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Purification and characterization of the acyltransferase involved in biosynthesis of the major mycobacterial cell envelope glycolipid – Monoacylated phosphatidylinositol dimannoside

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ABSTRACT

Phosphatidylinositol mannosides are essential structural components of the mycobacterial cell envelope. They are implicated in host-pathogen interactions during infection and serve as a basis for biosynthesis of other unique molecules with immunomodulatory properties – mycobacterial lipopolysaccharides lipoarabinomannan and lipomannan. Acyltransferase Rv2611 is involved in one of the initial steps in the assembly of these molecules in *Mycobacterium tuberculosis* – the attachment of an acyl group to position-6 of the 2-linked mannosyl residue of the phosphatidylinositol mannoside anchor. Although the function of this enzyme was annotated 10 years ago, it has never been completely biochemically characterized due to lack of the pure protein. We have successfully overexpressed and purified *MSMEG_2934*, the ortholog of *Rv2611c* from the non-pathogenic model organism *Mycobacterium smegmatis* mc^2155 using mycobacterial pJAM2 expression system, which allowed confirmation of its *in vitro* acyltransferase activity, and establishment of its substrate specificity.

Introduction

The cell surface of *Mycobacterium tuberculosis* is responsible for initial interaction of the bacillus with the host immune system and it therefore plays a crucial role in the pathogenesis of tuberculosis. From a large number of mycobacterial immunomodulators the mannosylated glycoconjugates of the mycobacterial cell envelope – lipoarabinomannan (LAM)[1], lipomannan (LM) and phosphatidylinositol mannosides (PIMs), represent the most prominent molecules proposed to significantly affect the course of infection [1–3].

Monoacylated phosphatidylinositol dimannoside (Ac_1PIM_2) is the most abundant mycobacterial cell envelope lipid from the family of PIMs [4] – phospholipids derived from 1,2-diacyl-*sn*-glycero-3-phospho-1-D-*myo*-inositol (PI), which is decorated with one to six mannosyl residues and one or two additional acyl groups. This molecule represents both an end product and an intermediate in the metabolic pathway leading to LAM and LM [3]. Biosynthesis of Ac_1PIM_2 in *M. tuberculosis* involves an action of three essential enzymes [5,6], two mannosyltransferases – PimA (Rv2610c) [7] and PimB (previously known as PimB′) (Rv2188c) [8], and acyltransferase Rv2611c [9] (Fig. 1). PimA catalyzes the transfer of the mannosyl residue from GDP-mannose to position-2 of the *myo*-inositol ring of PI producing phosphatidylinositol monomannoside (PIM_1). A second mannose is added to position-6 of the *myo*-inositol ring of PIM_1 by PimB enzyme, giving rise to phosphatidylinositol dimannoside (PIM_2). *Mycobacterium smegmatis* mc^2155 counterparts of both these mannosyltransferases have been successfully produced and purified from *Escherichia coli* hosts. This allowed thorough structural and mechanistic characterization of PimA protein [10–13] and in-depth investigation of PimB enzyme activity [8]. In contrast, acyltransferase Rv2611c has been only partially characterized. The enzyme was shown to attach palmitoyl group from its CoA carrier to position-6 of the 2-linked mannosyl residue in both PIM_1 and PIM_2 in the cell free assays performed with the crude mycobacterial membrane fractions [9], but the latter substrate was later proposed to be the preferred one [8]. Closer investigation of this acyltransferase has been precluded due to lack of the pure and active enzyme. In our previous work we attempted to produce Rv2611c using several expression systems in *E. coli* hosts (unpublished results), as well as from *M. smegmatis*

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[1] *Abbreviations used:* LAM, lipoarabinomannan; LM, lipomannan; PIMs, phosphatidylinositol mannosides; Ac_1PIM_2, monoacylated phosphatidylinositol dimannoside; PIM_1, phosphatidylinositol monomannoside; PIM_2, phosphatidylinositol dimannoside.

