SHORT COMMUNICATIONS

The Polar Lipids of Some Species of *Nocardia*

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Mannophosphoinositides appear to be widespread among the Actinomycetales. Besides the well-known mycobacterial source, they are also present in streptomyces, microbispora (Kataoka & Nojima, 1967; Tabaud, Tisnovska & Vilkas, 1971), nocardias (Yano, Furukawa & Kusunose, 1969) and corynebacteria (Brennan & Lehane, 1971) and a related phosphorus-free mannoinositide is prominent in propionibacteria (Prottey & Ballou, 1968; Shaw & Dinglinger, 1969). Prompted by the unusual observation of a monomannophosphoinositide in *Corynebacterium aquaticum* (Khuller & Brennan, 1972) we have examined in more detail three species of *Nocardia*, and find that mannophosphoinositides, which are always of the dimannosyl type, are barely evident in *Nocardia polychromogenes*, whereas in *N. coeliaca*, they are among the most obvious phospholipids. Glucose-containing phospholipids and glycolipids are always prominent.

Bacteria used were: *Nocardia polychromogenes* Jensen from the Shinogi Research Laboratories, Osaka, Japan; *N. polychromogenes* NRRLB-1513 from the Department of Antibiotics, National Institutes of Health, Japan; and *N. coeliaca* ATCC 13181. They were grown in a medium containing glucose, polypeptone and yeast extract (Yano *et al.* 1969). Lipids were extracted with chloroform + methanol (2:1, v/v) and were then divided into acetone-soluble and acetone-insoluble fractions. All other chromatographic and analytical procedures used have been described before (Khuller & Brennan, 1972).

Glycerol, mannose, glucose, inositol and ethanolamine were major components after acid hydrolysis of the total lipids from the three strains, as shown by paper chromatography, and of these only glycerol and glucose were present in the acetone-soluble lipids. The products of mild alkaline hydrolysis of the acetone-soluble lipids of *Nocardia polychromogenes* were glycerol, glucose and a component corresponding in R_F value to diglucosylglycerol (authentic diglucosylglycerol was supplied by Dr N. Shaw) (Fig. 1, left). This latter material, isolated by preparative paper chromatography using butan-1-ol + pyridine + water (6:4:3, by vol.), revealed only glucose and glycerol after paper chromatography of acid hydrolysates.

The occurrence of free glucose after mild alkaline treatment of the acetone-soluble lipids from the three strains of *Nocardia* indicated the presence of acylglucoses. A pure lipid was isolated from both *Nocardia polychromogenes* Jensen and *N. coeliaca* by preparative t.l.c. as described previously (Brennan, Lehane & Thomas, 1970). Each showed only glucose and fatty acids on alkaline hydrolysis and their chromatographic properties and fatty acyl to glucose ratios indicated a triacylglucose in *N. polychromogenes* Jensen and a diacylglucose in *N. coeliaca*.

Chromatography of the deacylated acetone-insoluble lipids from *Nocardia polychromo-*

Fig. 1. Paper chromatography of the deacylated lipids of *Nocardia polychromogenes* Jensen. Left: chromatography of the deacylated acetone-soluble lipids in butan-1-ol + pyridine + water (6:4:3, by vol.). A, glycerol; B, diglucosyl glycerol; C, glucose; D, deacylated acetone-soluble lipid. Right: chromatography of the deacylated acetone-insoluble lipids in propan-2-ol + aq. NH_3 (2:1, v/v). 1, deacylated acetone-insoluble lipids; 2, glucose (top) and glycerylphosphorylinositol; 3, trehalose.

genes Jensen in propan-2-ol + aq. NH_3 (2:1, v/v) showed four components (I, II, III, IV) (Fig. 1, right). Components I, II and IV were isolated by preparative paper chromatography. Component III was not always obvious and was not further examined. Paper chromatography of I in ethyl acetate + pyridine + water (10:4:3, by vol.) and in methanol + aq. NH_3 + water (6:1:3, by vol.) showed that it was identical to α, α' -trehalose and acid hydrolysis revealed only glucose. This evidence suggested the presence of an acyltrehalose. Ionedá, Lederer & Rozanis (1970) have described a dicorynomycyltrehalose from *N. asteroides*. Its structure appears to be identical with that previously isolated from *Corynebacterium diphtheriae* and which is acetone-insoluble (Senn, Ionedá, Pudles & Lederer, 1967). Acid hydrolysis of II and paper chromatography showed only glycerol and glucose. It also contained phosphorus as shown by its reaction with the Hanes–Isherwood reagent (Hanes & Isherwood, 1949). Quantitative analysis of component II for phosphorus, glucose and glycerol demonstrated their presence in the molar ratio of 1:1:2.2. These results could suggest a phosphatidyl

derivative of a monoglucosyl diglyceride of the type identified in *Pseudomonas diminuta* (Wilkinson & Bell, 1971).

The most abundant component of the deacylated phospholipids (IV in Fig. 1) corresponded to glycerylphosphorylinositol in ethyl acetate+pyridine+water (5:3:2, by vol.). There was no obvious evidence for glycerylphosphorylinositol mannosides among the products from *Nocardia polychromogenes* Jensen. However, glycerylphosphorylinositol dimannoside became evident in area V of Fig. 1 (right) when considerably larger amounts of the deacylated lipid fraction were chromatographed. Its identity was confirmed by chromatography in ethyl acetate+pyridine+water (5:3:2, by vol.). Lipids from *N. polychromogenes* NRRLB-1513 and *N. coeliaca* yielded a related type of pattern. However, in the latter case glycerylphosphorylinositol dimannoside was very much in evidence. Phosphatidylethanolamine and bisphosphatidyl glycerol were also identified by chromatography of the intact acetone-insoluble lipids from each of the three nocardias. Phosphatidylcholine was found only in *N. coeliaca*, thus confirming a previous report (Yano *et al.* 1969).

The usefulness of an examination of the polar lipid patterns of bacteria has recently been demonstrated by Minnikin, Abdolrahimzadeh & Baddiley (1971) who showed the complementary presence of neutral and acidic polar lipids in bacterial membranes. Our work indicates that in at least some nocardias these requirements are met by the diglucosyl diglyceride, acylglucoses and phosphatidylethanolamine (or phosphatidylcholine) as the principal neutral polar lipids and as the acidic complementation, phosphatidylinositol (the glucose-containing phosphoglyceride) and, particularly in the case of *Nocardia coeliaca*, dimannophosphoinositide. The presence of glycosyl diglycerides in some nocardias, compared with their apparent complete absence in mycobacteria, is a feature which may be of some phylogenetic significance. Tabaud *et al.* (1971) have recently discussed the role of other lipids in the classification of the Actinomycetales.

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