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Thermo-rheological behaviour of native silk feedstocks

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ABSTRACT

The rheology of native silk protein feedstock specimens was characterised by shear and oscillatory measurements, over the temperature range from 2 to 55 °C, producing no evidence of thermally-driven phase change behaviour. All specimens exhibited flow characteristics typical of a concentrated polymer solution, with visco-elastic behaviour dominated by two main relaxation modes exhibiting time constants around 0.44 and 0.055 s at 25 °C. The specimens showed well-behaved temperature dependence following the Arrhenius equation, consistent with the kinetics being governed by an activation energy of flow, which ranged from 30.9 to 55.4 kJ mol⁻¹ based on oscillatory data. Consequently, for the first time, it was possible to compile master-curves for native silk feedstock specimens following the principles of time-temperature superposition, using oscillatory data demonstrating visco-elastic behaviour typical of a polymer solution across a wide temperature range. Our work has highlighted the processing range of natural silks and furthered our stance on the molecular mechanisms governing the flow behaviour of these interesting and important materials.

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1. Introduction

The ability to spin silk fibres is a remarkable feature of many types of arthropods [1], exhibited by numerous species of insects [2–4], notably Lepidoptera larvae (*i.e.* caterpillars) [4–8], and is a defining characteristic of spiders [9,10]. In spite of the diversity of animals with this ability, however, in all cases, the fibres are produced in a similar way, from protein feedstock that is synthesised and stored in special glands inside the body, then spun, solidifying ‘on demand’ [11–14].

As would be expected for a biological process, natural silk spinning uses an aqueous medium at body temperatures (*i.e.* essentially ambient temperatures for ectothermic animals). This is in stark contrast to industrial fibre spinning, which typically involves esoteric or harmful process chemicals (for wet- or dry spinning) or high temperatures (for melt spinning) [15,16], in order to engineer sufficiently large changes in physical conditions or chemical composition to drive solidification and stabilise the nascent fibres.

Clearly, the conversion mechanism from liquid feedstock to solid fibre under such mild conditions must play a key role in natural silk spinning. This appears to occur by a flow-induced phase change, as the silk feedstock passes through the spinning duct or is drawn into a fibre, on leaving the body [10–14,17–27]. Notwithstanding numerous investigations, however, some of the structural mechanisms for this proposed phase change can appear contradictory or counter-intuitive, thus leaving the precise details somewhat conjectural. In particular, several authors have based their explanations on the proteins (*i.e.* fibroin or spidroin) adopting folded structures in the silk feedstock, which are unfolded by flow stress, promoting coagulation through hydrophobic interactions [23] or ionic crosslinks [25,26], followed by hydrogen bonding and culminating in β -

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sheet formation. Yet the idea of silk proteins having folded tertiary structures akin to those seen in other *in vivo* globular proteins appears to be contradicted by experimental evidence from Raman spectroscopy [24], circular dichroism (CD) studies [28–32], small-angle neutron scattering (SANS) [32] and nuclear magnetic resonance (NMR) spectroscopy [33–36]. Hence, as we have argued previously [37], the fibroin in native silk feedstock appears more similar to the recently defined class of intrinsically disordered proteins (IDP) [38,39], rather than a precisely-folded globular protein. This forms the basis for our investigations into the rheology and phase change behaviour of native silk feedstocks.

It is generally accepted that the rheology of the native silk feedstock is an important factor in the spinning process, which has led to numerous publications [37,40–51]. Recent detailed investigations from our group into the rheology of native silk feedstocks from the domesticated mulberry silk moth (*Bombyx mori*) larvae at 25 °C [37,40] confirmed it is a viscoelastic material with properties typical of a polymer solution. These studies also highlighted considerable natural variability of rheological properties, however, notwithstanding the consistent methods used to obtain the specimens [40]. Even more remarkably, in spite of this rheological variability, it was found that normalising with respect to the cross-over modulus (G_x) and angular frequency (ω_x) consistently reduced the oscillatory data onto master-curves of $\log(G'/G_x)$ and $\log(G''/G_x)$ against $\log(\omega/\omega_x)$ [37]. For the first time this demonstrated a 'rheological simplicity' between the data and excluded potential explanations such as incipient gelation, which would have changed the shape of the master-curves.

