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Review

Structure–function–property–design interplay in biopolymers: Spider silk ☆

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ABSTRACT

Spider silks have been a focus of research for almost two decades due to their outstanding mechanical and biophysical properties. Recent advances in genetic engineering have led to the synthesis of recombinant spider silks, thus helping to unravel a fundamental understanding of structure–function–property relationships. The relationships between molecular composition, secondary structures and mechanical properties found in different types of spider silks are described, along with a discussion of artificial spinning of these proteins and their bioapplications, including the role of silks in biomineralization and fabrication of biomaterials with controlled properties.

1. Introduction

The order *Araneae* (spiders) contains over 37,000 species that have ability to produce silk, a key protein for spider survival [1]. Spiders use silk for a variety of practical purposes, including arresting a fall, swathing prey, building a web, lining burrows or making egg cases [2,3]. Diverse uses of *Araneae* silks originate from the outstanding physical properties that are tailored for specific needs, resulting in variation of mechanical properties. Mechanical properties of spider silks have not yet been duplicated by man-made materials. For instance, major ampullate silk (also known as dragline silk) of *Caerostris darwini* has a maximum strength (the strength needed to break the fiber) of up to 1.7 GPa, which is in the range of that of high-tech materials. Nevertheless, man-made materials such as steel (1.5 GPa) and Kevlar (3.6 GPa) present higher strength and stiffness; the overall toughness (energy required to break the fiber) depends on the extensibility. In these terms, high-tech fibers appear to be more brittle compared to the more extensible dragline threads, making spider dragline silks the strongest material [4–6]. In addition to this, spider dragline silks are light-weight materials that can undergo supercontraction when hydrated [7]. On an equal weight basis, spider silk has a higher toughness (the amount of energy absorbed per volume before breakage) than steel and Kevlar due to extensibility [8,9]. Moreover, the other types of silk demonstrate unique mechanical properties as well. For example, flageliform silk (also known as a capture spiral silk) is over ten times more extensible than dragline silk; on the other hand flageliform silk is neither as strong as nor as stiff as dragline silk [10]. Little force is required to extend flageliform silk 200–300% of its original length until the fiber gradually stiffens under the increasing strain. The remarkable mechanical properties of spider silks originate from their unique amino acid sequences that have remained almost unchanged for more than 100 million years [11]. Moreover, spider silk primary amino acid sequences have been shown to be organized in block (or domain) copolymer-like arrangements, in which two basic sequences, crystalline and less crystalline polypeptides, alternate. At least seven different types of silk proteins are known for one species of spider [12]. They differ in primary sequence, physical properties and functions [1]. The complex spinning process allows spiders to transform water soluble silk proteins into solid fibers at ambient temperature and pressure, giving rise to an environmentally safe, biodegradable and high performance material [13–15].

Diverse and unique biomechanical properties together with biocompatibility and a slow rate of degradation make spider silks excellent candidates to be studied as biomaterials for tissue engineering, guided tissue repair and drug delivery, for cosmetic products (e.g. nail and hair strengthener, skin care products) and industrial materials (e.g. nanowires, nanofibers, surface coatings) [14–18]. A complete understanding of the anatomy of spider glands, the silk spinning process and the role of silk primary structural elements and their contributions to the physical and

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biological properties of the biopolymer are important in order to fully understand the materials. Several reviews have focused on the structure and properties of spider silks, with the main focus on dragline silks [3,16–18]. The structure–function relationships in other types of silk polymers are not as fully understood and this gap in knowledge can result in limitations in the use of these materials. In this review paper, the goal is to provide information on the current understanding of the structure and properties of various types of spider silks, with the main focus on the interplay among structure, architecture and function, and how these attributes are being used in biomaterial designs.

2. Anatomy and physiology of the spider spinning apparatus (*Nephila clavipes*)

Orb web spiders produce silk in seven distinct pairs of glands. However, it is thought that all spider glands evolved from a single type of gland which diverged in anatomy, morphology and luminal content [20]. The presence of different amino acids in the luminal contents within each gland is responsible for the secretion of defined silk fibers with specific functions. Today, most research is focused on the major ampullate gland, which produces dragline silks.

In *Nephila clavipes* the silk formation glands are located within the abdomen of the spider and each type of gland occurs in pairs with bilateral symmetry [12]. The major ampullate gland can be schematically divided into four zones, as shown in Fig. 1: the tail zone, responsible for synthesis and secretion of spider silk proteins; the lumen (also known as the sac), involved in protein accumulation; the spinning duct responsible for the alignment of silk fibers; and the spigot for final fiber production [21].

