

SPIDER CAPTURE SILK: A hierarchical chain model
REPORT

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Complex Polymer Morphology
Winter 2006

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The report is a synopsis of the paper in ref [7]. The concept of the hierarchical chain model is proposed by the authors of ref [7].

INTRODUCTION

Spider capture silk is a fiber secreted by spiders. Its tensile strength is comparable to that of high-grade steel [1], and has a tensile strength of roughly 1.3 GPa, while one source [2] lists a tensile strength for one form of steel at 1.65 GPa. However, spider silk is much less dense than steel; its ratio of tensile strength to density is perhaps 5 times better than steel—as strong as aromatic nylon filaments, such as DuPont's Kevlar. It has been suggested that a pencil thick strand of silk could stop a Boeing 747 in flight.

This report discusses a simple hierarchical chain model that was proposed to understand and reproduce this striking observation. In the hierarchical chain model, a polymer is composed of many structural motifs which organize into structural modules and supramodules in a hierarchical manner. Each module in this hierarchy has its own characteristic force. The repetitive patterns in the amino-acid sequence of the major flagelliform protein of spider capture silk, is in support of this model.

The capture silk is a natural material produced by orb-web weaving spiders (see Fig.1). Spiders rely on it to entrap flying preys. Like the spider dragline silk and many other naturally occurring silks, the capture silk has a tensile strength that is comparable to steel; but, unlike steel, it is also extremely elastic, with the ability to be stretched to almost 10 times its relaxed contour length without breaking [3, 4]. This perfect combination of strength and extensibility conveys a high degree of toughness to the capture silk: its breakage energy per unit weight is more than 20 times that of high-tensile steel [3]. With the aim to produce synthetic silks with similar mechanical properties, materials scientists have devoted many experimental and computational efforts to the understanding of spider silk's structural organization [5]. Despite these painstaking efforts, the mechanism behind

spider silk's remarkable strength and elasticity is still largely missing, partly because of the difficulty to obtain high-quality crystallized structures of silk proteins.

Spider silk is made of complex protein molecules. This, coupled with the spider's preference—as a predatory animal—for isolation from other species, has made the study and replication of this substance quite challenging. Because of the repetitive nature of the DNA encoding the silk protein, it is difficult to determine its sequence, and the silk from only 14 species has been decoded. As of 2001 ten such sequences have been completed through collaboration between the University of California at Riverside and the University of Wyoming. In 2005 two biology researchers from the University of California at Riverside, Jessica Garb and Cheryl Hiyashi, uncovered the molecular structure of the gene for the protein that female spiders use to make their silken egg cases.

Although different species of spider, and different types of silk, have different protein sequences, a general trend in spider silk structure is a sequence of amino acids (usually alternating glycine and alanine, or alanine alone) that self-assemble into a beta sheet conformation. These "Ala rich" blocks are separated by segments of amino acids with bulky side-groups. The beta sheets stack to form crystals, whereas the other segments form amorphous domains. It is the interplay between the hard crystalline segments, and the elastic amorphous regions that gives spider silk its extraordinary properties.

ARTIFICIAL SPIDER SILK

Spider silk's properties have made it the target of industrial research efforts. It is not generally considered possible to use spiders themselves to produce industrially useful quantities of spider silk, due to the difficulties of managing large quantities of spiders. Unlike silkworms, spiders are aggressive and will eat one another, making it impossible to keep many spiders together. Other efforts have involved extracting the spider silk gene and using other organisms to produce the required amount of spider silk. In 2000, Nexia,

a Canadian Biotechnology company, was successful in producing spider silk protein in transgenic goats. These goats carried the gene for spider silk protein, and the milk produced by the goats contained significant quantities of the protein. However, attempts to spin the protein into a fiber similar to natural spider silk failed. The spider's highly sophisticated spinneret is instrumental in organizing the silk proteins into strong domains. Specifically, the spinneret creates a gradient of protein concentration, pH, and pressure, which drive the protein solution through liquid crystalline phase transitions, ultimately generating the required silk structure (which is a mixture of crystalline and amorphous biopolymer regions). Replicating these complex conditions in lab environment has proved difficult. Nexia attempted to press the protein solution through small extrusion holes in order to simulate the behavior of the spinneret, but this was insufficient to properly organize the fibers. Ultimately, Nexia was forced to abandon research on artificial spider silk, despite having successfully created the silk protein in genetically modified organisms.

