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Stretching of supercontracted fibers: a link between spinning and the variability of spider silk

G. V. Guinea, M. Elices*, J. Pérez-Rigueiro and G. R. Plaza

Departamento de Ciencia de Materiales, Universidad Politécnica de Madrid, ETS de Ingenieros de Caminos, Ciudad Universitaria, 28040 Madrid, Spain

*Author for correspondence (e-mail: melices@mater.upm.es)

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Summary

The spinning of spider silk requires a combination of aqueous environment and stretching, and the aim of this work was to explore the role of stretching silk fibers in an aqueous environment and its effect on the tensile properties of spider silk. In particular, the sensitivity of the spider silk tensile behaviour to wet-stretching could be relevant in the search for a relationship between processing and the variability of the tensile properties. Based on this idea and working with MAS silk from *Argiope trifasciata* orb-web building spiders, we developed a novel procedure that permits modification of the tensile properties of spider silk: silk fibers were allowed to supercontract and subsequently stretched in water. The ratio between the length after stretching and the initial supercontracted length was used to control the process. Tensile tests performed in air, after drying, demonstrated that this simple procedure allows to predictable reproduction of the stress–strain curves of either naturally spun or forcibly silked fibers. These results suggest that the supercontracted state has a critical biological function during the spinning process of spider silk.

Key words: spider, silk, *Argiope trifasciata*, spinning, tensile properties, supercontraction.

Introduction

The combination of high tensile strength and elongation exhibited by spider silks, specifically silk produced from the major ampullate gland of orb-web spinning spiders (MAS fibers), gives rise to extremely tough fibers (Kaplan et al., 1991; Viney, 2000) that are the basis of a new family of protein-based materials produced through genetic engineering (Lazaris et al., 2002). These outstanding properties are imparted to the silk by means of a complex semi-crystalline microstructure (Marsh et al., 1955; Simmons et al., 1996; van Beek et al., 1999; Jelinski et al., 1999), which is a result of the combination of genetically determined traits, encoded in the silk proteins (Work and Young, 1987; Craig, 1997), and of the processing conditions (Jin and Kaplan, 2003).

The spinning of spider silk faces two major technological challenges: (i) synthesising an insoluble fiber from an aqueous solution of extremely large proteins (Xu and Lewis, 1990) and (ii) ensuring the capability to modify the tensile properties of the fiber within a time scale of fractions of a second (Garrido et al., 2002a). Not surprisingly, spider silk glands have evolved as highly specialized and sophisticated systems, where some distinct anatomic and physiological features can be identified. The spinning of silk starts with a highly concentrated protein solution produced in a sac in the initial region of the silk glands (Vollrath and Knight, 2001). The sac is anchored by the funnel to an S-shaped duct that tapers down to the spigot. The initially isotropic protein solution acquires a liquid crystal structure in the funnel (Kerkam et al., 1991; Jin and Kaplan, 2003), so that it can flow more easily through the narrow duct. The liquid crystal structure could also promote the pre-alignment of the fibers prior to full fiber formation. The fiber is formed from the solution in the third limb of the duct, as is seen from the separation of the lateral surface of the fiber from the walls of the duct (Vollrath and Knight, 2001), and emerges through the lips of the spigot. The identification of several structures along the duct and in the spigot responsible for recovering water initially present in the aqueous solution suggests that water content might be a critical parameter during spinning.

