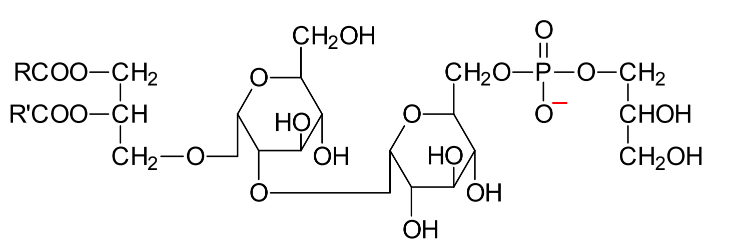
GLYCERO-GLYCOPHOSPHOLIPIDS

OCCURRENCE, COMPOSITION and BIOCHEMISTRY

As the name suggests, glycophospholipids are lipid molecules that contain both phosphate and carbohydrate as integral structural components. There are two main types of glycophospholipid based on a glycerol backbone, which with two exceptions discussed briefly below are exclusively of microbial origin. One type is derived biosynthetically from glycosyldiacylglycerols, in which the sugar moiety is phosphorylated, i.e. in which the carbohydrate moiety is linked to a diacylglycerol. The second group comprises more conventional phospholipids, with a phosphate moiety attached to a diacylglycerol, i.e. in which the phosphate is further glycosylated. The stereochemistry of the glycerophosphate unit can then often be a distinguishing feature, as this is dependent on the biosynthetic origin. A suggestion that the first group be termed '**phosphoglycolipids**' and the second '**glycophospholipids**' does not appear to have been widely adopted (see the review by Fischer cited below). However, they are used below for practical convenience. In addition, there are some lipids that at first glance appear to have features of both groups. It appears that most of these lipids occur in relatively small amounts in bacteria, and it has been suggested that they do not have an important role in membranes but may have some metabolic importance that has yet to be defined. Glycophospholipids are also found among the sphingolipids.

Phosphoglycolipids

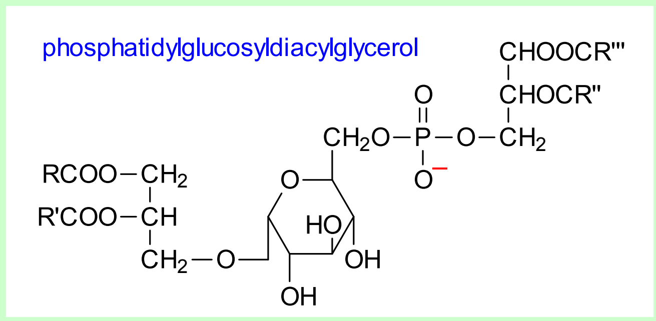
One of the first phosphoglycolipids to have its structure fully elucidated was found in *Streptococcus* and related bacterial species, i.e. 1,2-diacyl-3-[6''-(*sn*-glycero-1-phospho-)-α-D-kojibiosyl]-*sn*-glycerol. It is derived from a diglucosyldiacylglycerol, with the diglucoside unit equivalent to kojibiose in that it has an α-(1→2) linkage. The other distinctive feature is the stereochemistry of the glycerophosphate moiety attached to position 6 of the second glucose unit; this is linked via position *sn*-1 of glycerol rather than the *sn*-3 position as in most other phospholipids. Subsequently, an analogous lipid with a single glucose moiety was characterized from this organism, and a related lipid with a phosphorylated galactofuranosyl residue has been found in *Bifidobacterium bifidum*. Similar triglycosyl lipids or diglycosyl analogues with the *sn*-glycerol-1-phosphate residue in different positions from that illustrated or with more than one such substituent are also known. In some species, there is an alkenyl ether moiety in position *sn*-1 of the lipid component rather than a fatty acid.



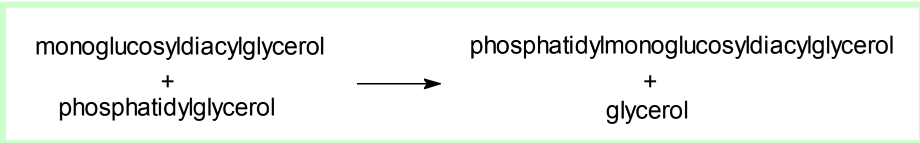
1,2-diacyl-3-[6''-(*sn*-glycero-1-phospho-)-α -D-kojibiosyl]-*sn*-glycerol

As in the biosynthesis of other glycosyldiacylglycerols (discussed elsewhere on this website), the first step in the biosynthesis of such lipids in *Streptococcus sp*. is a sequential reaction of 1,2-diacyl-*sn*-glycerol and UDP-glucose to yield first glucosyldiacylglycerol and then kojibiosyldiacylglycerol, both of which are also found in the organisms. The *sn*-1-glycerophosphate moiety is believed to be added last by an enzyme-catalysed trans-phosphatidylation reaction with phosphatidylglycerol as the donor molecule by analogy with comprehensive studies of the biosynthesis of phosphatidylglucosyldiacylglycerols discussed next.

