

Polymer Communication

Molecular deformation in spider dragline silk subjected to stress

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Received 15 February 1999; received in revised form 22 April 1999; accepted 22 April 1999

Abstract

It is demonstrated that well-defined Raman spectra can be obtained from the dragline silk of the spider *Nephila edulis*, and that the Raman bands also shift when the silk fibre is subject to mechanical deformation. This indicates that the protein molecules in the spider silk fibre undergo direct molecular deformation when the fibre is stretched. Hence, we demonstrate that the deformation behaviour of this natural polymer fibre is similar to that of high-performance synthetic polymer fibres. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Dragline silk; *Nephila edulis*; Raman spectra

Among the several types of structural proteins produced by nature, silk proteins are of particular interest because of their unique mechanical properties. The spider has evolved a wide range of silk fibres for many different purposes with surprisingly varied mechanical properties [1–3]. The dragline silk is a truly unique material. Although it is not as strong as some synthetic fibres, such as Kevlar [4,5], it is much more elastic [1] (necessary for a web silk to catch fast flying insects). This allows it to absorb more energy prior to breaking than any commonly used synthetic material. A full understanding of the relationship between the microstructure and the mechanical properties of natural silk fibres will lead to an increase in the understanding of the behaviour of polymeric fibres in general, potentially resulting in the development of new synthetic fibres for structural applications spun from aqueous solutions.

The fibre morphology of the dragline silk of *Nephila clavipes* has been investigated in detail by Grubb and Jelinski [6] through the use of a combination of wide-angle X-ray diffraction (WAXD) and small-angle X-ray diffraction (SAXD). They showed that the silk fibres contained about 10–15% of small (<10 nm) well-oriented crystals in a partially-oriented amorphous phase. They also found that, when the silk fibre was deformed, the orientation of the crystalline material improved. The mechanical behaviour of spider dragline silk has been modelled by Termonia [7] who envisaged the structure as consisting of a large number of small crystallites reinforcing amorphous regions

consisting of rubber-like chains. He demonstrated that the deformation of the silk fibre was similar to that of elastomers reinforced with carbon-black particles.

The spider's dragline reinforces the web and is the animal's lifeline. The most important mechanical property of such a fibre is its tensile stress–strain curve since it determines the response of the fibre to deformation along the fibre axis. The stress–strain response of a high-performance polymer fibre [5] is controlled by the ability of the molecules in the material to support the applied stress. State-of-the-art Raman spectroscopy allows us to follow the deformation process in a variety of high-performance synthetic fibres [5,8–13]. The wave numbers, $\Delta\nu$, of the bands in the Raman spectra of these materials shift under the action of stress or strain, which can be related directly to the deformation of the individual bonds in the materials [5]. The ability to obtain well-defined Raman spectra from spider silk filaments has been demonstrated by Gillespie, Viney and Yager [14] and this study is concerned with the application of Raman spectroscopy to a study of the deformation of single spider silk fibres.

The major ampullate dragline silk fibres of *Nephila edulis* were obtained by reeling directly from restrained but fully-awake spiders at 20 mm/s under room conditions (~25°C and 50% RH). The spiders had been raised in the laboratory and fed with houseflies. The silk fibres were collected and restrained on small plastic frames (100 × 100 mm) and were examined using Raman spectroscopy within one month of collection. In order to determine the stress in the fibres during deformation, the spider silk fibre diameters were measured accurately using a Philips XL-series FEG-SEM.