Interestingly, MAS fibers show a peculiar behaviour if unrestrained and submerged in water, known as supercontraction (Work, 1977). This effect is characterized by a shrinkage of the fiber, exceeding 50% of its initial length, and by a dramatic change in its mechanical behaviour. Dry fibers, either naturally spun (NS) or forcibly silked (FS), behave as glassy polymers (Fig. 1). Initially the stress–strain curve is stiff and shows a linear-elastic regime, up to a yielding point. At this point the slope of the curve decreases to a minimum and starts to increase gradually until breaking point. By contrast, supercontracted fibers tested in water (SCW) show a tensile behaviour that resembles that of an elastomer (Gosline et al., 1984), characterized by a very low initial elastic modulus (Fig. 1) and large (in excess of 100%) strain at breaking. When dried, supercontracted fibers show an initial high stiffness

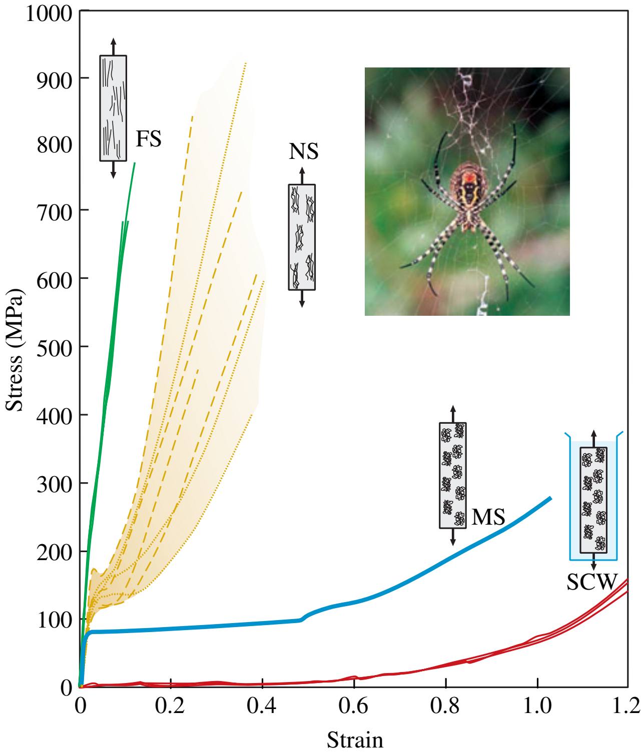

Fig. 1. Stress–strain curves of spider silk (MAS from *A. trifasciata*, see inset). FS, forcibly silked fibers. NS, naturally spun fibers, i.e. fibers retrieved either from the web or from the safety line. MS, maximum supercontracted fibers, i.e. fibers subjected to supercontraction and subsequent drying. All tests in air were performed at 20°C, 35%RH and strain rate 0.0002 s^{-1}. SCW, supercontracted fibers tested in water at 20°C and strain rate 0.0002 s^{-1}.

elastic regime with strain at breaking that exceeds 100%. This state is labelled as maximum supercontraction (MS). The different tensile properties of dry and supercontracted spider silk fibers have been modelled through a double network of hydrogen bonds and protein chains (Termonia, 2000). The model assumes that supercontracted fibers correspond to a state where the hydrogen bond network is molten and the tensile properties are controlled by the stretching of the protein chains, leading to the observed elastomeric behaviour. Stretching promotes the re-alignment of the protein chains, which can be frozen by re-establishing the hydrogen bond network. Despite its success in simulating the tensile properties of spider silk (Termonia, 2000), it was not clear whether this process did actually occur at any step during spinning or during the in-service life of the spider silk fiber, contributing to the controversy on the biological significance of the supercontraction effect (Bell et al., 2002).

The search for a biological role of supercontraction, possibly in combination with stretching, led us to consider the processing of the fiber as a situation where the conditions required by both effects meet. In this context, mimicking the range of tensile properties displayed by naturally spun silk fibers by stretching supercontracted fibers would support the hypothesis that the combination of a fiber under supercontrated state and simultaneous stretching play a significant role during the spinning of spider silk. In this work we show that the whole range of tensile properties exhibited by spider silk (Madsen et al., 1999; Garrido et al., 2002b) can be predictably reproduced by simply controlling the deformation of supercontracted fibers stretched in water. Since the fiber is subjected to similar influences during spinning, these results point to a critical biological function of the supercontracted state during the spinning process of silk fibers.

