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Orientational Order of Australian Spider Silks as Determined by Solid-State NMR

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Abstract: A simple solid-state NMR method was used to study the structure of ¹³C- and ¹⁵N-enriched silk from two Australian orb-web spider species, *Nephila edulis* and *Argiope keyserlingi*. Carbon-13 and ¹⁵N spectra from alanine- or glycine-labeled oriented dragline silks were acquired with the fiber axis aligned parallel or perpendicular to the magnetic field. The fraction of oriented component was determined from each amino acid, alanine and glycine, using each nucleus independently, and attributed to the ordered crystalline domains in the silk. The relative fraction of ordered alanine was found to be higher than the fraction of ordered glycine, akin to the observation of alanine-rich domains in silk-worm (*Bombyx mori*) silk. A higher degree of crystallinity was observed in the dragline silk of *N. edulis* compared with *A. keyserlingi*, which correlates with the superior mechanical properties of the former © 2006 Wiley Periodicals, Inc. *Biopolymers* 82: 134–143, 2006

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INTRODUCTION

Spider silk combines high tensile strength with light weight and extensibility to yield one of nature’s

strongest fibers.¹ Orb-web spiders (*Araneioidea*) can produce up to eight different kinds of silk and, of those, dragline, the most extensively studied silk, is considered to be the toughest natural fiber because of

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its unique combination of stiffness and extensibility. These unusual properties of spider silk are thought to be due to its unique molecular structure (e.g., 2,3).

Spider silks are semicrystalline biopolymers that consist of repeated sequences of amino acids. The two proteins secreted from the major ampullate gland that make up dragline silk, Major Ampullate Spidroin I and II (MaSp I, MaSp II), from *Nephila clavipes* exceed 700 kDa.⁴ Gly and Ala, the most common amino acid residues found in silk, are typically located in repeated blocks in dragline silk and, although dependent on the spinning process, the secondary structure contains a large proportion of β -sheet.^{5–9} The crystalline domains are thought to consist of Ala-rich, antiparallel β -sheets, the structure of which underpin the superior tensile strength of silk.^{10,11} From amino acid analysis of cDNA, a repeating unit made up of three segments (consisting of 6, then 13 and 15 amino acids in length) dominated by an Ala-rich sequence of 5–7 residues has been found.¹² Regions in which Gly is abundant are proposed to compose the amorphous domains that impart elasticity to silk,¹ while a pentapeptide GPGXX and a GGX repeat motif have been found in the noncrystalline regions of major ampullate silk.¹³ However, debate on the structure of the poly-Gly regions continues. Once thought to be entirely amorphous, the structure of the Gly-rich region may be semiordeed in a 3_1 helix^{14–16} or Type 1 β turn.^{11,17}

Silk from the silkworm *Bombyx mori* has been extensively studied and is a fibrous protein consisting of a repeating sequence of (GAGAGS) somewhat similar in composition to spider silk and contains Gly (43.7%), Ala (28.8%), and Ser (11.9%).¹⁸ NMR and other biophysical studies of *B. mori* and other silkworm silks have provided secondary structure information. In particular, solid-state NMR has been applied to silkworm silk to investigate molecular orientation using isotopically labeled amino acids: ^{13}C ,^{11,19–23} ^{15}N ,^{20,23} and ^2H to study the hydrogen bonding structure.²⁴

Amino acid composition of silk varies, however, from silkworm to spider, and a high degree of variation has been noted between individuals of the same species of spider.^{2,25,26} A strong relationship between structure and function in silk would suggest that silk from different species of spiders and different silk types may vary in amino acid composition and molecular properties. The mechanical strength of dragline silk can be explained in part by the Ala-rich sequences present in crystalline, β -sheet regions of the polymer. Although the amorphous, elastic regions of dragline silk are more difficult to assess due to their apparent lack of structure, coupling between the

highly ordered crystalline domains and the amorphous regions is believed to be responsible for producing an exceptionally strong biomaterial.¹ Solid-state NMR has been used to determine the amount of crystalline region in dragline silks and comparisons could be made between molecular structure and physical properties of silks from different spider species.

Studies of spider silks other than dragline have shown that different forms of silk have different properties. Flagelliform or spiral silk, which makes up the bulk of the orb-web, differs from dragline silk in amino acid content, secondary structure, and extraordinary extensibility.²⁷ The repeating GPGGX blocks, which were previously identified in dragline silk but not found in other less elastic silks, have been identified in flagelliform silk.²⁸ Protein and amino acid composition of silks from the black widow spider (*Latrodectus hesperus*) show variation between dragline, inner egg case, and scaffolding silks.²⁹ The inner silk of the egg case contains a higher proportion of Ala compared with Gly, possibly being high in crystalline β -sheet content for strength to protect the eggs. Both the scaffolding silk (used for structural purposes) and dragline silk contain higher percentages of Gly over Ala, as is the case for *Nephila clavipes* dragline silk.

