Structural characterization of a partially arabinosylated lipoarabinomannan variant isolated from a Corynebacterium glutamicum ubiA mutant

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INTRODUCTION

The Corynebacteriaceae represent a distinct and unusual group within Gram-positive bacteria, with the most prominent members being the human pathogens Mycobacterium tuberculosis and Mycobacterium leprae (Bloom & Murray, 1992). Non-pathogenic bacteria also belong to this taxon, such as Corynebacterium glutamicum, which is used in the industrial production of amino acids (Sahm et al., 2000). These bacteria collectively belong to the same suborder, and share a similar genome, cell envelope and corresponding cell wall biosynthetic enzymes (Dover et al., 2004; Stackebrandt et al., 1997).

The characteristic cell envelope of this distinct group of bacteria contains mycolic acids, arabinogalactan (AG) and peptidoglycan, which are covalently linked to each other to form the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex (Besra et al., 1995; Brennan & Nikaido, 1995; Brennan, 2003; Daffe et al., 1990; Dmitriev et al., 2000; Dover et al., 2004; McNel et al., 1990, 1991). In addition, they possess an array of cell-wall-associated glycolipids, such as phosphatidyl-myoinositol (PI), phosphatidyl-myoinositol mannosides (PIMs) and the lipoglycans lipomannan (LM) and lipoarabinomannan (LAM) (Brennan & Ballou, 1967, 1968; Chatterjee et al., 1991, 1993; Guerardel et al., 2002; Hill & Ballou, 1966; Khoo et al., 1995). As a result, arabinose is present in two polysaccharides with markedly different structures. The occurrence of Arabinan polysaccharide side-chains are present in both Mycobacterium tuberculosis and Corynebacterium glutamicum in the heteropolysaccharide arabinogalactan (AG), and in M. tuberculosis in the lipoglycan lipoarabinomannan (LAM). This study shows by quantitative sugar and glycosyl linkage analysis that C. glutamicum possesses a much smaller LAM version, Cg-LAM, characterized by single \( \alpha \)-(1→6)-linked mannan backbone. MALDI-TOF MS showed an average molecular mass of 13 800–15 400 Da for Cg-LAM. The biosynthetic origin of Ara residues found in the extracytoplasmic arabinan domain of AG and LAM is well known to be provided by decaprenyl-monophosphoryl-D-arabinose (DPA). However, the characterization of LAM in a C. glutamicum ::ubiA mutant devoid of prenyltransferase activity and devoid of DPA-dependent arabinan deposition into AG revealed partial formation of LAM, albeit with a slightly altered molecular mass. These data suggest that in addition to DPA utilization as an Ara donor, alternative pathways exist in Corynebacteriaceae for Ara delivery, possibly via an unknown sugar nucleotide.
arabinosyl-containing glycoconjugates in bacteria (Brennan & Nikaido, 1995), plants (Fincher et al., 1983) and protozoan parasites (Dobson et al., 2003; Goswami et al., 2006; Guha-Niyogi et al., 2001; Previato et al., 1982; Xavier Da Silveira et al., 1998) and their absence in mammalian cells is well known. Corynebacterianeae arabinan is an extremely complex homopolymer of D-arabinofuranose (D-Araf) residues based on discrete structural motifs; however, the complete structures and biosynthesis of these polymers are still to be established.

For LAM biosynthesis we initially proposed the following biosynthetic pathway PI→PIM→LM→LAM (Besra et al., 1997) in M. tuberculosis, which is now largely supported by biochemical and genetic evidence (Gurcha et al., 2002; Kordulakova et al., 2002; Kremer et al., 2002; Schaeffer et al., 1999). PimA catalyses the addition of ManP provided by GDP-mannose to the 2-position of the myo-inositol of PI to form PIM1 (Kordulakova et al., 2002), whereas PimB might be responsible for the addition of second ManP to the 6-position to yield Ac1PIM2 (Schaeffer et al., 1999; Tatituri et al., 2007). PimC has been demonstrated to allow further mannosylation to Ac2PIM3 (Kremer et al., 2002) and more recently, PimE has been shown to be involved in the biosynthesis of Ac2PIM5 (Morita et al., 2006). It has been proposed that PIM4 is the direct precursor of LM, characterized by a linear β(1→6)-linked mannan backbone linked with β(1→2) manno-lyranose side chains generated through a mannosyltransferase encoded by Rv2181 (Kaur et al., 2006). LM is then further glycosylated with arabinan to produce LAM, and finally ‘mannose-capped’ to produce ManLAM, a process initiated by the capping enzyme encoded by Rv1635c (Dinadayala et al., 2006). For the addition of arabinose residues into mycobacterial LAM, EmbC is required (Berg et al., 2005; Shi et al., 2006; Zhang et al., 2003), whereas AftA, EmbA and EmbB perform arabinan polymerization in AG (Alderwick et al., 2006b; Belanger et al., 1996; Escuyer et al., 2001). In contrast, C. glutamicum possesses a singular Emb, and an AftA orthologue, which are used in AG biosynthesis (Alderwick et al., 2005, 2006b; Seidel et al., 2007). It is interesting to note that the arabinan domains of AG and LAM utilize several different Araf linkages, which suggests that additional arabinofuranosyltransferases must be required to form AG and LAM, and still remain to be identified in Corynebacterianeae.

