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The interrelation of polar lipids in bacterial membranes

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SUMMARY

Bacillus subtilis W23 and *Bacillus cereus* T, grown under conditions of apparent phosphate starvation approaching stationary phase of growth, accumulate diglucosyl diglycerides while the proportion of phosphatidylethanolamine is reduced. The latter organism also produces under these conditions an acidic glycolipid which apparently partially replaces the acidic phospholipids, phosphatidylglycerol and diphosphatidylglycerol.

The membranes of bacteria contain both acidic polar lipids such as phosphatidylglycerol and diphosphatidylglycerol, and neutral polar lipids such as phosphatidylethanolamine and diglycosyl diglycerides. Phosphatidylethanolamine more typically occurs in Gram-negative bacteria[1], and glycosyl diglycerides are more characteristic in Gram-positive bacteria[2]; most simple bacteria contain one or other of these two lipids, and in certain Gram-positive organisms they occur together[3,4]. The observation that *Pseudomonas diminuta*, a Gram-negative organism which atypically lacks phosphatidylethanolamine, nevertheless contains glycosyl diglycerides[5], coupled with the absence of glycolipids from *Bacillus cereus* T, a Gram-positive bacterium which contains phosphatidylethanolamine[6], led to the suggestion that these two lipid types may have a similar and perhaps interchangeable role in bacterial membranes[6].

Further investigation of the lipids of *B. cereus* T indicated that in stationary phase cells a substantial quantity of diglycosyl diglyceride did in fact accumulate. It therefore seemed likely that a study of the lipid composition of bacilli during the growth cycle in batch culture might show whether the proportions of phosphatidylethanolamine and diglycosyl diglycerides are interrelated in these bacterial membranes. Since in Gram-positive bacteria the lipids are confined almost entirely to the cytoplasmic membrane, and as the operation of this membrane will depend upon the relative proportions of its components, the study has been confined to observed changes in proportions rather than absolute amounts of lipids.

B. cereus T and *B. subtilis* W23 were grown in the same medium as was previously employed for the former organism[6]. 1-l batches were innoculated from a Nutrient Agar slope and incubated on a gyrorotatory shaker at 37°. Exponential growth commenced after approximately 6 h and samples (250 ml) were then taken at regular intervals. Cells were harvested by centrifugation, lyophilized, and lipid was extracted with chloroform-methanol (2 : 1, v/v). Lipids were investigated by thin-layer chromatography on layers (0.4 mm) of Merck silica gel PF 254 impregnated with sodium acetate (0.5%, w/w) and identified by their staining properties with specific spray reagents and co-chromatography with standard materials isolated from other bacteria[6]. A two-dimensional solvent system using chloroform-methanol-water (65 : 25 : 4, by vol.) in the first direction followed by chloroform-acetic acid-methanol-water (80 : 18 : 12 : 5, by vol.) in the second direction gave excellent separations of the lipids of these bacteria[7]. All lipids were revealed by spraying with sulphuric acid diluted to 70% (w/v) with a saturated aqueous solution of potassium dichromate followed by charring at 200° for 45 min[8]. Visual inspection of the plates enabled variation in the relative proportions of the bacterial lipids to be assessed; more reproducible quantitative information was obtained by densitometry (Joyce-Loebl Chromoscan)[8]. The phospholipids of these bacteria contain only minor proportions of unsaturated fatty acids which are claimed to interfere with densitometric analysis[8]. The densitometric response of glycosyl glycerides has not been systematically investigated, so whilst the phospholipid proportions bear quantitative comparison with one another, the glycolipid data is probably in need of slight adjustment for the different charring properties of the sugar residues. This inexactitude, however, does not detract from the utility of the method in demonstrating the large changes in lipid composition observed in these studies.

