Spider Silk

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The Molecular Mechanism of Spider-Silk Formation**

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Spider silk, one of the most remarkable biomaterials, is made of fibers whose mechanical properties exceed those of steel once the smaller density of spider silk is taken into account: A spider silk fiber spun around the earth's equator would weigh not more than 500 grams.[1] Spider silk from *Araneus diadematus* spiders contains a polypeptide chain consisting of a so-called AQ repeat sequence of low complexity in its composition (A, hydrophobic polyalanine-rich motif: $GPYGPGASA_6GGYGPGSGQQ$; Q, hydrophilic glutamine- and glycine-rich motif: $(GPGQQ)_4$). The repeat sequence is a central part of the fiber and its role is reminiscent of the repeat sequence in collagen. Twelve AQ sequences are flanked by a secretion signal, along with non-repetitive N- and C-terminal domains, each of which is implicated in a number of different functions, including control of solubility and fiber formation.[2]

But how do the various structural elements accomplish the remarkable properties of spider silk? What is the molecular basis for spider-silk formation? How can silk be transformed from a soluble form stored in the lumen of the spider into an insoluble fibril with such unrivaled properties? In a recent report in *Nature*, the groups of Kessler and Scheibel have provided key insight into these questions by careful biophysical characterization of spider silk.[3] In particular, the structure of the C-terminal non-repeat (NR) domain of *Araneus diadematus* fibroin 3 (ADF-3) in solution has been solved by NMR spectroscopy (Figure 1). Two NR domains form a dimeric, highly symmetric structure. This structure constitutes a new fold characterized by a barrel-like shell enclosing two helices connected by a disulfide bridge. It is further stabilized by two salt bridges fixing helices H1 and H2 at one side of helix H4. Helix 1 (H1) of the first polypeptide chain is intertwined in a clamp-like manner with helix H5′ of the second chain. The barrel-like structure of the protein ensures that the hydrophobic residues are buried, whilst hydrophilic amino acids are positioned towards the solvent and ensure solubility of the protein.

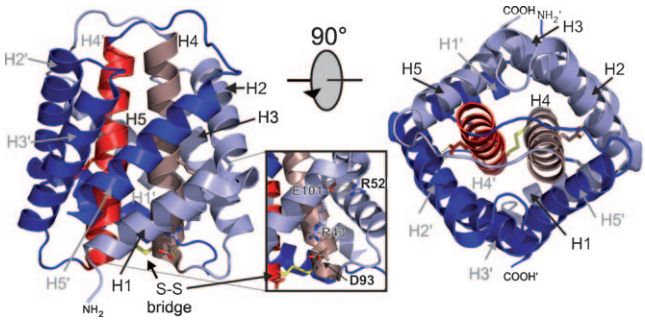

Figure 1. NMR structure of the C-terminal non-repetitive (NR) domain of *Araneus diadematus* fibroin 3 in solution. Secondary structure elements are indicated by H1-H5 and H1′-H5′. (PDB ID: 2khm; rendered with Pymol).

In the same issue of *Nature*, the groups of Johannson and Knight reported the X-ray structure of the N-terminal NR domain of major ampullate spidroin 1 (MaSp1) from *Euprosthenops australis* and unraveled its role by meticulous investigation of the pH dependence of silk-fiber formation (Figure 2).[4] Similar to the C-terminal NR domain, two N-terminal NR domains form a symmetric homodimer with a central pair of helices (H3 and H3′). The homodimer interface is largely hydrophobic, and the interface residues are highly conserved throughout known spidroins. The conserved sequence AxxxAxASS (residues 68–76 in H3) is crucial for dimer integrity because it ensures helices H3 and H3′ can pack tightly. The two types of charged residues (basic and acidic) are restricted to two regions on each dimer, respectively, giving the protein its dipolar character. Residues His6, Arg60, and Lys65, as well as Asp39, Asp40, Glu84, Glu85, and Asp134 are positioned at opposite sites of each monomer, and the monomer dipoles are arranged in opposite directions.

For the C-terminal NR domain, Scheibel and Kessler can show that helix H1 and the region around the salt bridges locally unfold first during chemical denaturation. Interestingly, the C-terminal NR domain shows its highest hydrophobicity in such intermediate state, underlining its template function in aggregation and fiber assembly. Acid- or high-salt-induced unfolding involves the formation of a molten globule state, as evidenced by binding of ANS (8-anilinonaphthalene-1-sulfonic acid) in fluorescence studies, which is characteristic of loss in persistent tight packing of the helical elements of the protein.

