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Lipoteichoic Acids from *Streptococcus sanguis*

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Two lipoteichoic acids, membrane (MLTA) and wall (WLTA), have been purified from *Streptococcus sanguis* by Sepharose and Ecteola-cellulose column chromatographies and concanavalin A-conjugated Sepharose affinity column chromatography. The teichoic acids were homogenous as judged by disc gel electrophoresis, column chromatography, and double diffusion tests. Both MLTA and WLTA consisted of glycerol, phosphate, glucose, and fatty acids in the ratios of 0.95:1:0.71:0.046 and 0.99:1:0.79:0.023, respectively. α-Glycerol-phosphate was obtained by the partial acid hydrolysis of the lipoteichoic acids suggesting that their backbone structure consists of the glycerol moieties linked by 1,3-phosphodiester bonds. Both WLTA and MLTA form aggregates, perhaps due to micelle formation, in concentrated solution. The aggregate form of MLTA dissociates to a much greater extent than that of WLTA under similar conditions.

The presence of a lipid-teichoic acid complex in the bacterial membrane was first demonstrated by Wicken and Knox (13) in *Lactobacillus fermenti*. A similar lipoteichoic acid was subsequently isolated by Toon et al. (12) from *Streptococcus faecalis*. Although lipoteichoic acids have been reported in some other gram-positive bacteria (2, 11), the purity of these substances has not been extensively investigated. To establish the presence of lipid moieties in the teichoic acid molecules, we have employed affinity chromatography and Triton X-100 in the purification of lipoteichoic acids from *Streptococcus sanguis*. Two lipoteichoic acids, membrane and wall (MLTA and WLTA), have thus been purified to homogeneity as judged by their chromatographic and electrophoretic behavior and appear to be separate molecular entities. This is the first instance in which lipoteichoic acid has been shown to be present in bacterial cell walls.

MATERIALS AND METHODS

Bacterial strains. *Streptococcus sanguis* ATCC 10556 was obtained from the American Type Culture Collection. This strain was routinely maintained in blood agar.

Materials. Todd-Hewitt broth was obtained from Difco Laboratories, Detroit, Mich., and Sepharose 6B and concanavalin A-conjugated Sepharose 4B (con A-sepharose) were purchased from Pharmacia, Piscataway, N.J. [^{3}H]glycerol (2Ci/mmol) was purchased from International Chemical and Nuclear Corp., Irvine, Calif. Fatty acids were obtained from Applied Science Laboratories, Inc., State College, Pa. All other chemicals were obtained from commercial sources.

Methods. Glucose was determined by the method of Radin et al. (10), phosphate by the method of Chen et al. (4) and glycerol by the method of Burton (3). Paper chromatography was carried out with Whatman no. 1 filter paper by using the following solvent systems: (i) pyridine-ethylacetate-acetic acid-water (5:5:1:3); and (ii) butanone-acetic acid-water (75:25:10). Sugars and glycerol on paper chromatograms were detected with silver nitrate-sodium hydroxide (7) and organic phosphate with molybdate-perchloric acid (1). ^{3}H-labeled compounds were located with a Packard model 7201 Radiochromatogram Scanner, Packard Instrument Co., Inc., Downers Grove, Ill. Fatty acids were converted to their corresponding methyl esters with boron trifluoride-methanol according to Metcalfe et al. (6). Gas chromatography of the methyl esters was carried out on a Perkin-Elmer 900 gas chromatograph (Norwalk, Conn.) equipped with a hydrogen flame detector. The flow rates of N_2 and temperature were maintained at 20 to 30 ml/min and 175 C (Diethylene Glycol Succinator column) or 190 C (Castor wax column), respectively. The amount of fatty acids was determined by comparing the peak areas with that of the external standard. The peak area was measured by triangulation. Polyacrylamide disc gel electrophoresis was performed in 7% acrylamide gel at pH 8.9 (5), and lipoteichoic acid was detected either by staining the gel with periodate-Schiff's reagent (9) or counting sliced gel in triton-toluene scintillation fluid after NCS (Amersham/Searle Co., Arlington Heights, Ill.) treatment (8). The radioactivity of the ^{3}H-labeled compounds was determined with a Tri-Carb liquid scintillation spectrophotometer model 3320 in triton-toluene scintillation fluid consisting of (grams per liter): 2,5-diphenyloxazole, 5.5; dimethyl 1,4-bis-2-

(5-phenyloxazolyl) benzene (POPOP), 0.1; Triton X-100, 333 ml; and toluene, 667 ml. Rabbit antiserum was prepared with formalin-treated *S. sanguis* cells serving as the antigen. Details of this procedure will be published elsewhere. Lipoteichoic acids were deacylated by treating with toluene-methanol-2 N KOH (5:14:1) at 0 C for 90 min., and the K^+ was removed as $KClO_4$ crystals after neutralization with $HClO_4$.