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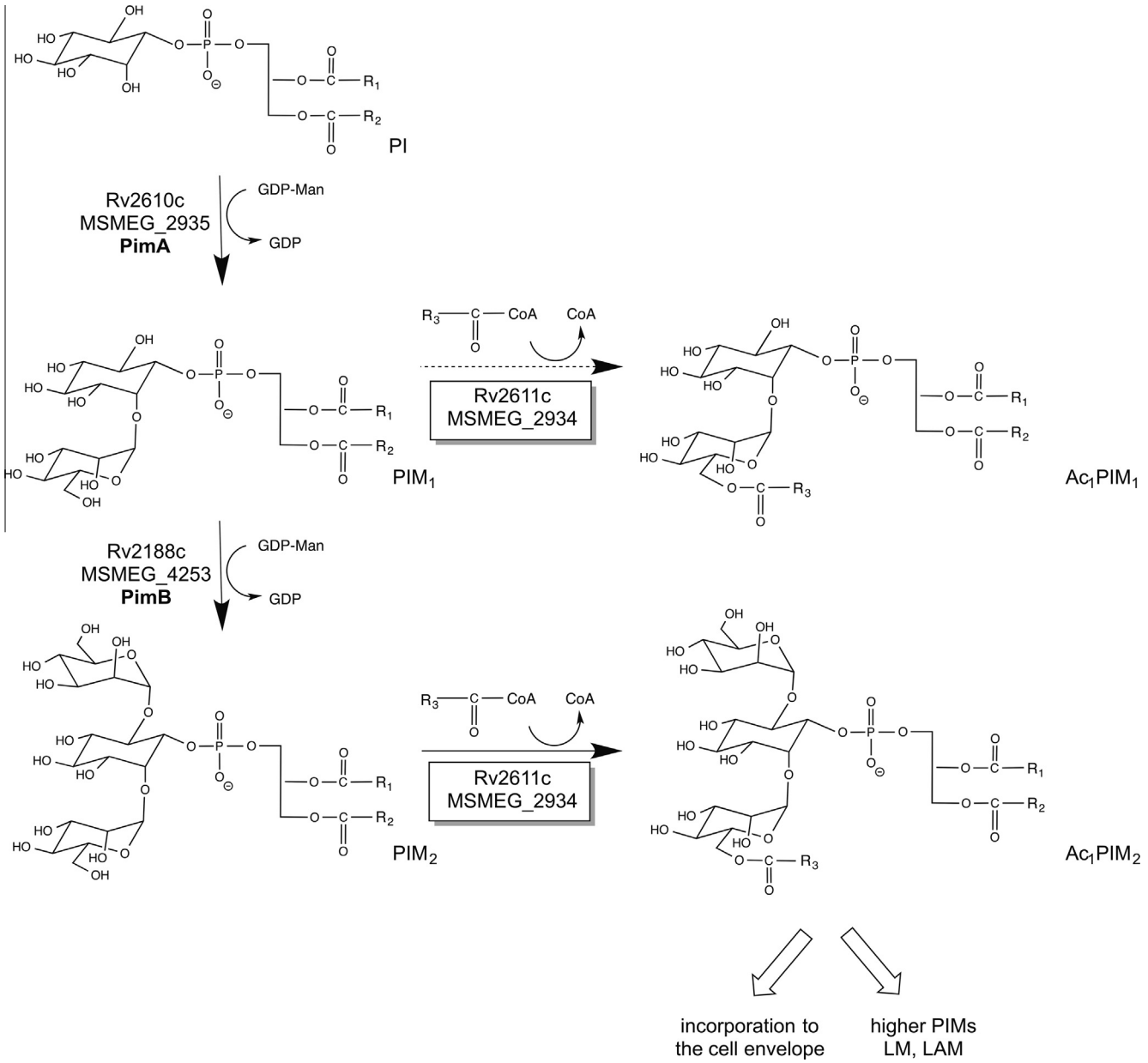

Fig. 1. Metabolic pathway for biosynthesis of Ac_1PIM_2 in mycobacteria. The enzymes catalysing the individual reactions in *M. tuberculosis* H37Rv and *M. smegmatis* mc^2155 are shown in the scheme. R_1, R_3–$C_{15}H_{31}$ (alkyl chain of palmitic acid), R_2–$C_{18}H_{37}$ (alkyl chain of tuberculostearic acid) represent the most abundant substituents at the specified positions [4].

strain constitutively expressing the recombinant His-tagged Rv2611c protein [9]. Since our efforts to obtain sufficient amounts of highly purified enzyme, suitable for further biochemical characterization from these sources failed, we decided to clone, express and purify an ortholog of *Rv2611c* from *M. smegmatis* mc^2155 – MSMEG_2934 protein.

The most successful approach, which we describe in this report, takes advantage of the acetamide-inducible expression vector pJAM2 designed for efficient production of recombinant C-terminally His-tagged proteins in *M. smegmatis*. The plasmid, derived from the *E. coli* – mycobacteria shuttle vector pJEM12 [14], contains 1.5 kb upstream of the acetamidase coding region from *M. smegmatis* NCTC8159, DNA encoding the first six amino acids (Met, Pro, Glu, Val, Val, Phe) of the acetamidase gene, the sites for the restriction enzymes *Bam*HI, *Sca*I, *Xba*I and a sequence for 6-histidine residues [15]. This expression system, described already in 1998 [15], has been used for a number of applications, but as far as we are aware, this is the first report of its exploitation for successful purification of the mycobacterial protein since the original publication. The identity and integrity of the produced protein was confirmed by mass-spectrometry and its activity, as well as specificity was examined with a range of anticipated natural substrates.

Materials and methods

Bacterial strains and culture conditions

E. coli XL1-Blue (Stratagene) used in cloning experiments was routinely grown in Luria–Bertani (LB) medium at 37 °C. *M. smegmatis* mc^2155 was cultivated in the LB medium supplemented by 0.05% Tween 80 at 37 °C. The mycobacterial mutant strain