Spider Silk Proteins – Mechanical Property and Gene Sequence

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[REVIEW]

Spider Silk Proteins – Mechanical Property and Gene Sequence

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ABSTRACT—Spiders spin up to seven different types of silk and each type possesses different mechanical properties. The reports on base sequences of spider silk protein genes have gained importance as the mechanical properties of silk fibers have been revealed. This review aims to link recent molecular data, often translated into amino acid sequences and predicted three dimensional structural motifs, to known mechanical properties.

Key words: spider silk, MaSp1, MaSp2, structure, function

INTRODUCTION

Spiders like a few other arachnids as well as many insects produce silk. Silk typically is defined as a protein polymer extruded into a fiber (Gosline *et al.,* 1996; Vollrath, 1992). Silks are composed principally of proteins with a predominance of alanine, serine and glycine and silk proteins are able to undergo irreversible transformations from soluble protein to insoluble fibres. The constituent proteins are normally synthesised in specialised glands where the epithelial cells are responsible for the biosynthesis. The proteins are subsequently secreted into the glandular lumen where they are stored until extrusion. Unlike silkworms which have only two, orb weaving spiders have seven sets of silk-producing glands - each producing a different type of silk (Vollrath, 1992). Spider silks are characterised by highly specialized properties including extreme strength and toughness, as well as a high capacity to adapt to environmental changes. This raises questions concerning the effect of different internal (e.g. starvation) and external (e.g. temperature and humidity) conditions on the spider's control of silk expression.

Diverse and unique biomechanical qualities allow those silks to be used for a variety of practical purposes ranging from arresting a fall to building a web for the capture of insects. Over the last decade, interest has focussed on dragline silks, mainly because it is thought that such silks have the most desirable properties to be copied and used in a variety of commercial applications. Spider dragline silk is produced by the major ampullate gland and possesses a rare combination of biological properties. It has low density, high strength and a considerable elongation to break. Together these give a toughness (as defined by energy stored before fracture per unit mass) which is superior to the best synthetic fibre material (Gosline *et al.,* 1999).

Molecular studies have determined the partial DNA-sequence for two dragline silk proteins termed Major Ampullate Spidroin 1 (MaSp1) and Major Ampullate Spidroin 2 (MaSp2). These proteins consist of repetitive sequences with poly-alanine regions which vary between four and ten alanine residues sandwiched between sequences that are rich in glycine. Structural studies revealed that the protein fiber is predominantly beta sheet interspersed with regions of undefined structure. Recently, sequence studies have also begun on other silks such as major ampullate silk, flagelliform silk and cylindriform silks. Thus a substantial

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data set has been collected on the structural motifs of spider silk proteins. Such data are being supplemented by a host of physical studies such as tensile testing, Raman spectroscopy, X-ray diffraction, Circular Dichroism and NMR studies. This paper aims to review recent studies and attempt link them to predicted mechanical function. For the purpose of simplicity, we have concentrated on the protein components of two main silks, from the major and minor ampullate glands from two species, *Nephila clavipes* (MaSp1 and MaSp2 from the Major Ampullate gland and MiSp1 and MiSp2 from the Minor Ampullate gland) and *Araneus diadematus.* (ADF-3 and ADF-4 from the Major Ampullate gland and ADF-1) from the Minor Ampullate gland.

SPINNING FIBERS FROM A SPINNING DOPE

The spider has different sets of glands, each set spinning a different silk. We may assume that the different silk glands developed during evolution from one progenitor gland (Vollrath, 1992). A spider is also capable of spinning more than one silk simultaneously. (Foelix,1996) Even though the glands produce different silks, the major ampullate gland can be used as a model system for the production of the silk and its control (Vollrath and Knight, 2001), and this review will concentrate on this particular gland.
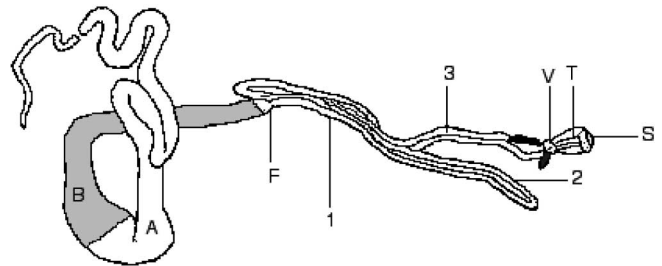
The gland

The secretory pathway through the gland begins with a secretory part, which consists of two zones, the A-zone and the B-zone. (Vollrath and Knight, 1999) The A-zone includes the tail and the first part of the sac. It is highly elongated and diverging with a single type of tall columnar secretory epithelium (Vollrath and Knight, 1999) though ultrastructural evidence suggests that the secretory activity may be higher in the glands tail than in the sac. The epithelium secretes the spinning dope consisting of 25–30% protein, The main components, the silk proteins MaSp I and MaSp 2 make up the bulk of the fiber (O'Brien *et al.,* 1998) as well as the feedstock and are dissolved in an aqueous and highly viscous solution (Hijrida *et al.,* 1996) produced mainly in the tail (i.e. the beginning) of the A-zone (Vollrath and Knight, 1999).

