Analysis of Structure/Property Relationships in Silkworm (Bombyx mori) and Spider Dragline (Nephila edulis) Silks Using Raman Spectroscopy

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Received October 7, 2002; Revised Manuscript Received December 16, 2002

The molecular deformation of both silkworm (Bombyx mori) and spider dragline (Nephila edulis) silks has been studied using a combination of mechanical deformation and Raman spectroscopy. The stress/strain curves for both kinds of silk showed elastic behavior followed by plastic deformation. It was found that both materials have well-defined Raman spectra and that some of the bands in the spectra shift to lower frequency under the action of tensile stress or strain. The band shift was linearly dependent upon stress for both types of silk fiber. This observation provides a unique insight into the effect of tensile deformation upon molecular structure and the relationship between structure and mechanical properties. Two similar bands in the Raman spectra of both types of silk in the region of 1000–1300 cm\(^{-1}\) had significant identical rates of Raman band shift of about 7 cm\(^{-1}\)/GPa and 14 cm\(^{-1}\)/GPa demonstrating the similarity between the silk fibers from two different animals.

Introduction

Over recent years considerable research has focused on spider silks\(^1^−3\) because of their unique properties—good extensibility combined with high levels of tensile strength. Spiders produce a wide variety of silks for many particular purposes coupled with a wide variety of mechanical properties,\(^4\) and it is thought that a better understanding of structure/property relationships in silk could be used to develop superior fibrous materials, be they man made or of natural origin. At present the commercial silk industry is almost entirely based upon material produced by an insect—the silkworm, Bombyx mori. Thus, among the wide variety of silks available in nature, the cocoon silk produced by Bombyx mori has been investigated the most extensively.\(^5^−10\)

Bombyx mori silk comprises about 65−70% crystalline material, with the remaining 30−35% material being amorphous with hydrogen-bond linkages.\(^6\) Early X-ray diffraction analysis\(^1^1\) implied that the molecules in the crystalline regions must be present as extended chains in a zigzag form with an axial repeat of 0.7 nm, corresponding to two amino acid residues.

Comparable to worm silk, the microstructure of a spider dragline silk fibril is at present viewed as a microcomposite of small, rigid, inextensible \(\beta\)-sheet crystallities embedded in a matrix of an amorphous rubbery polypeptide.\(^2\) The crystalline domains are presumed to be occupied by polypeptide segments consisting of about 15 amino acid units that are glassy when dry and rubbery when swollen in water.

The fibroins in both B. mori silks and N. edulis silks consist of alternating sequences of segments likely to form \(\beta\)-sheets interspersed with segments likely to remain in random coil or to form either \(\alpha\)-helical or 3(1)-helical structures. However, the lengths of these segments may be much shorter in spider silks.\(^1^2\)

It seems that different silks have unique amino acid compositions not only between but also within species. All spider silks show a predominance of glycine, alanine, and glutamine; there are also substantial amounts of leucine and tyrosine, which are among the larger amino acids.\(^1^3,1^4\) The structure of spider silk fibroins appears to be much less regular than that of silkworm silk, and spider silks tend to have repeats of Gly-Gly-X, rather than the Ala-Gly-Ala-Gly-Ser-Gly sequences found in B. mori silk.\(^1^2\)

Raman spectroscopy is a most useful method for evaluating changes that occur in a fiber structure subjected to stress or strain. It allows us to examine the molecular deformation mechanisms of the polymeric chains manifested through the large stress-induced Raman band shifts that can occur during deformation. Raman microscopy has been applied successfully to study deformation processes for a wide variety of high-performance polymeric fibers, such as aramid (poly-(p-phenylene terephthalamide)) fibers,\(^1^5\) rigid-rod polymer fibers, e.g., PBT (poly(p-phenylene benzobisthiazole))\(^1^6\) and PBO (poly(p-phenylene benzobisoxazole))\(^1^7\) fibers, and gel-spun polyethylene fibers.\(^1^8\) It has been found that the magnitude of the wavenumber shift of the Raman-active bands upon the applied stress or strain depends on both the chemical structure of the polymer molecules and the morphology and microstructure of the fibers.