While the crystalline, Ala-rich regions of silk have been characterized by a variety of techniques, including NMR, the amorphous, Gly-dominated regions have proven difficult to analyze. While it is generally agreed that the two-phase model of silk, crystalline and amorphous, is oversimplistic, a third observed phase has been more difficult to characterize. An additional oriented phase for silk fibers is proposed by a number of groups,^{1,6,30,31} with local structure unlike either crystalline or “normal” amorphous domains. Evidence from Rotational Echo Double Resonance NMR experiments¹⁷ suggests that this third phase forms compact turn-like structures. Two-dimensional (2D) spin diffusion NMR has demonstrated that the Gly-rich regions are ordered and form 3_1 -helices,¹⁴ but these experiments, however, do not distinguish between ordered Gly-rich regions from Gly-rich amorphous regions.¹¹ While most NMR results conclude that the Ala-rich regions form β -sheet structures, Jelinski and coworkers,^{1,10} using carbon and deuterium NMR relaxation times of *N. clavipes* dragline silk, further described Ala as present in two distinct motional environments, one highly oriented (40%) and another poorly oriented (60%). In their model, 90% of the alanine residues are in β -sheets and 70% of glycine is in the amorphous regions.

To understand more fully the structure–function relationships in silk, we have characterized different

types of silk produced by the same as well as by different species of Australian spiders, since variation in the function of silk may be related to different molecular structure and physical properties. Proton and carbon NMR relaxation times show differences in molecular mobility between types of silk (dragline versus cocoon) and between species (*Nephila edulis* versus *Argiope keyserlingi*)³² and most probably reflect differences in molecular structure related to biological function. We have extended these studies and now report ¹³C and ¹⁵N NMR results using oriented dragline silk from two species of spiders fed on labeled Gly and Ala amino acids to determine differences in the amount of crystalline and amorphous phases.

Solid-state NMR is able to identify the secondary structure of polypeptides,^{19,33} and understanding the molecular structure may help explain the superior mechanical properties of spider silks. The methods used by Asakura and coworkers [34 and references therein] on silkworm silk have been applied to study uniaxially aligned dragline silk from two species of spiders to gain insight into the relationship between molecular structure and the function of different silk types. [1-¹³C]-glycine, [1-¹³C]-alanine, ¹⁵N-glycine, or ¹⁵N-alanine were orally administered to two species of Australian spiders, *N. edulis* and *A. keyserlingi*. Dragline silk from these spiders was wound around Teflon or glass blocks to produce oriented silk fibers. The fraction of oriented and nonoriented domains in the silk samples was determined from the ¹³C and ¹⁵N solid-state NMR spectra acquired with the fibre axes parallel or perpendicular to the magnetic field.²³

MATERIALS AND METHODS

¹⁵N- and ¹³C-Labeling of Silk

Dragline silk for solid-state NMR studies was reeled from adult female *N. edulis* and *A. keyserlingi* spiders collected from Queensland and Victoria, Australia. To isotopically label the silk, three to five *Nephila* spiders were hand-fed with a pipette a 30- μ L solution of 10% w/v ¹⁵N-labeled Gly (Cambridge Isotope Laboratories, USA, 98%) in water containing 10% w/v sucrose for at least 5 days before collecting dragline silk. *Argiope* spiders, being smaller, were fed about 10 μ L of the same solution each day. Initially, spiders were also fed one fly per day to ensure the survival of the spider. After 5 days, the fly diet was discontinued and the spiders were fed only labeled Gly solution for at least 10 days. The spiders did not lay cocoons until several weeks after the feeding began. After the ¹⁵N-labeled dragline and cocoon silks were collected, the *Nephila* spiders were fed a diet of 10% unlabeled Gly and 10% sucrose without any

flies for further studies of amino acid supplementation on silk composition.

Another group of *Nephila* and *Argiope* spiders was hand-fed 10% w/v ¹³C-labeled Gly (Cambridge Isotope Laboratories, 99%) in a 10% w/v sucrose solution plus one fly per day for at least 5 days. After 5 days the fly diet was discontinued before collecting silk and performing ¹³C NMR experiments. A similar procedure was used for ¹⁵N-labeled Ala and ¹³C-labeled Ala (Cambridge Isotope Laboratories, 99%).

For oriented samples, dragline silk was wound onto a Teflon or glass plate at a rate of 1–2 cm s⁻¹ for a total of about 60 m of silk. Samples were prepared by reeling dragline silk directly from the spider either parallel (Teflon) or perpendicular (glass) to the long axis of the plates, which were then inserted into an NMR tube. Teflon plates (7 \times 3 \times 2 mm) were originally used to avoid signal interference from carbon in cross-polarization (CP) experiments. However, due to the relatively large quantity of Teflon compared to silk and the large number of scans needed, Teflon gave a noticeable broad background signal in ¹³C CP spectra. Glass plates, 20 \times 8 \times 0.5 mm, were subsequently used for ¹³C (and ¹⁵N) experiments. Oriented spectra were acquired using a static NMR probe with a 10-mm coil perpendicular to the magnetic field and the silk sample aligned at 0 and 90° to the field by turning the glass plate within the coil.

