Structure of Model Peptides Based on *Nephila clavipes* Dragline Silk Spidroin (MaSp1) Studied by ^{13}C Cross Polarization/Magic Angle Spinning NMR

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To obtain detailed structural information for spider dragline spidroin (MaSp1), we prepared three versions of the consensus peptide GGLGGQGAGAAAAAAGGAGQGGYGGLGSQGAGR labeled with ^{13}C at six different sites. The ^{13}C CP/MAS NMR spectra were observed after treating the peptides with different reagents known to alter silk protein conformations. The conformation-dependent ^{13}C NMR chemical shifts and peak deconvolution were used to determine the local structure and the fractional compositions of the conformations, respectively. After trifluoroacetic acid (solvent)/diethyl ether (coagulant) treatment, the N-terminal region of poly-Ala (PLA) sequence, Ala^{8} and Ala^{10}, adopted predominantly the α-helix with a substantial amount of β-sheet. The central region, Ala^{15}, Ala^{18}, and Leu^{26}, and C-terminal region, Ala^{31}, of the peptide were dominated by either 3_1-helix or α-helix. There was no indication of β-sheet, although peak broadening indicates that the torsion angle distribution is relatively large. After 9 M LiBr/dialysis treatment, three kinds of conformation, β-sheet, random coil, and 3_1-helix, appeared, in almost equal amounts of β-sheet and random coil conformations for Ala^{8} and Ala^{10} residues and distorted 3_1-helix at the central region of the peptide. In contrast, after formic acid/methanol and 8 M urea/acetonitrile treatments, all of the local structure tends to β-sheet, although small amounts of random coil are also observed. The peak pattern of the Ala C_β carbon after 8 M urea/acetonitrile treatment is similar to the corresponding patterns of silk fiber from *Bombyx mori* and *Samia cynthia ricini*. We also synthesized a longer ^{13}C-labeled peptide containing two PLA blocks and three Gly-rich blocks. After 8 M urea/acetonitrile treatment, the conformation pattern was closely similar to that of the shorter peptide.

Introduction

The remarkable tensile properties of orb web spider dragline silks have attracted a great deal of interest.[1,2] Among spider silks, the dragline silk of the golden-orb weaver, *Nephila clavipes* (*N. clavipes*), has become the benchmark. Its superlative toughness appears to result from a combination of the design of its structural proteins (Spidroins)[3] and controlled processing.[4] *N. clavipes* dragline silk is secreted by the major ampullate gland and is composed mainly of two proteins, major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2).[5,6] These two proteins can be described as block copolymers with alternating Poly-Ala and Gly-rich blocks. In MaSp1, the most abundant sub-block is GGX,[5,7] where X is Leu, Gln, and Tyr. In contrast, in MaSp2, the Gly-rich blocks contain repeats of GPGXY with XY being QQ or QY.

The structure of native spider silk fibers has been characterized by several kinds of NMR and by X-ray scattering methods.[8−17] Conformation-dependent ^{13}C NMR chemical shifts of Ala residues,[8,10,13] DOQSY (double-quantum single-quantum correlation),[14] and 2D spin-diffusion NMR spectra[10] all show that the poly-Ala regions in the dragline silk fiber are predominantly in β-sheet conformation. From recent WISE (Wide-line separation) NMR spin-diffusion experiments,[15] the poly-Ala crystalline domain size was estimated to be 6 ± 2 nm in excellent agreement with the dimensions ($2 \times 5 \times 7$ nm) derived from wide angle synchrotron scattering.[17] Furthermore, it was found that the poly-Ala crystalline domains remain intact when the silk supercontracts in water.[17] This result is consistent with ^{2}H NMR spectra that showed no change in molecular motion when dry spider dragline silk was placed in water.[16]

In contrast to the poly-Ala regions, there is debate about the local structure of the Gly-rich region. The Gly-rich region has been described as an amorphous rubber from X-ray diffraction (XRD) studies.[18,19] However, 2D spin-diffusion NMR spectra suggested that 3_1-helical structures are present in the Gly-rich regions.[10] Recently, DOQSY and DECODER NMR spectra have shown that the Gly-rich regions are partially incorporated into β-sheets and partially form helical structures with an approximate 3-fold symmetry.[14] The difficulties in the structural determination of Gly-rich region are due to the heterogeneity in the repeated sequences of

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dragline silk. Recently, we have sought to overcome this difficulty using solid-state synthesis to prepare relatively short ^{13}C-labeled model peptide based on the repetitive poly-Ala and Gly-rich blocks in *N. clavipes* dragline silk.[20,21] The local secondary structural composition was determined by deconvolution of the relevant parts of the ^{13}C CP/MAS NMR spectra. Several different organic solvent treatments were performed to induce structural change in the model peptides. Thus, the use of selectively ^{13}C isotope-labeled model peptides coupled with solid-state ^{13}C NMR chemical shift analysis[20−28] was effective at providing evidence concerning local structure in both the poly-Ala and the Gly-rich blocks of MaSp1.

