LOCALIZATION OF GLYCEROL PHOSPHATE IN MESOSOMAL VESICLES OF STAPHYLOCOCCUS AUREUS

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SUMMARY: A major difference in chemical composition between purified mesosomal vesicles and plasma membranes of Staphylococcus aureus has been found. Approximately 15 to 20 % of the dry weight of mesosomal vesicles was glycerol while only 5 % was found in plasma membranes. Most of the glycerol in mesosomal vesicles was phosphorylated, with a glycerol-to-phosphate ratio of one. The glycerol phosphate was not associated with the lipid soluble fraction and could not be removed with any of the organic solvents used for lipid extraction. The data suggest that the glycerol phosphate is in the form of glycerol teichoic acid ("intracellular teichoic acid") which up to now has been associated only with the plasma membrane fraction of Gram-positive bacteria.

INTRODUCTION: Morphologically, the most distinctive features of Gram-positive bacterial membranes systems are the mesosomes. The structure of these unique organelles, which usually arise from the membranous septum preceding the cross-wall (1), have been described in detail in several bacteria (see review, 2). In contrast, their functional role in the cell and relationship to the plasma membrane is still unknown. Using plasma membranes as a basis for comparison, our approach was to characterize the chemical composition of mesosomes and attempt to correlate these findings to a possible function.

Staphylococcus aureus membranes consist mostly of protein and lipid (75 to 80 %) (3); however, the ratio of protein to lipid in plasma membranes was 2.24:1 whereas in mesosomal vesicles it was 1.21:1 (4). The higher concentrations of lipid (4) and fatty acids (5) we found previously in mesosomal vesicles led us to investigate these differences further. Here we report a major difference in glycerol phosphate content between the two membrane fractions. Approximately 15 to 20 % of the dry weight of mesosomal vesicles is glycerol while only 5 % is found in plasma membranes. The data indicate that the increased levels of glycerol phosphate in mesosomal vesicles is present as glycerol teichoic acid.

MATERIALS AND METHODS: Mesosomal vesicles and plasma membranes were isolated from S. aureus ATCC 6538P after protoplasting by differential and sucrose density gradient centrifugation (4). Purified cell walls were prepared by the method of Huff et al. (5).

Polyols, sugars and glycerol were determined by gas chromatography after acid hydrolysis. Hydrolysis for 1 hour in 2 N HCl at 100 C under argon was optimal for the release of glucose and ribitol. A 4 day hydrolysis at 100 C was optimal for the release of anhydroribitol and total glycerol. Total glycerol was also determined after various stages of hydrolysis by treatment with alkaline phosphatase (chicken liver phosphatase, Worthington Biochem. Corp., Freehold, N. J.) for 3 hours at 37 C. After hydrolysis, standards of mannitol, 6-deoxy-glucose and 2-hydrolylymethyl-2-methyl-1,3-propanediol were added, the mixture passed through a Dow (H^+) and Amberlite IRA 410 (acetate), and a stream of dry argon passed over the sample at 37 C until just dry. Alditol acetates were prepared by reduction with sodium borohydride and acetylation with acetic anhydride and pyridine (7). Samples were run in a Perkin Elmer 900 gas chromatograph using 3 % ECNSSN on Chrom Q (Applied Science Laboratories) in an 8 foot by 1/8 inch stainless steel column programmed from 140 to 200 C at 2.5 degrees/minute.

Glycerol was also determined enzymatically following neutralization of the acid hydrolyzed samples (Glycerol Stat-Pack, Calbiochem., LaJolla, Calif.). Inorganic and total phosphates were deterimed by the method of Ames (8).