NMR Spectroscopy

Carbon-13 wideline and ¹⁵N MAS NMR measurements were carried out on a CMX Infinity 400 spectrometer at a proton frequency of 398 MHz using a static HX Apex probe, modified to operate from 500 to 398 MHz proton frequency. Proton ramp CP³⁵ was used for ¹³C and ¹⁵N excitation. The 50 kHz ¹H excitation pulse was followed by 1 ms Hartmann–Hahn match and 40 kHz TPPM proton decoupling was applied during acquisition. The dwell time was 10 μ s and 512 points were collected in each experiment. Between 10,000 and 60,000 transients were averaged for each free induction decay with a 3.5-s interpulse delay (exceeding 5T₁ for ¹H) in all cases. All experiments were carried out at room temperature (22°C). All spectra were convoluted with a 100 Hz exponential during processing.

The spectra were processed using Spinsight (Chemagnetics, Fort Collins, CO, USA) and Felix (MSI, Cambridge, UK). Randomly oriented *N. edulis* and *A. keyserlingi* cocoon silk and powdered dry ¹³C₁-labeled valine were used to obtain reference powder lineshapes.

Oriented Studies

In a β -sheet, methyl groups and C=O bonds are oriented perpendicular to the long axis of the fiber.^{1,36} In an α -helix the C=O bonds are approximately parallel to a fiber's long axis while side chain groups typically extend outward from the helical axis.³⁶ In studies of ¹⁵N-enriched *Samia cynthia ricini* silkworm fibres oriented parallel to the magnetic

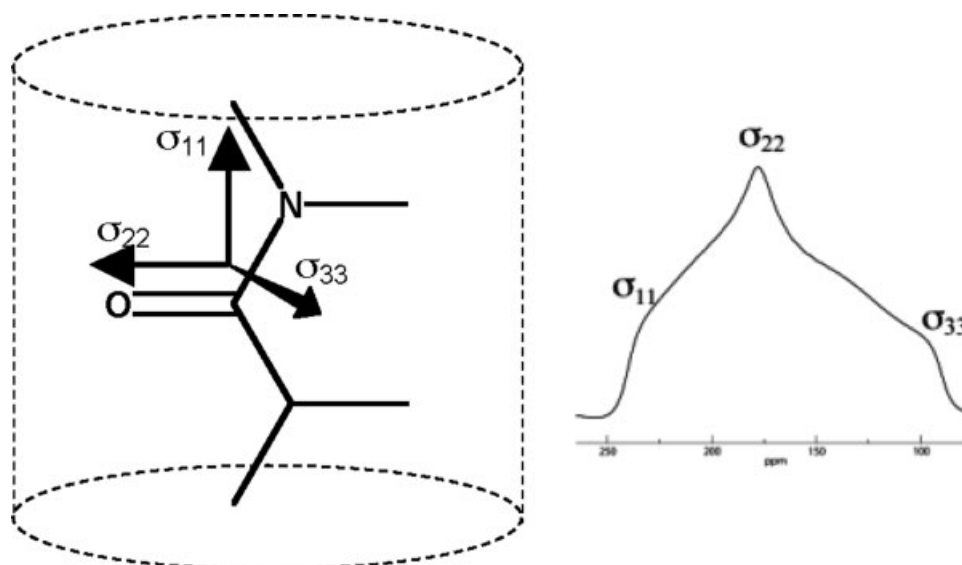


FIGURE 1 Carbonyl CSA tensor orientation within ordered antiparallel β -sheet domains in the spider dragline fibres (the notation follows reference 19). A simulated ^{13}C spectrum of $[1-^{13}\text{C}]$ -Gly silk using tensor values of reference 23 is shown for comparison.

field,^{23,34} the intensity of the most downfield chemical shift tensor element σ_{11} (~ 200 ppm) is small compared with σ_{33} (~ 30 ppm). As the oriented fibers are rotated through 90° to the field, σ_{11} increases in intensity, and the spectrum resembles a ^{15}N peptide powder pattern. In the case of ^{13}C -enriched silkworm fibers, when the fiber axis is parallel to the field, the intensity of the most downfield element σ_{11} (~ 240 ppm) is high compared with σ_{33} (~ 90 ppm). As the oriented fibers are rotated perpendicular to the field, σ_{33} increases in intensity and resembles a ^{13}C peptide carbonyl powder pattern. In this work we used the ^{13}C and ^{15}N chemical shift tensors given by Asakura et al.²³ for Gly and Ala residues. However, it should be noted that the tensor values and orientation are sensitive to the adjacent residue, as shown for glycine in dipeptides and tripeptides.³⁷

