Structure of a Novel Sulfate-Containing Mycobacterial Glycolipid†

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ABSTRACT: We described previously the unusual structures of the two major C-mycoside glycopeptidolipids from *Mycobacterium fortuitum* biovar. *peregrinum*. More polar glycolipids, potentially more interesting in terms of antigenicity, were also present in the strains. A combination of FAB mass spectrometry, NMR, chemical analyses, and radiolabeling was successfully applied to these glycolipids to arrive at the unexpected and novel structure for the more polar compound. This consisted of the "orthodox" basic structure of the apolar C-mycosides, modified at the alaninol end by the presence of a sulfate group on position 2 of a 3,4-di-*O*-methylrhamnosyl residue. This novel and second class of sulfate-containing mycobacterial glycolipid may provide a chemical basis for the differentiation and classification of members of the *M. fortuitum* complex, the main group causing human diseases among the many fast-growing mycobacteria widely distributed in nature.

Interest for mycobacteria other than *Mycobacterium tuberculosis* has been renewed by the increased frequency of clinical infections caused by these organisms, notably in patients with acquired immune deficiency syndrome (Wood & Washington, 1987). Among the rapidly growing species, most of the strains causing human diseases are members of the *Mycobacterium fortuitum* complex (Good, 1985), so called because biovariants and subspecies have frequently been misidentified and with difficulty recognized as distinct species. Strains belonging to this complex are known to be resistant to most antituberculous drugs, and their response to other antimicrobials depends on the species and biovariant or subspecies (Good, 1985). Consequently, a precise identification of clinical isolates is needed.

Because most of the nontuberculous mycobacteria are endowed with highly immunogenic species- or type-specific antigens, many attempts were made to chemically characterize these antigens for purposes of identification and classification of the strains. This approach led to the recognition of three classes of specific mycobacterial antigens: phenolic glycolipids, glycopeptidolipids (GPLs),[1] and lipooligosaccharides (Brennan, 1988).

On the basis of their glycolipid content, strains belonging to the *M. fortuitum* complex can be divided into two groups of organisms (Tsang et al., 1984). *M. fortuitum* biovar. *peregrinum* and *Mycobacterium chelonae* contain alkali-stable C-mycoside GPLs whereas *M. fortuitum* biovar. *fortuitum* contains several alkali-labile lipids tentatively assigned to lipooligosaccharide-type antigens. Recently, we established the structures of the two major relatively nonpolar GPLs of *M. fortuitum* biovar. *peregrinum* and demonstrated the occurrence of an unusual distribution of the saccharide moieties in these C-mycoside GPLs (López Marín et al., 1991). Because the apolar C-mycoside GPLs are thought to be nonantigenic whereas the polar GPLs, although relatively minor compounds, are known to be highly antigenic and species- or type-specific (Brennan, 1988), we deliberately looked for more polar C-mycoside GPLs in the biovar. *peregrinum*. The present study describes the structure of the two more polar GPLs, the most polar being highly characterized by the presence of a sulfate group. FAB-MS combined with MIKE and NMR analyses was used for the structural elucidation of the glycolipids.

MATERIAL AND METHODS

Strain and Growth Conditions. Strains of *M. fortuitum* biovar. *peregrinum* obtained from the American Type Culture Collection (ATCC 14467) and from the Institut Pasteur—Paris (IP 111) were grown on Sauton's medium (Sauton, 1912) as surface pellicules in 250-mL glass flasks at 33 °C for 2 weeks. These pellicule growth conditions allowed an easy harvest of cells by pouring off the medium, while the pellicules remained attached to the flasks.

Lipid Extraction. Wet cells were extracted first with $CHCl_3/CH_3OH$ (1:2, v/v) for 4 days and then with $CHCl_3/CH_3OH$ (2:1, v/v). Pooled lipid extracts were partitioned in $CHCl_3/H_2O$ (1:1, v/v), and the chloroform phase was evaporated to dryness.

Purification of GPLs. Purified GPLs were obtained by chromatography on a Florisil column (32 × 3.5 cm, 60–100 mesh) of deacylated crude lipids. Successive elutions (200 mL each) with increasing concentrations of CH_3OH in $CHCl_3$ were done. Fractionations were monitored by TLC on silica gel-coated plates (G-60, 0.25 mm thickness, E. Merck, Darmstadt, Germany) developed with $CHCl_3/CH_3OH$ (90:10, v/v) or with $CHCl_3/CH_3OH/H_2O$ (30:8:1, 65:25:4, or 60:35:8, v/v/v). Glycolipids were visualized by spraying plates with 0.2% anthrone in concentrated H_2SO_4, followed by heating.

