Location of the Mycolyl Ester Substituents in the Cell Walls of Mycobacteria*

Michael McNeil, Mamadou Daffe‡, and Patrick J. Brennan§
From the Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

(Received for publication, January 23, 1991)

The question of the precise location of mycolic acids, the single most distinctive cell wall entity of members of the Mycobacterium genus, has now been addressed. The free hydroxyl functions of the arabinogalactan component of the mycobacterial cell wall were O-methylated under conditions in which the mycolyl esters were not cleaved. Subsequent replacement of the mycolyl functions with O-ethyl groups resulted in an acid- and base-stable differentially O-alkylated surrogate polysaccharide, more amenable to analysis. Complete hydrolysis, reduction, acetylation, and gas chromatography/mass spectrometry revealed the unexpected finding that the mycolyl substituents were selectively and equally distributed on the 5-hydroxyl functions of the arabinogalactan. Each mycolyl substituent is clustered in groups of four on the previously recognized nonreducing terminal pentaarabinofuranosyl (Araf) residues. Further analysis of the O-alkylated cell wall through partial acid hydrolysis, NaB[2H]4 reduction, and gas chromatography/mass spectrometry demonstrated that the mycolyl units are clustered in groups of four on the previously recognized nonreducing terminal pentaarabinosyl unit [β-Araf-(1→2)-α-Araf]α-3,5-α-Araf. However, only about two-thirds of the available pentasaccharide units are so substituted. Thus, the antigenicity of the arabinan component of mycobacterial cell walls may be explained by the fact that about one-third of the pentaarabinosyl units are not mycolylated and are available for interaction with the immune system. On the other hand, the extreme hydrophobicity and impenetrability of the mycobacterial cell may be explained by the same motif also acting as the fulcrum for massive esterified paraffin residues. New fundamental information on the structure of mycobacterial cell walls will aid in our comprehension of its impenetrability to antibiotics and role in immunopathogenesis and persistence of disease.

| The insoluble matrix of the mycobacterial cell wall, namely the product remaining after removal of all soluble proteins, lipids, and carbohydrates, and variously known as cell wall skeleton (1) and cell wall core (2), has been implicated in an array of pathogenic and immunological events associated with tuberculosis and leprosy (3). Following on the fundamental structural work of many over a 50-year period (4), we termed this material the mycolylarabinogalactan-peptidoglycan-protein (mAGPP) complex and succeeded in defining the major structural features of the arabinogalactan entity (5), of a unique covalent linkage between arabinogalactan and peptidoglycan (6), and of the form and importance of the chemical association between certain proteins and peptidoglycan (7, 8). In particular, out of these more recent efforts arose the recognition of a pentaarabinofuranosyl motif (5) equating the nonreducing termini of the arabinan segment of arabinogalactan with the dominant antigenic determinant of arabinogalactan, if not of the whole mycobacterial cell. Recognition and definition of this entity provided the impetus to address another major structural issue in our quest of a chemical comprehension of the mycobacterial cell wall, namely, the precise location of the mycolate units. It is now demonstrated that these are attached to about two-thirds of the critical nonreducing pentaarabinosyl units, thus presenting an image of a structural arrangement which acts variously as a template for mycolate attachment, and, when not so substituted, as a powerful B-cell immunogen in mycobacterial infections.

EXPERIMENTAL PROCEDURES

Growth of Organisms and Production of mAGPP

Mycobacterium tuberculosis TMC 107 (Erdman), Mycobacterium bovis BCG (Danish strain), Mycobacterium leprae from armadillos, and Mycobacterium smegmatis TMC 607 were prepared in sizable quantity as described previously (5, 6). In all cases, mAGPP was prepared as described (5, 6); mAGPP is synonymous with the insoluble cell wall matrix.

Methylation of mAGPP

Intact cell walls in some instances were methylated by the base catalyzed procedure of Hakomori (9) as previously described (5). In other situations, cell walls were methylated with methyl trifluoro-methanesulfonic acid as described by Prehm (10) with modifications. The method was applied as follows. Five mg of cell walls were suspended in 1 ml of trimethylphosphate (Aldrich) followed by the addition of 300 μl of 2,5-di-tert-butylpyridine, double the usual amount in order to discourage acid-catalyzed cleavage of furanosides, and 100 μl of methyl trifluoro-methanesulfonate (both from Aldrich). The reaction mixture was stirred for 6 h at 50 °C, neutralized with pyridine, dialyzed, and freeze dried to recover the methylated mAGPP. Ethylation and concomitant demycolylation was performed as described (5) using C2H5I instead of C2[3H]3I.

