

# RECOMBINANT SPIDER SILK PROTEINS FOR COMPOSITE FIBERS

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# Recombinant Spider Silk Proteins for Composite Fibers

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## ABSTRACT

The use of genetic engineering for polymer synthesis provides many opportunities for control of chain composition, sequence, size, reactivity and stereochemistry. Silk proteins represent an unusual class of structural fibers with interesting mechanoelastic properties. Some types of spider silk exhibit improved mechanical properties when compared with silkworm silk. Therefore, spider silk was cloned and expressed in a bacterial host system. Research on selectively modifying composition at the genetic level to effect structural and functional changes in fiber properties is continuing. Applications in the material sciences are expected due to the versatility and properties of this class of fibers.

## INTRODUCTION

Silks represent an unusual class of fibers generally considered to be protein in composition. Unlike enzymes which are termed globular proteins, silks belong to the structural fibrous protein class which also includes keratin and collagen. Silk proteins exhibit a high degree of crystallinity which is derived from the anti-parallel beta sheet secondary structure. This crystalline array is stabilized by a combination of hydrogen bonding between anti-parallel chains, and hydrophobic interactions between the sheets or layers. These interactions result in a class of fibers with unusual and interesting properties, including high tensile strength.

In addition, there is evidence for noncrystalline or amorphous regions in the secondary structure of the silk and it is believed that these regions give rise to extensibility of the fiber and the resulting property of high energy absorption to break. It is the unusual combination of high strength and high extensibility that drives much of the interest in this class of fiber.

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In natural systems, the two common sources of silks are the domesticated silkworm, *Bombyx mori*, and the orb weaving spiders. The silkworm produces one type of silk used in spinning its cocoon during one stage in its life cycle. For domestic silk production the cocoon silk is boiled to remove the soluble sticky sericin protein, and the remaining fibroin portion of the silk is then unwound and used as silk fiber. Orb weaving spiders have the capability to produce many different silks, each of which is synthesized in a separate set of secreting glands in the abdomen. In addition some of the silks, such as the dragline, are produced continuously throughout the lifecycle of the spider. Each of the different silks exhibits different physical properties and functions. These differences are reflected in the amino acid composition of the silk. Some of the silks function in web construction/engineering, in egg cocoon structures, as adhesives, and in prey capture.

There has been some data collected on the physical properties of spider silks. Zemlin [8] and Work and Emerson [7] published data on the amino acid composition and mechanoelastic performance data of different spider silks. Dragline silk from *N. clavipes* has been reported to have a modulus of  $1 \times 10^{10}$  N/m<sup>2</sup>, tensile strength of  $1 \times 10^9$  N/m<sup>2</sup>, and energy absorbed to break value of  $1 \times 10^5$  J/kg. This compares with the silk worm which exhibits a modulus of  $1 \times 10^{10}$  and a tensile strength of  $7.4 \times 10^8$ . Both fibers exhibit about 18% elongation. Spider silks are of interest because the physical properties of the fibers appear superior to the silkworm silks.

For the silkworm, part of the fibroin gene has been mapped and partial sequencing of the 5' end of the gene was completed [2, 5, 6]. This information, in combination with X-ray data has provided the basic information on the protein structure which indicates discrete crystalline and amorphous regions as reflected in discrete coding regions in the silk gene. Genetic information on the silk worm is extensive because of the commercial interest in this material and in the translational controls over silk expression. No data on the organization of spider silk genes are available.

As in the silk worm, X-ray diffraction data on spider silk implies the presence of crystalline regions dispersed in a matrix of amorphous protein [3]. Additional data on conformation is being developed to further understand the relationships between primary and secondary structures for this class of proteins.

In addition, the capability of spiders to produce a multitude of silks with very different functions through changes in amino acid composition dictates this system as useful for genetic manipulation for fiber production. The first goal is to clone silk coding genes from the spider into a more useful expression system to increase available amounts of silk. The dragline silk, because of its high tensile strength, was chosen for cloning.

## RESULTS AND DISCUSSION

To accomplish the goal of increased silk production the silk gene was cloned. First, genetic libraries were constructed from the spider. High molecular weight genomic DNA was isolated and purified from *N. clavipes* and then partially digested with restriction enzymes to yield 25 kb fragments. These fragments were cloned into a Lambda phage vector to generate a genomic library. RNA was purified from the major ampullate gland of the spiders. The major ampullate gland is the site of dragline silk production. The mRNA was isolated by density gradient centrifugation and oligo (dt) column chromatography and then reverse transcribed to generate a cDNA library.

To screen these libraries, the native silk protein had to be solubilized, partially hydrolyzed, and sequenced [4]. This was accomplished and the sequence data developed was used to construct DNA probes. The protein composition data confirmed the high percent of short side chain amino acids (glycine, alanine, serine) which permits the close packing density of the beta sheets. The genomic and cDNA libraries were then screened with these probes. The probes were radiolabelled by the 5' end labelling method and hybridization was determined using autoradiography. Positive clones were then subcloned and expressed in a bacterial host system. Recombinant silk protein was produced and both the clones and the silk are being analyzed. This first phase of work provides the means to produce larger quantities of silk materials for study.

The second phase of the work which is also underway involves selective modification of the natural gene sequence to tailor silk structure and properties to specific functions. To accomplish this goal, molecular modeling studies are being conducted on silk protein sequences to understand the influence of primary sequence on secondary structure. The predicted secondary structure data must then be extrapolated to predicted functional properties of the fibers spun from these sequences. This extrapolation will be validated using protein engineering techniques to enact the sequence changes in the gene, expressing the modified silk proteins, spinning fibers, and then studying fiber properties from the modified proteins.

In general, fiber spinning from recombinant silk proteins will involve mimicking the natural process used by the spider which can be correlated to spinning lyotropic liquid crystals. In the silk gland, the translated product is present in a metastable state. As the material is processed and then spun at the spinnerette, there is a loss of water and the silk protein becomes highly ordered and crystalline. Physical processing appears to effect the major changes in the silk structure and there is no evidence for post-translational chemical modification of the protein. Of interest, based on the amino acid composition data collected, is the fact that spider silk fibers, despite containing a lower percentage of short side chain amino acids, exhibit superior strength properties. This would not be expected and therefore implies a significant role for the processing/spinning steps on resulting fiber properties.

Silk proteins may find application in a number of areas including composite materials. From a composites perspective, a high strength fiber such as silk with its ability to self-assemble into its secondary structure and its reactive functional groups, may provide some unique opportunities in processing and design. In addition, the environmental resistance of natural silks, presumably due to the high degree of crystallinity, would indicate that environmentally stable composite systems incorporating these materials could be considered. Illustrative of the resistance of silk fibers is the fact that proteolytic enzymes are not active in degrading silk proteins. Whether these fibers could also be incorporated into composites for biomedical implants remains to be demonstrated. Work on immobilized enzyme systems using silks for biosensor applications has already been demonstrated [1].

Through genetic engineering we can now consider large scale production of fibrous proteins such as silk. The increased availability of this material will lead to many material applications in the future. In addition, the ability to tailor these structures at the genetic level to meet specific functional requirements further amplifies the potential utility for this unusual class of fibers and for this approach to the material sciences.

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