The silk proteins are secreted into the lumen of the gland where they are stored as a highly concentrated (Hijrida *et al.,* 1996) liquid crystalline solution (Knight and Vollrath 1998, 1999b) important for the subsequent spinning from such large molecules (Knight and Vollrath, 1999b). The tertiary structure of the spidroin molecules in the dope at this stage is incompletely understood (Hijrida *et al.,* 1996) but unpublished low angle shadowing and AFM observations (Knight DP, unpublished) strongly indicate that the molecules are elongated flexible rods. AFM of solutions of recombinant spidroin reveals banded rod-shaped filaments (Orudjev and Hansma, 2002) while molecular force spectroscopy suggests these have a lemellar liquid crystal structure previously suggested for spidroin 1 and other silk proteins (Knight and Vollrath, 2002).

The epithelium of the proximal part of the A-zone also adds small mucopolysaccharide droplets, less than 1 um in diameter (Hijrida *et al.*, 1996), which are distributed throughout the silk feedstock, which otherwise is homogenous. As the droplets flow distally they coalesce into larger drops, up to 2.2 um diameter in the centre of the lumen. They seem to be larger in the centre of the lumen than closer to the walls. (Vollrath and Knight 1999; Frische *et al.* 1997). They undergo elongation during extensional low within the convergent spinning duct (Knight and Vollrath, 1999a, b).

The A-zone is followed by the B-zone, much smaller than the A-zone and converging, but still with one single type of epithelial cells, though the ultrastructure of the secretory vesicles changes somewhat with increasing distance from the start of the B-zone (Vollrath and Knight, 1999). These secrete a coat of another material onto the bulk from the A-zone. The coat persists all the way through to the final thread. (Vollrath and Knight, 1999) and makes up 5% of the Nephila dragline silk (Augusten *et al.*, 2000) The coat protein is already folded in short rods before secretion (Knight and Vollrath, 1999a). The secretory part is followed by a thickened cuticular structure called the funnel which connects the duct to the sac. As the duct is more extensible and mobile than the sac, the funnel may serve to anchor the duct to the ampulla. (Vollrath *et al.*, 1998).

Fig. 1. The spider major ampullate gland and associated duct. Zone A (A), zone B (B), funnel (F), limbs of duct (1,2,3), valve (V), terminal tubule (T), spigot (S). Modified from Vollrath and Knight (1999).

The Spinning Duct

The spinning duct is folded into S-shape and narrows progressively (Knight and Vollrath, 1999a)., The spinning dope is in liquid crystalline form in the first two limbs of the S (Knight and Vollrath, 1999b). In the first limb of the duct the spidroin molecules are thought to be anchored perpendicular to the cuticle lining but their long axis progressively bends over towards the centre of the lumen to become parallel to the long axis (Knight and Vollrath, 1999a; Bunning and Lydon, 1996). In the second limb of the duct, the protein molecules are bent alternatively forwards and backwards; in a cellular optical texture liquid crystalline pattern (Knight and Vollrath, 1999a; Bunning and Lydon, 1996). In this texture the slow axis of birefringence lies at right angles to the molecular axis and this is thought to result from the arrangement of the amphiphilic rod-shaped molecules into layered discs, the

molecules or parts of them lying perpendicular to the plane of the disk (Bunning and Lydon, 1996) possibly to prevent the dope from breaking into smaller domains (Knight and Vollrath, 1999a, b; Donald and Windle, 1992). The convergent shape of the duct elongates the dope at practically constant rate (Knight and Vollrath, 1999b). This generates slow and uniform strain. In analogy with comparable liquids (O'Brien *et al.*, 1998; Tirell *et al.*, 1994, Donald and Windle, 1992; Northolt and Sikkema, 1991) this might prevent local coagulation centres before the dope proteins have reached their optimal orientation for spinning. The small droplets secreted in the A-zone are also elongated at constant rate, to form the long canaliculi (Frische *et al.*, 1998; Vollrath and Knight, 1999), which are believed to contribute to the toughness of the thread (Shao *et al.*, 1999).