In the present paper, we report the results of further ^{13}C solid-state NMR chemical shift studies on synthetic model peptides labeled in different positions from those in our earlier study.[21] The Ala C_β and Ala C═O carbons show clear conformation-dependent chemical shift behavior, and therefore the introduction of these into the synthesized peptides was attempted. The Ala C_β peak gives more detailed structural information such as intermolecular arrangement as compared to the Ala C═O peak.[22] Leu C═O carbon was also used for the conformation analysis for the same reason.

Solvent treatments prior to the NMR measurements were performed to induce structural change of these model peptides. Trifluoroacetic acid treatment can induce the model peptide containing poly-Ala crystalline regions of *S. c. ricini* silk fibroin to take an α-helical conformation corresponding to the pre-spun state.[29] In contrast, the poly-Ala region in the synthetic silk protein tends to take β-sheet conformation after dissolution in 8 M urea, followed by precipitation in acetonitrile.[30] The β-sheet formation can be promoted for peptides containing both poly-Ala and repeated $(GGA)_3$ by dissolution in formic acid, and precipitation by methanol.[22] Although $(AG)_{15}$ takes exclusively a silk I-like structure after dissolution in 9 M LiBr followed by dialysis against water, the poly-Ala model peptide retains a β-sheet structure after the same 9 M LiBr/dialysis treatment.[22,29] Thus, the conformation depends on both the choice of sequence of the silk model peptides and the solvent treatment. In this paper, treatments with reagents including trifluoroacetic acid (solvent)/diethyl ether (coagulation solvent), formic acid/methanol, 8 M urea/acetonitrile, and 9 M LiBr/dialysis against water were performed to induce structural changes in the model peptides. The changes in the local structure perturbed by the solvent effect are discussed giving information on the inherent stability of the local structure in the peptides. The local structures in the short peptide were compared to those in a longer ^{13}C isotope-labeled peptide containing three Gly-rich and two Ala-rich blocks based on the consensus sequence for MaSp1.

Materials and Methods

Model Peptides. Using F-moc solid-phase synthesis as described elsewhere,[31−35] we prepared three different labeled versions (P1−P3) of a short peptide and a single version of a longer peptide (P4) (see Table 1) based on the consensus sequence of *N. clavipes*, MaSp1. The longer peptide had two poly-Ala blocks and was synthesized to compare the conformation with those of the shorter peptide possessing only a single poly-Ala block. After synthesis, the peptides were dissolved in (1) trifluoroacetic acid (TFA) followed by precipitation in diethyl ether and drying in air (TFA/diethyl ether), or (2) 9 M LiBr followed by dialysis against water and lyophilization (9 M LiBr/dialysis), or (3) formic acid (FA) followed by precipitation in methanol (MeOH) and drying in air (FA/MeOH), or (4) 8 M urea followed by precipitation in acetonitrile (AN) and drying in air (8 M urea/AN). An 8 M urea/AN treatment was also tried for the longer ^{13}C isotope-labeled peptide containing three Gly-rich and two Ala-rich blocks.

Table 1. The Sequence of Model Peptides Derived from *N. clavipes* Dragline Silk and Their ^{13}C Isotope-Labeled Model Peptides Together with Labeled Residues

P1	GGLGGQG[3-^{13}C]A^{8}G[1-^{13}C]A^{10}AAAAAGGAGQGGYGGLGSQGAGR
P2	GGLGGQGAGAAAAA[1-^{13}C]A^{15}GG[3-^{13}C]A^{18}GQGGYGGLGSQGAGR
P3	GGLGGQGAGAAAAAAGGAGQGGYGG[1-^{13}C]L^{26}GSQG[3-^{13}C]A^{31}GR
P4	GGLGGQGAGAAA[3-^{13}C]A^{13}AAGG[2-^{13}C]A^{18}GQGGYGGL[1-^{13}C]G^{27}SQGAGRGGQ[2-^{13}C]G^{37}AAAAAAGGAGQG

^{13}C CP/MAS NMR Observation. Solid-state ^{13}C CP/MAS NMR spectra were acquired on a Chemagnetics CMX-400 spectrometer operating at 100 MHz for ^{13}C nucleus. 30 mg of the peptide sample was contained in a cylindrical rotor with an outer diameter in 4 mm. CP was employed for sensitivity enhancement with high-power ^{1}H decoupling during the signal acquisition interval. A ^{1}H 90° pulse of 3 μs was used with a 1 ms contact time and a 3 s repetition time, and magic angle spinning at 10 kHz. A total of 10 000−25 000 scans were collected over a spectral width of 60 kHz. Chemical shifts were indirectly calibrated using the adamantane methylene peak observed at 28.8 ppm relative to TMS. The ^{13}C CP/MAS NMR spectrum of P4 after 8 M urea/AN treatment was obtained after subtracting the contribution of the natural abundance spectrum of the corresponding 49 mer without ^{13}C labeling. This was not necessary for the shorter peptides P1−P3, QGAGAAAAA-AGGAGAGGAGG^{20}A^{21}GGAGAGRGGLGG^{21}.

