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Polymer 40 (1999) 1799–1806

polymer

The effect of solvents on the contraction and mechanical properties of spider silk

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Received 22 January 1998; revised 10 March 1998; accepted 20 March 1998

Abstract

We examined the mechanical characteristics of four major ampullate (MA) dragline silks during and after submersion in a range of solvents. The silks were reeled from four very different spiders: *Araneus diadematus*, *Nephila edulis*, *Latrodectus mactans* and *Euprosthenops* sp. They displayed significant differences in behaviour in the native state as well as during and after supercontraction in solvents such as water, urea solution and a set of alcohols. The different polarities of the solvents are thought to affect different regions of the silk's molecular conformation. We hypothesise that the observed mechanical properties of dragline silks are those of a hard elastic polymer; and we explain the supercontraction of the silks as changes of orientation in the molecular chains.

Keywords: Hydrogen bond; Hard elastic biopolymer; Ultrastructure

1. Introduction

Spider silk is a most attractive biomaterial [1,2]. The major ampullate dragline silks of spiders such as *Araneus diadematus* and *Nephila clavipes* have the unusual combination of high strength and high stretch; this leads to toughness values rarely observed in synthetic high-performance fibres [3]. The amino acid composition and sequence of some silk proteins have been thoroughly investigated [4–7], and aspects of the fibre microstructure of several silks have been studied in some detail [8–11]. Still, there are more open than resolved questions concerning even the principal silks of benchmark spiders such as the golden silk spider *Nephila clavipes* or the garden cross spider *Araneus diadematus*. Note that there are at least 30 000 spider species of which all have more than one (and up to 7) different silks.

A common feature of typical dragline silks is their apparent ability to supercontract when submerged in a solvent such as water [12,13]. There are man-made polymers which exhibit supercontraction in organic solvents or when heated, but virtually none which will supercontract in pure water at room temperature [8]. Here we report on the mechanical behaviour of four different dragline silks reeled from four unrelated spiders, under controlled conditions. All silks were studied in air as well as during and after submersion in a range of solvents. We further attempt to compare and synthesise our experimental results in order to explain the mechanism of spider silk supercontraction.

2. Materials and methods

2.1. Spiders and silk preparation

The spiders we used were: *Araneus diadematus* (Araneidae), *Nephila edulis* (Tetragnathidae), *Latrodectus mactans* (Theridiidae) and *Euprosthenops* sp. (Pisauridae). These spiders belong to different families and are genetically separated by several to many million years. They all build webs: *Araneus* (a garden cross spider, Europe) and *Nephila* (a sub-tropical and moist-tropical golden silk spider, Australia) are orb web-weavers, *Latrodectus* (a sub-tropical black widow, North America) builds a tangle web and *Euprosthenops* (a dry-tropical nursery spider, Africa) builds a sheet web. All the spiders were kept in the laboratory on a diet of house flies. Silk was mechanically reeled under standard conditions at 2 cm per second [14]. This was comparable to the spider's natural spinning rates at room conditions (24 ± 3°C, 25 ± 3% rH) [15,16]. The silks were collected and restrained on small plastic frames (10 cm × 10 cm) and studied within a week of collection at most.

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PII: S0032-3861(98)00266-3

Table 1
The weight and silk diameter of spiders used

Spider	A1	A2	A3	A4	A5	N1	N2	L1	L2	L3	E1	E2
Weight (mg)	90	173	138	195	170	1456	1250	468	385	473	1031	526
Diameter of silk (μm)	2.4	3.0	2.2	2.7	3.0	2.7	2.7	2.3	3.2	2.1	2.0	2.0

Note: A = *Araneus diadematus*; N = *Nephila edulis*; L = *Latrodectus mactans* and E = *Euprosthenops* sp.

The diameters of five threads per spider were gold coated and measured in a JEOL 840 SEM at a magnification of around 3000 × ; we obtained values of 2.0 to 3.2 μm with a standard error of about 5%. All spiders were weighed to the nearest 1 mg, and it appeared that there was no fight correlation between spider weight and silk diameter in any of the species (Table 1).

