WISE NMR Characterization of Nanoscale Heterogeneity and Mobility in Supercontracted *Nephila clavipes* Spider Dragline Silk

Gregory P. Holland,*,[†,§] Randolph V. Lewis,[‡] and Jeff L. Yarger[†]

Contribution from Department of Chemistry and Department of Molecular Biology, University of Wyoming, Laramie, Wyoming 82071

Received December 23, 2003; E-mail: gpholla@sandia.gov

Abstract: The addition of water to spider dragline silk results in fiber contraction to 50% its initial length and significant changes to the mechanical properties of the silk. This event has been termed supercontraction. A decrease in strength and increase in elasticity have been reported when the silk is in contact with water. Two-dimensional wide-line separation (WISE) nuclear magnetic resonance (NMR) is implemented to correlate ^{13}C chemical shifts with mobility by observing the corresponding ^1H line widths and line shapes in water-saturated spider dragline silk. The WISE NMR spectrum of the native silk exhibits ^1H line widths that are ~40 kHz for all carbon environments characteristic of a rigid organic system. In contrast, the water-saturated case displays a component of the ^1H line that is narrowed to ~5 kHz for the glycine C_α and a newly resolved alanine helical environment while the alanine C_β corresponding to the β-sheet conformation remains broad. These results indicate that water permeates the amorphous, glycine-rich matrix and not the crystalline, polyalanine β-sheets. A delay time is added to the WISE NMR pulse sequence to monitor spin diffusion between the amorphous, mobile region and the crystalline domains. The time required for spin diffusion to reach spatial equilibrium is related to the length scale of the polyalanine crystallites. This technique is employed to measure crystalline domain sizes on the nanometer length scale in water-solvated spider dragline silk. These results provide further insight into the structure of spider silk and mechanism of supercontraction.

Introduction

Scientists have coveted spider silk for years because of its exceptional mechanical properties combining strength, elasticity, and toughness that surpass almost all synthetically produced fibers.[1,2] Significant advances have appeared recently in the literature regarding the production and spinning of artificial spider silk making industrial application a real possibility.[3-5] However, the molecular origin of the silk's superior mechanical properties is still not completely understood. Of particular interest is the supercontraction process that occurs when the silk is exposed to water. The mechanism and molecular structural rearrangement that occurs to aid this phenomenon remains a mystery.

It has been postulated by many that the unique mechanical properties of spider silk are related to its molecular structure.[6-10] The spider silk most commonly studied is the dragline silk that

---

† Department of Chemistry.

‡ Department of Molecular Biology.

§ Present address: Sandia National Laboratories, Albuquerque, NM 87185-1411.

(1) Gosline, J. M.; Denny, M. W.; DeMont, M. E. *Nature* **1984**, *309*, 551.
(2) Gosline, J. M.; Denny, M. W.; DeMont, M. E. *Endeavor* **1986**, *10*, 7.
(3) Vollrath, F.; Knight, D. P. *Nature* **2001**, *410*, 541.
(4) Lazaris, A.; Arcidiacono, S.; Huang, Y.; Zhou, J.-F.; Duguay, F.; Chretien, N.; Welsh, E. A.; Soares, J. W.; Karatzas, C. N. *Science* **2002**, *295*, 472.
(5) Jin, H.-J.; Kaplan, D. L. *Nature* **2003**, *424*, 1057.
(6) Hayashi, C. Y.; Shipley, N. H.; Lewis, R. V. *Int. J. Biol. Macromol.* **1999**, *24*, 271.
(7) Hinman, M. B.; Lewis, R. V. *J. Biol. Chem.* **1992**, *267*, 19320.

A. **MaSp1** GGAGQGGYGGLGGQGAGRGGLGGQGAG<u>AAAAAAAA</u>

MaSp2 (GPGGYGPGQQ)₂GPSGGPGS<u>AAAAAAAAAAAA</u>

B.
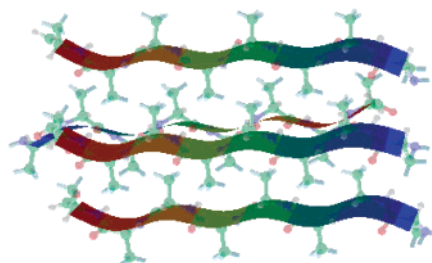
Figure 1. Consensus amino acid repeats (A) and polyalanine β-sheet conformation (B).

is collected from the major ampullate gland. It consists of two distinct proteins, major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2) (see Figure 1A), and is best described as a semicrystalline, biopolymer where crystallites composed of polyalanine β-sheets (see Figure 1B) are embedded in a glycine-rich, amorphous matrix.[11-14] The glycine-rich