1 The abbreviations used are: mAGPP, mycolyl-arabinogalactan-peptidoglycan-protein complex; mAGP, mycolyl-arabinogalactan-peptidoglycan complex; AG, arabinogalactan; GC/MS, gas chromatography/mass spectrometry; Ara, arabinosyl; f, furanosyl; BCG, Bacille Calmette-Guerin; m, multiplet; s, singlet.
**Preparation of Per-O-alkylated Oligoglycosyl Alditols—Glycosyl linkage compositional analysis of the per-O-methylated or the per-O-alkylated mAGPP was performed as described (5). The per-O-alkylated (i.e., partially O-methylated, partially O-ethylated) AGPP from *M. tuberculosis* was also partially hydrolyzed, reduced with NaBEt₄H, pentadeuterioethyalted, and the resulting fragments resolved by high performance liquid chromatography as described (5). The resulting per-O-alkylated oligoglycosyl alditols, now bearing ethyl groups at the position of the original mycolates, were analyzed by GC/MS as described (5), except that the ions m/z 194, 208, 244, 295, 303, 317, and 331 were selectively monitored.

**Smith Degradation of mAGPP—** mAGPP from *M. tuberculosis* (200 mg) was treated with 10 ml of 0.1 M NaIO₄ in 50 mM sodium acetate buffer, pH 5.0, and allowed to react for 4 days in the dark at room temperature. Periodate was removed from the insoluble residue by extensive washing with water, and the insoluble residue was reduced by stirring with 130 mg of NaBEt₄H, in 10 ml of water for 5 h. The reaction mixture was titrated to pH 5 with acetic acid, dialyzed extensively against water, freeze dried, treated with 1 M HCl at room temperature overnight, and the pellet washed with water. Further extraction of the pellet with CHCl₃ yielded 34 mg of lipid which was applied in CHCl₃ to a column (1 cm × 20 cm) of silica gel followed by a two-step elution regimen of 50 ml each of 2% CH₃OH in CHCl₃ and 6% CH₃OH in CHCl₃. Fractions (5 ml) from this column were examined by TLC in CHCl₃/CH₃OH (1:1). Analysis of the products arising from Smith degradation for arabinose and glycerol was accomplished by subjecting fractions to methanolsysis in 1 M HCl in CH₃OH at 85 °C for 1 h prior to trimethylsilylation and MS/MS analysis, as described (6).

**RESULTS**

**Application of Replacement O-Ethylation to Mycolyl Group Location—** Earlier work by others (11–13) had led to the present day concept (14) that cell wall mycolic acids of mycobacteria are attached as esters to position 5 of the terminal arabinosyl residues of arabinogalactan. Amar-Nacasch and Vilkas (15), in particular, had obtained a mycolate of arabinobiose from the cell walls of *M. tuberculosis* which evidence tended to support the then current idea that the side chains of arabinogalactan consisted of nonmycolyltriaerabinosyl arrangements. However, in light of the recent recognition (5) of several species of mycobacteria are shown in Table I. Products indicative of the presence of both a 5-O-mycyl-t-Araf unit, namely, 1,4-di-O-acetyl-2,3-di-O-methyl-5-O-ethyl-arabinobitol (m/z 118, 129, and 175) and a 5-O-mycyl-2-linked-Araf unit, namely, 1,2,4-tri-O-acetyl-3-O-methyl-5-O-ethyl-arabinobitol (m/z 129, 139, 175, and 190), were obtained from all four species of mycobacteria (Table I). The *M. tuberculosis* mAGPP complex used in the above analysis contained 35% mycolic acids, according to the weight of CHCl₃-soluble material obtained from methanolyses, and 25% of the mass consisted of arabinogalactan, as derived from a quantitation of alditol acetates. Calculations based on these percentages, assuming mycolyl substitution on the 5 positions of both the t-Araf and the 2-linked Araf, require that 76% of the total quantities of these 2 residues be mycolylated. The actual data

**Fig. 1.** The nonreducing terminal pentaarabinofuranosyl unit of the mAGPP complex of *M. tuberculosis* as deduced from earlier studies (5) and which is necessarily the site of mycolyl group location.

