Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

The rheological properties of native sericin

James Sparkes, Chris Holland *

Department of Materials Science and Engineering, The University of Sheffield, Sir Robert Hadfield Building, Mappin Street, Sheffield, S. Yorks S1 3JD, UK

ARTICLE INFO

Article history:
Received 18 October 2017
Received in revised form 13 December 2017
Accepted 16 January 2018
Available online xxxx

Keywords:
Sericin
Bombyx mori
Characterisation
Rheology
FT-IR spectroscopy

ABSTRACT

Unlike spider silk, spinning silkworm silk has the added intricacy of being both fibre and micron-thick glue-like coating. Whilst the natural flow properties of the fibre feedstock fibroin are now becoming more established, our understanding of the coating sericin is extremely limited and thus presents both a gap in our knowledge and a hindrance to successful exploitation of these materials. In this study we characterise sericin feedstock from the silkworm *Bombyx mori* in its native state and by employing both biochemical, rheological and spectroscopic tools, define a natural gold standard. Our results demonstrate that native sericin behaves as a viscoelastic shear thinning fluid, but that it does so at a considerably lower viscosity than its partner fibroin, and that its upper critical shear rate (onset of gelation) lies above that of fibroin. Together these findings provide the first evidence that in addition to acting as a binder in the construction of the cocoon, sericin is capable of lubricating the flow of fibroin within the silk gland, which has implications for future processing, modelling and biomimetic use of these materials.

Statement of Significance

This study addresses one of the major gaps in our knowledge regarding natural silk spinning by providing rigorous rheological characterisation of the other major protein involved – sericin. This allows progress in silk flow modelling, biomimetic system design, and in assessing the quality of bioinspired and waste sericin materials by providing a better understanding of the native, undegraded system.

1. Introduction

In nature silks can be spun under ambient conditions to achieve a wide range of strength [1–6], elasticity [7–9] and toughness [1,8,10–14], inspiring many to try and replicate these materials industrially [15–23]. However, a major limiting factor in furthering our understanding of silk production is that while much has previously been published on the function, properties, and structure of the primary proteins responsible for the fibrillar structure of silks (fibroins in silkworms and spidroins in spiders) [19,24,25], much less is known about the secondary proteins. Whilst not responsible for the impressive mechanical properties of a silk fibre per se, in their natural environment they perform a diverse range of functions that effectively extend the phenotype of silk by imbuing additional functionality, such as non-woven composite formation [26–29], water retention [30] or even adhesive qualities [31].

In this study we focus on the group of secondary silk proteins produced by *Bombyx mori,* known as sericins, which act as the binder between brins and adjacent cocoon fibres in their spun state [8,27,29,32,33]. These proteins account for approximately 30% of the spun silk fibre's weight [34], and thus represent a considerable resource investment for the silkworm, which pertains to their biological significance.

Sericins are water soluble, globular proteins [32,35]. They are most accurately described as a multi-component protein with an indefinite structure, which are secreted at various points along the gland, beginning in the middle posterior section [32,35–37]. These proteins have marginally different compositions, solubility, and levels of crystallinity [32,37]. However whilst we have insights into their structure, little is known about sericins' functions within the gland, with only suggestions or assumptions that they act as a lubricant for fibroin [38–41]. This may be in part due to the experimental difficulties in extracting and testing this material with minimal processing, as although fibroin can be readily extracted in its native state from mature *B. mori* larvae [42], isolation of native sericin in a similar fashion has thus far proved problematic.

Large quantities of sericin solution are produced industrially from wastewater in the cocoon degumming process [43–45] and find uses in the food [46–48], cosmetic [32,49–52], biomedical

* Corresponding author.
E-mail address: christopher.holland@sheffield.ac.uk (C. Holland).

https://doi.org/10.1016/j.actbio.2018.01.021
1742-7061/

[53–57], and health supplement industries [58]. Much of the recent work on sericins has focused on the extraction methods [32,59–62], characterisation of [48,59,63–68], and potential uses [32,51,55,62,69–73] for this by-product, with the primary aim of commercial diversification. Nevertheless, the combination of high temperature, pressure, and solvents typically used affects the quality of the sericin produced through changes to its conformation or molecular weight [32,35,57,63,73–76]. Therefore, while these industrially derived sericins represent promising advances in their respective fields, and a significant waste reduction in traditional silk industries [32,60,61,74,77], the mechanical [78–81] and rheological [35,47,78,79,82–84] sericin characterisation undertaken in these studies cannot be considered truly representative of the natural system.

Looking towards alternative methods for sericin extraction, early studies suggested that sericin could be extracted through time limited dissolution of *B. mori* glands [34,84], however this has proven difficult to control, as it is subject to contamination from fibroin and requires re-concentration. Other techniques, such as surgically removing the posterior (fibroin producing) section of the gland and dissolution of the resultant fibroin deficient feedstock [85,86], have not gained traction in the silk community due to the time-consuming, complex, and invasive nature of the operation. The use of the standard LiBr fibroin reconstitution method [87] has been employed with mutant *B. mori* strains to dissolve sericin only cocoons [68,88], but this can hardly be considered less degrading than wastewater extraction methods.

