

REVIEW

Structure, properties, and functional diversity of spider aciniform silk

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Abstract. Biomaterials are becoming increasingly popular in solving applied problems. Aciniform silk (wrapping silk) is one of the seven known types of silk secretions orb-web spiders produce. It has an impressive set of mechanical properties suggesting a high, hereto unexplored potential for textile and biomedical applications. Here we have summarized existing knowledge and identified the gaps in our understanding of the structure, functions, properties and biology of aciniform silk. Aciniform silk is composed of the protein aciniform spidroin (AcSp), which is characterized by relatively lower percentages of alanine and glycine than dragline silk. The specific mix of alpha helices and beta sheets is believed to bring about its great toughness and elasticity. The combination of high toughness and extensibility makes swathes of aciniform silk an ideal tool to keep the prey wrapped, but also for the protective encasing of spider egg clutches. Understanding of the relationship between the composition, properties and biological functions of aciniform silk is still rudimentary, as current studies are mostly mono-focal and barely apply an integrative approach. Closing this gap requires better integration of material science with ecological and evolutionary aspects. This will not only benefit a better understanding of why and how animals use silks, but it will also enable the production of better silk-inspired biomaterials with superior properties.

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Orb web spiders utilize their biological machinery to produce up to eight different types of proteinaceous fibers or adhesives commonly known as spider silk (Vollrath 1999; Foelix 2011). Spiders use silk for various purposes such as locomotion, prey-wrapping, egg-encasing and web construction (Foelix 2011) and produce major ampullate, minor ampullate, aciniform, flagelliform, aggregate, piri-form and tubuliform silks that each serve one or more specific functions and exhibit contrasting properties (Blackledge et al. 2011). Spider silks are composed of filamentous proteins (fibroins) of the spidroin group. Depending on the silk type, primary polypeptide chains are arranged into secondary structures forming sheets or helices in different proportions. The relative proportions and arrangements of such secondary structures are thought to be critical for setting the biomechanical properties, and hence, the biological function of each silk type (Gillespie et al. 1993; Hayashi et al. 1999; Römer & Scheibel 2008; Keten et al. 2010; Nova et al. 2010; Tokar-eva et al. 2014; Blamires et al. 2017). The structural characteristics of the proteins depend on their amino acid sequence and external environmental factors (Vollrath et al. 2001; Chen et al. 2006; Weatherbee-Martin et al. 2016).

Major ampullate silk, which is used in the draglines, the basic frame and the radial threads of the orb web, has been at the center of attention in spider silk research. Besides its remarkable mechanical properties, it can be easily isolated and harvested in sufficient quantities (Griffiths & Salantri 1980; Agnarsson et al. 2010). In contrast, other silk types are remarkably understudied, even though they could be better candidates as biomimetic models for specific applications.

One of these candidates is the silk produced in the aciniform glands. Previous studies have indicated that aciniform silk exhibits high toughness and extensibility that may even surpass some major ampullate silks (Hayashi & Lewis 1998; Blackledge & Hayashi 2006; Lefèvre et al. 2011; Xu et al. 2012b). Aciniform silk is one of the most ubiquitous and ancient types of silks and is produced by almost every species of spider. Yet, this type of silk has been largely

neglected by the spider silk research community, so that knowledge on its structure-function relationships and property variation is extremely limited. Spiders use aciniform silk to construct egg sacs (Vasanthavada et al. 2007), to wrap prey (Vasanthavada et al. 2007) and to build the stabilimenta of orb webs (Seah & Li 2002), among others. Here, we review the available published literature to present the state of knowledge on aciniform silk (AS) and to explore its potential in the applied sciences.

ACINIFORM GLANDS

The silk glands of spiders were initially believed to derive from coxal glands associated with appendages that evolved into spinnerets after losing their locomotory function (Bristowe 1932). However, more recent findings suggest that the silk glands are derived from epidermal invaginations (Kovoor 1987). According to fossil records, the first silk glands that evolved in spiders resembled aciniform glands of the extant species in form and structure (Shear et al. 1989; Selden et al. 2008).