Materials and methods

Silk fibers from the major ampullate glands *(*MAS*)* of *Argiope trifasciata* Forskäl spiders were used in this work. *A. trifasciata* is a common orb-web-building species in the Mediterranean coastal area that can be bred in captivity, and whose size allows easy manipulation. It has been shown that *A. trifasciata* silk fibers share most of their specific features with those obtained from a number of spiders (Work, 1976; Griffiths and Salinatri, 1980; Pérez-Rigueiro et al., 2001) such as *Araneus* sp. and *Nephila* sp., so the results reported below should also be extended to these species. Spiders were fed on a diet of crickets. Silk fibers were collected by FS (Work and Emerson, 1982), by pulling the silk fiber from the spider at a controlled speed of 1 cm s^{-1} at 20°C and 35% relative humidity (RH). The spider was not anaesthetised at any step of the process. The samples were stored at 20°C and 35% RH until testing. Details of the tensile testing procedure are given elsewhere (Guinea et al., 2003). Briefly, silk fibers (base length=20 mm) were mounted on aluminium foil frames and fixed in the grips of a testing machine (Instron 3309-622/8501, Canton, MA, USA) that was appended to an environmental chamber (Dycometal CCK-25/300, Barcelona, Spain). The fiber was surrounded with a silicone tube fixed to the lower grip, and water was added/removed to/from the silicone tube using a syringe. Forces were measured by a 100 mN load cell with 0.1 mN resolution (HBM 1-Q11, Darmstadt, Germany). The displacement of the grips was taken as a direct measure of the fiber's deformation, since the compliance of the fiber is 1000 times larger than that of the experimental set up (Pérez-Rigueiro et al., 1998). Only adjacent samples were used for the tests, to minimize experimental scatter (Work, 1977). In addition, reproducibility was checked by testing one out of every five samples as a control. No significant deviation was observed in the tensile properties of the control samples.

The experimental procedure for stretching supercontracted fibers is sketched in Fig. 2. Initially, an FS fiber glued by its ends to the aluminium foil frame is immersed in water at 20°C for 10 min. The fiber is allowed to contract unrestrained up to the supercontracted length, L_{SC} (Fig. 2A). The fiber is stretched up to the selected length, L_A, and the ends are clamped in this position (Fig. 2B). Water is removed after 10 min and the fiber allowed to dry overnight at 20°C and 35%RH (Fig. 2C). Stresses build up in the fiber during drying, probably due to

