Understanding the Mechanical Properties and Structure Transition of Antheraea pernyi Silk Fiber Induced by Its Contraction

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1. INTRODUCTION

Animal silks are unusual materials compared to other fibrous materials not only because they are born with comprehensive mechanical properties as indicated by an exceptional combination of strength, extensibility, and toughness that are inapproachable by most biomimetic and man-made materials not only because they are born with comprehensive mechanical properties as indicated by an exceptional combination of strength, extensibility, and toughness that are inapproachable by most biomimetic and man-made materials but also because they are discovered to be equipped with other properties, etc. On the other hand, animal silks are usual materials to scientists because their unusual properties are inescapable by most biomimetic and man-made materials but also because they are discovered to be equipped with other properties, etc. On the other hand, animal silks are unusual materials compared to other fibrous materials not only because they are born with comprehensive mechanical properties as indicated by an exceptional combination of strength, extensibility, and toughness that are inapproachable by most biomimetic and man-made materials but also because they are discovered to be equipped with other properties, etc. On the other hand, animal silks are unusual materials compared to other fibrous materials not only because they are born with comprehensive mechanical properties as indicated by an exceptional combination of strength, extensibility, and toughness that are inapproachable by most biomimetic and man-made materials. Among the most studied silk categories, the Chinese wild silkworm Antheraea pernyi silk has been the focus of attention in recent years, mainly owing to its peculiar role played in the whole family of animal silks. Specifically, it is used to construct a cocoon, like domesticated commercial Bombyx mori silk. Its amino acid sequence, especially the motifs, however, very much resembles the major ampullate silk (dragline silk) of a spider, with repetitive poly(alanine) regions of (Ala)$_n$ where $n = 10−14$, embedded into a semiamorphous matrix that consists predominantly of glycine residues. Up to now, a variety of experimental techniques, such as FTIR spectroscopy, Raman spectroscopy, as well as solid-state NMR, have been employed to investigate the structure of A. pernyi silks or their analogues. Nevertheless, most of these studies are based on degummed silk fibers, which neglect the effect of water content and orientation of both secondary structural components in silkworm fiber keep changed, no matter if it is native or contracted. 13C CP/MAS NMR results further indicate that the α-helix/random coil to β-sheet conformational transition that occurred in the silk of silkworm A. pernyi corresponds the Ala residues. Based upon these results, the detailed structure transition models of both as-reeled A. pernyi silk fibers during water contraction are proposed finally to interpret their properties transformation.

ABSTRACT: Like most major ampullate silks of spider, the length of Antheraea pernyi silkworm silk can shrink to a certain degree when the fiber is in contact with water. However, what happens in terms of molecule chain level and how it correlates to the mechanical properties of the silk during its contraction is not yet fully understood. Here, we investigate the water-induced mechanical property changes as well as the structure transition of two kinds of A. pernyi silk fiber, which are forcibly reeled from two different individuals (silkworm a and silkworm b; the silk fiber from either one represents the lower and upper limit of the distribution of mechanical properties, respectively). The tensile test results present that most of the mechanical parameters except the post-yield modulus and breaking strain for both silk fibers have the same variation trend before and after their water contraction. Synchrotron FTIR and Raman spectra show that the native filament from silkworm a contains more α-helix structures than that in silkworm b filament, and these α-helices are partially converted to β-sheet structures after the contraction of the fibers, while the order of both β-sheet and α-helix slightly increase. On the other side, the content and orientation of both secondary structural components in silkworm b fiber keep unchanged, no matter if it is native or contracted. $^{13}$C CP/MAS NMR results further indicate that the α-helix/random coil to β-sheet conformational transition that occurred in the silk of silkworm a corresponds the Ala residues. Based upon these results, the detailed structure transition models of both as-reeled A. pernyi silk fibers during water contraction are proposed finally to interpret their properties transformation.
penetration during degumming or softening process on the properties and structure of native silk fibers.

