Active Control on Molecular Conformations and Tensile Properties of Spider Silk

Z. J. Pan, C. P. Li, Q. Xu

College of Material Engineering of Suzhou University, Ganjiang East Road 178, Suzhou, Jiangsu 215021, China

Received 28 February 2003; accepted 11 November 2003

ABSTRACT: The mechanical properties of spider dragline silk vary with the spinning conditions, and molecular conformation is one of the important factors for the strength and strain of materials. Four kinds of *Araneus ventricosus* spider dragline silk fibers, measured by Raman microscopic spectrometry, were produced under different conditions: (1) reeled at the rate of 2 cm/s; (2) secreted by a dropping spider from a 100-cm-high table; (3) spun by spiders raised in two different containers. The Raman spectra of these fibers showed that the spinning method and growing environment of spiders had evident influences on the molecular conformations and tensile properties of dragline silk, and the dragline silk obtained from a dropping spider contained the greatest number of molecules with highly oriented β-sheet structures and gave higher stress/strain values. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 901–905, 2004

Key words: spider silk; conformational analysis; stress; strain; Raman spectroscopy

INTRODUCTION

Spider dragline silk is one of the high-performance fibers with excellent mechanical properties compared to those of *Bombyx mori* silk and chemical fibers such as Kevlar.[1–3] With the development of genetic engineering[4–6] and enhanced understanding of the spinning process, the development of artificial spider silk has progressed rapidly.[4,7–9] The spider is able to actively control mechanical properties during the spinning process,[10] by rapidly altering the diameters of dragline silk.[11] Molecular conformation is one of the important factors that determine the properties of materials. Structures higher in the hierarchy are not solely determined by the primary structure, but also by spinning conditions.[12,13] Therefore, it is important to investigate the relationships between spinning conditions and molecular structures of spider silk for the study of silklike high-performance material.

The molecular conformations of dragline silk fibers, secreted by *Araneus ventricosus* spiders raised in different containers and spun in various ways, were measured and studied. The tensile properties of these fibers were also investigated.

Correspondence to: Z. Pan (zhjpan@suda.edu.cn).

Contract grant sponsor: National Science Foundation of China.

Contract grant sponsor: Science and Technology Office of Jiangsu Province, China.

Journal of Applied Polymer Science, Vol. 92, 901–905 (2004)

EXPERIMENTAL

Four different dragline silk fibers of *Araneus ventricosus* spider were obtained in the following ways: (1) reeled at the rate of 2 cm/s[11]; (2) spun by spiders bred in a 5-cm-high carton; (3) spun by spiders bred in a 35-cm-high glass box; and (4) secreted by a dropping spider from a 100-cm-high table.

Samples were prepared in the following ways: (1) A live spider was placed on black paper, and when it crawled along the paper and dragline silk appeared, we fastened it to a glass plate with adhesive tape. Then, the end of the dragline silk was taped to a roller (diameter is 2 cm) and reeled at a controlled speed. About 3 cm silk was taken from the roller with a pair of bow compasses that had double-sided tape on the probes. (2) When a spider was dropped from a table, it secreted dragline silk and the sample was also taken by bow compasses. (3) The silk in the rearing container was directly taped with the compasses. These fibers were prepared on special frameworks for Raman spectra measurements.

The spectra of these samples were measured with a Labram 113 microscopic spectrometer system made by Dilov Corp. Ltd. (Lille, France).

The tensile properties of the samples were measured with an electronic tensile instrument for a single fiber with 0.01-cN and 0.001-cm resolutions at a tensile rate of 1 cm/min. The sample length was 1 cm. Each sample was measured 20 times and resulting averages were used for analysis.