A study by Gillespie and co-workers\(^1^9\) using Raman spectroscopy on the dragline silk of Nephila clavipes showed
that the β-sheet content in the silk fiber is about 22 ± 5%, which is significantly lower than that in silkworm silk (56 ± 5%). Spectra obtained using different polarizations of the laser beam also suggested that both the β-sheet and a small proportion of the α-helix are highly oriented along the fiber axis, whereas the disordered polypeptide chains are not preferentially aligned.

Sirichaisit, Young, and Vollrath²⁰ reported a significant stress-induced shift of the 1095 cm⁻¹ Raman band in spider dragline silk which they interpreted in terms of molecular deformation processes. In this present study a Raman microprobe was used to investigate and compare the molecular response of silkworm (Bombyx mori) and spider dragline (Nephila edulis) silks subjected to deformation processes such as tensile deformation, stress relaxation, and cyclic loading.

**Experimental Section**

The silkworm and spider silks used in this study were of the species Bombyx mori and Nephila edulis, respectively. The fibers of Bombyx mori were supplied by Professor K. Nakamae of Kobe University, Japan, and were characterized in the as-received state. The nominal diameter of each Bombyx filament tested was measured using an optical microscope calibrated with a graticule. The difficulties of determining the exact diameter of silk filaments have been discussed by Pérez-Rigueiro et al.²¹ Representative filaments were also examined in a Philips XL-series FEG-SEM and were shown to be made up of a number of individual fibers. The effective void content was estimated to be about 10%, and this was used to correct the filament diameter. Nevertheless, we anticipate that this could lead to a systematic error in the determination of filament stress.²¹

The major ampullate dragline silk fibers of Nephila edulis were obtained by reeling directly from restrained but fully awake spiders at 20 mm/s under room conditions (~25 °C and 50% RH). This reeling rate is similar to that used by the spiders in their natural environments. The spiders had been raised in the laboratory and fed with house flies. The silk fibers were collected and restrained on small plastic frames (100 mm × 100 mm) and were analyzed within 1 month of collection. The diameters of individual fibers were measured using a Philips XL-series FEG-SEM coated with carbon coating to avoid charging and calibrated using a calibration grid with 2160 lines/mm. The stress was calculated from the mean diameters of 10 fibers from the same batch.

Monofilaments of silkworm and spider dragline silks were mounted across cardboard windows using Ciba-Geigy LY 1927 two parts cold-curing epoxy resin. The specimens were left for at least 7 days for the adhesive to set completely prior to testing to minimize slippage errors. All specimens were tested using an Instron 1121 testing machine following the recommendations given in ASTM D3379-75. A Macintosh computer was connected to the tensile machine and both sides of the cardboard window were cut carefully. Specimens of silkworm and spider dragline silks with a gauge length of 50 mm were tested using cross-head speed of 0.5 mm/min. A standard weight of 1 N was used to calibrate the Instron load cell. A minimum of 10 specimens was tested at 23 ± 1 °C and 50 ± 5% relative humidity.

A Renishaw 1000 system Raman imaging microscope was used to record Raman spectra of both the silkworm and spider dragline silks. A low power of 25 mW He–Ne laser (632.8 nm) was employed with an intensity of about 1 mW when focused on the monofilaments. The laser beam was polarized parallel to the fiber axis. An Olympus BH-2 optical microscope was used to focus the laser beam on the specimen to a spot of ~2 μm diameter and to collect the scattered radiation. A highly sensitive Renishaw charge-coupled device (CCD) camera was used to collect the spectra. The band positions and intensities were analyzed using a Lorentzian fitting procedure.