Aciniform glands are smaller compared to ampullate glands (Eberhard 2020). While histochemical analyses of gland secretions suggest that aciniform glands are more closely related to ampullate glands than to other types of glands, the phylogenetic analysis of the gene sequence that encodes the c-termini of aciniform spidroins indicates that AS is more closely related to tubuliform and piri-form than to ampullate silk (Garb & Hayashi 2005; Correa-Garhwal et al. 2022). To date, the evolutionary origin of these glands is not resolved conclusively. As the name implies, aciniform glands have an acinous or “berry-like” morphology, where several lobe-like structures that bear a resemblance to short, small finger-like projections are clustered together (Kovoor 1987). Two distinct types of glands termed aciniform A and aciniform B are characterised based on the secretory cell types found in the gland lining (Kovoor & Peters 1988). Typically, the lining of the aciniform glands is

composed of two types of tall secretory cells (Kovoor 1987), although exceptions are found in some taxonomic groups that show either four types as e.g., in *Hypochilus* Marx, 1888 and *Dysdera* Latreille, 1804 (Kovoor 1987), three types as in *Hersilia* Audouin, 1826 (Kovoor 1984) or only a single cell type as in the family Gnaphosidae (Kovoor 1984, 1987). Aciniform spigots (i.e., the nozzle-like gland openings) are generally of a small diameter and slightly elongated, and can be separated from the piriform spigots, which are of similar size but are found on the anterior lateral spinnerets (Coddington et al. 2011). Aciniform glands occur in much higher numbers than other silk types (Coddington et al. 2011; Andersson et al. 2013). The aciniform spigots are located on the posterior median (PMS) and posterior lateral spinnerets (PLS) (Peters & Kovoor 1991; Blackledge et al. 2011). Many aciniform glands are arranged somewhat linearly along the spinneret in species with long PLS (Eberhard 2020). This arrangement reflects the use of AS in prey-wrapping and egg-encasing, which require applying a higher amount of silk over a large area in a short time.

COMPOSITION AND STRUCTURE

AS is a group of fine spider silks with an approximate diameter of 0.5 μm or less (Blackledge et al. 2005; Eberhard 2020). The presence of surface lipids or glycoproteins and exact numbers and composition of layers from the inner core to outer skin (if present) has not yet been described. AS is composed of a specific type of spidroin known as aciniform spidroin (AcSp) (Wang et al. 2012). Aciniform spidroin 1, characterised from orb web spiders (family Araneidae), is the main protein isolated from AS. Although the general transformation process of AcSp1 as a response to altered spinning duct conditions is similar to other types of silks, recent studies suggest that AcSp1 assembles to form large oligomers instead of dimers as seen in other spidroins (Chakraborty et al. 2020). The evidence suggests that there may be some variants of AcSp1 with continuously recurring sequence motifs. However, in general, the AcSp1 repetitive domains do not contain short repetitive motifs like (GA) $_n$, (GGX) $_n$, (GPGGX) $_n$ and (A) $_n$, typical for example, of ampullate spidroins (Lewis 2006; Ayoub et al. 2013; Chaw et al. 2014). AcSp1 in species of *Argiope* Audouin, 1826 has relatively lower percentages of alanine and glycine and the repetitive units are relatively longer (186–204 amino acids each) when compared to dragline silk (Ayoub et al. 2013; Chaw et al. 2014). In the liquid state (the dope stored in the silk glands), AS contains around 50% alpha helices and around 50% disordered regions, and in the solid fiber state it contains nearly 24% alpha helices and 15–30% moderately oriented beta-sheet-like nanocrystalline domains (Lefèvre et al. 2011; Tremblay et al. 2015, 2016; Addison et al. 2018). Moreover, both orb web spiders *Trichonephila antipodiana* (Walckenaer, 1841) and *Argiope trifasciata* (Forsskål, 1775) (Araneidae) have AcSp1 repeat units with two domains that differ in the number of alpha-helices (Wang et al. 2012; Xu et al. 2012b). Apart from AcSp1, another protein named aciniform spidroin 2 (AcSp2) was recently characterised from *Araneus ventricosus* (L. Koch, 1878) (Wen et al. 2020). This protein has repetitive sequences different from AcSp1. In AcSp2, serine, glycine, and alanine contents are lower and leucine content is higher than AcSp1 (Wen et al. 2018, 2020). Furthermore, a variant of AcSp2 has been identified from the same species (Wen et al. 2022). The repetitive region of this variant has cysteine residues, which is significant as this was the first time

that cysteine has been found in repetitive regions of spidroins. The implications of this difference on physical properties must be further studied as it largely depends on its chemical environment within the folded protein structure (Marino & Gladyshev 2012). However, the secondary structures of naturally secreted fibers are yet to be explored in detail. Nevertheless, studies on aciniform spidroins are limited to a few species of orb web spiders. The diversity of aciniform spidroin composition and structure may be much higher than known to date.

