Cell Wall Structure of a Mutant of *Mycobacterium smegmatis* Defective in the Biosynthesis of Mycolic Acids*

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A mutant strain of *Mycobacterium smegmatis* defective in the biosynthesis of mycolic acids was recently isolated (Liu, J., and Nikaido, H. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 4011–4016). This mutant failed to synthesize full-length mycolic acids and accumulated a series of long chain β-hydroxymeromycolates. In this work, we provide a detailed characterization of the localization of meromycolates and of the cell wall structure of the mutant. Thin layer chromatography showed that the insoluble cell wall matrix remaining after extraction with chloroform/methanol and SDS still contained a large portion of the total meromycolates. Matrix-assisted laser desorption/ionization and electrospray ionization mass spectroscopy analysis of fragments arising from Smith degradation of the insoluble cell wall matrix revealed that the meromycolates were covalently attached to arabinogalactan at the 5-OH positions of the terminal arabinofuranosyl residues. The arabinogalactan appeared to be normal in the mutant strain, as analyzed by NMR. Analysis of organic phase lipids showed that the mutant cell wall contained some of the extractable lipids but lacked glycopeptidolipids and lipooligosaccharides. Differential scanning calorimetry of the mutant cell wall failed to show the large cooperative thermal transitions typical of intact mycobacterial cell walls. Transmission electron microscopy showed that the mutant cell wall had an abnormal ultrastructure (without the electron-transparent zone associated with the asymmetric mycolate lipid layer). Taken together, these results demonstrate the importance of mycolic acids for the structural and functional integrity of the mycobacterial cell wall. The lack of highly organized lipid domains in the mutant cell wall explains the drug-sensitive and temperature-sensitive phenotypes of the mutant.

The lack of effective treatment for mycobacterial infections by most of the broad spectrum antibiotics has been largely attributed to the extremely low permeability of the mycobacterial cell wall (1–3). This cell wall has a unique structure, consisting of three covalently linked polymers: peptidoglycan, covalently linked arabinogalactan (AG),1 and mycolic acid (4). The peptidoglycan is attached to AG via a phosphodiester bridge (5). About two-thirds of the nonreducing termini of the AG polysaccharide are esterified with mycolic acids (6). This covalently linked skeleton of cell wall is often described as the mycolyl-arabinogalactan-peptidoglycan complex. One of the most remarkable features of the mycobacterial cell wall is that up to 60% of its weight is composed of lipids, including mycolic acids. In addition to lipids in the covalently linked skeleton, several types of “extractable lipids” are present in various mycobacterial species, including trehalose-containing glycolipids, phenolic glycolipids, glycopeptidolipids (GPLs), lipooligosaccharides (LOSes), phosphatidylinositol mannosides (PIMs), phosphatidylethanolamine (PE), and triacylglycerols (TAGs) (4, 7, 8).

*Mycobacterium* strains are long, complex α-alkyl-β-hydroxy fatty acids that are unique to mycobacteria and the closely related genera (3, 4, 9). Mycolic acids play a critical role in the structure and function of the mycobacterial cell wall. They constitute the inner leaflet of the lipid bilayer of cell wall and have extremely low fluidity (3, 10, 11). It is this nature of mycolic acids that accounts for the exceptionally low permeability of the mycobacterial cell wall and explains, in large part, the natural resistance of mycobacteria to many antibiotics and chemotherapeutic agents (1–3). This mycolic acid-based permeability barrier also shields mycobacteria from environmental stress and contributes to disease persistence during infection (12). In addition, mycolic acid-containing glycolipids such as trehalose di-mycolate (cord factor) have been implicated in the pathogenesis of *Mycobacterium tuberculosis* (9, 13).