Growth of culture. Glucose-supplemented Todd-Hewitt broth (Difco Laboratories) which was routinely used to grow the culture consisted of (grams per liter): infusion from 500 g of beef heart; Neopeptone, 20; glucose, 5; sodium chloride, 2; Na_2HPO_4, 0.4; and Na_2CO_3, 2.5. Two loopfuls of *S. sanguis* were each inoculated into two 2-liter Erlenmeyer flasks containing 1 liter of medium. The cultures were incubated overnight in a model G25 incubator shaker (New Brunswick Scientific Co., Inc.) at 35 C with shaking (100 rpm). These cultures were in turn used to inoculate 24 2-liter flasks each containing 1 liter of medium.

Two mCi of [^{3}H]glycerol (2mCi/mol) was added separately into four flasks to label the teichoic acids. After 16 h of shaking at 35 C the cultures were harvested by centrifugation at 8,000 × *g* for 10 min with a Sorvall RC2B centrifuge. The cells (approximately 40 g) were washed twice with cold saline and used for the preparation of lipoteichoic acids.

RESULTS

Extraction of lipoteichoic acids. The washed cells (40 g) were suspended in 100 ml of saline containing approximately 40 g of glass beads (5 μm in diameter). The cell suspension was divided into two batches which were sonically treated for 30 min in an ice-water bath with a sonifier cell disrupter (Heat Systems—Ultrasonics Inc., Plainview, L.I., N.J.). The sonicates were centrifuged at 31,000 × *g* for 20 min. The pellet was suspended in 80 ml of saline, sonically treated, and centrifuged again in the same manner. The procedure was repeated five times to minimize the possible contamination of membrane in the pellet. The pellet (crude cell wall) was used for the extraction of WLTA, which is described later. The preparation of MLTA is as follows. The supernatant fractions were combined (570 ml) and stirred with an equal volume of phenol at 4 C for 1 h. The mixture was centrifuged at 8,000 × *g* for 10 min, the aqueous phase was removed, and the combined phenol phases were again stirred at 4 C for 4 h with 500 ml of water. The aqueous phases were combined and dialyzed against 4 liters of cold water (4 C) with four changes of water over a total period of 3 days. The retentate was concentrated to 33 ml under reduced pressure at 35 C. A 2-ml amount of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (0.5 M, pH 8.0), 0.35 ml of 0.1 M $MgCl_2$, and 2 mg each of deoxyribonuclease (DNase) and ribonuclease (RNase) were added and the mixture was incubated at 37 C for 3 h. A 2-mg amount of trypsin and two drops of toluene were subsequently added and the incubation was continued for an additional 3 h. The resulting digest was then dialyzed overnight against 2 liters of cold water; the retentate was heated at 100 C for 5 min to inactivate the enzymes and was centrifuged at 31,000 × *g* for 20 min to remove the precipitated material. The supernatant fluid was concentrated to 17 ml (2.4×10^7 counts/min) and dialyzed 40 h against 4 liters of cold water with three changes of water, and the retentate was again evaporated to 6 ml. This material is designated crude MLTA. The crude cell wall was washed twice with cold saline, suspended in 100 ml of water, and extracted with an equal volume of phenol by using the same procedure described above for MLTA.

Purification of lipoteichoic acids. Sepharose column chromatography. The crude MLTA and WLTA were loaded separately onto a Sepharose 6B column (2.5 by 45 cm) equilibrated with 0.05 M Tris-hydrochloride (pH 7.5), containing 0.02% NaN_3 (buffer A), and eluted with the same buffer. The eluate was collected as 2.2-ml fractions after the first 31 ml of eluate emerged from the column. A 20-μliter portion of each fraction was counted.

The elution profile is shown in Fig. 1A and B. Fractions 21 to 47 of A and 16 to 36 of B were combined separately, concentrated, and dialyzed against 4 liters of water overnight.

Ecteola-cellulose column chromatography. The dialyzed Sepharose fractions of MLTA (1.8×10^7 counts/min) and WLTA (4.3×10^6 counts/min) were loaded onto an Ecteola-cellulose column (2.3 by 29 cm), washed with 200 ml of water, and eluted with a linear gradient of LiCl produced with equal volume of water and 1 M LiCl solution (pH 5.0) in the mixing chamber and reservoir, respectively. The elution profile is shown in Fig. 2A and B (in Fig. 2A, Triton X-100 was added to the eluants to the concentration of 1%). Fractions 30 to 52 of A and fractions 45 to 66 of B were combined separately. Triton X-100, if present, was removed by repeated extraction with an equal volume of toluene. Both fractions were concentrated and dialyzed overnight against 4 liters of water (total recovery: MLTA, 8.1×10^6 counts/min; WLTA, 9.4×10^5 counts/min).

Concanavalin A-Sepharose affinity column chromatography. Combined fractions from the Ecteola-cellulose column were applied to a concanavalin A-Sepharose 4B column (1 by