2.2. Stress–strain gauge

Because of the small diameter and weak absolute force of single MA silk threads, we used a custom built, highly sensitive, rapid response stress–strain gauge. This gauge was set up in such a way that we could measure fibres either in air or submerged full-length in a bath. The gauge with a FORT 10 force transducer (of World Precision Instruments) and linear extension mechanism (Pen Motor Assembly of Hewlett Packard) were driven and recorded using Lab View on Macintosh. It had a time resolution of a few ms and a force resolution of 30 μN. The silk was transferred from the holder to the gauge using our standard method [17]. Using cyanoacrylate adhesive, a single thread was mounted on the upper and lower bar of our gauge while taking care to avoid tensioning the thread. The fibres were stretched in air as well as submerged in selected solvents (the samples were given at least 10 mins to reach equilibrium after submersion) and the nominal stress–strain characteristics of fibre were calculated after normalisation for fibre diameter and initial length (usually 7.0 mm). The strain rate of the sample was 50%/min. The data shown give the average value of 3–6 measurement samples for each spider and each condition.

2.3. Shrinkage measurements of silk

We used distilled water and A.R. grade of all other agents for our experiments. A single thread was glued onto the two bars of our gauge and zero tension point attained (L_0 initial length, usually 10.0 mm). Then the bars were submerged in the solvent bath. Now the distance between the two bars was shortened until it was marginally longer than the (previously established) length of silk contracted in the particular solvent. After 10 mins, the bath was removed and the silk was dried in situ for 30 mins under room conditions. Then the silk was slowly stretched until it suddenly developed a stress which allowed us to know exactly the ‘new initial length (L_1)’ of the contracted silk. Thus, the shrinkage of silk in a selected solvent was defined:

$$\text{Shrinkage}(\%) = (\text{L}_0 - \text{L}_1)/\text{L}_0 \times 100\%$$

All measurements at each condition were repeated at least three times for each silk sample of each spider.

3. Results and discussion

3.1. Comparing the mechanical properties of virgin silk in air from the four species

The four spiders produced dragline silks that differed in basic mechanical properties in air (Fig. 1a–c). The stress–strain characteristics of *Araneus* and *Nephila* silk in air were consistent with previous results [3,17–19], although we used new, more sensitive, equipment. There were no published data for *Latrodectus* and *Euprosthenops*. The silk of *Araneus* was relatively weak, while the silks of *Latrodectus* and *Nephila* showed a good combination of stiffness and toughness. The dragline silk of *Euprosthenops* was by far the stiffest of the four silks; moreover, it had the largest Young’s modulus, the largest breaking strength and the lowest breaking elongation.

3.2. Effect of solvents effect on the mechanical properties

The effects of the full range of solvents used on the mechanical properties of all four silks are shown in Fig. 1a–d. Although some solvents such as water and methanol plasticised the silks and thus somewhat reduced modulus and strength, all silks still displayed high initial modulus, no clear yielding point, large breaking strength, typical extendibility, good recoverability and ‘energetic’ elasticity. Under a given strain (such as i.e. 18% extension), the stress of *Araneus* silk submerged in ethanol would relax to less than 40% until equilibrium, whereas the same silk in air would relax to only about 20%. This is evidence for a hard elastic material, where such behaviour has been explained using the mechanism of surface energy [20]. Thus our experimental results suggest that dragline silks are a natural polymer with hard elastic properties in a specific range.

The first hysteresis cycles of restrained *Araneus* silk in selected solvents are shown in Fig. 2. The other three silks showed similar hysteresis curves (not shown). All silks were affected similarly by the solvents and we deduce that there was no great difference in structure between them. In the following discussion, we present data on the silks of either of the benchmark spiders, *Araneus* or *Nephila*, as representatives for all four species.

