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Chemical, structural and thermal properties of *Gonometa postica* silk fibroin, a potential biomaterial

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ABSTRACT

In the present study, chemical, structural and thermal properties of fibroin from *Gonometa postica*, a wild silkmoth species were investigated. Silk from *Gonometa rufobrunnea* and *Bombyx mori* species were included in this study for comparison. The results indicated that *G. postica* and *G. rufobrunnea* silk exhibited similar properties whereas distinct differences were observed with *B. mori* silk. Amino acid analysis showed that glycine, alanine and serine accounted for more than 70% of the total amino acid content in all species. The amount of polar amino acids in *Gonometa* fibroin was significantly higher than for *B. mori* fibroin suggesting increased chemical reactivity of the former. The abundance of basic amino acids in *Gonometa* fibroin makes it a promising biomaterial in cell and tissue culture. Structural analysis revealed a unique β-sheet structure of *Gonometa* fibroin which is comprised of both poly-alanine and poly-glycine–alanine sequences. The maximum decomposition temperatures for *Gonometa* and *B. mori* fibroin were 350 °C and 320 °C respectively. The influence of amino acid composition on structural and thermal properties of the silks is also discussed.

1. Introduction

Silk is a natural protein fibre produced by a number of organisms including silkworms and spiders [1]. For centuries, mulberry (*Bombyx mori*) silk fibre has been the most important and common type of silk. However, wild silkworm species such as *Antheraea pernyi* and *Antheraea assama* species have become important sources of silk [2]. The increasing demand for functional biomaterials in biomedical and biotechnology fields has renewed scientific interest in silk due to its unique combination of mechanical strength and bio-properties such as biocompatibility, biodegradability as well as high oxygen and water permeability [3,4].

Fibroin has thus been successfully tailored into films, 3D scaffolds and nanofibres which have found application in enzyme immobilization [5], vascular grafts [6], supports for cell adhesion and growth [7] and bone regeneration [8]. Although *B. mori* fibroin has found most applications, noteworthy efforts are being made to utilize wild silk fibroin for biotechnological applications [9]. The attraction to fibroin as a biomaterial stems from its distinctive properties which are attributed to its chemical composition and structure.

Structural and functional studies primarily done on molecules isolated from *B. mori* species have long established that fibroin is the main silk protein comprising of heavy and light chain polypeptides linked by a disulphide bridge [10]. Fibroin possesses extraordinary mechanical strength due to its highly oriented β-sheet crystalline structure consisting of (Gly-Ala-Gly-Ala-Gly-Ser) repeat units found in the heavy chain. The fibroin amino acid composition is therefore dominated by glycine, alanine and serine.

The specific primary structure (i.e. sequence of amino acids) of fibroin may vary depending on the source species. X-ray diffraction analysis of fibroin from the wild or semi-domesticated *Saturniidae* silkworms showed existence of polyalanine repeats sequences different to the *B. mori* glycine–alanine repeat structure [11]. The crystalline region of fibroin is mostly hydrophobic and plays an important role in its physical properties thus influencing properties such as biodegradability and biocompatibility. For example, Acharya et al. reported that *A. mylitta* films had better support for cell growth as compared to *B. mori* films [12] because of the arganine–glycine–aspartic acid (Arg-Gly-Asp) tri-peptide sequence in the former which is not found in the fibroin of the latter. An extensive review on the potential of non-mulberry silk fibroin as a biopolymer was recently published [13] and highlights applications of wild silk fibroin in numerous fields along with its advantages over *B. mori* fibroin. As such, there has been a noticeable surge in the study of wild silks whose chemical and physical properties have not been reported [14].

Gonometa postica and *Gonometa rufobrunnea* (Lepidoptera: Lasiocampidae) are wild silkmoth species indigenous to Southern Africa which produce quality silk that has been successfully commercialized [15,16]. Despite similar cocoon features, there are differences between the two *Gonometa* species such as host plants

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and geographic location [17] which might influence their characteristics. Unlike *B. mori* and other wild silks which have been explored as biomaterials, both *Gonometa* species have not found applications beyond textiles to date. Their potential in biomedical and biotechnological fields can only be understood and explored if characteristics relating to applications are well investigated. These characteristics include procedures involved in the processing of the silks such as degumming and demineralization [18], as well as chemical and physical properties of the silks. Freddi et al. studied the chemical and physical properties of *G. rufobrunnea* proteins [19]. There has been a report on the chemical composition and rheological behavior of *G. postica* fibroin extracted directly from the silkworm glands [20]. However, an extensive study of chemical and physical properties of fibroin from the *G. postica* cocoons has not been reported. It is therefore fundamental to study the chemical and physical characteristics of *G. postica* fibroin in order to accurately assess its potential functionality and applicability in various fields.

The current study therefore focuses on determination of chemical, structural and thermal characteristics of fibroin from silkworm cocoons of the Southern African species *G. postica*. A comparison with *G. rufobrunnea* and the domesticated *B. mori* species is also presented. This work provides valuable information for further work which focuses on investigating possible utilization of these wild fibroins in a wide range of applications. Also, data from this study will add to and complement existing knowledge on silk proteins.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and reagents

Amino acid standards, sodium tetraborate decahydrate, boric acid, disodium hydrogen phosphate, triethylamine, methanol, phenylisothiocyanate (PITC), sodium carbonate and 32% hydrochloric acid were all purchased from Sigma Aldrich (St. Louis, USA) and were of analytical grade with a purity of at least 98%. Buffers and all aqueous solutions were prepared in Ultra-high purity water (18.2 mΩ) from a Millipore MilliQ water purification system (Molsheim, France).