To this end, we have opted to apply our existing techniques for native fibroin extraction [42,89] to a fibroin deficient strain of *B. mori,* thus providing a ready source of native sericin feedstock without the need for several interim processing steps. To our knowledge there is only one study which details the rheological properties of native sericin taken from the glands of a fibroin deficient *B. mori* strain, but the number of samples (n = 1) used, and the unlikely supposition that a polymer solution is a Newtonian fluid limits the applicability of this data [90]. Hence, looking to extend our understanding of this area, we present rheological data obtained from large sample sizes in an attempt to gain fundamental insights into this material. This work seeks to define native sericins' processing parameters and provide baseline data to both further our investigations into the recently discovered pultrusion dominated spinning mechanisms of silk (for it is pulled, not pushed from the body) [91], and address the major limitations identified in previous flow studies [38,39,42,92,93]. We show that sericin is a polymer solution which exhibits non-Newtonian, shear thinning behaviour; that its viscosity lies significantly lower than fibroin, and that its upper critical shear rate (onset of gelation) lies above that of fibroin. All of which allows us to conclude that as well as binding cocoon fibres together, native sericin can also act as a lubricant.

2. Materials and methods

2.1. Silkworm rearing

Fibroin deficient *B. mori* silkworms (Nd-s strain, supplied by *Silkworm Genetic Resource Database*, Kyushu University, Japan) were raised over a period of 6 weeks on black mulberry (*Morus nigra)* and silkworm chow (Silkworm store, UK) in a temperature and humidity controlled environment (28 °C, 80–85% RH, reduced to 26 °C, 70–75% in the final instar). When worms were observed to have begun wandering (loss of appetite, increased motion, voiding (at later stages) they were transferred to a cool (10 °C) environment to delay spinning.

The strain used has an incomplete penetrance of the phenotype responsible for the undeveloped posterior gland and resultant fibroin deficiency, meaning that not all silkworms of this strain produce sericin only. Identification of specimens carrying this phenotype was aided greatly by bimodal variation in their appearance (see Fig. 1) – *Dark*, with a mottled body (dark grey patches over a pale grey-white) and *Light* – which were a uniform pale grey-white (similar to regular *B. mori*).

2.2. Sample preparation

Feedstock was extracted from silk glands as per previously described fibroin extraction methods [42]. Adjacent portions of the same section of the middle division of the gland were used for rheological and concentration characterisation for all samples and assumed to be of equal concentration. Feedstock concentration was determined through evaporation to dryness and is expressed as mg/mg %.

2.3. Material identification

2.3.1. Spectroscopic characterisation

Attenuated Total Reflectance (ATR) Fourier-Transform Infra-Red (FT-IR) spectroscopy (Nicolet 380, Thermo Scientific, Waltham, USA) purged with dry air (118 ml/s Parker Balston, UK) was performed on the same samples as those used for amino acid analysis. Samples were progressively scanned as they were evaporated to dryness using a vacuum chamber mounted above the ATR crystal to leave a thin film on the ATR crystal. Spectra were obtained between 800 and 4000 cm^{-1}, performing 64 scans at 4 cm^{-1} resolution and are presented after correction for background absorbance and ATR sampling depth, and normalised against the amide I peak.

2.3.2. Amino acid analysis

Gland contents were dissolved in type I water and submitted for analysis in the PNAC Facility at the University of Cambridge. Samples were hydrolysed with 250 nmol norleucine standard, with 1% of the sample analysed using ion exchange liquid chromatography, and post column derivatisation achieved using ninhydrin (Biochrom 30, Biochrom, Cambourne, UK).

2.3.3. Molecular weight determination

Gland contents were dissolved in type I water, diluted to 0.1 and 0.05% (mg/mg) and reduced with an equal volume of 4%

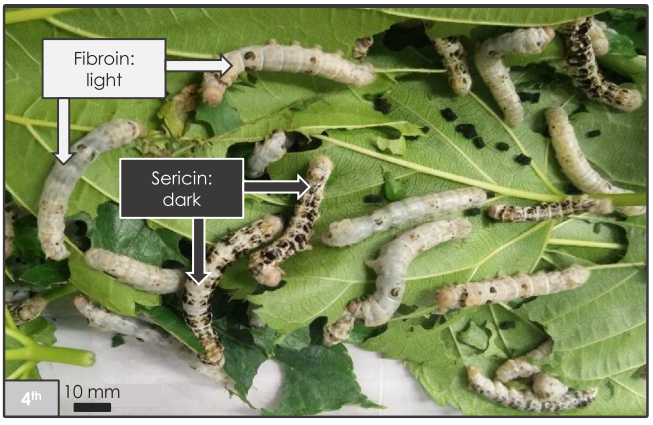

Fig. 1. Identification of fibroin deficient Bombyx mori samples. Individual sericin only mutants are readily identified through stark differences in patterning. Initially this was assumed to be variation in sex, but all colour pairings produced viable offspring. Confirmation of sericin/fibroin production is detailed in Section 2.3. In keeping with this natural colouring, all future graphs use dark colours to represent sericin data and pale grey for fibroin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: J. Sparkes, C. Holland, The rheological properties of native sericin, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.01.021