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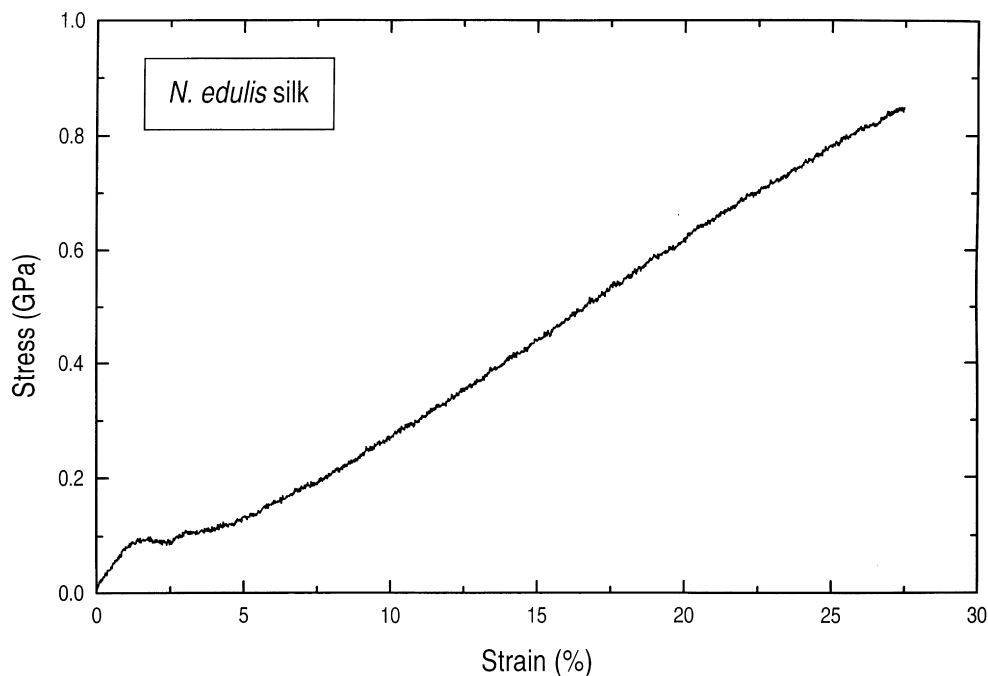


Fig. 1. A typical stress–strain curve for a single filament of the dragline silk of *Nephila edulis*.

They were coated with carbon and the SEM magnification was calibrated using a calibration grid with 2160 lines/mm. The silk fibres were found to range from 2–5 μm in diameter and to be approximately circular in cross-section. Single filaments of the dragline silk with a gauge length of 50 mm were deformed in tension in an Instron 1121 mechanical testing machine at a strain rate of 0.01 min^{-1} ,

a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $50 \pm 5\%$. The stress was calculated from the mean diameters of ten fibres from the same batch.

A stress–strain curve for a single filament of dragline silk is shown in Fig. 1. There is a pronounced deviation from linearity at a strain of about 2% corresponding to the yield point, leading to plastic deformation when the fibre is

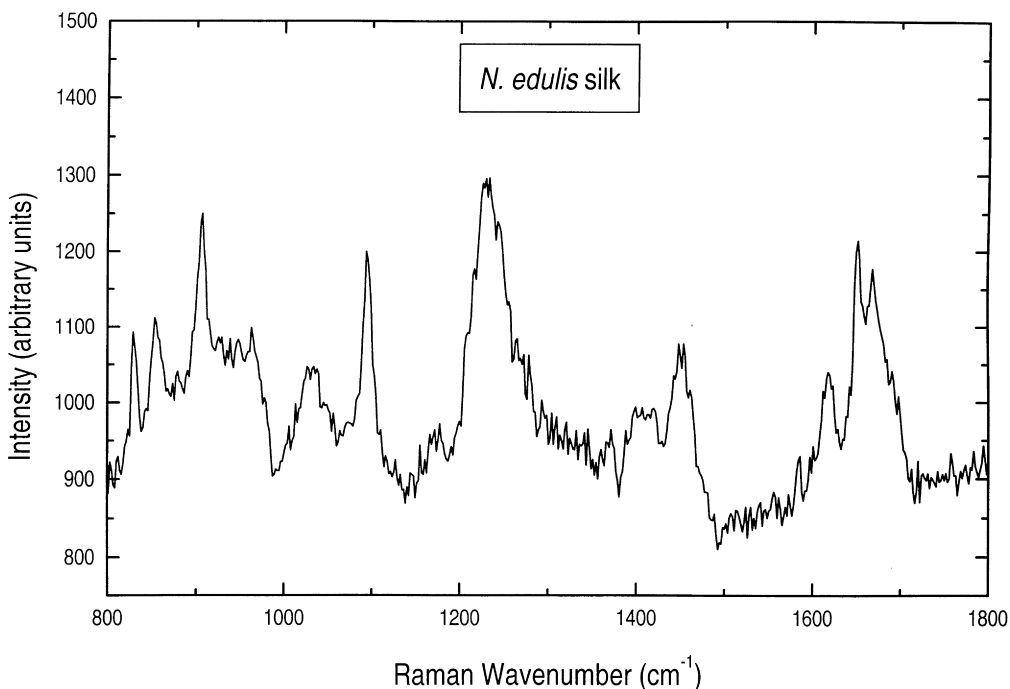


Fig. 2. Raman spectrum in the $800\text{--}1800 \text{ cm}^{-1}$ region for the dragline silk of *Nephila edulis*.