---

(8) Yang, Z.; Liivak, O.; Seidel, A.; LaVerde, G.; Zax, D. B.; Jelinski, L. W. *J. Am. Chem. Soc.* **2000**, *122*, 9019.
(9) Termonia, Y. *Macromolecules* **1994**, *27*, 7378.
(10) Becker, N.; Oroudjev, E.; Mutz, S.; Cleveland, J. P.; Hansma, P. K.; Hayashi, C. Y.; Makarov, D. E.; Hansma, H. G. *Nat. Mater.* **2003**, *2*, 278.
(11) Lewis, R. V. *Acc. Chem. Res.* **1992**, *25*, 395.
(12) Simmons, A.; Ray, E.; Jelinski, L. W. *Macromolecules* **1994**, *27*, 5235.

region, although amorphous by X-ray diffraction (XRD), was recently shown with nuclear magnetic resonance (NMR) techniques to have a preferred secondary structure where the chains orient predominantly along the fiber axis with an approximate 3-fold helical conformation.[15,16]

Exposure to water greatly affects the dragline silk causing the fiber to shrink 50% in length and swell in diameter.[17,18] This event has been termed supercontraction and results in rubber-like mechanical properties with an increase in elasticity and a decrease in strength and stiffness.[1,18,19] Raman spectra on supercontracted single fibers of *Araneus diadematus* dragline silk indicate an increase in the random coil conformation with a corresponding decrease in the presence of β-sheets.[19] This could possibly explain the observed increase in elasticity and decrease in strength, but there is no evidence of this occurring in *Nephila clavipes* silk. Jelinski et al. implemented ^{13}C cross-polarization magic angle spinning (CP-MAS) and ^{2}H NMR spectroscopy to show that a substantial fraction of the glycine, glutamine, tyrosine, serine, and leucine environments exhibit an increase in molecular motion when *N. clavipes* silk is in contact with water while the polyalanine, β-sheet region displayed no change in dynamics.[8] The latter is consistent with a prior study on *N. edulis* dragline where the presence of water had little affect on the β-sheet regions.[20]

The morphology and size of the β-sheet crystallites presumably influence the mechanical properties of the silk. There is a large discrepancy in the literature regarding the actual crystallite domain size in both the native and supercontracted states. Transmission electron microscopy (TEM) methods[21–23] measure crystalline domain sizes on the order of 70–500 nm while wide-angle X-ray diffraction (WAXD) techniques[24,25] estimate significantly smaller crystallites having dimensions that are $2 \times 5 \times 7$ nm. This discrepancy between XRD and TEM results has been explained as due to a nonperiodic lattice (NPL) where the larger domains observed with TEM are not completely ordered but are made up of the smaller, well-ordered polyalanine crystallites that are prevalent from XRD.[23,26,27] Molecular models favor the smaller crystallites however; additional experimental evidence is still required.[9]

Wide-line separation (WISE) NMR[28,29] is an excellent tool for correlating structure and mobility in polymers and has also been effectively applied to heterogeneous, biopolymeric systems.[30] Domain sizes and the degree of heterogeneity can be
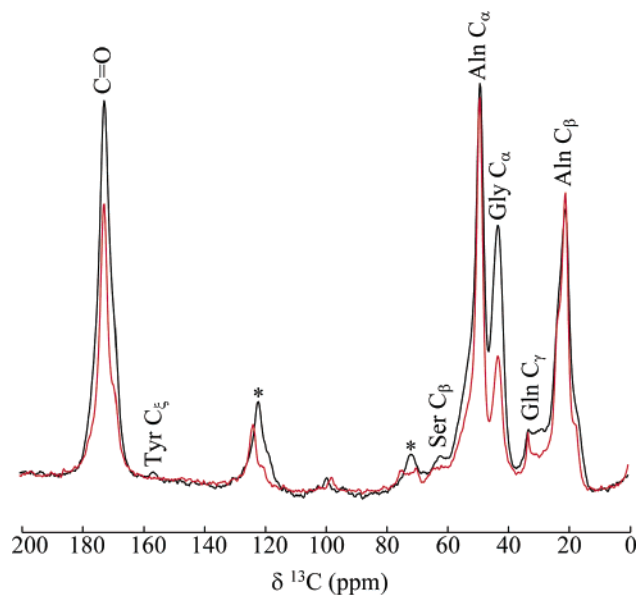
Figure 2. ^{13}C CP-MAS NMR spectra of dry (black) and wet, supercontracted (red) *N. clavipes* spider dragline silk. Spinning side bands are assigned an asterisk. The peaks representing different amino acids are indicated in the figure.

measured with the WISE method by monitoring ^{1}H spin diffusion between rigid and mobile regions. WISE is employed in this study to determine the structural and dynamic effects water has on spider dragline silk. Spin diffusion is coupled with the WISE technique to measure the size of the polyalanine, crystalline domains in water-saturated, supercontracted dragline silk.