When loosely immersed in water, spider dragline silk can shrink by up to about 50% of their stretched length, which is a phenomenon described as supercontraction.7 As a consequence, the dried-out supercontracted silk displays lower elastic modulus, breaking strength, and energy, but higher extensibility compared to the native silk, and the silk fiber comes back to a “reference state” that is mainly determined by its primary structure.53,12 Up to now, a number of studies have been conducted to understand supercontraction and the underlying mechanisms, and there is a consensus that supercontraction is a result of reorientation of both the aligned noncrystalline chains and the crystallites in the presence of polar solvents (usually water).13–36 B. mori silk, on the other hand, does not supercontract in water, and its mechanical properties remain unchanged after water treatment.37 Recently, Fu et al. found that forcibly reeled A. pernyi silk also can shrink when it is exposed to water, and the shrinkages are just around 7% or even lower.15 However, the detailed properties and structure transition of the A. pernyi silk during this process remains unclear. Additionally, though our recent work showed that the α-helix structures existed in forcibly reeled A. pernyi silk can be significantly decreased after water treatment,16 it is still an open question whether these disappeared α-helix structures are converted to β-sheet or random coil structure. On the other hand, forcibly reeled A. pernyi silks from different individuals display considerable structural variability,22 so it is interesting to find whether these different structures have varied responses to water treatment.

Based on these challenges, in the present work, the properties and structure transition of as-reeled A. pernyi silks from two different silkworms (these two kinds of silks have been found to represent the limits of the distribution of properties, which will be detailed in the Results and Discussion section) induced by water was investigated in detail. We first compared the mechanical properties of two as-reeled A. pernyi silks before and after water contraction. Then, the corresponding structure change was monitored by using synchrotron FTIR (S-FTIR), Raman spectroscopy combined with 13C CP/MAS NMR. Finally, the structure transition mechanisms of as-reeled A. pernyi silk fibers induced by water were suggested.

2. EXPERIMENTAL SECTION

2.1. Silk Fiber Preparation. Forcibly reeled silk fibers used here were all obtained in the same reeling speed (8 mm s−1),28 in laboratory conditions (20–25 °C, 40%–50% RH) from the mature A. pernyi silkworms, which previously lived on oak trees in a tussah field of Shandong province, China. The silks from the same silkworm were collected in the same spinning season on the same day. Forcibly reeling silks from the spinnneret of a mature A. pernyi silkworm is so convenient and controllable that it generates nearly uniform silks and extremely thin (100–200 nm) compared to the greater thickness of a single brin of A. pernyi silk (15–17 μm);12 (ii) the sericin coating remained intact after water treatment.32,35 With the normalization of cross-sectional area, the load–stress curve was converted to a stress–strain curve.

2.2. Cross-Sectional Area Measurement. To precisely measure the cross-sectional area of the native silk fiber, a length of silk fiber adhered onto our custom-built mold was sputtered with gold under 10 mA for 3 min, and then embedded in epoxy resin. After being dried in air overnight, the silk-embedded epoxy resin was fractured into six segments perpendicular to the silk fiber. The fracture cross section was sputtered with gold, and then observed by a Tescan 5136MM with secondary electrons at 10 kV. The gold layer between a silk fiber and resin effectively weakens the interface adhesion and gives an outline in the resin equal to the silk’s original cross section after the fiber was pulled out during the fracture. From this outline, we estimated the cross-sectional area of the silk fiber using the software Vega TC provided with the SEM. Usually, at least 5 well-defined cross-sectional images should be obtained from a silk fiber for area measurement. The average area was used as the cross-sectional area of adjacent as-reeled silk fibers. The area of the contracted silk fiber was calculated with the tested hypothesis that its volume was constant after contraction. We took no account of the effect of sericin coating on the cross-sectional area as well as the mechanical properties described below, because (i) the sericin coating on the fiber’s surface is uniform along the silk fiber and extremely thin (100–200 nm) compared to the greater thickness of a single brin of A. pernyi silk (15–17 μm);12 (ii) the sericin coating remained intact after water treatment.22,35 With the normalization of cross-sectional area, the load–stress curve was converted to a stress–strain curve.

2.3. Tensile Testing. Tensile tests were performed on Instron 5565, around 20–25 °C and 40–50% RH at a strain rate of 0.005 s−1. Before the test, the side support of the frame was simply cut away, so that the force was transmitted through the silk fiber. In loading–unloading tests, the sample was first loaded to a strain of 17% and immediately unloaded to zero strain. After 1 min recovery, the silk fiber was reloaded to its breakage. For the tensile test in water, a custom-built accessory,21 which can sustain the aqueous environment, was integrated into the Instron.

