Molecular basis for PNAG-dependent biofilm disruption by PgaB

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Poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG) is a major biofilm component of many pathogenic bacteria such as *Escherichia coli, Bordetella pertussis, Staphylococcus aureus, Staphylococcus epidermidis*, and *Klebsiella pneumoniae.* In Gram-negative bacteria like *E. coli* and *Bordetella* spp. PNAG production, processing and export involves the protein products encoded by the *pgaABCD* operon. PgaB is a two-domain periplasmic protein with a N-terminal domain responsible for de-*N*-acetylation of PNAG oligomers and a C-terminal PNAG binding domain critical for export. Here, we show that the C-terminal domains of both *Bordetella bronchisteptica* PgaB ($PgaB_{Bb}$, formerly known as BpsB) and *E. coli* PgaB ($PgaB_{Ec}$) are functional glycoside hydrolases that cleave de-*N*-acetylated PNAG (dPNAG) purified from *Staphylococcus aureus*. We also show that $PgaB_{Bb}$ disrupts PNAG-dependent biofilms formed by *B. pertussis*, *S. epidermidis*, *S. carnosus* and *E. coli*, and potentiates bacterial killing by gentamicin. To gain insight into the relationship between the PgaB de-*N*-acetylation and glycoside hydrolase functions, we developed an assay using $PgaCD_{Ec}$ for the *in situ* production of PNAG. Mass spectrometry (MS) analysis suggests that de-*N*-acetylation of this polymer is required prior to its hydrolysis by PgaB. The MS analysis of the PgaB-hydrolyzed dPNAG substrate showed a GlcN-GlcNAc-GlcNAc motif at the new reducing end of detected fragments. Our 1.76 Å crystal structure of the C-terminal domain of $PgaB_{Bb}$ reveals a central cavity within an elongated surface groove that appears ideally suited to recognize the GlcN-GlcNAc-GlcNAc motif. The structure, in conjunction with molecular modeling and site directed mutagenesis led to the identification of the dPNAG binding subsites and the probable catalytic acid. Our findings shed light on the role of PgaB within the PNAG production machinery and support a cleavage mechanism that specifically recognizes de-*N*-acetylated PNAG. Furthermore, the biofilm disruption and antibiotic potentiation activity identify PgaB as a possible therapeutic agent for treatment of PNAG-dependent biofilm infections.