High-Performance Fibers from Spider Silk**

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An insect trapped in a spider net has usually no chance of escape. Although a spider net is composed of the smallest amount of ultrathin fibers, it effectively captures rapidly flying insects without breaking, leaving the prey entangled. This ability is largely explained by the mechanical properties of the spider silk, the major component of the spider-net fibers. Spider silk is an unusually tearproof, strong, and elastic fiber protein that is only surpassed in these properties by some modern synthetic high-performance polymers.[1] Table 1 shows that the orb-web-spinning spider *Araneus diadematus* produces a silk for the dragline and the radiating spokes of the net the strength of which approaches that of high-tensile steel while being significantly more extensible, as well as lighter. Although highly ordered poly(*p*-phenyleneterephthalamide) fibers (Kevlar) are stronger than spider silk and almost as light, they are also significantly less elastic. Moreover, spider silk is hydrophilic, yet not water soluble, and biodegradable.

Table 1. Selected properties of some natural and synthetic fibers.[1]

	Density [g cm^{-3}]	Elasticity modulus [GPa]	Tenacity [GPa]	Strain break [%]	Toughness [MJ m^{-3}]
nylon 6,6	1.1	5	0.95	18	80
kevlar 49	1.4	130	3.6	3	50
dragline of *A. diadematus*	1.3	10	1.1	27	160
silk of the moth *B. mori*	1.3	7	0.6	18	70
wool	1.3	0.5	0.2	50	60
high-tensile steel	7.8	200	1.5	1	6

This comparison clearly demonstrates that spider silk is a highly interesting fiber for, for example, medicinal applications or the production of light and strong composite materials. In contrast to the textile silk that is traditionally produced by cultivation of the silk moth *Bombyx mori*, the isolation of larger amounts of spider silk from the natural resources is not feasible, however, because of the territorial nature of spiders. Alternatively, spider silk can be synthesized using microbiological methods but, in spite of various attempts, to date no material has thus been produced that is able to compete with natural silk. Only recently, researchers from Nexia Biotechnologies, in cooperation with the Materials Science Team of the U.S. Army, described the successful synthesis of a spider-silk protein with similar mechanical properties to the natural counterpart, by transfer of the corresponding gene into suitable mammalian cells.[2] A larger scale production of synthetic spider silk thus becomes practicable.

Orb-web spiders produce up to seven different types of silk, which are optimized for different applications in net spinning or cocoon wrapping.[1, 3] One of the best-investigated spider silks is the one in the dragline, because of its interesting mechanical properties. The nonprotein content of this silk is $<1\,\%$,[3] so that investigations on the molecular origins of its mechanical properties can focus on the fiber protein itself. The silk of a dragline usually contains more than one type of protein, however. For the dragline of the two most-studied orb-web-spinning spiders *Nephila clavipes* and *Araneus diadematus*, two and four protein components have been identified, respectively: spidroin I (MaSpI) and spidroin II (MaSpII),[4] from the former and ADF-1 to ADF-4 from the latter.[5] The amino acid sequence of all of these proteins has been elucidated with the help of recombinant DNA technology.[4, 5] In spite of differences in their exact primary structures, all protein chains feature common characteristic sequence motifs of alanine-rich regions that alternate with regions containing mostly glycine. X-ray diffraction and FT-IR spectroscopic investigations indicated that the alanine-rich regions are located in crystalline domains of the silk fiber with a β-sheet structure. The glycine-rich chain segments adopt a much less ordered secondary structure, in which turns or helices can occur locally that are stabilized by hydrogen bonds.[1, 4] Glutamine residues in the amorphous regions of the polymer chains are responsible for the hydrophilic nature of the spider silk.[1] The resulting overall protein structure resembles that of synthetic elastomers, in which rigid cross-linked sites alternate with flexible, randomly oriented polymer-chain segments. The origins of the elastic properties of spider silk and synthetic elastomers are therefore very similar: strain caused by an external source induces orientation in the flexible chain segments, whereby the degree of extension depends largely on the length of the chains in the amorphous regions. Releasing the strain causes the extended regions to return to the less ordered structure because of the associated

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entropy gain (Figure 1). In addition to the primary structure of the protein, the tertiary and quaternary structures that arise during the spinning process in Nature also strongly influence the properties of the silk, and all have to be reproduced optimally to prepare fibers with similar mechanical properties to spider silk.