HIERARCHICAL CHAIN MODEL

Single-molecule manipulation methods have recently been applied on spider silks to obtain very detailed information on their force-extension response [4]. In a recent experiment, Hansma and co-workers [4] attached capture silk mesostructures (probably composed of a single protein molecule) or intact capture silk fibers to an atomic force microscopy tip and recorded the response of the samples to an external stretching force. They found a remarkable exponential relationship between the extension x and the external force f ,

$$f \propto \exp(x/l)$$

where l , the length constant [4], is a fitting parameter whose physical meaning still needs to be decided. $l = 110 \pm 30$ nm for a capture silk molecule, and $l = 11 \pm 3$ mm for an intact capture silk fiber [4]. The length constant of a silk fiber is about 10^5 times that of a silk molecule; its relaxed contour length is also about 10^5 times that of a molecule [4]. It

appears that l holds an approximately linear relationship with the contour length. The exponential force-extension curve is significantly different from the data observed during stretching single double-stranded (ds) DNA molecules or single titin proteins. The behavior of dsDNA can be understood by the wormlike chain model of entropic elasticity and that of titin by a two-level system coupled with entropic elasticity.

Equation (1) seems to indicate the following: (i) Because the capture silk is highly extensible, a great amount of extra length must have been stored in its relaxed form. (ii) Since extension increases with force logarithmically, some fraction of the stored length must be easy to be pulled out, some fraction must be harder to be pulled out, and still some other fraction must be even harder to be pulled out.

To model this kind of cascading responses, a *hierarchical chain model* [7] (see Fig 2) for spider capture silk has been proposed. In the hierarchical chain model, the polymer is composed of many basic structural motifs; these motifs are then organized into a hierarchy, forming structural modules on longer and longer length scales. At the deepest hierarchy level h_m , the structural motifs could be β sheets, β spirals, helices [5], or microcrystal structures. The interactions among some of these motifs are much stronger than their interactions with other motifs; therefore, they form a structural module at the hierarchy level (h_m-1) . These level- (h_m-1) modules are then merged into level- (h_m-2) modules through their mutual interactions. This merging process is continued, and finally, at the global scale, the whole spider silk string is regarded as a single module of the hierarchy level $h = 0$. It has been found that the response behavior of such a model polymer is characterized by an exponential force-extension curve (fig 3).

In nature, the structures of many biomaterials are, indeed, hierarchically organized. As a composite material, the chromosome is a mixture of DNA, histone, and other nonhistone structural proteins. The DNA molecule first wraps onto histone proteins to form nucleosome particles, the basic units of chromosome. With the help of H1 histone, this linear sequence of nucleosomes are then coiled and folded to form the 30 nm chromatin fiber. With the help of other scaffold proteins, the chromatin fiber is then further coiled

and folded at several levels to form the compact chromosome structure. As another example, the amino-acid sequence of a protein first forms basic structural modules of α helix and β sheet, called secondary structures. By different ways of connections of these secondary modules, the protein constructs various tertiary topologies that are critical for its specific biological functions. In a higher level, these tertiary domains then form quaternary structures of protein complexes consisting of multiple chains. Recent experimental and theoretical studies have shown that the force-induced unfolding of protein molecules is indeed processed in a hierarchical way; i.e., the tertiary structure precedes the secondary structures to be pulled over at increasing external forces. The hierarchical chain model may also serve as a framework to understand the mechanical property of these biomaterial systems.

Hierarchical chains respond to external perturbations in a hierarchical manner. If the external force is small, only those structural units of length scale comparable to the whole polymer length will be displaced and rearranged; structural units at short and moderate length scales will remain unaffected. As the strength of the external perturbation is increased, additional structural units at shorter length scales are also deformed. Through such a hierarchical organization, a single polymer chain can respond to a great variety of external conditions; at the same time, it is able to keep its structural integrity even under strong perturbations. This hierarchical modular structure also indicates a broad spectrum of relaxation times. The modules at the shorter length scales will have much shorter relaxation times and will be refolded first when the external force decreases. This gap in relaxation times ensures that, after extension, the spider capture silk will return to its relaxed state gradually and slowly. This is a desirable feature for spider capture silk, because a too rapid contraction in response to the insect's impact would propel the victim away from the web.