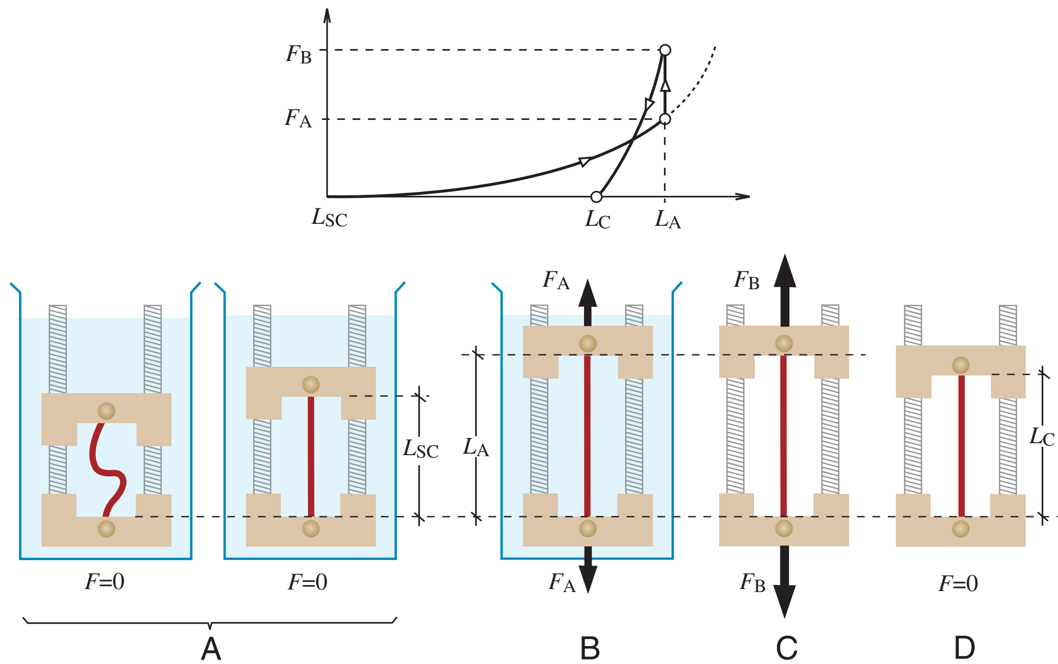

Fig. 2. Procedure for stretching supercontracted fibers. The fiber is allowed to fully supercontract in water at 20°C for 10 min down to a length L_{SC} (A). The fiber is stretched up to length L_A with a force F_A and their ends are clamped in this position (B). Water is removed after 10 min and the fiber is allowed to dry overnight. Stresses are built in up to a force F_B as a result of drying (C), and are allowed to relax by unloading to a final length L_C (D). The length L_C is considered as the base length to calculate strain. Stresses are calculated under the assumption that the volume remains constant throughout the process. The evolution of the forces during the process are sketched in the inset as an F–L plot. F_A, force required to stretch to length L_A; F_B, force built up in the fiber after drying.

the restoration of the hydrogen network that tends to shrink the fiber (Guinea et al., 2003; Savage et al., 2004). The fibers are allowed to relax by unloading down to the final length L_C (Fig. 2D).

Results and discussion

Fig. 1 compares the tensile behaviour of spider silk fibers obtained by FS, retrieved from the web or from the safety line (NS) (Garrido et al., 2002b), supercontracted and tested in water (SCW) and supercontracted and tested in air (MS) (Pérez-Rigueiro et al., 2003). All tests in air were performed under nominal conditions 20°C and 35%RH. Tests in water performed at 20°C. Engineering stress and strain, defined as the measured force divided by the initial cross-section, and length increment divided by initial length, respectively, are plotted. The cross-sectional area of FS and NS were assessed from diameter measurements at both ends of the fiber, assuming a circular shape (Pérez-Rigueiro et al., 2001). The cross section of supercontracted fibers (either SCW or MS) was computed from the cross section of the novel FS fiber after measuring the supercontracted length and assuming constant volume. The hypothesis of constant volume has been conventionally accepted both for silkworm silk (Pérez-Rigueiro et al., 1998) and spider silk, and recently been proved (Pérez-Rigueiro et al., 2003; Elices et al., 2004; Plaza, 2004).

Spider silk fibers tested in air behave as glassy polymers. This tensile behaviour can be explained as the result of a network of hydrogen bonds between chains (Termonia, 2000), which accounts for the high initial tensile modulus (E≈10 GPa) and breaks after yielding. Subsequent stretching leads to the extension of a network of protein chains and a rotation of microcrystallites, and induces a net molecular alignment parallel to the fiber axis (Gosline et al., 1999; Grubb and Gending, 1999). The stress–strain curve of supercontracted FS fibers tested in water (SCW) shows an elastomeric behaviour, which can be explained as the result of water molecules acting as a plasticizer (Gosline et al., 1984), by disrupting the network of hydrogen bonds. This explanation is supported by X-ray diffraction (Work and Morosoff, 1982; Grubb and Gending, 1999) and Raman spectroscopy data (Shao et al., 1999a,b). The chain conformation of proteins would be kept highly flexible in supercontracted fibers, but it could be frozen after drying by promoting the formation of protein–protein hydrogen bonds, thus re-establishing the hydrogen bond network and leading to behaviour observed in MS fibers.