Miscellaneous Analytical Techniques. Alkaline deacylation of crude lipid extracts was performed according to Brennan and Goren (1979): lipids were suspended in $CHCl_3/CH_3OH$ (2:1, v/v) and incubated at 37 °C for 60 min with an equal volume of 0.2 M NaOH in methanol. The reaction mixture was neutralized with CH_3COOH, concentrated, and extracted

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[1] Abbreviations: 2D-COSY, two-dimensional chemical-shift correlated spectroscopy; FAB-MS, fast atom bombardment mass spectrometry; GC, gas chromatography; GC/MS, gas chromatography coupled to mass spectrometry; GPL, glycopeptidolipid; MIKE, mass-analyzed ion kinetic energy; TLC, thin-layer chromatography.

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with $CHCl_3$. The $CHCl_3$ phase was washed with H_2O, dried, and chromatographed on a Florisil column as described above.

Alkaline-induced β-elimination was done by incubating 2 mg of GPL in CH_3OH/C_6H_6 (8:2, v/v) containing 1 M KOH for 4 h at 70 °C. The mixture was neutralized with CH_3COOH and extracted with $CHCl_3$. The $CHCl_3$ phase was washed, dried, and analyzed by TLC to check the complete elimination of the saccharide moiety linked to the threonine residue.

Sugar Characterization. Sugars were routinely released from GPLs with a 1 M CF_3COOH solution at 110 °C for 1 h. The hydrolysates were then partitioned between $CHCl_3$ and H_2O. The aqueous phase was dried under N_2 and analyzed by TLC as previously described (López Marín et al., 1991). Their trimethylsilyl derivatives (Sweeley et al., 1963) were also analyzed by GC using authentic sugars derived from previously characterized GPL I and II (López Marín et al., 1991). GC/MS of the alditol acetate derivatives of the sugars was performed to confirm the location of the methoxyl groups.

The D or L configuration of the monosaccharides was determined after their de-O-methylation by boron trichloride (Bonner et al., 1960). The retention times on GC analysis of the trimethylsilyl derivatives of their (−)-2-butyl glycosides were compared to those of (−)-2-butyl L-rhamnoside and (±)-2-butyl L-rhamnosides (Gerwig et al., 1978; Sharp et al., 1984).

GC was performed on a Girdel G30 apparatus equipped with a fused silica capillary column (25 m length × 32 mm i.d.) coated with OV-1 (0.3 mm film thickness). A temperature gradient of 100–280 °C (2 °C/min) was used for the separation of silylated monosaccharides whereas isothermal conditions (150 °C) were chosen for the analysis of butyl glycosides. For GC/MS analyses, a Hewlett-Packard 5890 gas chromatograph equipped with a 12 m HP-1 column (Hewlett-Packard, Avondale, PA) was used. The oven temperature was programmed to hold at 80 °C for 1 min followed by a 15 °C/min rise to 290 °C.

Identification of the Amino Compounds. Amino compounds were identified after treatment of the GPLs with 6 M HCl at 110 °C for 16 h. The hydrolysates were partitioned between $CHCl_3$ and H_2O. The aqueous phase was concentrated and analyzed by TLC on precoated cellulose plates (Merck) with 1-butanol/acetic acid/water (4:1:1, v/v/v) as solvent. Thr was differentiated from *allo*Thr using the upper phase from a mixture of 1-butanol/water/acetone/ammonium hydroxide (8:6:1:1, v/v/v/v) as the developing solvent (Shaw & Fox, 1953). The hydrolysates deriving from previously characterized C-mycosides (López Marín, et al., 1991; Lanéelle & Asselineau, 1968) were used as standard compounds.

The amino acid configuration was determined on chiral high-performance plates (Merck 14285) developed with acetone/methanol/water (10:2:2, v/v/v) according to the method of Günther (1988). Spots were detected by spraying with 0.2% ninhydrin in acetone followed by heating.

The Lipid Constituents. The chloroform extracts from the CF_3COOH hydrolysis were further hydrolyzed according to Crombie (1955) with concentrated HCl/ethanol (1:4 v/v) in order to cleave the very stable fatty acyl–Phe amide bond. The ethyl esters resulting from the Crombie reaction were refluxed with a 5% KOH solution in methanol/benzene (8:2, v/v) for 8 hours. After acidification and extraction with ether, the free fatty acids were first converted into their methyl ester derivatives using diazomethane and then analyzed by GC.

