Hierarchical Chain Model of Spider Capture Silk Elasticity

Haijun Zhou[1] and Yang Zhang[2]

[1]*Max-Planck-Institute of Colloids and Interfaces, 14424 Potsdam, Germany*

[2]*Center of Excellence in Bioinformatics, University at Buffalo, 901 Washington Street, Buffalo, New York 14203, USA*

(Received 8 July 2004; published 19 January 2005)

Spider capture silk is a biomaterial with both high strength and high elasticity, but the structural design principle underlying these remarkable properties is still unknown. It was revealed recently by atomic force microscopy that an exponential force-extension relationship holds both for capture silk mesostructures and for intact capture silk fibers [N. Becker *et al.*, Nat. Mater. **2**, 278 (2003)]. In this Letter a simple hierarchical chain model 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.

DOI: 10.1103/PhysRevLett.94.028104 PACS numbers: 87.15.−v, 78.40.Me, 81.05.Lg, 87.10.+e

The capture silk is a natural material produced by orb-web weaving spiders. Spiders rely on it to entrap flying preys [1]. 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 [2,3]. 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 a high-tensile steel [2]. 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 [4]. 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.

Single-molecule manipulation methods have recently been applied on spider silks to obtain very detailed information on their force-extension response [3,5]. In a recent experiment, Hansma and co-workers [3] 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,

$$f \propto \exp(x/\ell), \tag{1}$$

where ℓ, the length constant referred to in [3], is a fitting parameter whose physical meaning needs to be decided (see below). $\ell = 110 \pm 30$ nm for a capture silk molecule, and $\ell = 11 \pm 3$ mm for an intact capture silk fiber [3]. 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 [3]. 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 [6] or single titin proteins [7,8]. The behavior of dsDNA can be understood by the wormlike chain model of entropic elasticity [9,10], and that of titin by a two-level system coupled with entropic elasticity [11]. Similar exponential force-extension data were also observed by Dessinges *et al.* when they stretched a single-stranded (ss) DNA molecule at low salt conditions [12]. The data were explained as a result of the interplay of electrostatic repulsion and entropic elasticity [12,13]. However, the success of this model depends on the specific ionic concentration (i.e., low salt and high electrostatic interaction), and it could not naturally reproduce the exponential stretching data at extremely high force (i.e., when the extension is larger than 1.1 times the ssDNA backbone length) if higher order deformation energy terms are not included [12]. In the spider capture silk experiment, the exponential behavior was observed at both fluid and air within a force range from about 10^0 piconewton (pN) to about 10^6 pN [3].

Equation (1) indicates 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 heuristic cascading responses, here we propose a *hierarchical chain model* for spider capture silk. 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 [4], or microcrystal structures [14]. 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 $h = 0$.

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 [15]. 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 [16]. 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 [7,17]. Our hierarchical chain model may also serve as a framework to understand the mechanical property of these biomaterial systems.

Consider a polymer of contour length L_0. It is regarded as the module of hierarchy level $h = 0$ (the module M_0). M_0 is composed of a tandem sequence of m_0 subunits M_1 of contour length $L_1 = L_0/m_0$ (Fig. 1). Under the action of an external force field f, the extension of M_0 is denoted as $x_0(f)$. It was observed that water-induced protein mobility is a significant contribution to capture silk elasticity [18]. Therefore, we decompose x_0 into two parts: (i) the extension Δx_0 caused by the displacement and position rearrangement of these m_0 subunits, and (ii) the total extension $m_0 x_1$ caused by the inherent deformations of these m_0 subunits:

$$x_0(f) = \Delta x_0(f) + m_0 x_1(f). \tag{2}$$

Similarly, at the hierarchy level $h = 1$, each unit is itself composed of m_1 level-2 subunits M_2 of length $L_2 = L_0/(m_0 m_1)$. Therefore, $x_1(f)$ can be written as $x_1(f) = \Delta x_1(f) + m_1 x_2(f)$, where $\Delta x_1(f)$ is the contribution to the extension of a level-1 module due to the displacement and position rearrangement of its m_1 subunits, and $x_2(f)$ denotes the extension because of the inherent deformation of a level-2 subunit M_2. As this structural hierarchy is continued, we arrive at the following expression concerning the total extension $x(f)$:

$$x(f) = \Delta x_0(f) + \sum_{h=0} m_0 m_1 \ldots m_h \Delta x_{h+1}(f). \tag{3}$$