It has been shown that the activated Araf sugar donor in Corynebacterianeae is decaprenyl-monophosphoryl-D-arabinose (DPA) (Wolucka et al., 1994). It was also proposed that decaprenyl-monophosphoryl-D-ribose (DPR) could be an additional precursor involved in arabinan synthesis, via a 2’-epimerase (Wolucka & de Hoffmann, 1995). Recently, it was shown that 5-phosphoribofuranose pyrophosphate (pRpp) is converted to decaprenyl-monophosphoryl-5-phosphoribose (DPPR) via a 5-phospho-α-D-ribose-1-diphosphat-decaprenyl-phosphate 5-phosphoribosyltransferase (UbiA; Rv3806c and NCg2781 in M. tuberculosis and C. glutamicum, respectively) (Huang et al., 2005), which is then dephosphorylated to form DPR. DPR is then enzymically oxidized to form a keto-intermediate, DPX, followed by an enzymic reduction to DPA (Mikusova et al., 2005). Additionally, disruption in C. glutamicum of ubiA, encoding the first enzyme involved in the biosynthesis of DPA, resulted in a complete loss of cell wall arabinan (Alderwick et al., 2006a). To date, no sugar nucleotides of Araf have been identified. The key discovery that Araf residues in AG arise from DPA also raised the question whether mycobacteria neither produce nor require Araf sugar nucleotides. Herein, we now clearly show that an ubiA-disrupted mutant of C. glutamicum still produces a modified LAM variant (Cg-LAM), which is arabinosylated even in the absence of the sugar donor DPA (Alderwick et al., 2006a). Taken together, the data suggest that alternative pathways may exist in Corynebacterianeae, independent of DPA utilization, specifically involved in arabinosylation events in Cg-LAM biosynthesis.

**METHODS**

**Strains, construction of plasmids and culture conditions.** C. glutamicum ATCC 13032 (the wild-type strain, referred to for the remainder of the text as C. glutamicum) and Escherichia coli DH5αmcR were grown in Luria–Bertani broth (Difco) at 30 °C and 37 °C, respectively. The C. glutamicum::ubiA mutant was grown on the complex medium brain heart infusion (Difco). Kanamycin and ampicillin were used at a concentration of 50 μg ml⁻¹. The vector used for inactivation of C. glutamicum::ubiA was pCc::ubiA. It contained an internal ubiA fragment of 321 bp amplified with the primer pairs ATC TTC AAC CAG CGC ACG ATC and AAT ATC CAG CGC ACG ATC and AAT ATC GAT CAC TCG CAG ATG, which was ligated into the Smal site of the non-replicative vector pK18mob to yield pCg::ubiA. To enable chromosomal inactivation of ubiA, pCc::ubiA was introduced into C. glutamicum by electroporation (Alderwick et al., 2005).

**Extraction and purification of lipoglycans.** Purification procedures were adapted from protocols established for the extraction and purification of mycobacterial lipoglycans (Nigou et al., 1999). Briefly, 10 g of cells grown to an OD₆₀₀ of 1.0, were delipidated at 60 °C by using 500 ml CHCl₃/CH₃OH (1:1, v/v). The delipidated cells were resuspended in deionized water and disrupted by probe sonication (MSE Soniprep 150, 12 μm amplitude, 60 s on, 90 s off for 10 cycles, on ice) and the cell debris refluxed five times with 100 ml C₄H₄O/H₂O (1:1, v/v) at 68 °C, for 12 h intervals. The cell debris was removed by centrifugation and the supernatant containing lipoglycans, neutral glycans and proteins dried. The dried supernatant was then treated with a hot 80% (w/w) phenol/H₂O biphasic wash at 70 °C for 1 h, followed by several protease treatments, and the lipoglycan fraction was recovered following extensive dialysis against deionized water (Nigou et al., 1999).