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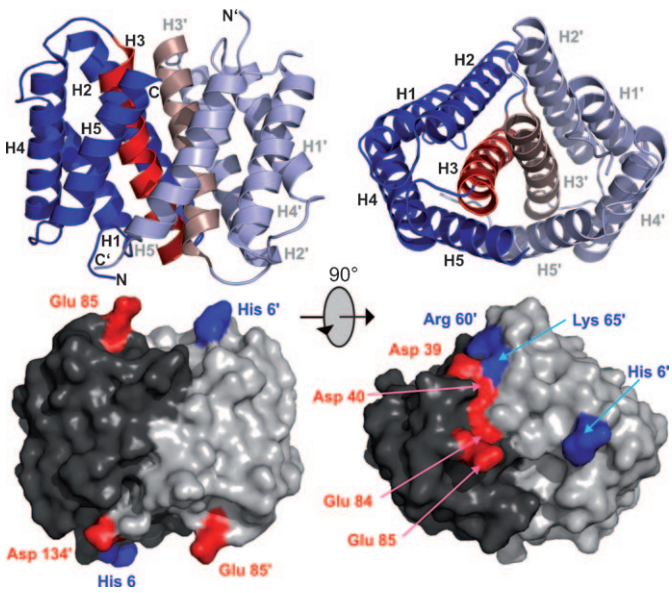

Figure 2. X-ray structure of the N-terminal NR domain of the major ampullate spidroin 1 (MaSp1) from *Euprosthenops australis*. Secondary structure elements are indicated by H1-H5 and H1′-H5′ (PDB ID: 3LR2; rendered with Pymol).

For the N-terminal NR domain, Johansson and Knight investigated the pH dependency of spidroin self-assembly. The experiments were conducted using so-called mini-spidroins, which are recombinant constructs of four repetitive segments flanked either by the N-terminal domain, the C-terminal domain, or both. In the presence of the N-terminal domain spidroin, self-assembly into macroscopic structures is greatly accelerated at about pH 6, whereas higher and lower pH levels, and high concentrations of salt, inhibit self-assembly. All effects were reversible and could also be demonstrated in isolated N-terminal domain dimers, suggesting a role of the highly conserved, titratable surface residues in the assembly process. While the C-terminal domain is a prerequisite for the formation of continuous silk fibers as opposed to amorphous molecular aggregates, it is the N-terminal domain which senses the pH and thereby appears to regulate the assembly process in vivo.

The spider-silk proteins are synthesized within the spider glands and are stored as self-assembled oligomers of high density as a micelle-like structure, forming a microemulsion. Each micelle consists of many spider silk proteins and is stabilized by the remarkable properties of the N- and C-terminal NR domains. The self-assembly is fully reversible, and the micelles form an isotropic sphere (Figure 3). Within the spider, fiber formation is not only governed by changes of the chemical environment, but also by mechanical stimuli in a rather unique synergy of microscopic and macroscopic effects (Figure 3 and Figure 4). To understand the fibril-forming properties of the polypeptide chain, the mechanical aspect of extruding the micelle supramolcular complex come into play: shear stress, water extrusion and ion exchange triggers the formation of silk fibers. The C-terminal structure plays a particular important role; polypeptide chains containing only the AQ repeat structure do not change in viscosity under shear. By contrast, the full-length polypeptide chain forms a