Quantitative analysis of the ^{13}C spectra was carried out by estimating the relative fraction of ordered material in the carbonyl component of the spectra. Experimental ^{13}C -carbonyl spectra of samples oriented with the fiber axis parallel to \mathbf{B}_0 were normalized to 100% \mathbf{M}_0 . Randomly oriented (powder) spectra, acquired from ^{13}C -labeled Gly or Ala enriched cocoon silk, were also normalized to 100% \mathbf{M}_0 . Fractions of the powder spectra were compared with the experimental spectra from oriented samples. The fractional powder contribution to the oriented spectra was assessed by matching spectral intensity in the region of σ_{33} (100–125 ppm), where the orientation of the carbonyl chemical shift anisotropy (CSA) tensor follows the notation in 21 (Figure 1). A Gaussian component was used to simulate the inhomogeneous spectral contribution from oriented material with a slight variation of local environment within and between individual crystallites across the sample. Distortions in the Gaussian shape toward higher frequencies, which may result from angular dispersion within the micro-

crystallite population, may contribute to the unassigned spectral intensity. The Gaussian with intensity

$$S = A \times \exp\left[-\frac{(\sigma_{11} - \omega)^2}{\delta^2}\right]$$

was added to the powder fraction and its parameters were adjusted to match the σ_{11} region of the experimental spectrum (220–240 ppm). The line was centered at σ_{11} while the parameter δ and amplitude A were adjusted to fit best the upfield region of the experimental ^{13}C spectra. The full width at half height of this Gaussian is $2\delta\sqrt{\ln 2} \approx 1.67\delta$. The integral contribution of the Gaussian was used as a measure of the oriented fraction in the $^{13}\text{C}=\text{O}$ spectra. The combined integral contribution of the powder component and the Gaussian did not exceed 95%. The residual intensity reflects a spectral contribution from approximately 6% silk oriented at 90° with respect to \mathbf{B}_0 (the glass slides, onto which the silk was wound, were approximately 8 mm wide and 0.5 mm thick).

Nitrogen-15 powder lineshapes were simulated using SIMPSON³⁸ on a i386 Linux RedHat 8 workstation. The CSA tensor elements used in the simulations were: $\sigma_{33} = 201$ ppm, $\sigma_{22} = 68$ ppm, and $\sigma_{11} = 34$ ppm for ^{15}N -alanine and $\sigma_{33} = 182$ ppm, $\sigma_{22} = 53$ ppm, and $\sigma_{11} = 21.0$ ppm for ^{15}N -glycine following Asakura and coworkers.²³ The isotropic chemical shifts of 101 ppm for ^{15}N -alanine and 85 ppm for ^{15}N -glycine were also taken from 23.

RESULTS AND DISCUSSION

Selectively ^{13}C -Ala- or ^{13}C -Gly-enriched dragline silk samples, collected from the Australian species of

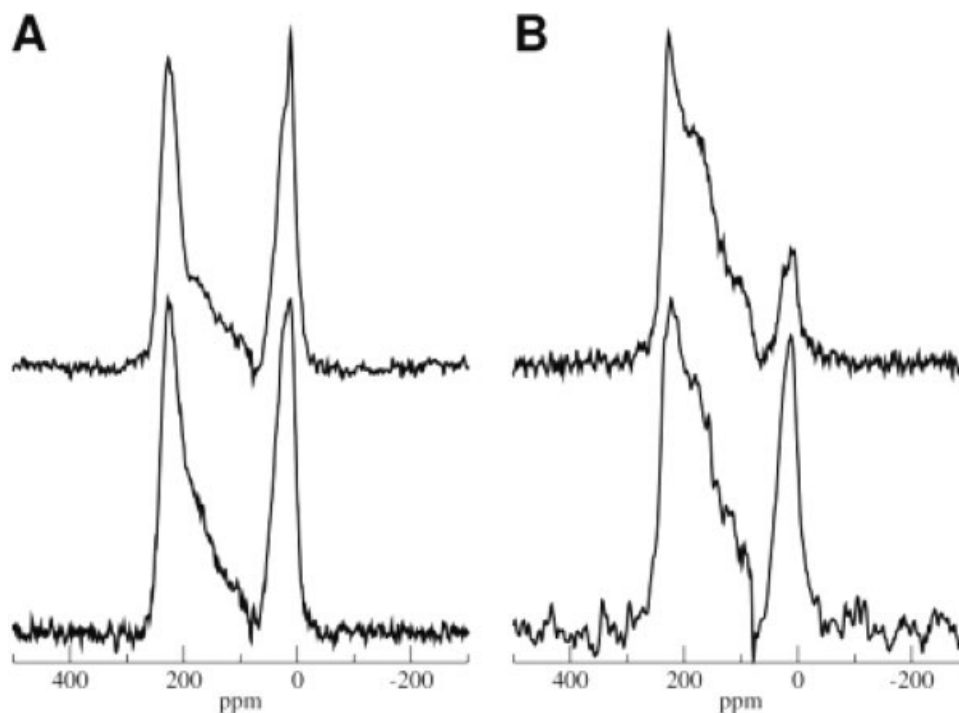


FIGURE 2 Carbon-13 NMR spectra of oriented dragline silk from *N. edulis* (top) and *A. keyserlingi* (bottom) spiders, fed on (A) enriched $[1-^{13}\text{C}]$ -Ala or (B) $[1-^{13}\text{C}]$ -Gly amino acid. Fibers aligned with the fiber axis parallel (0°) to the magnetic field.