To date, the majority of native silk rheology studies have yet to investigate the effect of temperature on the feedstock; those that have tended to focus on irreversible gelation effects brought about by raising temperature [43,47,50]. On the contrary, we believe focussing on changes in silk flow behaviour *below* the gelation temperature provides new perspectives onto the silk spinning process and is the subject of our present work.

Natural silk spinning can be observed over a relatively wide temperature range and, since all known producers are ectothermic, it follows that the environmental and feedstock temperatures during spinning are largely identical. For example, caterpillars of the arctic moth *Gynaephora groenlandica* spin silk cocoons and hibernacula at typical daytime (maximum) temperatures of 5–10 °C [52], while tropical species of silkworms [53] or spiders [54–56] may experience ambient temperatures above 35–40 °C. Clearly, compositional differences between the silk feedstocks of these species may facilitate spinning under these different conditions. Nevertheless, fibre spinning over significant temperature ranges has also been reported for single species in captivity (e.g. 5–30 °C for *Achaeareana tepidarium* [57]; 5–42 °C for *Araneus diadematus* spiders [58,59]; 22–38 °C for the silkworm *B. mori* [60]).

From a polymer science standpoint, changes in the flow behaviour of 'well-behaved' polymer systems at different temperatures generally follow the time-temperature superposition (TTS) principle [61–64]. Using the TTS principle, phase changes or other factors affecting the flow behaviour may be revealed in the temperature dependence (required to superimpose results onto a single master curve). Hence, for example, Liao and co-workers analysed the effects of monomer ratio in poly(hydroxybutyrate-co-hydroxyhexanoate) copolymers [65]; Osterwinter et al. observed the effects of hydrogen bonding in partially and permethylated polyglycerols [66] and Rwei and Lyu were able to locate the onset of liquid crystalline behaviour in a cellulosic system [67].

Thus, our present work seeks to examine in detail how the rheology of the liquid feedstock of *B. mori* changes with temperature. As silk flow behaviour is apparently governed by classical polymer rheology relationships [37,40], one might also expect to find that it exhibits TTS. Firstly, this will contribute to understanding how the ambient conditions may affect silk spinning. Secondly, studying changes in flow behaviour with temperature may help to explain the rheological variability observed for native silk feedstocks and the thermodynamic mechanisms behind its flow-induced phase change.

2. Experimental

Native silk feedstock specimens were obtained from 5th instar *B. mori* larvae (commercially bred four-way poly-hybrid cross of 2 Japanese and 2 Chinese strains) during early stages of cocoon construction and characterised using methods similar to those described previously [37,40]. In summary, silk glands were excised and the epithelial membrane was peeled off under cold (ca. 5 °C) distilled water, using fine tweezers and a dissection microscope. A portion (ca. 0.01–0.02 g) of the (predominantly) fibroin solution from the middle posterior section of the gland was carefully and promptly (within 30 s) transferred to a rheometer (Bohlin Gemini, Malvern Instruments Ltd. Malvern, UK), fitted with a Peltier heating and cooling stage and a CP1/10 geometry (10 mm diam. cone with 1° opening angle and 30 μm truncation). Excess water was removed from the surface of the specimen, by absorbing it into a small piece of tissue paper, before lowering the cone. The closing speed was reduced to the slowest (ca. 0.1 mm s⁻¹), to minimise the associated (shear and extensional) flow. The sample size was sufficient to completely fill the gap between the cone and plate; the loading was carefully observed by eye and any inadvertently under filled or unevenly filled samples were rejected. The small amount of excess material around the cone was not removed, to avoid any risk of flow-induced gelation. The entire sample area was flooded with distilled water, then enclosed within a loosely fitting cover to prevent drying and skin formation.

At the same time, a larger portion (0.1–0.2 g) of the native silk feedstock from the adjacent part of the gland was also removed and used to determine the concentration of solid residue (mainly fibroin [40]). In order to minimise possible weighing errors due to water evaporation, this specimen was weighed at the same time as the rheology specimen was loaded. (Hence, with practice, the weighing was generally performed within 1 min. after removing the gland epithelium.) After

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