Experimental Section

Silk Samples. *N. clavipes* dragline silk was ^{13}C labeled by mechanically silking the spider to remove stored silk proteins. The spiders were then fed the labeled amino acid in water. This process was repeated three times. For silk collected on the third silking, the ^{13}C enrichment is approximately 85%. Silk samples were exposed to D_2O to ensure that none of the observed ^{1}H WISE NMR signals originated from the water but rather ^{1}H environments on the chains.

NMR. Solid state [1]H → [13]C CP-MAS and WISE two-dimensional NMR experiments were performed on a 400 MHz NMR spectrometer equipped with a Tecmag Discovery Console and a Bruker 4-mm CP-MAS probe tuned to 400.1 and 100.3 MHz for the ^{1}H and ^{13}C channels, respectively. A MAS spinning speed of 5 kHz was applied in all experiments. The ^{13}C chemical shifts were referenced externally to TMS by setting the chemical shift of the methylene carbon resonance in adamantane to 38.6 ppm. Resonance assignments of the different amino acids in dragline silk were based on prior studies.[8,12,31] The increment time, t_1, was set to 5 μs for the two-dimensional experiments corresponding to a 200 kHz ^{1}H sweep width. The contact time was 1 ms for CP and WISE experiments and 300 μs for the WISE experiments with spin diffusion. The spin-diffusion mixing time, t_m, was varied between 1 and 150 ms. A recycle delay of 5 s was implemented in all experiments.

Results and Discussion

^{13}C CP-MAS NMR spectra of dry and wet *N. clavipes* dragline silk are presented in Figure 2. In the spectrum of the wet dragline some of the ^{13}C resonances display a decrease in intensity compared to the dry, untreated silk. This observation

(13) Warner, S. B.; Polk, M.; Jacob, K. *Macromol. Chem. Phys.* **1999**, *C39*, 643.
(14) Bram, A.; Bränden, C. I.; Craig, C.; Snigireva, I.; Riekel, C. *J. Appl. Crystallogr.* **1997**, *30*, 390.
(15) van Beek, J. B.; Hess, S.; Vollrath, F.; Meier, B. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10266.
(16) Kümmerlen, J.; van Beek, J. B.; Vollrath, F.; Meier, B. H. *Macromolecules* **1996**, *29*, 2920.
(17) Work, R. W. *Text. Res. J.* **1977**, *47*, 650.
(18) Work, R. W. *J. Exp. Biol.* **1985**, *118*, 379.
(19) Shao, Z.; Young, R. J.; Vollrath, F. *Int. J. Biol. Macromol.* **1999**, *24*, 295.
(20) van Beek, J. B.; Kümmerlen, J.; Vollrath, F.; Meier, B. H. *Int. J. Biol. Macromol.* **1999**, *24*, 173.
(21) Thiel, B. L.; Kunkel, D. D.; Viney, C. *Biopolymers* **1994**, *34*, 1089.
(22) Frische, S.; Maunsbach, A. B.; Vollrath, F. *J. Mirosc.* **1998**, *189*, 64.
(23) Thiel, B. L.; Guess, K. B.; Viney, C. *Biopolymers* **1997**, *41*, 703.
(24) Yang, Z.; Grubb, D. T.; Jelinski, L. W. *Macromolecules* **1997**, *30*, 8254.
(25) Grubb, D. T.; Jelinski, L. W. *Macromolecules* **1997**, *30*, 2860.
(26) Thiel, B. L.; Viney, C. *MRS Bull.* **1995**, *20*, 52.
(27) Barghout, J. Y. J.; Thiel, B. L.; Viney, C. *Int. J. Biol. Macromol.* **1999**, *24*, 211.
(28) Schmidt-Rohr, K.; Clauss, J.; Spiess, H. W. *Macromolecules* **1992**, *25*, 3273.
(29) Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic Press: London, 1994.

