GLYCOLIPIDS: a Colloquium organized on behalf of the Society and the Lipid Group by P. J. Brennan (Dublin and Denver)

Glycolipids in Prokaryotic Cells

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Glycolipids of the diacylglycosylglycerol type are common components of the bacterial membrane, particularly in Gram-positive bacteria (Shaw, 1975). Bacterial diacylglycosylglycerols show more structural diversity than do similar lipids in plants, where they were first discovered, and this has proved to be of value in taxonomic studies (Shaw, 1974). Notwithstanding their prevalence, however, no conclusive evidence has been presented to support a specific function for diacylglycosylglycerols. The recognition of the role of polyisoprenoid lipid intermediates in the synthesis of various bacterial polymers (Rothfield & Romeo, 1971) rules out for diacylglycosylglycerols such a function, which had been suggested earlier on the basis of certain structural similarities between the carbohydrate head groups of the glycolipids and some repeating units in polysaccharides present in the same organism. A new type of phosphoglycolipid in which a phosphatidyl or glycerophospho residue is linked to a diacylglycobilysylglycerol has been found in lipid extracts from several bacteria (Shaw & Stead, 1972) and these phosphatidyl or glycerophospho diacylglycobilysylglycerols are probably formed from the glycolipid by transfer of the appropriate phosphate residue from phosphatidylglycerol (Pieringer & Ganfield, 1975). These phosphoglycolipids are also integral components of the membrane teichoic acids, sometimes called lipoteichoic acids, present in Gram-positive bacteria (Lambert et al., 1977), but it seems unlikely that this is a major function for diacylglycosylglycerols, as the proportion of glycolipid present in these polymers represents a small fraction of the total cellular concentration (Toon et al., 1972). The major proportion is present in the membrane as free lipid, although in most bacteria this represents only a minor proportion of the total lipid.

There are two groups of micro-organisms where glycolipids are major components of the membrane: mycoplasma and L-forms, and thermophiles. A comparison of the lipid composition of various Gram-positive bacteria and their derived L-forms shows that in
L-forms the proportion of glycolipid is greatly increased to proportions similar to those found in many mycoplasma (Shaw, 1970; Smith et al., 1973). Thus a characteristic feature of the lipids from many organisms lacking a rigid cell wall is a high concentration of glycolipids.

Several reports indicate that there is a second group of micro-organisms, thermophiles, where glycolipids are major membrane components. The lipids of Sulfolobus acidocaldarius (Langworthy et al., 1974) and Thermoplasma acidophilum (Langworthy et al., 1972) contain 67% and 25% glycolipids respectively. Flavobacterium thermophilum, an extremely thermophilic organism isolated from a Japanese hot spring, also contains a high proportion of a glycolipid identified as a diacylglycotetraosylglycerol (Oshima & Yamakawa, 1972). We have examined the lipids of Thermus aquaticus YT1 and other related extremely thermophilic organisms isolated from Icelandic hot springs and domestic hot water supplies, and found high concentrations of diacylglycotetraosylglycerols similar to those present in Flavobacterium thermophilum. [The latter organism has now been reclassified as Thermus thermophilus (Oshima & Imahori, 1974).] All the isolates contained one major glycolipid that accounted for almost half of the total lipid. The glycolipids from the two domestic-hot-water isolates (D1 and NH) were both diacylgalabiosyl-(N-acyl)glucosaminylglucosylglycerols; the Icelandic strain J1 contained a diacylgalactosylglucosyl-(N-acyl)glucosaminylglycerol and strain H a diacylgalactosyl-(N-acyl)glucosaminylglycerol. Thermus aquaticus also contained a similar diacylglycotetraosylglycerol, with glucose and galactosamine as the constituent sugars. The glycolipids of these extreme thermophiles thus show several interesting features. First, the size of the hydrophilic groups, diacylglycotetraosylglycerols, have only been observed infrequently and then only as minor components; secondly, the presence of a hexosamine; and finally the presence of three acyl residues, one of which is linked to the hexosamine through an amide linkage reminiscent of the lipid-A moiety of the lipopolysaccharides in Gram-negative bacteria.