Radiolabeling. A 1 mCi portion of (^{35}S)sodium sulfate was added per liter of Sauton's medium and the sulfate content of the medium was lowered by substituting half of the magnesium sulfate by magnesium chloride (Goren, 1970; Lemassu et al., 1991). Radioactivity was located using an automatic TLC linear analyzer (Berthold LB 2832).

Spectroscopy. Infrared spectra of film samples were recorded on a Perkin-Elmer FTIR 1600 spectrophotometer.

^1H-NMR spectra were obtained in $CDCl_3/CD_3OD$ (2:1, v/v, D% = 99.9) on a Brucker AM 300 WB instrument at 25 °C. ^{13}C-NMR spectra were obtained in $CDCl_3/CD_3OD$ on a Brucker WM200 apparatus using a Brucker J-Mod sequence. The chemical shift reference used was that of tetramethylsilane. The homonuclear 2D-COSY spectra were performed using the previously described pulse sequence (Daffé & Servin, 1989) with 256 × 1024 matrix data points over a spectral range of 2092 Hz; the matrix data points were expanded to 1024 × 2048 by zero-filling. A sine-bell window function was used. A total of 256 experiments with 160 accumulations for each one were done.

Mass spectrometry was performed on a ZAB-HS reverse-geometry mass spectrometer (VG Analytical, Manchester, UK). FAB spectra were generated by an 8 keV xenon atom beam. Samples were dissolved in $CH_3OH/CHCl_3$ (1:1, v/v). A 1-μL sample of this solution was mixed on the probe tip with 1 μL of *m*-nitrobenzyl alcohol, and in the positive mode, 1 μL of a 10% NaI or KI solution was also added. Ten scans of 10 s/decade were accumulated to obtain a spectrum. The resolution of the instrument was set to 1500. MIKE spectra were measured by electrostatic voltage scanning (800 eV/s), while the parent ion resolution was kept to 1500. Between 20 and 40 scans were stored and accumulated for each experiment.

GC/MS was performed on a Hewlett-Packard 5989A mass-selective detector connected to a Hewlett-Packard 5890 gas chromatograph. The mass spectrometer was set to scan from 50 to 600 atomic mass units.

RESULTS

Isolation of the Polar GPLs. When the crude lipid extract of *M. fortuitum* biovar. *peregrinum* was analyzed by TLC on silica gel sheets, several carbohydrate-containing lipids were resolved (Figure 1). In addition to the two major relatively nonpolar glycolipids, named compounds I and II (López Marín et al., 1991), two other more polar glycoconjugates were detected. A comparative TLC analysis of the intact and mild-base-treated lipid extracts showed that the four glycolipids remained unaltered, indicating that an acyl ester group was probably not present in these molecules. Consequently, the total crude extract was treated with mild alkali prior to its fractionation, thus permitting an easier purification of the glycolipids since all the alkali-labile glycolipids (acyltrehaloses, phospholipids) were lysed. Fractionation of the deacylated crude extractable lipids by chromatography on Florisil allowed the recovery of the major glycolipids I and II in the 5% methanol in chloroform fractions whereas the more polar compounds III and IV were eluted with 20 and 50% methanol in chloroform, respectively.

Comparative analysis of the IR spectra of the four glycolipids demonstrated that all belonged to the C-mycoside GPL family and showed characteristic bands of amide-I (ν 1629 cm^{-1}), amide-II (ν 1589 cm^{-1}), and N-H (ν 3274 cm^{-1}).

Fatty Acid, Amino Acid, and Sugar Composition of the Polar GPLs. Acid cleavage of GPL III and IV according to Crombie (1955) led to the identification of 3-methoxylated long-chain fatty acids containing 26 and 28 carbon atoms, identified by comparison of their retention times on a capillary GC column with those of the authentic samples deriving from

