ELSEVIER

Polymer 41 (2000) 3487–3490

polymer

Polymer Communication

A 45 Å equatorial long period in dry dragline silk of *Nephila clavipes*

L.D. Miller[1], R.K. Eby*

Department of Polymer Science, The University of Akron, Akron, OH 44325-3909, USA

Received 18 September 1998; received in revised form 9 August 1999; accepted 18 August 1999

Abstract

A small-angle peak has been found by X-ray scattering measurements on the dry dragline silk of *Nephila clavipes*. The weak peak, located on the equatorial axis, yields a long period of approximately 45 Å. Probably it corresponds to the lateral spacing of the crystals and is determined kinetically during the formation of the nanofibers.

Keywords: Dragline silk; Small angle X-ray scattering; Dry

1. Introduction

The dragline silk of *N. clavipes* is a remarkable material with quite respectable properties. Some of the notable characteristics are its high compressive strength in relation to its tensile strength and the benefits of ambient temperature liquid crystalline spinning from an aqueous solution. Still in question are aspects of the larger-scale morphology that could answer some questions about the basis for the physical properties.

The study of silks has a long history. There is a wide variety of dimensions present in the fibers of *N. clavipes* dragline. The crystals exhibit equatorial dimensions of 70–100 nm and larger in dark field TEM [1]. Scherrer analysis of wide angle X-ray diffraction data yields crystal dimensions ranging from 2 to 7 nm as determined from the (002), (200) and (120) peaks [2,3]. Falling into Group 3b of Warwicker's classification [4], the unit cell has dimensions of $a = 10.6$ Å, $b = 9.44$ Å, and $c = 6.95$ Å [5]. Small angle X-ray scattering (SAXS), yielded [2] an axial period of ~79 Å direction which has been reconfirmed [6]. Larger dimensions have been found with atomic force microscopy and SAXS. They yield results consistent with nanofibrils having transverse dimensions and correlation lengths ranging from $\gg 59$ to 160 nm and in the direction of the fiber axis from $\gg 100$ to 230 nm [7–9]. New in this note are long exposure X-ray measurements on dry dragline silk which have led to the discovery of a new dimension which has not been detected in previous experiments due to the relatively weak scattering.

* Corresponding author. Tel.: +1-330-972-5397; fax: +1-330-972-6581.

[1] Present address: Gates Rubber Co., 31-2-1-C1, Denver, CO 80217-5887, USA.

0032-3861/00/$ - see front matter
PII: S0032-3861(99)00609-6

2. Experimental

The sample used was a bundle of 28 800 dragline silk fibers which had been reeled at a rate of 1.1 cm s^{-1} from female golden orb weaver spiders, *N. clavipes*. The cross-sectional area was 0.54 ± 0.07 mm [2,8] and the density was 1.35 ± 0.005 g/cm [2,10]. The calculated mass absorption coefficient was 7.46 cm^2/g [11]. The silk was stored in a vacuum and in the dark. A Rigaku RU-300 rotating anode generator was used with accelerating voltage and filament current settings of 40 kV and 300 mA, respectively. The exposure times were 5, 8 and 10 days. An evacuated Statton camera at ambient temperature was used with a pressure of 1 Torr and two 0.051 cm pinholes spaced about 15.2 cm apart. The range of 2θ attainable was 0.009–0.445 rad. Photographic detection was employed using Kodak TP4414 film. Densiometric measurements were performed with a Molecular Dynamics Personal Densitometer running ImageQuant software version 1.3. The resolution was 0.005 cm. Subtraction of any background scattering and inherent film density was performed using code written in Visual Numerics' PV-WAVE Command Language. Peak fitting was performed by using PeakFit™ version 4.00 by Jandel Scientific Software.

