Available online at www.sciencedirect.com
SCIENCE DIRECT
Polymer 46 (2005) 7909–7917
polymer
www.elsevier.com/locate/polymer

A new silk: Mechanical, compositional, and morphological characterization of leafhopper (*Kahaono montana*) silk

Jung C. Chang[a], Murray J. Fletcher[b], Geoff M. Gurr[c], Deborah S. Kent[d], Robert G. Gilbert[a,*]

[a]*Chemistry School F11, Key Center for Polymer Colloids, University of Sydney, NSW 2006, Australia*
[b]*New South Wales Department of Primary Industry, Orange Agricultural Institute, NSW 2800, Australia*
[c]*Pest Biology and Management Group, Charles Sturt University, NSW 2800, Australia*
[d]*Forests New South Wales, NSW DPI Science and Research, NSW 2119, Australia*

Received 5 February 2005; received in revised form 5 June 2005; accepted 20 June 2005
Available online 19 July 2005

Abstract

The mechanical properties, amino acid composition, internal morphology, and solvent-induced interaction of silk produced by the endemic Australian leafhopper, *Kahaono montana* Evans (Hemiptera: Cicadellidae) were studied. Ion plasma etching/scanning electron microscopy examination of the internal morphology revealed a skin–core structure, with bands in the core region aligned regularly in a transverse direction to the fibre axis, separated by a nominal spacing of 100 nm. The internal structure of the silk was compared with those from spider *Eriophora transmarina* (Keyserling) (Araneida: Araneidae) radial thread and silkworm (*Bombyx mori*). The amino acid composition of *K. montana* silk was determined using HPLC, and was found to be dominated by small amino acids: Serine, alanine and glycine. The silk–solvent interaction was tested using selected aqueous, organic and surfactant solutions, and the solubility of the silk was found depend primarily on the pH and ionic strength of the solvent. Tensile tests showed that the silk has considerably weaker mechanical properties than spider silk and silkworm silk. The differences in mechanical properties of *K. montana* silk compared with spider and silkworm silk are attributed to the distinction in amino acid composition ratio and internal morphology, and are likely to reflect the functions of the silks in these species.

Keywords: Biopolymer; Microstructure; Amino acid analysis

1. Introduction

Silk is a fibrous protein with unique physical and mechanical properties. The best studied silks are produced by spiders (Arachnida) and insects (Insecta), especially silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). The recent discovery of a silk produced by the endemic Australian leafhopper, *Kahaono montana* Evans [1], presents an opportunity to characterize the properties of a novel biopolymer. Previously described silks come from insects in several orders, but that from *K. montana* is remarkable for being the first known case of silk production by an hemipteran.

Characterization of a silk can be divided into five categories: Amino acid composition, sequencing, molecular orientation, structural conformation and mechanical properties; each category influences other categories in a hierarchical order. Thus the amino acid composition of silk has a direct influence on the sequence of the protein chains, which then determines protein orientation, conformation and lastly its physical and chemical properties [2–4]. The production mechanism of the silk is also of considerable interest. The rheology of the precursor liquid synthesized by the animal to spin the silk is also significant.

The amino acid ratio of familiar silks is dominated by small side chain amino acids [5], which is recognized as the major characteristic of the silk fibroins, where the total concentration of alanine, glycine and serine rarely accounts for less than 50% of the overall amino acid composition.

* Corresponding author. Tel.: +61 2 9351 3366; fax: +61 2 9351 8651.
E-mail address: gilbert@chem.usyd.edu.au (R.G. Gilbert).

0032-3861/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2005.06.077

Although only a few types of amino acid residues are dominant in silk, wide variations in composition are observed (and moreover variability can be influenced by the dietary intake of the animal and the environmental conditions during the spinning process [6–9]). For example, silk produced by insects in the orders Lepidoptera and Hymenoptera have an extremely high content of amino acids with small side chains (alanine, glycine and serine) [10–12], which may comprise up to 80% of the total composition. These small amino acids are crucial in forming the crystalline region of the silk: Where the polypeptides are incorporated largely in regular β-sheet structures in the form poly-Ala and poly-Gly-Ala, as well as in α-helical structure in the form of poly-Gly [3,13–15]. This crystalline region has been demonstrated to be responsible for the outstanding tensile strength and elasticity of spider silk fibres [16–18].

Silk may also contain relatively high concentrations of bulky amino acids such as glutamic acid, aspartic acid, proline and valine [3,19–21]. The presence of these amino acids is responsible for the formation of the amorphous part of the silk, which in turn affects the overall physical properties of the silk in association with the crystalline region [4].

The silk of *K. montana* aggregates on the leaf and forms a tent-like structure, characteristically coated by a heavy mixture of honeydew (a brown–yellow sugary substance secreted by various leaf-sucking insects), sooty mould (fungi, species *Capnodium*) and brochosomes (proteinaceous secretory particles produced by leafhoppers). Production of the tent usually occurs during winter and the growth of sooty mould on the honeydew imparts a black colouration to the tent, suggesting a thermoregulation benefit to the leafhopper [22]. A study of this novel biopolymer, being from a species which is taxonomically distant from previously studied silks, offers scope to more generally elucidate the structure–function relationships for all silks as well as elucidating the reasons for these materials evolving in widely separate taxa.