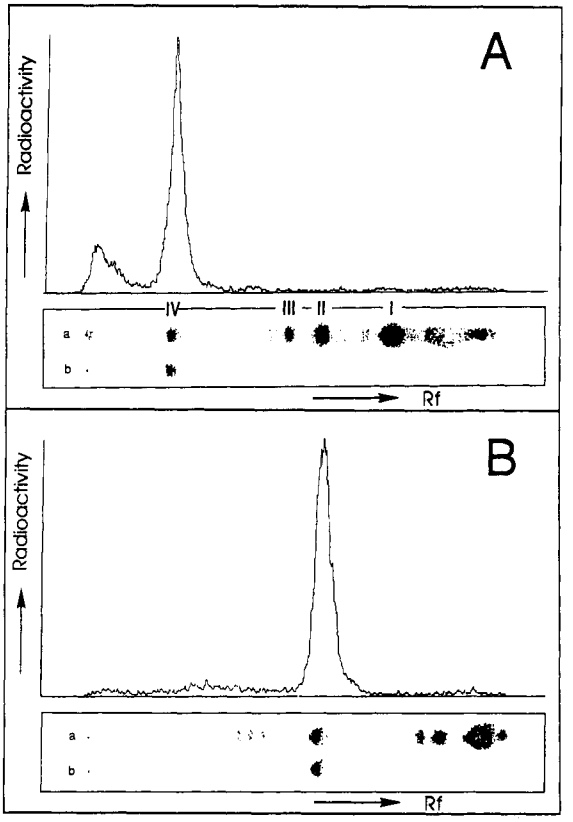

FIGURE 1: Radio-TLC of unfractionated lipids of *M. fortuitum* biovar. *peregrinum* (lane a) and of purified GPL IV (lane b) developed in chloroform/methanol/water 30:8:1 (A) and 60:35:8 (v/v/v) (B), I-IV refer to the different GPLs identified in the strains.

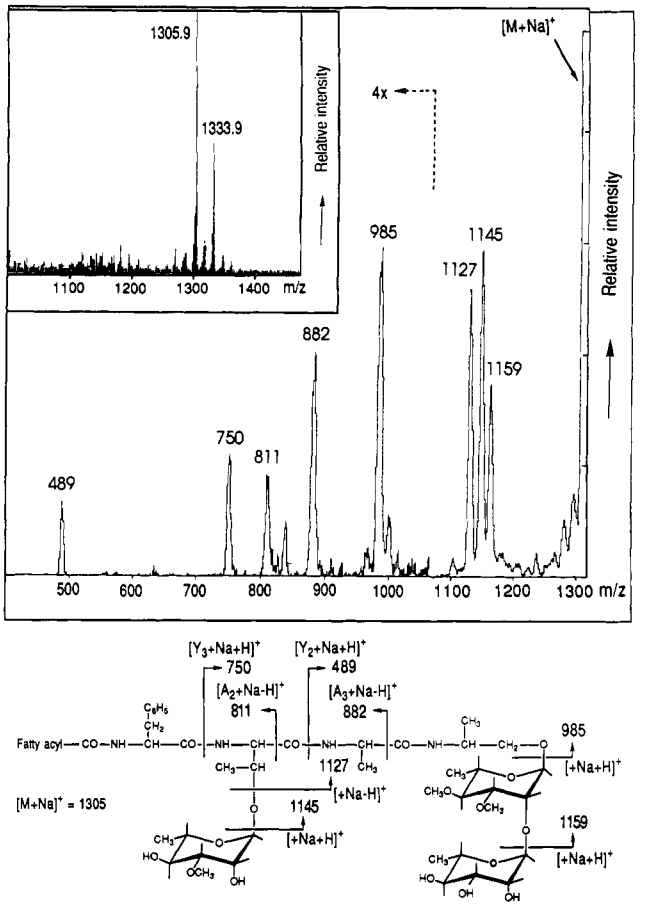

FIGURE 2: FAB mass spectrum (positive mode) of GPL III (inset) and FAB MIKE spectrum from the sodium cationized ion at m/z 1305 with the corresponding structure.

GPLs I and II (López Marín et al., 1991). Small amounts of nonmethoxylated, 3-hydroxylated homologues were also detected. Analysis of the ^{1}H-NMR spectra of the GPLs showed the presence of some double bond-containing molecules (multiplet at δ 5.2) commonly occurring in GPLs (Asselineau, 1991; Brennan, 1988). The amino acid compounds were identified as D-Phe, D-alloThr, D-Ala, and alaninol [the latter was assumed to belong to the L series, on the basis of the previous findings of Voiland et al. (1971)].

Mild acid hydrolysis (1 M CF_3COOH) released from GPL III three types of sugars identified as rhamnose, 3-*O*-methylrhamnose, and 3,4-di-*O*-methylrhamnose by GC/MS of their alditol acetate derivatives and TLC analysis. From acid treatment of GPL IV, the same partially methylated sugars, but not rhamnose, were identified. The series of the rhamnosides were determined by GC after O-demethylation of the sugars followed by the analysis of the TMS derivatives of the (±)-2-butyl glycosides. L-Rhamnoside was the only sugar constituent identified.