2.4. S-FTIR Microspectroscopy. The S-FTIR measurements were recorded at Beamline (BL01B) in the Shanghai Synchrotron Radiation Facility (SSRF). All the FTIR microspectra were acquired at 4 cm−1 resolution over the range of 4000–800 cm−1. A relatively large 20 × 20 μm square aperture was selected here due to the large width of flat-as-reeled A. pernyi silk (≥30 μm), and 256 scans were coadded. The square aperture was placed in the center of the fiber to avoid the possible diffraction and scattering of the infrared light. Before each test of silk fiber, a background spectrum was collected from an area free of sample. To minimize IR absorption by CO2 and water vapor in the ambient air, the sample was purged using dry N2 during measurement. For each silk sample (1 cm long), at least three different positions were analyzed, and if there is no variation in these three S-FTIR spectra, we took it as one effective datum. For the polarization experiment, a KRS-5 IR polarizer was inserted in the infrared beam to study the dichroism of certain absorption bands.

The data of all FTIR spectra were analyzed according to the method described in detail elsewhere.46 Briefly, the spectra were normalized using the band at about 1445–1455 cm−1 (variation along with the polarization angles) which is assigned to the CH3 in-plane bending vibration before data treatment. Deconvolution of amide III band (1300–1180 cm−1) was carried out using PeakFit v4.12. The number of peaks and their positions obtained from the second derivative spectra were fixed during the succeeding deconvolution process. A Gaussian model was selected for the band shape, and the bandwidth was automatically adjusted by the software. It should be pointed out that the data extracted from the deconvolutions (e.g., β-sheet content, etc.) were the average of more than five separate results from different positions and samples, although each spectrum shown in this paper was just from a single experiment.

The molecular order parameter (S2(θ)) of the corresponding secondary structural component was calculated as follows:44

\[ S_{2(θ)} = \frac{A_p(θ) - A_n(θ)}{A_p(θ) + 2A_n(θ)} \]
Nuclear Magnetic Resonance (\textsuperscript{13}C CP/MAS NMR) Measurement. Raman results on the silk during the measurement, which was indicated by the repeatable approximately 300 mW. There were no signs of structural damage 50 s using the 785 nm line of a semiconductor laser with energy of beam were collected. Spectra were obtained from ten acquisitions of parallel and perpendicular with to the vibration direction of the laser fiber samples.

2.5. Raman Spectroscopy. Polarized Raman spectra of as-reeled A. \textit{pernyi} silk in native and contracted (wet and dry) states were measured using a Renishaw inVia Reflex spectrometer coupled to a Lieca microscope. For each sample, both the spectra of fiber axis parallel and perpendicular with to the vibration direction of the laser beam were collected. Spectra were obtained from ten acquisitions of 50 s using the 785 nm line of a semiconductor laser with energy of approximately 300 mW. There were no signs of structural damage during the measurement, which was indicated by the repeatable Raman results on the silk fiber samples.

2.6. Carbon-13 Cross-Polarization Magic-Angle Spinning Nuclear Magnetic Resonance (\textsuperscript{13}C CP/MAS NMR) Measurement. The \textsuperscript{13}C CP/MAS NMR experiments were performed on a Bruker AVANCEIII spectrometer with a \textsuperscript{13}C frequency of 150.96 MHz. Commercial Bruker triple-resonance 4 mm MAS probes and 4 mm zirconia rotors were used for the experiments. The \textsuperscript{13}C chemical shifts were determined from the carbonyl carbon signal (\(\delta_{13C} = 176.5\) ppm) of glycine relative to tetramethylysilane (TMS). The spectra were recorded at 10 kHz spinning rate, the 90° pulse lengths were 4.0 ms. To obtain the sufficient silk samples for NMR test, mature A. \textit{pernyi} silkworm \(a\) was placed on a PET sheet so that it would spin a silk mat on the sheet in a natural "figure of eight", and the silk mat was removed from the plastic sheet carefully. For the water contracted samples, a certain number of mats were immersed in water for at least 24 h, then they were dried completely for NMR test.