The biosynthesis of mycolic acids has been the focus of intense research for a number of years, primarily because of the presumption that enzymes involved in the synthesis of this unusual lipid are attractive targets for the development of novel chemotherapeutic agents. Identifying new drug targets is now particularly important in view of the prevalence of multi-drug-resistant *tuberculosis* (14) and the increasing association of atypical mycobacteria such as the *Mycobacterium avium-intracellulare* complex with AIDS patients (15). In an effort to elucidate the biosynthetic pathway of mycolic acids, we recently isolated a mutant of *Mycobacterium smegmatis* that had a defect in the synthesis of mycolic acids (16). This mutant, 155NS1, was generated by chemical-induced random mutagenesis of the wild type (WT) strain mc²-155. The 155NS1 mutant was unable to synthesize full-length mycolic acids and accumulated a series of β-hydroxymeromycolates with sizes ranging from 36 to 48 carbons (16). This mutant was more permeable than the WT strain to hydrophobic agents and exhibited hypersensitivity to various hydrophobic compounds such as n-octanone, triacylglycerol; WT, wild type; HPLC, high performance liquid chromatography; ESI, electrospray ionization; MS, mass spectroscopy; MALDI, matrix-assisted laser desorption/ionization; TOF, time-of-flight; DSC, differential scanning calorimetry.
biocin, rifampicin, erythromycin, and crystal violet. Also, this mutant was temperature-sensitive; i.e. although it grew at a normal rate at 30 °C, it did not show any visible growth at 37 °C. In this work, the structure and composition of the cell wall of this mutant were studied in detail. We show that the β-hydroxymycolymycoclates that accumulate in the mutant strain are covalently attached to AG of the cell wall, but they do not form highly organized structural domains, and that mutant cells have an abnormal colony morphology and cell wall ultrastructure.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—The strain 155NS1 is a mycobacterial acid-defective mutant of \textit{M. smegmatis} mc2-155 isolated previously (16). Since this mutant cannot grow at 37 °C, strains 155NS1 and mc2-155 both were grown at 30 °C in all experiments described in this study. Mycobacteria were grown in Middlebrook 7H9 broth (Difco) supplemented with 0.2% glycerol and 10% Middlebrook OADC enrichment (Difco). Cells were harvested at midexponential phase.

Isolation and Fractionation of the Cell Wall—The isolation and purification of cell wall were as described previously (10, 17). Briefly, mycobacterial cells were disrupted by sonication, and the cell wall fraction was separated from the cytoplasmic membrane by centrifugation (27,000 \times g, 30 min). The supernatant was further purified by TLC (see below). Delipidated cell wall was treated twice with 2% SDS in 20 mM potassium phosphate buffer (pH 7.0) at 90 °C for 1 h and each time pelleted at 10,000 \times g for 30 min, which removed most proteins and lipoarabinomannan. The cell wall pellet was then washed successively with water, 80% acetone, and acetone to remove SDS. The resulting insoluble cell wall matrix is known as the mycolyl-arabinogalactan-peptidoglycan complex in WT cells, in which all soluble proteins, extractable lipids, and carbohydrates were removed (16, 18). Since we did not know whether the β-hydroxymycolymycoclates in the mutant strain were attached to the cell wall, we referred to this fraction of cell wall as the insoluble cell wall matrix.

HPLC and TLC Analysis—A previously described reverse phase high performance liquid chromatography (HPLC) method (16) was applied for quantitative analysis of mycolates and mycolic acids identified, which was achieved by fractionating the cell wall into several components and analyzing for the presence of mycolates in these different materials. We first examined whether the mycolates present in the cell wall of the mutant could be extracted with chloroform/methanol. Equal amounts of the extractable lipid fractions and the delipidated cell wall suspensions were saponified, derivatized quantitatively with p-bromophenacyl bromide, and analyzed by a reversed phase HPLC (16). The molar ratio of mycolates in various fractions was obtained directly from the integrated peak areas compared with that of an internal standard (e.g. oleic acid). Results showed that 48% of the mycolate residues in the original cell wall of the mutant were found in the organic phase, compared with 32% of mycolic acids in the cell wall of the WT strain in parallel experiments.

We further examined whether the mycolates are present in the insoluble cell wall matrix of the mutant. TLC showed that upon saponification and extraction with CHCl3, β-hydroxymycolymycoclates were released from the insoluble cell wall matrix of the mutant, in amounts comparable with that of mycolic acids released from the WT cell wall (Fig. 1). These results suggested that the β-hydroxymycolymycoclates accumulated in the mutant were attached to the cell wall, presumably by a linkage to AG. No mycolates were detected in the growth medium (data not shown).