The gland represents a reservoir of soluble silk proteins that are synthesized in specialized columnar epithelium cells in the tail zone and secreted into the lumen, where the proteins are stored as a highly concentrated liquid crystalline solution [22]. According to Vollrath and Knight [23], the tail zone is named the A-zone and the first part of the lumen is named the B-zone, based on the presence of the single type of cells known as the tall columnar secretory epithelium. The spinning duct follows the lumen, which is folded into an S-shape and narrows to the end [23]. Within this duct, silk proteins are present as liquid dope [22]. The spinning duct consists of three limbs. In each limb spidroin molecules have a different orientation. In the first limb, silk proteins are anchored perpendicular to the cuticle lining but parallel to each other [23]. In the second limb of the duct, silk molecules are bent in such a way as to allow the formation of layered discs made up of the amphiphilic rod-shaped molecules that are still connected to the ducts cuticle lining. Finally, in the third limb the silk dope pulls away from the cuticle lining, forming a draw-down taper [22]. At this moment, the liquid silk solution is converted into a solid thread surrounded by water and it is thought that β-sheet formation is initiated during this process by means of the rapid extension flow that pulls silk molecules close together and aligns them using hydrogen bonding into β-sheets crystals [24,25].

During the silk dope journey through the spinning duct, a number of changes in the chemical environment take place. These include lowing of pH from 6.9 in the first limb to 6.3 in the third limb, decreasing sodium and chloride concentrations and increasing potassium, phosphate and sulphate concentrations. At the same time, the drop in pH together with ionic changes can facilitate the neutralization of repulsive negative charges, aiding silk molecule alignment into β-sheets. The final part of the major ampullate gland is represented by the valve and the spigot. The valve is used as a clamping device to control spider dragline and as a pump to remove threads that are broken inside the spigot. The spigot (spinneret) is located at the very end of the gland from which the spider thread is drawn. It has been proposed that the spigot is also capable of further modifying the silk thread [22].

A spider's threads are only produced when the spider physically moves away from one attachment point to another. A combination of elongation flow and wall shear within the gland creates tensile strain that induces β-sheet transitions in silk proteins as they pass through the fluid-to-solid transition zone (the taper). Higher tension imposes greater molecular alignment in the thread, making the silk stiffer, stronger and less extensible. Lower tension makes the silk less stiff, and weaker but more extensible. It should be noted that at higher temperatures, spiders move faster and therefore spin silk faster. Faster-moving animals produce silk threads with different diameters and mechanical properties compared to slower moving animals. When the silk protein is drawn from the spinning duct all the way down to the spigot, silk becomes progressively more dehydrated, slightly acidified and birefringent, suggesting that the orientation and shape of silk molecules have been already programmed. Spiders can also apply a tension brake upstream from the valve that allows them to increase silk thread tension while the thread is still in the high humidity environment of the spigot. Fig. 2 depicts the natural spinning process in spiders.

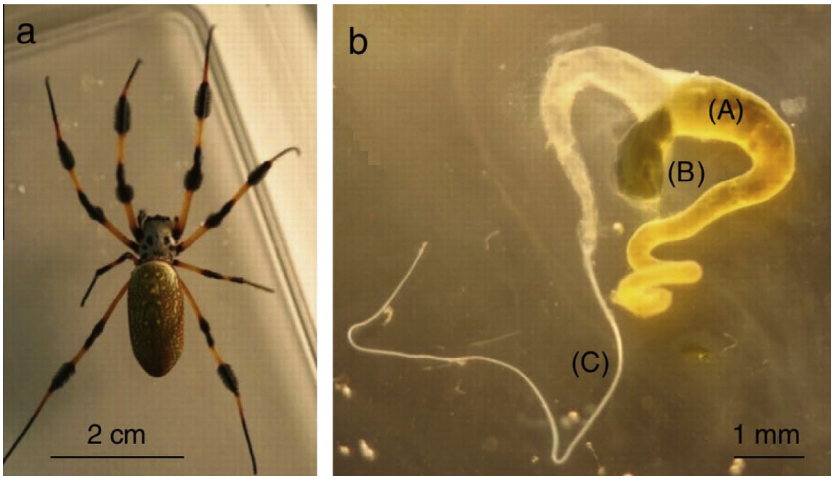

Fig. 1. (a) Adult female *N. clavipes* (golden-orb) spider provided by the Miami Metrozoo, Florida. (b) (A) Dissected major ampullate (MA) gland of the spider. The ~1 μl blob (B) protruding through a rupture of the gland wall near the spinning canal (C) was used for the rheology experiments. Modified from Ref. [113] with permission.