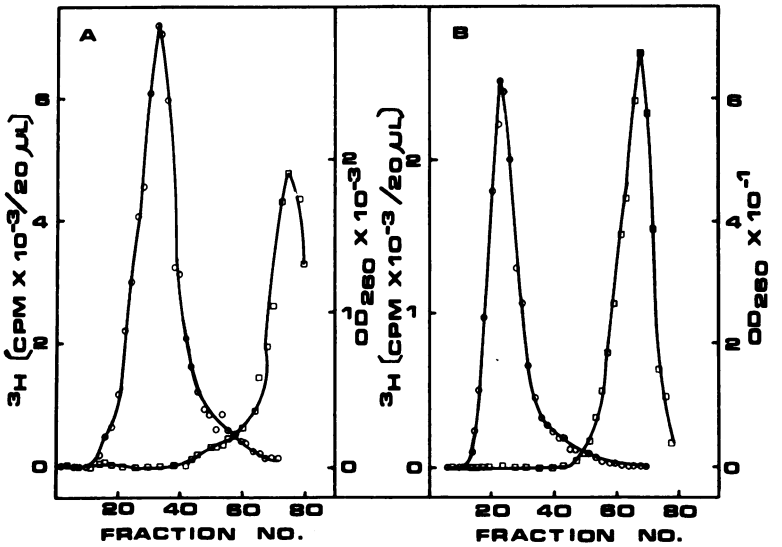

FIG. 1. *Sepharose 6B column chromatography. A 6-ml sample of crude membrane and wall lipoteichoic acids (MLTA, 2.4 × 10⁷ counts/min; WLTA, 7.7 × 10⁶ counts/min) were loaded separately onto a Sepharose 6B column and chromatographed as described in the text. Portions of each fraction were removed and counted in a scintillation counter (○). The absorbance at 260 nm was determined with a Beckman DBGT spectrophotometer (□). A, MLTA; B, WLTA.*

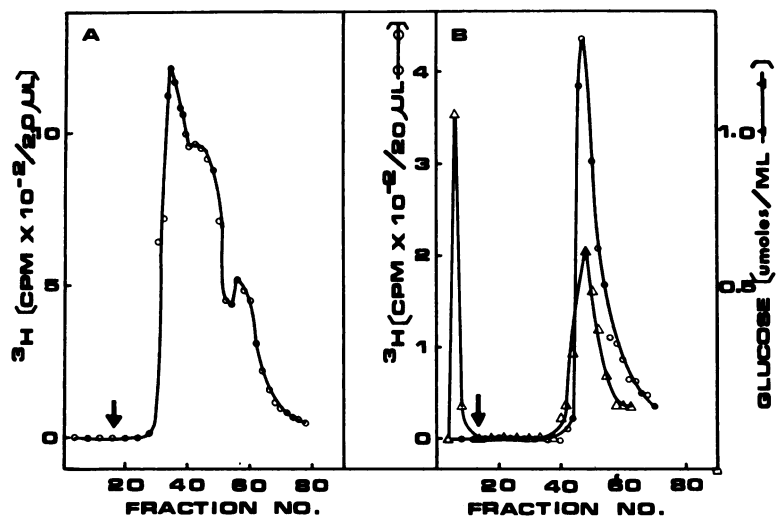

FIG. 2. *Ecteola-cellulose column chromatographic purification of membrane and wall lipoteichoic acids. The chromatographic procedures were carried out as described in the text. At the arrow, the LiCl gradient was initiated. Fractions of 7 ml were collected and portions were removed from every other fraction to determine the radioactivity (³H) (○) and glucose content (△), respectively. A, MLTA; B, WLTA.*

with 50 ml of buffer A. The column was then eluted in sequence with 30 ml each of buffer A containing 0.1 M α-methylglucoside in the absence and in the presence of 1% Triton X-100.

As shown in Fig. 3, about 30% of the radioactivity was not adsorbed by the column and very little radioactivity was eluted with α-methylglucoside in the absence of Triton X-100. Most of the radioactive compounds were contained in a sharp single peak after elution with α-methylglucoside in 1% Triton X-100. Fractions 37 to 41 (A) and 30 to 33 (B) were combined separately, extracted with toluene, and dialyzed overnight against 2 liters of distilled water. The recoveries were 4.2 × 10⁶ counts/min (MLTA) and 5.3 × 10⁵ counts/min (WLTA), respectively.

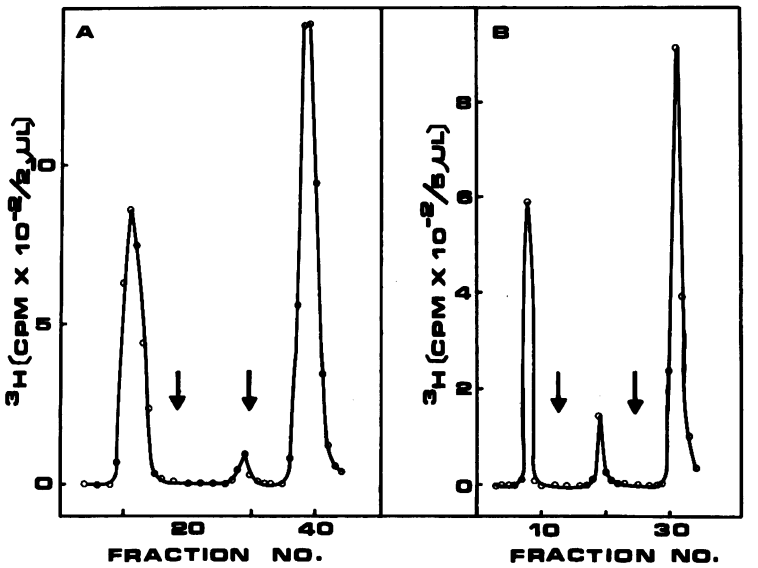

FIG. 3. *Concanavalin A-Sepharose 4B column chromatography. A 3-ml portion of MLTA (A) and WLTA (B) partially purified by Ecteola-cellulose chromatography were loaded onto a Con A-Sepharose column and chromatographed as described in the text. Fractions of 2 ml were collected and samples from each fraction were counted. At the first and second arrows, the elution buffer was changed to (a) buffer A containing 0.1 M of α-methylglucoside and (b) buffer A containing both 0.1 M of α-methylglucoside and 1% of Triton X-100, respectively.*

