Crystallographic and fluorescence-based ligand binding analysis of BshC: the putative cysteine ligase in bacillithiol biosynthesis

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Abstract

Bacillithiol is a compound found in Gram-positive bacteria and is responsible for redox homeostasis, detoxification of reactive oxygen species, and deactivation of electrophilic agents, such as the antibiotic fosfomycin. BshC is a cysteine ligase in the putative three-enzyme bacillithiol biosynthesis pathway. Interestingly, although the gene encoding BshC is necessary for bacillithiol production *in vivo*, cysteine ligase activity has not been demonstrated *in vitro*. Our structural investigations have revealed that BshC contains a putative active site within its core Rossmann fold as well as a second ligand binding pocket that may be involved in regulatory or catalytic functions. Additional X-ray crystallographic structures from our lab have shown that *Bacillus subtilis* BshC binds either AMP or ADP in the second binding pocket, suggesting that this pocket can accommodate various ligands. Forms of BshC in which this binding pocket has been disrupted via site-directed mutagenesis demonstrate an inability to bind these ligands. To corroborate our structural findings, we conducted intrinsic tryptophan fluorescence binding assays. The fluorescence assays indicate that wild-type BshC binds ATP preferentially over GTP or NADH, and that ATP binding is impaired in several site-directed mutant BshC enzymes. The second ligand binding pocket may simply be a vestige, but given the absence of catalytic function *in vitro*, it may play a pivotal role in enzyme function. Gaining a greater understanding of the structure of BshC, the ligands that bind to BshC, and the role of the second binding pocket will ultimately help in determining the factors necessary for BshC function and lead to the development of inhibitors to combat fosfomycin resistance among pathogenic Gram-positive organisms.