RESULTS AND DISCUSSION

Location of Meromycolates in the Cell Wall of 155NS1—We previously showed that the β-hydroxymycolymycoclates were accumulated in the 155NS1 mutant (16). To examine any potential involvement of the mycolates in the cell wall function, the localization of mycolates in mutant cells was determined, which was achieved by fractioning the cell wall into several components and analyzing for the presence of meromycolates in these different materials. We first examined whether the meromycolates present in the cell wall of the mutant could be extracted with chloroform/methanol. Equal amounts of the extractable lipid fractions and the delipidated cell wall suspensions were saponified, derivatized quantitatively with p-bromophenacyl bromide, and analyzed by a reversed phase HPLC (16). The molar ratio of meromycolates in various fractions was obtained directly from the integrated peak areas compared with that of an internal standard (e.g. oleic acid). Results showed that 48% of the meromycolate residues in the original cell wall of the mutant were found in the organic phase, compared with 32% of mycolic acids in the cell wall of the WT strain in parallel experiments.

We further examined whether the meromycolates are present in the insoluble cell wall matrix of the mutant. TLC showed that upon saponification and extraction with CHCl3, β-hydroxymycolymycoclates were released from the insoluble cell wall matrix of the mutant, in amounts comparable with that of mycolic acids released from the WT cell wall (Fig. 1). These results suggested that the β-hydroxymycolymycoclates accumulated in the mutant were attached to the cell wall, presumably by a linkage to AG. No meromycolates were detected in the growth medium (data not shown).
samples are fatty acids from whole cell of mc2-155 (mutant cell wall can be explained if we assume that the tuted with glycerol. The Smith degradation products of the trum of the Smith degradation products of the mutant cell wall and the resulting fractions were analyzed by mass spectros-
m/z acetate (9:1) and charred with 10% H2SO4. From methylated and separated on a TLC plate developed with hexane/ethyl lylglycerol, and the molecular weight of this b hydroxymeromyco-
b species detected in the mutant (16). The peak at mycolate is 605, which was the most abundant meromycolate
positions of the terminal- and 2-linked arabinofuranosyl resi-
droxymeromycolates in the mutant are attached to the C-5
adduct of sodium (M+H)+ to the molecular ions, respectively.
The most abundant ions, (M+H)+, are at m/z 666 and 680. The peaks at 688 and 702 correspond to adduct of sodium (M+23) to the molecular ions, respectively. The signal at m/z 680 corresponds to a β-hydroxymeromyco-
glycerol, and the molecular weight of this β-hydroxymeromy-
605, which was the most abundant meromycolate species detected in the mutant (16). The peak at m/z 666 may correspond to another β-hydroxymeromycolate (M, 591) substi-
tuted with glycerol. The Smith degradation products of the mutant cell wall can be explained if we assume that the β-hydroxymeromycolates in the mutant are attached to the C-5 positions of the terminal- and 2-linked arabinofuranosyl resi-
dues of AG (Fig. 2B). Accordingly, terminal- and 2- linked arabinofuranosyl units of AG substituted at C-5 with the meromycolate residues should produce CHCl3-soluble meromycolglycerols. The expected molecular weights of meromycolglycerols are in agreement with the result from MALDI-MS analysis; i.e. esterification of much of meromycolates with glycerol results in an increase in molecular weights by 75.
When the same sample was analyzed by ESI-MS with a negative ionization mode, more signals were evident (Fig. 2C). Two series of ions, (M-H)-, one at m/z 623, 651, 679, 707, 735, and 763, and the other at m/z 609, 637, 665, 693, 721, 749, and 777 were detected. Based on the above analysis, we believe that the series of peaks at m/z 623, 651, 679, 707, 735, and 763 correspond to β-hydroxymeromycolates with molecular weights of 549, 577, 605, 633, 661, and 689 (16) substituted with glycerol, respectively. We previously showed that these β-hydroxymeromycolates are likely to represent intermediates in the chain elongation cycles of the biosynthetic pathway of mycolic acids, and that each cycle adds a two-carbon unit to the meromycolates (16). The other series of ions at m/z 609, 637, 665, 693, 721, 749, and 777 in the ESI-MS spectrum may correspond to meromycolate homologs containing odd-num-ered carbons substituted with glycerol. These meromycolate species were observed in our previous study, although they were present in a lower abundance (see Fig. 5 of Ref. 16). In mycolic acids, the odd-numbered carbon-containing homologs represent mycolic acids that contain a distal cis-double bond and a proximal trans-double bond with an adjacent methyl branch in the meromycolate chain (9, 13). In the WT cell wall, it was previously shown that mycolic acids were attached to C-5
consistent with this result, MALDI-TOF showed major peaks at m/z 1211, 1225, 1239, 1253, 1267, 1281, and 1295 (data not shown), which correspond to the free mycolic acids at m/z 1138, 1150, 1164, 1178, 1192, 1206, and 1220 substituted with glycerol, respectively. Based on these results, we concluded that the meromycolates were attached at the same position (C-5 of the arabinofuranosyl residues of AG) in the mutant cell wall as mycolic acids in the WT cell wall (Fig. 2B).

