Poster Presentation

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De-N-acetylation and Export of the Biofilm Exopolysaccharide PNAG

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Bacteria embedded in a self-produced matrix of exopolymeric substances, or biofilm, represent a significant medical problem, as the bacteria are tolerant to antibiotics, protected from the environment, and isolated from the innate immune system. A key component required for the development of the biofilm in a wide variety of pathogenic bacteria is the exopolysaccharide poly-β-1,6-N-acetyl-D-glucosamine (PNAG). Four proteins, PgaA/B/C/D, are required for the polymerization, modification, and export of PNAG in Escherichia coli. PgaB is a two-domain outer membrane lipoprotein essential for the partial de-N-acetylation of PNAG (dPNAG); a process required for polymer export and subsequent biofilm formation. Here we report 1.9 Å and 1.8 Å crystal structures of PgaB and its isolated C-terminal domain in complex with a PNAG hexamer, respectively. Characterization of PgaB de-N-acetylase activity using PNAG oligomers reveals that the enzyme has low catalytic efficiency, and displays length- and metal-dependent de-N-acetylation activity specific for PNAG with preference for Fe^{2+}, Ni^{2+}, and Co^{2+}. Furthermore, tryptophan fluorescence quenching assays show the C-terminal domain binds PNAG oligomers with ~1-4 mM affinity. These data in combination with molecular dynamics simulations of PgaB with N-acetylglucosamine and glucosamine suggest PNAG de-N-acetylation occurs first, with subsequent binding of dPNAG to the C-terminal domain. We propose this concerted action plays a pivotal role in targeting dPNAG for export through the outer membrane porin PgaA.

Keywords: exopolysaccharide biosynthesis, biofilm, deacetylation