The crude lipoglycan extract was resuspended in buffer A (50 mM ammonium acetate and 15% propan-1-ol) and subjected to octyl-Sepharose CL-4B hydrophobic interaction chromatography (2.5 cm × 50 cm) as previously reported (Leopold & Fischer, 1993). The column was initially washed with 4 column volumes of buffer A to ensure removal of neutral glycans followed by an increasing gradient of propan-1-ol ranging from 25 to 65%, keeping the concentration of ammonium acetate constant. The eluates were collected and extensively dialysed against deionized water, concentrated to approximately 1 ml and precipitated using 5 ml C₄H₄O; the sample was freeze-dried using a Savant SpeedVac. The freeze-dried sample containing the...
retained material from the hydrophobic interaction column was then resuspended in buffer B (0.2 M NaCl, 0.25 %, w/v, sodium deoxycholate, 1 mM EDTA and 10 mM Tris/HCl, pH 8) to a final concentration of 200 mg ml$^{-1}$. The sample was gently mixed and left to incubate for 48 h at room temperature. The sample was then loaded onto a Sephacryl S-200 column (2.5 cm × 50 cm) previously equilibrated with buffer B. The sample was washed with 400 ml buffer C (0.2 M NaCl, 1 mM EDTA and 10 mM Tris/HCl, pH 8) at a flow rate of 3 ml h$^{-1}$, collecting 1.5 ml fractions using a Bio-Rad auto-sampler. The fractions were monitored by SDS-PAGE using either a silver stain utilizing periodic acid and silver nitrate (Hunter et al., 1986) or a Pro-Q emerald glycoprotein stain (Invitrogen), and individual fractions were pooled and dialysed extensively against buffer C for 72 h with frequent changes. The samples were further dialysed against deionized water for 48 h with frequent changes of deionized water, lyophilized and stored at −20 °C.

Glycosyl composition and linkage analysis of lipoglycans by alditol acetates. Lipoglycans from wild-type C. glutamicum and the C. glutamicum::ubiA mutant were hydrolysed using 2 M trifluoroacetic acid (TFA), reduced with NaBD$_4$, and the resultant alditols were per-O-acetylated and examined by GC (Tatituri et al., 2007). Lipoglycans (2 mg) were per-O-methylated using dimethyl sulfonil carbanion as described previously (Alderwick et al., 2005; Besra et al., 1995; Daffe et al., 1990). In this procedure, lipoglycan samples (2 mg) were resuspended in 0.5 ml DMSO (anhydrous) and 100 µl of 4.8 M dimethyl sulfoxide carbanion. The reaction mixture was stirred for 1 h and then iodomethane (50 µl) was slowly added and the suspension stirred for 1 h; this process was repeated for a total of three times. The reaction mixture was then diluted with an equal volume of water, dialysed against deionized water and dried. The resulting retentate from the hydrophobic interaction column was extensively against buffer C for 72 h with frequent changes. The retained material from the hydrophobic interaction column was then resuspended in buffer B (0.2 M NaCl, 0.25 %, w/v, sodium deoxycholate, 1 mM EDTA and 10 mM Tris/HCl, pH 8) to a final concentration of 200 mg ml$^{-1}$. The sample was gently mixed and left to incubate for 48 h at room temperature. The sample was then loaded onto a Sephacryl S-200 column (2.5 cm × 50 cm) previously equilibrated with buffer B. The sample was washed with 400 ml buffer C (0.2 M NaCl, 1 mM EDTA and 10 mM Tris/HCl, pH 8) at a flow rate of 3 ml h$^{-1}$, collecting 1.5 ml fractions using a Bio-Rad auto-sampler. The fractions were monitored by SDS-PAGE using either a silver stain utilizing periodic acid and silver nitrate (Hunter et al., 1986) or a Pro-Q emerald glycoprotein stain (Invitrogen), and individual fractions were pooled and dialysed extensively against buffer C for 72 h with frequent changes. The samples were further dialysed against deionized water for 48 h with frequent changes of deionized water, lyophilized and stored at −20 °C.