The lipid composition of *B. subtilis* W23 was similar to the published data[9]; diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, diglucosyl diglyceride and a trace amount of a lysine ester of phosphatidylglycerol (lysyl phosphatidylglycerol) occurred during all phases of growth. *B. cereus* T contained diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol and a small quantity of a ninhydrin-positive phospholipid at all times. This latter lipid, whilst having similar chromatographic behaviour to lysyl phosphatidylglycerol, reacted with the periodate-Schiff reagents and gave glucose on acid hydrolysis; phosphoglycolipids, free of amino-functions, have been detected in a variety of Gram-positive bacteria[10]. In the present experiments, involving shaken 1-l batches, diglycosyl diglyceride was detected during exponential and stationary phases of growth of *B. cereus* T; the late exponential (12-h) cells containing no glycolipid, described previously[6], were from 10-l stirred cultures. Stationary phase cultures of *B. cereus* T produced an additional lipid with the chromatographic mobility and staining properties of an acidic glycolipid. Since acidic glycolipids have been isolated from certain pseudomonads[5] the lipids of *Pseudomonas diminuta* (N.C.T.C. 8545) from a 16-h growth in Oxoid Nutrient Broth No. 2(2%) and glucose (1%) were obtained for comparison purposes. The chromatographic behaviour, particularly the greatly increased mobility on thin-layer chromatography in acidic solvents[11], of the acidic glycolipid from *B. cereus* T was identical to that of a lipid from *P. diminuta* presumed to be glucuronosyl diglyceride. Acid hydrolysis (0.5 M H_2SO_4, 6 h at 95°) of the total lipids of *B. cereus* T and *P. diminuta*, followed by paper

chromatography (Whatman No. 1) in ethyl acetate-pyridine-acetic acid-water (5 : 5 : 1 : 3, by vol.) of the hydrolysate, showed the presence of glucuronic acid and glycerol; the lipids of *B. subtilis* W23 produced no glucuronic acid on acid hydrolysis. This is, to our knowledge, the first acidic glycolipid detected in a simple Gram-positive bacterium.

Figs. 1 and 2 show the semi-quantitative analysis of the lipid of *B. cereus* T and *B. subtilis* W23 during progression from exponential to stationary phase of growth; only the major lipids have been studied quantitatively. Both organisms appear to require comparable amounts of neutral polar and acidic polar lipids. Within the class of neutral polar lipids phosphatidylethanolamine appears to balance diglucosyl diglyceride in both organisms and the proportion of acidic glycolipid is related to the acidic phospholipids (phosphatidylglycerol and diphosphatidylglycerol) in the acidic polar lipid class of