Results

Conformation of the Peptides 1−3 Treated with TFA/Diethyl Ether. Figure 1 shows the ^{13}C CP/MAS NMR spectra of ^{13}C-labeled Ala C_β and carbonyl regions of the model peptides after TFA/diethyl ether treatment. The fraction of each conformation was determined by peak deconvolution (Table 2). The intense C_β peak of Ala^{8} was observed at 15.9 ppm with a shoulder at 20.0 ppm in the lower field, indicating that Ala^{8} adopted predominantly an α-helical conformation with a small amount of β-sheet content. The Ala^{10} C═O peak deconvoluted into two peaks, assigned to a combination of α-helix (60%) and β-sheet (40%) conformations. The fractions of α-helix and β-sheet were almost the same (65% of α-helix and 35% of β-sheet) as that of Ala^{8} residue in the peptide, QGAGAAAA^{8}-AAGGAGAGGAGGAGGAGAGR-GGLGG, after the TFA/diethyl ether treatment reported previously.[21]

In contrast, a helical conformation, either α- or 3_1-helical, was found in the C-terminal end of poly-Ala blocks, in the

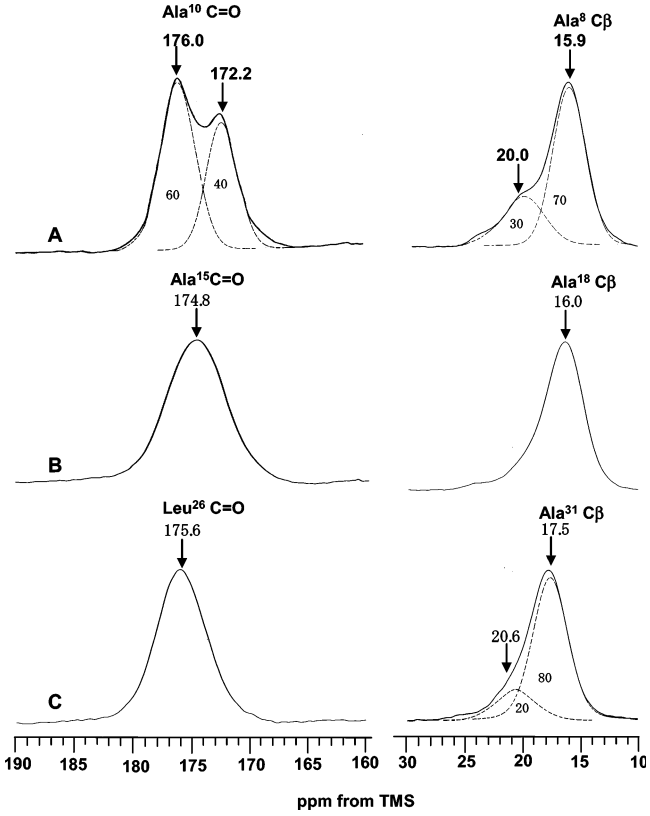

Figure 1. ^{13}C CP/MAS NMR spectra of ^{13}C-labeled model peptides (A) P1, (B) P2, and (C) P3 from spider dragline spidroin (MaSP1) after TFA/diethyl ether treatment.

central parts of peptide, and in the C-terminus of peptide (Ala^{15}, Ala^{18}, Lue^{26}, and Ala^{31}), although the peaks are broad (Table 2). The helical conformation in the GGL^{26} is consistent with the structure of GG^{20}A^{21} in our previous ^{13}C-labeled-model peptides, QGAGAAAAAAGGAGAG-GAGG^{20}A^{21}GGAGA GRGGLGG^{21}.