Protein conformation and internal morphology of silk can be characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [23–25], atomic force microscopy (AFM) [26,27], Raman spectroscopy [28–30] and X-ray scattering [10,23,31]. These have provided detailed information on the crystallites, nanofibril and microvoids for both silkworm and spider silks [27,32,33]. In this study, the properties of *K. montana* silk were examined in terms of its mechanical strength, internal structure, composition, and interaction with solvents. The mechanical property and amino acid studies utilized common methods including tensile testing [34–36] and high-performance liquid chromatography (HPLC) [6,7,21]. Ion etching was used to expose the internal structure [37,38], and silk–solvent interactions were determined based on the degree of solubility of silk in different solvents.

2. Experimental

2.1. Material and sample preparation

Raw silk samples were obtained from the leaves of *Eucalyptus robusta*, one of the host plants of *K. montana*, located in the Koala Browse Plantation of the University of Western Sydney, NSW, Australia. For mechanical property experiments, raw silk samples were used and were collected directly from the silken tents.

The following washing protocol was developed for experiments that required clean fibres. The silk was first rinsed with milli-Q water for 30 min to remove any water-soluble matter and loosely attached contaminants. The silk was then boiled in 0.4% sodium dodecyl sulfate (SDS) solution for approximately 8 h until the colouration associated with sooty mould was removed. The silk samples were then collected and rinsed with milli-Q water for 30 min and stored in milli-Q water prior to analysis. Washed samples were allowed to air dry for at least 12 h in a dust-free environment before any property characterization. SEM observations showed the silk, after being washed with the above procedure, to be free from brochosomes, sooty mould and honeydew residues, and also showed that the cleaning technique had caused minimal disturbance to the fibres.

2.2. Mechanical properties

Obtaining a single silk strand was impractical due to the small size of the strands (0.7–1 μm diameter), so samples of multiple strands consisting of 7, 17, and 24 strands were tested. Before the experiment, samples were examined under an optical microscope to measure the fibre diameters and to ensure sufficient spacing between the fibres, so that the result obtained would be the summation of individual fibres, not from a silk bundle. This eliminated artefacts such as friction or twisting forces. The mechanical properties of the silk were tested using an Instron 5567 tensile tester. Samples were prepared by carefully detaching raw silk fibres from a silken tent using forceps, and mounting onto a paper frame using double-sided tape to give a gauge length

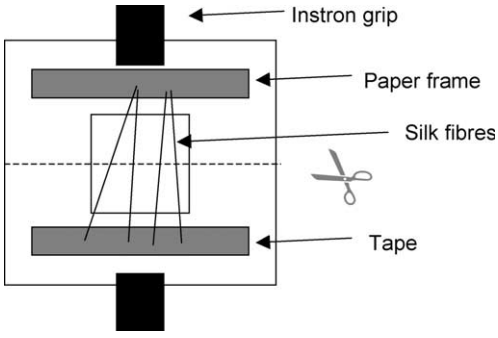

Fig. 1. Method for mounting *K. montana* silk specimens for Instron tensile testing.

of 15 mm, as shown in Fig. 1. After the sample was mounted into the Instron tester, the centre of the paper frame was cut and the stretching begun at a speed of 2 mm min^{-1}. A load cell of 2.5 N was used. The initial tension of the silk fibre was not determined.

The values of strain were measured as the fibre elongation divided by the initial length. Stresses were calculated as the load divided by the fibre cross sectional area, assuming the sample volume remained constant during the experiment. The Young's modulus, or the elastic modulus, was derived from the slope of the stress–strain curve. The diameter used for the calculations was 0.85 μm, the average diameter of the silk fibres estimated by SEM.

2.3. Ion etching

Ion etching is a technique for thinning specimens by slowly removing the surface material with ion plasma prior to examination by electron microscopy. Samples were prepared by attaching the silk specimens onto a SEM stub with double-sided carbon tapes, then sputtering with argon ions using a Dual Ion Miller, Gatan model 600. The pressure was reduced to 10^{-5}–10^{-6} Torr prior to bleeding argon gas into the chamber. Current and voltage were adjusted to give power of 1.05 W (0.3 mA, 3.5 kV) or 4 W (1 mA, 4 kV), for 3 or 5 min. The etched specimens were lightly coated with Au/Pt and examined with a Philips SEM 505.

2.4. Amino acid analysis

Two samples of approximately 0.5 mg cleaned silk were analysed commercially by Australia proteome analysis facility (APAF), Macquarie University, Australia. The samples were hydrolysed with HCl for a prolonged period [39], followed by derivatization of the hydrolyzed amino acid residues. Separation of the derivatized amino acids was performed using an HPLC equipped with a Waters AccQ-Tag system.

2.5. Silk–solvent interaction

Cleaned silk samples (~0.1 mg) were placed into a round-bottom flask with a magnetic stirrer. The solvents (10 mL) were then added into the flask and stirred at room temperature for at least 1 h. If the silk had not dissolved, the temperature was raised to 97 °C and stirred for a further hour.

3. Results and discussion

3.1. Mechanical properties

The aim of this paper is to establish the general properties of *K. montana* silk and compare these properties with those from silk from other species, with the eventual aim of elucidating the molecular structure, biological function and evolutionary role of this new biopolymer. As shown below, the dramatic qualitative feature of the mechanical properties of *K. montana* silk is its weakness compared to other silks: An important result for the broad aims of this work. Indeed, quantitative measurement of this mechanical strength encountered considerable technical difficulty, in particular with regard to sample handling and signal detection, due to the exceedingly small fibre diameter and its weak tensile strength. The inaccuracies in these quantitative measurements are, however, insignificant when viewed in the light of the importance of finding that *K. montana* silk is mechanically very weak.

