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Heterogeneous morphology of *Nephila edulis* spider silk and its significance for mechanical properties

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Dedicated to Professor Ronald K. Eby on the occasion of his 70th birthday

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Abstract

Previous work has shown that dragline silk of *Nephila madagascariensis* is not homogeneous in cross-section. We report here that the core of *Nephila edulis* silk also contains extremely fine elongated, electron lucent domains. These domains may contribute to the exceptional tensile strength and toughness of this material by acting as stress concentration parts or (and) fluid-filled canaliculi. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Spider dragline silk is an extremely strong biopolymer [1]. Its mechanical properties are usually explained by reference only to its properties at the molecular level by arguing that amorphous, disordered regions of the protein fibre provide extendibility while β-sheet crystallites form ordered regions, with numerous inter- and intramolecular hydrogen bonds providing a high initial stiffness and tensile strength (e.g. [2,3]). However, the benchmark of spider silks, the dragline silk of *Nephila* spp. shows a high degree of internal structural organisation including hierarchy [4]. Although many aspects of this microstructure are still unclear, the presence of distinct microfibrils organised into a complex bundle seems to be beyond doubt for the major ampullate silk of *Nephila* spp. [4–6]. Indeed, it now seems that *Bombyx* silk also contains tightly packed microfibrils [7]. Certainly, it is not unreasonable to assume that ultrastructure contributes substantially to the mechanical properties of these fibres. Indeed, the observed structural organisation may be at least as important as molecular structure and it is obvious that we must revise our understanding of silk form and function. There is considerable commercial interest in the use of hierarchically structured, energy dissipating ropes, sometimes liquid filled, constructed from synthetic microfibres; and spider silks might become important players in this field [8].

Here we present evidence for a novel mechanism by which the dragline silk of the spider *Nephila* might dissipate strain energy. We suggest that specific domains—resembling microscopic liquid filled channels or canaliculi—in the core material of the fibre bundle [9,10] aid in energy uptake by functional dissipation when the channels are compressed during extension and by forming numerous fine cracks (crazes). These effects are discussed in relation to the mechanical behaviour of multicomponent polymer fibres [8]. Note that Robson [11] and Akai et al. [12] observed comparable canaliculi in the silks of, respectively, the silkworms *Bombyx* and *Antheraea*.

2. Materials and methods

The major ampullate dragline silk was collected on a motorised rotating frame from *Nephila edulis* spiders at 2.0 cm/s which is comparable to the natural spinning rate [13].

2.1. Cryotome section

Bundles of silk were soaked in 2.3 M sucrose for 60 min to act as a cryoprotectant, and quenched in liquid nitrogen. 70 ~ 80 nm ultrathin sections were cut at -90 to -100°C using a LKB ultracryotome. The sections were collected and

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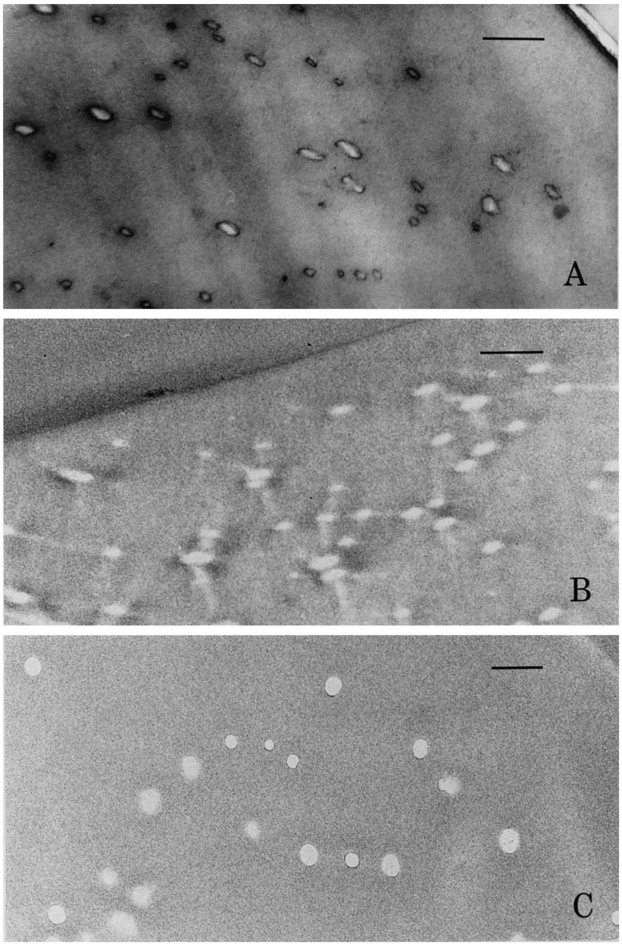

