Putting a New Spin on Spider Silk

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Materials scientists have long admired the properties of biological materials and materials systems. Constrained by a relatively limited set of starting materials (such as proteins, polysaccharides, and some simple minerals), plants and animals produce a diverse menu of hard and soft tissues that must meet a demanding set of performance requirements in order to ensure survival of the organism. Mechanical behavior, permeability, optical properties, and environmental sensitivity must be controlled within narrow limits, and processing into tissues of predetermined size and shape must be accomplished under ambient conditions, often without the intervention of external molds or energy sources. Comparable control of the properties of synthetic materials presents daunting challenges, and materials scientists are increasingly willing to turn to biology for lessons that might be transferred from natural to synthetic systems.

A substantial step forward along this path has been taken by Jelinski and coworkers, who report on page 84 an analysis of the molecular orientation of spider dragline silk (*1*). Dragline silk, used by spiders to build the frames of their webs and to drop in controlled fashion from high places, exhibits a combination of strength and toughness unmatched by other high-performance synthetic fibers (see figure). The origins of these unique mechanical properties have been of interest for some time but have remained obscure.

Not that there is a dearth of information about spider silk. Descriptions of its physical properties have been in the literature for nearly a century, and recent studies have revealed the compositions and, in part, the sequences of the most important silk proteins (*2*). What is new about the Jelinski *et al.* report is the insight it provides into the supramolecular architecture of the spider silk fiber and into the likely connections between supramolecular architecture and mechanical properties.

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The primary constituents of spider silk (as well as the more familiar silkworm silk used in textile manufacture) are the two simplest amino acids, glycine (42% of the structure) and alanine (25%). The remainder of the fiber consists of bulkier amino acids, with glutamine, serine, and tyrosine prominently represented. Much of the alanine is organized into short polyalanine sequences 5 to 10 residues in length, which have been shown to adopt a β-sheet conformation and are believed to account for most of the crystalline fraction (about 30%) of the fiber.

SPIDER VERSUS SYNTHETIC

Material	Strength (N/m^2)	Energy to break (J/kg)
Dragline silk	1×10^9	1×10^5
Kevlar	4×10^9	3×10^4
Rubber	1×10^6	8×10^4
Tendon	1×10^9	5×10^3

[Data from (*2*), based on (*6*)]

Tough silk. The high tensile strength of spider silk, coupled with a relatively high elongation-to-break, makes the silk remarkably tough in comparison to other macromolecular materials. [Photo Nuridsany et Pérennou/Photo Researchers. Inc.]

Jelinski and co-workers have focused on the organization of these polyalanine elements. By "hand-feeding" a solution of 10% perdeuteromethyl L-alanine to adult female spiders, the Cornell group was able to coax the spinning of fibers in which the level of deuteration of the alanine residues was about 4%, sufficient to obtain high-quality deuterium nuclear magnetic resonance spectra. For fibers aligned with the magnetic field, fitting of the observed spectral line shapes required consideration of two components, both consistent with chain alignment along the fiber axis but with very different angular distributions. One population, representing about 40% of the alanine residues, was found to be highly oriented, whereas a second was characterized by very weak orientation (the two populations were described by Gaussian orientation distributions with full widths at half maximum of 5° and 75°, respectively). The corresponding average orientation of the crystalline portions of the fiber agrees well with previous results from x-ray diffraction, but this is the first time that multiple crystallite populations have been proposed for spider silk.

Jelinski and co-workers further propose a structural model for the silk fiber, with highly oriented alanine-rich crystals, and with weakly oriented "protocrystals" contributing to the unusually high compressive strength of spider silk. In this model, glutamine and other bulky residues limit the growth of β sheets and force the formation of loops and tie chains that link crystals to one another and to the surrounding amorphous matrix. This picture is consistent with a theoretical model of spider silk elasticity developed recently by Termonia at DuPont (*3*), in which the fiber is represented by small crystallites embedded in a rubbery amorphous phase. A key feature of the Termonia model, which reproduces the stress-strain behavior of the fiber very nicely, is the presence of a thin layer within the amorphous matrix with a modulus higher than that of the bulk amorphous phase. Small crystallite size is advantageous in this description, as it allows the high-modulus "interphase" to occupy a large volume fraction in the semicrystalline fiber.

Does the limited length of the polyalanine sections in the primary sequence of spider silk represent an evolutionary solution to the problem of designing a tough, high-strength fiber? Can high compressive strength—an elusive property in synthetic fibers—be achieved by controlling the orientation of crystals with respect to the fiber axis? The example of dragline silk offers insights into the behavior of biological materials that will return practical dividends. Regardless of its evolutionary significance, the toughness of spider silk is a fact, and uncovering its origins will reveal valid lessons in materials design. Furthermore, we are now learning how to adapt biological processes to make new materials, making the prospects for exploiting biological design features more realistic. Spider silk analogs have now been expressed in genetically engineered bacterial cells (*4*), and a wide variety of biologically derived polymers with interesting materials properties have appeared within the last several years (*5*). Developments in biological synthesis and processing, coupled with studies of the kind reported by Jelinski and coworkers, offer promising new directions for materials research.