(30) Yan, B.; Stark, R. E. *Macromolecules* **1998**, *31*, 2600.
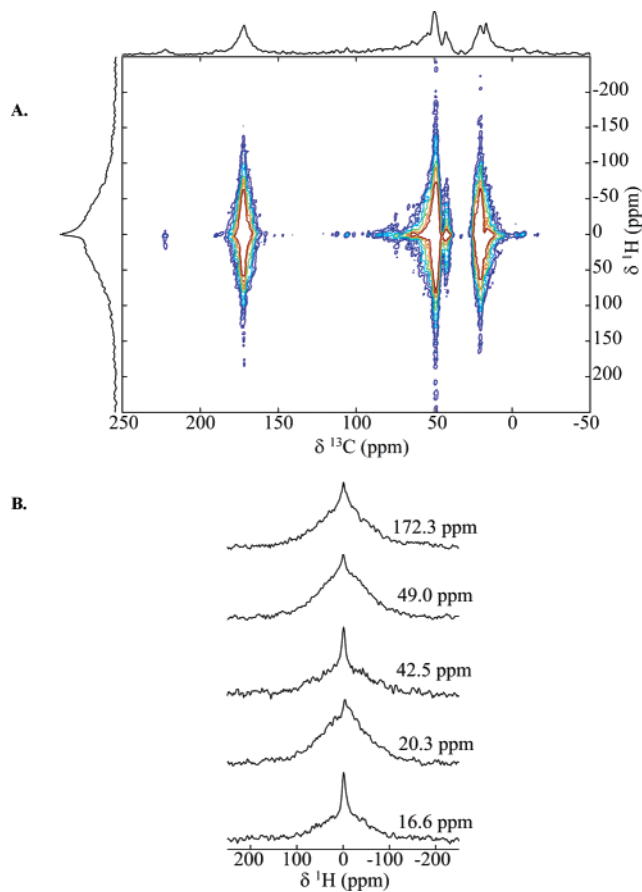(31) Saitô, H. *Magn. Reson. Chem.* **1986**, *24*, 835.
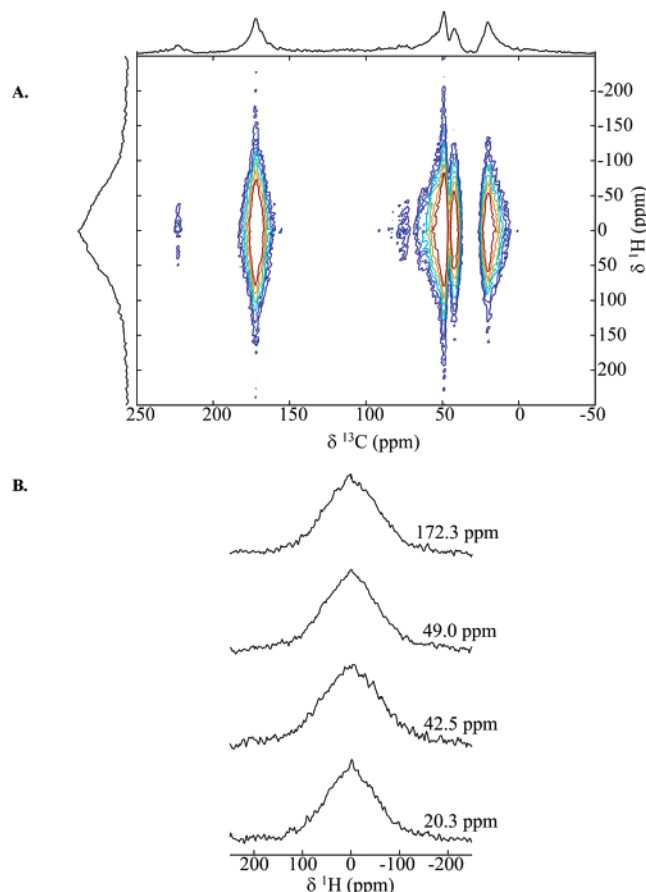
Figure 3. WISE NMR spectrum of untreated *N. clavipes* spider dragline silk (A) and ^{1}H slices at specified ^{13}C chemical shifts (B).

Figure 4. WISE NMR spectrum of wet, supercontracted *N. clavipes* spider dragline silk (A) and ^{1}H slices at specified ^{13}C chemical shifts (B).

is consistent with a previous study where the decreased intensity of the ^{13}C CP-MAS spectrum of wet dragline silk was attributed to an increase in chain dynamics.[8] The [1]H−[13]C dipolar coupling that facilitates the cross-polarization process is averaged due to an increase in chain mobility perceived to occur in the regions that accommodate water. The peaks corresponding to the polyalanine β-sheets at 20.3 ppm (C_β) and 49.0 ppm (C_α) show no change in intensity indicating that water does not penetrate the crystalline, polyalanine domains. Peaks representing glycine (Gly), glutamine (Gln), serine (Ser), and tyrosine (Tyr) environments all display a decrease in intensity, indicating an increase in chain motion that is caused by the presence of water.

WISE two-dimensional experiments were performed to correlate structural information from the ^{13}C chemical shift with mobility by indirectly observing the corresponding ^{1}H line widths. The WISE NMR contour spectrum of dry dragline silk is depicted in Figure 3A along with the ^{1}H slices at specified ^{13}C chemical shifts (Figure 3B). All the ^{13}C environments display very similar ^{1}H lines that are about 40 kHz broad. This is characteristic of a rigid organic system and indicates that there is no chain motion occurring on the time scale of the NMR experiment in the dry dragline silk.

