Molecular Basis of Secondary Cell Wall Polymer Pyruvylation

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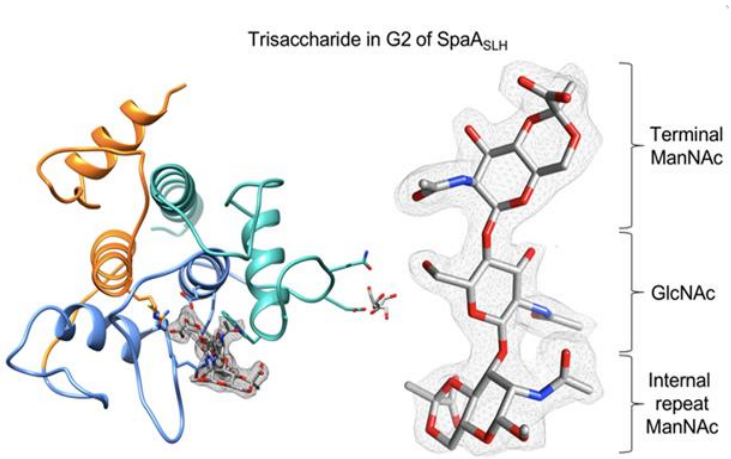
Non-reducing-end, 4,6-pyruvate-ketal-modified β-D-*N*-acetylmannosamine (Pyr-β-D-ManNAc) present on several peptidoglycan-bound secondary cell wall polymers (SCWPs) is a sufficient epitope for anchoring of proteins harboring a terminal S-layer homology (SLH) domain trimer to the Gram-positive cell wall. To investigate β-D-ManNAc pyruvylation, the SCWP of *Paenibacillus alvei* consisting of [4,6-Pyr-β-D-ManNAc-1,4-β-D-GlcNAc-1] repeats serves as a model. We propose that β-D-ManNAc pyruvylation is catalyzed by the CsaB enzyme, occurs at the stage of the lipid-linked SCWP repeat and might influence the ligation of SCWP to peptidoglycan.



Only the terminal monosaccharide of a synthetic SCWP tri-saccharide residue makes significant contact with groove 2 (G2) on the -layer protein SpaA$_{SLH}$. © Stephen V. Evans, University of Victoria, Canada.

In the proposed research, chemical, biophysical, microbiological, genetic, and crystallographic approaches will be synergistically employed in a series of *in vitro* experiments designed to elucidate the molecular basis of β-D-ManNAc pyruvylation and its status within SCWP biosynthesis. By using a bottom-up approach involving defined synthetic SCWP precursor fragments in concert with recombinant CsaB, the acceptor substrate for pyruvyl transfer will be defined allowing to infer the spatio-temporal organization of pyruvylation within SCWP biosynthesis. Insight into CsaB catalysis and kinetics will be obtained by determination of the activity of wild-type CsaB and rational mutants on an acceptor mimetic, complemented by X-ray crystallography of CsaB unliganded and liganded to the acceptor.

This study marks an important step towards understanding how the Pyr-β-D-ManNAc epitope is elaborated and at the same time informs about its role in SCWP-peptidoglycan tethering in Gram-positive bacteria. This may aid drug discovery and development programs focused on this important cell-wall biosynthetic pathway.