DESIGNING NEW BIOMATERIALS: Modifying a Spider Silk Gene For Efficient Bacterial Expression for Industrial Production

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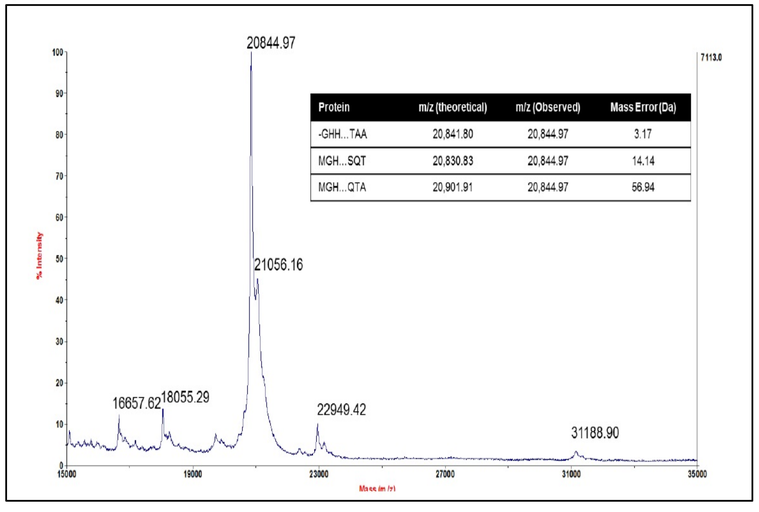
Introduction

Spider silks have remarkable physical properties due to a combination of strength and elasticity. In addition, spider silks are biocompatible and biodegradable. Our laboratory has shown that the strength of products, such as fibers, produced with other silk proteins correlates with the size of the silk protein. The aciniform silk protein (AcSp1), has been shown to produce the thinnest and strongest fibers of all the natural spider silks. Aciniform silk is composed of a nonrepetitive amino-terminal region, 14 repeats of approximately 200 amino acids each, and a nonrepetitive carboxy-terminal region. We have been able to produce different variants of this gene. All AcSp1 protein variants were able to express in *E. coli*. The bacteria expression of the AcSp1 protein is low and the protein is expressed not only as a full length polypeptide but also as fragments of the protein. We identified a sequence in the amino-terminal region of the first repeat of the AcSp1 gene that acts as an early termination sequence. Our objective is to modify this region on the gene to study changes in the expression efficiency of AcSp1.

A



B

MGHHHHHHHHHHSSGHIDDDDKHMTGGYPGGYPGGQGAGPLGGVPLVSQSLDNLGG

GGAQAGLISRVANALANTSTLRAVLRRGVSQNTVNNVVQRTVQSLANTLGVDGNNLAR

IASQAISQVPAGSDTNAYAQALSTANLVTGGILNERNIDSLGSRVLSAVLNGVSSAAQGL

GINVDTGNLQGDIRSSTGFLSTGSSSTILSQTAASTTSGAESTSGGYPGGYPGGQGAGP
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QSLANTLGVDGNNLARIASQAISQVPAGSDTNAYAQALSTANLVTGGILNERNIDSLGSR
VLSAVLNGVSSAAQGLGINVDTGNLQGDIRSSTGFLSTGSSSTILSQTAASTTSGAET

Figure 1: A) Mass Spectrometry chromatograph of the purified truncated polypeptide. The major peak indicates its molecular mass and allowed us to determine the sequence of the polypeptide. **B)** AcSp1 protein sequence (2 repeats) and site of early protein synthesis termination (red arrow).

Methods

An Aciniform gene was synthesized based on its protein sequence. The synthetic gene encodes for two identical repeats. Two new variants of the gene were generated by site directed mutagenesis which was performed using single stranded DNA (ssDNA). Two oligonucleotides (LoFi and NT1) were designed to introduce several mutations in the synthetic gene.