Testing multiple silk strands helped enhance the stress signal, although this induced greater experimental uncertainties. When testing multiple silk strands, the unevenness of silk length mounted on the paper was expected to give more than one maximum point on the load–strain curve. As shown in Fig. 2, all five curves showed multiple maximum points in the strain, indicating a series of breakage points during elongation. As the elongation reached about 6%, the forces dropped sharply, indicating the first breakage of the strands. The rest of the sample continued to elongate until another sharp decline on the load occurs. The average tensile strength for an individual silk fibre was determined as the total force at each reflection point divided by the total number of strands and the average diameter. The breaking strain was measured between the zero and the first break point. The noise apparent in the data is because the system is sensitive to small external forces such as air currents.

The average tensile strength of *K. montana* silk, 280±72 MPa, is several times weaker than that of silkworm silk [34] and spider silk [40,41], as shown in Table 1. The breaking strain of *K. montana* silk, 6.9±1.6%, was also significantly lower, while its Young's modulus, at 4.4 GPa, is in the medium range. The mechanical properties of *K. montana* silk exhibited degrees of variability, as also observed in silkworm silk and spider silk [34,42,43]. Because of the consistency in the sample preparations and measuring conditions, the discrepancies in the mechanical properties of the silk are regarded as reflecting variations

Table 1
Comparison of mechanical properties of *K. montana* silk with published values for silkworm silk *B. mori* [34], spider *A. diadematus* dragline silk [40], radial thread [41], and nylon

	Tensile strength (MPa)	Breaking strain (%)	Young's modulus (GPa)
K. montana silk	280.7±72.9	6.9±1.6	4.4±1.6
B. mori	650±40	12±1	5.1±1
A. diadematus (dragline)	1080±160	28±4	6.9±1.2
A. diadematus (radial thread)	1154±14	39.4±3.3	2.9
Nylon 6	76	50	1.4

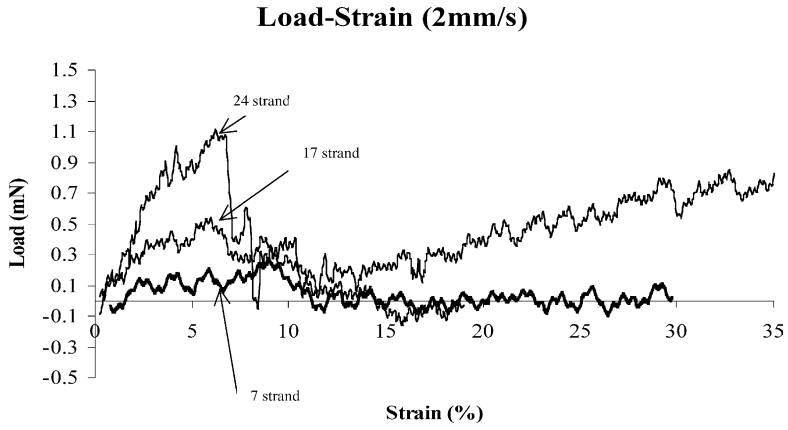

Fig. 2. Stress–strain curves for *K. montana* silk taken with 7, 17, and 24 strands at extension rate 2 mm s^{-1}.

within the silk itself, caused by changes in spinning conditions [8,44].

3.2. Internal morphology

Raw and cleaned silk samples showed similar behaviour when subjected to ion etching, although under similar conditions, cleaned silk appeared to etch more readily. At 1.05 W for 3 min, the surface topography of the silk fibres remained smooth and the plasma only caused distortion to some fibres, and also partially melted the brochosomes (Fig. 3(a)). The ion plasma appeared to remove the outer part of the fibre and to expose the presumably harder interior core (Fig. 3(b)). As can be seen from the image, the uppermost fibre shows a gradual removal of the silk surface from upper left to right, accompanied by a decrease in diameter. The irregularity on the edge of the etched silk indicates partial melting of the soft material, which disappeared (ablated) when treated under higher power and for longer duration.

Five minutes of etching reduced the fibre diameter to less than half its original size (<0.5 μm, Fig. 3(c)). The core section of the fibre appears regular in structure and is longitudinal to the fibre axis. Along the core there are evenly spaced transverse bands, which are continuous, periodic, slightly parabolic along the axis, and separated by $\sim$100 nm. After 3 min at 4 W the core showed signs of break-down and exposed what is interpreted to be an interior band (Fig. 3(d)) with a diameter of 0.3 μm. After 5 min at 4 W, the silk broke down further, becoming completely deformed and flattened (Fig. 3(e)). The presence of such bands is known in silkworm silk [23]. Computation and 2D modelling have shown [45] that parabolic or V-shaped bands can redistribute the axial stress from the centre to the edge of the fibre and may enhance the elastic modulus of the fibre without changing its yield strength.

It seems likely that the transverse bands have a direct relationship with the formation and production mechanism of the silk. Further investigation of this area appears warranted.