After the dope has travelled approximately three quarters of the way down the third limb of the duct it rather abruptly pulls away from the ducts cuticle lining to give a draw down taper (Knight, *et al.*, 2000; Gosline *et al.*, 1986). This initiates phase separation, where the liquid dope is converted into a solid thread surrounded by an aqueous phase (Knight *et al.*, 2000) with the water being taken up later in the duct (Knight and Vollrath, 2001). The formation of the beta sheet structures is also initiated here (Knight *et al.*, 2000). The rapid extension flow aligns the molecules and pulls them closer together so they can join by hydrogen bonds into beta crystallites (Knight *et al.*, 2000, Gosline *et al.*, 1986). Due to the taper nature of this duct the point of draw down can be moved, which allows the spider to control and modify thread diameter.

The extensional flow produces higher stresses in the skin than in the core of the thread, which gives rise to higher molecular alignment, which increases the formation of beta structures in the skin (Knight *et al.*, 2000).

The dope initially has a pH of 6.9, which decreases to 6.3 in the third limb. An ion exchange also takes place through the duct. Both sodium and chloride concentrations decrease, while the potassium, phosphate and sulphate increase. The potassium ions are more chaotropic than sodium ; this change gives a greater ability to convert structural water to bulk water. And may play a role in facilitating molecular aggregation into nanofibrils (Chen *et al.*, 2002). The pH decrease - in combination with ionic change - helps the molecular unfolding, and beta crystallisation by neutralising repulsive negative charges on the spidroin molecules encouraging the silk dope to gel (Knight and Vollrath, 2001; Chen *et al.*, 2002).

A final coat of the thread is also applied by flask shaped cells at the distal part of the duct, just after the draw down has been initiated. (Vollrath and Knight, 1999)

The Valve and spigot

The 'valve', a remarkable cuticular modification surrounded by tall columnar cells packed with highly orientated microtubules lies between the third limb of the S-shaped duct with a different cuticular lining. It is used as a breaking or clamping device by the spider to control its dragline as it descends, as the thread at this point is strong enough (Vollrath and Knight, 1999). The valve might also be used as a pump, to pump out a thread that is broken inside, so the spinning can be restarted (Vollrath and Knight, 1999). It lies in a similar location to the silk press of silkworms which may have similar functions. Finally there is the possibility that the valve acts as a clasp to allow post-extrusion draw-down. At the very end of the gland is the spigot, where the thread is drawn from the spider. It is possible that the spigot modulates the thread further mainly by stripping off surface water (Riekel *et al.*, 1999).

THE SILK; GENES AND PRIMARY STRUCTURE OF THE PROTEINS

Spider silk consists of proteins made up largely from non-essential amino acids (Andersen, 1970; Work and Young, 1987). Most spiders produce a variety of silks from different specialised glands. The phylogenetic divergence is reflected in the variability in silk properties in response to varying environmental conditions (Madsen *et al.*, 1999). It is under discussion whether the phenotypic differences in gland morphology and silk mechanics are the result mostly of differences in spinning conditions or in the protein mix of the spinning dope (Hinman and Lewis, 1992). With a variety of genes being cloned from different spiders, a complex pattern is beginning to emerge. In spite of considerable effort, we are still far from a complete picture of the silk genes. However, it has been suggested that the spider is making efficient use of the genetic blueprint to produce different proteins using differential splicing as well as potential post-translational modification mechanisms (Heslot, 1998).

Even though data are emerging from different spiders (Hayashi and Lewis, 2002), the bulk of experimental data has come from the golden orb web spider *Nephila clavipes* and the garden cross spider *Araneus diadematus*. Of particular interest from a molecular point of view has been the proteins that make up the major ampullate (dragline) and minor ampullate silk.

Spider dragline silk is composed of at least two different proteins, MaSp1 and MaSp2 in *Nephila clavipes* and ADF-3 and ADF-4 in *Araneus diadematus* (Guerette *et al.*, 1996; Hinman and Lewis, 1992). A partial cDNA sequence (2.4 kb) representing MaSp1 in *Nephila clavipes*, was first characterized by Xu and Lewis (1990). The total length of the transcript is estimated to be 12 kb (Hayashi *et al.*, 1999). The repeating sequence of MaSp1 is composed of stretches of poly-A or poly-GA sandwiched between segments rich in GGX (Xu and Lewis, 1990).

The total length of the MaSp2 transcript is estimated to be approximately 11.5 kb (Hayashi *et al.*, 1999). Hinman and Lewis (1992) succeeded in characterizing a partial cDNA sequence of MaSp2 which is approximately 2 kb (GenBank accession no. M92913). The predicted amino acid sequence contains highly conserved repetitive motifs. Segments which

are rich in the pentapeptide GPGXX leads into series of poly-A (4–10 residues) (Hinman and Lewis, 1992; Hayashi *et al.*, 1999).

