REVIEW LETTER

BACTERIAL GLYCOPHOSPHOLIPIDS

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1. Introduction

Glycophospholipids i.e. lipids containing both phosphate and carbohydrate residues* are now being isolated from a wide variety of bacteria [1–8]. Prior to these reports, representatives of this type of lipid were restricted to the family of phosphatidylinositol mannosides present in mycobacteria [9] and related organisms [10] and the glucosaminylphosphatidylglycerols present in *Bacillus megaterium* and *Pseudomonas ovalis* [11–13]. In both instances, carbohydrate residues are bound glycosidically to known phospholipids, phosphatidylinositol and phosphatidylglycerol, respectively. An analogous structure, diglucosylphosphatidylglycerol, has been proposed for the glycophospholipids subsequently isolated from several streptococci [1, 2, 5]. However, the structure of the glycophospholipid isolated from *Mycoplasma laidlawii* and originally called 'phosphatidylglucose' [14] has now been revised to glycerylphosphoryldiglucosyl diglyceride [3]. This is the first example of a new type of phosphoglycolipid, isomeric with diglucosylphosphatidylglycerol, but based upon a glycolipid, diglucosyl diglyceride. The structure of the glycophospholipid from *Streptococcus lactis*, originally thought to be a diglucosylphosphatidylglycerol [2, 15] has also been revised to glycerylphosphoryldiglucosyl diglyceride but containing an additional fatty acid residue [16]. We present here further evidence for the structure and distribution of this new type of phosphoglycolipid in bacteria and in light of these results to discuss other reports on bacterial glycophospholipids indicating where structural uncertainties may still exist.

2. The glycophospholipids of *M. laidlawii* and several streptococci

'Phosphatidylglucose' was tentatively proposed [14] as the structure of a glycophospholipid isolated from *M. laidlawii* but chromatographic comparison

* Note on nomenclature. In the past the terms 'phosphoglycolipid', 'glycophospholipid' and occasionally 'glycolipid' have all been used to describe carbohydrate containing phospholipids. With the diversification of structural types some systematic use of these terms is desirable. The IUPAC–IUB Commission on Biochemical Nomenclature of Lipids recommended that 'other generic terms may be employed and should be so constructed that prefixes denote substituting groups rather than define components already implied in the root name'. On this basis, those carbohydrate containing lipids based on known phospholipids e.g. phosphatidylinositol mannosides should be called glycophospholipids and those based on glycolipids e.g. glycerylphosphoryldiglycosyl diglycerides should be called phosphoglycolipids. Unfortunately, the problem is complicated by the isolation of lipids to which both terms could be applied equally e.g. phosphatidylglycosyl diglycerides. It is also convenient to have one all-embracing term to cover different structural types. We would tentatively suggest that the term 'phosphoglycolipid' be used exclusively for those lipids whose structure is based on known glycolipids containing additional phosphate or phosphatidyl residues e.g. glycerylphosphoryldiglycosyl diglycerides but that the term 'glycophospholipid' may be used to describe any lipid containing both carbohydrate and phosphate residues as the root name 'phospholipid' does not necessarily imply the presence of the phosphatidyl group (rule 5.1 of the IUPAC–IUB Commission). The term 'glycolipid' should only be used for non-phosphate containing glycolipids.

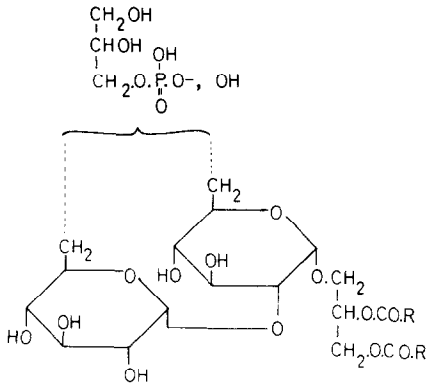

Fig. 1.

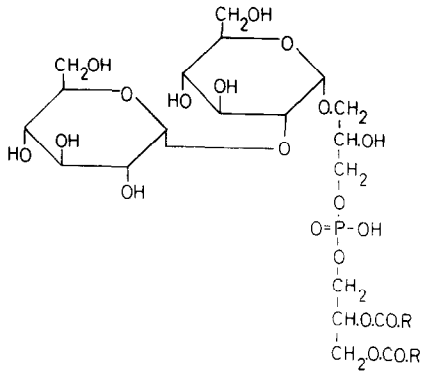

Fig. 2.