spiders *N. edulis* and *A. keyserlingi*, were oriented on glass slides and studied by ^{13}C and ^{15}N wide-line solid-state NMR. Carbon-13 and ^{15}N CP spectra from silk oriented along the external magnetic field are shown in Figures 2 and 3 respectively. Spectra from the same samples, oriented at 90° with respect to the field, were also acquired (not shown) and showed a strong orientational dependence but were not used in the quantitative analysis. All spectra are dominated by inhomogeneous broadening resulting from the orientation-dependent part of the CSA. The diagonal elements of the CSA tensor, σ_{11} , σ_{22} , and σ_{33} , for the $1-^{13}\text{C}$ of alanine and glycine have been determined previously.²¹

The ^{13}C spectra from oriented draglines reveal overlapping spectral contributions from at least two distinct environments with different orientational order. A spectral fraction from randomly oriented (powder) material can be distinguished clearly. This covers spectral contributions within the entire range between σ_{11} (ca. 245 ppm) and σ_{33} (ca. 99 ppm).²¹ The contribution from this randomly oriented material is higher in our study than in results reported by others for silkworm silk.³⁴ The silkworm silk was washed in their instance, while in our case the spider silk fibers have been investigated in their native form. Interestingly, our results are closer to earlier work for

N. clavipes,¹ which also reported up to 60% randomly oriented component.

Another spectral contribution can be resolved in spectra from material oriented along \mathbf{B}_0 . Its spectral intensity appears at chemical shift values around σ_{11} and is associated with the presence of an oriented, possibly crystalline, material. As discussed earlier, spider draglines have been shown to contain significant amounts of ordered domains of antiparallel β -sheet oriented with the polypeptide backbone along the fiber axis.¹ Such domains present the carbonyl CSA tensors in an orientation so that the diagonal element σ_{11} is approximately aligned with \mathbf{B}_0 (following the convention adopted in reference 21). Spider draglines are extruded polymers, in which these ordered domains form the hard “filler” in a “plastic” glycine-rich and more disordered biopolymer. The cross-section of spider silks is heterogeneous,³⁹ which would suggest the existence of a range of microenvironments within the fiber. We have adopted a model for the oriented dragline material, in which environmental dispersion of microenvironments (possibly influenced by hydration levels, extrusion speed, and other factors) gives rise to inhomogeneously broadened spectral features and a Gaussian intensity distribution. It is reasonable to assume that some dispersion exists in the orientation of such domains and the

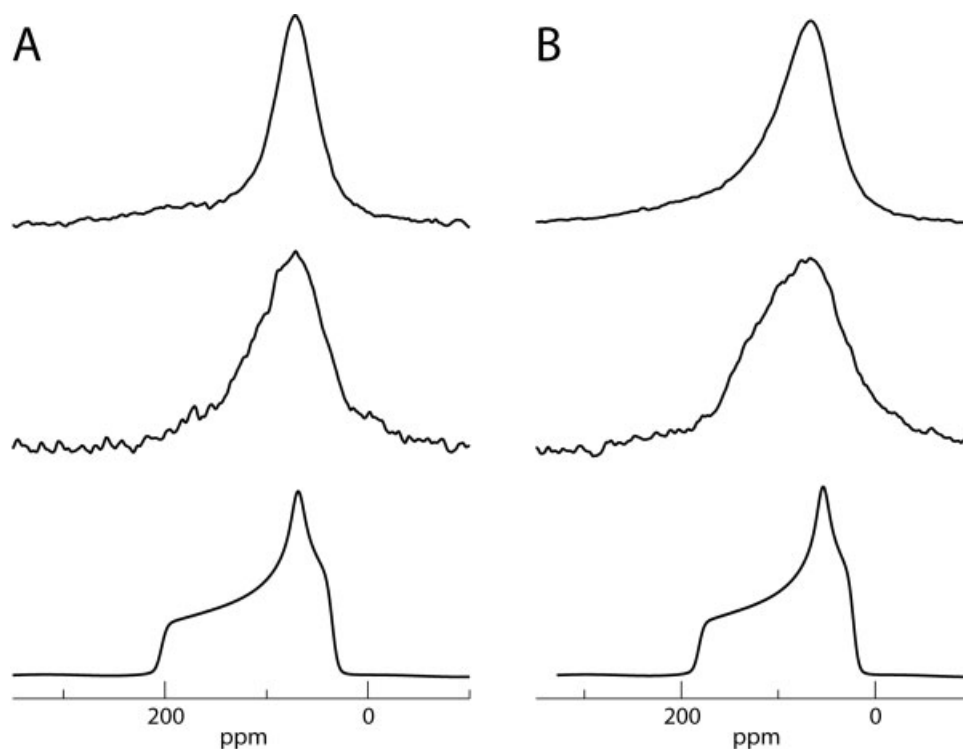


FIGURE 3 Nitrogen-15 NMR spectra of oriented dragline silk from *N. edulis* (top) and *A. keyserlingi* (middle) spiders, fed on (A) enriched ^{15}N -Ala or (B) ^{15}N -Gly amino acid. Fibers aligned with the fiber axis parallel (0°) to the magnetic field. Simulated powder spectra for ^{15}N -Ala (bottom, A) and ^{15}N -Gly (bottom, B) silk are shown for comparison. Nitrogen-15 CSA tensor elements used in the simulations were as reported by Asakura et al.²³

width of the distribution would depend on the extrusion speed, as does the mechanical strength of the fiber.⁴⁰ In our model such variations in domain alignment would contribute to spectral intensity in the region of the Gaussian with statistical emphasis toward higher frequencies and appear to make a small contribution toward the unassigned spectral intensity.

