The Interrelation of Phosphatidylethanolamine and Glycosyl Diglycerides in Bacterial Membranes

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Acidic phospholipids such as phosphatidylglycerol and diphosphatidylglycerol occur in the membrane of probably all bacteria, where in many cases they are major constituents. Among other constituents that have been found in significant amounts are phosphatidylethanolamine, a neutral ionic polar lipid occurring widely and often as a major component in Gram-negative bacteria (Ikawa, 1967), and glycosyl diglycerides, neutral non-ionic polar lipids that are more typically constituents of Gram-positive bacteria (Shaw & Baddiley, 1968; Shaw, 1970). However, species of Gram-positive bacteria have been shown to contain both glycosyl diglycerides and phosphatidylethanolamine (Op den Kamp, Redai & van Deenen, 1969; Short & White, 1970), and at least one Gram-negative organism, *Pseudomonas diminuta*, lacks phosphatidylethanolamine but contains glycosyl diglycerides (Wilkinson, 1968, 1969). We report here a Gram-positive bacillus that, although it contains phosphatidylethanolamine, does not contain detectable amounts of glycosyl diglycerides. This and the earlier observations suggest that in general bacterial membranes obtained during vegetative growth possess acidic and neutral polar lipids, and that the two neutral polar lipids, phosphatidylethanolamine and glycosyl diglyceride, are interrelated as essential complementary components with similar or identical functions.

Bacillus cereus T (kindly supplied by Dr G. W. Gould, Unilever Research Ltd., Sharnbrook, Beds., U.K.) was grown aerobically in submerged culture in a medium based on Nutrient Broth no. 2 (Oxoid) essentially as described by Chin, Younger & Glaser (1968) but with omission of calcium chloride and addition of glucose (1%, w/v) to suppress sporulation. Vegetative cells, obtained during the late exponential phase of growth (12h) at 37°C, were harvested by centrifugation, washed with water and freeze-dried. Lipids were extracted with chloroform-methanol (2:1 v/v), and non-lipid contaminants were removed by the procedure of Williams & Merrilees (1970). T.l.c. on plates of Merck silica gel PF254 (chloroform-methanol-water, 65:25:4, by vol.) revealed the presence of three major phospholipids, diphosphatidylglycerol,

phosphatidylethanolamine and phosphatidylglycerol. These lipids were identified by co-chromatography with authentic samples isolated from other bacteria and by the use of spray reagents specific for phospholipids (Dittmer & Lester, 1964), free amino groups (ninhydrin) and α -glycols (periodate-Schiff reagents; Baddiley, Buchanan, Handschumacher & Prescott, 1956); phosphatidylglycerol was the only lipid giving a positive reaction with the periodate-Schiff reagent, no diglycosyl diglyceride being detected even on co-chromatography with an authentic sample from *Staphylococcus aureus* (Shaw & Baddiley, 1968). The total lipid (400mg) was fractionated on a column of 20g of Merck silica gel by using the chloroform-acetone elution procedure of Vorbeck & Marinetti (1965), which allows recovery in the acetone fraction of diglycosyl diglycerides free from phospholipids. T.l.c. of the acetone fraction again failed to show the presence of a diglycosyl diglyceride. Hydrolysis of this acetone fraction and the total lipid with alkali gave no detectable diglycosylglycerol, the expected deacylation product of the glycolipids of bacilli (Shaw & Baddiley, 1968).

Most Gram-positive bacteria contain appreciable quantities of glycosyl diglycerides. However, species of bacilli usually contain both glycosyl diglyceride (Shaw & Baddiley, 1968) and phosphatidylethanolamine (Ikawa, 1967; Op den Kamp *et al.* 1969). The lipids of only one other strain of *B. cereus* (A.T.C.C. 4342) have been described in detail (Lang & Lundgren, 1970): glycosyl diglyceride and phosphatidylethanolamine were shown to be present during all stages of vegetative growth and sporulation. It is therefore suggested that in *B. cereus* T, as grown in the present investigation, phosphatidylethanolamine has taken on the function of the absent diglycosyl diglyceride. It is possible to extend this hypothesis and propose that membranes of bacteria generally have a requirement for both acidic polar lipids and neutral polar lipids. Glycosyl diglycerides and phosphatidylethanolamine are the neutral polar lipids most commonly found in bacteria and occur together in the bacilli (Op den Kamp *et al.* 1969), pseudomonads (Wilkinson, 1969) and *S. aureus* (Short &

White, 1970). They thus may have a similar and perhaps interchangeable role in bacterial membranes. The possibility of an interchangeable role of acidic glucuronosyl diglycerides and phospholipids has already been suggested (Wilkinson, 1968).

Although in Gram-positive bacteria the acidic phospholipids are usually dominant (Ikawa, 1967), in Gram-negative bacteria phosphatidylethanolamine often may constitute 75% or more of the total lipid (Randle, Albro & Dittmer, 1969). Gram-negative bacteria, however, possess at least two membranous layers containing lipids. In *Escherichia coli* it has been shown (Nagata, Shibuya & Maruo, 1967) that phosphatidylglycerol is almost entirely confined to the cytoplasmic membrane, whereas much of the phosphatidylethanolamine is associated with the external lipoprotein layer. Analysis of the cytoplasmic membrane of a Gram-negative pseudomonad (Martin & MacLeod, 1971) showed that, although phosphatidylethanolamine was a major lipid, phosphatidylglycerol was present in comparable amounts. Thus it is likely that the cytoplasmic membrane of Gram-negative bacteria may resemble that of the Gram-positive bacteria in its requirement for both neutral polar and acidic polar lipids.

If glycolipids and phosphatidylethanolamine are interchangeable in bacterial membranes, then similarities in their physical and chemical properties might be expected. Such similarities have been noted in ion-permeability studies, where bilayer membranes prepared from diglucosyl diglyceride or phosphatidylethanolamine were only slightly selective towards cations, whereas those constructed from phosphatidylglycerol and diphosphatidylglycerol were highly selective (Hopfer, Lehninger & Lennarz, 1970). Moreover, acidic phospholipids in several bacterial membranes bind Mg^{2+} ions, whereas phosphatidylethanolamine does not (Gordon & MacLeod, 1966; Rayman, Gordon & MacLeod, 1967). It is an essential pre-requisite for the proposed interchangeability of glycosyl diglycerides and phosphatidylethanolamine in bacterial membranes that the latter does not normally

form chelates with Mg^{2+} ions. It is known (Joos & Carr, 1967) that below pH 8 phosphatidylethanolamine does not bind bivalent cations; thus it is unlikely that this membrane component would bind such cations in living cells. As glycosyl diglycerides lack cation-binding centres their similarity in this respect to phosphatidylethanolamine is obvious.

Although the distribution of the neutral polar lipids among bacteria suggests that they may be interrelated and share an important role in the membrane, this view is difficult to confirm experimentally until the functions of these components have been established, and until the composition of the lipids of a wider range of bacteria has been investigated in greater detail.

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