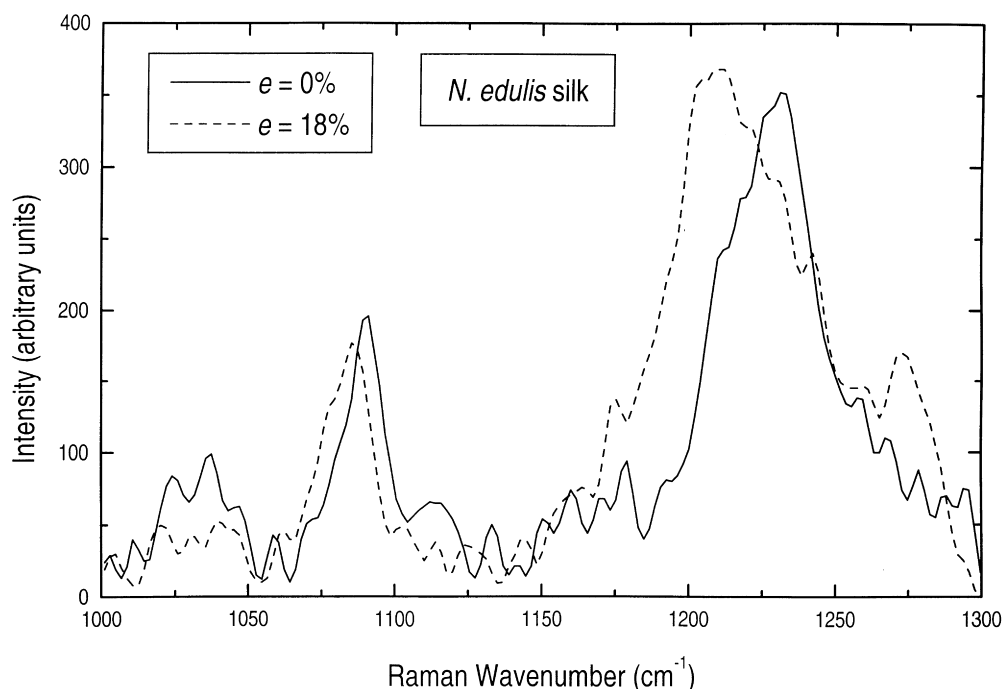


Fig. 3. Raman spectra obtained at strains of 0 and 18% showing the shift in the positions of the positions of the 1095 and 1230 cm⁻¹ Raman bands of the dragline silk subjected to stress.

subjected to further load. It is thought [7] that the yield point corresponds to the break down of a hydrogen-bonded network and the stress being transferred to the underlying amorphous chain structure. The tensile strength and failure strain of the dragline silk at a gauge length of 50 mm averaged from at least ten specimens were found to be 0.8 ± 0.4 GPa and $25 \pm 5\%$ respectively. The major difference between the spider silk and other high-strength materials, such as steel and aramid fibres, is not strength but the degree that the silk fibres extend before they break. This reasonable balance of stiffness, strength and extensibility makes the silk an ideal multipurpose material for advanced biological network construction. Spider silk is a semi-crystalline material [1,2] composed of both flexible amorphous regions (α -and/or 3(1) helices [15,16]), that are made up of disordered chains of amino acids, and strong/stiff crystals (β -pleated sheets), that consist of different sizes of amino acid groups arranged into an accordion-like formation. Additionally, the amorphous regions contain protein chains that are on average about 15 amino acid residuals long [17–19], extended and held in partial alignment with the fibre axis in the process of spinning [20]. Moreover, in air-dried silk the flexible part, poorly hydrated and occupying about 70% of the total volume [20], is reinforced by a reasonable quantity of stiff crystals. These structural combinations result in the extreme toughness and optimal extensibility [6,7] that the spider uses to such great advantage in its web.

As Raman spectroscopy is now established as an important tool for the study of the deformation of polymeric fibres [5,8–13] it was chosen to follow the deformation behaviour

of the dragline silk of *Nephila edulis*. The Raman spectra of single filaments of the silk were obtained using a Renishaw 1000 Raman microscope system with a He–Ne laser source with a power of approximately 1 mW on the fibre. An Olympus BH-2 optical microscope was used to both focus the laser beam on the specimen and to collect the scattered radiation. The laser beam was polarised parallel to the fibre axis and focused to give a spot size of 2 μm in diameter on the fibre surface. A highly sensitive Renishaw charge-coupled device (CCD) camera was used to collect the Raman spectra. The band intensity and band position were analysed using a Lorentzian fitting procedure.