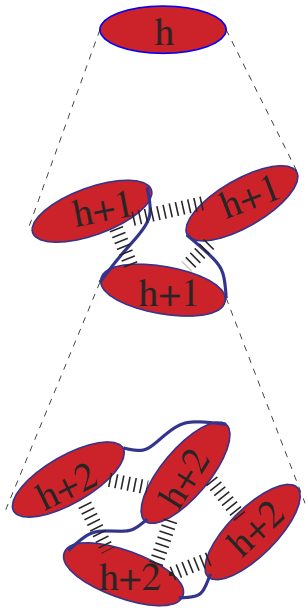

FIG. 1 (color online). The hierarchical chain model. At each hierarchy level h a structural module M_h is composed of a tandem sequence of m_h submodules M_{h+1} of hierarchy level $h + 1$. Under external stretching, M_h responds by (i) adjusting the arrangements of its m_h subunits and making them more 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.

It is reasonable that the average extension $\Delta x_h(f)$ of a M_h contributed by the reorientation or rearrangement of its level-$(h + 1)$ subunits is proportional to the relaxed contour length L_h of this module. To facilitate the following analytical calculation, let us assume $\Delta x_h(f)$ has the following nonlinear form (we show that the final force-extension relationship is not sensitive to the specific assumption made here):

$$\Delta x_h(f) = \begin{cases} \alpha L_h f/f_h, & f < f_h, \\ \alpha L_h, & f \geq f_h, \end{cases} \tag{4}$$

where α is a dimensionless proportional constant; f_h is the characteristic force needed to displace and rearrange the positions of the m_h submodules contained in a M_h (during this process some sacrificial bonds are broken). Equation (4) is in agreement with the experimental observation [3] that, between adjacent rapture events, a capture silk responds to external stretching in a linear way. Denote ΔE_h as the energy cost of breaking all the sacrificial bonds between a level-h module's m_h subunits. From Eq. (4) we

know that $f_{h+1}/f_h = m_h \Delta E_{h+1}/\Delta E_h$. Consider a level-$(h+2)$ module M^a_{h+2}: it is in M^a_{h+1} which in turn is in M^a_h. M^a_{h+2} feels an *internal* energy ϵ due to its interaction with other subunits in M^a_{h+1}, and it feels an *external* energy ϵ' due to its interaction with other subunits in M^a_h but not in M^a_{h+1}. Based on Fig. 1, we know that $\Delta E_{h+1} = m_{h+1}\epsilon/2$ and $\Delta E_h = m_h m_{h+1}\epsilon'/2$. The hierarchical organization of the polymer requires that $\epsilon > \epsilon'$, so as to ensure that structural modules of shorter length scales are formed earlier. Based on these considerations, we arrive at the following self-similar scaling form:

$$f_{h+1} = (\epsilon/\epsilon')f_h = \beta f_h \qquad (\beta \equiv \epsilon/\epsilon' > 1). \tag{5}$$

The parameter β characterizes the degree of coherence in the modular organization of the polymer: a large β value means that a submodule has much stronger internal interactions compared with its external interactions.

From Eqs. (3) and (4) we find that when $f_{h-1} < f \leq f_h$

$$\frac{dx(f)}{df} = \sum_{h'=h} \frac{\alpha L_0}{f_{h'}} = \frac{\alpha\beta L_0}{\beta - 1} f^{-1}. \tag{6}$$

Equation (6) therefore recovers the experimental exponential force-extension relationship of Eq. (1) with

$$\ell = \frac{\alpha\beta}{\beta - 1} L_0. \tag{7}$$

The length constant ℓ is proportional to the relaxed contour length L_0 of the whole polymer, consistent with Ref. [3].

Figure 2 demonstrates the numerically calculated force-extension curve based on Eqs. (3)–(5). As a comparison, the experimental data [3] on intact spider capture silk is also shown. As $\beta \simeq 2$ and the experimental exponential force range is roughly from $6 \times 10^{-5}N$ to $10^{-3}N$, it appears that 4–5 levels of hierarchy were probed.

