

Abstract of the Thesis

Staphylococcus aureus (*S. aureus*), a human pathogen, produces an arsenal of virulence factors for the survival of the bacterium inside the host. Amongst the repertoire of staphylococcal exotoxins, fourteen staphylococcal superantigen-like proteins (SSLs) are located within a mobile genetic element called pathogenicity Island in *S. aureus* genome. SSLs share similar structural architecture of classical bacterial superantigens; although they are functionally distinct. Previous study shows that SSLs have host immune evasion properties and target different component of host immune system, plasma proteins and receptors. SSL3 has been shown to bind the Toll-like receptor 2 (TLR2) and inhibit TLR2 activation. SSL7 has been reported to bind complement component C5 and inhibit membrane attack complex (MAC) formation and lysis of bacteria.

This study is focused on the crystal structure determination and functional characterization of SSL1. The 2.5 Å crystal structure of SSL1 reveals the presence of N-terminal β -barrel and C-terminal β -grasp domain adopting the classical superantigenic folds. A unique N-terminal dimer is found in the SSL1 structure in which β -barrel domains of each monomer is forming the dimeric interface. Glu70, His77, Thr112, Glu94 and Arg109 have been identified as dimeric interface residues in SSL1. Site directed mutagenesis of SSL1 (Glu70Ala, His77Ala, Glu94Ala and Arg109Ala) has been performed and a SSL1 mutant containing all four mutations has been purified as monomer in the size exclusion chromatography indicating the roles of these residues in dimer formation. A partially conserved glycan binding site has been identified in SSL1 structure in which a glycerol moiety is placed forming hydrogen bonds with the conserved glycan binding residue (Arg208) and His196 of SSL1. This indicates that the partially conserved glycan binding site of SSL1 can accommodate new ligand molecule. It has also been revealed that SSL1 induces the release of tumor necrosis factor α (TNF- α), a proinflammatory cytokine from macrophage cells (Raw 264.7) in a dose dependent manner and a subtle decrease in TNF- α level in macrophage culture filtrate after incubation with SSL1 monomer (mutant form) is observed when compared with the dimeric form (wild-type). Moreover, human mitogen activated protein kinase ERK2 (hERK2) has been identified by pull down assay and peptide mass fingerprinting analysis as interacting partner protein of SSL1, SSL7 and SSL10. Pull down and reverse pull down of SSLs with cloned and purified hERK2 confirms this interaction. Furthermore, it has been shown by nanoLC coupled MALDI MS/MS and peptide mass fingerprinting that a post-translationally modified form of plasma IgA1 with hydroxylation at Pro285 is affinity purified with SSL7 conjugated sepharose. But the role of hydroxyproline post-translational modification in SSL7-IgA1 interaction remains elusive and needs to be characterized in future.