FUNCTIONAL DIVERSITY AND ASSOCIATED PROPERTIES

AS is used in a range of ecological contexts, including wrap attacks (Walter et al. 2008), encasing eggs (Vasanthavada et al. 2007) and decorating webs (Lubin 1974; Walter et al. 2008) and (Fig. 1). This functional diversity is enabled by the specific set of properties possessed by AS, allowing for combining more toughness than other silks with high extensibility as described in the orb web spiders *Argiope argentata* (Fabricius, 1775) and *Argiope trifasciata* (Hayashi et al. 2004; Blackledge & Hayashi 2006; Craig et al. 2020) (Fig. 2). Species of Araneidae, as well as distantly related families of spiders such as Pholcidae and Theridiidae, use the wrap-attack method with AS for prey capture, instead of pluck-out or bite-attack methods (Olive 1980; Japyassú & Macagnan 2004; Hajer & Hrubá 2007). The high toughness and extensibility make AS an ideal material for swathing large and strong struggling prey, such as orthopterans and coleopterans (Robinson et al. 1969; Olive 1980). These properties are particularly important for certain groups of spiders such as *Argiope* spp., which rely on wrap-attack to immobilize the prey without delivering a bite at the start (Robinson et al. 1969). The role of AS in forming the outer casing of egg sacs might also benefit from the high toughness of AS, which may act as the primary barrier to egg predators and withstand elemental forces. Selection on toughness may be more pronounced in species that directly attach their egg sacs to the substrate surface, where they are easily accessible by predators, and in species whose egg sacs need to endure for long periods (e.g., overwintering).

The ability to reflect ultraviolet (UV) light is another important property of AS (Blamires et al. 2008). The stabilimentum is an enigmatic web decoration that some orb weavers apply to their webs. These stabilimenta are made of AS and their ability to reflect UV evidently facilitates the attraction of insect prey and/or deters larger animals that would damage the webs and/or prey upon spiders (Eisner & Nowicki 1983; Bruce et al. 2001; Zschokke 2002; Abrenica-Adamat et al. 2015; Walter 2018; Tugwell et al. 2021). AS is also a part of the ballooning (Peters 1990; Cho et al. 2018) and bridging lines (Wolff et al. 2014) used in dispersal, web initiation, and movement between distant objects. The thin AS fibers are thought to enhance the aspect ratio of the thread to facilitate wind drag and to enhance adhesion/attachment of the thread to distant surfaces (Wolff et al. 2014; Cho 2021). Furthermore, AS is also a part of the attachment discs of many spiders (Wolff 2020). This addition of aciniform fibers might reinforce the dragline anchorage due to the high toughness of AS (Wolff 2020).

Furthermore, the elongated PLS of sheet web spiders in families such as Agelenidae suggests that AS may play an important role in the construction of capture sheets, as these spiders lack primary adhesive silks such as cribellate or aggregate silk. The



Figure 1.—Functional diversity of spider aciniform silk. (a) A stabilimentum of *Argiope bruennichi* (Scopoli, 1772), (b) An *Argiope bruennichi* spider wrapping a prey, (c) An egg case of *Argiope bruennichi* that is composed of both aciniform and tubuliform silk, (d) Scanning electron microscopic view of the distal part of the bridging line collected from *Larinioides sclopetarius* (Clerck, 1757) (Araneidae); Ac = aciniform silk, Ma = minor ampullate silk, (e) A video still from comparative micro-high-speed video recordings of attachment disc formation by *Kukulcania hibernalis* (Hentz, 1842) (Filstatiidae). The arrow indicates the release of silk from Posterior Lateral Spinneret (PLS), where aciniform glands are located; ALS = anterior lateral spinneret, PMS = posterior median spinneret.

dense sheets of very thin silk are likely to entangle prey and could even enhance adhesion, though this has not been tested yet (Eberhard 2020).