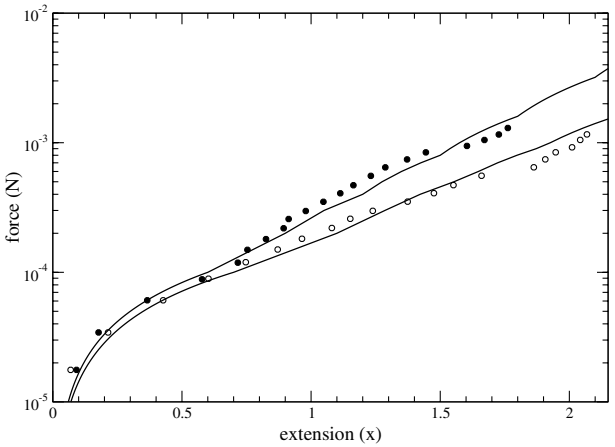

FIG. 2. Exponential force-extension relationship for the hierarchical chain model. Equation (4) is used in the numerical calculation. The parameters are $f_0 = 10^{-4}$ N, $\alpha = 0.3$, and $\beta = 2$ (the upper curve) or $\beta = 1.75$ (the lower curve). Extension is in units of L_0. Symbols are experimental data from Fig. 4 of Ref. [3].

The exponential relationship shown in the figure is insensitive to our particular assumption in Eq. (4), as long as the elastic response at each hierarchy level is nonlinear and bounded. As an example, the solid curves in Fig. 3 show the resulting force-extension relationship when Eq. (4) is replaced by

$$\Delta x_h(f) = \alpha L_h[1 - \exp(-f/f_h)]. \tag{8}$$

The same exponential behavior as in Fig. 2 is obtained. However, the hierarchical scaling form Eq. (5) is needed for the exponential force-extension correlations. For example, when Eq. (5) is replaced by a power law, $f_h \propto f_0 h^\gamma$, the response is not exponential (the dotted line in Fig. 3). We also noticed that, when in Eq. (5) the parameter β is not a constant but fluctuates over some finite range of $\beta > 1$, the resulting force-extension curve is still exponential (Fig. 3, dashed lines).

In summary, we have developed a hierarchical chain model to understand the strength and elasticity of spider silks. Remarkably, this simple model was able to reproduce the peculiar exponential force-extension response of spider capture silk reported by Becker *et al.* [3]. The model can also be used as a framework to understand the elasticity of other spider silks and other biopolymers with hierarchically organized structures.

Becker *et al.* [3] have proposed an alternate and interesting idea to model the spider silk as a branched network

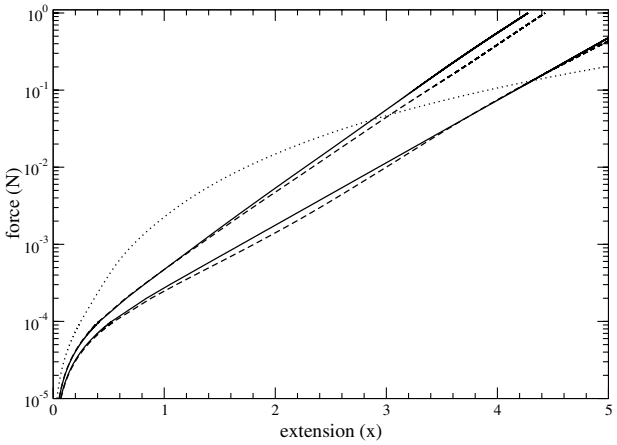

FIG. 3. The force-extension relationship of a hierarchical chain is insensitive to the assumption made to the response $\Delta x_h(f)$ of Eq. (4), but is sensitive to the hierarchical scaling form of the characteristic force f_h. The solid lines are obtained by assuming $\Delta x_h(f)$ has the form of Eq. (8), while other parameters are the same as those in Fig. 2. The dotted line shows the change in the force-extension curve when additionally a power-law form of $f_h = f_0 h^\gamma$ with $\gamma = 3.0$ is assumed for f_h. The dashed lines are obtained by assuming Eq. (8) and Eq. (5), with β fluctuating uniformly within $[1.25, 2.25]$ (the lower curve) and within $[1.5, 2.5]$ (the upper curve).