For comparison, spider silk was examined using the same technique and instruments as used for the silk of *K. montana*. Two samples of silk from the spider *Eriophora transmarina* (Keyserling, family Araneidae) were treated at 4 W for 5 min. It was seen that spider silk has a rather different internal morphology to *K. montana* silk, showing a nanofibril type of morphology (Fig. 3(f)). The nanofibrils were 25–40 nm in diameter, smaller than the result reported from fractographic analysis and atomic force microscopy [27,32,46]. Apart from the presence of nanofibrils, ion etching of spider silk did not show signs of the transverse bands, which is consistent with the result obtained from TEM observations [24]. The skin–core structure of spider silk reported in the literature [32,33] was not observed in the present study, presumably due to plasma-induced damage.

The difference in the internal morphology of the two silks provides a possible explanation for the differences in the mechanical properties. The presence of scattered crystallites on the spider silk nanofibrils is consistent with previous studies [33,47]. The soft skin–hard core structure of *K. montana* silk gives it a degree of mechanical strength, but in spider silks, crystallites in the numerous nanofibrils are likely to be responsible for its exceptional mechanical properties. Silkworm silk shows similar nanofibril structure, with diameters approximately 100 nm [27,32].

3.3. Amino acid analysis

During HCl hydrolysis, several amino acid residues undergo various degrees of degradation and conversion due to chemical instability and side reactions [39]. (a) Most tryptophan residues are destroyed, whilst methionine, cysteine and tyrosine residues are partially destroyed in the ratios of >50, >30, and $>20\%$, respectively [48]. The final concentrations of these amino acid residues after hydrolysis were expected to be very low, perhaps beyond the detection limit of the HPLC. (b) Asparagine and glutamine are completely hydrolyzed to their derivatives aspartic acid and glutamic acid; the amino acid analysis was

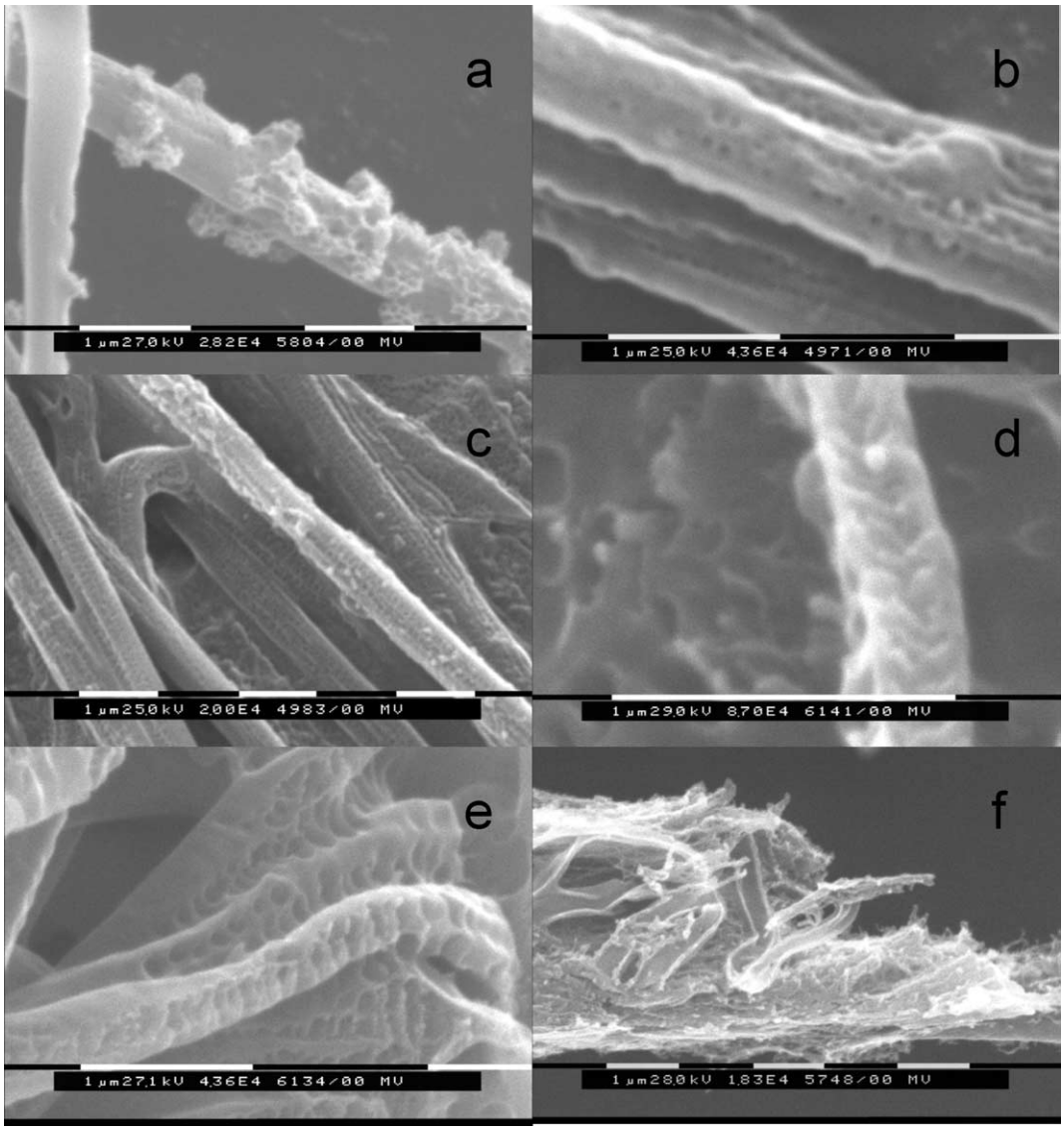

Fig. 3. Ion etched *K. montana* silk. (a) raw silk, 1.05 W, 3 min; (b) washed silk, 1.05 W, 3 min; (c) washed silk, 1.05 W, 5 min; (d) washed silk, 4 W, 3 min; (e) washed silk, 4 W, 5 min; (f) radial thread of spider silk (*E. transmarina*), 4 W, 5 min.

therefore considered to give the total of the corresponding acids and amides within the silk sample.

