Spider Silk Proteins

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RESEARCH OVERVIEW AND OBJECTIVES

Spider silks have the potential to provide new bio-based materials for numerous applications ranging from protective clothing to medical products to composite materials. A number of spider silk genes have been cloned and sequenced revealing the basis for understanding the key elements of spider silk proteins relative to their materials performance. In particular, specific amino acid motifs have been identified which have been conserved for over 125 million years in all spiders using their silk to physically trap their prey. ***The key element in taking the next step toward generating bio-based materials from spider silks will be to move from the current descriptive data to predictive knowledge.*** No one has systematically varied the sequence motifs in the spider silk proteins and determined how this influences the mechanical properties of the resulting fibers. These experiments will provide the predictive knowledge enabling the design of materials with very specific elastic and strength properties for each application.

Specific Aims

This proposal is designed to test three basic hypotheses and engineering concepts.
1) Amino acid sequence motifs from spider silk assembled into a protein can be used to create self-assembling elastic materials.
2) The material's elasticity will be proportional to the number of elastic motifs in the protein.
3) Varying the sequence of the elastic regions will vary the elastic (Young's) modulus.

SIGNIFICANCE

This project is highly significant for several reasons. First it will provide a basic understanding of elasticity and tensile strength in spider silk proteins. Specifically, it will reveal what controls the amount of elasticity and elastic modulus and if these two factors can be varied in a predictable way.

Second this project will advance our ability to use spider silk as a biomaterial. If our hypotheses are correct we will learn how to control the elasticity and other materials properties by controlling the protein sequence. Since these protein nano-materials will self-assemble into fibers or films they can be used in any application where the unique materials properties of these silks will be of advantage. Possible applications of spider silk are widespread both for military and civilian purposes. Uses range from artificial ligaments to protective clothing to to composite materials. In addition, there is substantial interest in these materials for coatings where they would assemble into a functional surface layer, thus the interest in films.

Third, students (undergraduate, graduate and postdoctoral) will be trained in a much broader way than is usually the case for the biological sciences. They will be involved in molecular biology, biophysical analyses, and materials science.

Finally, the broader impacts for this research, in addition, to the development of novel biomaterials are the general education efforts being done by our laboratories. These include visits to and from groups ranging from kindergarten students to organizations of retired persons, at least ten per year. A number of television programs, both news and feature, have been made at the Lewis and Nexia laboratories, including PBS, National Geographic and Discovery. Print media have also utilized a number of people in these laboratories for their stories, both as quotes and as background information.

Brief Work plan

1) Genes will be constructed with variations in the number and sequence of elastic motifs, based on naturally occurring spider silk sequences and the encoded proteins expressed in a suitable host system.

2) Each of these different proteins will be used to make both fibers and thin films.

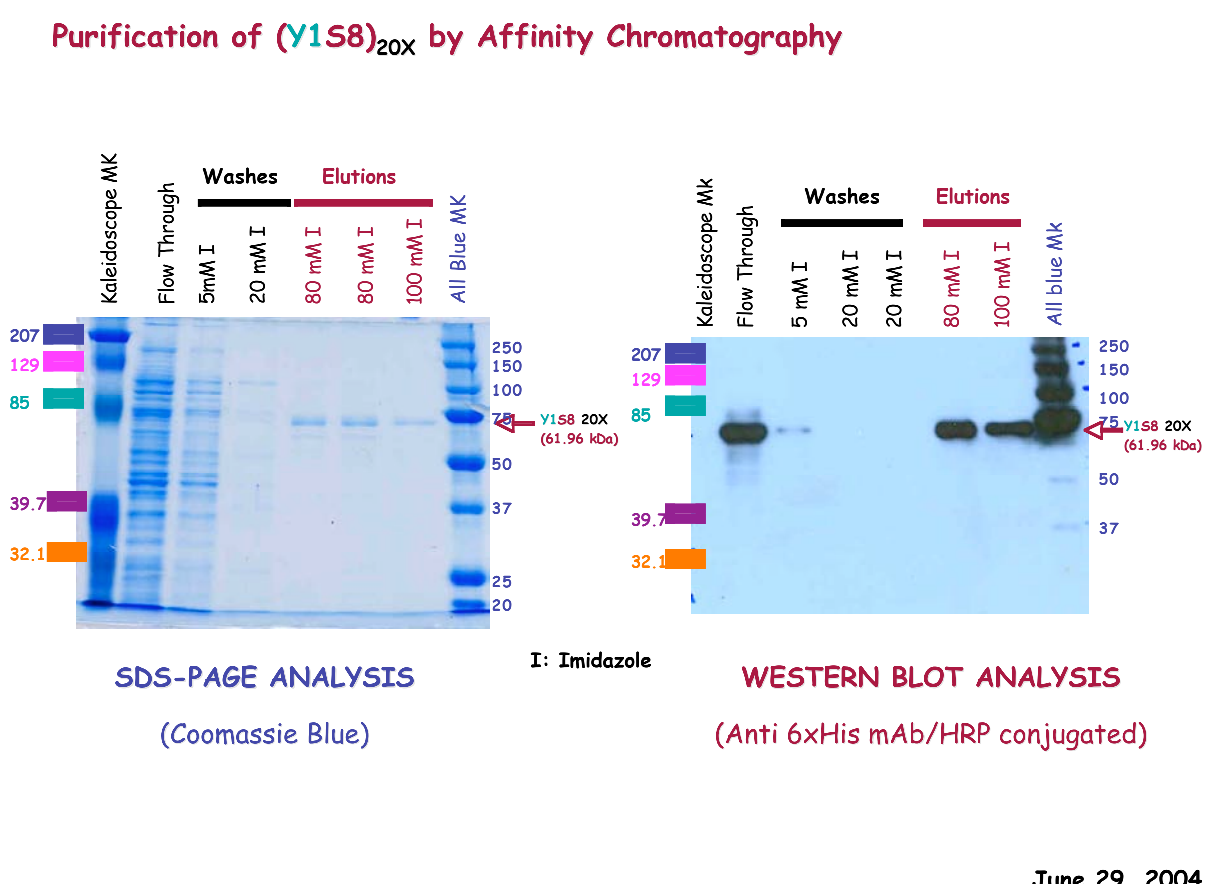
3) The films and fibers will be tested for their mechanical properties. The properties to be measured will be tensile strength, elasticity (recoverable elongation), total elongation, energy to break and elastic modulus.