3. RESULTS AND DISCUSSION

3.1. Tensile Performance of As-Reeled A. \textit{pernyi} Silks in Native and Contracted States. In our recent work, we found that as-reeled A. \textit{pernyi} silks from different silkworms (more than 10 silkworms) show significant variability of properties, and they can be grouped into four generic types of samples mainly according to their different tensile responses to methanol and ethanol.\textsuperscript{22} Later, the corresponding structure origin of variability of properties of these silks was studied in detail,\textsuperscript{22} and the results indicated that hydrogen bond stacking state in the amorphous regions and the overall ordered fraction are dramatically different for silks from different individuals. Here, silk fibers from two individuals (labeled artificially as silkworm \(a\) and silkworm \(b\)), which represent the lower limit (silk from silkworm \(a\)) and upper limit (silk from silkworm \(b\)) of the distribution of properties and then structures, were chosen to investigate their properties and structure changes induced by water.

The stress–strain curves of those as-reeled A. \textit{pernyi} silks before and after contraction in water are shown in Figure 1, and the detailed mechanical properties are illustrated in Table 1. In native state, the two kinds of silk fiber present prominently different mechanical properties. Specifically, silk fiber from silkworm \(b\) displays higher modulus, yield and breaking stress, resilience, and shrinkage, but lower extensibility than those from silkworm \(a\), which indicate noticeable variability of properties and are consistent with our previous observations.\textsuperscript{21,22} After being preimmersed in water for 24 h, similarly, the contracted silk fibers show largely decreased initial modulus, yield stress, breaking stress, and resilience compared to those in native state. However, the change of post-yield modulus and breaking strain are obviously different for these two kinds of silk fibers. For silks from silkworm \(a\), the post-yield modulus increases and the breaking strain decreases after contraction in water, but they are just the reverse for samples from silkworm \(b\). Undoubtedly, these significantly different property changes during water contraction derive from the intrinsic distinct structures of these two silk fibers (for example, the content of different secondary structures and the orientation of molecular chains), and they should have different responses to water, which is described and analyzed in the following section.

3.2. Structure Transition of As-Reeled A. \textit{pernyi} Silks with Water Treatment Monitored by S-FTIR and Raman Methods. The structure difference between these two as-reeled A. \textit{pernyi} silks and their structure change induced by

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**Table 1. Mechanical Properties of As-Reeled A. \textit{pernyi} Silks from Two Different Individuals in Native and Contracted States**\textsuperscript{a}

<table>
<thead>
<tr>
<th>samples</th>
<th>initial modulus (GPa)</th>
<th>post-yield modulus (GPa)</th>
<th>yield stress (MPa)</th>
<th>breaking stress (MPa)</th>
<th>breaking strain (%)</th>
<th>resilience (%)</th>
<th>shrinkage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>silkworm (a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>native ((n = 10))</td>
<td>10.4 ± 0.4</td>
<td>0.20 ± 0.04</td>
<td>203 ± 14</td>
<td>509 ± 48</td>
<td>69.6 ± 5.2</td>
<td>35.6 ± 0.3</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>contracted</td>
<td>8.8 ± 0.2</td>
<td>0.63 ± 0.04</td>
<td>148 ± 2</td>
<td>495 ± 32</td>
<td>61.1 ± 4.4</td>
<td>34.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>silkworm (b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>native ((n = 9))</td>
<td>12.4 ± 1.2</td>
<td>1.52 ± 0.15</td>
<td>220 ± 28</td>
<td>636 ± 41</td>
<td>30.7 ± 4.8</td>
<td>44.1 ± 0.8</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>contracted ((n = 6))</td>
<td>9.1 ± 0.4</td>
<td>1.34 ± 0.05</td>
<td>155 ± 10</td>
<td>560 ± 28</td>
<td>38.7 ± 2.4</td>
<td>39.0 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}All values measured were expressed as mean ± standard deviations (SD).

\(A_\nu (\nu)\) and \(A_\nu (\nu)\) are the absorbance values at wavenumber \(\nu\) of a certain band with light polarized parallel and perpendicular to the fiber axis, respectively.