Conformation of the Peptides 1−3 after 9 M LiBr/Dialysis. Figure 2 shows the ^{13}C CP/MAS NMR spectra of the model peptides, P1−P3, treated with 9 M LiBr/dialysis. The chemical shifts and shapes of the peaks depend on the position of the ^{13}C labeling. Peaks within the Ala C_β resonance region were assigned as β-sheet structure (20 ppm), random coil (16.3−16.6 ppm), and 3_1-helix (17.3 ppm). In addition, the broadening of the peak assigned to 3_1-helix indicates distortion of this conformation.[21] Table 2 shows the fraction of each conformation determined by deconvolution together with the half-height widths of the peaks. The Ala^8 C_β and Ala^{10} C═O peaks deconvoluted into two peaks assigned to a combination of random coil and β-sheet conformations. Judging from the chemical shifts, 174.7−8 ppm for both Ala^{15} and Leu^{28} C═O broad peaks, these residues mainly take the distorted 3_1-helix. After 9 M LiBr/dialysis treatment, the central residue, Ala^8, in the $(Ala)_6$ block, within the peptide, QGAGAAAA^{8}AAGGAGAGG-AGGAGGAGAGRGGLGG, was reported[21] to take 65% β-sheet. The fraction of β-sheet decreased slightly to 55% at Ala^{10} and a further decrease in the β-sheet content to 45% at Ala^8. Thus, the β-sheet content decreased slightly toward the N-terminus of the peptide. On the other hand, the Ala^{15} at the C-terminus of the poly-Ala block adopted the 3_1-helix, similar to that of the adjacent GGA^{18} sequence (mainly 3_1-helix). The sequence, GGL^{26}, also mainly adopted the distorted 3_1-helix judging from the chemical shift and the peak broadening of Leu^{26} (Table 2). The structure became mainly random coil at Ala^{31} residue.

Conformation of the Peptides 1−3 Treated with FA/MeOH. As reported previously in a similar model,[21] FA/MeOH treatment promoted β-sheet formation. As shown in Figure 3, the Ala C_β peak of the Ala^8 residue shifted to lower field, 20.1 ppm, indicating that Ala^8 adopted a predominantly β-sheet conformation after this treatment. However, there remained a small shoulder at the high field of the main peak, implying the presence of small amounts of random coil conformation. After FA/MeOH treatment, Ala^{10} (the N-terminal Ala of the poly-Ala block), gave a single sharp peak at 171.9 ppm as listed in Table 2, indicating an exclusively β-sheet conformation. In addition, the C═O carbon of Ala^{15} (the C-terminal Ala of the poly-Ala block) also showed a sharp peak at 172.4 ppm, indicating an exclusively β-sheet conformation. The central residue, Ala^8, in the peptide, QGAGAAAA^{8}AAGGAGAGGAGGGGAG-AG-RGGLGG, adopted predominantly a β-sheet structure as described in the previous paper.[21] After the same treatment, Ala^{18} showed an intense Ala C_β peak at 20.4 ppm, indicating a predominant β-sheet conformation, although the small shoulder at the high field indicates a small proportion of random coil conformation. Ala^{31} showed peaks at 19.9 and 16.3 ppm, indicating a combination of β-sheet and random coil conformations. The C═O peak at 173.0 ppm of Leu^{26} can be assigned to β-sheet structure. However, it was very broad (Table 2), indicating the possibility of distribution of the torsion angles (φ and ψ).

Conformation of the Peptides 1−3 Treated with 8 M Urea/AN. Figure 4 shows the ^{13}C CP/MAS NMR spectra of the peptides P1−P3 treated with 8 M urea/AN, while the chemical shifts of the labeled residues together with the fractions of each conformation are listed in Table 2. The spectral patterns of the peptides P1−P3 after 8 M urea/AN treatment are similar to those after FA/MeOH treatment. The conformations were almost entirely β-sheet. However, the two treatments produced some differences. After 8 M urea/AN treatment, the Ala C_β peak of Ala^{18} deconvoluted to a predominant β-sheet, a small random coil peak, and a further small peak on the lower field not observed after FA/MeOH treatment. The fraction of random coil was larger for 8 M urea/AN treatment than that for FA/MeOH. Deconvolution of the Ala C_β peak of Ala^{31} also gave a broad lower field peak at 22.8 ppm. These additional lower field peaks at 22.5−22.6 ppm indicate the coexistence of β-sheet structures with different intermolecular packing arrangements as already reported for *B. mori* silk fibroin fiber after spinning,[36] or the crystalline fraction of *B. mori* silk fibroin[32] or the model peptide $(AG)_{15}$ in silk II form.[32] The peaks of carbonyl carbons of Ala^{10} and Ala^{15} at 171.9 and 172.7 ppm, respectively, were single and sharp, indicating that these residues take exclusively a single type of β-sheet structure after both treatments. For the Leu^{26} C═O carbon, an asymmetric peak was observed, although the peak was still broad. The peak at 172.9 ppm can be assigned to a β-sheet

Table 2. ^{13}C CP/MAS NMR Chemical Shifts (in ppm from TMS) of the ^{13}C-Labeled Carbons in Four Peptides[a] in Three Treatments (^{13}C Chemical Shifts of Ala, Gly, and Leu Residues with Typical Conformtion Are Also Listed)