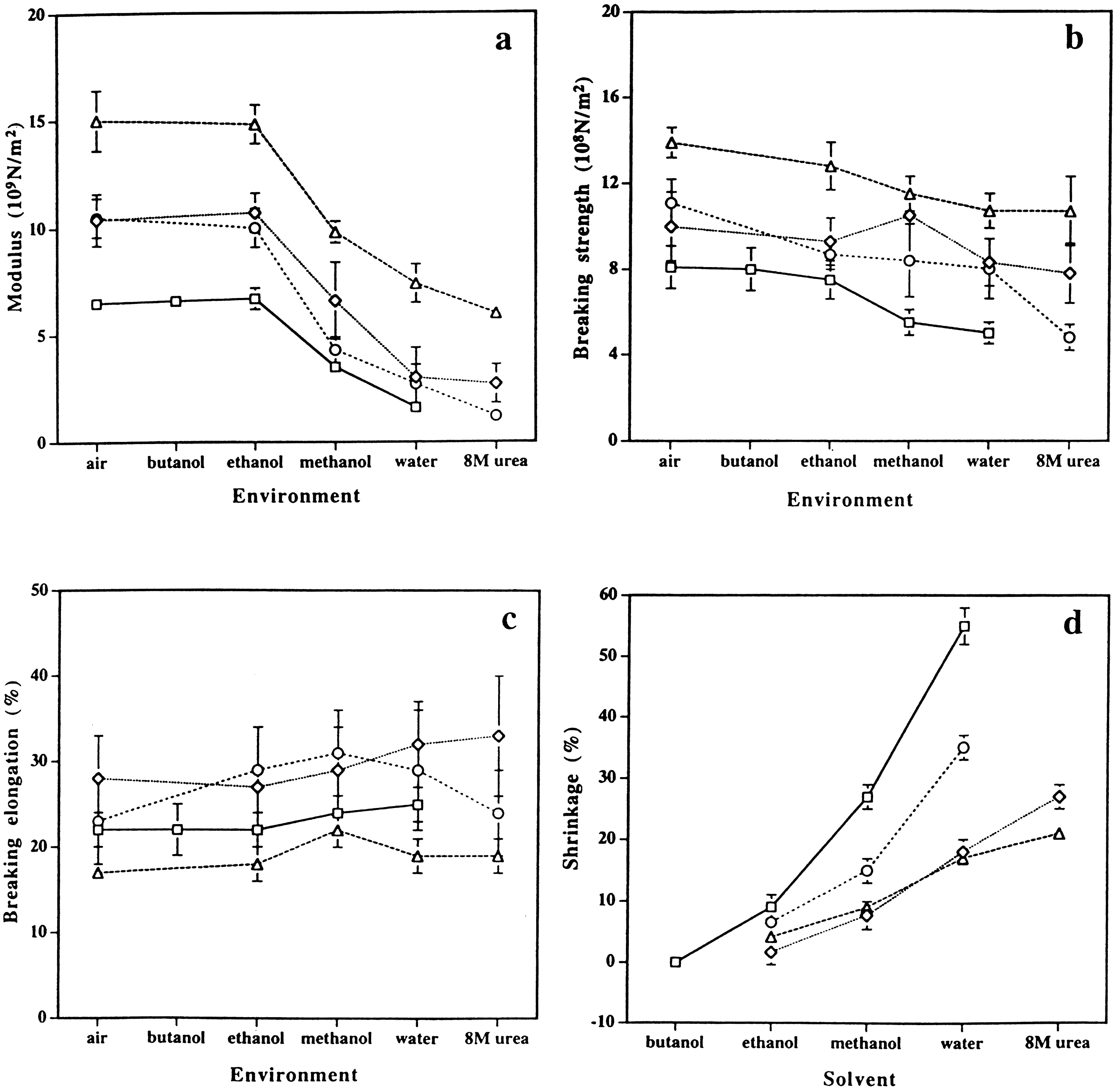

Fig. 1. Mechanical properties of our four spider silks. (a) Young’s modulus; (b) breaking strength; (c) breaking elongation and (d) shrinkage in various solvents. — □ — *Araneus*, · ·◇ · · *Latrodectus*, - - ○ - - *Nephila*, - - △ - - *Euprosthenops*.