where \(\lambda_\nu (\nu)\) and \(\lambda_\nu (\nu)\) are the absorbance values at wavenumber \(\nu\) of a certain band with light polarized parallel and perpendicular to the fiber axis, respectively.
water were first investigated by the S-FTIR and Raman methods. S-FTIR spectra show that as-reeled *A. pernyi* silk from silkworm *a* (Figure 2a) contains more α-helix structures (peaks at 1332, 1306, 1265, and 1105 cm⁻¹) than the silk fiber from silkworm *b* (Figure 2b). After being contracted in water, the peaks assigned to α-helix structures notably decrease for silk fiber from silkworm *a*. However, the spectrum of contracted silk fiber from silkworm *b* is quite similar to that of the native sample. These are consistent with the Raman spectra results (Figure 2c,d) that contracted silk fiber from silkworm *a* shows dramatically decreased α-helix structure with peaks at 1658 and 1105 cm⁻¹ compared to the native silk, and there is no observable spectral difference between native and contracted silk fibers from silkworm *b*. Besides, S-FTIR spectra of silk fiber from silkworm *a* show that the β-sheet structures (965 cm⁻¹) increase slightly after water treatment.

It is generally accepted that *A. pernyi* silk mainly contains three conformations: β-sheet, α-helix, and random coil. In order to analyze in detail the content of these three different secondary structural components before and after water treatment, amide III bands in FTIR spectra were deconvoluted according to the peak assignment of these three conformations described elsewhere. Specifically, the peak at 1222 cm⁻¹ is assigned to β-sheet conformation, while the peaks at 1241 and 1265 cm⁻¹ are assigned to the random coil and α-helix, respectively. The detailed results of the peak recognition and deconvolution are shown in Figure S1, and the content of these different conformations in native and contracted states are summarized in Table 2. For the silk fiber from silkworm *a*, α-helix conformation declines, and β-sheet conformation increases significantly after being contracted in water, while the random coil conformation increases slightly. This indicates that transition from α-helix to β-sheet is induced by water treatment. However, these three conformations almost remain unchanged before and after water contraction for silk fiber from silkworm *b*, which means there is no definite conformational transition. It should be mentioned that we did not consider the contribution of *A. pernyi* sericin coating on the content of different conformations of silk fiber because of its slight content and uniform distribution on the surface of silk fiber, as described above. In fact, the XRD result shows that *A. pernyi* sericin mainly takes an amorphous structure (Figure S2a), which fits in well with the XRD result reported by Yang et al. In addition, the S-FTIR result further excludes the existence of α-helix structure in *A. pernyi* sericin (Figure S2b).

We also compared the S-FTIR microspectra of as reeled *A. pernyi* silks in native and contracted states obtained from different polarization angles (0° to 90°). As for silk fiber from silkworm *a*, it shows that α-helices are nearly distributed evenly within native silk fiber (Figure S3a) and they all decrease dramatically after water treatment (Figure S3b). However, the absorption peaks at 1222 and 965 cm⁻¹ assigned to β-sheet structure all become gradually smaller from 0° (parallel to the fiber axis) to 90° (perpendicular to the fiber axis) for both native and contracted silks, indicating parallel dichroism. Additionally, we can clearly see from peak at 965 cm⁻¹ that β-sheet structure of different angles all increase after water treatment, further indicating the conformational transition from α-helix to β-sheet. However, for the sample from silkworm *b*, its S-FTIR microspectra at different polarization angles are quite different compared to that of *a*. This indicates that sericin coating on the surface of *A. pernyi* silk fiber is quite different from that of native silk fibers.

![Figure 2. S-FTIR microspectra (a,b) and Raman spectra (c,d) before and after contraction in water: as-reeled *A. pernyi* silks from silkworms *a* (a,c) and *b* (b,d). A silk fiber was aligned either parallel (solid line) or perpendicular (dashed line) to the vibration direction of the laser light for Raman characterization. The same legend is used for panels a and b, and panels c and d.](image)

Table 2. Comparison of Secondary Structures of As-Reeled *A. pernyi* Silks from Two Different Silkworms in Native and Contracted States

<table>
<thead>
<tr>
<th>Conformation</th>
<th>silkworm <em>a</em> (n = 5)</th>
<th>silkworm <em>b</em> (n = 5)</th>
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<tbody>
<tr>
<td></td>
<td>native</td>
<td>contracted</td>
</tr>
<tr>
<td>β-sheet</td>
<td>20 ± 1</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Random coil</td>
<td>46 ± 2</td>
<td>48 ± 1</td>
</tr>
<tr>
<td>α-helix</td>
<td>34 ± 2</td>
<td>24 ± 1</td>
</tr>
</tbody>
</table>

All values measured were expressed as mean ± standard deviations (SD).
similar to those of contracted silk fiber from silkworm a (Figure S3b), and they remain unchanged after contraction in water.

