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Multiaxial anisotropy of spider (*Araneus diadematus*) cocoon silk fibres

J.Y.J. Barghout[a], J.T. Czernuszka[a], C. Viney[b,*]

[a]*Department of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, UK*
[b]*Department of Chemistry, Heriot-Watt University, Edinburgh EH14 4AS, UK*

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Abstract

We report a remarkable feature of the cocoon silk spun by *Araneus diadematus* spiders, as revealed by electron diffraction: *transverse* sections of the fibres exhibit significant microstructural anisotropy. Although the mechanical processes of fibre spinning and drawing are uniaxial, the spider is able to achieve multiaxial control of molecular order. In contrast, complex flow patterns are used to obtain multiaxial control of molecular order in synthetic polymer extrudates or fibres. Possible origins and consequences of the transverse anisotropy in *A. diadematus* cocoon silk are considered briefly. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Multiaxial anisotropy; *Araneus diadematus*; Spider silk

1. Introduction

Studies of spider and insect silks are stimulating new approaches to the production of high-performance fibres [1–4]. Particular attention has been focused on major ampullate (drag line) spider silk, because it exhibits impressive values of tensile strength, stiffness and toughness. All natural silks are spun under environmentally benign conditions and are biodegradable. The potential exists to use the techniques of genetic engineering to tailor specific silk properties [5]. The crystal-like reinforcing regions in spider silk arise from statistical rather than perfect matches between adjacent chains [6–8], so the effectiveness of reinforcement is insensitive to both the rate of fibre spinning and the ambient conditions. Spinning is accompanied by formation of a liquid crystalline phase [9,10], facilitating the flow-induced physical transition to water-insoluble fibre.

Here we describe another instance of a spider silk exhibiting characteristics that are difficult to achieve when synthetic polymers are processed. Electron diffraction (performed in a transmission electron microscope (TEM)) reveals significant microstructural anisotropy in *transverse* sections of *Araneus diadematus* (garden spider) cocoon fibre. In other words, the spider is able to achieve multiaxial control of molecular order, even though the silk fibre is spun and drawn uniaxially and is therefore approximately cylindrical. To obtain multiaxial control of molecular order in synthetic polymer extrudates [11–13], it has been necessary to resort either to complex flow patterns (yielding a product that has a pronounced rectangular cross-section), or to rotating dies. Alternatively, use of a magnetic field at 90° to the machine direction has been proposed as a means of generating transverse anisotropy in liquid crystalline polymer extrudates [14].

2. Experimental

A. diadematus cocoons were collected from a natural habitat. Fibre was recovered from the inner layer; any debris was removed with surgical tweezers under a stereomicroscope, taking care to avoid damaging the silk. The fibre was then dehydrated in dry acetone, rolled into a loose ball, and embedded in epoxy resin (Hard Plus grade, TAAB Ltd, Aldermaston, UK). Samples were oven cured at 60°C for 24 h. We do not expect this resin curing treatment to affect the silk microstructure: previous work has shown that the molecular organisation in samples of silk fibroin is unaffected by exposure to 100°C temperatures for times exceeding 50 h [15]. A Sorval MT5000 ultra-microtome (DuPont, Stevenage, UK) equipped with a diamond knife was used to cut sections 60 nm thick, which were floated onto 400 mesh 3 mm diameter copper grids (Agar Scientific, Stansted, UK). Because dimensional changes are constrained by the presence of the mounting medium, we do not expect the silk to exhibit significant water uptake or any tendency to supercontract [16]. The sections were examined in a JEOL

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* Corresponding author. Tel.: +44-131-451-3759; fax: +44-131-451-3180.
E-mail address: c.viney@hw.ac.uk (C. Viney).