Fig. 1. TEM images of cross section of *Nephila edulis* dragline silk after different treatments. (A) Silk was cut under cryoprotection and stained by uranyl acetate (scalebar: 0.2 μm). (B) Silk was stretched until rupture, without staining. Note the cracks (or crazes) between the electron light domains (scalebar: 0.5 μm). (C) Silk remained unbroken after 25% stretching followed by relaxation of the sample, without staining. Note the two different types of domains indicating that these domains are not simple cavities (scalebar: 0.2 μm).

thawed on Formvar coated grids. They were stained with uranyl acetate, supported by methyl cellulose and examined with a Hitachi 7000 TEM operated at 75 kV.

2.2. Ultrastructural effects of mechanical stress

We subjected silk to mechanical stress prior to conventional Epon embedding. Threads were reeled and glued directly on to the points of a pair of dividers. The screw of the dividers was used to control fibre stretch. After allowing the silk to dry, it was stretched to various extension. The silk was fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 25°C for one week. After dehydration in ethanol series (20 min in each 90, 96 and 99.9 %) at room temperature, the material was embedded in Epon and ultra-sectioned with a diamond knife. Unstained sections were examined with a Philips 208 TEM at 80 kV.

2.3. Tensile testing

Single threads of reeled silk was mounted with cyanoacrylate adhesive onto the cross-heads of a custom-built, tensile testing instrument (time resolution <5ms for 1 mN, force resolution 30 μN [13]). The initial length of silk was 7 mm and the strain rate was 50%/min. Nominal stress was plotted against strain after determining the fibre diameter (about 3.3 μm) by SEM.

3. Results and discussion

We report here further work on the structure and function of electron-lucent, extremely fine elongated canaliculi previously described for *Nephila* spp. in both nascent [10] and fully formed [9] major ampullate dragline threads. Transverse sections of *Nephila edulis* silk, both in cryosection and Epon section, show circular profile electron lucent regions with diameters varying from 20 to 100 nm distributed irregularly in the matrix (Fig. 1). Serial sectioning revealed that these arose as cross sections of fine elongated canaliculus-like domains. Longitudinal ultrathin Epon section confirmed the presence of numerous such domains oriented parallel to the long axis of the fibre. In frozen sections, the interface between domain and matrix stained with uranyl acetate. The contents of the canaliculi showed little or no staining (Fig. 1(A)).

We hypothesised that these domains might be stress concentration points and work as crack initiators inhibiting the spreading of cracks. As such they should initiate cracks when a fibre is stretched, comparable to the behaviour of rubber toughened plastic [14]. Such localised cracks would take up energy and delay rupture of the fibre. We tested this hypothesis by ultra-structural investigation of a series of carefully stretched silk samples and predicted a positive correlation between stretching and crack formation at the domains. Indeed, we observed that the silk stretched until breaking typically showed distinct cracks between the electron lucent domains (Fig. 1(B)). Although we found no correlation between the extension and the number of cracks, we only ever observed cracks in fibres stretched to rupture (i.e. in fibres that broke) and in such samples cracks were often abundant (Fig. 1(B)). This suggested to us that crack formation may only come into play when the maximum elongation of a silk fibre is reached, i.e. just before rupture.