To further explore the variation of orientation of β-sheet structure during water treatment, we focused our analysis on the single peak at 965 cm\(^{-1}\) because it uniquely represents the β-sheet structure.\(^{20}\) As shown in Table 3, in native silk from silkworm a, the molecular order parameter \(S^\text{mol}\) value is 0.83 ± 0.03, but it increases to 0.87 ± 0.02 after water contraction, suggesting that the β-sheet structure in native silk fiber from silkworm a is poorly oriented along the fiber axis, and contraction slightly increases its order. Nevertheless, the order parameter \(S^\text{mol}\) of β-sheet structure of native silk from silkworm b reaches 0.93 ± 0.01 and it remains constant at 0.93 after water treatment. In addition, there is nearly no absorption appearing at 90° (Figure S4a,b). These facts indicate that the β-sheet structure in this kind of sample is almost perfectly oriented along the fiber axis, and it is immune to water treatment. It should be noted that the influence of water treatment on the orientation of β-sheet structure of A. pernyi silk either from silkworm a or b is inconsistent with that of spider (e.g., Nephila edulis) dragline silk.\(^{42}\) The orientation of β-sheet structure of spider dragline silk is decreased after supercontraction in water, compared to that of native silk. This inconsistency may be attributed to the distinguishing ordered fractions of these two kinds of silks.\(^{15}\) A. pernyi silk shows higher ordered fraction (or degree of crystallinity) than spider dragline silk. As such, the β-sheet structure of A. pernyi silk is difficult to move to change its orientation during water treatment. For the silk from silkworm a, the increase of orientation of β-sheet structure should arise from the conformational transition during water treatment.

Unlike β-sheet conformation, there is not a single and whole absorption peak in the S-FTIR spectrum of A. pernyi silk that only provides unambiguous information for either random coil or α-helix conformation. Therefore, we used amid III band to investigate the change of orientation of random coil and α-helix conformational transition during water treatment, like that determined from 965 cm\(^{-1}\) band. The deconvolution results of the amid III band acquired with the IR beam both parallel and perpendicular to the fiber axis are illustrated in Figure S5, and the calculated \(S^\text{mol}\) values are summarized in Table 3. For the sample from silkworm a, the order parameter for random coil conformation decreases slightly from 0.15 to 0.10 after water contraction, while it increases from 0.20 to 0.23 for α-helix. Besides, the order parameter of the β-sheet component increases slightly after water treatment, like that determined from 965 cm\(^{-1}\) band. We speculated that the change of \(S^\text{mol}\) for silk fiber from silkworm a may arise from the structure transition induced by water. However, the \(S^\text{mol}\) values of these three conformations in contracted silk from silkworm b are nearly the same as those in native silk fiber, which strongly indicates that contraction has no detectable effect on the orientation of different conformations in silk fibers from silkworm b. Noting that the \(S^\text{mol}\) value determined from the amide III band is calculated using the relative intensity of each conformation (dividing the area of each conformation by the peak area at ~1450 cm\(^{-1}\)) at 0° and 90°.\(^{20}\)

It also can be seen from the Raman spectra in Figure 2c that the band at 905 cm\(^{-1}\), which is associated with the poly(alanine) segments,\(^{28}\) exhibits stronger polarization dependence after water treatment, demonstrating that the poly(alanine) blocks have a higher orientation in contracted silk than that in native silk from silkworm a. However, the orientation of poly(alanine) blocks in silk fiber from silkworm b is already high and nearly remains unchanged after water contraction (Figure 2d). These facts agree well with the FTIR results mentioned above, because poly(alanine) blocks are mainly involved in the β-sheet and α-helix conformation.
3.3. Structure Transition of A. pernyi Silks from Silkworm a with Water Treatment Monitored by 13C CP/MAS NMR Method. We performed a 13C CP/MAS NMR test on the A. pernyi silk fibers (silk mat) from silkworm a in both native and contracted states to investigate the structure transition associated with the amino acid residues, especially the alanine (Ala) residues. The change in 13C CP/MAS NMR spectra is presented in Figure 3a,b, and the specific chemical shifts as well as the corresponding local conformation are summarized in Table S1.40 It should be mentioned that the secondary structures and tensile properties of silk mat used here are quite similar to those of as-reeled silk fibers from silkworm a.