Silk, including AS, was suggested to possess antimicrobial properties. However, Fruergaard et al. (2021) critically reviewed the evidence and found methodological shortcomings in the studies which claimed existence of such properties. Thus, no acceptable empirical evidence is available to support antimicrobial properties of AS.

STRUCTURAL DETERMINATION OF BIOMECHANICAL PROPERTIES

Mechanical properties of protein-based materials primarily depend on the macroscopic structure of the material, arrangement of the amino acids in the peptides as well as their secondary and tertiary structures (Keten et al. 2010; Voet et al. 2017). Thin materials with high aspect ratio (the proportional relationship between width and height of an object, which is extreme in nanofibers) generally tend

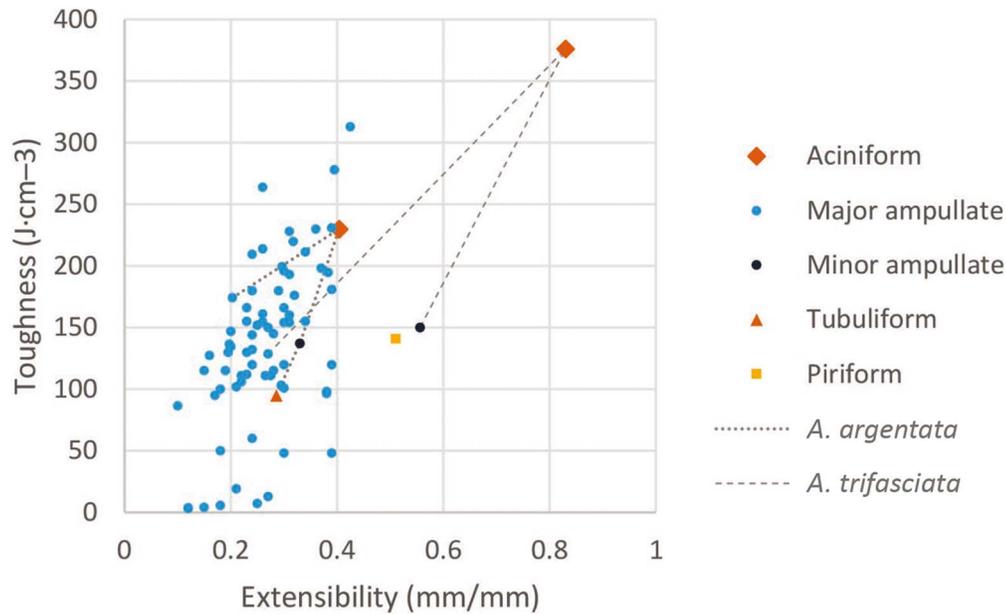


Figure 2.—Mechanical properties of different silk types produced by araneomorph spiders, measured in tensile tests of isolated threads or fibers. Each dot represents the mean of all per-species data for each silk type from one or several studies. For the orb web spiders *Argiope argentata* and *Argiope trifasciata* the data of several silk types are shown (indicated by lines between data points). Data were taken from Blackledge & Hayashi (Blackledge & Hayashi 2006), Craig et al. (Craig et al. 2020), and Wolff et al. (Wolff et al. 2017a). Please note that the tensile tests on native aciniform silk have thus far only been performed in two species of *Argiope*, which are highlighted here: to compare the properties of the different silk types the data points of the same species are connected by dashed lines.

to be tougher than bulk material (such as thick fibers or a sphere of the same material) (Gao et al. 2003) but none-the-less, the thinner a fiber, the lower its breaking force. This might be overcome by the bundling of many of such tough nanofibers, as usually is the case for AS. Beta-sheets improve silk strength and stiffness, while alpha-helices provide elasticity (Gosline et al. 1986, 1999; Guerette et al. 1996; Simmons et al. 1996; Hayashi et al. 1999; Savage & Gosline 2008). AS exhibits high toughness and elasticity in comparison to the other six silk types. The combination of alpha-helices, beta-crystalline and disordered (amorphous) structures in AS is believed to correlate with these superior properties (Hayashi & Lewis 1998; Lefèvre et al. 2011; Xu et al. 2012b).

