{21}(\phi, \psi)$
P3	(E) $\square$ GGLGGQGA[1- ^{13}C]G[1- ^{13}C]AGQGQGYGG	Ala $^{26}(\phi, \psi)$

Figure 1 The histogram of the fraction of random coil conformations of each residue in the model peptide for the MaSp1 silk protein of the *N. clavipes* dragline silk with poly(glutamic acid) at the amino terminus, (E) $\square$ GGLGGQGA[1- ^{13}C]G[1- ^{13}C]A AAAAGGAGQGQGYGG, at 'without' (gray) and 'with acid treatment' (black) states, as determined from our previous solid-state NMR studies.⁸ The double ^{13}C -labeling sites are marked by circles. The notations, P1–P3, refer to different ^{13}C selectively labeled versions for the peptide. A full color version of this figure is available at *Polymer Journal* online.

solid phase method using a PE Pioneer Peptide Synthesizer (Applied Biosystems, Tokyo, Japan), as described previously.^{18,19} The obtained peptides were purified by reverse-phase high-performance liquid chromatography using a 0.1% TFA/H₂O and 0.08% TFA/acetonitrile gradient with a PEGASIL ODS-II, C-18 column. Two states of the peptides, 'without acid treatment' and 'with acid treatment', were prepared as described previously.⁸ The solid-state NMR experiments were performed on Varian Unity INOVA 400 NMR spectrometer equipped with a 7 mm Jakobsen-type double-tuned MAS probe. The chemical shifts were indirectly calibrated using the adamantane methylene peak observed at 28.8 p.p.m. relative to the TMS peak. For the 2D spin-diffusion NMR experiments, the OMAS condition was $\theta_m = 6.7^\circ$, where θ_m is the magic angle, with sample spinning at 6 kHz. The scaling factor of the 2D spin-diffusion spectra is then $(1/2)(3 \cos^2(\theta_m - 6.7^\circ) - 1) = 0.172$. For cross polarization in the 2D experiments, a 7.0- μs ^1H $\pi/2$ pulse followed by a 1.5-ms contact time with a 5-s recycle delay was used. The other NMR experimental conditions and the method for the spectral simulation are as described previously.¹⁰ The data were processed by applying 100 Hz Gaussian line broadening to both time domains and zero-filling with a final size of 512×512 data points. We simulated the 2D spin-diffusion NMR spectra using a program developed in our laboratory. For simulation of the 2D spin-diffusion spectra, the calculations were performed using a grid of 10° for the torsion angles, ϕ and ψ values. To quantitatively demonstrate the difference between the observed and calculated spectra, the root-mean-square deviation (RMSD) for each (ϕ, ψ) pair was defined as

$$\text{RMSD}(\phi, \psi) = \sqrt{\sum_{i=1}^N [E_i - \lambda(\phi, \psi) S_i(\phi, \psi)]^2 / N}$$

where N is the number of intensities analyzed, QUOTE are the intensities of the experimental spectra, $S_i(\phi, \psi)$ are the calculated intensities, and $\lambda(\phi, \psi)$ is a scaling factor for minimizing the RMSD at each (ϕ, ψ)

RESULTS AND DISCUSSION

Figure 1 shows a histogram of the fraction of random coil conformations of each carbon in the model peptide, E $\square$ GGLGGQGA

Figure 2 (a) Observed and (b) best-fit simulated 2D OMAS spin-diffusion spectra of the model peptide P2 with ^{13}C double-labeled carbonyl carbons of Ala 20 –Ala 21 . The simulated spectrum is a mixture of the 80% β -sheet with a torsion angle of $(\phi, \psi) = (-150^\circ, 150^\circ)$ and 20% random coil patterns. (c) simulated spectra as a function of the torsion angles, ϕ and ψ of the Ala 21 residue in the energetically favored region of β sheet, $-180^\circ < \phi < -90^\circ$, $60^\circ < \psi < 180^\circ$.

GAAAAAGGAGQGGYGG, in the 'without acid treatment' (gray) and 'with acid treatment' (black) states, as determined from our previous 1D ^{13}C cross polarization MAS analysis.⁸ Thus, the fraction of random coil conformations of the ^{13}C labeled Ala residue in the model peptides P1–P3 with acid treatment were ~45%, 20% and 60%, respectively. We used these values as a constraint during the simulation of the following ^{13}C proton-driven 2D spin-diffusion analysis. For the spin-diffusion analysis, we should determine the principal values of the chemical shift tensors of the carbonyl ^{13}C in the Ala and Gly residues, because these values are directly reflected in the simulated peak positions. To adjust the peak positions, we slightly modified the principal values that were reported previously.¹¹ The values, in p.p.m., were $\sigma_{11}=247$, $\sigma_{22}=180$, $\sigma_{33}=90$ for Ala, and $\sigma_{11}=242$, $\sigma_{22}=177$, $\sigma_{33}=95$ for Gly. Hereafter, we use these values for the 2D spin-diffusion analysis in this paper. Figure 2a shows the experimental ^{13}C 2D spin-diffusion spectrum of the acid-treated P2, where the carbonyl carbons in the Ala²¹ and Ala²² residues in the center of the poly-Ala domain were ^{13}C double labeled. To determine the torsion angles in the β -sheet fraction, we calculated 2D spectra by considering a 20% random coil fraction. The assumed 2D spectral pattern of the random coil conformation was calculated as a superimposition of the calculated spectral patterns of the Ala residues, which were selected from the energetically allowed region in a Ramachandran map on the basis of stereo-chemical criteria of van der Waals contacts.²⁰ Then, the calculated spectrum for comparison with the observed one was calculated by considering an assumed random coil spectral pattern (20%) and β -sheet spectral pattern (80%), where the latter was calculated as a function of the torsion angle (ϕ , ψ) of the Ala residue selected from the β -sheet-favored region ($-180^\circ < \phi < -90^\circ$, $60^\circ < \psi < 180^\circ$). The calculated spectral patterns significantly changed with the torsion angles, ϕ and ψ , as shown in Figure 2c. The RMSD between the calculated and observed spectra was shown as a contour map in Figure 3. The best fitted spectrum (Figure 2b) was obtained with a torsion angle of (ϕ , ψ) = (-150° , 150°) in the β sheet region, as observed in the contour map. The obtained torsion angle is almost the same as the previous one.¹¹ Here, the experimental error was $\pm 10^\circ$.

