Novel Methods to Produce Large Recombinant Spider Silk Proteins via Polymerization

04/30/2018

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Full Abstract

Spider silk has long been a subject of scientific research due to its remarkable mechanical properties. Until recently, there has been no way to effectively obtain spider silk except by harvesting it from individual spiders. With advances in technology, the genes that code for the individual spider silk proteins have been isolated and genetically engineered into other hosts to produce recombinant spider silk proteins (rSSp) of varying molecular weights, ranging from 10 kDa to 250 kDa. Larger molecular weight rSSp have correspondingly greater mechanical properties that will be endowed to any ensuing products. When utilizing current production methods, larger molecular weight rSSp cannot be produced in commercially viable quantities while simultaneously maintaining low levels of impurities. Small molecular weight rSSp (10 - 80 kDa) are easier to produce in genetically engineered systems while maintaining favorable yields and purities. To alleviate current production constraints, the expression of small molecular weight rSSp can be achieved by utilizing current production methods that result in large quantities of proteins with a high degree of purity. After the small molecular weight rSSp are expressed and purified, they can be polymerized to form larger molecular weight rSSp.

There are two unique systems that can cause this polymerization via the autocatalytic nature of the Spy System peptides and inteins. These two systems require no external cofactors or enzymes and occur spontaneously once initiated. The expression and purification of rSSp from both of these systems have been characterized. When utilizing the intein system, yields of approximately 5 g/L have been achieved. The rSSp from this system has been made into biomaterials, such as films, gels, and aerogels. The mechanical properties of these biomaterials are comparable to biomaterials from other heterologous expression systems and show statistically significant differences in material properties when compared to biomaterials from other heterologous systems. Utilizing the intein system commercially viable yields of rSSp have been achieved, thus lowering the cost of this remarkable biomaterial.