It is now well established that the fatty acid composition of lipids is greatly influenced by temperature, and this has been related to membrane fluidity (Chapman, 1975). Thus as the temperature is decreased, the proportion of unsaturated fatty acids increases and/or chain length decreases. Studies on concomitant changes in polar lipid composition have received little attention, and in view of the high glycolipid content of many thermophilic bacteria we have investigated the changes in polar lipids of some mesophilic bacteria grown at various temperatures in the expectation that glycolipid concentration might increase with temperature. Thus far we have been unable to find experimental support for this hypothesis. Streptococcus faecalis was grown over a range of temperatures from 12 to 45°C and the lipids were extracted from freeze-dried organisms. The purified lipid extracts were analysed quantitatively for glucose and phosphorus as a measure of glycolipid and phospholipid content respectively. Neither changed significantly over this temperature range, although the proportion of C18 : 1 fatty acid increased from 20% to 50% at the lowest growth temperature. Isolation of the individual lipid components showed that this increase in unsaturation was a general phenomenon and not restricted to any particular lipid. The most significant change was the decrease in total lipid content as the temperature increased, e.g. from approx. 3.5% dry cell weight at 20°C to 1.6% at 45°C. A similar decrease in total lipid content as growth temperature increases has been reported for Bacillus stearothermophilus (Wisdom & Welker, 1973). Thermus aquaticus when grown over a range of temperatures from 55 to 75°C also gave similar results. Thus the polar lipid composition did not alter significantly, but the fatty acid composition changed in the expected manner, the ratio of C15 to C17 branched-chain fatty acids decreasing with temperature.

The ability of Thermus aquaticus and related organisms to maintain the structural integrity of their membranes at such high temperatures cannot be ascribed to their fatty acids; C15 and C17 branched-chain fatty acids are commonly found in many mesophilic bacteria. The stabilizing feature therefore appears to be the high concentration of glycolipids. Sharom et al. (1976) have examined the effects of glycolipids in phospholipid bilayer model membranes. Using spin labels, they have shown that the addition of
gangliosides (10–40%) to phospholipid bilayers markedly increases their rigidity. Preliminary experiments with a series of bacterial diacylglycosylglycerols have produced similar results (F. J. Sharom & C. W. M. Grant, personal communication) and have shown that this rigidifying effect is most dramatic with the diacylglycotetraosylglycerol from *Thermus aquaticus*. This is the first direct evidence for a specific function for diacylglycosylglycerols, namely the maintenance of the structural integrity of the membrane in an adverse environment, e.g. at extreme temperatures in the case of some thermophiles or the absence of a rigid cell wall in the case of mycoplasma and L-forms. Although the overall quantity of diacylglycosylglycerols in most bacteria is small, it is possible that their location at a few specific areas in the membrane would provide a sufficiently high concentration to enable this rigidifying effect to become operable and thus provide the organism with an alternative method for regulating the fluidity of specific areas within the membrane.

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**Glycolipids of Fungi and Plants**

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Numerous individual glycolipids have been isolated and identified from plants and fungi, particularly from the latter. In this paper, I shall deal with the occurrence and metabolism (where known) of the major glycolipids. Since little is known of their function in vivo, such remarks as are made will generally be of a speculative nature. For a fuller discussion of the structure and synthesis of these compounds, I refer the reader to the excellent reviews by Brennan et al. (1974), Hitchcock & Nicols (1971) and Mudd & Garcia (1975).

So far as higher plants are concerned, the prevalent glycolipids can be conveniently classified into four groups; the diacylgalactosylglycerols, steryl glycosides, the sphingolipids and the sulpholipids. Table 1 shows the lipid composition of a number of different plant tissues and the quantitative importance of glycolipids will be readily seen. Chloroplastic tissue contains approx. 50% of its lipids as glycolipid, whereas fruit tissue yields 20–30%.

**Diacylgalactosylglycerols**

The pioneering work from Carter’s (Carter et al., 1956, 1961) and Benson’s laboratories (Benson et al., 1958, 1959b) elucidated the structures of diacylgalactosylglycerol