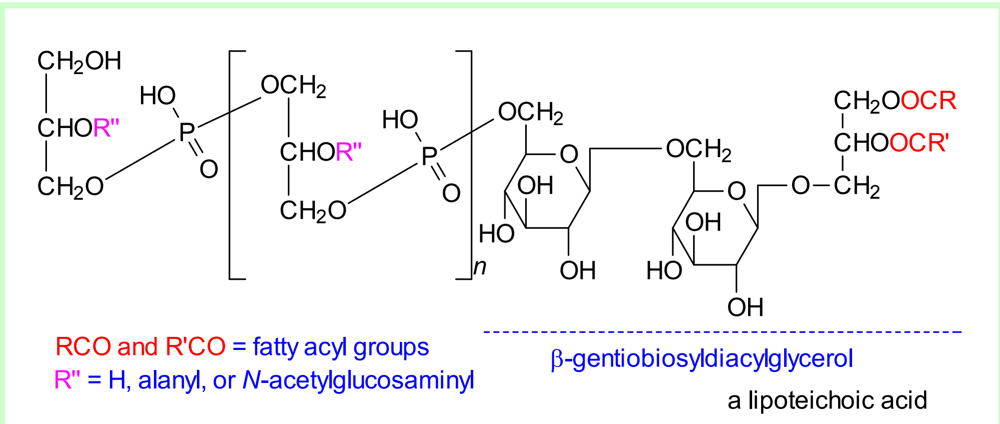
Some lipids occur with both a glycosyldiacyl moiety and a phosphatidyl group within a single molecule. For example, phosphatidylglucosyldiacylglycerol or 3-*O*-[6'-*O*-(1'',2''-diacyl-3'-phospho-*sn*-glycerol)-α-D-glucopyranosyl]-1,2-diacyl-*sn*-glycerol (illustrated) and the analogous diglycosyl phosphatidylkojibiosyldiacylglycerol have been found in some *Streptococcus* species and in *Pseudomonas diminuta*.



The key to the classification of these lipids within this first type of phosphoglycolipids has come from biosynthetic studies, which have shown that they are synthesised by an enzyme-catalysed transphosphatidylation of monoglucosyldiacylglycerols with phosphatidylglycerol as donor of the phosphatidyl group, rather than by a glycosylation reaction, for example.

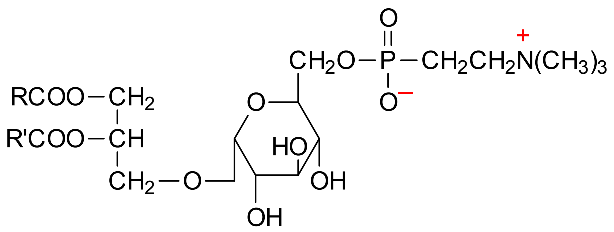


Glycosyldiacylglycerols are the lipid unit at the terminal end of the **lipoteichoic acids**, which are complex phospho-polysaccharides associated with the cell walls of Gram-positive and some Gram-negative bacteria. The lipid components are linked to a polymer of *sn*-glycerol-1-phosphate residues, which may be substituted with glycosyl (often *N*-acetylglucosamine) or alanyl residues. For example, that from *Bacillus subtilis* (illustrated below) has β-gentiobiosyldiacylglycerol, i.e. with a β(1→6) linkage between two glucose units, as the lipid component. The lipoteichoic acid from *Streptococcus faecium* contains 28 glycerophosphate moieties attached to the lipid component, for example.



The diacylglycerol unit serves as an anchor to hold the molecule in the membrane of the cell wall by hydrophobic interactions. While this is believed to be the main type of lipoteichoic acid, exceptions are known to exist with poly(digalactosylglycerophosphate), or ribitol or choline phosphate in place of the glycerophosphate residues.

A choline-containing phosphoglycolipid, i.e. 6'-*O*-phosphocholine-α-glucopyranosyl-(1'→3)-1,2-diacyl-*sn*-glycerol, from the human pathogenic bacterium *Mycoplasma fermentans* is distinctive for a number of reasons. In structural terms, it differs from most of the other phosphoglycolipids described here in that the phosphate moiety on position 6' of glucose is linked to a choline moiety, rather than to glycerol. In biological terms, it has been suspected of involvement in the pathogenesis of rheumatoid arthritis and of acquired immunodeficiency syndrome (AIDS), as it is a major immunological determinant for the organism in infected tissues. A second phosphoglycolipid with strong antigenicity found in the organism has a related if more complex structure, with position 6' of glucose linked to phospho-1,3-dihydroxy-2-aminopropane and then to the phosphocholine moiety.