Second Ecteola-cellulose column chromatography. The combined MLTA from concanavalin A-Sepharose column chromatography was loaded again onto an Ecteola-cellulose column (2.3 by 29 cm), washed with 200 ml of water, and eluted with a linear gradient produced by 200 ml each of water and 1 M LiCl. After washing the LiCl solution away from the column with 500 ml of water, the column was subsequently eluted with a linear gradient of LiCl in 1% Triton X-100 as described above. As shown in Fig. 4, the lipoteichoic acid was further resolved into two fractions. The majority was eluted with LiCl only in the presence of Triton X-100. Fractions 28 to 35 were combined, extracted with toluene to remove Triton X-100, and dialyzed (2.2×10^6 counts/min recovered).

Characterization of lipoteichoic acid. The purified membrane lipoteichoic acid formed a single precipitin band against both concanavalin A (Fig. 5) and the antiserum prepared against formalin-treated *S. sanguis*, whereas two bands were detected when the same tests were applied to purified wall lipoteichoic acid. When both teichoic acids were subjected to acrylamide disc gel electrophoresis, WLTA migrated as a single band, and MLTA as two bands. In contrast to the latter, only a single band was detected after electrophoresis of deacylated MLTA. The slow-moving band was not observed (Fig. 6). The PAS-stained bands coincide with ^{3}H-peaks suggesting that both glucose and glycerol are present in the same molecule.

When MLTA (4.6×10^4 counts/min or 1.3 µmol in glycerol) was loaded onto the Sepharose 6B column and eluted with buffer A, nearly all of the radioactivity was eluted as a single symmetric peak at fraction no. 54 (Fig. 7). However, if large amounts of MLTA (3.6×10^6 counts/min or 100 µmol of glycerol) were loaded onto the same column, a single peak appeared much earlier, at fraction 29. This suggests that the lipoteichoic acid forms aggregates at high concentration. When small amounts of MLTA and WLTA (1.6×10^4 counts/min and 2.3×10^4 counts/min, respectively) were mixed and chromatographed on the same column (2.5 by 45 cm), the lipoteichoic acids were resolved into two peaks, one at fraction 25, the other at 54, in the relative quantities of approximately 1:3. When the same amount of WLTA was chromatographed in the same manner, two symmetrical peaks, roughly equal in size, were observed (Fig. 8). However, when larger quantities of WLTA were chromatographed, the majority appeared in the first peak. This result indicates that WLTA is less dissociable than MLTA. This was further confirmed by chromatographing lipoteichoic acid extracted from intact cells. As

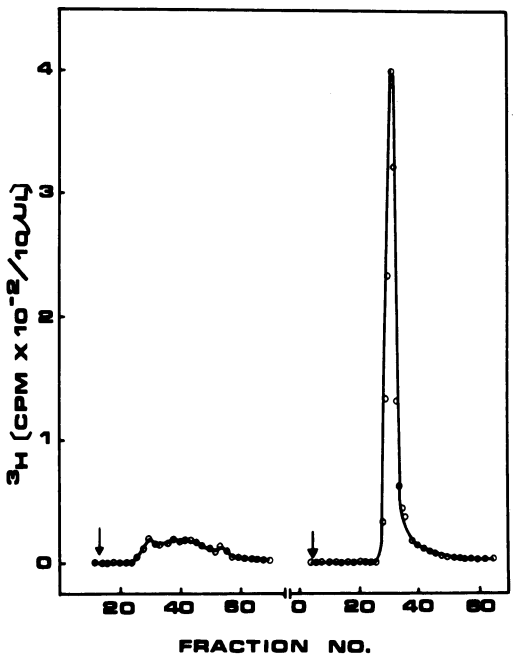

FIG. 4. *Second Ecteola-cellulose column chromatographic purification of membrane lipoteichoic acid. The lipoteichoic acid fraction obtained by Con A-Sepharose chromatography was chromatographed on an Ecteola-cellulose column as was described in the text. At the first arrow, a linear gradient of H_2O-LiCl (1 M) was started. The column was then washed with water to remove LiCl and a gradient of 1% Triton X-100-LiCl (1 M) in 1% Triton X-100 was initiated at the second arrow.*

shown in Fig. 9, the teichoic acids were resolved into two peaks, one at fraction 27, the other at 56. When the first peak, presumably a WLTA, was rechromatographed, the majority of lipoteichoic acid appeared in the first peak even though MLTA was completely dissociated at a much higher concentration. A similar phenomenon was observed when the first peak was rechromatographed (Fig. 9).

