Title:

Versatile Substrates and Probes for IgA1 Protease Activity

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Abstract:

Bacterial meningitis is an often-fatal disease caused by diverse Gram-negative and Gram-positive bacteria, including Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae. Though several virulence factors associated with the bacterial pathogenesis have been identified, the specific roles of many of these proteins are not yet well understood. One such protein, IgA1 protease (IgAP) is over-produced by meningitis-causing bacteria. IgAP exclusively cleaves the hinge region of the major immunoglobulin on mucosal surfaces, IgA1. It has been hypothesized that the proteolysis of IgA1 helps in immune suppression and immune evasion by the bacteria. Recent literature shows that IgAP also cleaves host proteins involved in extrinsic apoptosis and lysosomal trafficking, providing additional evidence to support IgAP as a virulence factor. The lack of IgAP inhibitors has hindered research on IgAPs and their roles in bacterial virulence. In our current work, we identified peptide sequences that are self-cleaved by membrane-bound IgAPs during secretion from N. meningitidis and H. influenzae IgAPs. These peptides were synthesized, and tested as substrates for IgAPs from diverse human pathogens. A single peptide, NG2, was found to be a surprisingly common substrate for all IgAPs tested. A Förster resonance energy transfer (FRET) probe, F2.2, was synthesized based on this peptide. High-throughput assays using probe F2.2 were also developed in anticipation of the first screening effort for the discovery of small-molecule inhibitors of IgAPs. By developing novel probes and inhibitors, we will be able to explore the roles of IgAPs in adherence, colonization and infection of epithelial cells by these pathogens, and determine the value of inhibiting IgAP as part of a combination antibacterial strategy. This probe was also used to demonstrate sensitive IgAP detection in buffer and in human cerebrospinal fluid, which may be useful in developing a diagnostic assay for rapid diagnosis of bacterial meningitis.