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The conserved C-termini contribute to the properties of spider silk fibroins

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Abstract

Spider silk fibroins can adopt different structural states at high protein concentrations. They are soluble within the spinning dope of the glands, but readily converted into insoluble polymers upon extrusion. A contribution of the C-termini to the maintenance and conversion of these states is suggested by their predicted secondary structures and biochemical behavior in vitro. Special sequence parts endow the C-termini with the capability to promote both the solubility and aggregation of the fibroins depending on the environmental conditions.

Keywords: Spider fibroins; Spidroins; Structural proteins; Secondary structure predictions; Atomic force microscopy

Derived orb-weaving spiders (Aranoidea) produce up to seven different silk fibers and silk glues in specialized glands [1,2]. Their major constituents are structural proteins designated as silk fibroins or spidroins [3,4]. The spidroins from the major, minor, and flagelliform glands identified so far are composed of short common peptide motifs that are repeated multiple times and variously combined to form repetitive structural modules [5–9]. A different blueprint is seen with spidroins synthesized in the aciniform and tubuliform glands, which are made up by long repeat units [10]. How the specific arrangement of the modules gives rise to the observed mechano-physical properties of the respective silks is still poorly understood. Alanine-rich motifs are considered to form β-sheet crystalline domains responsible for the high tensile strength of the respective fibers [11–15]. In contrast, the more hydrophilic glycine rich regions can adopt 3_1-helical conformations forming amorphous matrices [16] or β-turn spirals [17]. Both conformations are believed to confer elasticity to the fibre [7,15]. The spinning process itself and environmental conditions impinge on the nature of silk as well probably by inducing alterations in the amino acid composition of the spidroins and changes in the hierarchical order of the modules [12,18–23].

Besides the high variety in their repetitive sequences nearly all spidroins contain conserved non-repetitive C-termini [5,10,24,25]. Excepted spidroins do not represent major products of their associated glands but are rather specialized adaptations [26]. Variations within the same species do occur in the C-terminal sequences, but are extremely rare compared to the corresponding repetitive parts [27]. This conservation hints to an important function these sequences play for the spidroins. In analogy to *Bombyx mori* C-terminal fibroin sequences, the spiders' counterparts might be implemented in the solubility of these proteins within the spinning dope and their conversion between different physical states during the spinning process [28,29]. It has been demonstrated that the solubility of Baculovirus expressed spidroins decreases significantly in the presence of the C-termini, suggesting that they play a role in the solidification of silk [30].

In this report, we have analyzed the C-termini of spidroins derived from the major ampullate gland of spiders of

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the genus *Nephila*. Their secondary structures and biochemical data of expressed fusion proteins predict that the presence of C-termini influences the conformation and solubility of spidroins.

Material and methods

Spiders. The animals were kept at 80% humidity in little cages. For the experiments adult female animals at the 6th to 9th instar states were used.

Sequence analysis. Hydropathy analysis and secondary structure predictions were all carried out with the Lasergene Protean v3.08 program (DNA Star). Hydropathy analysis was done according to Kyte and Doolittle [31]. Alpha/beta/turn structures, amphipathicity and flexibility predictions were according to Chou and Fasman [32], Eisenberg et al. [33], and Karplus and Schulz [34], respectively.

Gel electrophoresis. Threads were dissolved overnight at room temperature in saturated (9 M) lithium bromide (LiBr) at 10 mg/ml. The sample was centrifuged to remove any remaining particulate matter and dialyzed against 8 M urea containing 1 mM EDTA and 10 mM Tris–HCl, pH 8. Gland secretion material approximately from six glands after removal of the gland epithelium was extracted over night in 100 μl of 8 M urea. Any debris were removed by sedimentation for 10 min at 10,000*g*. Laemmli sample buffer [35] with and without the reducing agent β-mercaptoethanol was added to the sample. Samples (approximately 10 μg LiBr dissolved thread and 20 μg gland extract) were separated by 5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; 16 cm plates) after heat denaturation. Gels were stained with Coomassie brilliant blue R250.

Cloning, expression, and purification of repetitive spidroin sequences. The cloning, expression, and purification of major ampullate spidroin 1 and 2 (MaSp 1 and 2) recombinant clones H_6-S1C (amino acids 645–768 of MaSp 1), H_6-S2C (amino acids 521–627 of MaSp 2), H_6-S1R (amino acids 565–645 of Masp 1), H_6-S2R (amino acids 410–472 of MaSp 2), and H_6-S2CR (amino acids 477–527 of Masp 2) have been described earlier [36,37]. The "C" and "R" stand for conserved C-terminal and repetitive sequences, respectively. "CR" refers to a less repetitive sequence in MaSp 2 that bridges the highly repetitive sequences and the C-terminus. Using the published primer pairs in different combinations we cloned additional constructs termed H_6-S2R/CR, H_6-S1R/C, and H_6-S2R/CR/C that comprise the designated combined sequences. Ten micrograms of fusion proteins was separated on 12.5% pre-cast SDS gels using the Mini-Protean II system (Bio-Rad) [35]. The gels were stained in Coomassie brilliant blue.