The two amino acid analyses showed acceptable consistency. The amino acid composition of *K. montana* silk samples is dominated by serine, glycine, and alanine, representing about 57% of the total amino acid concentration (Table 2). Glutamic acid, aspartic acid, leucine, proline and threonine account for the second highest amount of residues, 32%, followed by valine, isoleucine, methionine, lysine, arginine, histidine, phenylalanine and tyrosine, 11%. Other amino acids are either in trace quantities or absent from the silk fibroin.

Serine, glycine, and alanine are the major constituents of most biological silks and are the three amino acids with the smallest side chains [3,5]. It has been demonstrated that these amino acids are responsible for the formation of crystallites within the silk fibroins [49–51], consisting of β sheet structures stacked parallel to the silk axis. The crystallites, or the crystalline regions, usually take up more than 50% of the silk and are considered to be the major reason for silk's mechanical strength. The second highest set of amino acid concentrations in the *K. montana* silk include glutamine/glutamic acid, asparagine/aspartic acid, leucine and threonine, ranging from 6 to 11% of the total amino acid content. These residues have relatively long side chains that inhibit formation of helices or sheets, and therefore exist in a less regular arrangement, making up the amorphous region of the silk. The presence of proline may introduce turns to peptide arrangements in the silk fibroin.

Despite the predominance of serine, glycine and alanine, the amino acid proportion of silk can vary considerably from species to species [5,52]. Table 3 shows the concentration of the nine most commonly occurring amino acids of several silks, arranged by the taxonomy of the silk

Table 2
Amino acid composition of *Kahaono montana* silk fibroin

Amino acid	Sample 1	Sample 2
Non-polar		
Gly	13.9	11.3
Ala	10.6	12.7
Val	3.3	3
Leu	7.3	6.2
Ile	2.5	2.2
Pro	4.2	3.5
Met	0.3	0.2
Polar		
Ser	34	30.8
Thr	7.2	6.8
Cys	–	–
Acidic		
Arx	3.9	7.2
Glx	6.6	10.9
Basic		
Lys	0.5	0.8
Arg	2.2	1.7
His	0.3	0.5
Aromatic		
Phe	2.3	1.5
Tyr	0.9	0.6
Trp	–	–
Total	100	100

producers. The great variety in the amino acid concentration ratios suggests a wide diversity in silk composition: No two types of silk are the same. The variability is not only dependent on the family and species of the producer, but is also strongly dependent on the function of the silk. For example, proline is one of the major constituent in spider draglines and capture threads, because it promotes the formation of the β-turn spiral structure that is believed to govern the elasticity and retraction force [3,16]. Insect silks, as opposed to those of most spiders, are mainly used for protective purposes, which do not require properties that absorb impact forces. Therefore a lower concentration of proline in leafhopper silk compared with that in spider silks is not unexpected. The reason for the observed higher proline content in leafhopper silk compared with other insect silks is not known at this stage.

The LC/SC (small R group:bulky R group) ratio is a structural index that gives a primary means of comparing amino acid residues taking part in the crystalline regions of the silk [12,53], based on the assumption that smaller amino acids are more likely to form regular packing arrangements. The LC/SC ratios therefore correspond to the relative degrees of crystallinity and can reflect the rigidity of the silk fibre. From Table 3, the silk of *K. montana* has the highest value of LC/SC among the silks listed, 0.71. It is 1.5 times greater than the LC/SC value for spider silk and 4.5 times greater than silkworm silk. Other silks represented in the table also showed higher LC/SC ratio than the *K. montana* silk, suggesting a much lower structural regularity in the *K. montana* silk and hence lower rigidity. This index, however, only represents the primary effect based on the chemical composition of the silk. In order to fully understand the relationship between the silk fibre and its mechanical properties and function, the specific protein secondary structure, besides the rigidity of the silk, needs to be determined, especially for spider silk, where β-turns have an important role in enhancing the mechanical properties.

Serine is an important distinguishing factor for the *K. montana* silk, as it represents the highest fraction of the amino acids. Past research has indicated a strong correlation between cocoon silks and the presence of high serine concentrations. As shown in Table 3, silken cocoons produced by lacewings, weevils and spiders all contain a high amount of serine [5,20,52]. Silkworm silk is an exception as it contains more alanine and glycine. Serine is a readily obtainable residue in the insect's food source, which can be synthesized from metabolites such as glucose [54–56]. A higher fraction of serine compared to those of alanine and glycine indicates a lesser degree of regular

Table 3
Composition of the nine predominant amino acids in silk fibroins [5,52]