The cross-sectional images of dragline silk fibers were recorded by a Hitachi S-520 SEM (Hitachi, Osaka, Japan). Computer image manipulation soft-

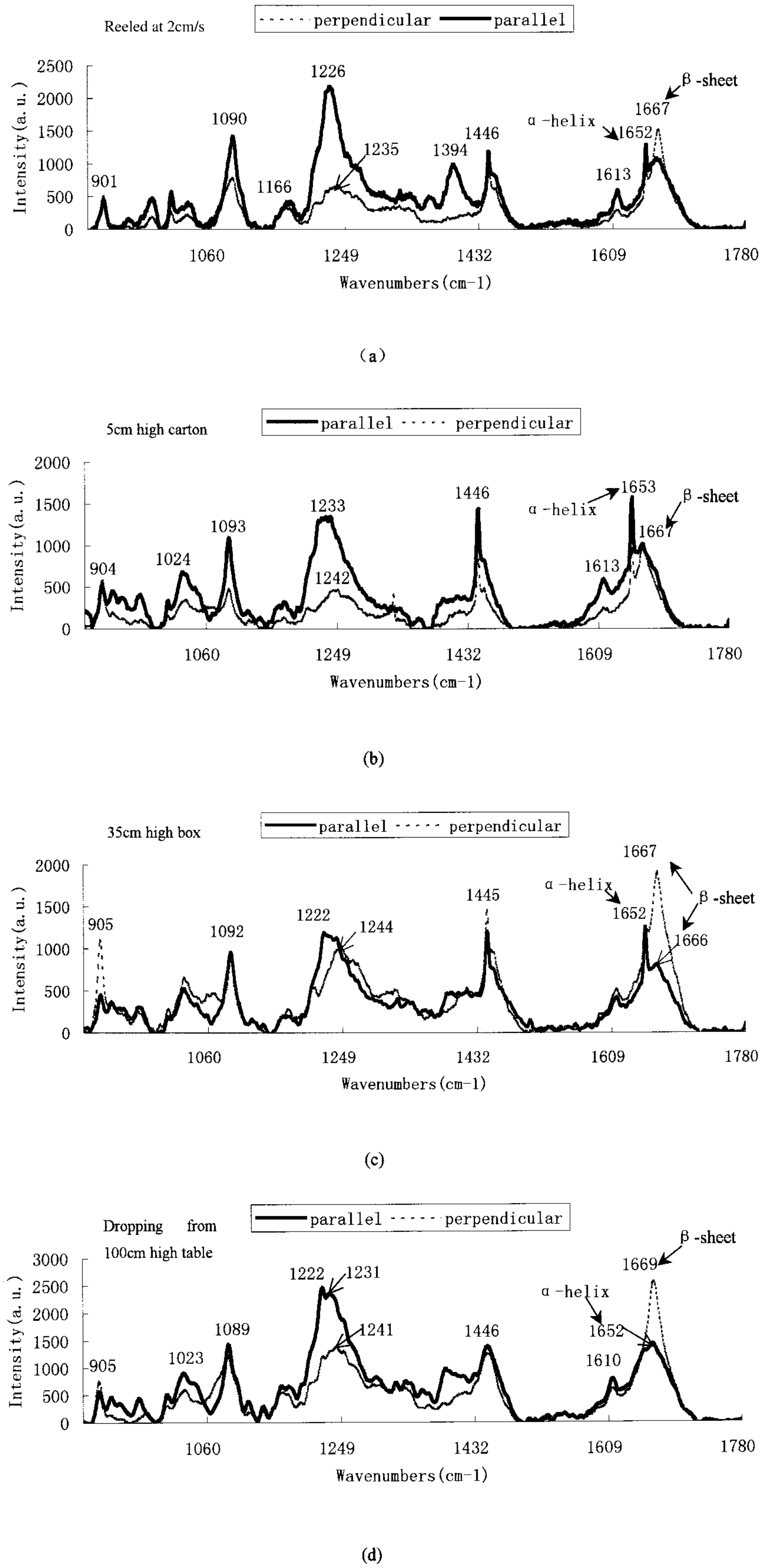

Figure 1 Raman spectra of *Araneus ventricosus* spider dragline silk: (a) dragline silk reeled at a rate of 2 cm/s; (b) dragline silk secreted by spiders raised in a 5-cm-high carton; (c) dragline silk secreted by spiders raised in a 35-cm-high glass box; (d) dragline silk produced by a dropping spider from a 100-cm-high table (—, laser beam parallel to the fiber axis; · · ·, laser beam perpendicular to the fiber axis).