Both membrane (3.7×10^4 counts/min) and wall lipoteichoic acids (1.5×10^4 counts/min) were hydrolyzed with 2 N HCl at 100 C for 2 h and were evaporated to dryness in a vacuum desiccator containing NaOH pellets. The residues were dissolved in 0.1 ml of water and chromatographed in solvents A and B for 15 h (Fig. 10). Both teichoic acids yielded three similar radioactive peaks in solvent A with mobilities corresponding to glyceroldiphosphate (pk-2; Rglc, 0.18), α-glycerolphosphate (pk-3; Rglc, 0.51), and glycerol (pk-4; Rglc, 1.53), (A). A slow-moving peak (Rglc, 0.1), in addition to the three peaks with mobilities corresponding to those of glyceroldiphosphate (Rglc, 0.62), α-glycerolphosphate (Rglc, 1.2) and glycerol (Rglc, 3.1), was detected in solvent B (B). No amino acids or other ninhydrin-positive compounds were detected. Peak 2 of A can be further resolved into peaks 1 and 2 in solvent B. In both solvent systems, a spot with mobility equivalent to that of glucose was detected with silver nitrate-NaOH spray in both WLTA and MLTA hydrolysates. When peak 3 was eluted and rechromatographed in solvent B over 40 h, it migrated like α-glycerolphosphate and was well separated from β-glycerolphosphate (C). Glycerol was released from the eluates of peak 2 of B and peak 3 of A and B, upon alkaline phosphatase treatment (D).

These results indicated that the products of acid hydrolysis were indeed glyceroldiphosphate, α-glycerolphosphate, glucose, and glycerol. Since α-glycerolphosphate, but no β-glycerolphosphate, was released in acid hydrolysis, the glycerol moieties of both teichoic acids are probably linked by 1,3 phosphodiester bonds, which constitute the backbone of the teichoic acids.

When MLTA and WLTA, after repeated extraction with *n*-heptane to remove possible

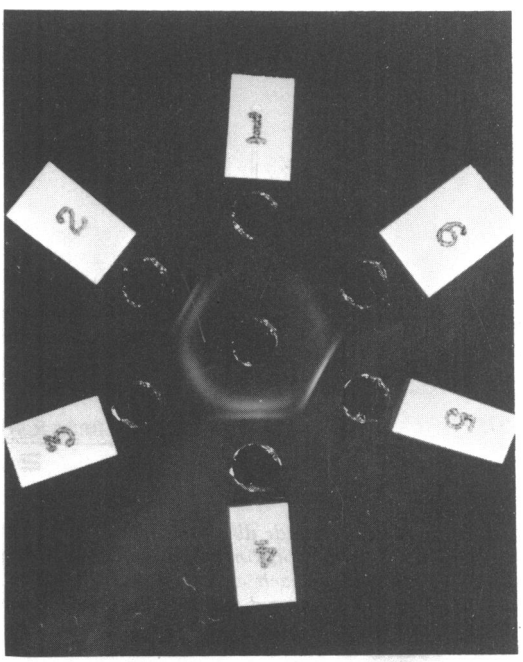

FIG. 5. *Double diffusion test of purified lipoteichoic acids. Center well, con A; 1, WLTA; 2, MLTA, partially purified by Con A-Sepharose chromatography; 3, MLTA, pk-1 of second Ecteola-cellulose column; 4, MLTA, the same as 2; 5, MLTA, purified; 6, diluted WLTA.*