2.1.2. Silkworm cocoons

Samples of *G. postica* cocoons were collected from Eastern Cape Province in South Africa. *G. rufobrunnea* cocoons were obtained from two different geographic locations: Gwanda, Zimbabwe [*G. rufobrunnea* (Zim)] and from Shashe in Botswana [*G. rufobrunnea* (Bots)] and *B. mori* cocoons were donated by Elster Vermeulen.

2.2. Procedures

2.2.1. Degumming

The *Gonometa* cocoons were degummed using a two-step boiling procedure, firstly with 1.1 g/L Na_2CO_3 for 1 h followed by 0.5 g/L Na_2CO_3 for another hour. For *B. mori* cocoons, degumming was achieved by boiling in 1.1 g/L Na_2CO_3 solution for an hour. The cocoon to water ratio was 1:40 in both cases. The fibres were thoroughly rinsed with warm distilled water prior to air drying.

2.2.2. Cocoon structure and fibre morphology

Efficiency of degumming, cocoon surface characteristics, fibre size and morphology were examined using Scanning electron microscopy (SEM). Images of undegummed and degummed silk fibres were obtained after gold sputtering using a TESCAN Vega VG1760481J (Brno, Czech Republic) scanning electron microscope at 20 kV. Fibre diameters were obtained by randomly measuring 25 fibres from different SEM images.

2.2.3. Amino acid quantification using MEKC

Degummed silk fibres were analysed for amino acid composition. For each silkworm species five replicates of 5 mg fibres were hydrolyzed using 6 M hydrochloric acid in an oven at 110 °C for 24 h. 50 μL aliquots of the amino acid hydrolysates were dried then derivatized using phenylisothiocyanate (PITC) according to the procedure described by Zahou et al. [21]. An Agilent 7100 capillary electrophoresis system (Waldbronn, Germany) equipped with a DAD detector at 210 nm was used for micellar electrokinetic chromatography (MEKC) analyses. Optimized separation conditions were achieved with a 32 mM/0.5 mM/172 mM phosphate–borate–SDS buffer at pH 6.70, an applied voltage of +26 kV and a temperature of 27 °C. Instrument control, data collection and processing were achieved using Agilent Chemstation (DE, Germany). Norleucine was used as an internal standard.

2.2.4. Structural and conformational analysis

X-ray diffraction studies were conducted to elucidate the physical structure by identifying the positions of the diffraction peaks. Diffraction curves were recorded using a PANalytical X'pert Pro X-ray diffractometer (Almelo, Netherlands) with Cu (Kα) radiation (λ = 1.54 Å) from 1° to 70° with a scan step size 0.02626° (2θ). Voltage and current of the X-ray source were 45 kV and 40 mA, respectively. Data and quantitative phase analysis was done using HighScore Plus v3.0e software (Almelo, Netherlands).

FTIR analysis was done using a Perkin Elmer RX1 spectrometer (MA, USA) with a Miracle^{TM} ATR attachment. Spectra were acquired in transmittance mode with 32 scans at a resolution of 2 cm^{-1} over the 4000–600 cm^{-1} spectral region.

FT-Raman spectra were obtained using a Brucker MultiRAM FT-Raman spectrometer (Ettlingen, Germany) with a germanium detector. Silk samples were mounted vertically in front of a mirror and the spectra were accumulated from 512 scans at a 4 cm^{-1} resolution with 0.45 W laser power.

2.2.5. TGA and DSC analyses

Thermal properties were studied using a TA Instruments TGA Q500 (DE, USA). Samples (10–15 mg) were loaded into a platinum crucible. The samples were heated under nitrogen from 25 to 1000 °C at a heating rate of 10 °C min^{-1}.

Differential scanning calorimetric (DSC) analysis of 4.5 mg of fibres was done using a TA Instruments DSC Q2000 (DE, USA) with a scanning rate of 10 °C min^{-1} (between 25 and 450 °C) and nitrogen and air gas flow rate of 50 mL min^{-1}.

3. Results and discussion

3.1. Cocoon and fibre morphologies

Fig. 1 shows photographs of cocoons from the different species and degummed *G. postica* silk fibres. *G. postica* and *G. rufobrunnea* cocoons showed similarity in shape and both had highly variable sizes, though *G. postica* cocoons were generally larger. Both wild species cocoons were tougher than those of *B. mori* thus requiring harsher degumming conditions than the latter.

SEM images of undegummed fibres for both *Gonometa* species were noticeably cemented together with sericin unlike the *B. mori* fibres where sericin appeared to be a non-uniform coating on the fibres (Fig. 2A(i)–D(i)). Cocoons for *Gonometa* species had a white deposit on the surface, a phenomenon reported elsewhere and ascribed to calcium oxalate from the silkworm excretions [22]. After degumming, silk fibres were examined and showed no