BMB Graduate Group PhD Defense

Friday, March 27 at 1:00 p.m.

Chalmers Hall Room 36 (Johnson Cancer Research Center conference room)

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Staphylococcal Peroxidase Inhibitor: Structure/Function Analysis and Studies on Host-Species Specificity

Human neutrophils are the primary responders in the innate immune defense against bacterial pathogens. They employ a diverse arsenal of defenses including proteases, lysozymes, antimicrobial peptides, as well as an oxidative killing system to combat infections. The oxidative killing system is centered on the heme-containing enzyme, myeloperoxidase (MPO), the most abundant protein within the neutrophil. MPO consumes H_2O_2 as an oxidant to convert halide and pseudohalide anions into cytotoxic hypohalous acids. Following phagocytosis, *Staphylococcus aureus* secretes a potent MPO inhibitor, SPIN, as part of its immune evasion repertoire. The matured SPIN polypeptide contains two functional domains: a 60-residue C-terminal helical bundle domain responsible for MPO binding and a 13-residue N-terminal domain for MPO inhibition.

While the SPIN helical domain is responsible for its initial binding to MPO, less is known about the contribution of individual residues within this domain. We therefore performed a residue-level structure/function analysis of the *S. aureus* SPIN helical bundle. Using sequence conservation and existing structures of SPIN bound to human MPO, we selected residues L49, E50, H51, E52, Y55, and Y75 for interrogation by site-directed mutagenesis. We found that loss of L49 or E52 reduced SPIN activity by roughly an order of magnitude, but that loss of Y55 or H51 caused greater loss of inhibitory potency. SPR analysis showed that loss of inhibitory potency resulted from a diminished association rate between the inhibitor and MPO. Together, our studies provided new insights into the structure/function relationships of SPIN and identified positions Y55 and H51 as critical determinants of SPIN function.

SPIN binds MPO via its C-terminal helical bundle but requires formation of an N-terminal β-hairpin to inhibit MPO. To investigate this structure-function relationship in more detail, we introduced two cysteine residues into the SPIN N-terminal region (SPIN-CYS) to trap it in its MPO-bound conformation. Although SPIN-CYS adopted an ensemble of constrained lariat-like conformations rather than a defined β-hairpin, it showed enhanced inhibitory potency against human MPO. This persisted even in the presence of deleterious mutations within the C-terminal helical bundle domain. SPR analysis revealed that this gain of function was driven by increased apparent affinity, also primarily through a faster association rate. This work provided new insights into the coupled binding and folding events underlying SPIN activity.

S. aureus SPIN cannot inhibit MPO from many non-human species. Conversely, SPIN orthologs from other staphylococcal species show reduced potency against human MPO. We therefore hypothesized that staphylococci adapted to other hosts produce SPIN variants with binding preferences for MPO from those hosts. To test this, we isolated canine MPO (cMPO) from abscess samples obtained in both winter and summer 2025 and assessed its inhibition by various SPIN orthologs. Consistent across both winter and summer trials, we found that SPIN proteins from known canine pathogens bound and inhibited cMPO more potently than hMPO. These gains in potency were driven by increased association rate constants. Comparison of the 2.1 Å resolution crystal structure of SPIN-delphini bound to cMPO with that of SPIN-delphini bound to hMPO provided a physical basis for interpreting our biochemical observations. Together with our prior SPIN/MPO studies, these findings provide a basis for understanding the structure/function principles underlying host-specific evolution of virulence proteins.