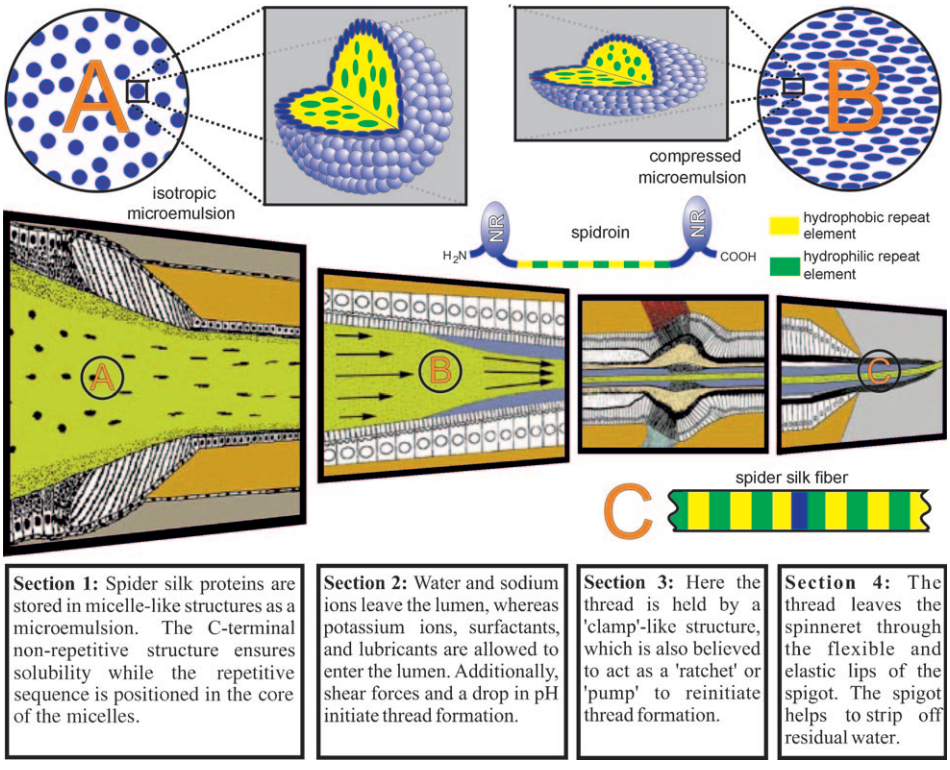

Figure 3. Representation of macroscopic processes in the dragline spinneret of a spider. Adapted by permission from Macmillan Publishers Ltd 2001.[5]

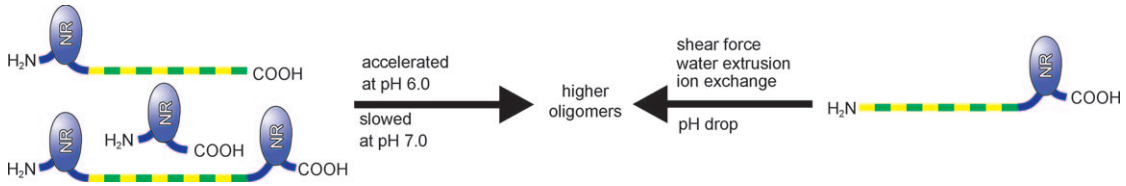

Figure 4. Representation of microscopic processes in the dragline spinneret of a spider for different investigated constructs. In the presence of the N-terminal domain spidroin, self-assembly into macroscopic structures is greatly accelerated at about pH 6, whereas higher and lower pH levels and high concentrations of salt inhibit self-assembly. All of these effects were reversible and could also be demonstrated in isolated N-terminal domain dimers, which suggests a role of the highly conserved, titratable surface residues in the assembly process.

molten-globule state under shear, and well-defined fibrous aggregates form only if the C-terminal NR domain is present, as shown by polarized FTIR spectroscopy.

Taken together, the spider produces a very specific protein to make silk. The protein is a fusion of an amphiphilic block copolymer and two dimeric α-helical barrels. In the spider gland, it forms a supramolecular microemulsion assembly. The spider then applies strong shear forces when extruding the polypeptide chain from its glands. Such extrusion is accompanied by a dehydration of the microemulsion and leads to a partial unfolding of the C-terminal barrel structure, turning hydrophobic residues inside out; a macroscopic change in the shape of the emulsion results, finally leading to the specific polymerization to form spider silk with its remarkable mechanical strength.

Apart from unraveling the mechanisms of spider silk formation, the findings have important implications beyond the peculiar way of how spiders build mechanical devices to angle their prey. The conformational transition from a highly soluble supramolecular complex stored as micelles in the glands of the spider to form a fiber provides key insight into understanding general properties of proteins. Protein fibril formation is often associated with protein misfolding and its deadly consequences in the context of protein-folding diseases. Nature, however, also uses fibril formation to produce some of the most stable materials from the amino acid monomers that can be made.

Both articles suggest a number of interesting questions: Do the N-terminal and C-terminal domains operate cooperatively or do they react on different chemical stimuli? And how does the supramolecular assembly assure cross-linking of individual strands to form a continuous thread that can be more than a meter long? Another question yet to be answered is how a set of few specific silk glands meets the demand for a wide variety of different thread types with highly diverse mechanical properties.[4] In a typical *Araneidae* orb weaver for example, seven different highly specialized glands produce several silk components, enabling the spider to generate threads for numerous purposes. Silk quality is modulated by spinning conditions and subtle environmental factors, such as the spider's dietary composition. However, the properties of a given thread of silk is ultimately linked to the amino acid compositions of the spinning dopes produced by the particular set of silk glands it originated from. A full structural understanding of how the interplay of the different silk components translates into the final thread property therefore remains one of the pivotal questions in biomaterial science.

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