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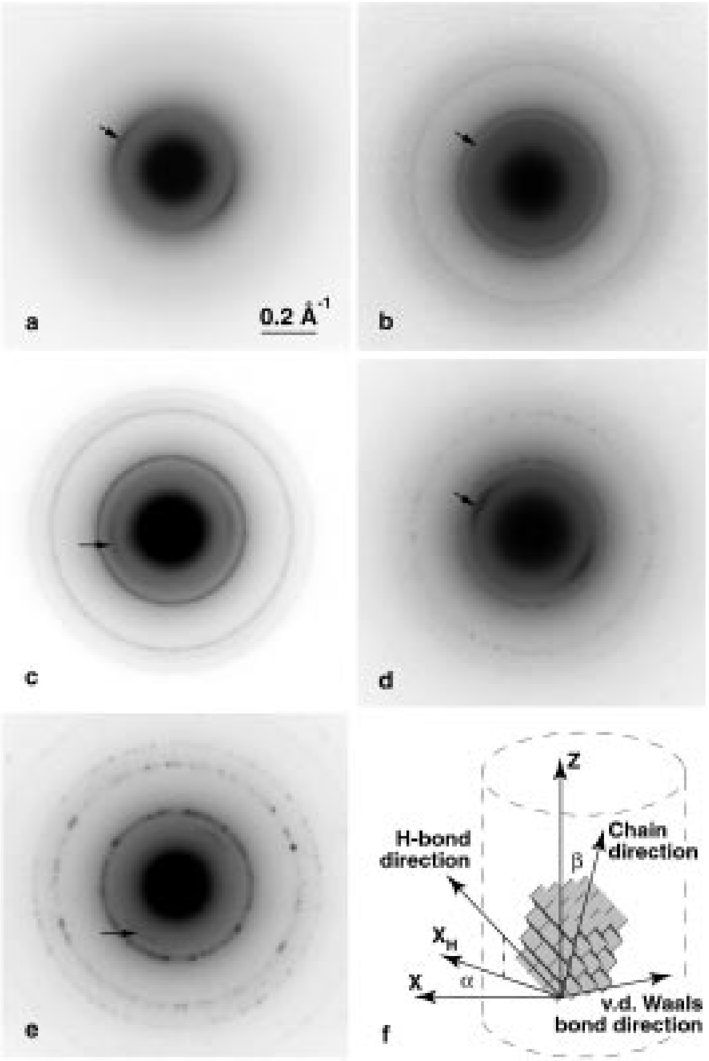

Fig. 1. Arrows indicate arcs — or a ring in the case of pattern (b) — that arise from diffraction by silk. The composite figure was compiled digitally from scanned diffraction patterns. (a) Diffraction pattern obtained from a transverse section of cocoon silk; parallel beam. (b) Diffraction pattern obtained from a transverse section of major ampullate silk; parallel beam. (c) Diffraction pattern obtained from an off-longitudinal section of cocoon silk; parallel beam. (d) Local diffraction pattern obtained from a transverse section of cocoon silk; convergent beam. (e) Local diffraction pattern obtained from an off-longitudinal section of cocoon silk; convergent beam. (f) Part of a randomly-chosen β-sheet crystal within a cocoon silk fibre, with significant directions identified. Z is parallel to the fibre axis. X is an arbitrary but consistently-chosen direction lying in the transverse section of the fibre. X_H is the projection of the hydrogen bond direction onto the transverse section. The angle α between X_H and X is defined within the transverse section; the angle β defines the tilt of the chain direction relative to Z. Neighbouring crystals would be characterised by values of α and β similar to those exhibited by the example shown. Crystals further away would still exhibit similar values of α, but their values of β would vary more widely.

4000EX TEM operating at 400 kV and 128 μA and equipped with a liquid nitrogen cooled stage (Gatan, Corby, UK). The safe exposure levels for this combination of imaging conditions and specimen type have been assessed previously [8]. Parallel and focused beams were used to characterise global and local crystalline order, respectively. For parallel beam work, the whole fibre section was illuminated. Focused beam studies were performed by illuminating particular areas of the specimen with a 0.7 μm diameter spot. Diffraction patterns were recorded on Agfa Scientia EM film (Agfa, Brentford, UK).

The sample preparation method provides no control over the orientation at which a given fibre is cut, but it does yield TEM specimens in which several fibre sections can be located easily before significant beam damage occurs. Transverse sections are encountered frequently; they are approximately circular and well supported by the embedding medium, and they remain intact during sample preparation. In contrast, thin longitudinal sections are less well supported, and so are extremely susceptible to pull-out or creasing. Results described here were obtained from transverse sections and off-longitudinal sections, the latter having normals inclined within the range $60° \leq \phi \leq 80°$ relative to the fibre axis. The angle ϕ was estimated from the dimensions of the fibre section, using $\phi = \cos^{-1}(x/y)$ where x and y, respectively, are the width (fibre diameter) and length of the section.

Most diffraction patterns contained sharp rings populated by diffraction spots due to polycrystalline ice. These rings can be indexed accordingly, and so provide a convenient internal calibration. We expect the ice to associate preferentially with the amorphous regions in silk [8]. Formation of ice can be avoided by working at temperatures above -113°C at the prevailing pressure of 10^{-7} Torr, but this is accompanied by a decrease in specimen lifetime and therefore in the quality of the diffraction detail obtained from the specimen. Alternatively, a jacket and cold trap can be provided in the vicinity of the specimen; however, this severely restricts the freedom to vary the specimen tilt relative to the beam.