The structural determinants of ultraviolet reflectance properties of AS are not very well studied. Here we use major ampullate silk as a model to understand structure property associations of AS.

The UV reflectance of AS may derive at least partly from the lower abundance (or absence) of certain amino acids with thiol groups—such as tryptophan, tyrosine and cysteine—in the spidroin polypeptide chain (Beaven & Holiday 1952; Millington 2012). Additionally, or alternatively, the ultraviolet reflection may depend on the structural arrangement of the aciniform spidroin. It is known that the alteration of surface properties of protein-based fibers by physical or/and chemical means affect their reflection properties (Bhoyro et al. 2001; Levene & Shakkour 2008). According to Blamires et al. (2020) the UV reflectance properties of major ampullate spider silk is more likely to be due to the surface structure and the way the silk threads are arranged. More generalized studies suggest that variation in the spectral properties of silk likely results from the specific amino-acid sequences in the different polypeptide subunits (Xu & Lewis 1990; Hinman & Lewis 1992). The conditions under which the protein is produced are also known to affect the spectral properties of spider silk (Kerkam et al. 1991). The latter may be due

to its effects on the secondary structure of the silk protein. However, specific secondary structures responsible for such properties are not clearly characterized to date.

ENVIRONMENTAL DETERMINANTS OF THE STRUCTURE AND PROPERTIES

Selection acts on traits to adapt to different living conditions within the boundaries set by design constraints (Ridley 2004). These constraints can be of many types, namely, phylogenetic, ontogenetic, metabolic, mechanical, structural, or biochemical. Selection favours those individuals that maintain the best balance between these constraints (Vollrath 1999). Within an individual lifetime, phenotypic plasticity is a well-known strategy to cope with environmental uncertainty. Silk properties vary with differences in the internal environment under which the silk dopes are spun and the external conditions under which the fibers are released from spinnerets (Boutry & Blackledge 2008). The liquid silk dope stored in the gland passes through the spinning duct before exiting as a solid fiber through the spigot of the spinneret. This liquid dope turns into a solid fiber through various physical and biochemical processes that include pH changes (Vollrath & Knight 2001; Dicko et al. 2004; Gaines et al. 2010) as well as water and ion re-absorption (Vollrath & Knight 2001). Variation in any of these factors could affect the material properties of the silk (Blamires et al. 2017).

The biological plasticity of AS properties is not very well explored, let alone considering the ecological context in the field or laboratory-based observations. Spiders adjust the architecture of their capture webs to prey types by varying mesh size (Schneider & Vollrath 1998; Wijerathna et al. 2019) and can modify the chemical composition and mechanical properties of their silk within specific boundaries (Madsen et al. 1999; Tso et al. 2007; Blamires & Tso 2013). Other ecological

parameters such as wind speed and temperature are also known to cause plastic adjustments in spider silk properties (Vollrath et al. 2001; Liao et al. 2009; Blamires et al. 2012, 2013). Differential expression of spidroins, post secretion glandular processes, and forces that act during spinning, drawing, and post-spinning are the main processes that induce plastic variations in protein structure or amino acid alignment (Vollrath et al. 2001; Blamires et al. 2012).

During prey capture, the wrapping and containing of different prey types such as flies, bees, or locusts will likely require different levels of elasticity and strength in AS. Therefore, based on the observations on major ampullate silk properties and their flexible adjustment to prey, ontogenetic stage, habitat type, physiology (e.g., hunger level), adaptive modifications of AS properties likely occur as well to meet differences in the challenges imposed by different prey types and sizes (Blamires et al. 2007, 2017; Harmer et al. 2011; Sensenig et al. 2011; Vollrath & Samu 1997). Variation in MaSp silk composition is associated with the type of prey rather than the amount of food intake (Craig et al. 2000). These effects were observed mainly in the amorphous region, which is responsible for the elasticity of the silk, but not in the crystalline region corresponds to the strength of the silk (Craig et al. 2000). Silk of spiders fed with a high protein diet are shown to have a high percentage of proline, which may result in a disruption of hydrogen bonds in the secondary structures of the protein (Blamires et al. 2015). This is supposed to increase the extensibility of the silk, while decreasing its stiffness. However, it was found that this increased amount of proline is known to increase both the stiffness and extensibility, probably due to its influence of ion concentrations and pH in the spinning apparatus (Blamires et al. 2015). Therefore, we might assume that high protein diets may increase the extensibility of AS, but experimental evidence is required before further conclusions can be drawn.

