TURNOVER OF PHOSPHATIDYLGLYCEROL IN STREPTOCOCCUS SANGUIS

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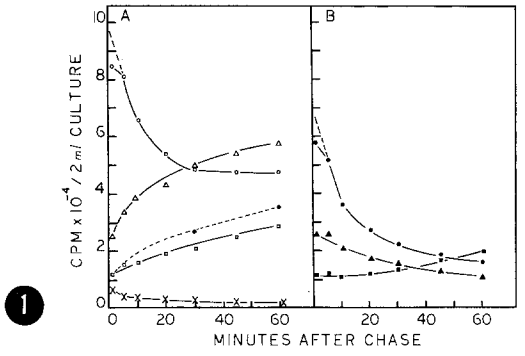
SUMMARY: When S. sanguis was pulsed with [2-^{3}H]glycerol for 10 min and chased for 60 min, 52% of the [^{3}H]lipids were chased into exocellular compounds (34%) in the culture medium and cold phenol-extractable cell bound complex saccharides (18%). The apparent rapid turnover of the lipid fraction was found to be due to phosphatidylglycerol. The phenol extractable compounds have been purified and characterized as lipoteichoic acids. The exocellular compounds were fractionated with a Bio-Gel P-2 column into three peaks (Pk's 1, 2 and 3, based on descending order of molecular size). Pk 1 was characterized as a lipoteichoic acid-like compound containing glycerolphosphate and possibly glucose; Pk 2 as a mixture of glycerolphosphate and a small molecular weight compound, and Pk 3 as glycerol.

The rapid turnover of phosphatidylglycerol has been reported in a wide variety of gram-positive and gram-negative organisms (1, 2, 3, 4). White and Tucker (4) found that the turnover rate of the unacylated glycerol and phosphate moieties of phosphatidylglycerol was three times faster than that of the diacylated glycerol in Haemophilus parainfluenzae. Subsequent studies in Escherichia coli showed that a portion of phosphatidylglycerol was incorporated into cardiolipin and the remainder possibly incorporated into nonlipid compounds (2, 5). More recently Van Golde et al. (6) reported that phosphatidylglycerol and/or cardiolipin serves as a precursor of water-soluble oligosaccharides containing glycerol, phosphate, glucose and succinic acid in E. coli. We have found in S. sanguis that phosphatidylglycerol can also serve as a precursor of both water-soluble exocellular compounds and cell-bound lipoteichoic acids. The lipoteichoic acids were composed of glycerol, phosphate, glucose and covalently linked fatty acids. The lipoteichoic acids have been found to be present in significant amounts in the cell wall and membrane of S. sanguis (7).

MATERIALS AND METHODS: The sources of S. sanguis, Todd-Hewitt broth and [2-^{3}H]glycerol were the same as previously reported (7). All other chemicals

were obtained from commercial sources. Polyacrylamide disc gel electrophoresis, preparation of toluene-Triton scintillation fluid, radioactive measurements and deacylation of lipids have been described (7). Thin layer chromatography was performed on Absorbosil-5 thin layer plates (0.25 mm thick) (Applied Science) in chloroform-methanol-water (65:25:4) (solvent A). The radioactive compounds on thin layer plates were located by dividing the silica gel on the plate into 1 cm segments which were scraped into scintillation vials and counted in toluene-Triton scintillation fluid. Reference compounds were detected with 50% H_2SO_4 (8). Paper chromatography was carried out on Whatman 3 MM paper and developed in either pyridine-water-ethyl acetate-acetic acid (5:3:5:1) (solvent B) or ethanol-1 M ammonium acetate (7:3) (solvent C). [^{3}H]labeled compounds were located with a Packard model 7201 Radiochromatogram Scanner. Sugars on paper chromatograms were detected with silver-nitrate sodium hydroxide (9), and organic phosphates with molybdate-perchloric acid (10).