Structure of Mycoside GPL III. The FAB spectrum of mycoside III (Figure 2) showed two main sodium cationized molecule peaks at m/z 1305.9 and 1333.9 (Figure 2, inset). The 14 mass unit difference between this pair of ions and that of mycoside II (López Marín et al., 1991) suggested the presence of three sugar units on the peptidolipid core, with a difference in O-methylation. From the selected pseudomolecular ions, sequencing of the constituents was obtained by MIKE spectrometry. Both spectra exhibited a loss of 146 mass units, which was attributed to the loss of anhydrorhamnose (peaks observed at m/z 1159 and 1187, respectively). A loss of an anhydro disaccharide unit containing a rhamnosyl and a 3,4-di-*O*-methylrhamnosyl unit was also observed from the cationized mycoside III (Figure 2). The A and Y cleavages types (López Marín et al., 1991) established a conserved peptidolipid structure and the unusual location of the dirhamnosyl on the alaninol residue. This location was confirmed by the analysis of products deriving from the β-elimination of GPL III. Acid hydrolysis liberated rhamnose and 3,4-di-*O*-methylrhamnose from the β-eliminated GPL, but not 3-*O*-methylrhamnose. Indeed Phe, Ala, and alaninol, but not alloThr, were identified. As expected, an amino acid migrating as 2-aminobutyric acid was detected on the chromatogram instead of *allo*Thr. Thus, 3-*O*-methylrhamnose was linked to the allothreonine side chain in GPL III as previously demonstrated for GPLs I and II.

^{13}C-NMR analysis of GPL III confirmed the proposed structure in that the spectrum was found to be very similar to that of mycoside II (López Marín et al., 1991). The main difference was observed in the number of carbon resonance signals attributable to methoxyl groups (50–60 ppm) and to O-substituted sugar ring carbons (75–85 ppm). Four signals corresponding to methoxyl resonances (including that located on the lipid chain) and three substituted C3/4 signals were present in the ^{13}C spectrum of GPL III, instead of five and four for GPL II, respectively (López Marín et al., 1991).

The anomeric configuration of the three rhamnosyl units was determined by measuring the $J_{C1,H1}$ values, since the $^3J_{1,2}$ values are of no use in the *manno* series (Kasai et al., 1979). The $J_{C1,H1}$ values were found to be equal to 169 ± 1 Hz, establishing an α configuration for the three rhamnosides (Kasai et al., 1979).

It follows then that GPL III differs from GPL I and II only by the number of methoxyl groups located on the terminal rhamnosyl unit in the disaccharide (see Figure 2).

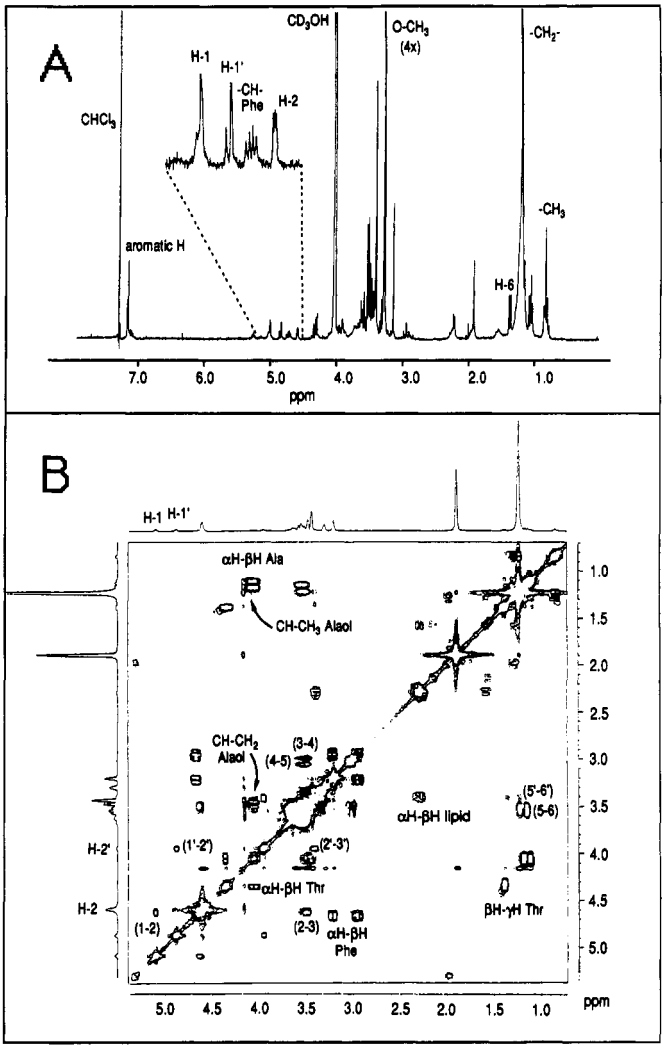

FIGURE 3: ^{1}H-NMR spectra of GPL IV; (A) 1D and (B) 2D-COSY spectra in $CDCl_3/CD_3OD$.