Carbon-13 spectra of aligned $[1-^{13}\text{C}]$ -Ala-enriched *Nephila* dragline silk (Figure 2) show high orientational order and are similar to those of aligned $[1-^{13}\text{C}]$ -Ala *S.c. ricini* silkworm silk.^{23,34} On the other hand, the ^{13}C spectra of Gly-enriched *Nephila* silk are less ordered and resemble more closely those of oriented $[1-^{13}\text{C}]$ -Gly *B. mori* and *S.c. ricini* silkworm fibers.^{21,23,34} One noticeable difference is the higher degree of enrichment that the spider silk appears to have compared with the silkworm silk^{19,33}—the aliphatic peak in our studies has significantly less intensity than the carbonyl peak in the ^{13}C -Gly-enriched spectra (Figure 2). The apparent degree of label incorporation is different for $1-^{13}\text{C}$ alanine and $1-^{13}\text{C}$ glycine (Table I) as the aliphatic-to-carbonyl intensity is approximately twice as large in the former case. This is likely to arise from metabolic

migration of label from 1-C into overlapping spectroscopic positions 2-C and 3-C for alanine and at the same rate from glycine 1-C into position 2-C only.

Metabolic migration of ^{13}C away from 1-C can take place during bioincorporation of alanine and glycine, as both amino acids are nonessential. An important biosynthetic and degradation pathway for alanine involves interconversion into pyruvate and subsequently to oxaloacetate.⁴¹ The important metabolic role and ubiquity of pyruvate allow a route for isotopic migration from 1-C to positions 2-C and 3-C. In the case of glycine biosynthesis, serine is the precursor, with the main catabolic pathway leading to CO_2 , NH_4 , and water. Despite the ubiquity of pyruvate, there is no major metabolic crossover between the alanine and glycine ^{13}C pathways.

Migration of $1-^{13}\text{C}$ labels between alanine and glycine cannot be excluded, however. The relative intensity in the carbonyl to aliphatic regions in the ^{13}C spectra from ^{13}C -Ala and ^{13}C -Gly silk (90–240 and 0–60 ppm, respectively, in Figure 2) provides some indication of the metabolic crossover between the two pathways. Complete metabolic leakage would provide equal populations of all alanine and glycine aliphatic