Raman spectra in the region of 1000–1300 cm⁻¹ were obtained from monofilaments of silkworm and spider dragline silks using the same types of specimens as described in the mechanical testing section. Single fibers with a 50 mm gauge length were deformed using a stressing rig that fitted directly on to the microscope stage. The rig had a transducer to monitor the load and a micrometer to control the displacement, from which the strain was determined. The monofilaments were stretched up to failure in steps of the order of 0.5–1.0% strain, and this setup allowed a precision of ±0.05% for strain measurement. The exposure time needed to collect the spectrum for each deformation step was 180 s for the silkworm silk and 300 s for spider dragline silk. The silkworm silk gave a stronger spectrum as a result of the larger diameter of its filaments.

For the stress relaxation experiments, monofilaments of both kinds of silks were stretched rapidly to fixed displacements corresponding to 15% strain. The shift of the Raman band was then recorded over a period of about 8000 s by obtaining a series of spectra at 300 s intervals from approximately the same positions on the fibers. The cyclic deformation behavior of both types of silk was also investigated. Monofilaments of the two types of silk with a 50 mm gauge length were loaded to about 0.4 GPa, then unloaded to zero stress, and finally stretched up to failure.

**Results and Discussion**

**Tensile Deformation.** Scanning electron microscopy (SEM) image analysis showed that the Bombyx mori silks had irregular cross sections. The average effective diameter of the silkworm silk filaments used in this study was about 50 ± 7 μm. In contrast to the irregular diameter of the silkworm silk fibers, the spider dragline silk fibers (N. edulis) showed circular cross sections with diameters in the range 2–3 μm.

Typical stress/strain curves for both the silkworm and spider dragline silks are shown in Figure 1. There was a pronounced deviation from linearity at about 3% strain for the silkworm silk and about 2% strain for the spider silk.
The tensile strength and failure strain for the spider dragline silk were 0.8 ± 0.4 GPa and 25 ± 5%, while for the silkworm silk they were 0.4 ± 0.1 GPa and 22 ± 4%, respectively. These values are similar to those quoted in the literature for the two types of fiber. It should be noted, however, that a recent study has shown that the mechanical properties of silkworm silk can approach those of spider dragline silk when reeled under controlled conditions.

Investigations of silk structure have shown that both silkworm and spider silk are semicrystalline materials. They are both composed of amorphous flexible chains that are made up of strands of disordered amino acid chains. Such regions of the fibers are reinforced with strong and stiff crystals (β-pleated sheets) that consist of different size of amino acid groups arranged in an accordion-like formation. It has been suggested that the segments with bulky side groups, such as tyrosine, would not crystallize easily and would therefore form the major part of noncrystalline regions whereas the crystalline regions would be made up from the parts of the molecules with simpler, more easily packed side groups, with, for example, glycine, alanine, and serine sequences.

When tension is applied, the initial deformation could be expected to occur primarily in the amorphous regions. The relatively high initial modulus of silk indicates that the strength of the combined interacting forces between chains in the amorphous regions and between chains interacting with crystalline regions is also quite high. If the application of force is continued, interchain bonds are broken and the randomly arranged chains extend and may slide past each other and past the crystalline regions, resulting in plastic deformation. This is characterized by the yield point in the stress/strain curve (Figure 1). As more chains are extended further, it is thought that the load will begin to be taken up by the crystalline regions. When the load can no longer be accommodated, the fiber ruptures and interchain hydrogen bonds are finally broken. These processes are discussed further following the analysis of the deformation processes through the use of Raman spectroscopy.

Raman Spectroscopy and Deformation. Raman spectroscopy is now established as an important tool for the study of the deformation of polymeric fibers with well-defined Raman spectra being obtained from high-modulus, highly oriented polymer fibers. When such polymers are deformed, the bonds in the backbone chains are strained leading to a decrease of wavenumbers of Raman-active bands by an amount, Δν, depending on the material, the modulus, and structure of the fibers and the band under consideration.