NMR Analysis of the Arabinogalactan—To examine whether the structure of AG in the cell wall of the mutant was altered, AG released from the cell wall skeleton by treatment with NaOH was analyzed by 1H NMR. For comparison, AG from the cell wall of the WT strain was solubilized and analyzed in the same way. The proton NMR spectrum of the AG in the cell wall of the mutant was identical to that of the WT strain (data not shown), suggesting that the bulk structure of AG in the cell wall of the mutant is the same as that in the WT strain. An interesting question arising from these results is which en-
ymes are responsible for the transfer of meromycolates to AG of the cell wall. The antigen 85 complex (Ag85A, -B, and -C) is known to possess mycolyltransferase activity and has been shown to catalyze the transfer of mycolic acids to trehalose (29). It was suggested that the Ag85 complex was also responsible for deposition of mycolic acids on AG, although direct evidence has yet to be shown. Inactivation of the Ag85C gene was shown to transfer 40% fewer mycolates to the cell wall (30). It is not clear at present whether the same enzyme is responsible for the transesterification of the meromycolates to AG in the mutant cell wall.

TLC Analysis of CHCl3-extractable Lipids—The organic sol-
vent-extractable lipids of the cell wall were analyzed by TLC. It was shown that the cell wall of M. smegmatis contains GPLs, LOSs, PIMs, PE, DPG, and TAGs (4, 7, 8). To determine whether these lipids are present in the mutant, we applied TLC developing systems that were previously established to examine each group of lipids (see “Experimental Procedures”). Two-dimensional TLC system A was used for analysis of apolar lipids (e.g., TAGs). For analysis of polar lipids such as PIMs, PE, and DPG, two-dimensional TLC developed with solvent system B was used. In all experiments, lipids extracted from the cell wall of the WT strain under the same conditions were com-
pared. The results are summarized in Table I. The cell wall of the mutant contained some of the extractable lipids including PIMs, PE, TAGs, and DPG but lacked GPLs and LOSs.

DSC of Cell Wall from the Mutant—We previously showed that the mycobacterial cell wall forms an asymmetric lipid bilayer with a mycolic acid-containing inner leaflet covered by an outer leaflet composed of extractable lipids (10, 17). We have
FIG. 2. Mass spectra of Smith degradation products. The insoluble cell wall matrix isolated from the 155NS1 mutant was subjected to Smith degradation (see “Experimental Procedures”) and extracted with CHCl₃, which was then analyzed by mass spectrometry. A, positive MALDI-TOF mass spectrum of CHCl₃-soluble fraction of Smith degradation products of the mutant cell wall matrix. Peaks at 688 and 702 are sodium adducts to the molecular ions. B, diagram illustration of the effects of Smith degradation on the terminal arabinofuranosyl units of the insoluble cell wall matrix of the mutant. The β-hydroxymeromycolates are attached at the C-5 position of the terminal- and 2-linked arabinofuranosyl residues. Production of CHCl₃-soluble β-hydroxymeromycolylglycerol that corresponds to the ions in A and C is illustrated. R represents β-hydroxymeromycolates in the mutant and mycolic acids in the WT strain. C, ESI-MS spectrum of CHCl₃-soluble Smith degradation products of the cell wall matrix isolated from 155NS1. The spectrum was acquired at a negative ionization mode.
shown above that the \( \beta \)-hydroxymeromycolates accumulated in the mutant were attached to AG of the cell wall and that the mutant cell wall contained some of the extractable lipids. To examine whether these lipid components form an organized structure in the mutant cell wall, DSC analysis of the purified cell wall of the mutant was performed. No cooperative thermal transitions were observed in the mutant cell wall (Fig. 3A), indicating that lipids in the cell wall of the mutant did not form highly organized structural domains. DSC of the WT cell wall had major peaks between 30 and 60 °C and reflected the melting of tightly packed mycolic acids (Fig. 3B; also see Refs. 10, 11, and 27).