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Ecdysis Control Sheds Another Layer

James W. Truman

The dramatic escape of a butterfly from its chrysalis is one of the most commonly photographed behavioral sequences in biology. The apparent abruptness of this ecdysis, however, is misleading because the transformation actually takes days or weeks but is hidden from sight within the cuticle of the chrysalis. The steroid hormone ecdysone and its metabolites cause the epidermis of the chrysalis to detach from its overlying cuticle and begin to form the butterfly. As this molting process progresses, the inner layers of the old cuticle are digested, leaving it vulnerable to the forces that will eventually cause it to rupture. The forming new cuticle is soft and pliable, a condition that allows it to be slipped out from the confining sheath of the old cuticle. Only when the developmental events are complete are the ecdysial processes then called into play to bring about the shedding of the old cuticle and the expansion and hardening of the new one. The triggering of ecdysis itself is hormonally controlled, in a process that just became more complex. In this issue, Žitňan and colleagues report the existence of a second ecdysial hormone—Mas-ETH (*Manduca sexta* ecdysis-triggering hormone)—to add to the existing eclosion hormone (*1*).

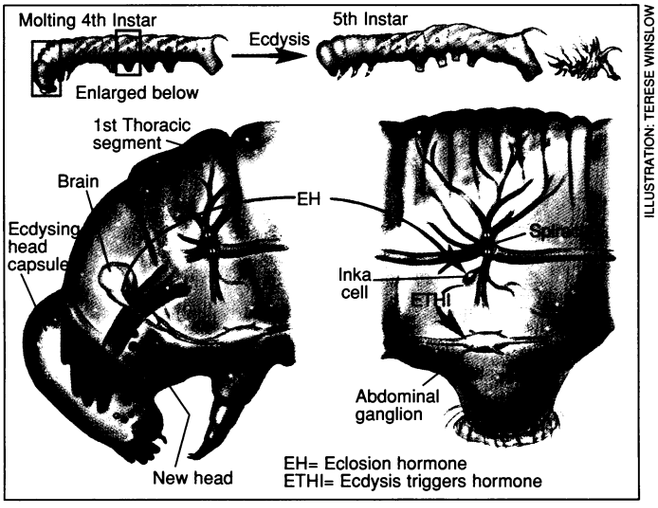

ILLUSTRATION: TERESE WINSLOW

Mechanism of ecdysis control. The pathway for triggering ecdysis behavior originates in the brain but is relayed through the Inka cells, a set of glands in the periphery.

Although in a sense a conjurer's illusion, ecdysis is nevertheless a process worthy of fascination. It includes a sequence of complex behavioral programs that enable the insect to shed its entire body surface, including the cuticular linings of its fore- and hindgut and tracheal system (*2*). Many of the behaviors seen during ecdysis are unique to this event and, indeed, after the final molt to the adult stage, the neurons dedicated to these behaviors then die (*3*). A successful ecdysis requires that these specialized behaviors be closely coordinated with ongoing physiological and developmental processes (*2*). Because of irreversible changes within the new cuticle, the behavior can only be performed once. Hence, a failure in this coordination results in crippling deformities or in insects being fatally trapped in their old skin, and each ecdysis is a potential crisis point in an insect's life history.

The hormonal control of ecdysis was initially inferred from experiments on moths that showed that the circadian timing of this behavior could be transferred from one species to another by transplanting the brain (*4*). These experiments led to the isolation of a peptide from the brain that could induce precocious ecdysis behavior and was called eclosion hormone (EH). In more striking studies in the moth *Hyalophora cecropia*, EH was shown to act on an isolated chain of abdominal ganglia with its accompanying tracheal supply to release the complete preecdysis and ecdysis motor programs (*5*). This 62–amino acid peptide was purified and sequenced from the moths *M. sexta* (*6*, *7*) and *Bombyx mori* (*8*).

The new report by Žitňan *et al.* (*1*) indicates that the long-held view that EH is the sole mediator of ecdysial behavior and physiology is too simplistic. They have found a second peptide from *M. sexta*, an ecdysis-triggering hormone (Mas-ETH), which can also trigger the preecdysis and ecdysis behaviors. This peptide is released from a set of peripheral secretory cells, Inka cells, that are situated on ventral tracheal trunks near each spiracle. When injected into molting animals, the Inka peptide induces the rapid onset of preecdysis and ecdysis behaviors. The sequence of this 26–amino acid peptide is novel and is structurally unrelated to that of EH.

What is the functional relation of Mas-ETH to EH? The authors describe an insightful set of experiments on the isolated central nervous system (CNS) of *M. sexta*. The isolated CNS, without its tracheal supply, does not respond to EH, but it does respond to Mas-ETH by generating both the preecdysis and ecdysis motor programs. The isolated CNS can be made to respond to EH in vitro if freshly dissected Inka cells are also placed in the bath. These findings on *Manduca* call into question the interpretation of the need for the tracheal system in the original studies on the isolated CNS of *H. cecropia*. Ironically, the ventral tracheal trunk that leads to each ganglion is also the one on which the Inka cell resides.

As depicted in the figure, these new results suggest that a peripheral step involving the Inka cells is interposed between the secretion of EH and the subsequent triggering of the ecdysial motor programs from the CNS. Because a successful ecdysis requires that the old cuticle be sufficiently digested to be shed, it seems appropriate that there is peripheral input in deciding the timing of ecdysis. It is especially intriguing that the Inka cells are located on the trachea near each spiracle, the opening through which the tracheal linings are withdrawn. These linings are the most fragile parts of the old cuticle, and if they are torn and left behind during ecdysis, the tracheae to that region are obstructed and oxygen delivery is impaired. Thus, the Inka cells are in an excellent position to monitor the changes in the tracheae during the molt. Sitting at this most vunerable site in the periphery, they might serve as a final checkpoint to ensure that the old cuticle is ready before the insect irreversibly commits itself to ecdysis.

Although the relation depicted in the figure seems likely, some observations remain unexplained. For example, experiments on pupal ecdysis in *Manduca* show that ecdysis still occurs when the secretion

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