Structure of GPL IV. Evidence and Nature of the Additional Acidic Group in GPL IV. The most polar GPL was analyzed by ^{1}H-NMR spectroscopy (Figure 3) and FAB mass spectrometry (Figures 4 and 5). Both techniques showed that it contained only two rhamnosyl residues per molecule: two narrow doublets at δ 4.9 and 5.1 (1 H, each; Figure 3A) and intense pseudomolecular ion peaks at m/z 1261.8 and 1289.8 (Figure 4 inset). This result was unexpected in view of the chromatographic behavior of the compound which exhibited a significantly higher polarity than those of the major three-sugar-containing GPLs I and II (Figure 1). In fact, a two-sugar-unit, three-methoxyl-group-containing GPL might have an R_f similar to that of the so-called apolar GPLs, higher than those of GPL I and II. Consequently the presence of an additional polar group was suspected and its nature was investigated.

Comparison of the FAB mass spectra of GPL IV in the presence of sodium or potassium iodide showed a 32 mass unit shift of the pseudomolecular ion peaks instead of the 16 mass unit shift usually observed for previously analyzed GPLs (Daffé et al., 1983, López Marín et al., 1991). This data indicated the presence of two cations per molecule. In agreement with the above data, FAB mass spectrometry in the negative mode showed two pseudomolecular ions $(M - H)^-$ at m/z 1215.6 and 1243.7 (Figure 5, inset), i.e. 46 mass unit (2 Na) lower than in the positive mode (Figure 4, inset). The ^{1}H-NMR spectrum (Figure 3A) also supported the presence of a chemical

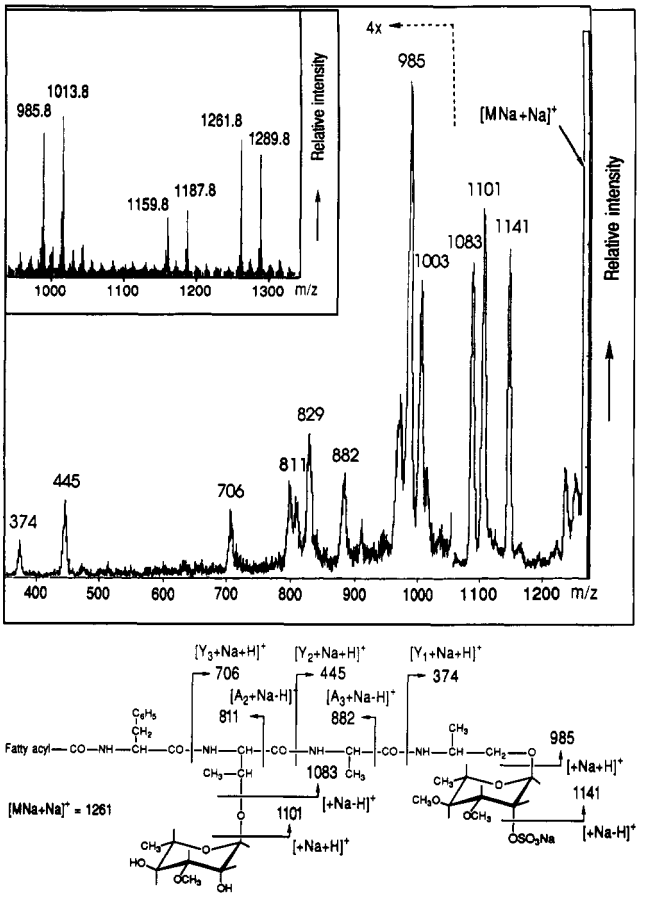

FIGURE 4: FAB mass spectrum (positive mode) of GPL IV (inset) and FAB MIKE spectrum from the sodium cationized ion at m/z 1261 with the corresponding structure.

group inducing the deshielding of a sugar ring proton as indicated by the observed multiplet at δ 4.6. As GPL IV was shown to be alkali-stable (see above), the deshielding was necessarily due to the presence of an alkali-stable polar substituent. The other deshielded multiplet at δ 4.8, also observed in the spectra of GPL I and II, was assigned to the signal resonance of the methine proton of Phe.