RESULTS

Disruption of Cg-ubiA

The ubiA gene product was shown in prior work to synthesize DPPR, which is converted to DPA, thus supplying the substrate for the 'priming' arabinosyltransferase AFA (Alderwick et al., 2006b). These initial Ara residues ‘prime’ the galactan backbone for further attachment of α(1→5)-linked Ara residues. These reactions require the arabinofuranosyltransferase activities of Mt-EmbA and Mt-EmbB, or Cg-Emb, respectively, and also the utilization of DPA (Alderwick et al., 2005; Escuyer et al., 2001; Seidel et al., 2007), to eventually result in mature AG. In light of our present studies demonstrating the occurrence of Cg-LAM we inactivated the mycobacterial orthologue of C. glutamicum, NCgl2781, by transforming the wild-type to kanamycin resistance conferred by the vector-borne aph gene product of pCG::ubiA with a view to generating a clear C. glutamicum LM-only phenotype. The vector, as previously described (Alderwick et al., 2005), was integrated into the chromosomal ubiA gene, thus disrupting ubiA, as confirmed by two independent PCR analyses with two different primer pairs (Alderwick et al., 2005). As expected, the resulting strain C. glutamicum::ubiA exhibited strongly reduced growth (Alderwick et al., 2006a).

Purification of lipoglycans from C. glutamicum and C. glutamicum::ubiA

Analysis of the crude phenol-extracted lipoglycans from C. glutamicum (Fig. 1a) and C. glutamicum::ubiA (Fig. 1b) visualized on SDS-PAGE revealed two closely migrating lipoglycans. A two-step purification protocol was performed to fractionate the crude lipoglycans. In the first step, the neutral glycans and nucleic acids were eliminated by using an octyl-Sepharose CL-4B column (Amersham Biosciences). In the second step, the lipoglycans were individually purified using a Sephacryl S-200 column (Amersham Biosciences). Fractions containing the lipoglycans were monitored by SDS-PAGE stained with either

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silver nitrate or Pro-Q emerald glycoprotein stains and pooled accordingly to afford lower (1) and upper (2) lipoglycans (Fig. 1a, b).

General structural features of lipoglycans from C. glutamicum and C. glutamicum::ubiA

The lower lipoglycans (1) from both C. glutamicum and C. glutamicum::ubiA were similar and exhibited the basic components of a structure related to mycobacterial LM (now termed Cg-LM) and contained solely mannopyranose (Manp). Per-O-methylation analysis of Cg-LM from both strains indicated the presence of t-Manp, 2-Manp, 6-Manp and 2,6-Manp residues (Tatituri et al., 2007). GC analysis of the total acid-hydrolysed upper lipoglycan (2) from C. glutamicum showed that it contained arabinose and mannose in a ratio of 23:77 and was related to mycobacterial LAM (Fig. 2a). Per-O-methylation analysis of wild-type Cg-LAM indicated the presence of t-Araf, t-Manp, 2-Manp, 6-Manp and 2,6-Manp residues (Fig. 3a). Interestingly, Cg-LAM of the wild-type strain was previously shown to be composed of a PI anchor linked to an α(1→6)Manp backbone substituted at most of the O-2 positions by the structural motifs t-Araf, t-Manp, t-Araf(1→2)-Manp and t-Manp(1→2)-Manp units (Fig. 4e) (Tatituri et al., 2007). Accordingly, the 1D ^1H-NMR anomeric region (Fig. 4a) exhibited a complex pattern of overlapping resonances corresponding to Araf and Manp units. The different spin systems were characterized by $^1H$-$^13C$ HMQC NMR (Fig. 4b) and anomeric resonances were attributed as follows: δH_1C_5.52/112.2 (I_1) to two overlapping t-Araf units (see below) and 5.13/112.0 (II_1) to a third t-Araf unit, 5.06/105.2 (III_1) to t-Manp units, 5.12/101.4 (IV_1), 5.07/101.7 (V_1) and 5.04/101.9 (VI_1) to 2,6-Manp units, 5.06/105.2 (VII_1) to 6-Manp units, and 5.00/104.9 (VIII_1) to 2-Manp units.