In the pulse-chase experiments, a 25 ml mid-log phase culture of S. sanguis grown in Todd-Hewitt broth was pulsed for 10 min with 500 μCi of [2-^{3}H]glycerol (1 mCi/μM). Bacterial cells were collected on a Millipore filter, washed with 10 ml of culture medium (25°C), resuspended in 25 ml of fresh Todd-Hewitt broth supplemented with 0.1% cold glycerol, and incubated with shaking (150 rpm) at 37°C. At various time intervals, 2 ml aliquots of the culture were filtered on a Millipore filter and washed with 1 ml of the culture medium (25°C). The filtrate and wash were combined (filtrate fraction). The bacteria were suspended in 2 ml of distilled water. Eight-tenth ml was mixed with an equal volume of 90% phenol, stirred at 0-4°C for 60 min and centrifuged at 900 x g for 10 min. The aqueous phase was removed and the phenol phase washed with 1 ml of water and centrifuged again. The original aqueous layer and wash were combined (phenol extract). Another 0.8 ml of cell suspension was stirred with 20 ml of chloroform-methanol (1:1) at room temperature for 60 min. Eight ml of 0.85% NaCl con-



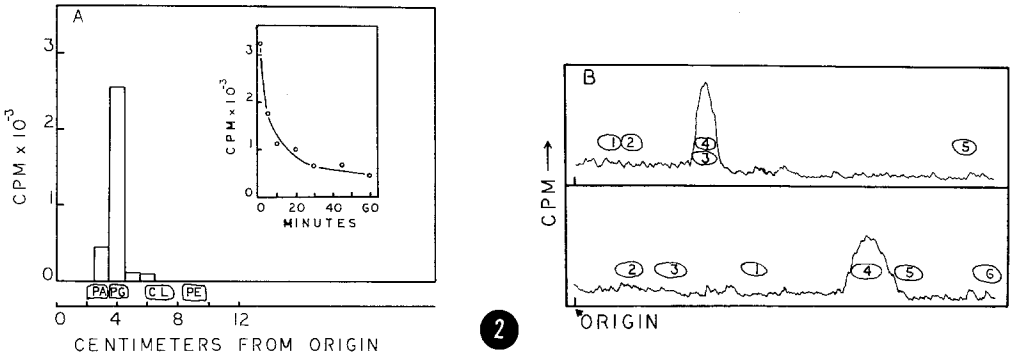


Fig. 1. A, The kinetics of incorporation of [2-^{3}H]glycerol into lipid and nonlipid compounds. S. sanquis was pulsed, chased and fractionated as described in Materials and Methods. The optical density at 620 nm of the culture increased from 0.34 to 0.63 during the 60 min chase. ○ , total lipid; △ , filtrate fraction; □ , phenol extract; X , saline fraction; ◆ , phenol extract plus Pk 1 (see figure 4). B, Silicic acid column chromatography of the total lipid. The total lipids of each time point were dried and fractionated on a silicic acid column (4 x 130 mm) as described in Materials and Methods. ▲ , neutral lipid; ■ , glycolipid; ● , phospholipid.

Fig. 2. A, Thin layer chromatography of [^{3}H]phospholipids from the 0 min chase period. PA, phosphatidic acid; PG, phosphatidylglycerol; CL, cardiolipin; PE, phosphatidylethanolamine. Insert, upper right, the kinetics of PG turnover based on thin layer chromatography. Thin layer chromatography was carried out as described in Materials and Methods. B, Paper chromatography of the mild alkaline hydrolysate of phospholipids. [^{3}H]phospholipids of 0 min chase were deacylated and chromatographed in solvent B (top) and C (bottom). 1, glycerolphosphorylglycerolphosphorylglycerol; 2, glycerol 1,2-diphosphate; 3, α-glycerolphosphate; 4, glycerolphosphorylglycerol; 5, D-glucose; 6, glycerol.

taining 0.01 M HCl was added. The mixture was vortexed, chilled in an ice bath and centrifuged. The aqueous phase was carefully removed, neutralized, evaporated to dryness in a flash evaporator and the residue dissolved in

1 ml of water (saline fraction). The chloroform layer was also evaporated to dryness in a flash evaporator, and the lipid was dissolved in 1 ml of chloroform (total lipid). Small portions of each fraction were removed, blown dry in scintillation vials with nitrogen, and counted.