of interconnected springs. In their model, the system responds to external stretching by first unfolding the single spring at the root level, then the m springs at the first branching level, then the m^2 springs at the second branching level, and so on. The hierarchical chain model developed here is different from the model of Becker *et al.*. First, the molecule in the hierarchical chain model consists of a tandem sequence of structural modules of different length scales. Therefore, no assumption of branching structure is made in our model. Second, the response of the chain to external perturbations is 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 intact. As the external perturbation is elevated, 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 degree of structural integrity as high as possible. 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 contract following 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 [19–21]. At the basic level, the flagelliform sequence is consisted of three repetitive modules (motifs): (i) the GPGGX motif (Gly-Pro-Gly-Gly-X, $X \in$ {Ala, Ser, Tyr, Val}); (ii) the GGX (Gly-Gly-X, $X \in$ {Ala, Ser, 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 [19]. 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 [19], which may encode important structural information. The structures of spider capture silks (and other spider silks) therefore have the potential to be hierarchically organized.

For spider capture silk, one important experiment will be to check the validity of Eq. (5) by single-molecule force spectroscopy. The characteristic forces f_h may correspond to the forces at the saw-tooth rapture events observed by Hansma and coworkers [3]. Although the experimental curves of Ref. [3] is in agreement with Eq. (5), more systematic investigations are necessary to draw a solid conclusion.

We are grateful to H. Hansma for communicating her experimental results with us prior to publication and for the experimental data used in Fig. 2, and to D. E. Makarov for comments on the manuscript. Thanks are also due to R. Lipowsky, Z. C. Ou-Yang, and J. Skolnick for their support.

[1] F. Vollrath, Sci. Am. **266**, No. 3, 70 (1992).
[2] J. M. Gosline, P. A. Guerette, C. S. Ortlepp, and K. N. Savage, J. Exp. Biol. **202**, 3295 (1999).
[3] N. Becker, E. Oroudjev, S. Mutz, J. P. Cleveland, P. K. Hansma, C. Y. Hayashi, D. E. Makarov, and H. G. Hansma, Nat. Mater. **2**, 278 (2003).
[4] *Silk Polymers: Materials Science and Biotechnology*, edited by D. Kaplan, ACS Symposium Series (American Chemical Society, Washington, DC, 1994).
[5] E. Oroudjev, J. Soares, S. Arcidiacono, J. B. Thompson, S. A. Fossey, and H. G. Hansma, Proc. Natl. Acad. Sci. U.S.A. **99**, 6460 (2002).
[6] S. B. Smith, L. Finzi, and C. Bustamante, Science **258**, 1122 (1992).
[7] M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub, Science **276**, 1109 (1997).
[8] K. S. Z. Kellermayer, S. B. Smith, H. L. Granzier, and C. Bustamante, Science **276**, 1112 (1997).
[9] J. F. Marko and E. D. Siggia, Macromolecules **28**, 8759 (1995).
[10] H. Zhou, Y. Zhang, and Z.-C. Ou-Yang, Phys. Rev. Lett. **82**, 4560 (1999).
[11] M. Rief, J. M. Fernandez, and H. E. Gaub, Phys. Rev. Lett. **81**, 4764 (1998).
[12] M. N. Dessinges, B. Maier, Y. Zhang, M. Peliti, D. Bensimon, and V. Croquette, Phys. Rev. Lett. **89**, 248102 (2002).
[13] Y. Zhang, H. Zhou, and Z.-C. Ou-Yang, Biophys. J. **81**, 1133 (2001).
[14] F. Vollrath and D. P. Knight, Nature (London) **410**, 541 (2001).
[15] K. E. van Holde, *Chromatin* (Springer-Verlag, New York, 1989).
[16] C. Branden and J. Tooze, *Introduction to Protein Structure* (Garland Publishers, New York, 1999), 2nd ed.
[17] H. Lu, B. Isralewitz, A. Krammer, V. Vogel, and K. Schulten, Biophys. J. **75**, 662 (1998).
[18] K. M. Bonthrone, F. Vollrath, B. K. Hunter, and J. K. M. Sanders, Proc. R. Soc. London B **248**, 141 (1992).
[19] C. Y. Hayashi and R. V. Lewis, J. Mol. Biol. **275**, 773 (1998).
[20] P. A. Guerette, D. G. Ginzinger, B. H. F. Weber, and J. M. Gosline, Science **272**, 112 (1996).
[21] C. Y. Hayashi and R. V. Lewis, Science **287**, 1477 (2000).