		TFA/diethyl ether			9 M LiBr/dialysis			FA/MeOH			8 M urea/AN							
carbon	^{13}C-labeled residue	chemical shift	fraction	width (Hz)	chemical shift	fraction	width (Hz)	chemical shift	fraction	width (Hz)	chemical shift	fraction	width (Hz)	dragline silk[8]	random coil[b]	α-helix[35]	β-sheet[23]	3_1-helix[23]
Ala Cβ	Ala[8]	15.9	[70%]	342	16.3	[55%]	364	16.3	[15%]	309	16.8	[15%]	222	20.6[c]	16.6	15.7	16.7[d]	17.4
		20.0	[30%]	378	20.1	[45%]	418	20.1	[85%]	324	20.3	[72%]	279				19.6	
											22.6	[13%]	185				22.2	
	Ala[18]	16.0[d]		411	17.3	[80%]	430	16.4	[20%]	280	16.9	[30%]	262					
					21.3	[20%]	368	20.4	[80%]	340	20.4	[48%]	250					
											22.5	[22%]	232					
	Ala[31]	17.5	[80%]	345	16.6	[85%]	426	16.3	[60%]	286	16.3	[60%]	217					
		20.6	[20%]	380	20.5	[15%]	286	20.3	[45%]	288	20.4	[30%]	250					
											22.8	[10%]	273					
	Ala[13]										16.6	[14%]	302					
											20.4	[67%]	273					
											23.0	[19%]	163					
Ala Cα	Ala[18]										49.0			49.0	50.0	52.5	48.7	48.9
Ala C═O	Ala[10]	172.2	[60%]	320	172.3	[45%]	328	171.9		190	171.9		192	172.3	175.5	176.5	171.8	174.6
		176.0	[40%]	344	176.0	[55%]	403											
	Ala[15]	174.8		580	174.7		573	172.4		180	172.7		212					
Leu C═O	Leu[26]	175.6		497	174.8		536	173.0		546	175.6	[58%]	536			175.7	171.8	
											172.9	[42%]	376					
Gly Cα	Gly[37]										42.4			43.2	42.9	44.0	42.4	41.6
Gly C═O	Gly[27]										168.8			172.3	171.3	172.3	169.1	171.3
											171.5							

[a] The Ala[8] and Ala[10] residues are in P1, GGLGGQG[3-^{13}C]A[8]G[1-^{13}C]A[10]AAAAAGGAGQGGYGGLGSQGAGR. The Ala[15] and Ala1[8] residues are in P2, GGLGGQGAGAAAAA[1-^{13}C]A[15]GG[3-^{13}C]A[18]GQGGYGGLGSQGAGR. The Leu[26] and Ala[31] residues are in P3, GGLGGQGAGAAAAAAGGAGQGGYGG[1-^{13}C]L[26]GSQG[3-^{13}C]A[31]GR. The Ala[13], Ala[18], Gly[27], and Gly[37] residues are in P4, GGLGGQGAGAAA[3-^{13}C]A[13]AAGG[2-^{13}C]A[18]GQGGYGGL[1-^{13}C]G[27]SQGAGRGGQ[2-^{13}C]G[37]AAAAAAGGAGQG. [b] The random coil chemical shifts were obtained from the solution NMR.[23] [c] The peak of Ala Cβ was broad and asymmetric. [d] The peak at 16.7 ppm was assigned to distorted β-turn.[23]

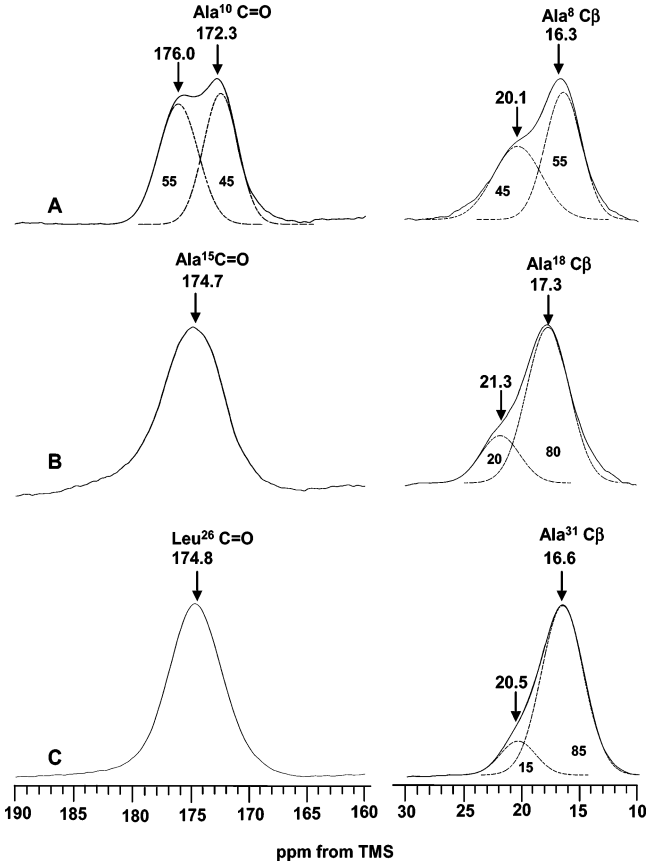

Figure 2. ^{13}C CP/MAS NMR spectra of ^{13}C-labeled model peptides (A) P1, (B) P2, and (C) P3 from spider dragline spidroin (MaSP1) after 9 M LiBr/dialysis treatment.