In *Araneus diadematus*, the partial cDNA sequences ADF-3 and ADF-4, show large quantities of transcript in the major ampullate gland and they are thought to be major constituents of dragline silk. The approximate transcript size for ADF-4 is 7.5 kb. ADF-3 has two transcripts (approximately 9.0 and 4.4 kb) which suggests the presence of either a splicing variant or heavy and light chain forms. Both proteins are rich in proline and encode crystal-forming poly-A blocks and repeat blocks of GPGXX (Guerette *et al.*, 1996).

In *Nephila clavipes* the major ampullate gland secretes a protein complex which is approximately 320 kD (Candelas *et al.*, 1983; Xu and Lewis, 1990; Vollrath and Knight 2001). It is chiefly made up of two different proteins MaSp1 and MaSp2 and is about 5% glycosylated. The MaSp1 and MaSp2 chains that form the native protein identified on SDS-PAGE are held together by three to five disulphide bonds. It may polymerise in solution to form the initial oligomers, which are a prerequisite for successful spinning. The nucleotides G and C are abundant in the coding DNA sequence, which results in high amounts of alanine and glycine in the protein. Both MaSp1 and MaSp2 show a similar codon usage with a preference for A or T as the third nucleotide. This is likely to reduce the high degree of secondary structure of the transcript (Mita *et al.*, 1988; Hinman and Lewis, 1992). (At least this has been observed in the silkworm where stretches of G and C are thought to create recombination hotspots, something which could destabilise the genes [Mita *et al.*, 1994].) When MaSp1 sequences from different individuals are compared substantial allelic variation is displayed by the gene; this could be a result from replication slippage and unequal crossing-over. The size of each section of repeats appears not to be under stringent control. Blocks can be duplicated and subject to repositioning in its immediate vicinity. The sequence control tightens nearer the C-terminal, and this can also be observed in different regions along the gene. Each block nearly always begins with GGAGQGGY and ends with GQGAG prior to the start of the poly-alanine region (Beckwitt *et al.*, 1998).

MaSp2 and ADF-3 (as well as the MaSp2-gene from *Araneus bicentenarius*, ABF-1) display differences that appear in each block of repeats. How this regularity is maintained is not understood, the changes can not develop independently. It has been proposed that some fundamental mechanism as mis-matched recombination or gene conversion will result in an advantageous track that spreads throughout the gene (Beckwitt *et al.*, 1998).

In summary, there are strong similarities between the four genes cloned from *Araneus diadematus* and the *Nephila clavipes* genes, MaSp1 and MaSp2. They are thought to be members of the same gene family, since they all exhibit a pattern of alternating alanine-rich blocks with glycine-rich repeats of similar size. Furthermore, all spider silk proteins´ C-terminal domains show strong identity (40 to 91% strict identity and 63 to 95% functional identity), and they contain a conserved cysteine residue that may participate in interprotein disulfide cross-linking (Guerette *et al.*, 1996). This part of the protein seems highly conserved, not only between the different silk proteins of one species, but between different species as well (Beckwitt and Arcidiacono, 1994).

The minor ampullate silk of *Nephila clavipes* consists of two different proteins, MiSp1 and MiSp2 (Colgin and Lewis, 1998). The size of the transcripts is estimated to 9.5 respectively 7.5 kb (Hayashi *et al.*, 1999). In *Araneus diadematus*, ADF-1 is expressed in very large amounts the minor ampullate gland, and is believed to be a major constituent of the minor ampullate silk (Guerette *et al.*, 1996). All of these three minor ampullate proteins share the same amino acid motifs. Poly-A and poly-GA regions interspersed with segments rich in the GGX-motif form the repetitive regions. These regions are interrupted by highly conserved, nonrepetitive and serine-rich spacer regions. The spacer sequence differs from the typical amino acid composition of spider silks, and its function and structure is unknown (Colgin and Lewis, 1998). In contrast to the major ampullate proteins, where the crystal-forming elements occupy approximately 25%, the minor ampullate proteins seem to have a much larger proportion crystal-forming blocks (68% in ADF-1) (Gosline *et al.*, 1999; Guerette *et al.*, 1996; Colgin and Lewis, 1998).