RESULTS AND DISCUSSION: The kinetics of incorporation of [2-^{3}H]glycerol into lipid and nonlipid fractions is shown in fig. 1A. Sixty-seven percent of the [2-^{3}H]glycerol taken up during the 10 min pulse was found in the total lipid. A rapid turnover of lipid was observed following an initial 5 min lag. The lag was probably due to the presence of residual [^{3}H]lipid synthesis at the beginning of the chase. If the lipid turnover was extrapolated from the 5 min period back to 0 min, it was found that 52% of the [^{3}H]label in total lipid was chased into the phenol extract (18%) and the filtrate fraction (34%). When the total lipid was fractionated into neutral lipid, glycolipid and phospholipid with a silicic acid column according to the method of Lennarz and Talamo (11), 11, 27 and 62% of the [^{3}H]label appeared in the glycolipid, neutral lipid and phospholipid, respectively at the beginning of the chase. A decrease in the neutral lipid, during the chase, was accompanied by a corresponding increase in the glycolipid (fig. 1B). A seventy-four percent decrease of the [^{3}H]phospholipid was detected during the chase.

When the [^{3}H]phospholipids from each chase period were chromatographed on thin layer plates in solvent A, more than 95% of the labeled compounds had the same mobility as authentic phosphatidylglycerol. A representative profile is shown in fig. 2A. The amount of turnover of [^{3}H]phosphatidylglycerol based on thin layer chromatography was found to be 87% (fig. 2A). When the [^{3}H]phospholipids from 0 min chase were deacylated, and chromatographed on Whatman #3 MM paper in solvents B and C, a single [^{3}H]labeled compound with the same mobility as that of authentic glycerolphosphorylglycerol was detected in both solvents (fig. 2B). These findings indicate that the apparent rapid lipid turnover is due primarily to the turnover of phosphatidylglycerol.

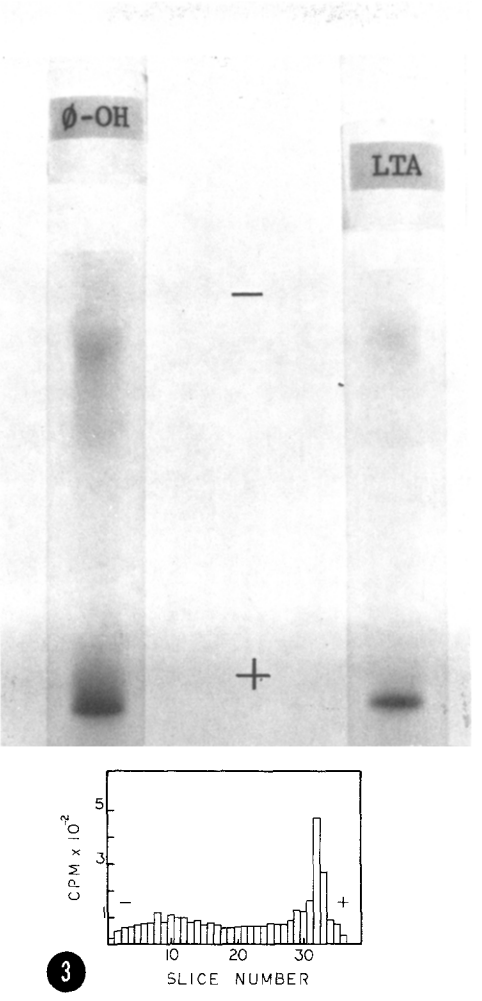


Fig. 3. Polyacrylamide disc gel electrophoresis of the phenol extract. Duplicate samples of purified phenol extract (ϕ-OH) were electrophoresed separately along with a membrane lipoteichoic acid (LTA) in 7% polyacrylamide gels. One gel of the phenol extract was sliced and counted (bottom) and the other gels stained with periodate-Schiff reagent (top) as described previously (7).