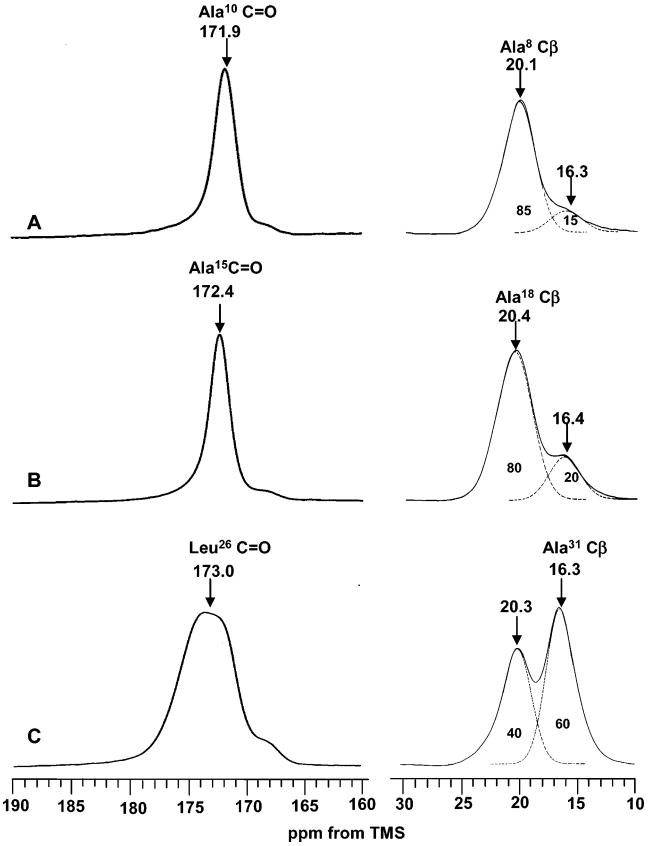

Figure 3. ^{13}C CP/MAS NMR spectra of ^{13}C-labeled model peptides (A) P1, (B) P2, and (C) P3 from spider dragline spidroin (MaSP1) after FA/MeOH treatment.

structure, and the peak at 175.6 ppm can be assigned to a random coil.

Conformation of Peptide 4 Treated with 8 M Urea/AN. To investigate whether the conformation of the shorter peptide is likely to be representative of the structure in the very large molecule of natural silk heavy chain fibroin, we synthesized a longer peptide with 49 amino acid residues (P4) and compared the local structure of the former and later peptides after 8 M urea/AN treatment. The natural abundance ^{13}C peak will make an appreciable contribution to the peak intensity of the ^{13}C-labeled site in the lengthened peptide. Therefore, the contribution from ^{13}C in the natural abundance spectrum was subtracted from the ^{13}C CP/MAS NMR spectrum of ^{13}C-labeled P4. Figure 5 shows the relevant parts of the ^{13}C CP/MAS NMR spectra for the ^{13}C-labeled Ala^{13}, Ala^{18}, Gly^{27}, and Gly^{37} in the larger peptide (P4). Ala^{13} (the central Ala residue in the poly-Ala block) took a β-sheet conformation as evident from the chemical shift at 20.4 ppm for the main peak of Ala C_β carbon. The additional peak at 22.5 ppm indicates heterogeneity in the β-sheet structure as discussed above. The peak at 49.0 ppm of Ala^{13} can be assigned to β-sheet and/or 3_1-helix conformations. However, the Ala^{18} residue of the peptide 2 after 8 M urea/AN treatment clearly indicated the predominance of β-sheet structure as judged from the chemical shift of the [3-^{13}C]-Ala peak (Figure 4B). It is, therefore, likely that the addition of a second poly-Ala block does not affect the β-sheet conformation of Ala^{18} after 8 M urea/AN treatment. Gly^{37} showed a C_α chemical shift, 42.4 ppm different from that of a 3_1-helix or an α-helix. Further discrimination was not possible because the C_α chemical shifts of the β-sheet structure and random coil conformations are similar. The residue Gly^{37} is located at the N-terminal of the second poly-Ala block in P4 in a position somewhat analogous to that of Ala^8 in the shorter peptide. As shown in Figure 4A, Ala^8 adopted predominantly a β-sheet conformation with a small random coil component. For the Gly^{27}, there was an intense peak at 171.5 ppm with a shoulder at 168.8 ppm, the former corresponding to 3_1-helix and/or random and the latter to β-sheet. Thus, the local structure of P4 (the longer peptide) after 8 M urea/AN treatment is close to that of the shorter peptide. This provides some evidence for the validity of using the shorter peptide as a model for the repetitive blocks in MaSp1.