Order	Hemiptera	Lepidoptera	Lepidoptera	Neuroptera	Hymenoptera	Coleoptera	Araneae		
Family	Cicadellidae (leafhoppers)	Saturniidae (moths)	Bombycidae (silkworms)	Chrysopidae (lacewigs)	Sphecidae (wasps)	Curculionidae (weevils)	Argiopidae (spiders)		
Average (mol%)							Cocoon	Dragline	Capture thread
Gly	12.6	28.6	44.5	24.6	13.7	10.5	14.1	34.3	29.1
Ala	11.7	42.5	29.3	21.2	34	22.8	25.6	26.2	26.5
Val	3.1	1.1	2.2	–	3.9	4.9	4	1	1.3
Leu	6.8	1.5	0.5	–	3.6	3	4	1.2	2.1
Pro	3.9	0.3	0.3	–	2.4	2.2	2.2	9	14.3
Ser	32.4	9.8	12.1	42.7	13.1	28.6	23.7	6.8	6.6
Arx	5.6	4.5	1.3	6	7.2	7.7	4.4	1.6	1.7
Glx	8.7	1.4	1	0.8	13.8	7.5	10.1	11.1	10
Thr	7	0.4	0.9	3.9	2.7	4.4	2.9	1.7	1.2
LC/SC	0.71	0.23	0.16	0.13	0.65	0.62	0.58	0.49	0.44

LC/SC provides a rough estimate on the degree of crystallinity based on the proportion of small amino acids to large amino acids. SC, small R group (alanine, glycine, serine); LC, bulky R group (other amino acids).

β-sheet formation, primarily consisting of poly-Ala and poly-Gly-Ala [57]. The hydroxyl group of serine, however, plays an important role in stabilizing the overall protein structure by providing hydrogen bonds to neighbouring backbone N–H and C=O groups.

3.4. Silk–solvent interaction

Silks are strong, tough and insoluble in water and dissolve only in concentrated salt, acid, base and, sometimes, $NaHCO_3$ solutions [58–60]. Silk behaves differently in solvents with different properties, and investigating this solubility behaviour will aid understanding the chemical nature of the silk.

Solubilities of *K. montana* silk from the present work are shown in Table 4. Of the solvents used, only concentrated NaOH, HCl and $NaHCO_3$ were able to dissolve the silk. Organic solvents and surfactant solutions showed little or no effect. The solubilities of silk in various aqueous solutions suggest that the bonding force in its tertiary structure is primarily electrostatic and not hydrophobic. Changing the pH alters the degree of ionization of amino acid side chains, which disrupts the charge distribution of the protein and hence breaks the intermolecular interactions. The higher solubility in alkaline solutions presumably arises from ionization of acidic residues: Aspartic and glutamic acids. The insolubility of *K. montana* silk in organic solvents and surfactants suggests that the polypeptides maybe arranged in conformations where only the hydrophilic amino acid side chains are on the surface. The dissolution of silk in mild $NaHCO_3$ (0.3 wt%) implies that the ionic strength of the solution is crucial in the solvation mechanism. At similar concentrations, $NaHCO_3$ solution gives slightly lower ionic strength than Na_2CO_3 solution (0.036 and 0.084 M at 0.3 wt%, respectively), which then gives $NaHCO_3$ a higher activity coefficient (0.80) than Na_2CO_3 (0.51). The larger activity coefficient will lead to more interactions between peptide chains and water molecules, suggesting that the 'salting in' effect for silk occurs at a very low concentration. When the ionic strength is raised above ~0.036 M, water molecules are sufficiently bound up in the ion hydration shells to reduce the solubility of the silk. The optimum conditions for solubilizing *K. montana* silk, therefore, can be achieved through combining mild alkaline conditions (pH 8–9) and low salt concentrations [58,60]. Fig. 4(a) shows the change in morphology of the silk after being treated with dilute $NaHCO_3$ (0.03%).

Table 4
Solubility of *K. montana* silk in various solvents

Solvent	20 °C	97 °C
Aqueous		
H_2O (pH 7)	−	−
HCl 1 M (pH 0.1)	−	−
HCl 2 M (pH −0.3)	−	Partial
HCl 3 M (pH −0.5)	−	+
NaOH 0.1 M (pH 13)	−	−
NaOH 0.2 M (pH 13.3)	−	+
NaOH 0.5 M (pH 13.7)	Partial	+
NaOH 1 M (pH 14)	+	+
Na_2CO_3 0.5% (pH 10.8)	−	−
$NaHCO_3$ 0.03% (pH 8.5)	−	Partial
$NaHCO_3$ 0.3% (pH 8.3)	−	+
Organic		
Ethanol	−	
DMSO	−	
Surfactant		
SDS 0.4% (pH 9.7)	−	−
SDS 1% (pH 9.9)	−	−
Aq+surfactant		
SDS 0.4%+Na_2CO_3 0.5% (pH 10.8)	−	+
SDS 0.4%+$NaHCO_3$ 0.03% (pH 9.8)	Partial	+

Symbols + and − indicate soluble and insoluble, respectively.

Surfactant (SDS) did not dissolve the silk at either 0.4 or 1 wt%. At 1 wt% SDS, the silk showed large-scale swelling (Fig. 4(b)) but no dissolution was observed. Spider and silkworm silks, in comparison, showed higher resistance to the solvents than *K. montana* silk [58,59].