3. Results and discussion

As noted above, there is a peak on the meridian at a spacing of 76 Å. Fig. 1 shows a low angle exposure containing this peak. A similar meridional spacing has been reported for the cocoon silk of *B. mori* [12]. The 76 Å spacing is qualitatively consistent with the length of a

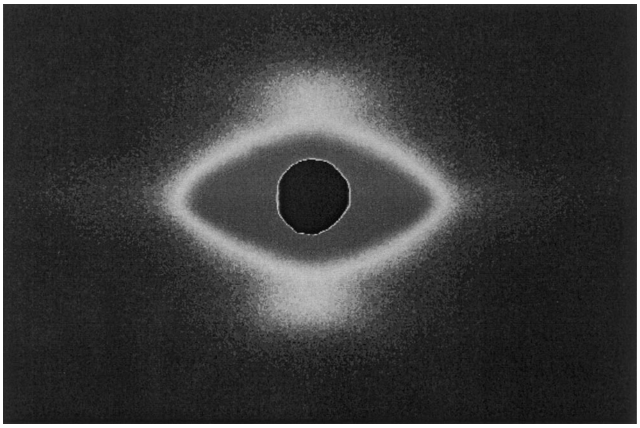

Fig. 1. Small angle exposure of *N. clavipes* dragline silk. The fiber axis is parallel to the meridian. Note the 76 Å peak along the meridion. The dark region at the center of the image is the beam stop. The exposure time was ten days.

genetically determined "crystallizabile" sequence followed by a "non-crystallizable" sequence of amino acids in the molecule [2]. With long exposure times, a new peak located at ~45 Å on the equator can be seen (Fig. 2) for dry dragline silk. Due to its closeness to the small angle scattering region, careful measurements were necessary. In fact, the peak is a shoulder of the small angle region. Fig. 3 shows the scattered intensity as a function of 2θ. Subtraction of an appropriately scaled exposure without the sample in place was performed to remove background scattering. Another correction was made for film darkening not from the actual scattering experiment. This could be of the form of film fog resulting from a poor seal of the film cassette from light during the exposure. Inherent darkening of the film was corrected for by subtracting a term assumed to be constant over the area of the film.