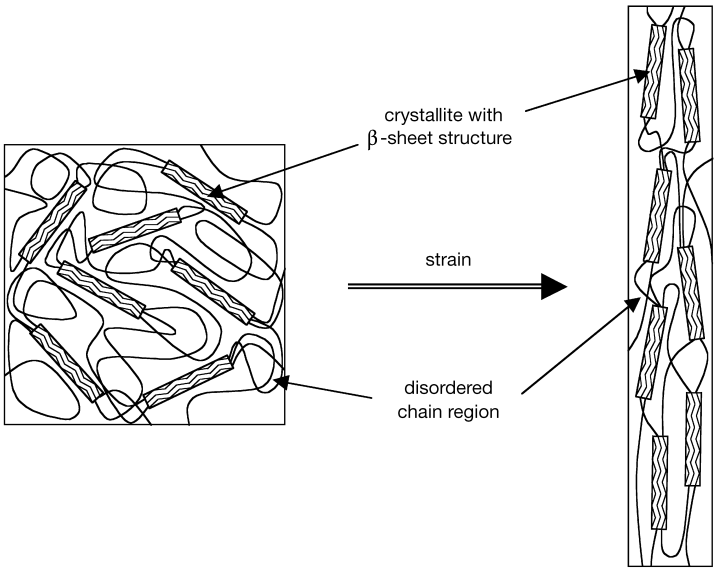

Figure 1. Schematic representation of the three-dimensional structure of a spider-silk dragline, and the effect of mechanical strain on this structure.

Although microbiological syntheses of proteins are state of the art today, the production of fiber proteins with a highly repetitive amino acid sequence remains problematic.[6] One reason is the occurrence of repetitive sequences in the gene for the protein causes a genetic instability. Furthermore, repetitive sequences in the complementary mRNA chains can induce undesirable secondary structures that hinder translation. Finally, fiber proteins often contain certain amino acids in high amounts (in the spidroins, alanine and glycine) and cells therefore have to provide an extensive pool of the corresponding tRNAs for effective protein synthesis. These limitations cause the molecular weight distribution of spider-silk proteins synthesized by transgenic *E. coli* bacteria to be highly heterogeneous with a significant amount of prematurely terminated chains.[7] Transfected yeast[8] or plants such as tobacco or potatoes[9] have been shown to be more suitable for a synthesis of the spider-silk protein, but all these strategies had the disadvantage that the product accumulated inside the cell, for example, in the endoplasmatic reticulum of plant cells, which necessitated a number of steps for isolation. The strategy proposed by the team of Nexia Biotechnologies to use suitable mammalian cells specialized for the secretion of proteins for the synthesis of spider silk thus represents a significant advancement over previous work.[2] These cells have the advantage that they release the protein during synthesis into the surrounding solution, from which it can be isolated easily. Moreover, the cells are more closely related to the epithelial cells that produce silk proteins in the glands of the spiders, and they are therefore better suited to an error-free protein synthesis.

The mammalian cell lines used consisted of bovine mammary cells (MAC-T) and hamster kidney cells (BHK), into which partial cDNA encoding a spider-silk protein (MaSpI, MaSpII, or ADF-3) was transferred.[2] Afterward, these cells were able to produce the corresponding protein, however, the combination of BHK and ADF-3 gave the best results. Because the cells were transfected with partial cDNA, the molecular weight of the protein synthesized was 60 kD, and was thus significantly lower than the average molecular weight (M_W) of natural spider-silk proteins, which can reach 740 kD.[10] Although a transfer of cDNA multimers into the cells resulted in the expression of proteins with the expected higher molecular weights, synthesis proceeded less effectively, an observation that was ascribed to the above-mentioned reasons. The investigations on fiber processing thus focused on ADF-3 weighing 60 kD. This protein was produced continuously in a hollow-fiber culture system in a total amount of 12 g (ca. 20 μg per 10^6 cells per day). Afterward, it was enriched by precipitation with ammonium sulfate, and purified chromatographically. The product was readily soluble in phosphate-buffered saline, an important advantage for processing, and a significant difference to spider-silk proteins produced by *E. coli* or yeast.[7, 8]