Raman spectra of single fibers of silkworm and spider dragline silks in the range of 800–1800 cm⁻¹ are shown in Figure 2. The most prominent Raman-active bands are located approximately at 1085, 1232, and 1667 cm⁻¹ for the B. mori silk and at 1095, 1230, 1652, and 1684 cm⁻¹ for the N. edulis silk. These bands have been assigned in previous studies and the assignments are given in Table 1. The two prominent and intense bands at 1085 and 1232 cm⁻¹ for silkworm silk and at 1095 and 1230 cm⁻¹ for spider dragline silk were chosen to follow the molecular deformation processes as they have been assigned to skeletal backbone vibrations.
Monofilaments of both silkworm and spider dragline silks were deformed up to failure in steps of 1.0% strain. We found that the Raman bands at 1085 and 1232 cm\(^{-1}\) for \textit{B. mori} silk and at 1095 and 1230 cm\(^{-1}\) for \textit{N. edulis} silk all shifted to lower wavenumber when the fibers were deformed in tension as shown in Figure 3. Similar behavior has been found for a number of different types of high-performance fibers.\(^{15-17}\) No significant stress-induced band shift was observed in the 1450–1800 cm\(^{-1}\) region of the Raman spectrum for either type of silk fiber.

Figures 4 and 5 show the change of the Raman wavenumber of the peak position for the two different bands in the two types of silk fiber as a function of fiber strain. The error bars were determined from the standard deviation of 10 measurements of the band positions in undeformed fibers. It can be seen that, as the strain is increased, the band positions shift to lower wavenumbers. Thus, when the macroscopic stress is applied, it is transformed directly into stressing of the covalent bonds in the molecules of the fibers resulting in alignment of the molecules along the direction of extension. The deformation of the oriented molecules in both the crystalline and the amorphous regions cause the change in bond lengths, bond angles, and internal rotation angles and, therefore, change in the corresponding force constants which can be monitored precisely through measuring the frequency shifts.\(^{25,26}\)

Figure 4 shows that the shift of the Raman bands to lower wavenumber is nonlinear with strain for the silkworm silk. Similar behavior has been reported for as-spun PBT fibers,\(^{16}\) as-spun-ABPBO fiber,\(^{27}\) and PET fibers,\(^{29}\) all of which undergo yield during tensile deformation. In fact the shape of the curves in Figure 4 mirrors that of the stress–strain curve for the silkworm silk fiber in Figure 1. It appears that up to yield the molecules in the fibers undergo significant chain stretching, but after yield there is less chain stretching, with tensile deformation proceeding probably by the molecules sliding past each other.

In contrast, over the same range of deformation the spider dragline silks show an approximately linear relationship between Raman wavenumber and strain as can be seen in Figure 5. A yield point is found in the stress/strain curve for this fiber at about 2% strain (Figure 1), but there is also significant strain hardening which makes the curve approximately linear. The Raman spectra for the spider silk have lower intensity and less well-defined peaks than for the silkworm silk due to their smaller diameter. Hence there is more scatter in the spider data (Figure 5) than in the moth data (Figure 4). This means that the linear plots of band shift against strain in Figure 5 are consistent with the stress/strain curve for the spider silk fiber in Figure 1 since any corresponding nonlinearity in the Raman data would not be detected.

Figure 6 shows the shift of the peak positions of the Raman bands at 1085 cm\(^{-1}\) for \textit{B. mori} silk and at 1095 cm\(^{-1}\) for \textit{N. edulis} silk with stress. It can be seen that the two sets of data fall on straight lines with a similar slope of the order of \(-8\) cm\(^{-1}\)/GPa. Similar behavior is found for the other main Raman band. Figure 7 shows the shift of the peak positions of the Raman bands at 1232 cm\(^{-1}\) for \textit{B. mori} silk and at 1230 cm\(^{-1}\) for \textit{N. edulis} silk with stress. These two sets of
data also fall on straight lines with slopes in this case of the order of \(-15\) cm\(^{-1}\)/GPa. There are two important points to note:

1. Even though the dependencies of the peak positions upon strain for the \textit{B. mori} silk are not linear, they are linear when plotted against stress. We note that the shifts in the Raman band peaks are related to the fiber stress rather than the fiber strain, which has been reported for poly(ethylene terephthalate) (PET)\(^{28,29}\) and ABPBO fibers.\(^{27}\) The significance of this will be discussed later.