Fig. 2 shows the Raman spectrum obtained from a single filament of the dragline silk of *Nephila edulis*. It can be seen that a number of well-defined intense peaks can be obtained between 800 and 1800 cm⁻¹ with the Raman bands located approximately at 854, 1095, 1230, 1450 and 1667 cm⁻¹ being particularly well resolved. The Raman bands at 1095 and 1230 cm⁻¹ are the most prominent in the spectrum and were therefore chosen to follow the molecular deformation. Although a full band assignment has not been undertaken for spider silk, it is likely that the 1095 cm⁻¹ band can be assigned to a disordered conformation of the $\nu(\text{CC})$ skeletal band using the analogy of the 1085 cm⁻¹ band in polypeptide chains [21]. The other band located at about 1230 cm⁻¹ in the amide III region [14] is thought to be the same band as that found in silkworm silk (*Bombyx mori*) related to a disordered conformation [21] or a β -conformation in synthetic polypeptides [22].

Single-fibre deformation, again using a 50 mm gauge length, was carried out using a deformation rig which is

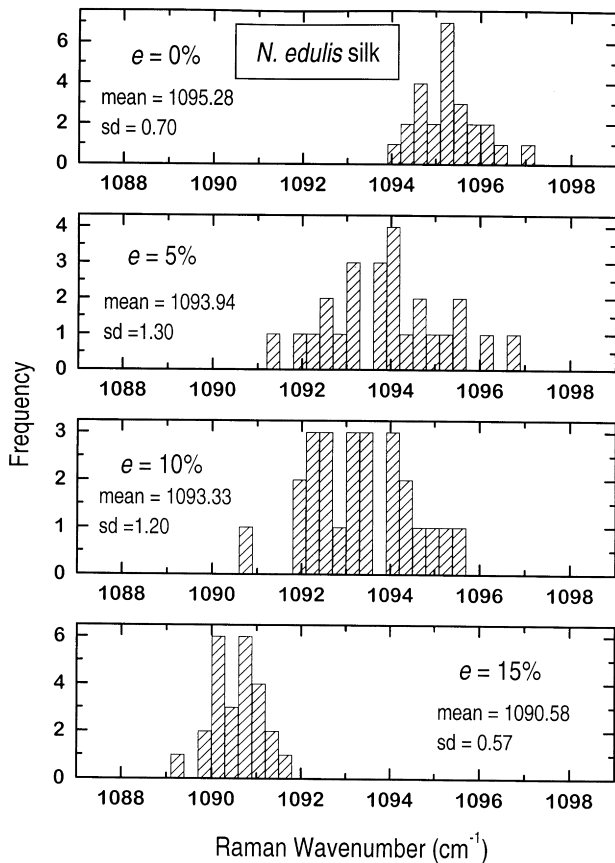


Fig. 4. Histograms showing the distributions of the peak position of the 1095 cm⁻¹ Raman band obtained at random along a single dragline silk fibre subjected to strains of 0, 5, 10 and 15%.

fitted directly onto the Raman microscope stage. The fibre was connected to a sensitive load cell to measure the applied load and attached to a micrometer to control the fibre displacement. The effect of deformation upon the positions of the 1095 and 1230 cm⁻¹ Raman bands is shown in Fig. 3 at fibre strains of 0 and 18% where shifts in band position can be seen clearly. The shift in the wave number of the 1095 cm⁻¹ Raman band is represented in Fig. 4 where peak position distributions are shown for 25 random measurements taken at different positions along a deformed dragline silk filament at 5% strain intervals. A similar distribution was also found for the 1230 cm⁻¹ Raman band. In both cases, the distributions shift to lower wavenumbers as the strain is increased. It can be seen from Fig. 4 that although there is some scatter in the data due to both instrumental factors and local structural variations in the natural fibre, the mean in the band position distributions shift significantly by up to 5 cm⁻¹ at 15% strain. The dependence of the peak position of the 1095 cm⁻¹ Raman band upon stress is shown in Fig. 5 where it can be seen that there is an approximately linear shift in band position up to the point of fibre fracture at about 1 GPa. Based upon similar measurements upon five nominally-identical dragline silk fibres, the average rates of Raman band shift with stress ($d\Delta\nu/d\sigma$) were found to be -7.3 ± 1.8 cm¹/GPa and -14.8 ± 2.2 cm¹/GPa for the 1095 and 1230 cm⁻¹ bands respectively.

