The Occurrence of Acylated Sugar Derivatives in the Lipids of Bacteria

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Glycolipids of the diglycosyl diglyceride type are widely distributed in Gram-positive bacteria but so far have not been isolated from Gram-negative organisms (Brundish, Shaw & Baddiley, 1966; Shaw & Baddiley, 1968). On the other hand, the work described below shows that lipid preparations from several Gram-negative bacteria contain glycolipids that are not diglycosyl diglycerides but are acylated sugar derivatives; a similar glycolipid has also been isolated from a Gram-positive organism. Mycobacteria are known to contain esters of mycolic acids and various carbohydrates, e.g. cord factor (Lederer, 1967), and an extracellular rhamnolipid has been isolated from the culture medium of *Pseudomonas aeruginosa* (Jarvis & Johnson, 1949), but as far as we are aware no information is available on the occurrence of acylated sugar derivatives in other bacteria. Four methods of extraction have resulted in the isolation of these lipids from freeze-dried cells: (1) chloroform-methanol (2:1, v/v) at 21°; (2) extraction of cells defatted by procedure (1) with boiling chloroform; (3) phenol extraction followed by acid hydrolysis of the lipopolysaccharide (0.1 N-HCl at 100° for 30 min.) and extraction of the hydrolysate with chloroform; (4) acid hydrolysis (0.1 N-HCl at 100° for 30 min.) of cells defatted by procedure (1),

followed by extraction of the hydrolysate with chloroform.

The first procedure is that normally used for the extraction of lipids from bacteria, and only small amounts of the glycolipids were removed by this method; considerably higher yields were obtained by procedure (2). The third method is that generally used for the isolation of lipid A (Westphal & Luderitz, 1954; Burton & Carter, 1964), crude preparations of which contain considerable amounts of acylated sugars other than glucosamine. Procedure (4) has been used in this Laboratory as an alternative method for the isolation of lipid A. The yields of glycolipids obtained were approx. 1 mg./g. of dry cells. Non-lipid contaminants were removed by the method of Wells & Dittmer (1963), and thin-layer chromatography on silicic acid in chloroform-methanol-water (65:25:4, by vol.) provided a convenient method for separating these lipids; in this system they all have R_F values approaching unity and are usually well separated from other lipids. The products of hydrolysis with acid (2 N-HCl for 3 hr. at 100°) and alkali (Marinetti, 1962) were examined by paper chromatography in the system butan-1-ol-pyridine-water (6:4:3, by vol.) and were detected with alkaline AgNO_3 reagent (Trevelyan, Procter & Harrison, 1950). The

Table 1. *Acylated sugar derivatives from the lipids of various bacteria*

For details of methods of extraction and hydrolysis see the text.

	R_F in chloroform-methanol-water	Methods of extraction	Products of acid hydrolysis	Products of alkali hydrolysis
<i>Aerobacter aerogenes</i>	0.8-0.9	2, 3	Glucose	Glucose
<i>Pseudomonas fluorescens</i>	0.8	3	Glucose	Glucose
	0.9-1.0	3	Glucose, galactose	Disaccharide of glucose and galactose
	0.9-1.0	3	Glucose, galactose, arabinose	Trisaccharide of glucose, galactose and arabinose
<i>Escherichia coli</i> 086	0.8-0.9	3, 4	Glucose	Glucose
	0.9-1.0	3, 4	Glucose, glycerol	Glucosylglycerol
	0.9-1.0	3, 4	Glucose, galactose	Glucosylgalactose
	0.9-1.0	3, 4	Glucose, galactose, glycerol	Oligosaccharide of glucose, galactose and glycerol
<i>Escherichia coli</i> B	0.8-0.9	2, 3, 4	Glucose	Glucose
	0.9-1.0	2, 3, 4	—	Oligosaccharides
<i>Streptococcus faecalis</i>	0.6	1	Glucose	Glucose

sugars in the glycolipid from *Pseudomonas fluorescens* were also identified by gas-liquid chromatography of their trimethylsilyl derivatives (Sweeley, Bentley, Makita & Wells, 1963). The results are given in Table 1.

Hydrolysis of the glycolipids with alkali gave in most cases a mixture of mono-, di- and tri-saccharides and a wide range of fatty acids. The apolar nature of the glycolipids, as shown by their behaviour on thin-layer chromatography, suggests that most or all of the available hydroxyl groups carry fatty acid residues. The individual components of the glycolipids of *Escherichia coli* 086 were separated by chromatography on columns of silicic acid and Sephadex LH-20 into at least nine fractions. Hydrolysis of the four major fractions with alkali gave the products shown in Table 1. One of the lipid components has been tentatively identified as an acylated glucosylglycerol, but its behaviour on thin-layer chromatography is different from the monoglycosyl diglycerides found in some Gram-positive bacteria. Moreover, it was not isolated by the procedures usually used for these compounds. Two of the minor fractions contained glucosamine and may have arisen by degradation of lipid A. Thin-layer chromatography of the mixture in the system light petroleum (b.p. 40–60°)–acetone (4:1, v/v) gave a similar resolution of components. Gas-liquid chromatography of the acylated glucose from *Escherichia coli* B gave at least five fractions, each presumably representing a different fatty acid distribution on the sugar.

During the isolation of the diglucosyl diglyceride from *Streptococcus faecalis* (Brundish *et al.* 1966) a small amount of another glycolipid was isolated that had a similar R_F value (0.6) on thin-layer chromatography to the diglucosyl diglyceride in the

system chloroform-methanol-water (65:25:4, by vol.) but did not contain glycerol. Hydrolysis with alkali yielded glucose, lauric acid and acetic acid. The lauric acid/acetic acid molar ratio was about 1:3, as determined by gas-liquid chromatography, and comparison of the mass spectrum of the lipid with those of authentic acylated mono- and disaccharides (cf. Biemann, DeJongh & Schnoes, 1963) indicated the probable structure 3,4,6-tri-*O*-acetyl-2-*O*-laurylglycopyranose. Smith & Koostra (1967) have reported the presence of a similar glycolipid in *Mycoplasma* sp. avian strain J. This glycolipid also gave glucose on hydrolysis with alkali and did not contain glycerol.

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