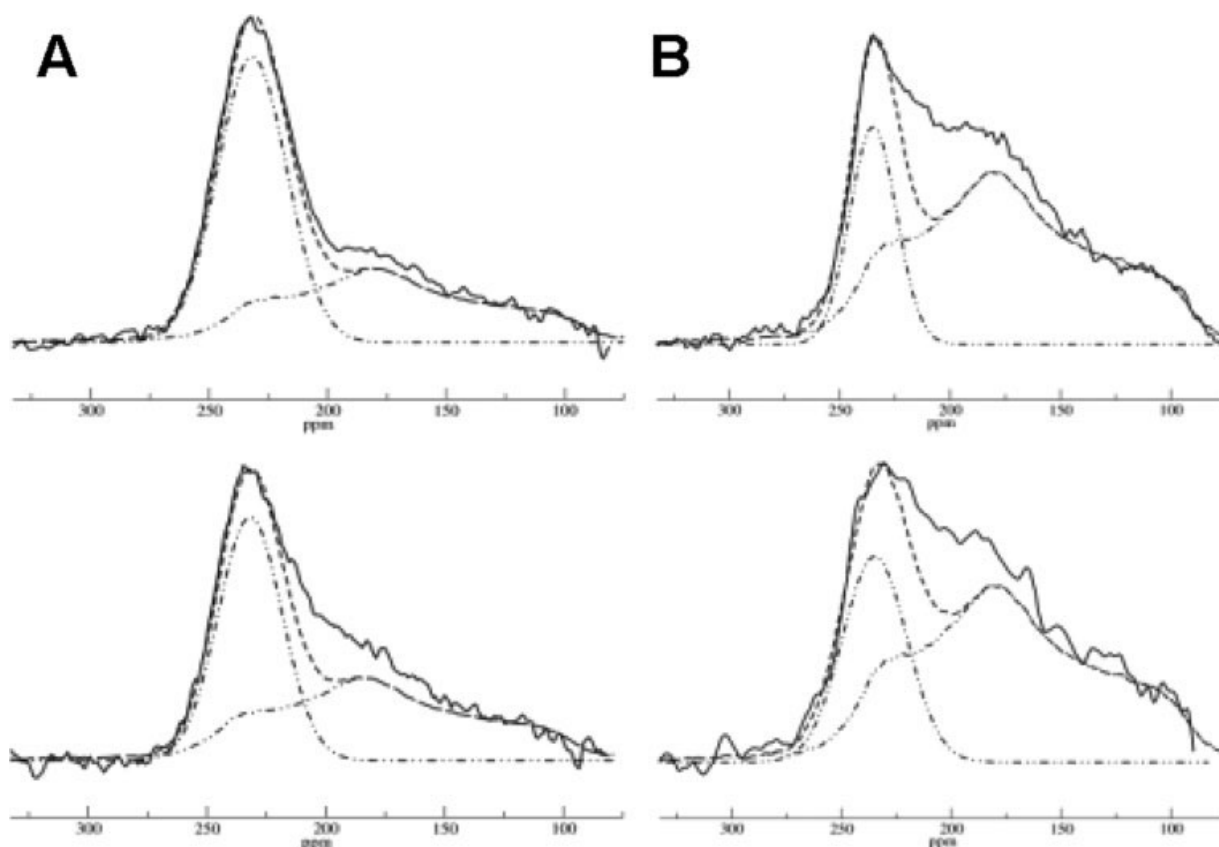


FIGURE 4 Carbonyl ^{13}C NMR spectra (solid lines) of oriented dragline silk from *N. edulis* (top) and *A. keyserlingi* (bottom) spiders, fed on (A) enriched $[1-^{13}\text{C}]$ -Ala or (B) $[1-^{13}\text{C}]$ -Gly amino acid, aligned with the fiber axis parallel (0°) to the magnetic field. Experimental powder spectra and simulated Gaussians (both dash-dot) are shown together with their sum (dash). Details of the simulations are described in the text and in Table I.

sites regardless of the amino acid supplement and comparable ratios between 1-C and 2-C/3-C intensities. Isotopic migration from 1-C within the individual pathways populates 2-C and 3-C for alanine but 2-C only for glycine. Our results showed approximately twice as

high 1-C/(2-C + 3-C) ratio for alanine compared with the 1-C/2-C ratio for glycine (Table I) and indicate a relatively low cross-amino acid migration. This allows us to interpret the distinct differences between the spectral fractions from oriented material in alanine

Table I Parameters and Gaussian Line Widths for the Simulated ^{13}C Spectra shown in Figure 4^a

Spectral Component	Alanine <i>A. keyserlingi</i>	Alanine <i>N. edulis</i>	Glycine <i>A. keyserlingi</i>	Glycine <i>N. edulis</i>
Powder (%)	45	40	65	65
Gaussian (%)	43	55	27	21
Simulation (%)	88	95	92	86
δ (ppm) ^b	19	21	21	15
C_α intensity (%) ^c	38	38	27	17

^a For each experimental spectrum, the carbonyl resonance has been normalized to 100% and the intensity is given as a fraction of the experimental spectrum obtained with the silk fiber axis aligned parallel to the magnetic field. Although the percent contribution of each spectral component has been calculated, the large background signal from the natural abundance ^{13}C allows only qualitative comparison of the amount of oriented material.

^b The full width at half height of Gaussian is $2\delta\sqrt{\ln 2} \approx 1.67\delta$.

^c Fraction of the total carbonyl spectral intensity.

and glycine-fed animals as contributions from conservatively metabolized 1-C in both amino acids.

Van Beek et al.¹¹ report migration of ¹³C from position 1-C in alanine to 1-C in glycine at levels lower than 20%. We estimate internal label migration of approximately 20% from 1-C into each aliphatic site (Table I), the higher value for alanine resulting from migration into 2-C and 3-C. Since direct 1-C to 1-C metabolic contacts between alanine and glycine do not exist⁴¹ any 1-C to 1-C migration between amino acids would occur at lower rates. This is not the case for ¹⁵N, which can exchange between alanine and glycine during alanine deamination in a branch reaction of the citric acid cycle.⁴¹ Notwithstanding this label exchange, the alanine and glycine ¹⁵N spectra (Figure 3) show noticeable differences. However, because of this migration we focused our quantitative analysis on ¹³C spectra only.

In the case of ¹³C-Ala-labeled silk, 55% of the spectral intensity appears to originate from ordered material in β -sheets parallel to the fiber axis while for ¹³C-Gly about 21% is ordered (Table I). Nevertheless, despite any migration of labels and natural abundance background signal in our spectra contributing to the nonaligned component, qualitatively it does appear that there are differences in the oriented components in Ala and Gly between spider species. Our results can be compared to Jelinski and coworkers who found using solid-state NMR that in *N. clavipes* dragline about 40% of the alanine is highly ordered and 60% less ordered¹ while 70% of glycine is in the amorphous phase.¹⁰