Potential effects of environmental light and humidity conditions on spider silk properties are not well investigated for any of the silk types. While the elasticity of dragline silk is known to increase under high humidity (Vehoff et al. 2007), low humidity has been suggested to increase toughness of draglines silk through its impact on spinning conditions (Yazawa et al. 2020). Temperature tolerance of spider silk is high and it can withstand very high and low temperatures. Hence environmental temperatures are not expected to elicit large variation in silk properties (Yang et al. 2005; Aparicio-Rojas et al. 2020; Gu et al. 2020). Targeted studies on AS are needed to uncover how it will be influenced by environmental variables and changes.

CAVEATS AND THE POTENTIAL OF ACINIFORM SILK FOR THE APPLIED SCIENCES

AS could be an interesting candidate for more focussed applied research. If compared to the better known ampullate silks, it exhibits an impressive combination of toughness and high extensibility. Especially in combination with the low density and biodegradability of spider silks, AS-based polymer products could strongly enhance fiber-based materials. Further, AS stands out by its UV reflection ability, which is a property worth further investigation. While several studies provide valuable information on AS, further studies are required to transfer this knowledge to real world applications.

Natural AS could be used for small-scale applications. The methods to harvest suitable quantities from the spiders must be developed. However, the recombinant production of proteins is

the only way to achieve an industry-scale production and industry-grade composition of AcSp2-based materials (Ghimire et al. 2024). Despite the successful expression of silk proteins within a range of host organisms, producing spider silk-inspired biomaterial remains a grand challenge. The primary obstacles are the limitations of the spinning process (Blamires 2024) and the challenges related to upscaling and standardization of the artificial spider silk production (Wolff et al. 2017b), which must be resolved to manufacture functional materials.

Spider silks, which are made of proteins, might elicit an immunological response when used as a biomaterial. Even though studies suggest that major ampullate silk is biocompatible (Schäfer-Nolte et al. 2014; Steins et al. 2015), such evidence from only one silk type cannot be generalised (Vepari & Kaplan 2012). Therefore, assessment of the biocompatibility of AS must be addressed if a biomedical application is the target.

The physical properties of spider silk are equally affected by the molecular level organization of major protein components and the hierarchical organization of the silk fibers, which consist of other non-peptide compounds. Ampullate silks have been shown to comprise several layers from an inner core to outer skin (Li et al. 1994; Gould et al. 1999; Vollrath & Knight 1999), but the ultrastructural composition of AS is still unclear. The exceptional properties of spider silk, though significantly affected, are not solely derived from its core (Lazaris et al. 2002; Xu et al. 2012a). Therefore, clarifying the hierarchical organization and identifying the chemical compositions of each layer (if present) is useful as they may play an important role in the exceptional physical properties of natural AS fibers.

The stabilimenta of orb webs reflect ultraviolet (UV) light (Zschokke 2002; Blamires et al. 2008) and have been studied widely for their functional significance, with still debated findings (Eisner & Nowicki 1983; Blackledge 1999; Kok et al. 2003; Li et al. 2004). However, the exact structural or chemical factors that are responsible for UV reflectance properties have received very little attention. Such knowledge could be used to manufacture UV protective clothing, cosmetics, personal accessories, and laboratory personal protective equipment such as gloves, lab coats and goggles.

The effects of external environmental variables on AS properties are poorly studied when compared to other major silk types. Considering the importance of the properties for specific functions of silk materials, investigating ecological and evolutionary determinants of AS properties would close a major gap.

CONCLUSIONS

Spider aciniform silk is an impressive biomaterial with exceptional biomechanical properties, which are critical for the survival of spiders. Despite the available evidence on the biomimetic potential of this silk type, only a handful of studies have explored the structure and property variation of aciniform silk. The external and internal factors involved in the determination of silk properties remain unexplored. Further studies might enable the development of silk-inspired biomaterials with superior properties.

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