4) The structure of the protein in solution, films and fibers will be determined by Fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD) and by solid state NMR.

5) The elasticity and elastic modulus data will be correlated with the number and sequence of each motif to produce a prediction algorithm for elastic and other materials properties.

Accomplishments to date

We have constructed 18 different genes with varying amounts of tensile strength and elasticity elements. They have been inserted into bacteria and protein has been produced and purified. We are also inserting them into yeast to try to get the protein secreted into the media for easier purification. The proteins from bacteria have been purified and are undergoing chemical and biophysical analyses currently. The figure below shows an example of one construct and its protein.



We are now gearing up to spin fibers in conjunction with Dr. Karatzas at Nexia Biotechnologies and then will be conducting mechanical testing. We have now obtained a custom built 5 gram load cell that allows us to measure elongation and strength on a single fiber accurately. Using computer modeling we have tested single molecules and assemblies of molecules and compared that to the natural fibers. The values found are within a factor of two of each other suggesting that the proteins are the key factor in the mechanical properties. The data are shown below in the figure.

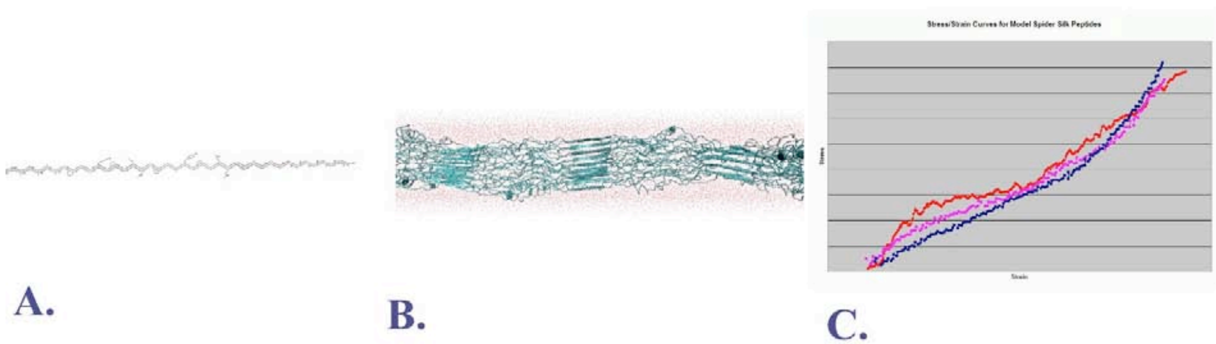


Figure 4. A. is the model of a single spider silk protein molecule. B. is an assembly of molecules and C. shows the stress-strain curve for the single molecule (blue) and assembly (pink) from computer simulation and fiber (orange) from mechanical testing.

References

[1] For further information contact Randy Lewis at silk@uwyo.edu or the website at http://coblewis.uwyo.edu/RVLLab/DesktopDefault.aspx?tabindex=0&tabid=1