From the Ala Cα and Cβ peaks shown in Figure 3a, we can see that the Ala residues in the poly(alanine) segments44 in native silk fibers almost equally take both β-sheet and α-helix structures. In addition, the chemical shifts values of two Ser carbons (Cα and Cβ) also indicate that some Ser residues in native silk fibers take α-helix structures. However, after water contraction, the Ala Cα, Cβ, and C==O peaks at 52.9, 15.7, and 176.4 ppm (α-helix), respectively, decrease dramatically, and the Ala residues in the silk fiber take mainly β-sheet (20.5, 22.8, 49, and 172.4 ppm) conformation (Figure 3b). The Ser Cα and Cβ peaks also shift from 59.6, 60.9 ppm (α-helix) to 54.8, 63.5 ppm (β-sheet), respectively, although these peaks were broad.44 These results undoubtedly suggest that α-helix is converted to β-sheet for both Ala and Ser residues after water treatment. The Ser residues in A. pernyi silk fibroin are located at the position adjacent or close to poly(alanine) segments, and thus Ser residues are incorporated into α-helix or β-sheet conformation, which is consistent with the results reported previously.44

We also collected the 13C CP MAS NMR spectrum of contracted silk fibers in wet state and found that it significantly differs from that in dry state (Figure S6). Particularly, the wet silks show a clear decrease in the intensity for most of the peaks, with an exception for Ala Cβ peak that associated with the β-sheet structure. This demonstrates that water mainly penetrates the glycine rich regions and the poly(alanine) regions that adopt α-helix and random coil conformations rather than the poly(alanine) β-sheet domains in A. pernyi silks. The regions attacked by water display an increase in molecular chain mobility, which leads to a loss in CP signal.45 However, there is a decrease in the intensity for Ala Cα peak that is associated with the β-sheet structure, which should be caused by the loss of intensity of its low and high ppm shoulder.

To detail the conformation transition related to Ala residues, the Ala Cβ peak was expanded and decomposed (Figure 3c,d), and the extracted results are summarized in Table 4. After water treatment, the fractions of α-helix and random coil conformation decrease, while the fraction of β-sheet conformation increases dramatically compared to those before water treatment (Table 4), indicating that conformation transition from α-helix and/or random coil to β-sheet is induced by water for Ala residues. We speculate that the conformation transition related to the Ala residues is focused on the poly(alanine) regions, because the Ala residues in the glycine rich domain always adopt random coil conformation.44 In fact, the fraction of random coil component in contracted silks is in agreement with the content of Ala residues in the glycine rich domain (related to all Ala residues) that is predicted from the A. pernyi fibroin amino acid sequence.45 This further proves that the Ala residues in the poly(alanine) segments in native A. pernyi silk fibers from silkworm a take three conformations simultaneously, while those in contracted silk fibers just take both β-sheet and α-helix conformations.

3.4. Structural Model during Water Contraction. The three different analytical methods, i.e., S-FTIR, Raman, and 13C CP/MAS NMR, clearly show that the structure transition occurs remarkably for the silk fibers from silkworm a during water treatment. However, no observable structure change occurs for silk fibers from silkworm b. Obviously, the different structure variations of these two silk fibers when interacted with water arise from their intrinsic differences of structures, and S-FTIR results indeed show that the content and orientation of secondary structures (especially α-helix and β-sheet) is different for these two samples (Tables 2 and 3). Additionally, the different capacities of shrinkage of these two silks (Table 1) also suggest the different orientations of isolated amorphous regions, and it is much higher for silk fiber from silkworm b, as it shows higher shrinkage than silk fiber from silkworm a.8 Furthermore, our previous study on hydrated A. pernyi silk suggests that water primarily penetrates into relatively disordered regions (amorphous regions) and breaks the hydrogen bonds consequently and improves the mobility of chains within these regions, while the well-defined crystalline regions remain rigid and intact.21

Table 4. Fractions of Different Conformations in Ala Cβ Peak before and after Water Contraction

<table>
<thead>
<tr>
<th>conformation</th>
<th>Ala fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>native</td>
</tr>
<tr>
<td>β-sheet</td>
<td>41</td>
</tr>
<tr>
<td>random coil</td>
<td>23</td>
</tr>
<tr>
<td>α-helix</td>
<td>36</td>
</tr>
</tbody>
</table>

