

Structural and Functional Analysis of the Bacillithiol Biosynthesis Enzyme BshA Supports the S_{Ni}-like Retaining Mechanism

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Bacillithiol (BSH) is the major low molecular weight thiol in many gram-positive organisms. This compound is involved in the detoxification of xenobiotic compounds, the maintenance of redox homeostasis, and is the preferred cosubstrate for the fosfomycin resistance enzyme FosB. Organisms with knocked-out BSH biosynthesis genes have demonstrated an increased sensitivity to fosfomycin, making BSH production a logical target for inhibition. In the first committed step of the BSH biosynthesis pathway, the enzyme BshA catalyzes the formation of *N*-acetylglucosaminylmalate (GlcNAc-Mal) via an activated sugar donor and l-malate acceptor. BshA is a GT-B retaining glycosyltransferase and likely utilizes an S_{Ni}-like retaining mechanism. To further characterize BshA and other GT-B retaining glycosyltransferases, we embarked on a structural and functional analysis of BshA. Our lab has determined several X-ray crystallographic structures of wild-type BshA from *Bacillus subtilis* with products bound. In addition, we have determined an initial structure of the S17A mutant of BshA from *Bacillus subtilis* that contains the substrate UDP-*N*-acetylglucosamine bound within the active site. These structures provide compelling evidence for the proposed S_{Ni}-like mechanism. We also report progress on the structural and functional analysis of the BshA enzyme from *Staphylococcus saprophyticus*. Together, these analyses further our understanding of BshA and other GT-B retaining glycosyltransferases, which may be used in the future to model BshA inhibitors and increase the effectiveness of fosfomycin antibiotics.