The wet dragline silk displays a very different WISE spectrum than the dry dragline, as demonstrated in Figure 4A. The resonance corresponding to the alanine C_β environment in the[13]C projection displays two distinct resonances at 20.3 and 16.6 ppm. This contrasts with the ^{13}C projection in the WISE NMR spectrum of the dry silk where there is only one distinct ^{13}C resonance at 20.3 ppm corresponding to the polyalanine β-sheet

Table 1. Alanine [13]C Chemical Shifts and Conformation

	dragline	fibroin	silk I	α-helix	3_1-helix	β-sheet
C_β	20.3	16.6	16.6	14.9	17.4	19.9
C_α	49.0	50.3	50.5	52.4	48.7	48.2
C=O	172.3	175.5	177.1	176.4	173.2	171.8

conformation as assigned previously by comparison with ^{13}C chemical shifts observed for model proteins (see Table 1).[31,32] Two alanine components were observed previously in the ^{2}H NMR spectrum of wet, supercontracted *N. clavipes* dragline silk. However, previous NMR studies have never resolved two distinct resonances in the ^{13}C spectrum of the native or supercontracted dragline.[33] Further evidence of two distinct alanine environments comes from ^{13}C NMR relaxation measurements where the C_β resonance of alanine at ~20 ppm required a fit to two distinct spin−lattice (T_1) relaxation times indicating a rigid and mobile alanine region.[12] This is consistent with the observations made previously in the ^{2}H NMR spectrum of wet dragline which clearly displayed a mobile and rigid alanine fraction.[33]

In this study a distinct ^{13}C resonance is resolved at 16.6 ppm for the first time in the ^{13}C projection of the WISE NMR spectrum of the wet, supercontracted silk. This chemical shift is identical to that observed in the major ampullate silk fibroin for the C_β alanine resonance where no indication of the ~20.0 ppm resonance corresponding to the polyalanine β-sheet con-

(32) Bax, A.; Spera, S. *J. Am. Chem. Soc.* **1991**, *113*, 5490.
(33) Simmons, A. H.; Michal, C. A.; Jelinski, L. W. *Science* **1996**, *271*, 84.

formation was observed.[34] This environment was assigned to a dynamic loose helical structure in silk gland fibroin and is also displays a comparable chemical shift to the silk worm Silk I protein (see Table 1).[35] Observation of this environment in the WISE NMR spectrum of the wet, supercontracted dragline silk indicates a reintroduction of this dynamic loose helical alanine environment when the silk is in contact with water. The ^{1}H slice taken at this 16.6 ppm ^{13}C resonance clearly displays a significant narrow component with a ∼5 kHz line width. This shows that this alanine environment becomes mobile in contrast with the broad ^{1}H slice taken at 20.3 ppm indicating that the polyalanine β-sheets remain rigid when in contact with water. This is consistent with a previous Raman spectroscopic investigation on wet, supercontracted *A. diadematus* silk where an increase in alanine helical conformations was observed.[19]

The only environment that shows no indication of a narrowed ^{1}H line width in the WISE NMR spectrum of the wet dragline silk is the polyalanine β-sheet conformation at a ^{13}C chemical shift of 20.3 ppm. This indicates that the water does not affect the crystalline β-sheet domains while the glycine C_α at 42.5 ppm clearly displays a narrowed ^{1}H line width. This shows that the water enters the glycine-rich amorphous region and not the crystalline polyalanine β-sheets. In addition, the ^{1}H slices of the C_α alanine and carbonyl environments at ^{13}C chemical shifts of 49.0 and 172.3 ppm, respectively, clearly show a small narrow component in the ^{1}H resonances. The carbonyl ^{13}C resonance represents all the carbonyl environments, and therefore, a combination of narrow and broad components is expected. The combination of mobile and rigid components displayed by the C_α alanine environment is probably due to convolution of the dynamic helical domain and the β-sheet conformation (see Table 1). The C_α of the alanine helical conformation is expected to occur at 50.3 ppm while the corresponding β-sheet environment at 49.0 ppm. These two environments are not resolved and appear to overlap in the WISE spectrum unlike the alanine C_β where there are clearly two resolved ^{13}C resonances at 20.3 and 16.6 ppm corresponding to the polyalanine β-sheet crystallites and a dynamic alanine helical environment, respectively.