Additional structural data on the nature of the acidic substituent was obtained by the analysis of the FAB mass spectrum in the positive mode (Figure 4 inset), which showed the presence of fragment ion peaks at m/z 1159 and 1187, corresponding to the loss of 102 mass units from the pseudomolecular ion peaks. These fragment ion peaks were assignable to the loss of a sodium sulfite or phosphate group normally existing as its sodium salt in the corresponding pseudomolecular species (Dell & Thomas-Oates, 1989).

The nature of the anionic substituent was then determined by spraying the TLC plates with a phosphorus-specific reagent (Dittmer & Lester, 1964) and by radiolabeling the growing cells with ^{35}S. GPL IV was found to be labeled by ^{35}S (Figure 1) but was not revealed by the phosphorus reagent (data not presented).

Location of the Sulfate Group. The acidic group was located by 2D-COSY of the native GPL IV (Figure 3B), which showed a cross peak between the H1 resonance at δ 5.1 and a deshielded H2 resonance at δ 4.6. Analysis of the peracetylated GPL IV by 2D-COSY (data not shown) indicated no significant deshielding of the H3 and H4 resonances of the sulfated sugar residue; the sulfate was then located at position 2 of the 3,4-di-*O*-methylrhamnoside.

Sequencing of the Constituents of GPL IV. Structural data which established the sequence of the constituents of GPL IV

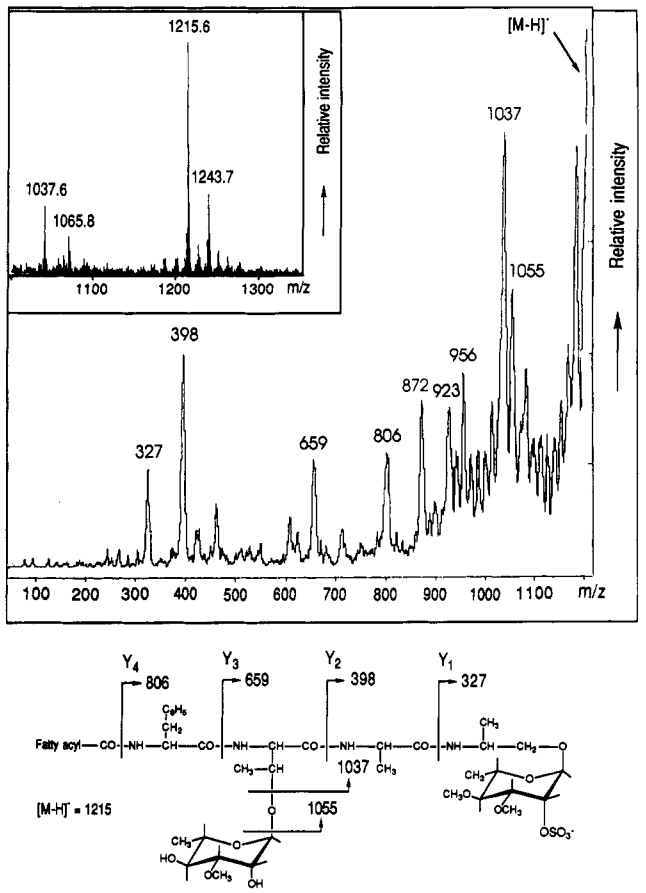

FIGURE 5: FAB mass spectrum (negative mode) of GPL IV (inset) and FAB MIKE spectrum from the ion at m/z 1215 with the corresponding structure.

were obtained by analyzing the MIKE spectra from the two pairs of pseudomolecular ions obtained by FAB using both positive and negative modes. Figure 4 shows the spectrum from the sodium-cationized ion at m/z 1261. The presence of fragment ion peaks at m/z 811, 882 (A-type fragments), and 985 indicated a conserved structure for the threonyl-linked monoglycosyl peptidolipid core (López Marín et al., 1991). The 260 mass unit difference between the Y-type fragments (m/z 706 and 445) and the loss of a mono-*O*-methylrhamnosyl unit, both as an intact and an anhydro sugar residue (m/z 1083 and 1101, respectively), supported the location of the 3-*O*-methylrhamnosyl residue on the *allo*Thr. These data suggested that the sulfated di-*O*-methylrhamnosyl unit was linked to the alaninol end. Indeed the successive losses of the sulfate group and of the sulfated di-*O*-methylrhamnosyl unit from the intact molecule (observed at m/z 1141 and 985, respectively), confirmed the proposed sequence. Furthermore, the observed Y_1-type fragment peak (m/z 374), absent from the previous MIKE spectra of neutral GPLs (López Marín et al., 1991), clearly indicated the location of the sulfated sugar residue.