RESULTS AND DISCUSSION: Gas chromatographic analysis on the glycerol content of purified mesosomal vesicles and plasma membranes is shown in Table 1. Approxi-

TABLE 1

Gas chromatographic analysis for glycerol, sugars and polyols in mesosomal vesicles, plasma membranes and cell walls of Staphylococcus aureus

	Percent of Dry Weight		
	Mesosomal Vesicles	Plasma Membranes	Cell Walls
Glycerol[a]	15-20	5.4-5.5	1.1
Anhydroribitol[a]	0.38	0.41	4.8
Ribitol[b]	0.43	0.32	2.0
Glucose[b]	0.91	0.90	0.14

[a] 4 day acid hydrolysis

[b] 1 hour acid hydrolysis

mately 15 to 20 % of the dry weight of mesosomal vesicles consisted of glycerol. This concentration of glycerol was three to four times greater than that found in the plasma membranes. Glucose, ribitol and anhydroribitol were also present,

but each to an extent of 1% or less. Similarly analyzed cell walls contained only 1 % glycerol. These results also verified that S. aureus ATCC 6538P cell walls contain a ribitol teichoic acid (9, 10).

Of particular interest was the pattern of glycerol release from the two membrane fractions (Fig. 1). Initially, there was a rapid release (first 6 hours)

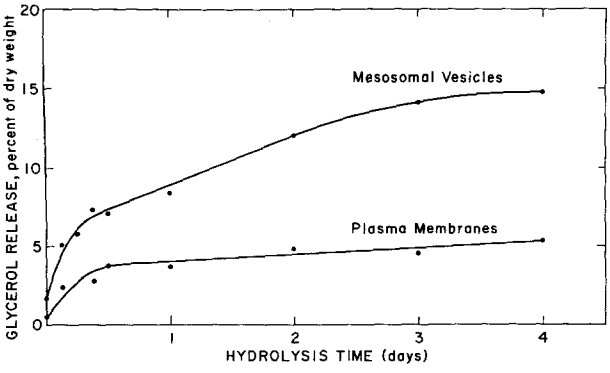

Fig. 1 Gas chromatographic analysis of glycerol released during acid hydrolysis of mesosomal vesicles and plasma membranes of Staphylococcus aureus.

which accounted for 80 % of the plasma membrane glycerol and 40 % of the mesosomal vesicle glycerol. This was followed by a much slower rate of release which was complete after 4 days of hydrolysis. Such resistance to hydrolysis might be expected to occur if glycerol were present in a phosphorylated form. On further analysis, it appeared that most of the glycerol in mesosomal vesicles and plasma membranes was present as glycerol phosphate (Fig. 2). In these experiments the the glycerol determined by gas chromatography was also verified by enzymatic analysis. The release of inorganic phosphate (Pi) paralleled the release of glycerol and throughout the hydrolysis an equimolar ratio of glycerol to Pi was found. Of the total glycerol detected in mesosomal vesicles (1.8 μmoles/ mg dry weight), 1.5 μmoles appeared to be phosphorylated. Similarly in plasma membranes, which contained only one-third to one-fourth of the glycerol present in mesosomal vesicles, the glycerol was also phosphorylated. The possibility

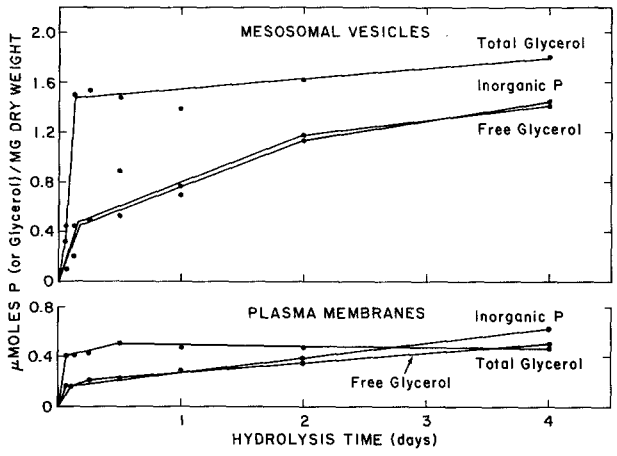

Fig. 2 Release of total glycerol, free glycerol and inorganic phosphate during acid hydrolysis of mesosomal vesicles and plasma membranes of Staphylococcus aureus. Total glycerols determined after alkaline phosphatase treatment of acid hydrolyzed samples.

that the Pi released during acid hydrolysis was due to contamination by nucleic acids and ribosomes was not likely since the two membrane fractions were treated with DNase, RNase and purified on sucrose gradients. Also, the purity of the mesosomal and plasma membranes was routinely monitored by electron microscopy.