SECONDARY STRUCTURE OF SILK PROTEIN

Spider silk proteins and their genes are only partly characterised (Grip, 2003). It is however generally concluded that spider silk is made up from four different motifs; (i) an elastic beta spiral that is composed of multiple GPGXX motifs where X varies within or between proteins, (ii) crystalline beta-sheet that is rich in alanine, (iii) tight amino acid repeats (GGX) that probably forms a helical structure and (iv) spacer regions with hitherto unknown function (Gosline *et al.*, 1999). The structure of the spacer sequence is unknown, but the other three motifs are thought to be parts of specific tertiary and secondary forming structures within the protein (Hayashi *et al.*, 1999). Interestingly, these motifs appear to be conserved within a variety of spider species (Gatesy *et al.*, 2001). A key element in known sequences are the crystalline poly-alanine and the less crystalline glycine-rich motifs. Even though it is accepted that the silk protein secondary structure is a function of interplay between these regions (Craig and Riekel, 2002), the link to mechanical function is still far from clear. Parts of the sequences have a higher probability to form alpha-helices whereas others are more prone to establish beta-sheets. The interplay between these two secondary forms is probably the most likely explanation for the rapid transformation from dope to fiber .

The GPGXX penta-peptide repeat may conform to a spiral that is similar to the beta turn of elastin. In a predicted model where the spiral was formed by the GGPGGSG-

PGGY, two features were noticeable (Urry *et al.,* 1995). First, the spring-like structure could have an elastic function in the fibre, and the proline residue would be important for the retraction of the fibre when stretched. The hydroxyl groups in serine and tyrosine that form hydrogen bonds with downstream glycine residues are probably also important in the formation of a secondary structure. As a general rule, the major ampullate silk has up to nine beta turns in succession before they are interrupted by another motif. This is sometimes attributed to a 35% extension - as opposed to be the 200% extension measured of flagelliform silk, which has 43 continuously linked beta turns in its spring like spirals (Hayashi and Lewis, 2000; Gosline *et al.,* 1996). We note, however, that the enormous extensibility of several hundred percent of the flag-silk is only shown in the normally wet state (and major ampullate silk is also highly extensible when wet) while in the dry state (normal for ampullate silk) both silks are comparable in showing the much lower extensibility (Vollrath *et al.,* 2001; Vollrath, 2002).

The poly A and poly GA regions probably gives the fibre its tensile strength and constitute the crystalline beta sheets in the silk. The rigid structure of the poly-A segments is accomplished through hydrophobic interactions between alanine residues on alternate sides of a backbone leading to a zipper like structure. The poly GA regions will not display the same hydrophobic interactions and will therefore not yield the same tensile strength.

In theory it is possible for the GGX repeat to have either a beta sheet or a helical conformation. The latter appears more likely since it is corroborated by spectroscopic data (e.g. Chen *et al.,* 2002; reviewed by Vollrath and Knight, 2001). GGX is supposed to have a tight three amino acid helical structure that could serve as a link between crystalline beta sheet regions within the protein and between neighbouring GGX helices in adjacent protein molecules, keeping the fibre aligned.

Very little is known about the spacer regions, other than that they are highly conserved, non-repetitive and rich in serine. Two types of spacers are recognized and even though they differ in amino acid sequence, they are both relatively long, charged and complex (Colgin and Lewis, 1998). The structures formed by the spacer regions in the minor ampullate proteins are still unknown, but a number of possible functions have been put forward; (i) keeping the fiber in an alternative conformation while it is stored in liquid form, this might prevent premature fiber formation in the silk glands, (ii) alignment of crystalline or other structural regions among individual protein molecules, (iii) provide surface regions that interact with other components of the silk, and (iv) serve as a matrix for embedding the crystalline regions (Colgin and Lewis, 1998). The spacer regions seem only to exist in minor ampullate silk. However, other silk proteins might have segments with similar function but different sequences (Colgin and Lewis, 1998).
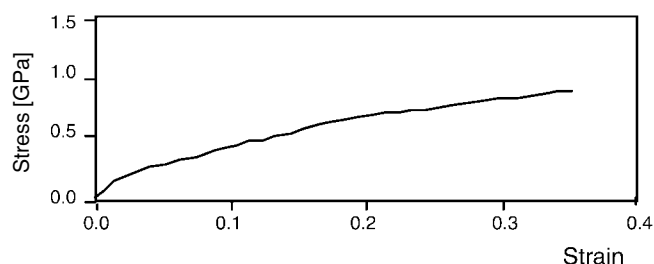
Fourier transform infrared spectroscopy (FTIR) studies indicate that the beta sheets are oriented parallell to the axis of the fiber and suggest that rotation of the sheets may be responsible for contraction of the fiber in water (reviewed by Heslot, 1998). In short, these studies formed the basis for the current structural model which proposes that the poly-alanine regions form the beta sheets which pile up to produce crystals in an glycine rich matrix. NMR studies of the major ampullate dragline silk are perfectly consistent with this model.