Atomic force microscopy. Recombinant proteins were diluted to an end concentration of 0.1 mg/ml in dilution buffer (20 mM Hepes–KOH, pH 6.8; 1 mM EDTA, and 10 mM NaCl) and 10 μl spotted onto freshly cleaved glimmer. After 2 min of adsorption, the liquid was carefully removed by blotting and samples were washed four times with 30 μl water. Samples were dried under vacuum and analyzed by atomic force microscopy using a NanoScope (Digital Instruments) in tapping mode with a sampling frequency of approximately 1.65 Hz as described previously [38].

Results

Secondary structure predictions of C-termini

The *Nephila clavipes* major ampullate spidroin 1 and 2 (MaSp 1 and 2) hydropathy plots show an alternation of hydrophilic and hydrophobic stretches in their repetitive sequence parts that are approximately equal in size (Fig. 1, panel A). The C-termini of MaSps, starting with amino acid 651 for MaSp 1 and 531 for MaSp 2 of the published sequences [11,12], also show these alterations, but less regular and the extent of hydrophobic blocks dominate (panel B). The C-termini contain approximately in their middle the highest hydrophobic patch of the published sequences.

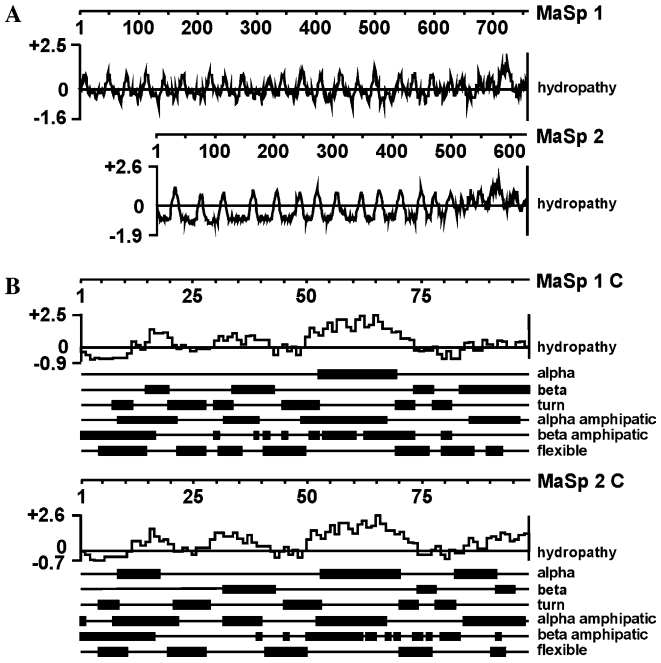

Fig. 1. Hydropathy plots and secondary structure predictions of MaSps. (A) Depicted is a Kyte and Doolittle scale mean hydrophobicity profile (scan-window size of 7) of the available *Nephila clavipes* MaSp 1 and 2 sequences. Scores above 0 indicate hydrophobicity, scores below 0 hydrophilicity. The size of the sequence in amino acids is indicated. (B) Hydropathy plot and secondary structure predictions for the C-terminal sequences of MaSp 1 and 2 from *N. clavipes*. The extent of the sequence in amino acids is indicated.

All α-helices, β-strands, and β-turns contribute to the C-terminal sequences in MaSps (panel B). Strikingly, the highest hydrophobic patch might adopt an amphipathic α-helix for both spidroins (Fig. 2 panel CR). This stretch contains the negatively charged residues aspartic acid (D) and glutamic acid (E). Sequences near the ends of the conserved C-terminus possess also amphipathic character (panels NT and CT). The helix NT contains the positively charged arginine (R), whereas no charged residues are found within the CT sequences. The most pronounced flexible regions can be found close to the ends and in the regions framing the highest hydrophobic patch.

Size determination of ampullate spidroins

The size distribution of spidroins isolated from the major and minor ampullate glands was investigated under non-reducing and reducing conditions by denaturing gel electrophoresis (Fig. 3). As is evident, high molecular weight material exists in the MaSps of *Nephila edulis* in the size range of 420–480 kDa that is converted into a lower molecular weight material of 250–320 kDa upon reduction, suggesting disulfide bridge formation in these proteins (compare lanes 1 and 2). The MaSps of other spiders like *Nephila clavipes* [36,39] and *Argiope aurantia* showed the same outcome (data not shown). In contrast,