**Altered Colony Morphology of Mutant Cells**—The cell morphology and the cell wall ultrastructure of the mutant were directly examined by transmission electron microscopy (Fig. 4). Mutant cells appear to have a significantly larger average radius and tend to be more amorphous and less rodlike than WT cells (data not shown). The cytoplasm of mutant cells was centered with large electron-translucent voids, and they were often detached from the cell wall materials (Fig. 4). Such features were not evident in WT cells. The cytoplasm of WT cells possessed a uniform appearance of heavily stained ribosomes that were difficult to distinguish in the cytoplasm of mutant cells. These differences in cytoplasmic ultrastructure were previously observed in WT cells processed by different techniques \( \text{e.g.} \) dehydration with acetone rather than ethanol during the embedding of the specimens and reflected perturbation of the ultrastructural features during sample preparations (31). These data suggest that the structural integrity of mutant cells is compromised by the fixation procedure, which is expected, considering the altered cell wall of the mutant (see below).

There were striking differences in the cell envelope profiles between the mutant and the WT strain. Although mutant cells had a similar plasma membrane and a peptidoglycan layer as WT cells, they appeared to lack the typical thick electron-transparent zone outside of the plasma membrane (Fig. 4). The electron-transparent zone constitutes the hydrophobic domain of the cell wall and has been thought by many workers to correspond to mainly the mycolic acids covalently bound to AG (3, 4, 31). Transparency to electrons in this ultrastructure is explained by the extremely hydrophobic nature of mycolic acids, which excludes water-soluble, electron-dense heavy metal salts such as the uranyl acetate used in this study. In our previously proposed cell wall bilayer model (10), the mycolic acids extend upward from AG to fill the electron-transparent zone of the cell wall and interact with the extractable lipids. The arabinan chains are anchored fairly close to the reducing end of galactan, which itself is linked to peptidoglycan via linker disaccharide phosphate (3, 10). This model is in agreement with the results presented here. The disappearance of the electron-transparent zone in the mutant cells agrees with the appearance of disorganized fibrils on the cell surface (Fig. 4). The identity of these thin fibrils is unknown, and they are probably composed of the \( \beta \)-hydroxymeromycolates accumulated in mutant cells, which were shown above to attach the cell wall onto AG in the same manner as mycolic acids. This is somewhat different from the situation of \textit{M. avium} treated with isoniazid described above (26), where the loss of the electron-transparent zone did not coincide with the appearance of obvious shedding materials (the samples were processed for electron microscopy in identical ways; see Ref. 26). Instead, a more densely staining thick layer appeared. Correspondingly, no fatty acid intermediates with lengths equivalent to the \( \beta \)-hydroxymeromycolates accumulated in the 155NS1 mutant were detected in the cell wall of \textit{M. avium} treated with isoniazid (26). In addition to meromycolates, some
of the extractable lipids including TAGs, PIMs, DPG, and PE were also present in the mutant cell wall (Table I), and they are likely to be components of the fibrils in mutant cells. In WT cells, there is an outermost electron-dense layer that was also absent in mutant cells (Fig. 4). This layer was visualized by staining with ruthenium red in earlier studies (3, 4). It varies in thickness (from negligible to massive), electron density, and appearance (fibrillar, granular, or homogeneous), which is attributable to differences in species, growth conditions, and preparation methods for microscopy (3, 4). The fact that ruthenium red allowed this layer to be consistently visualized suggests that the minimal structure is probably composed of negatively charged head groups of lipids. In the cell wall bilayer model, such lipids include many of the extractable lipids like GPLs, LOSs, and PIMs, which interact with mycolic acids and constitute the outer leaflet of the cell wall bilayer (3, 10). Loss of the outermost electron-dense layer in the mutant may correlate with the lack of GPLs and LOSs in the cell wall (Fig. 4, Table I).