Polar solvents strongly and predictably affected the mechanical behaviours of all four dragline silks studied (Figs 1 and 2). With increasing solvent polarity, the Young’s modulus and breaking strength of the silks gradually decreased, while their breaking elongation increased slightly, although there was some abnormal experimental data, especially with methanol. In 8 M urea solution, the tension of *Araneus* silk (and also one of the *Nephila* samples) could not be detected even by our sensitive machine. Moreover, we believe that silks were partly dissolved in this environment because the silk surface developed bubble-like protuberances after submersion in 8 M urea solution (unpublished observations on AFM).

The effect of water on silk is generally called plasticisation [21,22]. A plasticiser insinuates itself into the polymer and reduces the interaction between polymer chains; thus it decreases the modulus and strength of the polymer. We showed that solvents with a wide range of polarities and molecular sizes affected the dragline silks in varying degrees. This suggests to us that inter- and/or intra-molecule hydrogen bonds have been damaged to differing degrees by the different solvents. This might indicate differences in silk

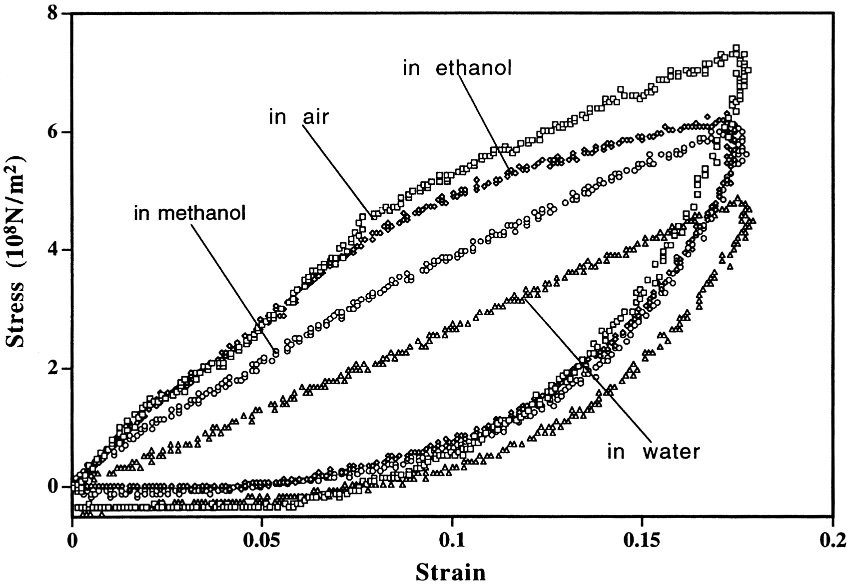

Fig. 2. Loading–unloading cycles of reeled *Araneus diadematus* dragline silk in the different environments: air, ethanol, methanol and water.

molecular and micro-structure (conformations). Furthermore, these microstructures seemed to change gradually (rather than drastically) with polarity.

After stretching, all four silks displayed high elasticity as well as a certain amount of 'permanent' set. The elastic recoverability of all four silks was about 80%–90%, after they had been stretched to about 70% of breaking elongation. Note that this kind of set should be termed 'temporary' rather than 'permanent' because it could be removed by submersion in water and some others solvents. Most illuminating, however, was the behaviour of such 'stretched and water-recovered' silks when they were stretched again after drying. Then their stress–strain curves (loading-unloading cycles) were rather different from comparable behaviour of the same silk in its virgin state (Fig. 3). Comparing three such loading-unloading cycles, we noted that their initial moduli were nearly identical (Fig. 3a). However, hysteresis cycles of recovered silk covered about 50% less area than cycles of virgin silk although they covered 30% more area than cycles of unrecovered silk. Silk measured while submerged in water showed much less hysteresis in its second loading-unloading cycle (Fig. 3b).