4. Conclusions

K. montana silk was found to have tensile strength and elasticity considerably weaker than those of spider silk and silkworm silk. This may be due to its completely different internal morphologies. Spider radial thread contains numerous nanofibrils and crystallites, whereas *K. montana* silk appears to have a simple soft skin–hard core structure; these morphological differences may arise from differences in primary structure (amino acid composition) and/or physical production of the silk fibres. *K. montana* silk has a high amount of small amino acids (alanine, glycine and serine). The ratio between the amino acid composition of *K. montana* silk and other silks is distinguished by a high ratio of serine, which is a characteristic feature for the cocoon silks produced by other insects. The solubility of silk was found to be strongly dependent on the pH and the ionic strength of the solvent. Its solubility in basic aqueous solution is presumably due to ionization of the aspartic and glutamic acid residues that make up the exterior of the silk.

The different compositions and properties of leafhopper, spider and silkworm silks reflect the various biological purposes of these silks. Spider silks require high elasticity as well as high tensile strength to absorb the impact of flying prey, whereas silkworm silk does not have such elasticity, but has high enough strength to serve its protection purpose. As a result of less complex internal structure, leafhopper silk is weak in both elasticity and tensile strength, indicating that the silk is unlikely to be used for physical protection, but rather to be used for thermoregulation or even camouflage purposes.

Besides the physical and chemical properties explored in the study, future work is required to elucidate the origin and the production mechanism of the *K. montana* silk. Silk

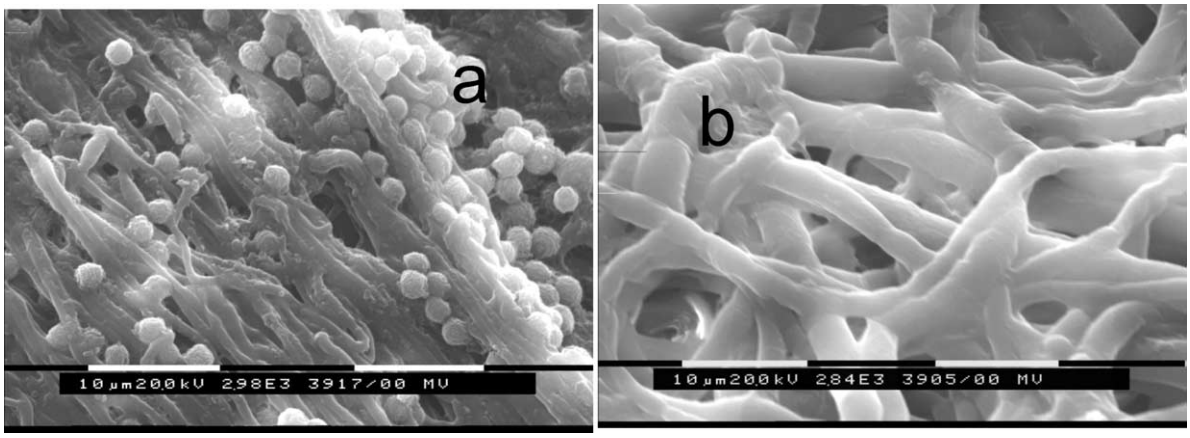

Fig. 4. Solvent and silk interaction. Raw silk washed in (a) 0.03% $NaHCO_3$ at 97 °C; and (b) SDS 1%, 97 °C.

produced by animals from different taxa are from a range of morphological origins, for example abdominal glands (spiders) labial glands (silkworm), and colleterial glands (honeybee) [61]. In the case of *K. montana* silk, it is most likely to be the Malpighian tubules, although the origin remains to be confirmed. Differences in origins may affect the kinetics and mechanism of silk production, which has a direct influence on the conformation of protein structures.

This work has focussed on examining the differences in structure and properties of a new biopolymer: Silk from the insect *K. montana*, compared to silks from other species. The profound differences in mechanical properties of *K. montana* silk compared with spider and silkworm silk, manifested by *K. montana* silk having much weaker mechanical properties, are attributed to the differences in amino acid composition ratio and internal morphology, and are likely to reflect the functions of the silks in these species.

Acknowledgements

The authors acknowledge the facilities as well as the technical assistance from Trevor Shearing and Tony Romeo in Mechanical Engineering and the Electron Microscope Unit, the University of Sydney. The Koala Browse Plantation of the University of Western Sydney kindly provided samples of silk. This research has been facilitated by access to the Australian Proteome Analysis Facility established under the Australian Government's Major National Research Facilities program. The authors also acknowledge the Rural Management Research Institute, the University of Sydney. The Key Centre for Polymer Colloids is established and supported by the Australian Research Council's Research Centres program.