A further verification that most of the glycerol present in mesosomal vesicles was phosphorylated is also shown by the results in Fig. 2. Approximately 85 % of the total glycerol found after 4 days of hydrolysis could be detected after a 3 hour acid hydrolysis if the samples were treated with alkaline phosphatase. This amount of glycerol approximated the free glycerol and Pi released after 4 days of hydrolysis. Likewise in plasma membranes, over 90 % of the total glycerol could be detected after a 3 hour hydrolysis and phosphatase treatment. The above results were also obtained under conditions of alkaline hydrolysis with 1 N NaOH.

The phosphorylated forms of glycerol known to occur in S. aureus membranes are; two major phospholipids (phosphatidyl glycerol, glycerol:Pi = 2:1; and cardiolipin, glycerol:Pi = 1.5:1) and to a lesser extent phosphatidyl glucose (glycerol:Pi = 1:1), all of which are present in the lipid fraction (11,12); and, glycerol teichoic acid (glycerol:Pi = 1:1) which is not extractable with

lipid solvents (13). To ascertain what percentage of the glycerol was associated with the lipid fraction of mesosomal vesicles and plasma membranes, we extracted the lipids with butanol-pyridine-acetic acid (100:22:31) and chloroform-methanol (2:1) and analyzed for glycerol and Pi following acid hydrolysis and phosphatase treatment. The glycerol content of the lipid soluble fraction of mesosomal vesicles and plasma membranes was 0.6 and 0.25 μmoles/ mg dry weight; respectively, with a glycerol to Pi ratio of 1.7:1. Assuming there was an equal mixture of the two major phospholipids in each membrane fraction, the glycerol to Pi ratio we obtained was in close agreement with the expected values. This data also substantiated our earlier findings pertaining to the increased lipid and fatty acid content we found in mesosomal vesicles (4,5). The most important aspect of this study; however, was the amount of glycerol present in the non-lipid fraction of the mesosomal vesicles. Of the total glycerol found (1.8 μmoles/ mg dry eight, Fig. 2), 1.2 μmoles could not be extracted by routine lipid extraction procedures whereas only 0.2 μmoles was left in the defatted plasma membranes. The glycerol to Pi ratio for the two membrane fractions was one. The data strongly suggests that the glycerol phosphate present in mesosomal vesicles after the lipid has been extracted may be in the form of glycerol teichoic acid (also referred to as intracellular teichoic acid, membrane teichoic acid or lipoteichoic acid). This is quite unique since up to now glycerol teichoic acid of non-wall fractions has been reported only in the plasma membrane fraction of Gram-positive bacteria (13-17). This apparently erroneous situation may be explained by a general failure to utilize reported techniques for isolation and separation of purified plasma membranes and mesosomal fractions (1,4,18,19); and the consequent failure to recognize that, depending on centrifugal forces used, mesosomal vesicles were either discarded in large part in the supernatant fraction or were included in differing amounts in what was analyzed as the plasma membrane fraction.

Although it is now believed that all of the non-wall glycerol teichoic acid occurs as lipoteichoic acid (15,16), proof that the non-lipid glycerol phosphate of mesosomal vesicles is glycerol teichoic acid will depend upon the isolation

and identification of diglycerol triphosphate after alkaline hydrolysis of this material (20,21).

It has been proposed that the glycerol teichoic acid functions as a sequestering agent of Mg^{++} which are needed in high concentrations for the activation of enzymes involved in membrane and cell wall biosynthesis (22,23). Ideally, a mesosomal site would be the most suitable for this type of function since, these organelles arise from the membranous septum and precede the formation of crosswall.

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