PHYSICAL PROPERTIES OF SPIDER SILK PROTEIN

Major ampullate dragline silk is 5 times stronger than steel by weight and has physical properties comparable to those of the synthetic fibre Kevlar. Both dragline silk and Kevlar have strengths of approximately 4 GPa, but differ in elasticity and energy to break. Dragline silk elasticity is up to 35% whereas Kevlar elasticity is only 5%. The energy to break reaches 1×10^5 J/kg in dragline silk as compared to 3×10^4 J/kg in Kevlar. Minor ampullate silk is not as strong as dragline silk, with a strength of approximately 1 Gpa (Hinman *et al.,* 2000).

The properties of spider silk are expressed in so called stress-strain curves, where 'stress' defines the strength ($\sigma = F/A$, i.e. force per cross-sectional area for silk averaged over the fibre length) and 'strain' the extensibility ($\varepsilon = \Delta L/L_0$, where L_0 is the fibre's initial length and ΔL is the change in fibre length). Silk diameters vary greatly and are a function of silk type and spider size but most silks studies range from 0.5–3 μm. The slope of the stress-strain curve gives the stiffness. The area under the curve can be defined as the energy required to break the material, i.e. the toughness of the material. Another advantage of spider silk is its ability to transform a great part of the kinetic energy (65%) into heat instead of catapulting the prey out of the web (advantageous hysteresis). A material with great toughness therefore shows a balance of strength, extensibility and visco-elasticity (Gosline *et al.,* 1999).

Fig. 2. Example of a stress-strain curve for major ampullate silk (Madsen *et al.,* 1999).

The different physical properties in the various silks could be explained by the silk specific amino acid motifs. Major ampullate silk is thought to be a hetero or homo-dimer of two different proteins encoded from the MaSp1 and MaSp2 genes. MaSp1 gives rise to a protein with a crystalline beta-sheet structure composed of poly-A or poly-GA that

are repeatedly alternated by segments rich in GGX. This gives a fiber that is strong, rigid and tensile but with limited elasticity. The MaSp2 protein is more elastic, due to a higher degree of elastic β-spirals that are built up by GPGXX repeats. It is slightly stronger than the MaSp1 protein, which could be explained by a higher number of poly-A repeats.

Unlike the major ampullate silk proteins, proteins from minor ampullate glands are not simply homogenously repetitive. Instead, highly conserved non-repetitive serine rich spacer-regions are alternated with repetitive regions (Colgin and Lewis, 1998). The lack of GPGXX regions in both MiSp1 and MiSp2 may explain the significantly lower degree of elasticity in silk from the minor ampullate gland (5% as compared to 35% in major ampullate silk, *Nephila clavipes*). (Gosline *et al.*, 1986; Stauffer *et al.*, 1994). Prior to breaking MiSp silk deforms upon stretch, whereas MaSp silk regains some its original shape after it breaks. This may be attributed to the irreversible extension of the spacer regions that are only found in MiSp. MiSp1 and MiSp2 are structurally very similar. They constitute mainly of poly-A, poly-G, GGX and spacer regions. The minor ampullate silk contains less poly-A regions than the silk from the major ampullate gland, which explains minor ampullate silk's lower tensile strength value (Colgin and Lewis, 1998) (1 Gpa, as compared to 4 Gpa in Ma silk (*Nephila clavipes*)). The high crystal forming content of MI silk proteins makes them less prone to supercontract in water (Gosline *et al.,* 1999) and also gives them a specific role to absorb kinetic energy in the web (Heslot, 1998).

The major and minor ampullate silks (ADF-3, ADF-4 and ADF-1 respectively) from the spider *Araneus diadematus* have similar motifs and properties as the *Nephila clavipes* silks and display only slight differences in biomechanical parameters (Guerette *et al.*, 1996). It must however be noted that there is significant variability in determining the mechanical properties of the silks, not only within the same species but also for the same individual (Vollrath *et al.*, 2001). This is may often due to gross defects causing fibre failure (Cuniff *et al.,* 1994); however it may also be a result of spider diet (Vollrath 1999) or other internal physiological variables (Madsen *et al.*, 1999; Madsen and Vollrath, 2000; Vollrath *et al.*, 2001). Strength values reported for *Nephila clavipes* MA silk are 4.4–4.8 GPa, as compared to 0.91–1.1 GPa in *Nephila clavipes* MI silk. Elasticity was estimated to 18–28% in *N. clavipes* MA silk and 22–28% in *N. clavipes* MI silk (Stauffer *et al.*, 1994). Other authors report 12% (Gosline *et al.,* 1999) up to 35% (Hayashi *et al.*, 1999; Hinman *et al.,* 2000; Cuniff *et al.*, 1994) elasticity in *N. clavipes* MA silk, versus 5% in *N. clavipes* MI silk (Hayashi, 1999; Hinman, 2000). In major ampullate silk of *Araneus diadematus* the measured strength is estimated between 0.95 and 1.39 GPa and the elasticity between 24–35% (Madsen *et al.,* 1999; Gosline *et al.*, 1999; Cunniff *et al.*, 1994). No values are reported for *Araneus diadematus* minor ampullate silk (See Table 1). We note that measuring conditions are crucial for accurate data on this visco-elastic and extremely thin fibre where the limits of measurability are reached. Therefore many of the measurements accounted for above must be taken with 'a pinch of salt'.