When a high-performance polymer fibre is deformed, the stress applied is transformed directly into deformation of the covalent bonds in the molecules aligned in the fibres [5]. The deformation of these oriented molecules in both the crystalline and the amorphous regions of the fibre cause a

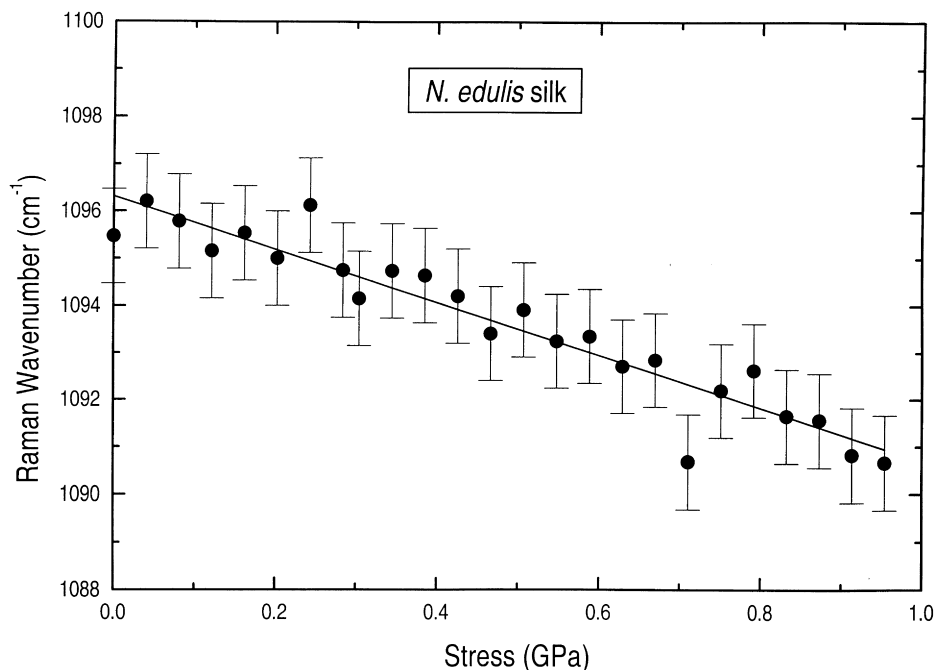


Fig. 5. Variation of the peak position of the 1095 cm⁻¹ Raman band for the dragline silk with applied stress.

change in bond lengths, bond angles and internal rotation angles, which leads to a shift in the Raman frequency for the particular vibrational modes [5,23]. It should be noted that the significant rates of Raman band shift, $d\Delta\nu/d\sigma$, for the dragline silk suggests that a high level of molecular stressing takes place in the materials during deformation. In fact the shift of $-7.3 \pm 1.8 \text{ cm}^1/\text{GPa}$ for the 1095 cm^{-1} Raman band in the dragline silk is similar to the value of $-6 \pm 1 \text{ cm}^1/\text{GPa}$ found for the 1130 cm^{-1} C–C stretching band in polyethylene fibres [13] implying that a similar molecular deformation process occurs in the two very different fibres. The larger stress-induced shift of the 1230 cm^{-1} Raman band in the amide III region [14,24], on the contrary, may be related to changes in the H-bonded network or other structural reorganisation. There is, however, no significant stress-induced change in the spectrum in the amide I region [14] ($1600\text{--}1700 \text{ cm}^{-1}$). In fact the 1230 cm^{-1} band is probably made up of a number of peaks and the shift may be due to changes in band intensity as well as peak position. This is clearly an area for further study.

The linear dependence of the 1095 cm^{-1} Raman band position upon axial fibre stress shown in Fig. 5 is also very similar to the behaviour of a number of high-performance polymer fibres such as aramids [5,8,9], polyesters [10], gel-spun polyethylene [13] and rigid-rod polymer fibres [11,12]. This linear dependence upon stress has been interpreted [10,25] in terms of the microstructure in the synthetic high-performance fibres being subjected to an overall uniform stress during deformation, even though local variations of stress may exist. Hence, it appears that a similar continuity of stress must also pertain in the microstructure during the deformation of the dragline silk fibres of the spider *Nephila edulis*. Indeed this is a fundamental assumption in the model of Termonia [7] that has been used to predict successfully the stress–strain curve of spider silk fibres. Since the different components in the silk microstructure—the flexible chains and stiff crystals—have very different properties a uniform stress means that the strain in the microstructure must be highly non-uniform.

In summary, it appears that the unique mechanical properties of spider silk, good extensibility combined with high strength, are a result of a microstructure in which the molecules are able to extend whilst also being capable of

supporting high stress. The stress-induced Raman band shifts are direct evidence for molecular deformation taking place within the fibres.

Acknowledgements

This study was supported by a research grant from the EPSRC Materials Programme and one of the authors (J.S.) is grateful to the Government of Thailand for financial support.

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