**Fig. 2.** Explanation of the analytical approach to locating the mycolyl substituents on the cell wall of mycobacteria. The actual proposed structure is demonstrated in order to illustrate the approach. The strategy involved replacement of the mycolyl esters with ethyl ethers and then the determination of the positions of the ethyl groups by formation and analysis of partially acetylated, partially ethylated, and partially methylated alditols. Only the structures of the alditol acetates of residues originally substituted with mycolyl groups are shown. CH₃SO₂CF₃, methytrifluoromethanesulfonate; CH₃SOCH₃, dimethylsulfinycarbanion.
Location of Mycolic Acids

The general strategy employed in the replacement of mycolates with ethyl groups and the preparation of alditol acetates for analysis is described in the legend to Fig. 2.

### Table I

Application of mycolyl replacement ethylation to the question of mycolyl placement on the arabinogalactan of Mycobacterium ssp

| Product identified* | Deduced structure | Total glycosyl residuesb |
|---------------------|------------------|--------------------------|
|                     |                  | M. tuberculosis | BCG | M. leprae | M. smegmatis |
| 2,3,5-Tri-O-CH₃-Ara | Nonmycolylated t-Araf | 4.3 ± 0.38 | 7 | 9 | 8 |
| 2,3,6-Tri-O-CH₃-5-mono-C₂H₅-Ara | 5-Mycolyl-t-Araf | 7.6 ± 0.98 | 5 | 6 | 7 |
| 3,5-Di-O-CH₃-Ara | Nonmycolyl-2-linked-Araf | 4.7 ± 1.1 | 7 | 7 | 6 |
| 3-Mono-CH₃-5-mono-C₂H₅-Ara | 5-Mycolyl-2-linked-Araf | 8.3 ± 1.75 | 5 | 5 | 7 |

* For clarity, only the Ara residues with a free OH group at C-5 (i.e. 2-linked-Araf and t-Araf) are listed. The remaining glycosyl residues, i.e. 5-linked-Araf, 3,5-linked-Araf, 5-linked-Galp, 6-linked-Galp, and 5,6-linked-Galp, were detected as their methylated derivatives with no ethyl substituents and in the proportions originally reported (5). Small amounts (<1% of the total glycosyl residues) of 5-linked-Araf residues substituted with O-ethyl groups at C-2 and/or C-3 and 5,5-linked-Araf residues with an O-ethyl at C-2 were also detected. However, the amounts of these derivatives were variable and were probably due to incomplete methylation; nevertheless, the possibility of small amounts of additional acyl group substitution (either mycolyl or otherwise) on these residues cannot be ruled out.

b The standard deviation was obtained for the M. tuberculosis product only, based on analysis of four different samples. The figures for the other species are based on analysis of but one sample from each and are rounded to the nearest whole figures.

![Fig. 4. TLC of the CHCl₃-soluble fractions arising from Smith degradation on mAGPP of M. tuberculosis. Fractions A-D resulted from the application of the CHCl₃-soluble material to a column of silicic acid as described in the text. Solvent, CHCl₃-CH₃OH (95:5). Spray, 10% H₂SO₄ at 110 °C for 5 min.](image)

![Fig. 3. Explanation of the effects of Smith degradation on the terminal tetramycolylpentaarabinofuranosyl unit of mAGPP of M. tuberculosis. The production of CHCl₃-soluble mycolyglycerol (fractions C and D in Fig. 4) and the CHCl₃-soluble triarabinosylglycerol (fractions A and B in Fig. 4) are illustrated.](image)
periodate labile mycolylarabinofuranosyl residues, i.e. 5-O-mycolyl-t-Araf, in the original mAGPP (Fig. 3). Fraction C also co-chromatographed on TLC with authentic 1-O-mycoc- 
yllylycerol, and "H NMR analysis showed the same cyclopro- 
pyl signal that characterized fraction A (6 -0.35, 0.6) as well as two doublet of doublets (6 4.23, 12 and 4 Hz; and 6 4.15, 12 and 7 Hz) attributable to the primary H's which used to be 
attached to C-5 of t-Ara and now are the hydrogens attached 
to the mycolyl-substituted primary glycerol carbon.