(a) Glycerophosphate on internal glucose.
(b) Glycerophosphate on terminal glucose.

of both the lipid and its water soluble deacylation product with synthetic materials showed that this was incorrect [3, 17] and the structure has now been revised [3] to glycerylphosphoryldiglucosyl diglyceride. This is a new type of phosphoglycolipid in which a glycerophosphate residue is linked to the 6-hydroxyl of one of the glucoses in diglucosyl diglyceride (fig. 1). Although conclusive evidence for the location of the fatty acid residues was not obtained, the resistance of the lipid to hydrolysis by phospholipases and the presumed biosynthetic relationship to the diglucosyl diglyceride present in the same organism [18] supported the proposed structure. The authors also suggested that the glycophospholipids found in *S. faecalis, S. lactis* and *S. hemolyticus* to which diglucosylphosphatidylglycerol structures (fig. 2) were assigned [1, 2] may be of the same type as the preliminary evidence presented could be applied equally to both. Acid hydrolysis of fig. 1 and fig. 2 will yield glucose and α,β-glycerophosphates and both on alkaline hydrolysis will yield diglucosylglycerol and α,β-glycerophosphates, although fig. 2 might be expected to give detectable amounts of diglucosylglycerol phosphate. However, as discussed by Shaw et al. [3], the two structures may be readily distinguished by oxidation with sodium periodate; the deacylation product of fig. 2 would liberate one mole proportion of formaldehyde whereas fig. 1 yields two mole proportions. Subsequently the structure of the lipid in *S. lactis* has been revised from fig. 2 to fig. 1 although it also contains an additional fatty acid residue, presumably located on the glycerophosphate as oxidation of the lipid itself with periodate does not liberate formaldehyde [16]. Treatment with α-glucosidase liberated one mole of glucose thereby locating the glycerophosphate on the internal glucose (fig. 1a). We have been unable to repeat this experiment on the deacylated lipid from *M. laidlawii* so it is possible that this lipid has the isomeric structure (fig. 1b). The location of fatty acid residues has been established by hydrolytic studies with hydrogen fluoride. This reagent is known to cleave phosphodiester linkages in teichoic acids without hydrolysis of glycosidic linkages [19, 20] and treatment of the lipid from *M. laidlawii* with hydrogen fluoride at 0° for 8 hr gives a good yield of diglucosyl diglyceride. Fatty acid ester linkages are relatively stable under these conditions and model experiments have shown that although 1,2-diglycerides are extensively isomerised to 1,3-diglycerides, there is little hydrolysis to monoglycerides or glycerol. This result conclusively establishes diglucosyl diglyceride as the basic structural unit. We have also isolated the more highly acylated phosphoglycolipid from *S. lactis* and hydrolysis with hydrogen fluoride gave diglucosyl diglyceride and a mixture of mono- and diglycerides. No evidence was obtained for a mono-acyl derivative of diglucosylglycerol (*lyso*-diglucosyl diglyceride). Thus it appears a series of phosphoglycolipids exists containing two, three or four fatty acids, glycerylphosphoryldiglucosyl diglyceride, mono-acylglycerylphosphoryldiglucosyl diglyceride and phosphatidyldiglucosyl diglyceride.

During investigations of the biosynthesis of glyco-

lipids in *S. faecalis* Pieringer [21] was able to demonstrate the transfer of glucose from UDP-glucose first to diglyceride and then to glucosyl diglyceride but was unable to show the biosynthesis of a triglucosyl diglyceride. A third glucose containing lipid was formed from diglucosyl diglyceride but apart from demonstrating its anionic nature, it was not further identified. By analogy with the lipid from *M. laidlawii,* Shaw et al. [3] suggested that this lipid was probably a glycerylphosphoryldiglucosyl diglyceride and this has now been established by Ambron and Pieringer [8]. The lipids of the same strain of *S. faecalis* have been studied independently by Dos Santos Mota et al. [5]. They have also isolated a glycophospholipid but suggested the isomeric diglucosylphosphatidylglycerol structure (fig. 2). The evidence presented by Ambron and Pieringer, in particular the elegant use of degradations with the lipid separately labelled with ^{32}P, ^{14}C-glycerol and ^{14}C-glucose, clearly establishes structure (fig. 1a) for their lipid. In contrast Dos Santos et al. rely on the hydrolysis of their lipid by phospholipase D to yield a phosphatidic acid. Unfortunately, as pointed out by Ambron and Pieringer [8] the amount of phosphatidic acid released or the identity of the other product was not recorded. Neither were studies with periodate carried out, experiments which, as discussed above, would immediately distinguish between the alternative structures.

3. The glycophospholipid from *Staphylococcus aureus*

A 'phosphatidylglucose' structure has recently been proposed again [4] for a glycophospholipid present in *S. aureus.* During studies on the phosphoglycolipid in *M. laidlawii* [3] the three most probable deacylation products of 'phosphatidylglucose' namely glucose-6-phosphorylglycerol and α,β-glucose-1-phosphorylglycerol have been synthesized. The deacylation product of the lipid from *S. aureus* did not correspond on paper chromatography to any of the synthetic materials, but it closely resembled the glycerylphosphoryldiglucosyl glycerol from *M. laidlawii.* These results indicate that this lipid is not a 'phosphatidylglucose' and may be a glycerylphosphoryldiglucosyl diglyceride. If further work substantiates this premise, an interesting structural feature will be the location of the glycerophosphate residue. Presuming a structral relationship between the phosphoglycolipid and the diglucosyl diglyceride present in *S. aureus* as has already been established in *M. laidlawii, S. lactis* and *S. faecalis,* the glycerophosphate cannot be located on the 6-hydroxyl of the internal glucose because this is the position of the disaccharide linkage.