As noted above, the meridional correlation could be the

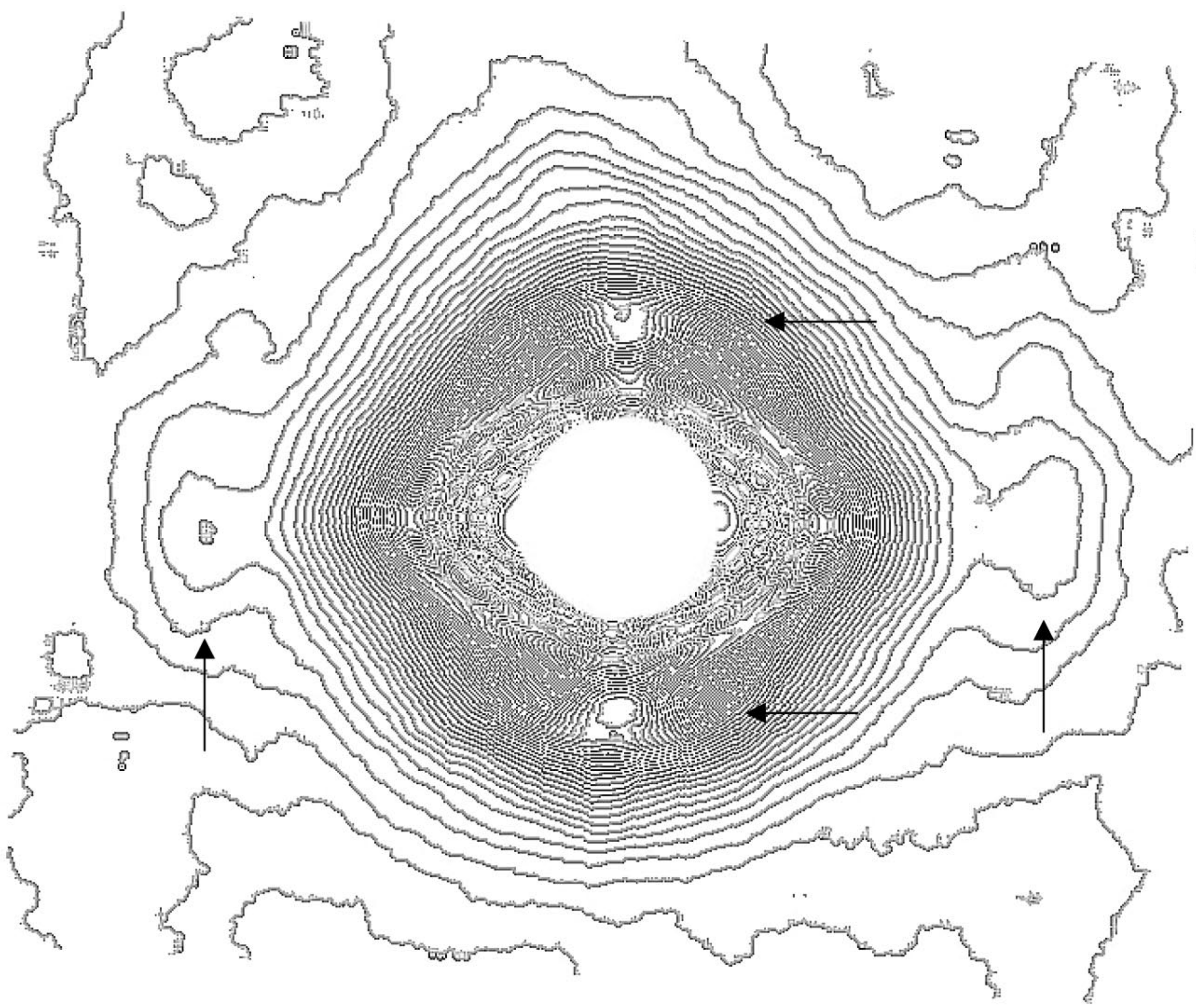

Fig. 2. Iso-intensity contours of small angle scattering from *N. clavipes* dragline. Arrows oriented vertically point toward the 45 Å peak. Arrows oriented horizontally point toward the 76 Å peak. The exposure time was 8 days.

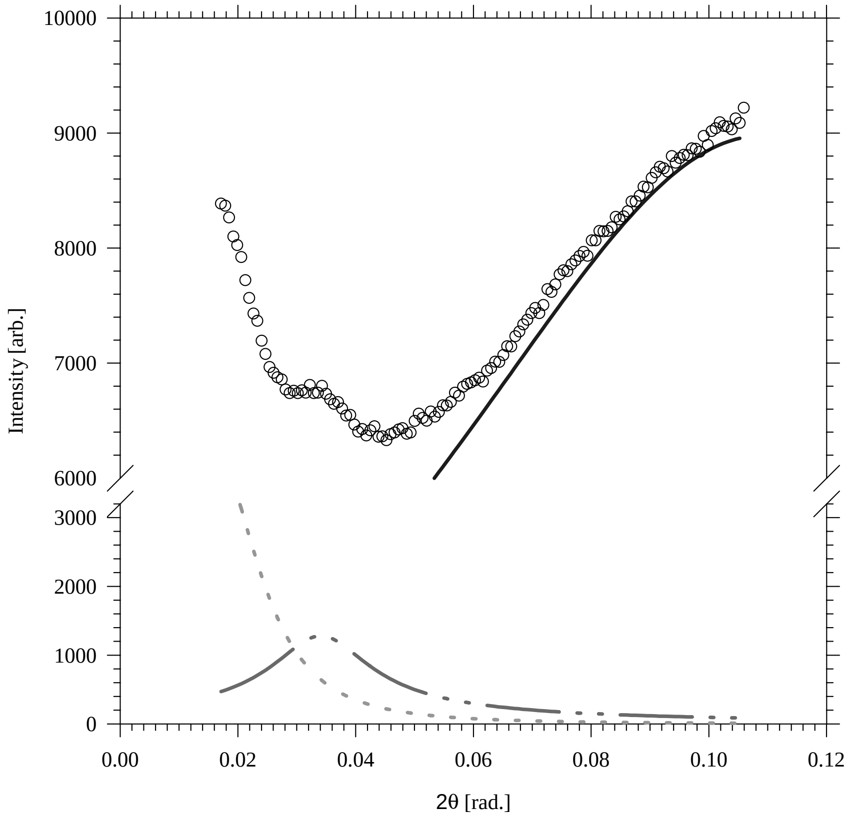

Fig. 3. Equatorial profile of intensity. The peak centered near 0.0345 rad, has the 45 Å long period. The two additional lines were used to fit the scattering at higher and lower angles. The exposure time was 5 days.

genetically determined spacing of the crystals. The equatorial correlation, on the contrary, would probably be determined kinetically during the formation of the nanofibrils, which appear to fill much of the interior of silk fibers [7–9,13–15]. Thus, the meridional might be expected to be the stronger one. Although it is possible that there is a regular three-dimensional array of the crystallites, this does not seem likely. If this were the case, other equatorial and/or off-axis peaks might be expected. Such peaks could be “hidden” behind the small angle region, coincident with the peak at 45 Å or lie at greater angles. However, the results are probably more consistent with a more regular meridional and a more irregular equatorial arrangement of the crystallites such as that developed from the patterns resulting from etching the silk with Argon ions [16]. The relationship of the 45 Å spacing in dry fibers to the 60 Å spacing reported for wet fibers is not clear [17]. They might be from related morphological features since the unrestrained dragline silk of *N. clavipes* has been reported to swell in diameter by about 30% when wetted [18]. More work is needed.