Evidence for the Clustering of Four Mycolyl Groups on Single Pentaarabinosyl Units—The studies described above demonstrated that most of the available terminal Araf and penultimate 2-linked Araf units are mycolylated, and, previously (5), we had shown that these 2 sugar residues invariably occur together as part of the pentaarabinosyl reducing end motif of arabinogalactan (Fig. 1). Nevertheless, several alternative mycolyl substitution patterns could be conceived within the framework of this motif, all consistent with both the methylation and periodate oxidation data; some of these possibilities are illustrated in Fig. 6. In addition, a random distribution of mycolyl substitution was possible.

In order to determine which of these patterns prevailed, mAGPP was methylated followed by replacement of the mycolyl groups with ethyl ethers, as outlined in Fig. 7. From our previous study, we knew that the per-O-methylated terminal pentaarabinosyl unit yielded Fragments IIIa, IVa, and Va when the permethylated arabinogalactan component was subjected to partial acid hydrolysis, NaB["H]", reduction, and pentadeuterioethylation (5). In the present instance, the 5-position of the t-Araf and the 2-linked Araf residues should be labeled with C₂H₅ or CH₃ groups, reflecting the presence or absence, respectively, of a mycolyl ester. This form of dual labeling should result in the generation of families (III, IV, and V) of products (Fig. 7), and the relative amounts of the four products within each family should reflect the mycolyl substitution patterns within the intact mAGPP.

The identity of the actual experimentally generated products was determined by GC/MS analysis. The GC retention time and mass spectra of products IIIa, IVa, and Va were already known (5). The remaining three members of each family were identified based on the fact that for every exchange of an ethyl for a methyl group, the retention time of a given alkylated oligoglycosylaliditol is increased. Also, the presence of alkyl groups results in an increase in the m/z value of some but not all of the mass spectral ions in a predictable fashion (Fig. 7).

Selected ion monitoring GC/MS analysis for the expected members of families III and IV is shown in Fig. 8A; ions at m/z 303, 317, 331, and 295 were selectively sought and monitored (see Fig. 7 for the origin of these ions). Product IVa was readily recognized from its retention time (16.0 min) and ion profile (m/z 303 and 295). Product IIIa was also recognized from its retention time (16.62 min) and ion profiles (m/z 303 and 295); these products were also identified by a direct comparison of the ion profiles and retention times to those of standards available from previous work (5). Product IVd was readily identified by its retention time (16.60 min; 0.60 min later than IIIa) and its characteristic ion profile (m/z 295 and 331). Product IIId was recognized by its retention time (17.25 min; 0.65 min later than IIIa) and its characteristic ion profile (m/z 295 and 331). The lack of appreciable amounts of products with a m/z 317 ion profile (Fig. 8A) was indicative of the absence of products IIIb, IIIc, IVb, or IVc. These results combined are consistent with the mycolyl substitution pattern shown in Fig. 6A.

Analysis of the products of family V is shown in Fig. 8B. Product Va, previously characterized (5), was identified based on its retention time of 16.10 min and ion profile of m/z 244 and 194.² Product Vd was identified by its retention time

² The closely related compound, labeled as 20 in the previous report (5), arises from internal segments of arabinan. The pentadeu- 
terioethyl groups found at C-2 on the terminal t-Araf's in product Va (compound 19 in Ref. 5) are instead found on C-6 in compound 20. Compound 20 elutes at 16.42 min and has the same ion profile as compound 19/Va.
FIG. 7. The application of chemical derivatization, partial fragmentation, and GC/MS analysis to identification of the mycolylated Araf residues within the nonreducing terminal pentasaccharide unit of mycobacterial cell walls. The results of this approach as applied to the mAGPP complex of M. tuberculosis is discussed in the text.

The generation of products IIIa, IVa, IIId, and IVd (Fig. 8A) and Va and Vd (Fig. 8B) (see also Fig. 7) unequivocally demonstrated that the mycolylated Araf residues are arranged as shown in Fig. 6A. Approximately two-thirds of the terminal nonreducing pentaarabinosyl units (Table I) are mycolylated in this fashion, and consequently about one-third of the terminal pentaarabinosyl units are free of mycolyl residues (Fig. 6A).