Lack of a highly organized lipid bilayer in the mutant cell wall explains many of the physiological phenotypes of mutant cells. Thus, mutant cells became hypersensitive to various hydrophobic drugs and were extremely permeable to hydrophobic compounds (16). The structural characteristics of the mutant cell wall may also explain why mutant cells cannot grow at 37 °C. We previously showed that mycobacteria maintain proper cell wall fluidity by changing the structure and composition of mycolic acids in response to growth temperature. For example, an increase in the growth temperature from 20 to 45 °C increased the chain length of mycolic acids by 2–4 carbon atoms and the percentage of trans-mycolate from 19 to 59% in M. smegmatis (11). Both of these changes resulted in a decrease in the cell wall fluidity (11). Yuan et al. recently showed that M. tuberculosis H37Rv and M. bovis BCG strains that lack ketomycolates were severely defective for growth within macrophages, establishing a critical role for mycolate composition in proper cell wall function (32). The β-hydroxymycolymycocytolates in the cell wall of the mutant, with sizes ranging from 36 to 48 carbons, did not form a organized structure and thus are unlikely to maintain the proper cell wall fluidity to support the growth of mutant cells at 37 °C (16). On the other hand, the fact that the mutant did grow at a lower temperature, e.g. 30 °C, is intriguing. It is generally thought that the structural integrity of the mycobacterial cell wall is essential for the survival of mycobacteria. This is true in M. tuberculosis. Isoniazid is a potent drug with exquisite specificity for M. tuberculosis. In M. tuberculosis, isoniazid inhibits mycolic acid synthesis by interfering with enzymes involved in the chain elongation reaction (16, 33, 34). The lethal effect of isoniazid on mycolic acid synthesis parallels the time course of loss of M. tuberculosis viability. However, it was recently shown that M. avium formed colonies at 37 °C at isoniazid concentrations that are inhibitory for mycolic acid biosynthesis (26). Thus, M. smegmatis appears to like M. avium and is able to compensate for the loss of mycolic acids by unknown mechanisms.

In summary, the availability of a mycolate-defective mutant allows us to directly study the structure-function relationship of the mycobacterial cell wall in great detail. The results presented in this paper demonstrate the importance of mycolic acids for the structural and functional integrity of the mycobacterial cell wall. Our better understanding of the structure of the mycobacterial cell wall, and of the contribution of various cell wall components to the cell wall structure and its role in drug resistance, will help in designing a strategy to overcome this permeability barrier.