The WISE NMR spectrum displayed in Figure 5 was collected on supercontracted silk that was allowed to dry for 2 weeks under ambient conditions. All the ^{13}C environments have corresponding ^{1}H components (Figure 5B) that have broad line widths equal to ∼40 kHz identical to the WISE spectrum of the native silk shown in Figure 3. This shows that the dynamics displayed in the WISE spectrum in Figure 4 only occur when the silk is water-saturated. This could explain the broad ^{1}H lines previously observed in the WISE NMR spectrum of supercontracted silk that was allowed to dry under nitrogen prior to the NMR measurements.[20] It should also be noted that the C_β resonance observed at 16.6 ppm and assigned to a dynamic helical conformation disappears when the silk is allowed to dry. These findings show the reversibility of the process. Additionally, the presence of this dynamic alanine helical environment in the wet silk could explain the decrease in stiffness and rubber-like mechanical properties observed for the supercontracted dragline silk.[1,18,19] This alanine environment probably represents
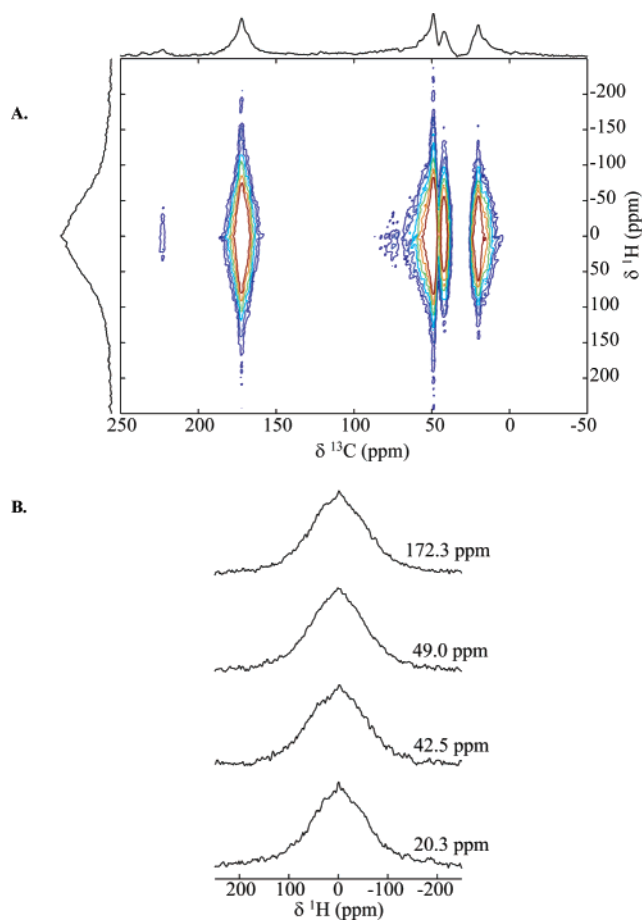
Figure 5. WISE NMR spectrum of *N. clavipes* spider dragline silk exposed to water and allowed to dry for 2 weeks (A) and ^{1}H slices at specified ^{13}C chemical shifts (B).

the interphase that links the glycine-rich, helical region with the polyalanine β-sheet crystallites.[36] Returning to the consensus protein sequence in Figure 1, the region that likely corresponds to this alanine environment is the GAG repeat that terminates the polyalanine runs. The chain mobility displayed by this environment (Figure 4B) could aid the crystallite rotation observed by XRD when the silk is exposed to water.[37] The results displayed in Figures 3−5 show that this alanine environment reversibly changes conformation from rigid, β-sheet to mobile, helical environment in the dry and water-saturated dragline silk, respectively. This conformational change could be the key driving force in the supercontraction process.

Spin diffusion was monitored between the mobile regions and the polyalanine β-sheet crystallites by including a mixing period in the WISE NMR pulse sequence. The ^{1}H line width of the alanine C_β representing the β-sheet conformation (20.3 ppm) was monitored as a function of τ_m as shown in Figure 6. The narrowing of the ^{1}H line widths with increasing mixing time is due to ^{1}H magnetization from the mobile region participating in the CP process as a result of spin diffusion. The τ_m where this process comes to spatial equilibrium is related to the length scale of the polyalanine crystalline domains. In Figure 6 the ^{1}H line widths are clearly broad for $\tau_m = 1$ ms (A), at 5 ms the line widths are beginning to narrow (B), at 20

(34) Hijirida, D. H.; Do, K. G.; Michal, C.; Wong, S.; Zax, D.; Jelinski, L. W. *Biophys. J.* **1996**, *71*, 3442.
(35) Asakura, T.; Watanabe, Y.; Uchida, A.; Minagawa, H. *Macromolecules* **1984**, *17*, 1075.

