Unraveling a Tangled Web: A Synthetic Route to Spider Silk

Li Huey Tan Literature Seminar Oct 7, 2010

The spider silk, 4 μm in diameter and capable of spanning more than 10 m across rivers (Figure 1), is an attractive material with outstanding mechanical properties.[1] The tensile strength of spider silk is comparable to that of steel (1.5GPa) and the toughness is superior than any of the high performance synthetic material made to date like Kelvar (50 MJm^{-3} as compared to 180 MJm^{-3}).[2] Due to its high toughness and biocompatibility, spider silk is a suitable candidate to replace the current petroleum-based polymer used in the fabrication of bulletproof vest, parachutes and sutures or tissue scaffolds in biomedical applications.[2] Unfortunately, it is difficult to produce huge amount of spider silk due to the territorial and cannibalistic nature of spiders. The current methods to obtain spider silk involve forcibly silking of the spider or killing the spider.[3] In order to obtain large amounts of spider silk and avoid the complicated procedures in harvesting spider silk, there have been great efforts to synthetically produce the spider silk via chemical synthesis or biosynthesis.

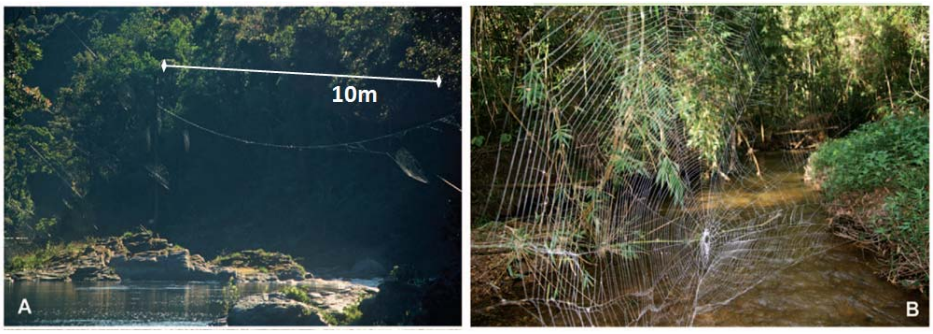

Figure 1. *C. darwini* spider webs. a) spanning across a river in Ranomafana; b) an orb-web over a stream in Andasibe-Mantadia NP. [1]

To reproduce spider silk artificially without compromising its mechanical properties, it is important to first understand the structure and composition of the spider silk. The toughest silk of the spider, the major ampullate spidroin (MaSp), was found to be mainly constructed from two proteins of approximately 250 to 352 kDa.[4] Analyzing these huge proteins, the amino acid sequence of MaSp were found to have two non-repetitive peptides of about 100 amino acids at the end of the N- and C-terminus. In between the two terminal peptides is a sequence containing highly repetitive blocks of peptides that are responsible for the unique properties of the spidroin (Figure 2a). The strength of the spidroin originates from extensive crystalline blocks of β-sheets formed from long stretches of alanine repeats.[5] The extensibility of the spidroin, on the other hand, originates from the blocks of unique Glycine-Glycine-X and Glycine-Proline-Glycine-X-X motif where X mainly consist of amino acids like glutamine, tyrosine and alanine giving 3_1-helix[6] and a beta-turn spiral[7] respectively.

The two current ways to synthesize spidroin mimics are via the chemical route and the biosynthesis route. The chemical route involves mimicking the structure of the spidroin of having block domains and flexible domains.[8] The block domains are made up

of repeating units of alanine while the flexible domains are replaced with polyethylene glycol repeating units. (Figure 2b) The polymers that were formed from this synthesis demonstrated similar extensibility before yield but the tensile strengths were about two magnitudes lower than the native spidroin.[8] In the biosynthesis route, recombinant DNA technology was used. The gene responsible for the expression of spidroin was reengineered and incorporated in *E.coli*.[9] Despite the initial challenges to express such a highly repetitive protein sequence, Xia et al. successfully expressed recombinant spidroin of 284 kDa in *E. coli* which is of similar length to the native silk (Figure 2c). The tensile strength of this recombinant protein is by far the highest and closest to the native spidroin (580 MPa as compared to 1100MPa).

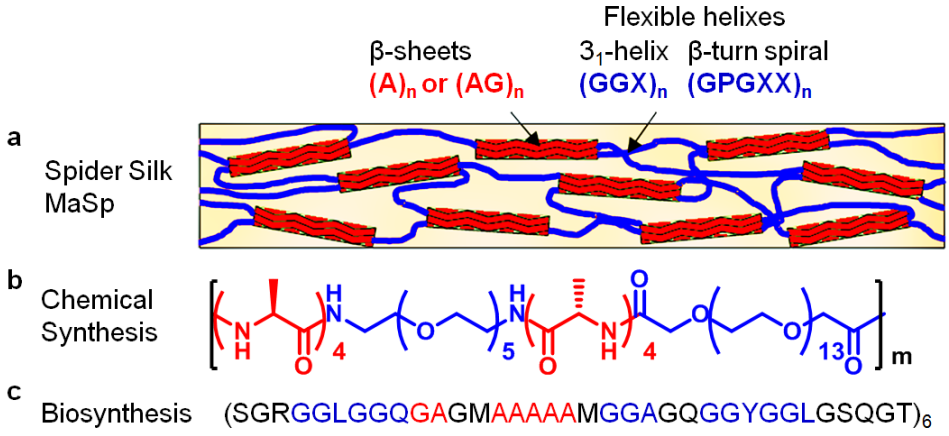

Figure2. a) Repetitive blocks of β-sheet domain consisting of alanine and alanine-glycine repeats (in red) and flexible domain consisting of glycine-glycine-X and glycine-proline-glycine-X-X motifs where X are mainly alanine, glutamine, and tyrosine (blue);[4] b) Chemical structure of spider silk mimic, with alanine blocks (red) and polyethylene glycol as the flexible domain (blue);[8] c) Sequence of recombinant spider silk expressed from *E. Coli* (blue and red regions correspond to respective domains on the spider silk).[9]

Synthesizing the protein is only half of the fiber producing mechanism in a spider. This was demonstrated by the significant drop in mechanical properties of reconstituted silk.[10] Changing the spinning speed at which silkworm silk is produced, the mechanical properties can be tuned and even mimic the toughness of spider dragline.[11] Before the MaSp is spun into fiber the spider stores high concentration of protein >50% w/v and as it is the protein excreted the protein. This solubility which is absent in recombinant silk may be provided by the role of the C- and N-terminus peptide. Analyzing the structure of both the C-terminus peptide[12] and the N-terminus peptide[13], the two terminus peptides were found to play important roles in inducing the aggregation of the silk protein upon salt and pH trigger respectively in addition to the solubility of the protein.

With the current progress in recombinant DNA technology to synthesize spider silk in *E. coli*, it is promising that we could reproduce the full spider silk protein and even introduce additional functions with chemical modification. The technology in spinning the protein into fiber however has to be optimized further in order to fully reveal the excellent mechanical properties of the spider silk.

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