Discussion

The liquid silk stored in the spider silk gland has been reported to be in a dynamic loose helical structure[37] or in a random coil conformation[38] on the basis of the conformation-dependent Ala C_β chemical shift in the ^{13}C solution NMR spectrum of native liquid silk. On the other hand, the α-helical structure of the poly-Ala chain has been reported for *S. c. ricini* silk fibroin stored in the silk gland, where the number of Ala residues in poly-Ala region is about 12, roughly 2 times longer than that of poly-Ala in spider dragline silk. The peptide, $GGAGGGYGGDGG(A)_{12}GG$-AGDGYGAG, dissolved in TFA has been used as a model

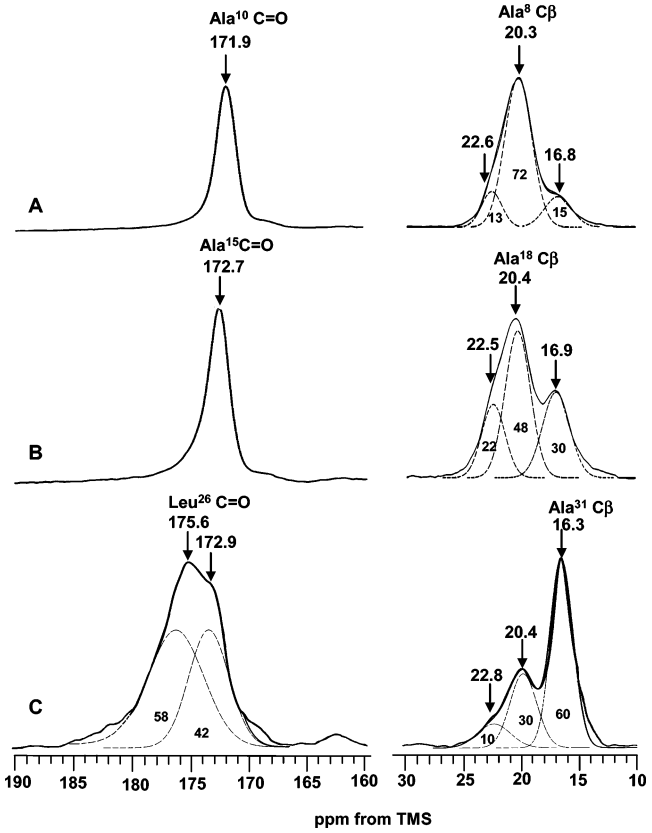

Figure 4. ^{13}C CP/MAS NMR spectra of ^{13}C-labeled model peptides (A) P1, (B) P2, and (C) P3 from spider dragline spidroin (MaSP1) after 8 M urea/AN treatment.

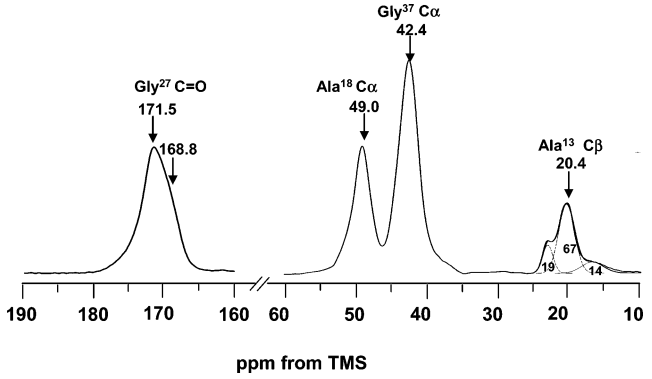

Figure 5. ^{13}C CP/MAS NMR spectra of ^{13}C-labeled model peptide, P4, from spider dragline spidroin (MaSP1) after 8 M urea/AN treatment.

for the determination of local structure of the silk fibroin stored in the *S. c. ricini* silk gland.[29,34,35] Accordingly, in the present study we used the same solvent for the structural study of the model peptide from spider dragline silk. As an approximation, the conformation of the model peptide in TFA is predominantly α-helical except for the Ala^{31} residue, but there are local structure variations in conformations depending on the position. The Ala^{8} and Ala^{10} of the poly-Ala region showed 30−35% β-sheet. In our previous report,[21] the local structure of the central Ala^{8} residue in another model peptide of spider dragline silk, QGAGAAAA^{8}AAGGA-GAGGAGGAGGAGAGRGGLGG, after TFA/diethyl ether treatment was reported to be 65% α-helix and 35% β-sheet.[21] Thus, the fractions of α-helix and β-sheet were almost the same in the central and N-terminal Ala residues of the poly-