These assignments were supported by the analysis of the MIKE spectra in the negative mode (Figure 5), which showed, as expected, only Y-type fragmentations from the peptidic backbone.

Anomeric Configuration of the Rhamnosides. All attempts to establish the anomeric configurations of the two rhamnosides by ^{1}H-NMR, including 2D-NOESY, failed probably due to the very low solubility of GPL IV in chloroform. Addition of methanol also failed to give the expected 1–2 or 1–3 and 1–5 intraring connectivities in the dipolar correlated spectra (Yu et al., 1986). Although the chemical shifts of H1s (δ 4.9 and 5.1) suggested an α configuration for the rhamnosides, further indications were needed. The $^3J_{1,2}$ values (ca. 1.5 Hz) were consistent with both anomers of rhamnosides. Consequently, GPL IV was analyzed by ^{13}C-NMR and the $J_{C1,H1}$ values were found to be $\geq$168 Hz, clearly establishing an α configuration for the two rhamnosides (Kasai et al., 1979).

DISCUSSION

The present study demonstrates that the more polar glycolipids of *M. fortuitum* biovar. *peregrinum* belong to the C-mycoside GPL family in that the same basic invariant core, fatty acyl-D-Phe-D-*allo*Thr-D-Ala-alaninol *O*-(3,4-di-*O*-methylrhamnoside), is present. They share with the GPLs previously isolated from the same species the presence of a 3-*O*-methylrhamnosyl unit linked to the *allo*Thr residue, a characteristic feature not found in GPLs isolated from other mycobacterial species (Asselineau, 1991; Brennan, 1988). More importantly, the novel structure established herein for the more polar GPL differs from all the previous ones by the occurrence of a sulfate group. To the best of our knowledge, this is the first report of a sulfated glycopeptidolipid in the microbial world.

Sulfated glycolipids are rarely encountered in bacteria (Kates, 1990). The first and extensively studied substances of this class were the sulfolipids of Goren, which are based on sulfated trehalose esterified by multi-methyl-branched long-chain fatty acids (for a comprehensive review, see Goren, 1990) and are confined in the tubercle bacillus. Although the presence of sulfur-containing substances has been reported in several mycobacterial species, including *Mycobacterium avium* and *M. chelonae*, they have been recognized only through incorporated ^{35}S and have been only minimally characterized (McCarthy, 1976; Tsukamura & Mizuno, 1981; Tsukamura et al., 1984). A survey of representative strains belonging to the above species showed that they were devoid of sulfated glycolipids (our unpublished work).

Recently, sulfate-containing lipooligosaccharides have been characterized in rhizobia, which specifically induced nodulation of leguminous plants (Lerouge et al., 1990; Roche et al., 1991a). Moreover, the sulfate group has been shown to be responsible for the host specificity (Lerouge et al., 1990; Roche et al., 1991b). In the case of mycobacteria, although a clear relationship between the presence of sulfated trehalose-containing glycolipids and virulence of tubercle bacilli has not been established, it has to be noted that these compounds possess biological activities of potential relevance for pathogenicity. For instance, they have been shown to inhibit the macrophage priming, a key step in host defense mechanisms (Pabst et al., 1988). In the case of the novel sulfated GPL, studies are in progress for evaluating its biological functions. Nevertheless, on the basis of the already demonstrated roles of GPLs in the inhibition of mitogens-induced proliferation of mononuclear cells (Brownback & Barrow, 1988), in mitochondrial oxidative phosphorylation, and in the drastic increase of passive permeability of membranes (Sut et al., 1990), it would be interesting to investigate the contribution of the sulfate group to such phenomena.

Strains of *M. fortuitum* biovar. *peregrinum* present serological cross-reactions with the two subspecies of *M. chelonae* (Tsang et al., 1984), suggesting that the implicated GPLs in these reactions share a common epitope despite their dissimilar TLC profiles. In the light of the present results and our recent data on *M. chelonae* (López Marín et al., 1992), the disaccharide α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-methyl-

α-L-rhamnopyranoside, common to both species, appears to be a good candidate for being responsible for the cross-reactions. Indeed, results of the serologic experiments in progress conducted on purified antigens isolated from both species and antisera raised against the whole cells will be of interest in establishing this point. Similarly, keeping in mind the difficulty in distinguishing members of the *M. fortuitum* complex, the antigenicity of the novel sulfated glycolipid as well as its specificity as a taxonomic marker within the mycobacterial genus is an important point to elucidate.

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