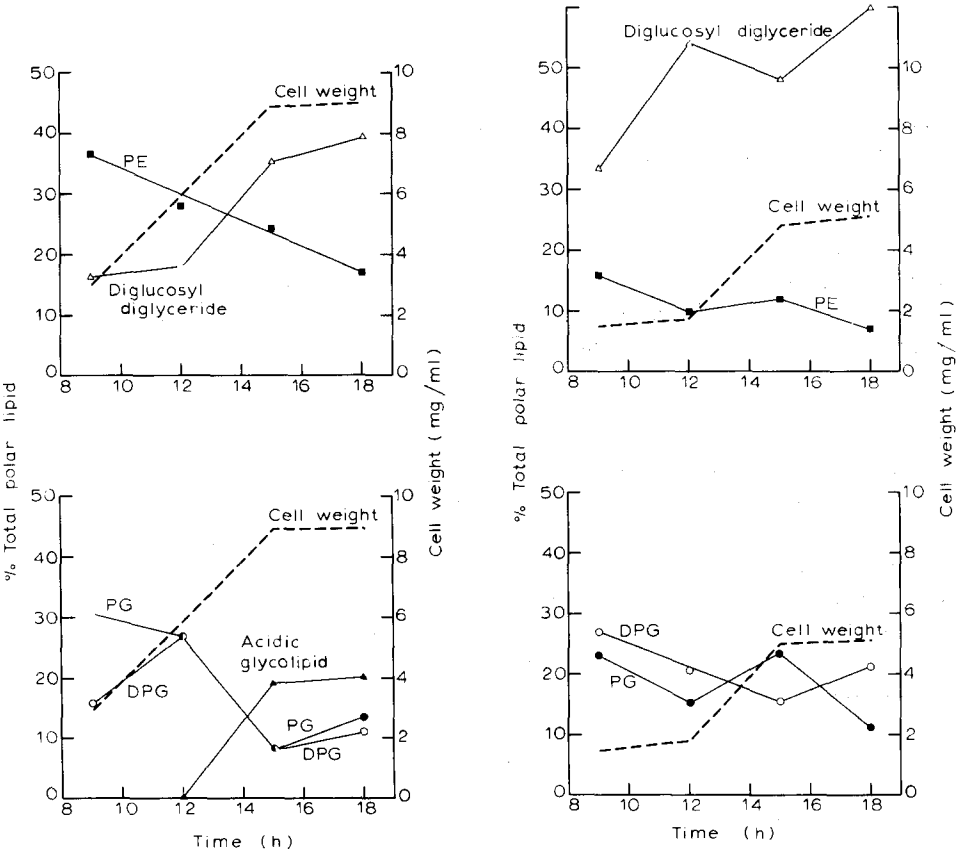

Fig. 1. Polar lipid composition of *B. cereus* T in exponential and stationary phases of growth. The upper part shows the relative proportions of neutral polar lipids and the lower part those of the acidic polar lipids expressed as a percentage of total polar lipid. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

Fig. 2. Polar lipid composition of *B. subtilis* W23; results are expressed in identical manner to those for *B. cereus* T in Fig. 1. Abbreviations: See Fig. 1.

B. cereus T. In *B. subtilis* W23, where no acidic glycolipid is produced, the acidic phospholipids are maintained at a fairly constant level. These results reinforce the proposed interchangeability of phosphatidylethanolamine and glycosyl diglycerides in bacterial membranes[6] and support the original suggestion of Wilkinson[12] that acidic glycolipids may replace acidic phospholipids. These conclusions suggest that growth conditions can affect the composition of membranes in a manner analogous to the change from teichoic acid- to uronic acid-containing polysaccharides in the walls of bacteria when grown under conditions of phosphate limitation[13].

A 1-l batch of *B. cereus* T was allowed to grow to early exponential phase (8 h) and then a sterile solution of KH_2PO_4 (1 g) and K_2HPO_4 (1 g) in water (50 ml) was added and incubation of the culture was continued to late stationary phase (18 h). Analysis of the lipids of this culture showed the absence of acidic glycolipid and a much reduced proportion of diglucosyl diglyceride (9.0%); the proportion of the phospholipids was correspondingly increased (phosphatidylethanolamine, 40.6; phosphatidylglycerol, 26.8; diphosphatidylglycerol, 23.6%) compared with the 18-h batch without added phosphate (Fig. 1). It is apparent, therefore that the increase in the proportion of glycolipids at the expense of phospholipids on proceeding to the stationary phase of growth may be due to phosphate starvation.

Changes in lipid composition with age of culture have been reported for a variety of bacteria. The most common trend appears to be accumulation of diphosphatidylglycerol in the stationary phase at the expense of the phosphatidylglycerol. *Escherichia coli*, however, shows such behaviour at 37° but the reverse at 27°, thus verifying that a complex combination of factors must operate in controlling the lipid content of bacteria[14].

Polar lipids having charged groups may be expected to play a role in the ion permeability of the membranes in which they reside. Recent quantitative experiments on model bilayer membranes prepared from bacterial phospholipids and glycolipids showed that those prepared from phosphatidylethanolamine and diglucosyl diglycerides have similar slight selectivity to cations, whereas those involving phosphatidylglycerol and diphosphatidylglycerol are highly cation selective[15]. More recently, it has been shown that acidic phospholipids such as phosphatidylglycerol and phosphatidylserine are capable in model membranes of discriminating between univalent cations, whereas neutral ionic phospholipids such as phosphatidylethanolamine and phosphatidylcholine are not[16]. It has been predicted that discrimination between monovalent cations could depend upon carboxyl as well as phosphate groups in biological membranes[16]. The acidic glycolipids described here and by Wilkinson[5,11,12] might at least be capable of carrying out an ion selectivity function similar to that demonstrated for phosphatidylglycerol and phosphatidylserine.

Most cell membranes contain an acidic phospholipid such as phosphatidylglycerol or phosphatidylserine, and these lipids are implicated in ion permeability. Similarly cell membranes usually contain neutral polar lipids (*e.g.* phosphatidylcholine, phosphatidylethanolamine or glycolipids) whose functions may be distinct from those of the acidic lipids. It is, therefore, possible that biological membranes in general have a requirement for one or more acidic polar lipid (*e.g.* phosphatidylglycerol, phosphatidylserine, phosphatidylinosine, diphosphatidylglycerol, acidic glycolipid) but, in addition, one or more neutral polar lipids (*e.g.* phosphatidylethanolamine, phosphatidylcholine, glycolipids) and that within each class the lipids may be to some extent interchangeable.

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