Based on these facts, we can now make a description of the molecular mechanism of the structure transition induced by water. When the silk fiber from silkworm a is submerged in water, largely agminated α-helix bundles in the silk fiber are easily attacked by water molecules, and they become more relaxed due to the breakage of the intrachain hydrogen bonds within single α-helix structure. This is confirmed by the fact that α-helix structures almost become invisible (their peaks decrease significantly) in the 13C CP/MAS NMR (Figure S6) and even Raman (Figure S7) spectra when the silk fibers are immersed in water. At the moment, the amorphous regions in the silk fiber behave like a rubber,21 and the stress–strain curve tested in water is nearly a straight line, with the modulus almost equal to the post-yield yield modulus of water-contraction A. pernyi silk tested in air (Figure S8a,b). Then, in the following drying process, the adjacent unconfined α-helix structures would be closer to each other, and thus gives rise to the structure transition from α-helix to β-sheet through the conversion of hydrogen bonding pattern from intrachain to interchain. The random coil conformation taken by a few parts of the poly(alanine) segments can also be converted to β-sheet with the aid of water molecules. Meanwhile, this structure transition would lead to a change in orientation of these secondary structural components (Table 3). For the silk fiber from silkworm b, it contains a small number of α-helix structures and they are isolated to each other. Although their intrachain hydrogen bonds can also be destroyed by water molecules, there is no new hydrogen bonding to be formed between different α-helix structures in the drying process, hence, no structure transition occurs. In addition to the α-helix
domains, water can also insinuate into the oriented amorphous regions of both silks and consequently cause the rearrangement of hydrogen bonding structures (disorientation of molecular chains), although these changes cannot be definitely determined by our methods used here.8,42 The detailed structure transition models are summarized in Figure 4.

![Figure 4](image-url)

**Figure 4.** Structure transition models of as-reeled A. pernyi silk fibers from two different silkworms during water contraction.

Next, we can interpret the mechanical response of these two kinds of silk fibers to water treatment. After water submersion, the chain segments that were penetrated by water molecules become more loosely packed, and then less strain energy is needed to unfold these chain segments when the silk fiber is subjected to tension.46 Therefore, the contracted silks display kinds of silk from two different silkworms during water contraction. After water submersion, the increase of orientation of both β-sheet and α-helix conformations after water treatment should be the origin of the increased post-yield modulus and decreased breaking strain of contracted silk compared to the native silk (Figure 1a and Table 1). However, for silk fiber from silkworm b, the increase of breaking strain should be attributed to the reduction of orientation of molecular chains in the amorphous regions.

4. CONCLUSIONS

In summary, we have investigated the water-induced properties and structure transition information on as-reeled A. pernyi silk fibers from two different individuals. Detailed mechanical properties data indicate that initial modulus, yield stress, breaking stress, and resilience all decrease after water contraction for these two kinds of silk fiber. However, post-yield modulus increases, and breaking strain decreases for silk from silkworm a, and they are just the reverse for silk from silkworm b. Both S-FTIR and Raman spectra show that silk fiber from silkworm a contains more α-helix conformation than silk from silkworm b, and these α-helices are partially converted to β-sheet structures after water treatment for silk fiber from silkworm a, but there is nearly no conformation transition for silk fiber from silkworm b. In addition, the orientations of both α-helix and β-sheet structures increase along with the conformation transition generated in silk fiber from silkworm a. 13C CP/MAS NMR results further suggest that structure transition from α-helix and random coil to β-sheet is induced by water for Ala residues for silk fiber from silkworm a and it mainly occurs in poly(alanine) regions. Additionally, similar structure transition from α-helix to β-sheet is also observed for Ser residues. Finally, the structure transition models of these two as-reeled A. pernyi silk fibers are described, and we propose that α-helix to β-sheet transition is driven by the conversion from intrachain hydrogen bonds to interchain hydrogen bonds. In combination with the previously published work, our results present here further shed light on the effect of water on the mechanical properties and structure of animal silk, and will provide valuable information for the interaction between water and other materials with hydrophilic groups.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.7b01691.

FTIR, XRD, Raman spectra, 13C CP/MAS NMR chemical shifts and spectra, and stress–strain curves (PDF)

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**Notes**
The authors declare no competing financial interest.

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