Table 1. Structural modules in Nephila spider silk proteins. MA is the major ampullate gland and MI the Minor ampullate gland. The third column represent transcript sizes and the fourth column summarises the modules present. ⊙⊙ = helical motif.

Nephila clavipes	MA	MaSp1	12.0	$(GA)_n$ and A_n GGX	beta-sheet ⊙⊙	(i).18–28% Elasticity (ii). Tensile strength 4.4–4.8 GPa
	MA	MaSp2	11.5	GPGXX A_n	helix beta-sheet	(iii). Energy to break 10×10^4 J kg^{-1}
	MI	MiSp1	9.5	$(GA)_n$ and A_n GGX Spacer	beta-sheet ⊙⊙ ?	(i). 22–28 % Elasticity (ii). Tensile strength 0.91-1.1 GPa
	MI	MiSp2.5	7.5	$(GA)_n$ and A_n GGX Spacer	beta-sheet ⊙⊙ ?	(iii). Energy to break 10×10^4 J kg^{-1}

〰〰〰 = beta-sheet. The table is based on data from Heslot, 1998; Gosline *et al.,* 1999; Hinman *et al.,* 2000; Vollrath and Knight, 2001.

Single fibres of Major Ampullate silk drawn from the major ampullate glands of mature females *Nephila edulis* (average weight 527±103 mg, mean±s.d.) drawn at highly controlled spinning conditions (a drawing speed of 20 mm s^{-1} and a temperature of 25°C) have an average silk diameter of 3.35±0.63 μm with a normalised average breaking strain of 0.39±0.08, a breaking stress of 1.15±0.20 GPa, an initial modulus of 7.87±1.85 GPa, a yield stress of 0.153±0.058 GPa and a breaking energy of 165±28 kJ kg^{-1} (Vollrath, 2000). Like most fibers, such silk has a moderate positive poisson ratio with a thinning ratio of ca 5% for each 10% of strain in a linear fashion until the maximum extensibility of about 40% when these fibres break. However, these typical mechanical properties of a fibre are greatly affected by the manufacturing conditions. For example, not only diameter but also most mechanical properties were affected significantly by both speed of spinning and temperature of the spider (Vollrath *et al.,* 2001). Micro-X-ray diffraction shows that spinning speed and temperature both affect the molecular structure of a filament (Riekel *et al.,* 1999; Riekel and Vollrath, 2001) which thus is responsible for the observed mechanical properties.

Madsen, Shao and Vollrath (1999), studied the intraspecific, interspecific and intraindividual variability in mechanical properties of silk from spiders of 4 different families. Silk of *Euprosthenops* and *Cyrtophora* were stronger than but not as elastic as silk from *Araneus* and *Nephila*. Silk from *Nephila* was more sensitive to changes in reeling speed than *Araneus*. Moreover, starvation had a negative effect on breaking elongation, whereas the breaking stress varies from day to day but does not seem to correlate with starvation. We note that starvation effects could be a result of other unknown factors, e.g. long duration between measurements. Nevertheless, it can be concluded that the spider silk synthesis is under constant adjustment properties. Hence, effects, like spider growth, food intake or drawing speed, may play a considerable role in mechanical properties of the spider silk. For example, observations on a group of 29 juvenile *Argiope keyserlingi* spiders in Brisbane, Australia, show that change in diet might slightly change the amino acid content of the dragline silk (Craig *et al.,* 2000). On the other hand, there was no observed correlation between certain vegetation and amino acid composition. Eighteen species of *Argiope argentata* from areas in the Caribbean Islands with various types of vegetation, beach-coconut, beach-acacia and mixed grasses, were studied (by Craig *et al.,* 2000). The amino acid composition of dragline silk in the different spiders turned out to be highly variable, particularly the concentration of glycine and serine. Nevertheless, all individual silks had the same *b*-poly-alanine structure. The silks from all groups of spiders contained mostly glycine (20–58%), alanine (6–34%) and serine (6–17%). Of the remaining amino acids of dragline silk, only 4–25% seemed to have been obtained from the prey. Variation in amino acid composition between observed species was believed to lie in the amorphous fraction, which is the fraction responsible for elasticity (Craig *et al.,* 2000).