Akin to the results from *Nephila*, the ¹³C NMR spectra from oriented *Argiope* dragline also reveal differential partitioning of Ala and Gly into the oriented fiber components. The oriented spectral contribution from [1-¹³C]-Ala is approximately 43% compared with 27% from [1-¹³C]-Gly. In comparison to the *Nephila* results, the alanine fraction in the oriented domains of *Argiope* dragline is lower, while that of glycine is higher (Table I). Within this simple two-component model of the fiber,¹ we can interpret the greater contribution from oriented alanine in *Nephila* dragline (55%) in comparison to *Argiope* (43%) as indicative of a greater proportion of crystalline domains in the former with a corresponding higher tensile strength and lower extensibility. The fraction of the amorphous, glycine-rich component is greater in *Argiope* dragline (27 versus 21% for *Nephila*), which would aid extensibility at lower ultimate tensile strength. Therefore, it can be speculated that the work function associated with mechanical failure of *Nephila* dragline⁴⁰ would be inferior to that of *Argiope* dragline as the expected total strain at the

point of failure of the latter would be greater. The results of recent work support this supposition by way of stress-strain curves that show significant differences between silk fibers from *Nephila* and *Argiope* with the average peak load stress and Young's modulus higher for the latter.⁴²

Electron micrographs of spider dragline cross-sections reveal heterogeneous morphologies,³⁹ which is likely to reflect a range of distinct local microenvironments. The distribution of such environments, as well as the axial degree of microdomain alignment, would be expected to vary as a function of extrusion speed, exposure to moisture and solvents, fiber strength, and between species. Surprisingly, we did not observe any significant correlation between species and the spectral dispersion, δ (Table I), for either amino acid. This may indicate that the ratio between ordered and disordered dragline fractions may be a key determinant of the mechanical properties of the fibers. While the contribution of local and angular variations toward the observed frequency distribution within the oriented component cannot be quantified by our study, from the value of δ a potential maximum contribution to the width of the Gaussian was estimated to arise from 15° mean spread due to variations in structure or environment.

Although metabolic migration of ¹³C takes place during incorporation of [1-¹³C]-alanine and [1-¹³C]-glycine into the dragline silk of *Nephila* and *Argiope*, as noted above (Figure 2), the ¹³C spectra indicate a relatively low cross-amino acid migration. On the other hand, ¹⁵N is readily transferred from alanine to glycine and to other amino acids during deamination of alanine to pyruvate so that quantitative interpretation of ¹⁵N spectra is more problematic. Wideline ¹⁵N NMR spectra from *Nephila* and *Argiope* draglines oriented along the external magnetic field are shown in Figure 3. While in this case spectral deconvolution and quantitative analysis are more complex than in the interpretation of ¹³C-carbonyl spectra, most likely as a result of a higher probability for ¹⁵N migration as already seen with *N. clavipes*,¹⁷ the presence of a spectral component from the oriented material can be identified clearly. The higher degree of orientational order in ¹⁵N-alanine than in ¹⁵N-glycine parallels the ¹³C-carbonyl results. In addition, the oriented material in the *Nephila* dragline appears to follow a narrower orientational distribution than that of *Argiope* dragline.

CONCLUSION

In our earlier NMR study,³² different spider silks were compared to determine the relationship between

the function of the silk to its structural and dynamic properties. NMR relaxation times of protons and carbons indicated that different types of silks have different molecular motion on both slower (kHz) and faster (MHz) timescales. Both the *Argiope* and *Nephila* silks showed different relaxation properties between dragline and cocoon silk with dragline relaxation times generally longer than cocoon. Relaxation times can distinguish between more mobile, flexible regions and crystalline, more durable, but less flexible areas. The generally shorter relaxation times of Gly compared with Ala groups support the model of silk in which Gly-rich blocks in silk constitute the amorphous, elastic regions and Ala-rich blocks dominate the more crystalline, structured areas. The more durable *Nephila* silk reveals higher crystalline content than the more fragile *Argiope* silk. X-ray diffraction results also suggest that *Argiope argentata* dragline silk contains a large amount of material with short-range order or noncrystalline components.⁹ Supporting data from positron annihilation spectroscopy, which gives quantitative information about the number and dimensions of free-volume sites, or amorphous regions,^{43,44} indicated that the *Nephila* silk was more crystalline than *Argiope* silk (A. Hill, personal communication). We have previously shown that diet can vary the amino acid content of *Argiope* dragline silk⁴⁵ and this can show great inter- and intraspecies variation.^{2,26,45} Significant mechanical differences are observed in *N. clavipes* dragline when spiders are fed diets deprived of Ala and Gly.⁴⁶

Nephila appears to be more ordered than *Argiope* dragline silk when considering the ¹⁵N-Ala spectra. The ¹⁵N NMR data suffers from less background signal than ¹³C and the alanine is more concentrated in the crystalline regions of dragline silk.¹ The more durable *Nephila* silk appears to possess a greater amount of crystalline β -sheet region than the more fragile *Argiope* dragline silk. Proton and carbon NMR relaxation times have shown differences in molecular mobility between types of silk (dragline versus cocoon) and between species (*Nephila* versus *Argiope*) and may reflect the relationship between molecular structure and biological function.³² Our new ¹³C and ¹⁵N NMR results using silk, derived from spiders maintained on labeled Gly and Ala amino acid diets, have revealed further differences in the crystalline, amorphous, and oriented amorphous phases of dragline silk between species, which may correlate with the different physical properties of the materials.

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