Synthetic Piriform Spider Silk Protein Production

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Abstract

Spider silks from N. clavipes have a range of useful properties in addition to remarkable mechanical properties. These silks are uniquely suited for biomedical applications as they are generally biocompatible and biodegradable. Piriform silk is an adhesive protein used by the spider to adhere its silk to a variety of substrates via a structure called an “attachment disk”. Sequencing of the silk mRNA extracted from the piriform silk gland of N. clavipes has revealed two unique amino acid motifs. The structural role of these motifs in the attachment disk is unknown. The specific aim of this project is to produce recombinant piriform spider silk in Escherichia coli. Three biochemical proteins will be produced, two based on individual amino acid motifs and one based on the overall piriform protein sequence. The resulting piriform-analogue proteins will be used to produce fibers, films and gels for mechanical testing. The structural role of piriform’s unique motifs will be characterized using X-ray Diffraction and Circular Dichroism on solid samples. Elucidating the mechanical and structural roles of these motifs will add to the existing repertoire of characterized motifs for the production of tunable, chimeric spider silk materials.

Introduction

Silk from Nephila clavipes has been shown to have extraordinary mechanical properties. Native silk has been shown to have a higher toughness than Kevlar and twice the extensibility of nylon.1–4 Of the seven types of silk produced by N. clavipes, Piriform remains the most uncharacterized. Silk is produced and stored in glands upstream from the spigot from which the fiber is drawn (Figure 1). By generating cDNA libraries from dissected glandular tissue, the gene sequence coding for the specific silk protein type can be elucidated.5 Because silks require no posttranslational modification, expression in a bacteria such as E. coli is possible. In order to generate a synthetic silk gene, the repetitive motifs of the silk are iterated to the extent of the bacteria’s metabolic capacity. Piriform silk is comprised of two structural motifs, PX(P motif) and QQA (Q motif, Figure 2). The structural roles of these motifs are not clear. Based on Raman Spectroscopy analysis, the Piriform silk structure has been shown to be similar to Aciniform silk. Based on this comparison, it will likely have similar mechanical properties.6

Methods and Materials

Synthetic piriform genes were optimized by Life Technologies® for expression in BL21 strain E. coli. The synthesized gene subunits shown in Figure 1 were iteratively-cloned to produce Piri4, P[16] and Q[18]. Each of these constructs were cloned into a modified pET_19b vector. Specifically, this vector includes a synthetic gene based on a conserved nucleotide motif found in MaSp2 C-terminal domains. Expression was accomplished using a custom dual-vector approach. A second vector was produced by modifying pACYC to include tRNA genes (GlyT, GlyL and ProM). Supplementing tRNA pools improves availability of tRNAs that are rare in E. coli but common to spider silks. Fermentation at 1L, 10L and 20L was done to obtain crude protein. To date, Piri4 and P[16] proteins have been produced and optimized. Purification was accomplished using a nickel-agarose chromatography procedure. A 10X polyhistidine tag was expressed in-frame with each protein. Cell pellets were resuspended in lysis buffer containing 8M urea as a denaturant, and processed by centrifugation. Supernatant containing solubilized protein was purified with a GE® AKTA FPLC purification system. Fractions containing purified protein were dialyzed and lyophilized, yielding raw protein for testing.

In order to produce fibers, gels and other testable substrates, silk proteins must be solubilized and subjected to specific biophysical conditions to induce self-assembly. A method for producing aqueous spider silk dopes has been developed by members of Lewis Lab, and used successfully to produce aqueous piriform dopes. Native piriform silk forms a staple-pin architecture from a multi-spigot gland that is difficult to replicate in a laboratory setting (Figure 1).

Results

Piri4[4] protein has been analyzed with Fourier transform infrared spectroscopy, both as a solubilized dope and as a dry powder. The results are shown in Figure 5. Dry piriform powder was found to have amide III and I at 1450 and 1650 cm⁻¹. The amide I band is likely indicative of beta sheet structures in the dry protein. The liquid samples produced significantly weaker signals, but similar amide I and II bands are visible.

Conclusions

Piriform spider silk is potentially useful as a material and adhesive for biomedical applications. Producing a synthetic version of piriform spider silk will allow for structural characterization of piriform’s unique PxP and QQAS motifs. Synthetic proteins based on piriform and its PxP motif have been successfully produced and purified. Initial success with solubilizing piriform into aqueous dopes is a key step towards producing piriform as a testable biomaterial. FTIR results indicate that the secondary structure of unassembled piriform protein contains beta sheet structures. Once the correct biophysical conditions for piriform self-assembly have been identified, a variety of testable substrates can be produced; including fibers, gels, films and mats. Further development of piriform may also lead to biocompatible adhesives for medical applications.

Future Work

• Produce and optimize Q[18].
• Identify biophysical conditions necessary for piriform self-assembly.
• Characterize structural motifs using XRD and NMR.
• Produce and test piriform-based materials for mechanical testing.
• Assess biocompatibility of piriform-like silk proteins.

References


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Figure 1. Staple-pin architecture of piriform and multi-spigot gland structure.

Figure 2. Amino acid sequences of P (blue) and Q (red) structural motifs, and full piriform cassette.

Figure 3. Western blot showing expression of 100kDa Piri4[4] protein.

Figure 4. Piri4[4] has a range of useful properties in addition to remarkable mechanical properties. These silks are uniquely suited for biomedical applications as they are generally biocompatible and biodegradable. Piriform silk is an adhesive protein used by the spider to adhere its silk to a variety of substrates via a structure called an “attachment disk”. Sequencing of the silk mRNA extracted from the piriform silk gland of N. clavipes has revealed two unique amino acid motifs. The structural role of these motifs in the attachment disk is unknown. The specific aim of this project is to produce recombinant piriform spider silk in Escherichia coli. Three biochemical proteins will be produced, two based on individual amino acid motifs and one based on the overall piriform protein sequence. The resulting piriform-analogue proteins will be used to produce fibers, films and gels for mechanical testing. The structural role of piriform’s unique motifs will be characterized using X-ray Diffraction and Circular Dichroism on solid samples. Elucidating the mechanical and structural roles of these motifs will add to the existing repertoire of characterized motifs for the production of tunable, chimeric spider silk materials.

Figure 5. FTIR spectroscopy results for solubilized and dry piriform powder.

FTIR

Liquid and Dry Piriform

FTIR

Solubilized Dopes

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