TABLE I
Raman Spectra Characteristic Peaks of Proteins with Special Conformations[a]

Conformation	Amide I (cm^{-1})	Amide III (cm^{-1})	Other sensitive peak (cm^{-1})
α-helix	1645–1658	1264–1310	890–945
β-sheet	1665–1680	1230–1245	1020–1060
Random coil	1660–1666	1242–1250	

[a] Ref. 14.

ware was used to calculate the average cross-sectional areas of the spider silk fibers.

RESULTS AND DISCUSSION

The Raman spectra of samples with the laser beam parallel and perpendicular to the silk axis were measured respectively. Some differences between parallel and perpendicular spectra attributed to the anisotropy of molecular arrangement could be found (Fig. 1). Because the protein usually gives a very complicated Raman spectrum, the Raman spectra of spider dragline silk were analyzed referring to the literature data and the characteristic peaks of protein Raman spectra (Table I).[14–16] It can be found from the spectra that the molecular conformations of these spider dragline silk fibers were mainly β-sheet and α-helix. The peak at 1666–1672 cm^{-1} is characteristic of β-sheet configuration for amide I of the polypeptide and reflects the contribution of β-turn structure. A band around 1652–1653 cm^{-1} evidences α-helix conformation. When the laser beam was perpendicular to the fiber axis, the peaks around 1245 cm^{-1} indicated the existence of random coil and, in the parallel spectra, the peaks around 1230 cm^{-1} were attributed to unordered, β-sheet and β-turn conformations. The peak around 1095 cm^{-1} might be regarded as a mixture of all conformations in spider silk.[15] It should be noted that there is a strong peak around 905 cm^{-1} for every *Araneus ventricosus* dragline silk in both parallel and perpendicular directions. The band around 905 cm^{-1} is the stretching mode of C—CH_3, and poly-L-alanine also had a peak at 907 cm^{-1}.[17] The special peak confirmed that the spider silk had a motif of $(Ala)_n$. Therefore, the conformations of polypeptides in the dragline silk, formed at every condition, were mainly β-sheet and α-helix, and had random coil and β-turn. However, Figure 2 shows that these fibers had differences