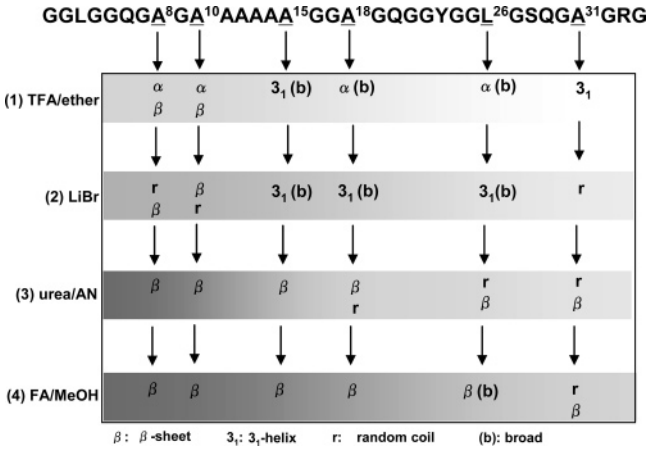

Figure 6. Summary of the structures of the model peptides, P1, P2, and P3, from spider dragline spidroin (MaSP1) after (1) TFA/diethyl ether, (2) 9 M LiBr/dialysis, (3) 8 M urea/AN, and (4) FA/MeOH treatments.

Ala block. In contrast, the alanine at the C-terminus of the poly-Ala region (Ala^{15}) or in the central region of the peptide (Ala^{18} and Leu^{26}) tended to take α-helix or 3_1-helix with no β-sheet structure. A similar tendency was observed after 9 M LiBr/dialysis treatment; we found 45% β-sheet and 55% random coil conformation in the N-terminal region (Ala^{8} and Ala^{10}) of the poly-Ala region. In our previous paper,[21] the local structure of the central Ala^{8} residue in another model peptide of spider dragline silk after 9 M LiBr/dialysis treatment has been reported to be 65% β-sheet and 35% other conformation. Thus, the partially β-sheet structure was present at the central and N-terminal Ala residues of poly-Ala region. However, at the C-terminal of poly-Ala region, Ala^{15}, or the central region of the peptide, Ala^{18} and Leu^{26}, tend to take 3_1-helix and no β-sheet structure. Thus, 9 M LiBr/dialysis induced conformations similar to those produced in *Nephila* spider dragline silk as a result of the natural spinning process. As shown above, the TFA/diethyl ether treatment of a model peptide of *S. c. ricini* silk fibroin induces a predominantly α-helical conformation, while 9 M LiBr/dialysis treatment induces predominantly the β-sheet structure. Thus, structural transition from α-helix to β-sheet occurs clearly when the treatment is changed from TFA/diethyl ether to 9 M LiBr/dialysis. The structural transition to the β-sheet form seems to occur more completely in *S. c. ricini* model peptides as compared to the *Nephila* models. This probably results from the fact that the poly(Ala) blocks in the former are approximately twice as long as in the latter. After 9 M LiBr/dialysis treatment, the shorter poly-Ala blocks of spider model peptides exist in several conformational states at the N-termini of the blocks, although some β-sheet conformation is always present in these regions. As summarized in Figure 6, helical structures such as α-helix and 3_1-helix are present in the central regions after both TFA/diethyl ether and 9 M LiBr/dialysis treatments, although peak broadenings indicate that the distributions of the torsion angles are relatively large for the Ala^{15}, Ala^{18}, and Leu^{26} residues. The C-terminal alanines (Ala^{15} and Ala^{18}) of the poly(Ala) block form structures similar to that of GGL^{26} in the Gly-rich region. We, therefore, suggest that the structure of the C-terminus of the poly(Ala) block is largely driven

by that of the Gly-rich region following it. After FA/MeOH treatment or 8 M urea/AN treatment, β-sheet structure prevails in the N-terminus of poly-Ala block, as well as the central regions of peptide (Ala^{15}, Ala^{18}, and Leu^{26}). In contrast, the C-terminus of peptides Ala^{31}, which forms 3_1-helix (TFA/diethyl ether treatment), or random coil structure (9 M LiBr/dialysis treatment), adopts predominantly random coil conformation with a small amount of β-sheet content after both FA/MeOH and 8 M urea/AN treatments. The peak pattern of the Ala Cβ carbon after 8 M urea/acetonitrile treatment is similar to the corresponding patterns of *B. mori* silk fiber[32,33] and *S. c. ricini* silk fiber,[29] although the Ala Cβ peak pattern could not be clearly observed for dragline silk fiber.[8,10,13]

Thus, changes in local structure produced by the various treatments provide information on the inherent stability of the local structure in both the poly-Ala and the Gly-rich blocks in two model peptides based on spider dragline spidroin with probable relevance to the parent protein (MaSp1). The difficulty in studying the detailed structure in the latter material results from the heterogeneity in the repeated sequences. The present study suggests that the study of ^{13}C conformation-dependent chemical shifts in relatively short ^{13}C-labeled model peptides based on the repetitive blocks of MaSp1 probably provides a model system to circumvent this difficulty.

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