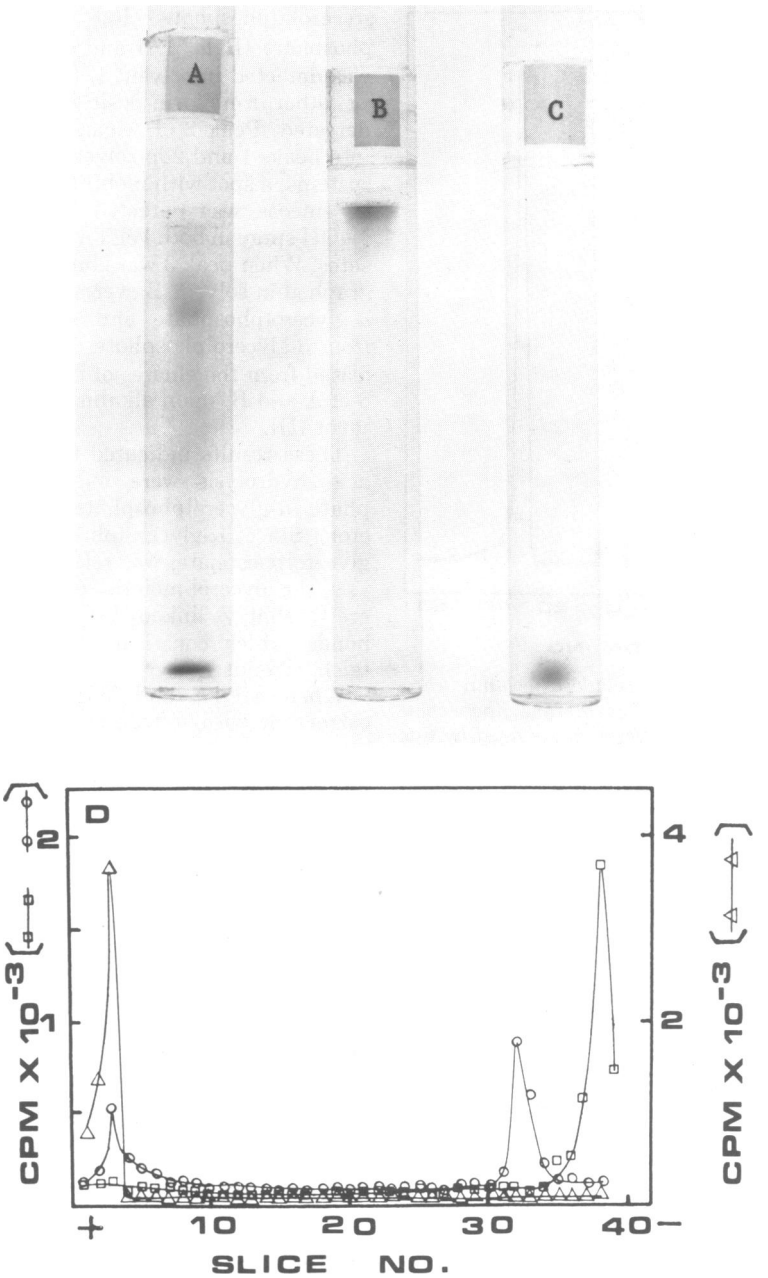

FIG. 6. *Polyacrylamide disc gel electrophoresis of the lipoteichoic acids. The electrophoresis was performed in 7% gel at pH 8.9. Approximately 20,000 counts/min of lipoteichoic acids and 60,000 counts/min of deacylated MLTA were used in each electrophoresis. A, MLTA, stained with PAS; B, WLTA, stained with PAS; C, deacylated MLTA, stained with PAS; D, gels were sliced and counted. Symbols: ○, MLTA; □, WLTA; △, deacylated MLTA.*

contaminant lipid, were esterified as described under Materials and Methods and subjected to gas liquid chromatography, six fatty acids, 14:0, 16:0, 16:1, 18:0, 18:1, and 18:2, were detected. The molar ratio of these fatty acids is shown together with the other components in Table 1. The ratio of glycerol to phosphate is approximately 1 in both teichoic acids. The glucose content is slightly lower than that of glycerol and phosphate, but is similar for both

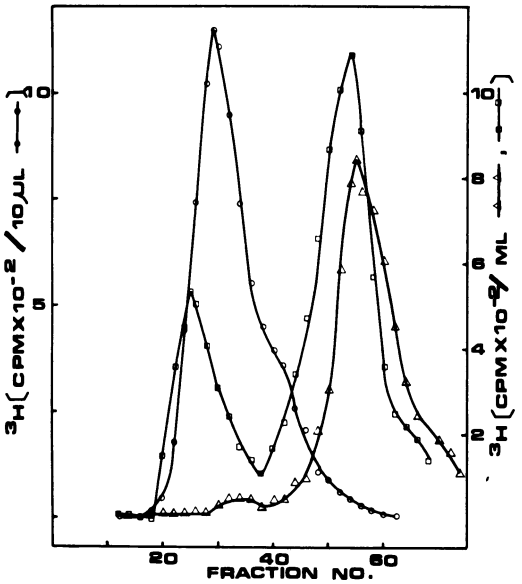

FIG. 7. *Sepharose 6B column chromatography of purified membrane and wall lipoteichoic acids. The column was eluted as was described in Fig. 1. The amounts of lipoteichoic acids loaded were:* (Δ) *4 × 10⁴ counts/min of MLTA;* (○) *3.6 × 10⁶ counts/min of MLTA;* (□) *1.6 × 10⁴ counts/min of MLTA + 2.3 × 10⁴ counts/min of WLTA.*

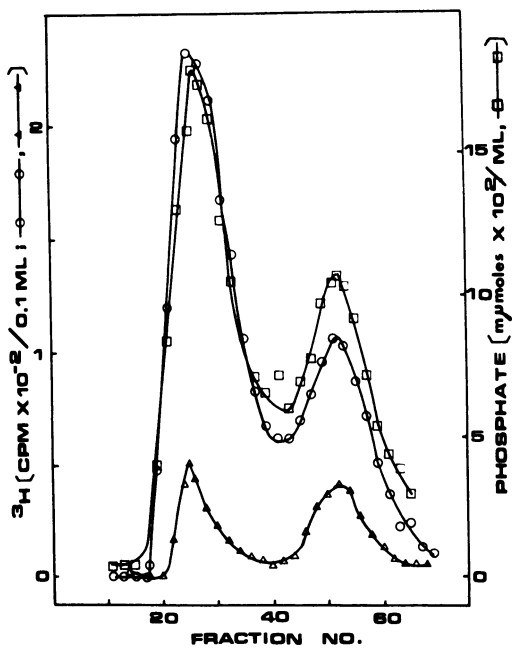

FIG. 8. *Sepharose column chromatography of wall lipoteichoic acid. The chromatographic procedures were identical to those described in Fig. 1. 2.3 × 10⁴ counts/min* (Δ) *and 3.4 × 10⁵ counts/min* (○, □) *of WLTA were loaded separately.*

teichoic acids. The fatty acid content of MLTA is 100% higher than that of WLTA.