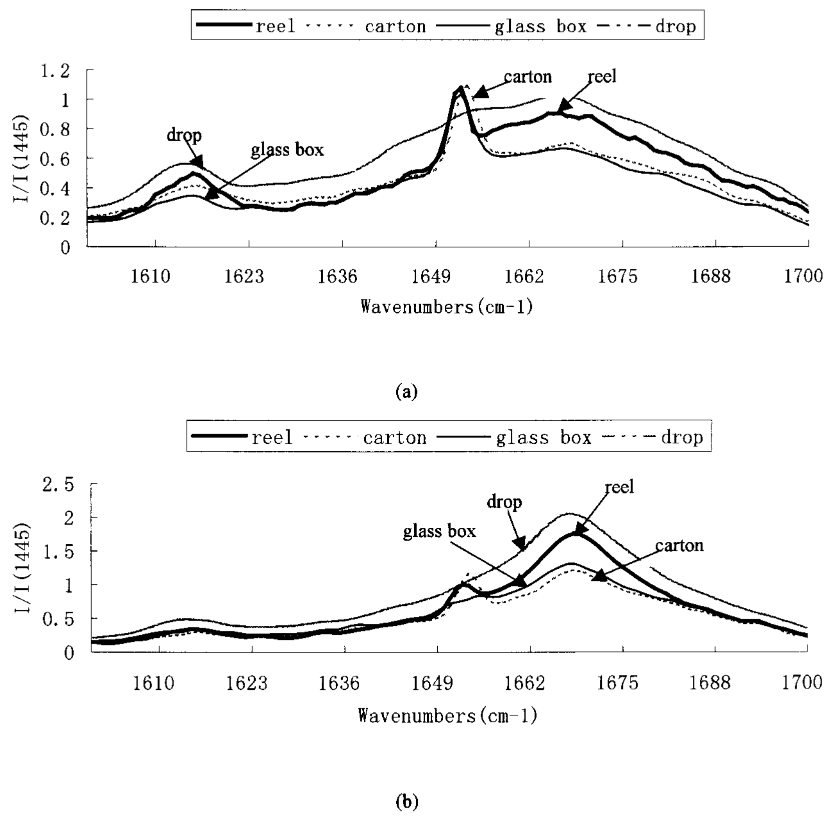

Figure 2 Raman intensity distributions from 1600 to 1700 cm^{-1}: (a) laser beam parallel to the fiber axis; (b) laser beam perpendicular to the fiber axis. $I(1445)$: Raman intensity at wavenumber 1445 cm^{-1}; reel: dragline silk reeled at the rate of 2 cm/s; carton: dragline silk secreted by spiders raised in a 5-cm-high carton; glass box: dragline silk secreted by spiders raised in a 35-cm-high glass box; drop: dragline silk produced by a dropping spider from a 100-cm-high table.

in the distributions of molecular conformations and orientations.

The peak at 1667 cm^{-1} is the C═O stretching vibration of amide I; the C═O is perpendicular to the axis of the β-sheet molecules. For the oriented molecules, if the laser beam is perpendicular to the axis of dragline silk, the polarization of the C═O will be increased and the Raman scatter intensity becomes higher. The intensity at 1445 cm^{-1} is assumed as the benchmark; the opposite intensity around 1667 cm^{-1} reflects the quantity of β-sheet molecules, and the ratio of perpendicular intensity to the parallel intensity shows the orientation of these molecules. The characteristic f_r data, which indicate the orientation of the dragline silk fibers, are listed in Table II.

Figure 2 shows that the opposite intensity at 1667 cm^{-1} varied with the spinning condition. The dragline silk from a dropping spider had the most β-sheet structure, with the highest orientation attributed to the spider's weight and high dropping velocity. Reeled spider silk ranked next with higher orientation attributed to the action of rolling tension. It was very surprising that the orientation of molecules with β-sheet structures varied greatly with the height of the box for spiders fed with the same food. Silk secreted by a spider in a 35-cm glass box had higher orientation and more β-sheet structure than that secreted by a spider in the 5-cm carton. The results imply that the security of the environment influences the molecular structure of spider silk. We discovered that spiders crawl along the top of a box and secret dragline silk in both the glass box and the carton. The spider is apparently more secure in the lower carton than in the higher glass box. When a spider is in danger, the spinning apparatus may have special effects on the silk protein solution, the muscle may tense, and the valve may increase in activity to avoid breaking the fiber.[18] The shear tension of silk increases and leads to a high order and orientation of molecules; thus study of the spider's spinning mechanisms is necessary for spinning a spider silklike fiber.

The peak at 1650 cm^{-1} is the α-helix structure character of amide I of polypeptide chain. Figure 1 and Figure 2 indicated that there were close relationships between the spinning conditions and α-helix structures of molecules of spider silk. There was not a clear peak around 1650 cm^{-1} in the Raman spectrum of dragline silk secreted by a dropping spider; in contrast, the spectrum of silk from the spider bred in a carton had a distinct peak at 1650 cm^{-1}. The opposite Raman intensity of the reeled silk was similar to that of the glass box sample. The strength and initial modulus increased with the reel velocity and strain gave the opposite trend,[19] which implied that the high velocity was advantageous to the stretching and ordering of molecules of dragline silk.

TABLE II
Characteristics of Orientation in Dragline Silk[a]

Variable	Sample			
	Reeled	Carton	Glass box	Dropping
$I_{\perp}$ (1670)/$I_{\perp}$ (1445)	1.709	1.170	1.244	1.984
$I_{\parallel}$ (1670)/$I_{\parallel}$ (1445)	0.887	0.663	0.637	0.988
f_r	1.927	1.765	1.953	2.01

[a] $f_r = X_{\perp}/X_{\parallel}$; $X_{\perp} = I_{|}$ (1670)/$I_{\perp}$ (1445); $X_{\parallel} = I_{\parallel}$ (1670)/$I_{\parallel}$ (1445).