Whilst observing spider silk through experimental methods, it is important to note that anesthesia highly affects the spider's natural spinning behaviour, as does, naturally, forced silking with a reeling speed decided by an investigator. Anaesthetized spiders produce a thread that has significantly lower breaking strain and energy (Madsen and Vollrath, 2000).

For better understanding of the spider silk protein and how it could be expressed in an artificial way, further studies, not only on a primary protein structure level, but also on internal (e.g. starvation) as well as external (e.g. humidity and temperature) controlling factors and their influence on the protein, are needed. Spider silk research has mainly focused on the resemblance in amino acid sequence of different spiders and spider species. It seems that environmental conditions might play a greater role in the turnout of

Table 2. Structural modules of Araneus silk proteins. MA is the major ampullate gland and MI the minor ampullate gland. The third column represent transcript sizes and the fourth column summarises the modules present. Ⓦ = helical motif.

MA	ADF-3	9.0 and 4.4	GPGXX A_n	(helical motif) (beta-sheet)	(i). 24–35% Elasticity (ii). Tensile strength 0.95–1.39 GPa
MA	ADF-4	7.5	GPGXX A_n	(helical motif) (beta-sheet)	
MI	ADF-1	8.5–9.5	$(GA)_n$ and A_n GGX Spacer	(beta-sheet) (helical motif) ?	---------

= beta-sheet. The table is based on data from Heslot 1998; Gosline *et al.,* 1999; Hinman *et al.,* 2000; Vollrath and Knight 2001.

the fiber than hitherto expected. Differential environmental factors might lead to differential regulation of gene expression, which will result in, for example, a stronger or more elastic fiber. The importance of these notions cannot be underestimated.

CONCLUSIONS

The ultimate aim of this review is to link structural data obtained from silk produced by the major ampullate and minor ampullate glands with their respective mechanical properties. One key structural element of the major ampullate silk from Nephila and Araneus silk is the GPGXX motif, It is present in MaSp2 but not in MaSp1. The GPGXX motif can be readily found in both ADF 3 and ADF 4. Successive head-to-tail arrangements of multiple GPGXX motifs are likely to form a spiral that is similar to the beta-turn spiral of elastin (Urry *et al.,* 1995). Since the GPGXX motifs can be found in major ampullate silk but not in minor ampullate silk, this probably accounts for the fundamental differences in mechanical properties between the two silks. The proposed beta spirals in major ampullate silk may provide one explanation for its high elasticity as compared with minor ampullate silk. Elasticity can be viewed as two counter-acting forces, one to extend and another to retract the fiber. How the arrangement of amino acids contributes to the development of these forces has been partly revealed. Proline residues in the GPGXX motif form the focal point for retractive forces after stretching. When a force is applied to Major ampullate silk, hydrogen bonds break as extension is provoked. After the initial stretching, proline bonds torque; a process that accumulates sufficient energy for retraction.

The predicted role of GPGXX for elasticity has been slightly obscured by the finding that Nephila major ampullate silk has a very low proline content (3%) in comparison with eg Araneus (16%). This is partly reflected by differences in mechanical properties between the dragline silk produced by the two species, but also by recent data suggesting that Nephila dragline silk in chiefly composed of MaSp1 which only contains minute numbers of proline residues. Taken together this data suggests that the pivotal role of GPGXX for the formation of elastic fibres is applicable for some but not all spider species.

The poly-A and poly-GA motifs are thought to form the beta-sheets in the fiber, which probably contributes to its tensile strength. Major ampullate silk is largely composed of poly-A regions whereas poly-GA is more abundant in the minor ampullate silk. The rigid structure of these motifs is accomplished through hydrophobic interactions between alanine residues on alternate sides of a backbone. The poly-GA regions will have less hydrophobic interactions due to the glycine residues which can not form the same interaction as the alanine residues. This probably gives the minor ampullate silk its lower tensile strength when compared with major ampullate silk.

Both the major and the minor ampullate silk contain the GGX motif, which might form a tight three amino acid helical structure. GGX could serve as a link between the crystalline beta-sheet regions within the protein and between neighbouring GGX-helices in adjacent protein molecules, keeping the fiber aligned.

One of the more fundamental features of the synthesis of spider silk is the rapid formation of a solid fiber from a liquid dope. This transformation is amongst other things dependent on the merger of the poly-A repeats by aligning them in an anti-parallel fashion. Even though poly-A repeats have an intrinsic tendency to form helices, this unusual structural behaviour probably is the key to our understanding of the spinning process. Experiments to elucidate this complexity are currently in progress.

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