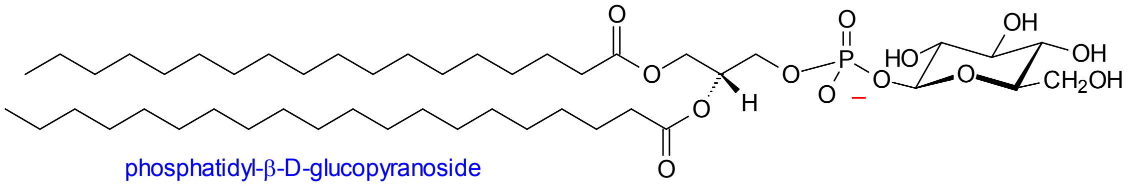
a choline-containing glycophospholipid

Glycophospholipids

Some of the lipids in the second group of glycerol-containing glycophospholipids defined above, i.e. with a phosphatidyl backbone, are discussed in relation to the parent phospholipids in the relevant pages of this website. They include the **glycosyl-phosphatidylinositols**, which are ubiquitous lipids that serve to anchor proteins in membranes. For some time, these were erroneously thought to be the only glycophospholipids found in higher organisms. The trace levels of glycosylated phosphatidylethanolamine and phosphatidylserine formed by a non-enzymatic Maillard reaction are not considered here. Complex **mannosyl derivatives of phosphatidylinositol** are typical components of the membranes of Actinomycetes and of coryneform bacteria.

Phosphatidylglucoside was first described from the bacterium *Staphylococcus aureus* in 1970, but doubts have been cast on the identification. The first definitive isolation and characterization of this lipid was as recently as 2001, when surprisingly it was found in mammalian cell types rather than in a microorganism.

Phosphatidyl-β-D-glucopyranoside was found first in human cord red cells, and while its fatty acid composition and their positional distributions were reportedly similar to the other phospholipids, it is now known that this was because the sample was contaminated. Subsequently, it was characterized from an erythroblastic leukemia cell line, and then from developing astroglial membranes of HL60 cells (together with phosphatidyl-β-D-(6-*O*-acetyl)-glucopyranoside). In the latter example, it was isolated by an improved procedure involving a specific monoclonal antibody, and it was shown to exist in the form of a single saturated molecular species with 18:0 at position *sn*-1 and 20:0 at position *sn*-2 of the glycerol backbone, a highly un-mammalian-like structure! In addition, it has been shown more recently to exist in enantiomeric forms, i.e. a small proportion has the phosphoglucose moiety attached to an *sn*-2,3-diacylglycerol backbone. Again, this feature appears to be unique to this lipid.



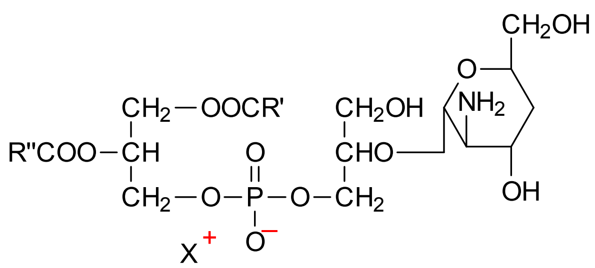
phosphatidyl-β-D-glucopyranoside

Although it is a minor lipid in quantitative terms, disaturated phosphatidylglucoside is believed to have biological importance in that it forms signalling microdomains, akin to **rafts**, where there is evidence that it is involved in cellular differentiation. It has a melting point of 73°C, similar to that of glucosylceramide, which is also located predominantly in rafts. Phosphatidylglucoside and its acetylated form are located predominately in the outer leaflet of the membrane. While phosphatidylglucoside might have a role in signal transduction, its acetylated form may be involved in extracellular signaling. It is not yet known how or where it is synthesised in cells.

This lipid may be more abundant than has been realized, since molecular species have the same mass numbers as for phosphatidylinositol on electrospray mass spectrometry and are easily missed.

Similar lipids but with cholesterol attached to the glucose moiety, i.e. cholesteryl-6'-*O*-phosphatidyl-α-glucoside, and cholesteryl-6'-*O*-lysophosphatidyl-α-glucoside, occur in the pathogenic bacterium *Helicobacter pylori*, together with simple cholesterol-α-glucosides.