DISCUSSION

Two lipoteichoic acids, MLTA and WLTA, have been isolated from cell membrane and cell wall. MLTA showed one precipitin band by double-diffusion tests against concanavalin A (Fig. 5) and antiserum. However, WLTA always showed two bands, one sharp and the other diffuse, by the same tests, perhaps due to an equilibrium between aggregated and dissociated forms.

Several lines of evidence suggest that fatty acids are covalently linked to the polymers. (i) The fatty acids are not extractable with heptane nor with chloroform-methanol (2:1). (ii) The majority of lipoteichoic acids are eluted only by the addition of Triton X-100 to the eluates after a certain stage of purification (Fig. 3 and 4). (iii) The chromatographic profile of the lipoteichoic acids always shows a peak with a long tail during purification. (iv) The lipoteichoic acids seem to form aggregates at high concentration and dissociate upon dilution (Fig. 7, 8, and 9). This is consistent with the two bands found upon polyacrylamide disc gel electrophoresis of intact MLTA as opposed to the single band

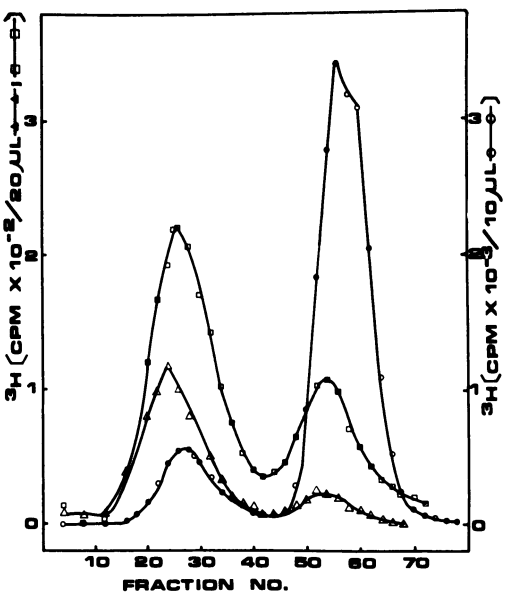

FIG. 9. *Sepharose 6B column chromatography of lipoteichoic acids extracted from intact cells. Crude lipoteichoic acid was loaded onto a Sepharose 6B column and eluted with buffer A* (○). *Fractions 20 to 38 were combined, dialyzed, concentrated to 3 ml, and loaded onto the same column and eluted in the same way* (□). *Fractions 20 to 38 were recombined and rechromatographed as above* (Δ).

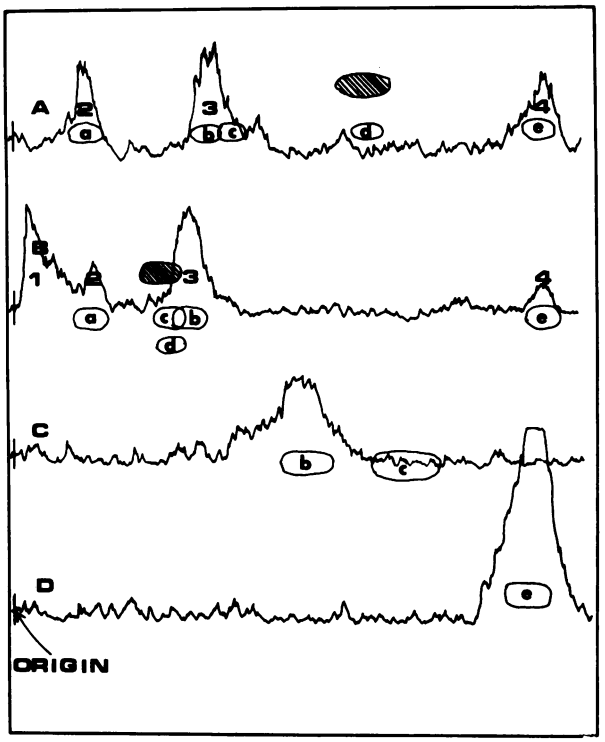

FIG. 10. *Paper chromatography of acid hydrolyzates of lipoteichoic acids. 5 × 10⁴ counts/min (³H) each of MLTA and WLTA was hydrolyzed in 2 N HCl at 100 C for 2 h and evaporated in a vacuum desiccator containing NaOH pellets. The residues were dissolved separately in 0.3 ml of water, spotted in duplicate onto Whatman no. 3 MM filter paper and developed separately in solvents A (A) and B (B). ³H-compounds were located with radiochromatogram scanner, and sugars by silver nitrate-NaOH (●). Similar profiles were obtained with both MLTA and WLTA. Peak 3 was eluted with water and rechromatographed in solvent B for 40 h (C). Eluates of peaks 2 and 3 were treated with alkaline phosphatase (E. coli) at pH 8.5 and rechromatographed in solvent B (D). (a) Glyceroldiphosphate; (b) α-glycerolphosphate; (c) β-glycerolphosphate; (d) glucose; (e) glycerol.*

TABLE 1. *Composition of membrane and wall lipoteichoic acids*

Components / Lipoteichoic acids	Phosphate (μmol)	Glycerol (μmol)	Glucose (μmol)	Fatty acids (μmol)						
				Total	14:0[a]	16:0	16:1	18:0	18:1	18:2
Membrane	1	0.95	0.71	0.046	0.002 (0.12)	0.017 (1)	0.009 (0.53)	0.002 (0.12)	0.015 (0.88)	0.001 (0.06)
Wall	1	0.99	0.79	0.023	0.003 (0.33)	0.009 (1)	0.006 (0.67)	0.005 (0.55)		

[a] Figures in the parentheses are the molar ratio of fatty acids with palmitic acid as 1.

obtained from deacylated MLTA under similar conditions (Fig. 6). The slow-moving band, presumably the aggregated form, was not detectable upon deacylation.