(36) Jelinski, L. W.; Blye, A.; Liivak, O.; Michal, C.; LaVerde, G.; Seidel, A.; Shah, N.; Yang, Z. *Int. J. Biol. Macromol.* **1999**, *24*, 197.
(37) Parkhe, A. D.; Seeley, S. K.; Gardner, K.; Thompson, L.; Lewis, R. V. *J. Mol. Recog.* **1997**, *10*, 1.
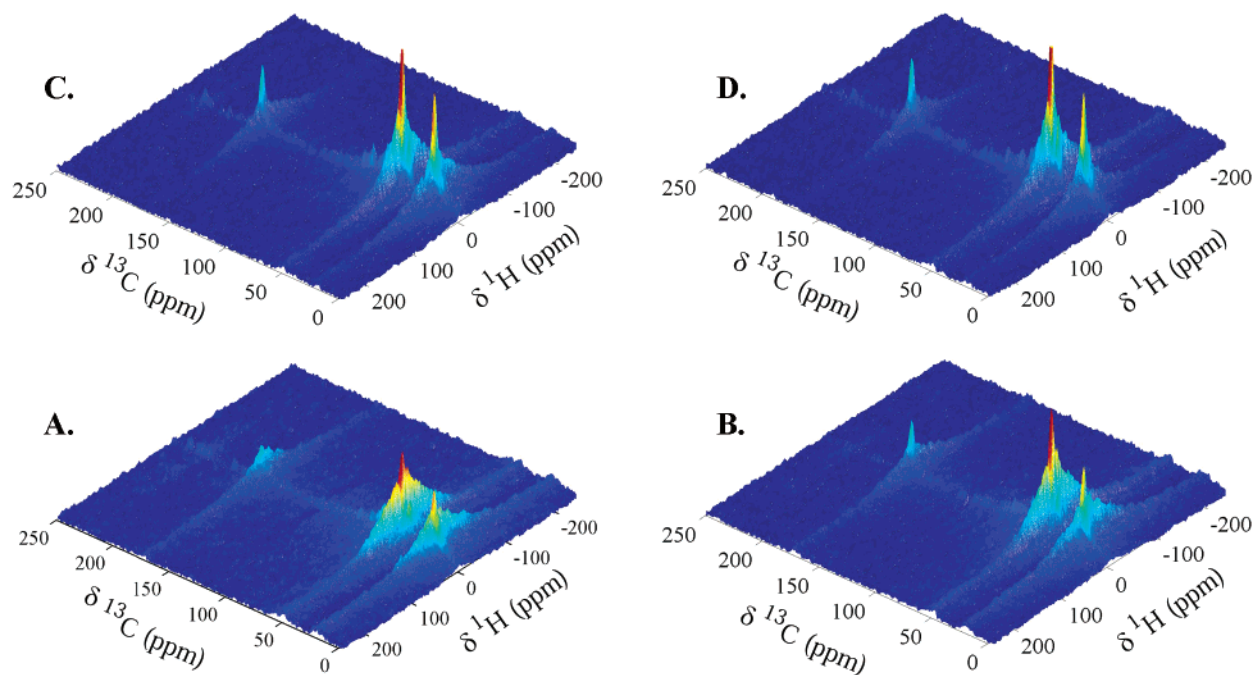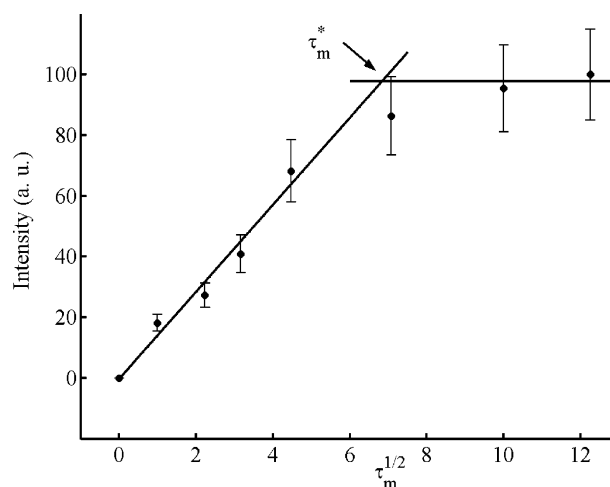
Figure 6. WISE NMR spectra of wet, supercontracted *N. clavipes* spider dragline silk with a spin-diffusion mixing period equal to 1 ms (A), 5 ms (B), 20 ms (C), and 50 ms (D).

ms the narrowing continues (C), and at 50 ms spatial equilibrium is reached (D). The mixing period was varied out to 150 ms to ensure that the spin-diffusion process was complete at 50 ms. Essentially no difference was observed in the silhouette of the WISE spectrum at $\tau_m = 50$ and 150 ms.