2. The dependence upon stress of the peak positions of similar Raman bands is similar for the silk of two animals as different as the spider and moth. The sensitivity of the two different bands to strain and stress in the two types of silk examined is summarized in Table 2 for similar measurements upon 10 filaments of each type of silk.

It is not clear why there is a higher rate of band shift for the 1232 and 1230 cm\(^{-1}\) Raman bands than that of the 1085 and 1095 cm\(^{-1}\) bands. A value of \(d\Delta\nu/d\sigma\) of approximately \(-4.0\) cm\(^{-1}\)/GPa was found for high-performance fibers such as PET (1616 cm\(^{-1}\) band),\(^{29}\) PPTA (Twaron and Kevlar) fibers (1610 cm\(^{-1}\) band),\(^{30}\) PBO fibers (1620 cm\(^{-1}\) band),\(^{30}\) and also PIPD or M5 fibers (1507 cm\(^{-1}\) band),\(^{31}\) even though different Raman bands are involved. This suggests that silk fibers subjected to a given macroscopic stress encounter similarly high levels of local molecular stress.

**Stress Relaxation.** No significant changes in the Raman band positions for the silkworm and spider dragline silks, within the range of experimental scatter, were found with time for the undeformed fibers. The application of load corresponding to 15\% strain led to an instantaneous shift of both bands to lower wavenumber for both fibers. There were no subsequent significant changes in the two band positions with time (up to 8000 s) for either fiber implying a lack of stress relaxation of the experiment. This can be contrasted with PET fibers\(^{29}\) where a small shift to higher wavenumber is observed over a similar period of time for the 1616 cm\(^{-1}\) Raman band. Thus both silk fibers showed less stress relaxation than PE or PET fibers. This could be due to the presence of intermolecular bonding—hydrogen bonding between polymer chains in the silk restricting the molecules sliding past each other.

**Cyclic Deformation.** Cyclic loading behavior and associated Raman band shifts were investigated for both Raman bands for the two types of silk. In both cases the fibers were loaded up to about 0.4 GPa, unloaded, and then reloaded.
the case of the *B. mori* silk, the fiber underwent yielding so that, when the fiber was unloaded, there was permanent plastic deformation in excess of 10% strain (Figure 8a). On reloading the stress increased along a similar path to the unloading curve. No correlation was found between the 1085 and 1232 cm\(^{-1}\) Raman band positions and strain for the *B. mori* silk. However, when the positions of the two bands were plotted against stress as shown in parts b and c of Figure 8, the data for both the first and second loading were found to fall on the same straight line.

The cyclic deformation behavior of the *N. edulis* silk fiber was similar. Figure 9a shows the effect of loading, unloading, and reloading a fiber to failure. There was a permanent plastic deformation of the order of 10% strain, and the reloading curve joined up with the original loading curve. Again there was a close correlation between the peak positions of the 1095 and 1230 cm\(^{-1}\) Raman bands and stress for both the first and second loading with both sets of data in parts b and c of Figure 9 falling on straight lines. The results shown in Figures 8a,b and 9a,b confirm an earlier observation that there is a close correlation between the Raman band positions and stress (rather than strain).

The widths of the Raman bands of many high-performance fibers typically increase during tensile deformation. We
Deformation Mechanisms

The Raman deformation studies for the moth cocoon silk (Bombyx mori) and spider dragline silk (Nephila edulis) suggest that the different mechanical properties of both silks result in different relationships between Raman peak position and strain (Figures 4 and 5). Moth silks showed pronounced yielding compared to the much more linear relationship between stress and strain for the dragline spider silks. In contrast, there was no significant difference between the stress-induced band shift rate of the Raman bands at ~1085 and ~1230 cm$^{-1}$ for both kinds of silk (Figures 6 and 7, Table 2). The shift rate of these two Raman bands with stress was ~7 cm$^{-1}$/GPa and ~14 cm$^{-1}$/GPa for the ~1085 and ~1230 cm$^{-1}$ bands, respectively.