WLTA differs from MLTA in the following characteristics. (i) WLTA is larger than MLTA as judged by Sepharose column chromatography (Fig. 7) and disc gel electrophoresis (Fig. 6). Since both lipoteichoic acids are highly negatively charged, the slow-moving characteristics of WLTA in disc gel electrophoresis can be attributed to its size. (ii) MLTA is easily dissociable, while WLTA is only slightly dissociated under similar conditions (Fig. 7). The chemical basis for such differences is not known. Based on these differences it is concluded that WLTA is indeed present in the cell wall and is not due to the contamination of

membrane in our cell wall preparation. Due to long trailing of peaks in column chromatography, our recoveries of lipoteichoic acids are generally low. However, based on the ^{3}H-counts in the crude preparation and assuming 20% of the wet weight of cells as dry weight, the amount of lipoteichoic acids present in the cells may reach as high as 1.32 mmol of glycerol per 8 g (dry weight) of cells or 4.6% of the total dry weight.

Since α-glycerolphosphate is the only glycerolmonophosphate released by acid hydrolysis of MLTA and WLTA, and no glucose phosphate could be detected in the hydrolyzate, we propose that the backbone of the lipoteichoic acids consists of glycerolphosphate repeating units in which glycerol moieties are linked by 1,3 phosphodiester bonds. Both lipoteichoic acids react with concanavalin A, suggesting the presence of terminal glucose moieties. As shown in Table 1, the molar ratios between fatty acids and phosphate are 0.046 and 0.023, respectively, for MLTA and WLTA. If it is assumed that two fatty acids are covalently linked to a terminal glycerol moiety, the chain lengths for MLTA and WLTA are 43 and 87 glycerolphosphate repeating units, respectively.

Wicken and Knox (13) reported the presence of higher apparent molecular weight (HT) and lower apparent molecular weight (LT) forms in their membrane lipoteichoic acid extracted from *L. fermenti* with phenol. We have found that our MLTA could be present in two forms, perhaps equivalent to HT and LT, at appropriate cencentration. On the other hand, WLTA was always present in higher and lower molecular weight forms. We have not succeeded in separating these forms by repeated chromatography of WLTA on Sepharose 6B column. Perhaps the apparent two forms are due to the presence of an equilibrium between the aggregated and dissociated forms of WLTA.

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LITERATURE CITED

1. Bandurski, R. S., and B. Axelrod. 1951. The chromatographic identification of some biologically important phosphate esters. J. Biol. Chem. **193**:405–410.
2. Briles, E. V., and A. Tomasz. 1973. Pneumococcal forssman antigen, a choline-containing lipoteichoic acid. J. Biol. Chem. **248**:6394.
3. Burton, R. M. 1955. Glycerol dehydrogenase from *Aerobacter aerogenes*, p 397–400. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 1. Academic Press Inc., New York.
4. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
5. Davis, B. J. 1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. U.S.A. **121**:404–427.
6. Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. **38**:514–515.
7. Partridge, S. M. 1948. Filter-paper partition chromatography of sugars. I. General description and application to the qualitative analysis of sugars in apple juice, egg white and foetal blood of sheep. Biochem. J. **42**:238–250.
8. Paus, P. N. 1971. Solubilization of polyacrylamide gels for liquid scintillation counting. Anal. Biochem. **42**:372–376.
9. Price, W. H., H. Harrison, and S. H. Ferebee. 1964. Disc electrophoresis on polyacrylamide gels on serum mucoids of individuals with selected chronic diseases. Ann. N.Y. Acad. Sci. U.S.A. **121**:460–469.
10. Radin, N. S., J. R. Brown, and F. B. Lavin. 1956. The preparative isolation of cerebrosides. J. Biol. Chem. **219**:977–983.
11. Sharpe, M. E., J. H. Brock, K. W. Knox, and A. J. Wicken. 1973. Glycerol teichoic acid as a common antigenic factor in lactobacilli and some other Gram-positive organisms. J. Gen. Microbiol. **74**:119–126.
12. Toon, P., P. E. Brown, and J. Baddiley. 1971. The lipid-teichoic acid complex in the cytoplasmic membrane of *Streptococcus faecalis*. N.C.I.B. 8191. Biochem. J. **127**:399–409.
13. Wicken, A. J., and K. W. Knox. 1970. Studies on the group F antigen of lactobacilli: isolation of a teichoic acid-lipid complex from *Lactobacillus fermenti* NCTC 6991. J. Gen. Microbiol. **60**:293–301.