One of the first glycophospholipids of with a phosphatidyl backbone to be discovered was in a *Bacillus* species, and was characterized as a glucosaminylphosphatidylglycerol in which the glucosaminyl residue is linked to position 2' of the *sn*-glycero-1-phosphate moiety of phosphatidylglycerol. Soon thereafter, an isomeric compound with the galactosamine residue in position 3' was discovered, and analogous lipids, with *N*-acetyl-galactosamine and glucose attached to phosphatidylglycerol in a similar manner have been isolated from other bacterial species.



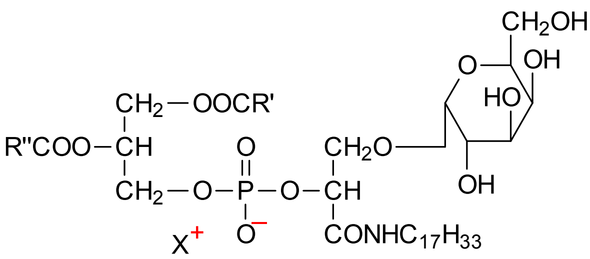
1'-(1,2-diacyl-*sn*-glycero-3-phosphoryl)-2'-*O*-
-β-(2-amino-2-deoxy-D-glucopyranosyl)-*sn*-glycerol

With such compounds, it is known that pre-formed phosphatidylglycerol is glycosylated by an enzyme that transfers a glycosyl unit from the appropriate uridine 5-diphosphate(UDP)-hexose as in the glycosylation of many other complex lipids, such as in the biosynthesis of the **mono- and digalactosyldiacylglycerols**.

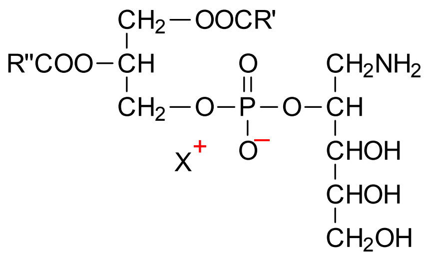
A related lipid, diphosphatidylglycerol (cardiolipin) containing an α-D-linked glucopyranose unit in position 2', was first found as a minor component of certain *Streptococci*. Subsequently, it was detected in the thermophilic bacterium *Geobacillus stearothermophilus,* where it was suggested that it might stabilize the membranes when they were exposed to high temperatures.

As further examples, a major phosphoglycolipid from *Deinococcus radiodurans*, a Gram-positive bacterium, has been shown to be 2'-*O*-(1,2-diacyl-*sn*-glycero-3-phospho)-3'-*O*-(α-galactosyl)-*N*-D-glyceroyl alkylamine, i.e. in which a phosphatidic acid moiety is linked to glyceric acid and thence to a long-chain amine and to galactose.

Related lipids have been found in bacterial thermophiles of the genera *Thermus* and *Meiothermus*. Other glycophospholipids are known with carbohydrate moieties linked to position 2 of glyceric acid. *Hydrogenobacter thermophilus*, an extremely thermophilic hydrogen bacterium, contains a phospholipid with a 1-amino-pentanetetrol moiety, i.e. 1,2-diacyl-3-*O*-(phospho-2'-*O*-(1'-amino)-2',3',4',5'-pentanetetrol)-*sn*-glycerol. Similar lipids but with alkyl moieties rather than fatty acids and tertiary and quaternary amine groups have been found in the archaeon *Methanospirillum hungatei*. Many more such glycophospholipids are known from extremophile bacteria.



glycophospholipid containing galactose, glyceric acid and an alkylamine



glycophospholipid containing 1-amino-2,3,4,5-pentanetetrol

Analysis

Analysis of glycophospholipids is not straightforward as no standards are available, and structural characterization is a task for the specialist, especially as the stereochemistry of the glycerol and carbohydrate moieties is of great importance for biological activity. High-performance thin-layer chromatography is the single techniques used most often for isolation purposes, though HPLC in the adsorption and ion-exchange modes also has considerable value. Modern techniques of nuclear magnetic resonance spectroscopy and of mass spectrometry are indispensable aids.

Suggested Reading

- Fischer, W. Bacterial phosphoglycolipids and lipoteichoic acids. In: *Handbook of Lipid Research 6. Glycolipids, Phosphoglycolipids and Sulfoglycolipids*, pp. 123-235 (ed. M. Kates, Plenum Press, NY) (1990).
- Nagatsuka, Y. and Hirabayashi, Y. Phosphatidylglucoside: a new marker for lipid rafts. *Biochim. Biophys. Acta*, **1780**, 405-409 (2008).
- Pieringer, R.A. Biosynthesis of non-terpenoid lipids. In: *Microbial Lipids. Volume 2*, pp. 51-114 (ed. C. Ratledge and S.G. Wilkinson, Academic Press, London) (1989).

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