Expression, Purification, And Characterization Of Elastin-Like Polypeptides Containing Chondroitin Sulphate Binding Domains

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Introduction:

The development of small-diameter artificial blood vessels that mimic the properties of natural blood vessels has proven to be a clinical challenge. While autologous vessels are the current standard, they can be difficult to obtain and require invasive surgeries. Synthetic materials have been successful in large diameter applications, but they have been unsuccessful in small-caliber environments due to a number of factors including thrombus formation, intimal hyperplasia, and infection. Intimal hyperplasia, of particular interest in this study, involves the build up of smooth muscle cells (SMCs) in the intimal layer of the artery due to abnormal migration and proliferation. Therefore, the development of a new polymer that has the potential to function as an intimal/medial component of a small-diameter blood vessel is of great interest.

The overall objective of this project is to develop and characterize a new elastin-like polypeptide-chondroitin sulphate binding domain (ELP-CSBD) block copolymer that holds the potential to gel in the presence of chondroitin sulphate (CS). In evaluating this novel polypeptide, the specific aims of this study are: 1. To express (in an *E. coli* bioreactor system) and purify novel elastin-like polypeptides modified to incorporate chondroitin sulphate binding domains (CSBD1 and CSBD2); 2. To characterize these novel polypeptides using mass spectrometry and amino acid analysis to assess purity, and coacervation to evaluate self-assembly; and 3. To investigate the influence of the glycosaminoglycan (GAG) chondroitin sulphate on the self-assembly of the polypeptides.

Materials and Methods:

The expression of the ELP1-CSBDs was accomplished using *E. coli* BL21 cells in a 10L bioreactor system. The polypeptides were purified using dialysis and ion exchange chromatography, and expression and purity were characterized using mass spectrometry and amino acid analysis.

Coacervation was used to determine the self-assembly characteristics of ELP1-CSBD1 by using a Thermo Evolution 300 spectrophotometer equipped with a temperature controller (Figure 1). ELP1-CSBD1 was dissolved in coacervation buffer (50 mM Tris, 1.5 M NaCl, 1 mM $CaCl_2$) at concentrations ranging from 2 - 100μM. Coacervation was also used to investigate the influence of CS on the propensity of ELP1-CSBD1 to self-aggregate. CS was added to the coacervation solutions maintaining a 1:1 molar ratio with the ELP1-CSBD1.

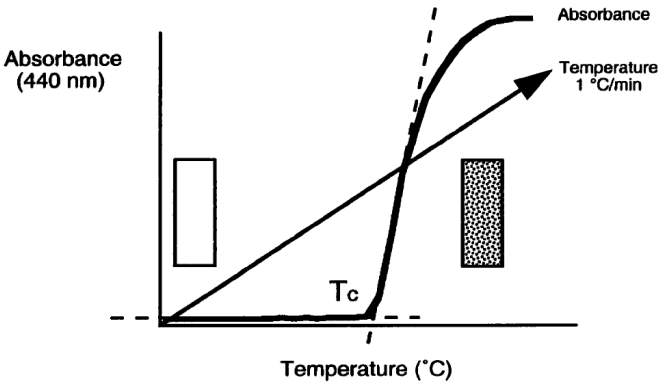

Figure 1: The process of coacervation, studied by monitoring turbidity by light scattering at 440nm over a range of temperatures. The temperature is increased at a rate of 1°C per minute. The coacervation temperature, T_c, is defined as the onset of turbidity appearing as an increase in absorbance. Adapted from Bellingham (2001).

Results:

Both ELP1-CSBD1 and ELP1-CSBD2 were successfully expressed using the methods described, with ELP1-CSBD1 being produced at up to 95% purity. ELP1-CSBD1 was able to undergo coacervation *in vitro* at concentrations between 6.25µM and 100µM, suggesting that ELP1-CSBD1 is able to self-assemble in a manner similar to native elastin (Figure 2). In the presence of CS, the temperature of coacervation of ELP1-CSBD1 is increased, the rate and extent of coacervation is decreased, and aggregates remain in solution even at higher temperatures (Figure 3).

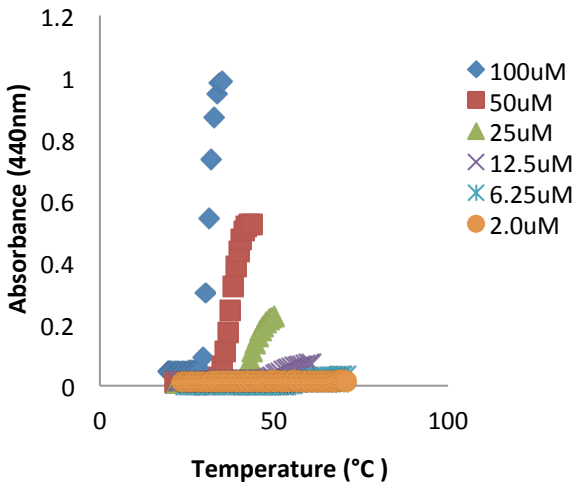

Figure 2: Effect of polypeptide concentration on coacervation temperature of ELP1-CSBD1. Average coacervation curves are shown for each concentration.

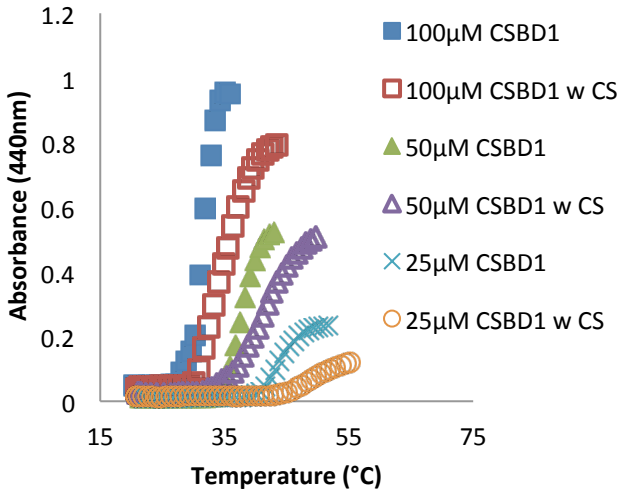

Figure 31: Effect of CS on coacervation temperature of ELP1-CSBD1.Average coacervation curves are shown.

Discussion:

To our knowledge, this is the first reported study to successfully incorporate chondroitin sulphate binding domains into an elastin-like polypeptide. It proves that the crosslinking regions of ELPs can be manipulated to include a binding region that has the ability to bind to chondroitin sulphate while maintaining similar properties to native elastin. These results also indicate that there is little effect on the propensity for self-assembly when replacing the crosslinking domains with ELP1-CSBDs. While the nature of the interaction between the ELP1-CSBDs and CS has yet to be confirmed, based on previous research it can be assumed that binding is likely occurring; however, further investigations into the mechanisms driving this behaviour are required. These ELP-CSBD polypeptides have significant potential in many clinical areas where other synthetic polymers have failed, including in small-diameter applications as a component of a composite TEBV. It is anticipated that when combined with CS, ELP1-CSBD1 will gel, forming a basis for an intimal/medial layer of a TEBV that will modulate SMC response and increase graft integrity.

References:

Bellingham, C. M. *Self-assembly of Recombinant Human Elastin Polypeptides with Potential for Use in Biomaterials Applications*. Thesis. University of Toronto, 2001.