The effect of stretching on supercontracted fibers and subsequent drying is illustrated in Fig. 3 (test conditions: 20°C, 35%RH, strain rate 0.0002 s^{-1}). Engineering strain is calculated from the length of the unloaded fiber after drying, L_C, and the engineering stress is calculated under the assumption that volume remains constant throughout the process. The stretching process is characterized by the alignment parameter defined as $\alpha = L_C/L_{SC} - 1$. Very good

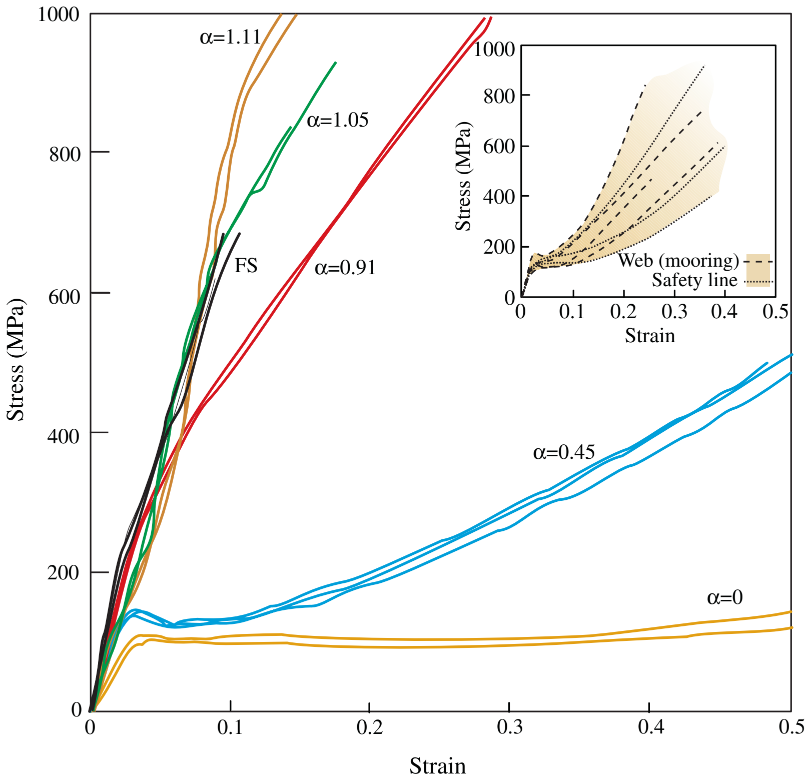

Fig. 3. Stress–strain curves of supercontracted fibers subjected to stretching in water and drying (tensile testing conditions 20°C, 35%RH, strain rate 0.0002 s^{-1}). The stretching process is characterized by the alignment parameter, α, as defined in the text. At least two samples were tested for each alignment parameter, showing the remarkable reproducibility of the process. Stress–strain curves of forcibly silked (FS) fibers are shown for comparison. The inset shows the whole range of curves produced by the naturally spun (NS) silk when building the web, or from the safety line.

repeatability (unusual when dealing with spider silk fibers) was achieved using this process, as is shown by the similar stress–strain curves presented by the three fibers tested for each alignment parameter.

A value of zero for the alignment parameter corresponds to dried supercontracted fibers, i.e. supercontracted fibers not subjected to stretching. The tensile behaviour of dried supercontracted fibers (labelled as MS) have been described previously (Pérez-Rigueiro et al., 2003), and it has been found that MS fibers represent a lower limit of the tensile properties that can be reached by spider silk fibers tested in air, regardless of the previous loading history of the fiber. Stretching the fiber leads to an increase in L_C and, consequently, of the alignment parameter. The collection of stress-strain curves displayed by NS fibers (see inset in Fig. 3) is regained for alignment parameters ranging from α≈0.4 to α≈0.8, and it is illustrated by the curves corresponding to α≈0.45. Alignment parameters in the range from α≈0.9 to α≈1.1 yield fibers with stress-strain curves similar to those of forcibly silked fibers (FS; shown to allow direct comparison). These values of the alignment parameter indicate that the FS fiber length is double that of supercontracted fibers, as expected from the approximate 50% reduction of the FS fiber length when subjected to supercontraction. Consistent with the hypothesis that stretching leads to a better alignment of the protein chains, it is apparent from Fig. 3 that overall stiffness increases monotonically with the alignment parameter, although the behaviour of the elastic modulus and the proportional limit is slightly more complex (see Table 1). Tensile strength follows a comparable trend, despite the low Weibull modulus of spider silk (Pérez-Rigueiro et al., 2001) that implies a large scattering on the values of this parameter.