This behavior is entirely consistent with the deformation of the fibers following a uniform stress series model. There is good evidence that the microstructure of silk fibers consists of an amorphous phase reinforced with crystals, similar to other synthetic polymer fibers. The relatively stiff crystallites reinforce the soft amorphous phase. As a consequence of this, the fibers when deformed show similar stresses in the crystalline and amorphous phases but very different strains (due to the difference in silk moduli). The tendency of the band shifts to follow fiber stress rather than fiber strain is a confirmation that the Raman band shifts follow the stress in the fiber microstructure.

The silk fibers can be assumed as an aggregate of a series of anisotropic units with a uniform orientation distribution, which are subjected to a uniform stress throughout the aggregate along the orientation axis. Then the macroscopic modulus can be simply expressed by the following equation:

$$
\frac{1}{E} = \frac{1}{E_c} + \frac{\langle \sin^2 \theta \rangle}{G}
$$

where $E$ is the macroscopic Young’s modulus, $E_c$ is the average chain modulus, $G$ is the shear modulus, and $\langle \sin^2 \theta \rangle$ is the mean value of orientation over the chains in the units about orientation axis. This indicates that tensile deformation in highly oriented fibers and tapes takes place by a combination of crystal stretching (first term) and crystal rotation (second term). The Raman band shift is a clear indication of the levels of axial stressing on the molecular chains; hence the Raman band shift reflects only chain stretching. The Raman band shift should therefore correlate directly with stress rather than with overall strain, which can be dominated by chain rotation.

It appears, therefore, that the stress applied to silk fibers is approximately uniform within the silk microstructures during molecular deformation. In this case the rates of stress-induced Raman band shift are ~7 cm$^{-1}$/GPa and ~14 cm$^{-1}$/GPa for the ~1085 and ~1230 cm$^{-1}$ Raman bands, respectively, for both silkworm and spider silks. Hence similar levels of band shift are obtained for silk fibers from two different animals. Furthermore, cyclic loading experiments on both types of silk showed that the Raman wavenumber followed the applied stress on both the first and second loading cycle as shown in Figures 8b,c and 9b,c. This is despite a large level of plastic deformation in the fibers and after the first cycle confirms the relationship between Raman peak position and fiber stress even for cyclic loading.

Raman band broadening during tensile deformation was found in both the moth and spider silks, although it was more pronounced for the spider silk. As the tensile stress was increased, the ~1085 and ~1230 cm$^{-1}$ Raman bands broadened toward lower wavenumbers. The broadening is an indication of a local stress distribution within the molecules occurring while the silk fiber is deformed, even though the overall deformation occurs at uniform stress. The band broadening was more apparent in the spider silk because this silk sustained higher levels of stress. There was, however,
more band broadening in the spider silk even at the same stress level. This may indicate high levels of local stress in the silk microstructure and is consistent with the hypothesis that the crystallites in spider silk act as cross-links in an amorphous network.\textsuperscript{37} It is clear that a more detailed investigation of the Raman band broadening could yield interesting information.

Conclusions

We have provided comparative data showing that Raman spectroscopy is a powerful technique for the detailed analysis of the deformation of Bombyx and Nephila dragline silks at the molecular level. Raman spectroscopy can provide a unique insight into the effect of macroscopic deformation upon the molecules in the microstructure and allows the relationship between structure and mechanical properties to be studied quantitatively. In both types of silk the macroscopic deformation of the fiber leads to specific and significant Raman band shifts that indicate high levels of molecular deformation in the fibers. The Raman band shifts follow fiber stress rather than strain, consistent with the deformation of the microstructure following a uniform stress series model. However, stress-induced Raman band broadening indicates significant local variation in molecular stress during tensile deformation.

Acknowledgment. This study was supported by two research grants from the EPSRC Materials Program (R.J.Y.) and Life Sciences Interface Program (F.V.). J.S. is grateful to the Government of Thailand for financial support, and V.L.B is grateful to the EPSRC for a research studentship. The Silk Network of the European Science Foundation provided a helpful platform for discussions.

References and Notes


BM0256956