Stretched but unbroken silk never showed cracks but rather resembled unstretched silk (Fig. 1(C)). In these samples the electron lucent regions appeared to occur in two different forms, one of irregular shape and diffuse border and one with regular shape and distinct border. The latter is probably formed during sectioning by the microtome knife inducing holes at the position of the fine canals.

The cross sectional diameter of the domains was so small that usually an electron lucent region appeared to initiate or stop only one crack in one cross section (Fig. 1(B)). Similar

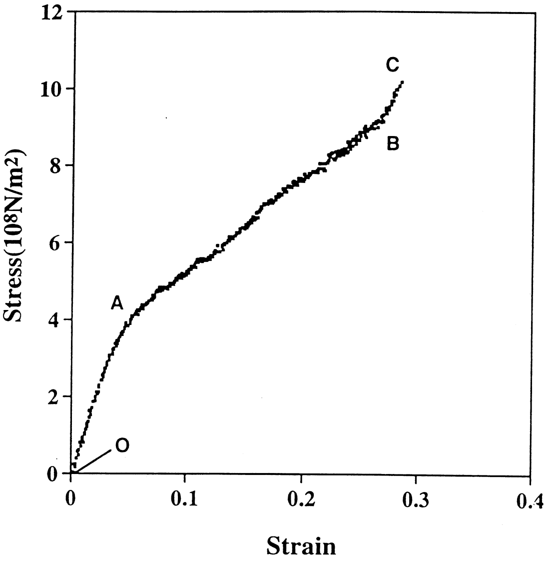

Fig. 2. The typical stress–strain curve of *Nephila edulis* dragline silk. Note that the slope of BC deviates considerably from AB which is the type of curve generally seen.

patterns are known from synthetic multi-component polymers like acrylic rubber-modified epoxy resins in which the cracks are deflected by the rubber particles [15]. In silk, like in synthetic materials, this process would create new surface to take up strain energy. Thus the observed domains would toughen the silk and thus prevent the spiders safety line from breaking easily and help the web threads in their task of resisting the impact of prey with high kinetic energy.

4. Conclusions

We measured the mechanical properties of the studied silk in our custom-built rapid response stress–strain gauge. Sometimes we observed abnormal stress–strain curves (Fig. 2). We divided the curve into three sections: OA, AB and we suggest that the initial linear part of the curve (OA) correspond to the ideal elastic deformation of silk. The AB part of the curve might then correspond to the plastic deformation of the material when the molecular chains of silk are oriented under stress and the hydrogen bonds gradually break. Normally, the silk fails at point B, but occasionally (about one in ten samples) the failure happens at C. Thus *Nephila* dragline silk would occasionally show in its stress–strain curve a sudden rise just before breaking. We hypothesise that it was in this moment that the cracks were formed.

Of course, there are other explanations for the fact that cracks were never observed in samples stretched well below the limit and only in two of the eight samples that were stretched over 25% until rupture. Firstly, the canaliculi might be reservoirs of liquid fibroin that solidifies in areas of high stress to self-repair cracks: uranyl-acetate staining suggests an encapsulating boundary layer (Fig. 1(A)), which could indicate the silk protein's hydrophilic domains which prevent precocial solidification. Secondly, the canaliculi may contain substances ensuring hydration of the silk proteins and proper fibre plasticisation [16,17]; this could be related to the change in behaviour of silk with hydration [2,13,16]. Thirdly, the longitudinally oriented canaliculi might act as areas of lateral force distribution by dispersion of hydrodynamic pressure or as fluid-filled shock absorbers, such as their roles in some collagens [18] and tooth enamel [19]. Fourthly, in analogy to oil filled tennis strings the liquid filled domains could act as areas of lubrication, reducing inter-fibrillar friction. Finally, the presence of our electron lucent regions may be nothing but a production constraint and represent enclaves of solvent left after the liquid-solid transition of fibroin in the process of spinning the fibre [10]. But Nature generally has her way of minimising such constraints, or turning them to her favour as spider silk has demonstrated abundantly [16].

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