We performed a similar approach for the acid-treated P1 and P3 samples. Namely, the ^{13}C double-labeled carbonyl carbons are the Gly¹⁷-Ala¹⁸ part at the amino terminus of the poly-Ala region and the Gly²⁵-Ala²⁶ part in the sequence, GGA adjacent to the carboxy-terminal end of the poly-Ala domain, respectively. Figure 4a shows the observed 2D spin-diffusion spectra of P1 and P3. The S/N ratio of the P1 spectrum was low because of the lack of sample amount, and therefore, the spectral pattern looks slightly asymmetric because of the difficulty with phase correction. The RMSD between the calculated and observed spectra was shown as a contour map in Figure 5. Minimal RMSD values were distributed from the typical torsion angle of the β -sheet, (ϕ , ψ) = (-150° , 150°), to the energetically allowed region other than the β -sheet-favored region. This result is different from the case of P2, which is in the center of the poly-Ala region. However, because of the low quality of the spectral pattern for P1, it appears difficult to clearly describe this difference. In the case of P3, where the observed spectral pattern is relatively good, a similar tendency was also observed, indicating a distribution in the energetically allowed region other than the β -sheet-favored region. Thus, this tendency seems common in both P1 and P3. The best-fitted spectra were obtained from the contour plot of RMSD shown in Figure 4(b). The torsion angles in the β -sheet-favored region were (ϕ , ψ) = (-130° , 150°) for P1 and (ϕ , ψ) = (-180° , 140°) for P3. As expected, these values are different from the typical values of a β -sheet, (ϕ , ψ) = (-150° , 150°), which were obtained in the center of

Figure 3 RMSD contour plot for the 2D spin-diffusion of P2 in the energetically allowed region. The torsion angle (ϕ , ψ) = (-150° , 150°) gave a good agreement between the observed and calculated 2D spin-diffusion spectra (Figure 2).

the poly-Ala region, as mentioned above. This result also seems to be related with the difference in the random coil fraction, that is, P1 (45%) and P3 (60%), compared with P2 (20%). These results provide insight into the detailed structure of the Ala residues around the β -sheet poly-Ala region. When apart from the poly-Ala region, distortion of the torsion angle from the typical β -sheet structure occurred together with an increase in the random coil fraction, as is expected. This paper more quantitatively analyzed the phenomena that occurred locally along the chain of spider silk.

SUMMARY

The local structure of the acid-treated model peptides, (E)₈GGLGGQGAG(A)₆GGAGQGGYGG, that were derived from the consensus sequence of the *Nephila edulis* fibroin MaSp1 was determined using 2D proton-driven spin-diffusion solid-state NMR under OMAS coupled with ^{13}C isotope double labeling of specific residues. We estimated the torsion angles of the Ala residue in the center of poly-Ala region and of the Ala residues around the poly-Ala region. The positional dependence in the torsion angles of the Ala residues was obtained. These results, together with previous data of the random coil fraction, could more quantitatively indicate that the phenomena occurred locally along the chain of spider silk.

Figure 4 (a) Observed and (b) simulated 2D OMAS spin-diffusion spectra of the model peptide P1 and P3 with ^{13}C double-labeled carbonyl carbons of Gly¹⁷-Ala¹⁸ and Gly²⁵-Ala²⁶, respectively. The simulated spectra are mixtures of the 55% β -sheet with a torsion angle of $(\varphi, \psi) = (-130^\circ, 150^\circ)$ and 45% random coil patterns for P1, and 40% β -sheet with a torsion angle of $(\varphi, \psi) = (-180^\circ, 140^\circ)$ and 60% random coil patterns.

Figure 5 RMSD contour plot for the 2D spin-diffusion spectra for (a) P1 and (b) P3 in the energetically allowed region. The torsion angles $(\varphi, \psi) = (-130^\circ, 150^\circ)$ for P1 and $(-180^\circ, 140^\circ)$ for P3 gave agreement between the observed and calculated 2D spin-diffusion spectra (Figure 4) in the β -sheet-favored region, although the agreement is relatively poor for P1.

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