Investigations into a novel hypothetical virulence factor from Group A Streptococcus

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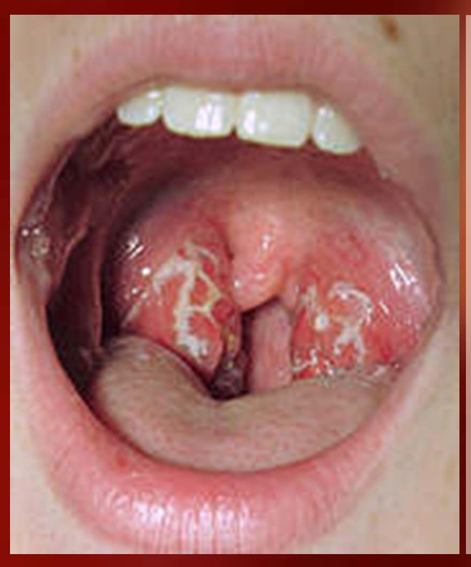
Introduction



Group A Streptococcus (GAS) or *Streptococcus pyogenes* is a major human pathogen that causes a range of diseases, from minor skin and throat infections such as impetigo and pharyngitis, to severe invasive infections such as streptococcal toxic shock syndrome and necrotising fasciitis. This is facilitated by a large arsenal of virulence factors that contribute to colonisation of host tissue, invasive spreading and immune evasion. Since the completion of the first GAS genome sequencing project in 2001, the number of novel virulence factors is constantly growing. We have identified an open reading frame encoding for a hypothetical 221 amino acid protein that is predicted to be secreted by the bacteria. The Spy0136 protein is highly conserved in GAS, but not found in related species suggesting a more specialised function. The overall goal of this study is to characterise this novel potential virulence factor and demonstrate how it might interact with human immune factors to evade immune responses. This project will also analyse the suitability of the putative virulence factor as a target for therapeutic intervention or vaccine development.

Methods and Results

Expression and purification of MBP-Spy0136:

Spy0136 protein fused to Mannose Binding Protein (MBP) with a N-terminal (His)6-tag from pROEX-HTb vector was produced in *E.coli*, and purified by immobilised metal-chelate affinity chromatography (IMAC) using Ni^{2+}-NTA

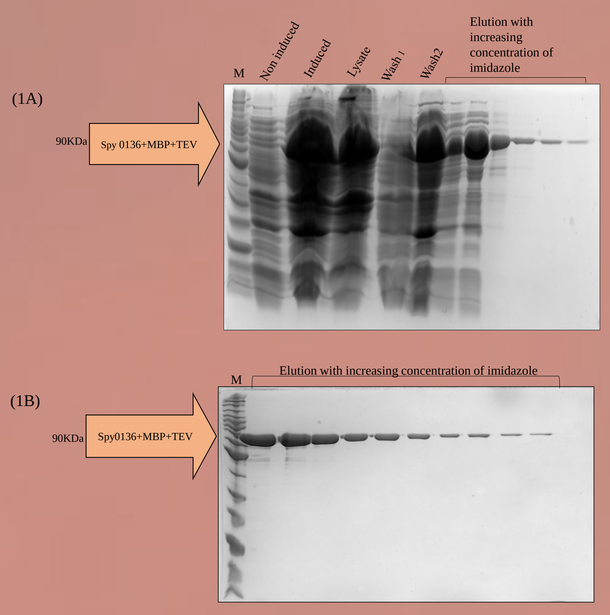


Figure 1 A & B: SDS-PAGE gel images showing expression and purification of Spy0136 by IMAC

Removing MBP by rTEV protease and further purification of Spy0136 with size exclusion chromatography:

Purified fusion protein was cut by rTEV protease. Spy0136 protein was separated on a Ni^{2+}-NTA column and by size exclusion chromatography

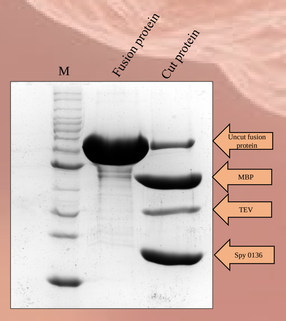


Figure 2: MBP-Spy0136 fusion protein after purification was treated with rTEV protease

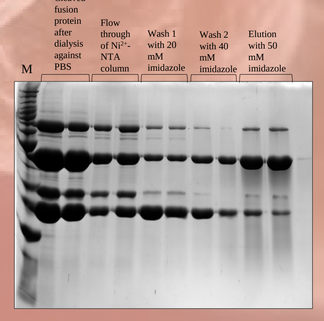
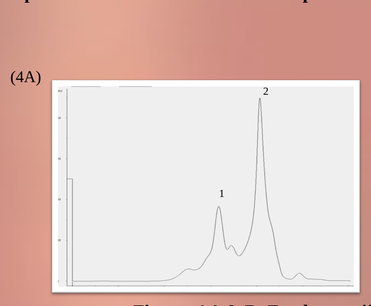
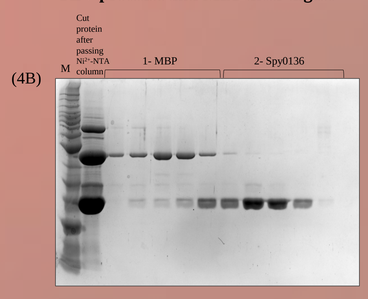


Figure 3: Using IMAC for removing TEV protease and MBP after digest





Figures 4 A & B: Further purification of Spy0136 by size exclusion chromatography

Pull-down Assay

The purified GAS protein was investigated for specific binding to host factors using pull-down experiment with human plasma. Several proteins were found to specifically bind to Spy0136 and identified by mass spectrometry

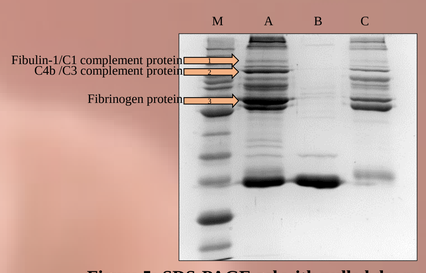


Figure 5: SDS-PAGE gel with pulled down proteins (A) rSpy0136-CNBr-Sepharose-plasma (B) rSpy0136-CNBr-Sepharose (C) inactivated CNBr-Sepharose-plasma

CH50 Assay

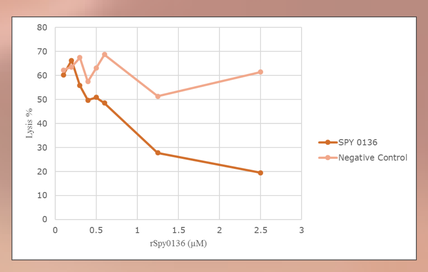


Figure 6: Spy0136 inhibits the lysis of sensitised sheep red blood cells indicating interference with complement pathway

Conclusion and Future Directions

Spy0136 from GAS can be produced as a soluble recombinant protein which interacts with human plasma proteins and inhibits complement-mediated lysis.

The purified GAS protein is currently being investigated for specific binding to human complement proteins by ELISA. Spy0136 may be an important immuno-modulatory virulence factor and putative candidate for therapeutic intervention or vaccine development.