We assume that the microstructure of the silks changed during stretching, comparable to some high molecular weight poly(amino acid) fibres [23]. We envision that silks, when first under strain, take up energy because molecular chains reorient and even slip against each other when hydrogen bonds break. After stretching, the molecular chains settle in stable conformations and the friction between chains and some reformed hydrogen bonds induce permanent set and prevent full recovery at relaxation. Water (or some other polar solvents) might enable the molecular chains to disorient (rather than recover their original conformation), followed, during evaporation of the water, by the forming of new hydrogen bonds. Note that the unloading curves of recovered and unrecovered silks overlap which indicates that, when stretched a second time, recovered silk dissipates more energy than unrecovered silk. This could be due to the fact that both the orientation changes of molecular chains and the formation of hydrogen bonds releases energy.

Evidence of slip in the molecular chains under strain came from a separate experiment. Here we observed that *Nephila* silk, which had been pre-stretched in water to 70% of its breaking elongation, shrank only about 25 ± 1% ($n = 4$). This must be compared to 30 ± 2% ($n = 4$) shrinkage of unstretched silk with the same initial length.

3.3. Supercontraction in solvents

All four dragline silks studied contracted not only in water but also in a range of other polar solvents, although the level of contraction varied with spider and solvent. Fig. 4 shows the typical stress–strain curves of *Araneus* silk during and after contraction. We confirmed that the elastic modulus of contracted silk decreased drastically when submerged in water [3,21,24]. Moreover, we showed that the elastic modulus also changed with submersion in other solvents with different polarities and a range of molecular sizes. The nominal initial modulus of re-dried silk was not affected by supercontraction in any solvent. The shapes of these stress–strain curves of re-dried silks were more like those of semi-crystalline polymers (the original state of silk when dry) rather than rubbers (the state of silk when wet).

The results of three cycles of extension and relaxation for contracted *Nephila* silk with 10 min recovery between cycles are shown in Fig. 5. Although the silk showed similar initial moduli in each of the cycles, it also showed a large degree of permanent setting after the first cycle. In this cycle the silk was initially extended to its original length before

