Abstract:

Recombinant purification of Asmp15b; a protein responsible for the functional properties of a tough biological glue

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The slug Arion subfuscus secretes a defensive glue that is very strong despite being composed of 97% water. The overarching goal for analyzing this glue is to guide the development of a biomimetic hydrogel to replace staples and stitches in surgical practice. Previous research has identified certain proteins that are unique to the glue and are integral for adhesion. This particular research aims to purify one of these proteins, Asmp15b (Arion subfuscus mucus protein, size 15kD) by using a recombinant method. Ligation independent cloning was used to insert cDNA coding for this protein into a plasmid vector. The plasmid also contained a His-tag to facilitate purification, and the coding sequence for Protein G. Appending a cleavable Protein G domain markedly improved the tolerance of the bacteria for the recombinant protein. After amplification and purification of the recombinant plasmids, the plasmids were used to transform an expression vector designed to facilitate disulfide bond formation (SHuffleT cells, NEB). After triggering overexpression, the protein could be isolated by binding to nickel resins. The purified proteins will be further analyzed to identify specific domains that aid adhesion, identify cross-linking sites, and to determine its binding partners.