4. The occurrence of phosphoglycolipids in other organisms

The structural relationship already established between glycerylphosphoryldiglycosyl diglycerides and diglycosyl diglycerides suggests that the former may be as widely distributed as the latter. Glycerophosphate derivatives of β-diglucosyl diglyceride have been isolated from *Cellulomonas biazotea* and *C. fimi.* The location of the glycerophosphate presents a similar problem to that discussed above for *S. aureus*; the disaccharide linkage is β(1-6). Hydrolysis of the lipid with hydrogen fluoride yields diglucosyl diglyceride. *Leuconostoc mesenteroides* and *Listeria monocytogenes* both contain glycerylphosphorylgalactosylglucosyl diglycerides. Alkaline hydrolysis yields the same glycoside, *O*-α-D-galactopyranosyl-(1-2)-*O*-α-D-glucopyranosyl-(1-1)-glycerol as obtained from the glycolipid in these organisms. The location of the glycerophosphate group has not been established but more highly acylated derivatives are also present in *L. monocytogenes.*

Monoglycosyl diglycerides are known to be the biosynthetic precusors of the diglycosyl diglycerides and although they do not usually accumulate in significant quantities it would be reasonable to predict the possible occurrence of glycerophosphate derivatives of monoglycosyl diglycerides. Wilkinson and Bell [7] have now presented evidence for the first example of this derivative in the lipids from *Pseudomonas diminuta.* The structure proposed is phosphatidylglucosyl diglyceride (fig. 3) and the evidence presented clearly distinguishes it from a diacyl derivative of the isomeric structure, glucosylphosphatidylglycerol (fig. 4). However, Preleg and Tietz [6] have suggested this structure for a glycophospholipid from a halophilic bacterium. Their proposed structure is based mainly upon the results of enzymatic hydrolysis;

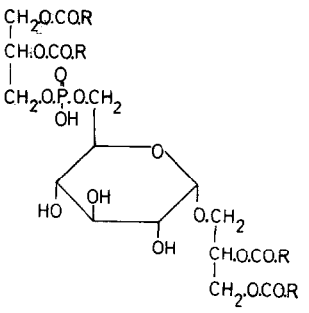

Fig. 3.

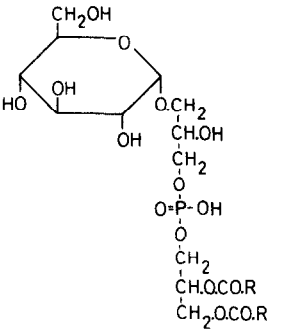

Fig. 4.

treatment with phospholipase C gave diglyceride and a water soluble product which liberated glycerophosphate on acid hydrolysis. This result is consistent with (fig. 4) and not (fig. 3) and although further evidence is desirable, it would appear that this lipid is a glucose analogue of the glucosaminylphosphatidylglycerol found in *P. ovalis* and *B. megaterium* [11–13].

5. Biosynthesis and function

Ambron and Pieringer [8] have shown that particulate enzyme preparations of *S. faecalis* catalyse the conversion of diglucosyl diglyceride to the phosphoglycolipid although the source of the glycerophosphate has not been established. CDP-glycerol has been postulated [3] as the source for the phosphoglycolipid in *M. laidlawii* and this would lead to a *sn*-glycerol-3-phosphate derivative which has been shown experimentally. The occurrence of more acylated forms suggests that the initial product of biosynthesis may be a phosphatidyldiglucosyl diglyceride and this is probably the case in *S. faecalis* [8]. The required substrate would be the ubiquitous CDP-diglyceride to give the phosphatidyldiglucosyl diglyceride which may then lose one or both of its acyl groups. Alternatively the additional acyl residues may be added after transfer of the glycerophosphate: an analogy of this route is the *in vitro* enzymic acylation of phosphatidylinositol mannosides to yield tri- and tetra-acylated products [22].

The recent isolation from lactobacilli [23] and *S. faecalis* [24] of lipoteichoic acids, which, like the lipopolysaccharides from Gram-negative bacteria, contain covalently bound lipid has suggested a possible function for phosphoglycolipids. Alkaline hydrolysis of the lipoteichoic acid yields a diglycosylglycerol identical to that produced by deacylation of the glycolipid present in the same organism and treatment of the lipoteichoic acid from *S. faecalis* with hydrogen fluoride yields diglucosyl diglyceride together with diglyceride and monoglyceride [24]. The presence of the neutral lipids suggests that the lipid component may be a phosphoglycolipid rather than the glycolipid itself. The lipid component could intersperse with other membrane lipids and thereby anchor the 'intracellular' polymer to the surface. The unbound phosphoglycolipids are only present in very small quantities in most bacteria with the exception of *M. laidlawii*, where it is a major component.

Acknowledgements

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