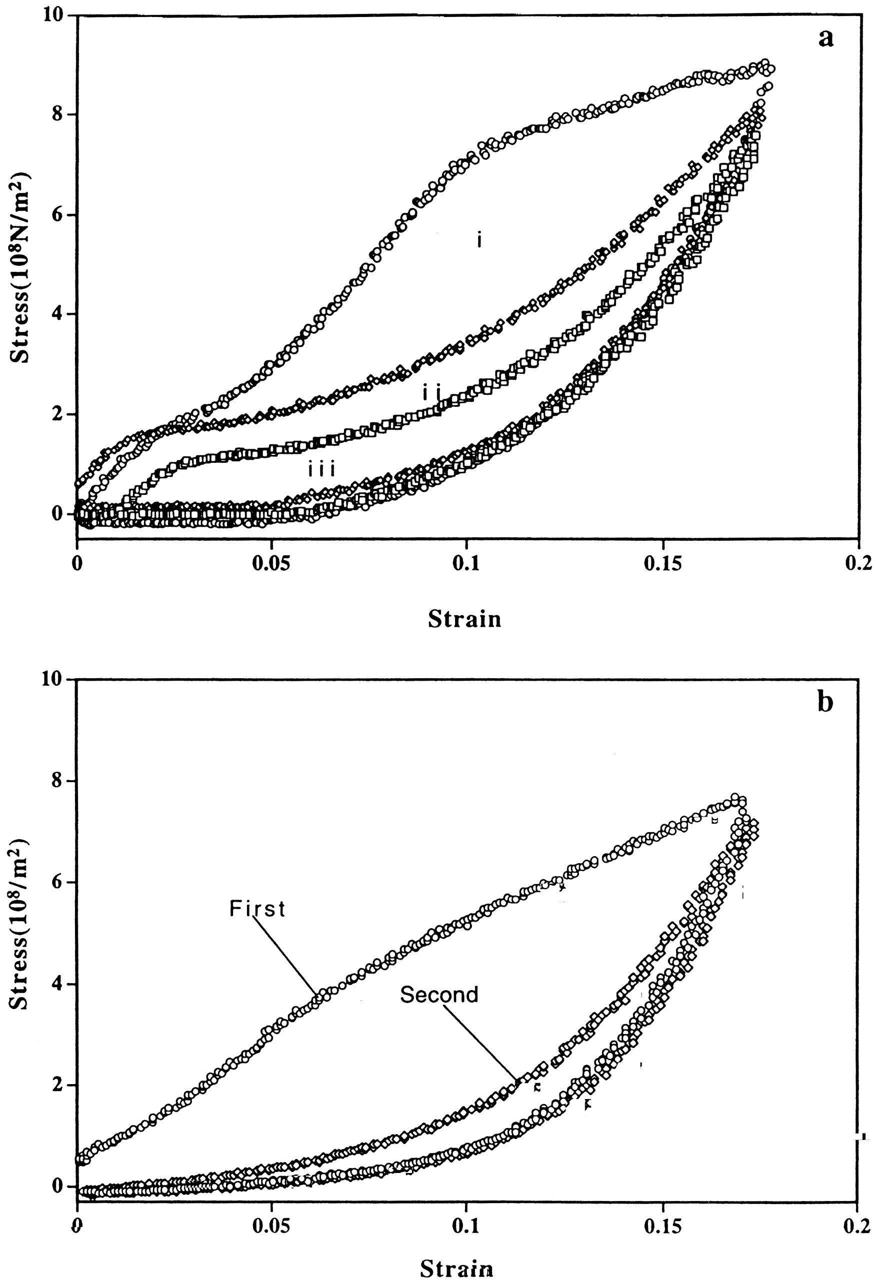

Fig. 3. Multiple loading–unloading cycles of *Nephila edulis* silk. (a) (i) first stretching of normal (virgin) silk; (ii) after stretching, the silk was recovered to its initial length by soaking in water for 2 min and drying in air for 20 min, followed by another stretching; (iii) after the initial first stretching, the silk was allowed to recover in air for 20 min before it was stretched again. Room conditions: 23°C, 49% rH. (b) the first and second loading-unloading cycles of *Nephila edulis* silk in water.

supercontraction. This experiment suggests that the silk lost a greater part of its elasticity after supercontraction. In other words, the microstructures of original and contracted silk must differ considerably.

Fig. 1d shows the shrinkages of our silks in the selected solvents. Shrinkage, like the softening of silk fibre, always increased along the sequence: ethanol, methanol, water and urea. As already mentioned, *Araneus* and *Nephila* silk partly dissolved in 8 M urea, this prevented the measurement of their shrinkage. However, light microscopy showed that in this solvent they contracted more than 60% [9]. We found that highly concentrated guanidine hydrochloride solution totally dissolved our spider silk. We noticed further that silks wetted by submersion in water, methanol and ethanol shrank by about 3%–8% of initial length during subsequent drying in air. This also indicates that the silks swelled to varying degrees in these solvents.

Guanidine hydrochloride is a known destroyer of hydrogen bonds. These bonds (both within and between molecular chains) play a crucial role in the structure of proteins [25]. When the spider spins its silk through its production line, most, if not all, of the molecular chains (or segments) will