Neutral lipid was reported to be a precursor in the biosynthesis of glycolipids (11, 12, 13). Thus, the amount of turnover of neutral lipid is not enough to account for the synthesis of [^{3}H] labeled compounds in the phenol extract or the filtrate fraction in addition to glycolipids. Nucleotide sugars, like CDP-glycerol, should be present in the saline fraction. The amount of radioactivity in this fraction is very small compared with that in the phenol extract or filtrate fraction. Therefore, phosphatidyl-

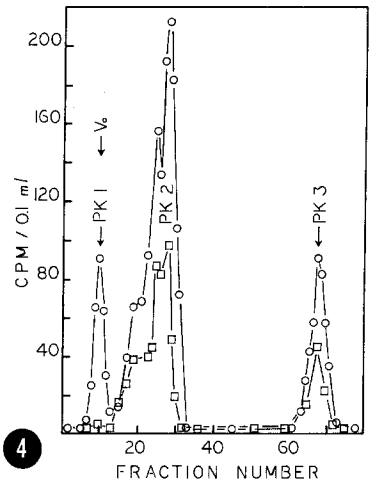


Fig. 4. Incorporation of [2-^{3}H]glycerol into the exocellular compounds. The filtrate fraction of each time point were chromatographed separately on a Bio-gel P-2 column. The effluent was collected in 0.3 ml fractions at a rate of 0.15 ml/min. ◘ , 5 min filtrate fraction; o , 60 min filtrate fraction; V_0, void volume.

glycerol is the only compound which contains sufficient radioactivity to account for the synthesis of [^{3}H]labeled compounds in the phenol extract and filtrate fraction.

The phenol extract was purified by Bio-Gel P-10 (2.5 x 45 cm) and Concanavalin A-Sepharose 4B column (1 x 48 cm) chromatography similar to that reported previously (7). An overall recovery of 60% was obtained. This purified phenol extract yielded one major and two minor periodate-Schiff positive bands in polyacrylamide gel electrophoresis (fig. 3). The bands coincided with the radioactive peaks. All except one minor band exhibited the same electrophoretic mobilities as those of the membrane lipoteichoic acid (fig. 3). The molar quantities of glycerol, glucose, phosphate and fatty acid (i.e. 14:0, 16:0, 16:1, 18:0, 18:1, and 18:2) are similar to those reported previously for membrane lipoteichoic acid (7).

The filtrate fraction of each time point were loaded separately on a Bio-Gel P-2 column (1 x 48 cm) and eluted with distilled water (fig. 4). Three distinct peaks, Pk 1, 2 and 3 on the basis of the descending order of

molecular size could be detected after 30 min chase. Only Pk 2 and 3 were detected between the 0 and 20 min chase. By paper chromatographic analysis, Pk 3 was identified as glycerol and Pk 2 as a mixture of glycerolphosphate and an unknown compound. The acid hydrolysate of Pk 1 yielded glycerol, glycerolphosphate and glyceroldiphosphate. The Concanavalin A-Sepharose 4B column chromatographic profile of Pk 1 was identical to that of purified membrane lipoteichoic acid (7). Pk 1 also formed a single band of identity with purified membrane lipoteichoic acid in a double diffusion test against Concanavalin A in agarose gel. These results suggest that Pk 1 is a lipoteichoic acid-like compound possibly derived from the cell bound lipoteichoic acid. Based on the kinetics of incorporation of [2-^{3}H]glycerol into both cell bound lipoteichoic acid and Pk 1, as shown in fig. 1, it appears that only the turnover of phosphatidylglycerol is great enough to account for the biosynthesis of these compounds. These results, though indirect, suggest that phosphatidylglycerol is a precursor of cell bound lipoteichoic acid and its exocellular derivative, Pk 1. The exocellular glycerol (Pk 3) and glycerolphosphate (in Pk 2) formed during the pulse-chase experiment probably resulted from the degradation of phosphatidylglycerol.

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