3. Results and discussion

3.1. Parallel beam conditions

Diffraction patterns for transverse sections of cocoon silk are characterised by a well-defined pair of arcs that correspond to an interplanar spacing of 4.6 ± 0.05 Å as read from the film (Fig. 1a). This observation of a crystallographic texture in *transverse* sections of a fibre is surprising. Fibres that are spun and drawn artificially have uniaxial symmetry, with the unique axis lying along the fibre. We are confident that the unexpected anisotropy of the transverse sections of cocoon silk is not an artefact of the specimen preparation technique. A study of transverse sections taken from 10 different fibres showed that there is no correlation between the cutting direction and the orientation of the arcs in the corresponding diffraction pattern. Also, we note that transverse sections of *A. diadematus* major ampullate (drag line) silk, prepared and examined in the same way as the sections of cocoon silk, yield diffraction patterns containing the uniform rings characteristic of a uniaxial fibre (Fig. 1b).

The lattice parameters of crystals in *A. diadematus* cocoon silk have yet to be measured, so we cannot definitively assign indices to the 4.6 Å reflexion. However, noting that the observed reflexions should be restricted to the simple cases of *hk*0 in transverse sections, and given the homology of diffraction patterns obtained from related fibroins [17], we tentatively associate our 4.6 Å reflexion with the very strong 4.72 Å X-ray peak which has been obtained from *Nephila senegalensis* cocoon silk and indexed [17] as 020. Under current convention, this peak is indexed as 200, where the crystallographic x-axis is assigned to the direction of hydrogen bonds between chains in a β-sheet, and the y-axis corresponds to the inter-sheet direction. The *h*00 spacings are expected to show little variation between fibroins from different sources.

Diffraction patterns from the off-longitudinal sections also showed well-defined arcs (Fig. 1c). The occurrence of a texture in such off-longitudinal sections is unremarkable, as spinning and drawing achieve a degree of molecular alignment along the fibre axis. Crystal reflexions from off-longitudinal sections are not restricted to simple subsets of *hkl*, and, because the lattice parameter b in fibroin crystals is sensitive to the local side chain chemistry [6,16,18], the reflexions should not be identified by analogy with indexed patterns from other silks.

3.2. Focused beam conditions

For transverse sections, the anisotropy observed under parallel beam conditions is still apparent when a convergent (focused) beam is used (Fig. 1d). Neither the azimuthal spread of the 4.6 Å (or 200) arcs, nor the preferred orientation direction defined by these arcs, varies significantly across the transverse sections, i.e. over an area of approximately 5 μm^2. The local diffraction patterns always resemble the pattern obtained when the entire section is illuminated. There is a wider variation in the azimuthal spread of diffraction arcs, as well as in the preferred direction of orientation defined by the arcs, across off-longitudinal sections. The azimuthal spread in local diffraction patterns (Fig. 1e) is always less than the spread in the corresponding global pattern. Although chain orientation persists over distances that correspond to a few times the 0.2 μm maximum crystal size, the alignment is significantly less well preserved across the specimen as a whole.

3.3. Process-related considerations

Fig. 1f summarises the results described above. The preferred orientation of the intracrystalline hydrogen bonds (and of the intracrystalline van der Waals bonds, along a different direction) in transverse sections of cocoon silk demonstrates that three-dimensional (3D) control of molecular order in semicrystalline polymer fibres is possible. 3D tailoring of mechanical properties can therefore be realised in these fibres. The mechanism by which the spider achieves such control remains to be determined; any explanation must be able to account for the absence of transverse anisotropy in major ampullate silk as well as its presence in cocoon silk. A possibility for consideration is that the removal of water from the incipient fibre, which occurs through the cuticle lining the duct [19], could be made to occur at different rates in different radial directions in the cocoon silk. Anisotropic rates of water removal have previously been suggested as the cause of complex orientation patterns in dogfish egg case [20] (an extruded collagenous composite). Alternatively, the anisotropy may arise from the geometrical changes induced during silk flow through the duct/spinneret system. There is also the question of why (or even whether) the spider might benefit from the transverse sections of major ampullate and cocoon silks exhibiting such divergent characteristics. The principal role of major ampullate silk is to carry tensile loads, in which case there is no need for transverse anisotropy.

Cocoon silk is used to construct a tangled mat of material that surrounds and protects the eggs. At any point along its length, the cocoon fibre must be able to bend easily in one plane but otherwise resist bending or stretching; a multiaxially anisotropic microstructure of the type described here can impart these mechanical properties.

4. Conclusions

1. Transverse sections of *Araneus diadematus* spider cocoon silk fibres exhibit significant microstructural anisotropy.
2. Neighbouring β-sheet crystals in this silk are orientationally correlated as regards: (a) their chain axis direction; and (b) their rotations about the chain axes. The latter correlation persists over longer distances than the former.
3. This anisotropy is not observed in transverse sections of major ampullate silk produced by the same species of spider.

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