4. Conclusions

Through long exposures, a new equatorial period of ~45 Å has been found for dry dragline silk of *N. clavipes*. It and the previously reported period of ~76 Å [2] are qualitatively consistent with a morphological model based on the results of etching the silk by Argon ions [17].

Acknowledgements

For help in acquiring and using X-ray time, we acknowledge W. Wade Adams, Douglas S. Dudis, and Gary Price of the Materials Directorate, Wright Patterson Air Force Base. We are in debt to Mark K. Stowe of the Department of Zoology at the University of Florida, for providing spiders.

References

[1] Thiel BL, Kunkel DD, Viney C. Biopolymers 1994;34:1089 See also Thiel BlL, Guess KB, Viney C. Biopolymer. 1997;41:703.
[2] Mahoney DV, Eby RK. In: Kinlock AJ, editor. Proceedings of the Ninth International Conference on Deformation Yield and Fracture of Polymers. 13-1, Institute of Materials, London. 1994. See also Eby RK. Am Crystallographic Assoc, Series 2, vol. 23, 1995. p. 68. See also Mahoney DV. PhD dissertation. The University of Akron, 1994, p. 115.
[3] Grubb DT, Jelinski LW. Macromolecules 1997;30:2860–7.
[4] Warwicker JO. J Mol Biol 1960;2:354.
[5] Becker MA, Mahoney DV, Lenhert PG, Eby RK, Kaplan DL, Adams WW. In: Kaplan DL, Adams WW, Farmer BL, Viney C, editors. Silk polymers, ACS Symposium Series 544, 1994. p. 185.

[6] Grubb DT, Jackrel D, Jelinski LW. Polym Prepr 1997;38(2):73–4.
[7] Li SFY, McGhie J, Tang SL. New internal structure of spider dragline silk revealed by atomic force microscopy. Biophys J 1994;66:1209–12.
[8] Mahoney DV, Vezie DL, Eby RK, Adams WW, Kaplan DL. In: Kaplan DL, Adams WW, Farmer BL, Viney C, editors. Silk polymers, ACS Symposium Series 544, 1994. p. 196.
[9] Miller LD, Puttanarat S, Eby RK, Adams WW. Int J Biol Macromol 1999;24:159.
[10] Zemlin JC. A study of the Mechanical Behavior of Spider Silks. Technical report to the US Army Natick Laboratories (report 69-29-CM), Collaborative Research, Inc., September 1968.
[11] Mahoney DV. PhD dissertation. University of Akron. 1994. p. 81.
[12] Fraser RDB, MacRae TP. Conformation in fibrous proteins and related synthetic polypeptides, New York: Academic Press, 1973. p. 314–5.
[13] Vollrath F, Holtet F, Thorgersen HC, Frische S. Proc R Soc London. Series B, Biological Science 263, 1996;1367:147.
[14] Putthanarat S, Eby RK, Adams WW, Liu GF. Pure Appl Chem 1996;A33(7):899 See also Putthanarat S. MS thesis. The University of Akron, 1995.
[15] Putthanarat S, Stribeck N, Fossey SA, Eby RK, Adams WW. Submitted for publication.
[16] Kitagawa M, Kitayama T. J Mat Sci 1997;32:2005 See also Kitagawa, Sasagawa H. In: Kinlock AJ, editor. Proceedings of the Ninth International Conference on Deformation Yield and Fracture of Polymers. 39-1 Institute of Materials, London. 1994. See also Kitagawa M, Katsumi S, Wakoh Y. J Soc Mat Sci Jpn. 1997;46:470.
[17] Yang Z, Grubb DT, Jelinski LW. Macromoluecules 1997;30:8254.
[18] Fornes RE, Work RW, Morosoff N. J Polym Sci: Polym Phys 1983;21:1163.