The average size of the dispersed polyalanine crystallites (d_{dis}) can be estimated from the following equation:

$$d_{dis} = \frac{\rho_A^H \phi_A + \rho_B^H \phi_B}{\phi_A \phi_B} \frac{\sqrt{D_A D_B}}{\sqrt{D_A}\rho_A^H + \sqrt{D_B}\rho_B^H} \frac{4\epsilon\phi_{dis}}{\sqrt{\pi}} \sqrt{\tau^*_m} \quad (1)$$

where ρ^H, ϕ, D, and ϵ are the proton density, volume fraction, spin-diffusion coefficient, and dimensionality, respectively.[38] The value τ^*_m is extracted by plotting the magnetization versus $\tau_m^{1/2}$ and extrapolating the initial spin-diffusion behavior to longer mixing times as shown if Figure 7. The intensity was defined as the fraction of mobile component in the ^{1}H line width of a slice taken at a ^{13}C chemical shift of 20.3 ppm (polyalanine β-sheet). The fraction of mobile component was extracted by fitting the ^{1}H resonance to two components, a broad (44 kHz) and narrow (5 kHz) component for the various mixing times. This plot yielded a $\tau^*_m = 45$ ms. The proton densities, volume fractions, and spin-diffusion coefficients implemented to calculate the domain size are listed in Table 2. The proton densities for the mobile glycine-rich region and the polyalanine crystallites were calculated from the density of glycine and density of polyalanine in β-sheets, respectively.[39] The volume fraction used for the polyalanine crystallites was based on an XRD study where ~15% of *N. clavipes* spider dragline silk was reported to be crystalline.[25] The spin-diffusion coefficients of the two regions were estimated from the ^{1}H line width ($\Delta v_{1/2}$) and average minimum proton distance (r_{HH}) with the following

(38) Clauss, J.; Schmidt-Rohr, K.; Spiess, H. W. *Acta Polym.* **1993**, *44*, 1.
(39) Gold, D. G.; Miller, W. G. *Polymer Data Handbook*; Oxford University Press: New York, 1999.

Figure 7. Spin-diffusion buildup of the narrow component in the ^{1}H slice taken at a ^{13}C chemical shift of 20.3 ppm (polyalanine β-sheet) in the WISE NMR spectra as a function of mixing time.

Table 2. Parameters for Calculating Domain Size from Spin Diffusion

	ρ^H (g/cm³)	ϕ	D (nm²/ms)
polyalanine β-sheet	0.107	0.15	0.9
glycine-rich region	0.078	0.85	0.1

proportionality as previously described:[38]

$$D \sim \Delta v_{1/2}(r_{HH})^2 \quad (2)$$

The spin-diffusion coefficient of PS-PMMA is accurately known to be 0.8 ± 0.2 nm²/ms with a ^{1}H line width of 38 kHz and an average proton minimum distance of 2.5 Å. This spin-diffusion coefficient can be employed with the proportionality in eq 2 to estimate the spin-diffusion coefficients for the mobile and rigid domains in spider dragline silk. Using this methodology, the spin-diffusion coefficient for the mobile, glycine-rich region and

the rigid polyalanine crystallites were estimated to be 0.1 ± 0.03 nm^2/ms and 0.9 ± 0.2 nm^2/ms, respectively.

An average polyalanine crystallite domain size of 6 ± 2 nm is calculated with eq 1. A dimensionality of 2 was used since the crystallite domains are best described as cylindrical in shape.[24,25] This value is in excellent agreement with WAXD experiments that measured crystallites having dimension of $2 \times 5 \times 7$ nm.[24,25] This shows that the polyalanine crystallites remain in tact upon wetting and supercontraction. The smaller domains measured in this study show that water enters the larger 70−500 nm domains observed by TEM.[21−23] This proves that these larger domains are in fact polycrystalline as previously proposed.

Conclusions

The broad ^{1}H line widths observed in the WISE NMR spectrum of the dry *N. clavipes* dragline silk show that the system is completely rigid on the time scale of the NMR experiment prior to hydration. In wet dragline the β-sheet domains remain rigid while significant chain motion occurs in the glycine-rich region and a newly resolved alanine helical environment. The mobile alanine section has an identical chemical shift to the silk fibroin and is assigned to a loose helical environment that probably links the crystalline and amorphous domains and could facilitate the crystallite reorientation observed by XRD. The appearance of this mobile alanine helical environment in the WISE spectrum of the wet silk could explain the increase in elasticity and decrease in stiffness observed for wet, supercontracted dragline silk. Furthermore, it was shown that this process is reversible since this resonance disappears when the silk is allowed to dry and all the ^{1}H line widths return to ∼40 kHz. This alanine environment is thought to represent the GAG repeat that terminates the polyalanine runs. The reversible conversion of this environment between β-sheet and helical conformation could provide the driving force for the supercontraction process. Finally, spin-diffusion experiments estimate average polyalanine crystalline domains that are 6 ± 2 nm in excellent agreement with the WAXD measurements on dry silk which shows that the crystallites remain in tact when the silk is in contact with water. The small crystallites measured here indicate that the water enters the larger domains observed by TEM, proving that these domains are in fact polycrystalline. These results provide convincing structural and dynamic evidence that may help explain the supercontraction process and changes in mechanical properties observed in wet dragline silk. Further work is needed, particularly in the area of molecular modeling, to obtain a complete quantitative understanding of the relationship between structural dynamics and the mechanical properties.