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Grant Title: Unravelling the early steps in the biosynthesis of the mycobacterial MGLP

Abstract

Mycobacteria synthesize intracellular methylglucose lipopolysaccharides (MGLPs) with a reducing end glucosylglycerate (GG), the likely biosynthetic primer (1). MGLPs have been identified in the 1960s' and proposed to regulate fatty acids synthesis, but the biosynthetic pathway remained elusive for decades (2). Although *M. tuberculosis* genome has been sequenced years ago, many genes remain to be associated to a genuine function, which protracts the path toward new therapies for emerging drug-resistant strains. We have identified genes for the initiation steps of the MGLP pathway (GG synthesis), and characterized the corresponding enzymes (3). Although genes for further glycosylation and methylation of MGLP have been deduced from genomic context and knock-out studies (2), little is known about the regulation of their expression. Since GG levels in *M. smegmatis* cytoplasm were recently found to negatively correlate with nitrogen availability (4), we examined the intracellular GG pools in *M. hassiacum* growing in media with variable content in assimilable nitrogen and identified an enzyme for the specific hydrolysis of GG. To circumvent protein stability issues we also sequenced the genome of this thermophilic organism, amplified and expressed the corresponding gene in *E. coli*, and characterized the recombinant stable enzyme. We found that the endogenous expression of this gene in *M. hassiacum*, as examined by qRT-PCR, was regulated by nitrogen availability. In addition to this novel GG hydrolase (GgH), the present study was crucial for the identification of an additional GG processing activity. Our initial proposal linking GpgS and GpgP to the early steps of MGLP biosynthesis has now been reconfigured to accommodate GgH and a novel enzyme. Although further studies are required to elucidate the function of GG in the mycobacterial response to nitrogen stress, we anticipate an important role for GgH during MGLP assembly and survival of mycobacteria coping with nitrogen fluctuations.

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