References

[1] Fletcher MJ, Kent DS. Aust Entomol 2002;29:115.
[2] Gosline JM, DeMont ME, Denny MW. Endeavour 1986;10:37.
[3] Lewis RV. Acc Chem Res 1992;25:392.
[4] Jelinski LW. Curr Opin Solid State Mater Sci 1998;3:237.
[5] Lucas F, Rudall KM. Compr Biochem 1968;26B:475.
[6] Craig CL, Hsu M, Kaplan D, Pierce NE. Int J Biol Macromol 1999;24:109.
[7] Craig CL, Riekel C, Herberstein ME, Weber RS, Kaplan D, Pierce N E. Mol Biol Evol 2000;17:1904.
[8] Shao Z, Vollrath F. Nature (London) 2002;418:741.
[9] Vollrath F, Knight DP. Int J Biol Macromol 1999;24:243.
[10] Fraser RDB, MacRae TP. Conformation in fibrous proteins and related synthetic polypeptides. New York: Academic Press; 1973.
[11] Freddi G, Bianchi Svilokos A, Ishkawa H, Tsukada M. J Appl Polym Sci 1993;48:99.
[12] Freddi G, Gotoh Y, Mori T, Tsutsui I, Tsukada M. J Appl Polym Sci 1994;52:775.
[13] Craig CL, Riekel C. Comp Biochem Physiol, Part B: Biochem Mol Biol 2002;133B:493.
[14] van Beek JD, Hess S, Vollrath F, Meier BH. Proc Natl Acad Sci 2002;99:10266.
[15] Van Beek JD, Beaulieu L, Schifer H, Demuras M, Asakura T, Meier B H. Nature (London) 2000;405:1077.
[16] Parkhe AD, Seeley SK, Gardner K, Thompson L, Lewis R. J Mol Recognit 1997;10:1.
[17] Grubb DT, Jelinski LW. Macromolecules 1997;30:2860.
[18] Grubb DT, Ji G. Int J Biol Macromol 1999;24:203.
[19] Vollrath F, Knight DP. Nature (London) 2001;410:541.
[20] Andersen SO. Comp Biochem Physiol 1970;35:705.
[21] Casem ML, Turner D, Houchin K. Int J Biol Macromol 1999;24:103.
[22] Ruf C, Fiedler K. J Therm Biol 2002;27:493.
[23] Shen Y, Johnson MA, Martin DC. Macromolecules 1998;31:8857.
[24] Thiel BL, Kunkel DD, Viney C. Biopolymers 1994;34:1089.
[25] Robson RM. Int J Biol Macromol 1999;24:145.
[26] Gould SA, Tran KT, Spagna JC, Moore AM, Shulman JB. Int J Biol Macromol 1999;24:151.
[27] Miller LD, Putthanarat S, Eby RK, Adams WW. Int J Biol Macromol 1999;24:159.
[28] Rousseau M-E, Lefevre T, Beaulieu L, Asakura T, Pezolet M. Biomacromolecules 2004;5:2247.
[29] Shao Z, Vollrath F, Sirichaisit J, Young RJ. Polymer 1999;40:2493.
[30] Sirichaisit J, Brookes VL, Young RJ, Vollrath F. Biomacromolecules 2003;4:387.
[31] Asakura T, Yamane T, Nakazawa Y, Kameda T, Ando K. Biopolymers 2001;58:521.
[32] Poza P, Perez-Rigueiro J, Elices M, Llorca J. Eng Fract Mech 2002;69:1035.
[33] Frische S, Maunsbach AB, Vollrath F. J Microsc (Oxford) 1998;189:64.

[34] Perez-Rigueiro J, Viney C, Llorca J, Elices M. J Appl Polym Sci 2000; 75:1270.
[35] Perez-Rigueiro J, Viney C, Llorca J, Elices M. Polymer 2000;41:8433.
[36] Osaki S, Ishikawa R. Polym J (Tokyo) 2002;34:25.
[37] Nadiger GS, Bhat NV. J Appl Polym Sci 1985;30:4127.
[38] Blakey PR, Alfy MO. J Textile Inst 1978;69:38.
[39] Fountoulakis M, Lahm H-W. J Chromatogr, A 1998;826:109.
[40] Madsen B, Shao ZZ, Vollrath F. Int J Biol Macromol 1999;24:301.
[41] Kohler T, Vollrath F. J Exp Zool 1995;271:1.
[42] Madsen B, Vollrath F. Naturwissenschaften 2000;87:148.
[43] Marszalek P, Li H, Fernandez JM. Nat Biotechnol 2001;19:258.
[44] Garrido MA, Elices M, Viney C, Perez-Rigueiro J. Polymer 2001;43: 1537.
[45] Johnson MA, Martin DC. Int J Biol Macromol 1999;24:139.
[46] Li SF, McGhie AJ, Tang SL. Biophys J 1994;66:1209.
[47] Thiel BL, Viney C. J Microsc (Oxford) 1997;185:179.
[48] Molnar-Perl I. J Chromatogr, A 1994;661:43.
[49] Asakura T, Kuzuhara A, Tabeta R, Saito H. Macromolecules 1985;18: 1841.
[50] Nadiger GS, Bhat NV, Padhye MR. J Appl Polym Sci 1985;30:221.
[51] Warwicker JO. J Mol Biol 1960;2:350.
[52] Kenchington WJ. Insect Physiol 1981;29:355.
[53] Lombardi SJ, Kaplan DL. J Arachnol 1990;18:297.
[54] Gilmour D. The metabolism of insects. Edinburgh: Oliver and Boyd; 1965.
[55] Pate J, Shedley E, Arthur D, Adams M. Oecologia 1998;117:312.
[56] Brodbeck BV, Mizell III RF, French WJ, Andersen PC, Aldrich JH. Oecologia 1990;83:338.
[57] Hayashi CY, Shipley NH, Lewis RV. Int J Biol Macromol 1999;24: 271.
[58] Hazan A, Gertler A, Tahori AS, Gerson U. Comp Biochem Physiol, Part B: Biochem Mol Biol 1975;51:457.
[59] Sashina ES, Novoselov NP, Heinemann K. Russ J Appl Chem (Translation of Zhurnal Prikladnoi Khimii) 2003;76:128.
[60] Shaw JTB, Smith SG. Biochim Biophys Acta 1961;46:302.
[61] Craig CL. Annu Rev Entomol 1997;42:231.