Our previous experiments demonstrated that the C. glutamicum::ubiA mutant failed to synthesize DPA (Alderwick et al., 2006a) and possessed an arabinan-deficient cell wall phenotype (Alderwick et al., 2005). As a consequence, we anticipated that the Cg-LAM from the mutant would be devoid of arabinose. Surprisingly, glycosyl compositional analysis of Cg-LAM from the C. glutamicum::ubiA mutant showed that it contained arabinose, albeit in substantially smaller amounts, and mannose in a ratio of 4.5:95.5 (Fig. 2b). Per-O-methylation analysis of Cg-LAM from the mutant indicated the

![Fig. 1. Lipoglycan profiles of wild-type C. glutamicum (a) and C. glutamicum::ubiA (b). Lipoglycans were analysed using SDS-PAGE and visualized using the Pro-Q emerald glycoprotein stain (Invitrogen) specific for carbohydrates. Individual lipoglycans (1 and 2) were purified as described in Methods. C, crude lipoglycan; M, molecular mass markers (kDa).](image)

![Fig. 2. Glycosyl compositional analysis of lipoglycan-2 from wild-type C. glutamicum (a) and C. glutamicum::ubiA (b). Samples of individually purified lipoglycans (2) were hydrolysed with 2 M TFA, reduced and per-O-acetylated. Alditol acetates were subjected to GC analysis. Peak area integration shows that the arabinose content is 23% in wild-type C. glutamicum (a) and 4.5% in the C. glutamicum::ubiA mutant (b). Ara, arabinose; Man, mannose.](image)
presence of t-Araf, t-Manp, 2-Manp, 6-Manp and 2,6-Manp, although the relative abundance of t-Araf residues was reduced (Fig. 3b). In agreement with the glycosyl compositional and linkage analysis data indicating a reduced amount of t-Araf units, the 1D $^1$H anomic region of Cg-LAM from the glutamicum::ubiA mutant (Fig. 4c) was simpler than that observed for Cg-LAM from wild-type C. glutamicum (Fig. 4a). Resonance I1, corresponding to a t-α-Araf unit, was dramatically reduced, suggesting two overlapping t-Araf signals. This was further confirmed by $^1$H-$^1$C HMQC analysis (Fig. 4d). Indeed, the relative intensity of the correlation at $\delta_{H1C1} 5.13/112.0$ (I1) was much weaker and correlation at $\delta_{H1C1} 5.20/112.2$ (I1) of the third t-Araf residue was absent.

The native Cg-LAMs from wild-type C. glutamicum and the C. glutamicum::ubiA mutant were analysed by MALDI TOF MS. MALDI spectra were performed in the linear negative mode. The mass spectra show broad unresolved molecular ion envelopes due to the heterogeneity of the Cg-LAMs and the relatively low resolution of this type of MS. The native Cg-LAM from wild-type C. glutamicum shows an average molecular mass of around 15 400 Da (Fig. 5a). The native Cg-LAM from the C. glutamicum::ubiA mutant shows a somewhat lower average molecular mass in the region of 13 900 Da (Fig. 5b). Samples of Cg-LAM from wild-type C. glutamicum and C. glutamicum::ubiA were subjected to per-O-methylation and analysed by MALDI TOF MS (data not shown). Per-O-methylation replaces fatty acyl substituents with methyl groups as well as methylating all the free hydroxyl groups. The average molecular mass of the derivatized Cg-LAM from wild-type C. glutamicum was observed around 15 600 Da, whilst the molecular mass of the corresponding Cg-LAM from C. glutamicum::ubiA was observed around 15 200 Da.

Altogether, these data indicate that the C. glutamicum::ubiA mutant produces a slightly truncated Cg-LAM with a severe reduction in t-Araf content but maintaining and possibly extending its mannan core. In addition, the presence of UbiA and the biosynthesis of DPA appear to be essential for the higher arabinosylation of Cg-LAM. Moreover, these findings suggest that arabinose deposition in the C. glutamicum::ubiA mutant is a result of incorporation of t-Araf through another arabinose substrate, and one which is not DPA dependent.