TABLE III
Tensile Properties of Dragline Silk of *Araneus ventricosus*

Property	Sample			
	Reeled	Carton	Glass box	Dropping
Breaking stress (Gpa)	1.07 ± 0.34	0.71 ± 0.26	1.11 ± 0.43	1.78 ± 0.30
Breaking strain (%)	26.29 ± 8.15	24.41 ± 8.48	25.23 ± 5.16	34.62 ± 5.72

The distribution of scatter intensity of amide III at 1220–1250 cm^{-1} reflected results similar to those of amide band I. The wavenumbers of peaks of dragline silk from the dropped spider were about 1222 and 1223 cm^{-1}, meaning that the polypeptide chains had β-sheet structures. In the perpendicular direction, there was a peak around 1240–1250 cm^{-1} for every sample, but the location changed under different spinning conditions. The amount of random coil molecules of dragline silk increased with the security of the growth environment.

It could be concluded that the spinning conditions and rearing environment have measurable effects on the molecular conformation of dragline silk. The safer the spinning environment of a spider, the more random coil and α-helix structures and the less β-sheet and lower-orientation molecules in the dragline silk.

The mechanical properties are consistent with these results. Table III shows the tensile properties of these dragline silk fibers. The silk from a dropping spider gave the highest strength and toughness, which was consistent with its comparatively oriented β-sheet structure. With an increase in environmental security, the breaking stress and toughness of spider dragline silk are apparently reduced.

CONCLUSIONS

A detailed study of the relationship between silk morphology and spinning conditions elucidates understanding spider silk properties, which is important for the production of man-made spider silk. It can provide the basis for designing methods and technology for the preparation of high-performance protein fibers.

Financial support for this work was provided by the National Science Foundation of China and Science and Technology Office of Jiangsu Province. The authors thank Prof. Z. Z. Shao of Fudan University, Shanghai, China, for help with Raman spectroscopy measurement and analysis.

References

1. Denny, M. J Exp Biol 1976, 65, 483.
2. Griffiths, J. R.; Salanitri, V. R. J Mater Sci 1980, 15, 491.
3. Kitagawa, M.; Kitayama, T. J Mater Sci 1997, 32, 2005.
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. Fahnestock, S. R.; Irwin, S. R. Appl Microbiol Biotechnol 1997, 47, 23.
6. O'Brien, J. P.; Fahnestock, S. R.; Termonia, Y.; Gardner, K. H. Adv Mater 1998, 10, 1185.
7. Liivak, O.; Blye, A.; Jelinski, L. W. Macromolecules 1998, 31, 2947.
8. Seidel, A.; Liivak, O.; and Jelinskit, L. W. Macromolecules 1998, 31, 6733.
9. Seidel, A.; Liivak, O.; Clave, S.; Adaska, J.; Ji, G.; Yang, Z.; Grubb, D.; Zax, D. B.; Jelinski, L. W. Macromolecules 2000, 33, 775.
10. Garrido, M. A.; Elices, M.; Viney, C.; Pérez-Rigueiro, J. Polymer 2002, 43, 1537.
11. Calvert, P. Nature 1998, 393, 309.
12. Vollrath, F.; Knight, D. P.; Hu, X. W. Proc R Soc Lond B Biol Sci 1999, 266, 817.
13. Madsen, B.; Vollrath, F. Naturwissenschaften 2000, 87, 148.
14. Shao, Z. Z.; Wu, D.; Li, G. X.; Peng, L. W.; Yu, T. Y.; Zheng, S. D. Chin J Light Scattering 1995, 7, 2.
15. Shao, Z. Z.; Vollrath, F.; Sirichaisit, J.; Young, R. J. Polymer 1999, 40, 2493.
16. Tsukada, M.; Freddi, G.; Monti, P.; Bertoluzza, A.; Kasai, N. J Polym Sci Part B: Polym Phys 1995, 33, 1995.
17. Pan, J. L. Application of Laser Raman Spectra in Organic Chemistry [M]; Chemical Industry Press: Beijing, 1986; p 121.
18. Knight, D. P.; Vollrath, F. Proc R Soc Lond B Biol Sci 1999, 266, 519.
19. Madsen, B.; Shao, Z. Z.; Vollrath, F. Int J Biol Macromol 1999, 24, 301.