DISCUSSION

The architecture of the cell wall of mycobacteria is central to our understanding of the pressing biological questions of our day such as drug and solute impenetrability (18), antigenicity, and immunoreactivity, notably the issue of antigen processing and presentation by accessory cells (19), immune complex deposition and sequelae (20), and other aspects of immunopathogenesis, such as granuloma formation in tuberculosis (21), and persistence and disease recrudescence in leprosy and tuberculosis. In a spate of intensive fundamental investigations during the period 1950–1970 conducted largely by French (22) and Japanese (23) workers, the fundamental structural features of the bound mycolic acids (17), the peptidoglycan framework (24), and the arabinogalactan heteropolysaccharide (25) were elucidated, and the concept of immunologically active cell wall-bound protein (26) was developed. We recently returned to these topics, in abeyance for 20 years, still without applicable degradative enzymes but in possession of the highly applicable tools of partial depolymerization of per-O-alkylated polysaccharides (5, 27), high resolution NMR, and selective ion monitoring GC/MS (28).

From these efforts emerged the realization that: (i) all glycosyl residues within arabinogalactan, arabinose, and galactose alike, are in the furanose form (5, 29); (ii) the homogalactan segment consists of linear alternating 5- and 6-linked β-D-Galf residues (5); (iii) the bulk of the homoarabinan chains is composed of linear 5-linked α-D-Araf residues with branching introduced by the existence of 3,5-linked α-D-Araf units substituted at both branched positions with 5-linked α-D-Araf residues (5); (iv) the nonreducing terminal segments of arabinan are mostly comprised of pentaarabinofuranosyl motifs with the structure [β-D-Araf-(1→2)-α-D-Araf]5-3,5-α-D-Araf (5); (v) the arabinan chains are attached to the galactan core through the C-5 of some of the 6-linked Galf units (5); and (vi) the galactan of arabinogalactan in turn is linked to C-6 of some of the muramyl residues of peptidoglycan by a special diglycosylphosphoryl bridge with the structure, L-Rhap-(1→3)-D-GlcNAc-(1→P) (6). Thus, between the old and the new, a considerable body of structural definition has now been conferred on the cell wall core of mycobacteria. The last remaining fundamental structural question concerned the location of the mycolyl residues on the arabinogalactan entity.

With the earlier isolation of d-arabinosyl-5-mycolate (11–13), the terminal segments of arabinogalactan clearly became implicated as the primary binding site for cell wall mycolates. However, these observations predated the recent surge of knowledge on the primary structure of arabinogalactan and, in particular, the recognition of a pentaarabinofuranosyl arrangement as representing the majority of the nonreducing termini of the arabinan component of arabinogalactan. Present evidence now clearly states that these units are fully

(16.75 min; 0.65 min later than IVa) and ion profile (m/z 244 and 208). No evidence for the presence of products Vb and Vc was found; such products should have eluted at about 16.5 min and yielded both m/z 208 and 194 ions (see Fig. 7). The generation of products IIIa, IVa, IIId, and IVd (Fig. 8A) and Va and Vd (Fig. 8B) (see also Fig. 7) unequivocally demonstrated that the mycolylated Araf residues are arranged as shown in Fig. 6A. Approximately two-thirds of the terminal nonreducing pentaarabinosyl units (Table I) are mycolylated in this fashion, and consequently about one-third of the terminal pentaarabinosyl units are free of mycolyl residues (Fig. 6A).
The mycolic acid types chosen for inclusion in this structure are those that are predominant in *M. tuberculosis* (17). The actual location of individual mycolic acid types is arbitrary.

**Acknowledgments**—We gratefully acknowledge the skilled technical assistance of Kimberly Robuck. We also thank Marilyn Hein for preparation of the manuscript and Carol Marander for the graphics.

**REFERENCES**

1. Azuma, I., Ribi, E., Meyer, T. J., and Zbar, B. (1974) *J. Natl. Cancer Inst.* 52, 95-103

2. Melancon-Kaplan, J., Hunter, S. W., McNeil, M., Stewart, C., Modlin, R. L., Rea, T. H., Convit, J., Salgame, P., Mehr, V., Bloom, B. R., and Brennan, P. J. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85, 1917-1921

3. Brennan, P. J., Hunter, S. W., McNeil, M., Chatterjee, D., and Daffe, M. (1990) in *Microbial Determinants of Host Response* (Ayoub, E. M., Cassell, G. H., Rea, T. H., Convit, J., Salgame, P., Mehr, V., Bloom, B. R., and Brennan, P. J. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85, 1917-1921)