Our results suggest that a similar stretching and drying process can account for the variability observed in the tensile properties of MAS fibers, and places supercontraction, or more accurately the supercontracted state of spider fibers, as a central feature of the spinning process. Although the details of the spinning process remain largely unknown, the results of the present work are consistent with the finding that spiders use a friction brake to control the force exerted on the fiber (Ortlepp and Gosline, 2004). In this context, applying a controlled stress to the fiber in the final stages of silk production, when the silk exits the spigot but is likely to remain hydrated for a short period of time, would lead to the observed variability in the tensile properties of spider silk. It has also been shown that the stresses exerted on the fiber by the friction brake can account for the stresses involved in the supercontraction process (Guinea et al., 2003; Savage et al., 2004). It is interesting to notice that the low forces involved in stretching supercontracted fibers up to large deformations (strain values up to 1.0) permit the modification of the tensile properties with a minimum expense of energy.

From a technological perspective, the stretching and drying process enables production of spider silk fibers with a tailored and pre-established stress–strain profile in a reliable and repetitive way. The procedure illustrated in this work casts light on the natural spinning process and should be helpful for the design of spinning processes currently being developed by

Table 1. *Tensile parameters computed from the stress–strain curves of Fig. 3*

Alignment parameter	E (GPa)	σ_p (MPa)	σ_u (MPa)	ε_u
0	3.2±0.4	100±20	440±20	1.2±0.1
0.45	5.6±0.4	135±4	600±200	0.6±0.1
0.91	8.6±0.6	300±20	900±200	0.25±0.08
1.05	7.1±0.2	142±8	800±200	0.13±0.06
1.11	7.3±0.6	130±2	1100±100	0.17±0.04
FS	10.4±0.6	260±10	690±60	0.11±0.02

Values are means ± 95% confidence interval.

E, elastic modulus; σ_p, proportional limit defined as the stress at the intersection point of the stress–strain curve and a straight line that starts at the origin and has a slope equal to 95% of the initial slope of the curve; σ_u, tensile strength; ε_u, strain at breaking point.

the biomimetics industry. It also offers the possibility of reproducing the full range of tensile properties exhibited by MAS fibers, so that the variability found in natural silk fibers should not represent a significant drawback in future research work.

Summary and conclusions

The whole range of tensile properties exhibited by either naturally spun or forcibly silked fibers can be predictably reproduced through a combination of supercontraction and stretching. The entire process is described by a single parameter, the alignment parameter, which is a measure of the length of the fiber after stretching and the initial length of the supercontracted fiber. It has been shown that fibers with similar alignment parameters yield similar stress–strain curves.

The combination of an aqueous environment and stretching is an essential feature of the spinning process of silk, and in consequence it is suggested that a similar process accounts for the broad range of tensile properties displayed by spider silk fibers. The ubiquitous presence of supercontraction and the simplicity of the process allows it to be implemented in the operation of spinning of artificial silk fibers, improving the reproducibility of the fiber tensile properties.

List of abbreviations

F	force
FS	forced silking
L_A	selected length
L_C	final length
L_{SC}	supercontracted length
MAS	major ampullate glands
MS	maximum supercontracted fibers tested in air
NS	naturally spun
RH	relative humidity
SCW	supercontracted fibers tested in water
α	alignment parameter

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