**DISCUSSION**

Understanding the biosynthesis of mycobacterial arabinan is paramount to identifying potential new drug targets for the treatment of tuberculosis. However, as discussed earlier our understanding of these pathways is far from complete. In this respect, C. glutamicum is a useful tool in understanding mycobacterial cell wall biosynthesis, since this organism possesses the core structural elements of Corynebacterianeae with few gene duplications, and deletion of orthologous genes, which is often lethal in mycobacterial species, is possible (Alderwick et al., 2005, 2006b; Gande et al., 2004). Specifically, the deletion of the DPA requiring arabinosyltransferase Emb in C. glutamicum resulted in a strongly abrogated arabinan domain of AG (Alderwick et al., 2005), and deletion of AftA also disabled attachment of the remaining singular t-Araf residues to galactan (Alderwick et al., 2006b). Furthermore, the loss of DPA synthesis in a C. glutamicum::ubiA mutant also resulted in the full loss of the arabinan domain in mature AG (Alderwick et al., 2005). In contrast, Cg-LM and Cg-LAM isolated from the corresponding C. glutamicum Δemb and ΔaftA mutants were similar to the wild-type glycans (data not shown). An absence of Araf residues was expected...
in Cg-LAM of the C. glutamicum :: ubiA mutant. Strikingly, a total absence of arabinan was not observed (Fig. 2), thus requiring an alternative source and mechanism of addition of this sugar which may be attributed to the presence of an uncharacterized nucleotide sugar donor in Corynebacterineae.

In contrast to mycobacteria, parasites do synthesize and utilize a sugar nucleotide to provide an activated arabinose sugar donor. Schneider et al. (1994) characterized GDP-α-D-Ara, the precursor of α-Ara in Leishmania major lipophosphoglycan. In trypanosomatids, this sugar nucleotide is believed to be synthesized in a two-step process.
through the combined activities of an Ara-1-kinase and GDP-Ara pyrophosphorylase, whereby arabinose is phosphorylated to form arabinose 1-phosphate and then activated to the nucleotide level by GDP-D-Arap pyrophosphorylase (Mengeling & Turco, 1999; Schneider et al., 1995). Compared with the many genes encoding glycosyltransferases in prokaryotic and eukaryotic systems, very little is known about those that encode arabinopyranosyltransferases. Dobson et al. (2003) identified two genes (SCA1/2) encoding arabinosyltransferases mediating scAra capping, and recently it was demonstrated by heterologous expression that Leishmania SCA1 encodes an arabinopyranosyltransferase (Goswami et al., 2006).

Glycosyltransferases belonging to one of the families, GT-A and GT-B, or both, may be responsible for the initial addition of t-Araf residues on to Cg-LAM in the absence of DPA. These enzymes may be present on the cytoplasmic side of the membrane and probably aid in the transfer of t-Araf residues from a putative sugar nucleotide donor. These results could explain the presence of t-Araf in Cg-LAM, even though DPA biosynthesis has been completely abrogated in the C. glutamicum::ubiA mutant (Alderwick et al., 2005). The mechanism by which these Araf residues are added is not clear. One possible explanation is that Araf residues from a nucleotide precursor are added in a step-wise fashion to Cg-LAM on the cytoplasmic side to give motif A proximal to PI, and then this complex polysaccharide is then probably flipped on to the periplasmic side. It is thought that the flipping mechanism is a prerequisite for arabinosylation to occur outside of the membrane, where the arabinan and mannan domains are formed through specific GT-C glycosyltransferases, by the addition of either t-Araf and t-Manp residues, from DPA or decaprenyl-monophosphoryl-D-mannose (DPM) to give motifs B, C and D (Figs 4e and 6) (Berg et al., 2007; Seidel et al., 2007; Tatituri et al., 2007). The biosynthetic origin of mycobacterial Araf residues is generally a poorly understood area. To date, no sugar nucleotides of Araf have been identified, and as a result, a number of theories regarding the generation of arabinan in mycobacteria have been put forward (Huang et al., 2005; Mikusova et al., 2005; Scherman et al., 1995, 1996). Depending on the organization of the arabinan chains, it is speculated that five or six arabinofuranosyltransferases are required for arabinan biosynthesis in mycobacteria. In this regard, it is interesting to note that an M. smegmatis embC mutant (Escuyer et al., 2001; Zhang et al., 2003) was found to be devoid of LAM but from the chemical analysis, the resulting LM precursor possessed two or three Araf residues. The possibility exists that these residues are initially added via a non-DPA-dependent glycosyltransferase; this requires further investigation.

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