4. Gremp, M., and Brennan, P. J. (1990) in *Tuberculosis* (Youmans, G. P., ed) pp. 69-193, W. B. Saunders, Philadelphia

5. Daffe, M., Brennan, P. J., and McNeil, M. (1990) *J. Biol. Chem.* 265, 6734-6743

6. McNeil, M., Daffe, M., and Brennan, P. J. (1990) *J. Biol. Chem.* 265, 18200-18206

7. Hunter, S. W., McNeil, M., Modlin, R. L., Mehr, V., Bloom, B. R., and Brennan, P. J. (1989) *J. Immunol.* 142, 2864-2872

8. Hirschi, D. R., McNeil, M., and Brennan, P. J. (1990) *J. Bacteriol.* 172, 1005-1013

9. Hakomori, S. (1964) *J. Biochem.* (Tokyo) 55, 205-208

"D. Chatterjee, M. McNeil, and P. J. Brennan, *J. Biol. Chem.* submitted for publication."
Location of Mycolic Acids

10. Prehm, P. (1980) *Carbohydr. Res.* **78**, 372–374
11. Azuma, I., Yamamura, Y., and Fukushi, K. (1968) *J. Bacteriol.* **96**, 1885–1887
12. Azuma, I., Yamamura, Y., and Misaki, A. (1969) *J. Bacteriol.* **98**, 331–333
13. Kanetsuna, F., Imaeda, T., and Cunto, G. (1969) *Biochim. Biophys. Acta* **173**, 341–344
14. Draper, P. (1982) in *The Biology of the Mycobacteria* (Ratledge, C., and Stanford, J., eds) Vol. 1, pp. 9–52, Academic Press, London
15. Amar-Nacasch, C., and Vilkas, E. (1970) *Bull. Soc. Chim. Biol.* **52**, 145–151
16. Camphausen, R. T., McNeil, M., Jardine, I., and Brennan, P. J. (1987) *J. Bacteriol.* **169**, 5473–5480
17. Minniken, D. E. (1982) in *The Biology of the Mycobacteria* (Ratledge, C., and Stanford, J., eds) Vol. 1, pp. 95–184, Academic Press, London
18. Jarlier, V., and Nikaido, H. (1990) *J. Bacteriol.* **172**, 1418–1423
19. Kaufmann, S. H. E. (1988) *Curr. Opin. Immunol.* **1**, 431–440
20. Bjorvatn, B., Barnetson, R. S., Kronvall, G., Zubler, R., and Lambert, P. H. (1976) *Clin. Exp. Immunol.* **26**, 388–396
21. Dannenberg, A. M. (1989) *Rev. Infect. Dis.* **11**, Suppl. 2, 5369–5378
22. Lederer, E. (1975) *Mol. Cell. Biochem.* **7**, 87–104
23. Kotani, S., Kato, T., Matsuda, T., Kato, K., and Misaki, A. (1971) *Biken J.* **14**, 379–387
24. Wietzerbin-Falszpan, J., Das, B. C., Azuma, I., Adam, A., Petit, J.-F., and Lederer, E. (1970) *Biochem. Biophys. Res. Commun.* **40**, 57–63
25. Misaki, A., Seto, N., and Azuma, I. (1974) *J. Biochem. (Tokyo)* **76**, 15–27
26. Petit, J.-F., and Lederer, E. (1984) in *The Mycobacteria: A Sourcebook* (Kubica, G. P., and Wayne, L. G., eds) Part A, pp. 301–313, Marcel Dekker, Inc., New York
27. Valenti, B. S., Darvill, A. G., McNeil, M., Robertson, B. K., and Albersheim, P. (1980) *Carbohydr. Res.* **78**, 165–192
28. McNeil, M., Darvill, A., Aman, P., Franzen, L., and Albersheim, P. (1982) *Methods Enzymol.* **83**, 3–45
29. McNeil, M., Wallner, S. J., Hunter, S. W., and Brennan, P. J. (1987) *Carbohydr. Res.* **169**, 290–308
30. Hunter, S. W., and Brennan, P. J. (1990) *J. Biol. Chem.* **265**, 9272–9279
31. Winder, F. G. (1982) in *The Biology of the Mycobacteria* (Ratledge, C., and Stanford, J., eds) Vol. 1, pp. 353–438, Academic Press, London
32. Goren, M. B., Cernich, M., and Brokl, O. (1978) *Am. Rev. Respir. Dis.* **118**, 151–154