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REFERENCES
1. Jarlier, V., and Nikaido, H. (1994) FEBS Microbiol. Lett. 123, 11–18
2. Jarlier, V., and Nikaido, H. (1990) J. Bacteriol. 172, 1418–1423
3. Liu, J., Barry, C. E., III, and Nikaido, H. (1999) in Mycobacteria: Molecular Biology and Virulence (Ratlledge, C., and Dale, J. W., eds) pp. 220–239, Blackwell Science, Oxford
4. Brennan, P. J., and Nikaido, H. (1995) Annu. Rev. Biochem. 64, 29–63
5. McNeil, M., Daffe, M., and Brennan, P. J. (1990) J. Biol. Chem. 265, 18200–18206
6. McNeil, M., Daffe, M., and Brennan, P. J. (1991) J. Biol. Chem. 266, 13217–13233
7. Minnikin, D. E., and Goodfellow, M. (1980) in Microbiological Classification and Identification (Goodfellow, M., and Board, R. G., eds) pp. 189–256, Academic Press, London
8. Ortalo-Magné, A., Lemassu, A., Laneelle, M. A., Bardou, F., Silve, G., Guonon, P., Marchal, G., and Daffe, M. (1996) J. Bacteriol. 178, 456–461
9. Barry, C. E., III, Lee, R. E., Mdluli, K., Sampson, A. E., Schroeder, B. G., Slayden, R. A., and Yuan, Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11254–11258
10. Liu, J., Barry, C. E., III, Besra, G. S., and Nikaido, H. (1996) J. Biol. Chem. 271, 29545–29551
11. Barry, C. E., III, and Mdluli, K. (1996) Trends Microbiol. 4, 275–281
12. Daffe, M., and Draper, P. (1998) Adv. Microbial Physiol. 39, 132–203
13. World Health Organization (1997) Anti-tuberculosis Drug Resistance in the World, World Health Organization, Geneva, Switzerland
14. Shafran, S. D., Singer, J., Zarowny, D. P., Phillips, P., Sulit, I., Walmsley, S. L., Fong, I. W., Gill, M. J., Rachlis, A. R., Lalonde, R. G., Fanning, M. M., and Tsoukas, C. M. (1996) N. Engl. J. Med. 335, 377–383
15. Liu, J., and Nikaido, H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4011–4016
16. Nikaido, H., Kim, S.-H., and Rosenberg, E. Y. (1993) Mol. Microbiol. 8, 1025–1030
17. Daffe, M., Brennan, P. J., and McNeil, M. (1990) J. Biol. Chem. 265, 6734–6743
18. Marques, M. A. M., Chitale, S., Brennan, P. J., and Pessolani, M. C. (1998) Infect. Immun. 66, 2625–2631
19. Dobson, G., Minnikin, D. E., Minnikin, S. M., Parlett, J. H., Goodfellow, M., Ridell, M., and Magnusson, M. (1985) in Chemical Methods in Bacterial Systematics (Goodfellow, M., and Minnikin, D. E., eds) pp. 237–265, Academic Press, Inc., London
20. Besra, G. S. (1996) Methods Mol. Biol. 101, 91–107
21. Barrow, W. W., and Brennan, P. J. (1983) J. Bacteriol. 150, 381–384
22. Daffe, M., McNeil, M., and Brennan, P. J. (1991) Biochemistry 30, 378–388
23. Dittmer, J. C., and Lester, R. L. (1964) J. Lipid Res. 5, 126–127
24. Jacin, H., and Mishkin, A. R. (1965) J. Chromatogr. 16, 170–173
25. Mdluli, K., Swanson, J., Fischer, E., Lee, R. E., and Barry, C. E., III (1998) Mol. Microbiol. 27, 1223–1233
26. George, K. M., Yuan, Y., Sherman, D. R., and Barry, C. E., III (1995) J. Biol. Chem. 270, 37282–37288
27. Besra, G. S., Khos, K.-H., McNeil, M. R., Dell, A., Morris, H. R., and Brennan, P. J. (1995) Biochemistry 34, 4257–4266
28. Belisle, T. J., Vissa, V. D., Sievert, T., Takayama, K., Brennan, P. J., and Besra, G. S. (1997) Science 276, 1420–1422
29. Jackson, M., Raynaud, C., Laneelle, M. A., Guillot, C., Winter, C. L., Ensengrueix, D., Gicquel, B., and Daffe, M. (1999) Mol. Microbiol. 31, 1573–1587
30. Paul, W., and Beveridge, T. J. (1992) J. Bacteriol. 174, 6508–6517
31. Yuan, Y., Zhu, Y. Q., Crane, D. D., and Barry, C. E., III (1998) Mol. Microbiol. 29, 1449–1458
32. Mdluli, K., Slayden, R. A., Zhu, Y., Ramaswamy, S., Pan, X., Mead, D., Crane, D. D., Musser, J. M., and Barry, C. E., III (1998) Science 286, 1607–1610
33. Yuan, Y., Mead, D., Schroeder, B. G., Zhu, Y. Q., and Barry, C. E., III (1999) J. Biol. Chem. 274, 21282–21290