In the silk gland of orb-web-spinning spiders, the silk protein is dissolved in a concentrated lyotropic liquid-crystalline solution. During extrusion, the silk assembles into fibers without the need for strong mechanical forces,[11] a process that differs from the industrial spinning of many synthetic polymers. Hereby, a polymer, often dissolved in an appropriate solvent, is extruded into a coagulation bath through an opening of suitable diameter, and the mechanical properties of the fiber formed are improved afterward by an external postspinning draw. Although the natural spider-silk protein can, in principle, be processed by a method similar to industrial spinning,[12] the fiber thus obtained possesses mechanical properties which are inferior to those of the natural starting material. This effect is partly attributed to the need to use the denaturing hexafluoroisopropanol as a solvent, which perturbs the secondary structure of the protein too strongly, and thus the preorganization of the protein chains during spinning. In contrast, an aqueous solution of the synthetic ADF-3 protein produced by BHK could be processed,[2] which resulted in a fiber with a diameter of 20 μm after postspinning draw, whose morphology, stiffness, and extensibility compared favorably with the ones of the natural dragline silk of *Araneus diadematus*. Only the strength tenacity remained lower, yet it did approach that of fibers spun from regenerated spider silk (Table 2).[12] Considering that the molecular weight of the protein used for the preparation of the fiber is lower than that of the natural fiber

Table 2. Comparison of selected properties of natural, regenerated, and synthetic spider silk.

	Elasticity modulus [GPa]	Tenacity [GPa]	Strain break [%]
dragline of *N. clavipes*[1]	22	1.3	12
dragline of *A. diadematus*[1]	10	1.1	27
regenerated silk of *N. clavipes*[12]	8	0.32	
synthetic ADF-3[2, 15]	13	0.26	43

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protein, and that only a single component of the dragline silk was used, the reported results are impressive. It is expected that, by optimizing the process, possibly in combination with a deliberate variation of the primary structure of the protein,[13] fibers that are almost indistinguishable from natural spider silk will be accessible. Nexia Biotechnologies manufactures these fibers under the brand name BioSteel. For the production of larger amounts, they plan to use transgenic goats that express the silk protein in their milk.[14] Applications of spider silk are anticipated in areas where high mechanical strength, in combination with biodegradability, of fibers and films produced thereof are advantageous, for example, in medicine. The presented results show that a production of high-performance polymers on the basis of spider silk is a promising prospect for the very close future.

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The Decaphosphatitanocene Dianion—A New Chapter in the Chemistry of Naked Polyphosphorus Ligands

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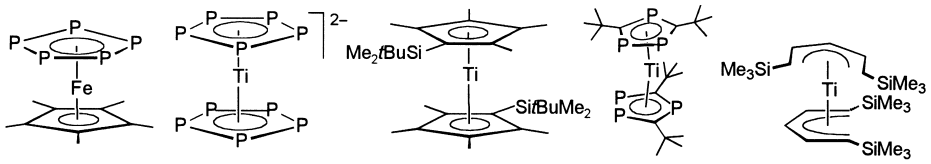

1 2 3 4 5

Even before those progressive researchers had thought about sandwich complexes, ferrocene had already formed from iron and cyclopentadiene, had been collected,[1] and was awaiting discovery. When $[(C_5Me_5)TiCl_3]$ was distilled from the products of a $TiCl_4$-catalyzed olefin isomerization reaction forty years ago,[2] the affinity of transition metals to this prototype of a pentaalkylcyclopentadienyl ligand became apparent and fueled the boom in cyclopentadienyl complex chemistry.

The interest in transition-metal-promoted self-assembly of five-membered-ring ligands extended to Group 15 at the end of the 1980s, when the dinuclear iron complex $[\{(C_5Me_5)Fe(CO)_2\}_2]$ was allowed to react with white phosphorus.[3] The resulting product, namely pentamethylpentaphosphaferrocene (**1**), stimulated the newly emerging area of P_n complexes and awakened the idea of bis(pentaphosphacyclopentadienyl) metal complexes (often referred to as decaphosphametallocenes).[4]

While steric protection is not available to prevent aggregation of decaphosphametallocenes, electrostatic repulsion is. When Ellis and Urnėžius et al.[5] treated the bis(naphthalene)titanium dianion with 2.5 equivalents of P_4 at low temperature, the decaphosphatitanocene dianion $[Ti(\eta^5\text{-}P_5)_2]^{2-}$ (**2**) was formed in high yield as the $2\,[Na([18]crown\text{-}6)]^+$ salt.[5]

Whereas the known metallocene dianions that have been generated by electrochemical reduction are highly labile species,[6] **2** is unreactive with carbon monoxide, xylyl isocyanide, or trimethylphosphite, is stable in solution under an atmosphere of pure dioxygen, can be handled in air, and is only slowly attacked by wet pyridine. Crystalline samples of $(PPh_4^+)_2$ and $(Ph_3PNPPh_3^+)_2$ salts of **2** melt at 213–215

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