The simple hierarchical chain model, while appealing, needs further experimental validation. This model seems to be supported by recent genetic sequencing efforts. By analyzing the cDNA sequence of the major protein of spider capture silk, the flagelliform protein; it was revealed that the amino-acid sequence of flagelliform has a hierarchy of

modularity [6]. At the basic level, the flagelliform sequence is consisted of three repetitive modules (motifs): (i) the GPGGX motif (Gly-Pro-Gly-Gly- X , $X \in \{\text{Ala}; \text{Ser}; \text{Tyr}; \text{Val}\}$); (ii) the GGX (Gly-Gly- X , $X \in \{\text{Ala}; \text{Ser}; \text{The}\}$); (iii) the highly conserved spacer motif of length 28 amino acids. At the next level, an ensemble motif is formed, which is a tandem array of 43–63 GPGGX followed by 6–12 GGX, the spacer and another GGX [6]. At the even higher level, the ensemble motif then repeats itself about 14 times to form the flagelliform monomer. The variable residues X are not randomly distributed along the protein sequence [6], which may encode important structural information. The structures of spider capture silks (and other spider silks) therefore have the potential to be hierarchically organized.

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FIGURES

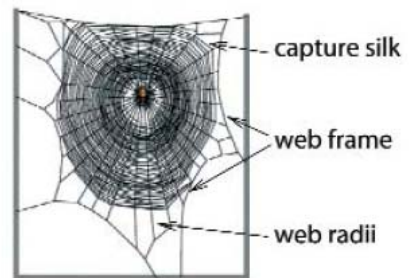
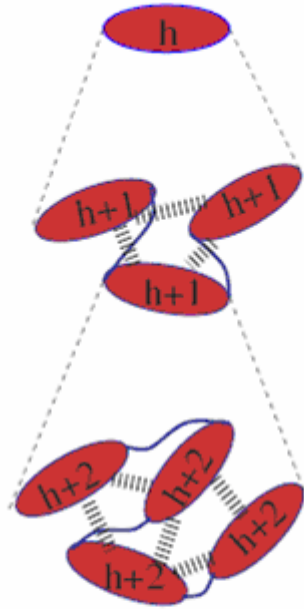
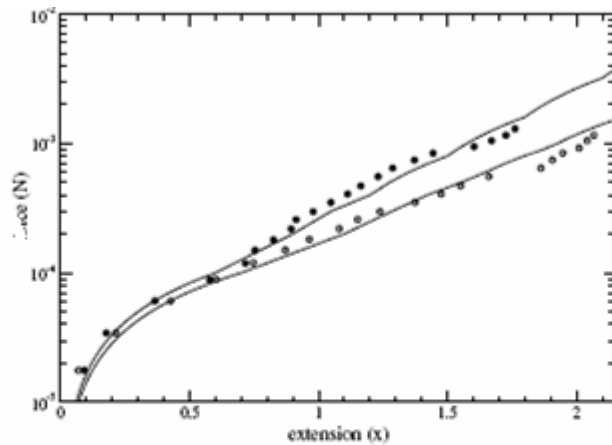


Fig. 1: The web of Araneus diadematus.



The hierarchical chain model. At each hierarchy level h a structural module M_h is composed of a tandem sequence of m_h submodules M_{h+1} of hierarchy level $h + 1$. Under external stretching, M_h responds by (i) adjusting the arrangements of its m_h subunits and making them more aligned along the force direction, and (ii) extending these m_h subunits. The thick broken lines between submodules of each hierarchy level indicate the existence of sacrificial bonds.

Fig 2 of Ref [7]



Exponential force-extension relationship for the hierarchical chain model. Equation (4) is used in the numerical calculation. The parameters are $f_0 = 10^{-4}$ N, $\alpha = 0.3$, and $\beta = 2$ (the upper curve) or $\beta = 1.75$ (the lower curve). Extension is in units of L_0 .

Fig: 3 Symbols are experimental data from Fig 4 of Ref [4]