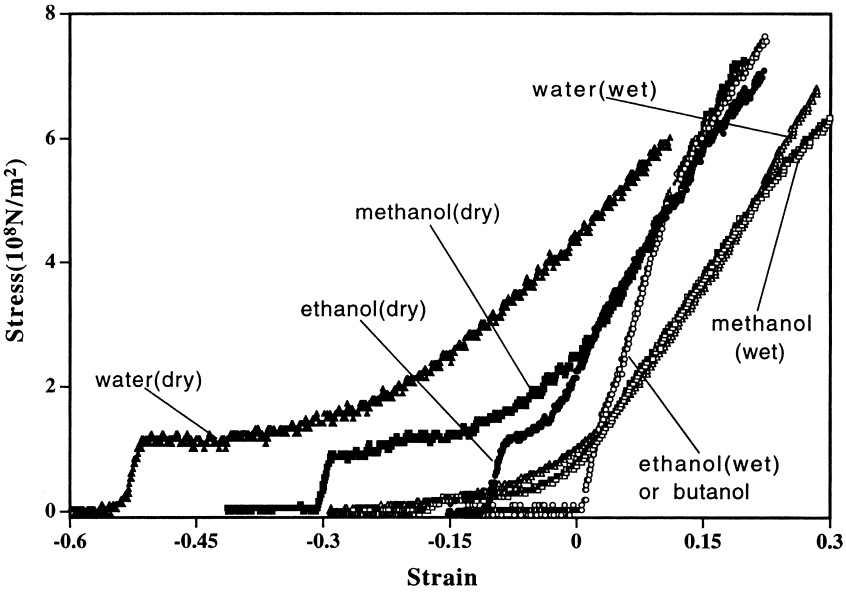

Fig. 4. Typical stress–strain curves of *Araneus diadematus* dragline silk during (wet state) and after (re-dried state) contraction in water, methanol, ethanol and butanol. Shrinkage was defined as negative strain; diameter and length of silk before contraction were used for normalising.

self-assemble to form liquid crystal [11]. Thus they uncoil from their relaxed state in the gland and in the spinning duct orient to varying degrees along the fibre axis forced or facilitated by the shear and stretch of the spinning process [26]. It seems that there is no clear boundary between the amorphous phase (with its relatively poor orientation and lower density) and the so called crystalline regions (with their clear orientation and higher density). This might be a reason why more than one kind of differently oriented crystalline region has been observed in dragline silk [10]. Since the glass transition temperature of spider silk is very low [27], we may assume that, at room temperature, the oriented chains (or segments) would be fixed by some intermolecular hydrogen bonds (probably some of them are 3_1-helix conformation [28]). When these bonds are gradually destroyed, for example by the different actions of our solvents, the molecular chains (or segments) would disorient step by step.

We found that stress–strain curves of contracted silk (re-dried) were similar to those of re-stretched silk (see Figs 3 and 4). Their shapes indicate the ductile behaviour of a polymer when cold-drawn this suggests that the molecular chains of the polymer orient and uncoil along the direction of stretch [29]. We further found that contraction in a solvent correlated positively with the plateau of the

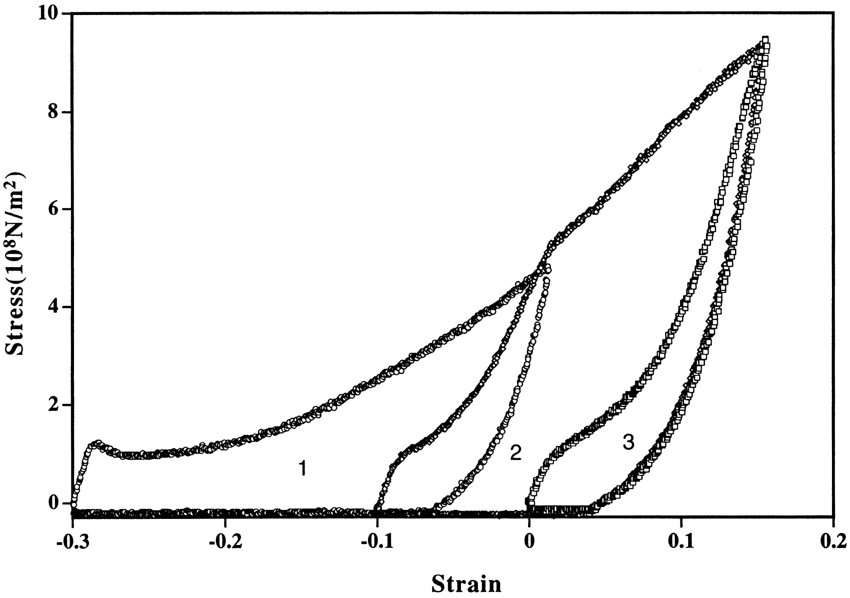

Fig. 5. Successive loading–unloading cycles of 30% contracted *Nephila edulis* silk at a constant strain rate of 50%/min. The specimen was allowed to recover for 10 min between cycles. *X* and *Y* axes are the same as Fig. 4. Room conditions: 25°C, 51% rH.

stress–strain curve. We explain the different swelling action of our principal solvents (8 M urea, water, methanol, ethanol and n-butanol) by their different effects on hydrogen bonds in the silk material. These differences are likely to be due to solvent polarity and/or molecule size. Thus the essence of spider silk supercontraction lays in the orientation change and coiling of the molecular chains.