AcSp1 gene

First repeat — Second repeat

NT1 | LoFi | NT1

High-usage (ratio) codons replaced by low-usage codons

Original sequence

Ratio		0.27	0.43	0.43	0.13	0.38	0.22	0.70	0.27	
	5'	AGC	ACC	ACC	AGT	GGT	GCA	GAA	AGC	**3'**
		S	**T**	**T**	**S**	**G**	**A**	**E**	**S**	

LoFi mutant

Ratio		0.13	0.13	0.21		0.13			0.13	
	5'	AG**T**	AC**T**	AC**T**	AGT	GG**G**	GCA	GAA	AG**T**	**3'**
		S	**T**	**T**	**S**	**G**	**A**	**E**	**S**	

N-terminus of second repeat replaced by N-terminus of first repeat

Original sequence

5'	GGC	GGT	TAT	CCT	GGC	GGT	TAC	CCA	GGC	GGA	**3'**
	G	**G**	**Y**	**P**	**G**	**G**	**Y**	**P**	**G**	**G**	

NT1 mutant

5'	GG**T**	GGT	TAT	CC**G**	GG**T**	GGC	TA**T**	CC**T**	GG**T**	GG**T**	**3'**
	G	**G**	**Y**	**P**	**G**	**G**	**Y**	**P**	**G**	**G**	

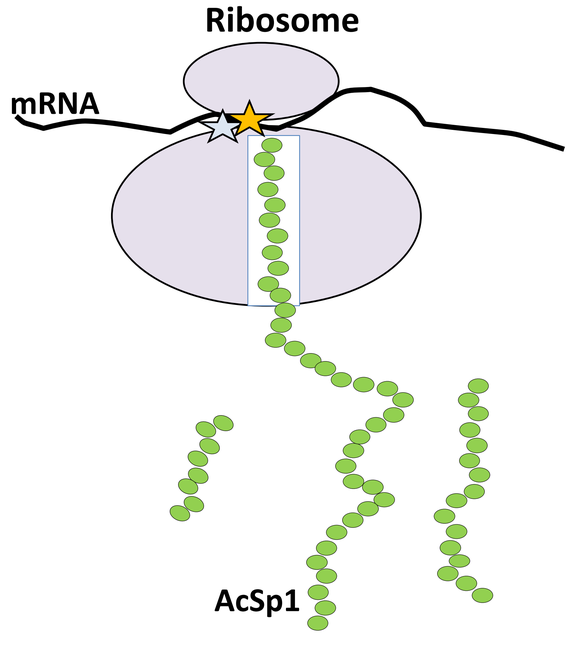

Figure 2: Model represents the site where the mRNA stalls during translation, resulting in an early termination of protein synthesis. Stars indicate sites modified by mutagenesis.

Results

We have generated two variants of the AcSp1 synthetic gene: LoFi and NT1. Both variants have a much higher expression than the original synthetic gene (Fig. 3). The changes have not reduced the number of additional incomplete polypeptides.

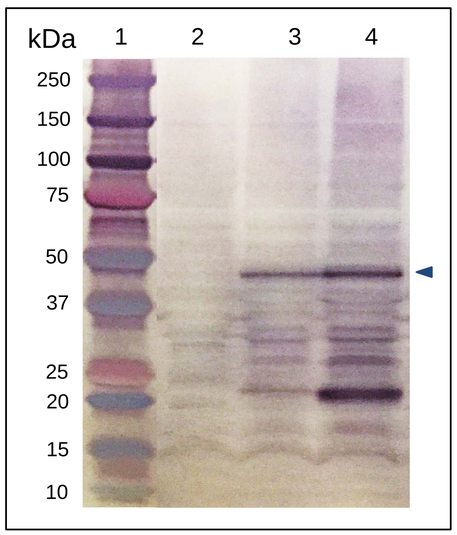

Figure 3. Western Blot of Aciniform 2 repeats. Lanes 1: MW markers, 2: AcSp1 synthetic gene 2 repeats, 3: AcSp1 LoFi mutant, 4: AcSp1 NT1 mutant. Samples were standardized by optical density (OD_{600}). Arrow indicates full length AcSp1.

Conclusions

- The strategy to convert high-usage codons into low-usage codons by *E. coli* increased the efficiency of AcSp1 expression in variant LoFi.
- Replacing the sequence of the N-terminus of the second repeat by the one of the first repeat resulted in a much higher expression of AcSp1 (NT1).
- Neither variant shows a reduction of the number of partial-length polypeptides of AcSp1.

Future work

Future work remains to be done in order to increase the expression of a full length protein.
- Study additional domains of the gene by mutagenesis.
- Express AcSp1 proteins with higher number of repeats.