It seems that there is competition between contraction and crystallisation in the molecular chains of the four dragline silks [30]. Highly oriented, uncoiled chains would easily form inter-molecular hydrogen bonds, causing close arrays of chains (or segments), thus further developing high density crystalline regions. A limit would be set by highly oriented β-sheet crystals [31] (there should be a strong effect of amino acids sequence). Solvents with lower polarity or bigger molecule size would find it difficult to creep into these tightly packed structures to damage hydrogen bonds and induce supercontraction. We hypothesise that this is the reason why *Euprosthenops* silk shrinks so much less than the others. After all, this silk also had the largest Young's modulus, the largest breaking strength and the lowest breaking elongation. We would predict that *Euprosthenops* will prove to have the highest degree of crystallinity in our four silks, followed by *Latrodectus* silk, which was the toughest (see Fig. 1).

The silks we studied have different amounts of secondary structures (unpublished Raman spectroscopy observation), possibly caused by sequence differences, amino acid differences and spinning differences. Thus, it is not surprising that the dragline silks we studied displayed the same trend in supercontraction; but to differing degrees. This difference could explain the observation that *Nephila* silk with high birefringence shrinks less in water than *Araneus* silk with low birefringence [32].

4. Conclusions

To elucidate the relationship between structure and function of the silk biopolymer, we measured the mechanical and supercontraction properties of dragline silks from four spiders under a range of conditions. We confirmed with our new state-of-the-art stress–strain gauge the excellent properties of the two benchmark silks reeled from the distantly related *Nephila* and *Araneus*. These properties were set against the, in some aspects even better, silks of the still more distantly related *Latrodectus* and *Euprosthenops*. All four draglines showed comparable combinations of tensile strength and extensibility, although there were illuminating differences between them.

All silks displayed mechanical behaviour suggestive of hard elastic polymer materials, even when submerged in some of the solvents tested. Moreover, several of these chemicals strongly affected the mechanical properties of the silks and there seemed to exist a relationship between contraction, mechanical properties and the polarity or molecular size of the solvent.

Supercontraction of spider silk is thought to be the result of hydrogen bonds breaking and molecular chains de-orienting and coiling. Thus, in a given solvent, the shrinkage of silk would depend on its degree of crystallisation (i.e. proportion of β-sheet stacking) and the ability of solvent to interact. The more hydrogen bonds that are affected, the larger the shrinkage of a silk, because of a tendency towards random coil conformation. In a strong solvent (such as 8 M guanidine hydrochloride solution) all silks were totally dissolved. In a weaker solvent (such as 8 M urea) *Araneus* silk was dissolved whereas the other silks were not.

A mechanical point of view would see supercontraction of spider silk as a constraint. An evolutionary point of view might see it differently as this property allows the spider to use silk more efficiently. Major ampullate silk is typically restrained when used in the web, and dew or rain water would easily run off. Total submersion, as in our experiment, is surely an extreme condition. Still, there could be occasions then it might be advantageous for the spider to have, under heavy dew loading, sharply decreased modulus and tensile strength but increased breaking elongation of particular members in the web. Indeed, there is evidence that the *Araneus* adapts silk properties and web engineering to ambient humidity and temperature [8]. On the other hand, instead of adaptations, the described properties might just be constraints inherent in the material, its evolutionary history, building blocks and production system. Whatever their origin, the observed properties under solvent wetting of our silks help to unravel these intriguing natural polymers.

Acknowledgements

ZS thanks the Carlsberg Foundation for a generous postdoctorate fellowship and FV thanks the Danish SNF for support of this study.

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