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Phosphoinositides of *Corynebacterium xerosis*

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Phospholipid fractions have been obtained from *Corynebacterium diphtheriae* containing mannose, inositol and glycerol (Asselineau, 1961; Gomes, Ioneda & Pudles, 1966), indicating the presence in corynebacteria of mannophosphoinositides of the type described for mycobacteria (Lee & Ballou, 1965). Recently, during investigations of the occurrence of trehalose in the soluble and lipid fractions of the saprophyte *Corynebacterium xerosis*, it was found that the phospholipid fraction on hydrolysis yields glucose, arabinose, mannose, inositol and glycerol. The present communication describes the isolation and characterization of two phosphoinositides from *C. xerosis*. The major one is a dimannophosphoinositide with apparently four acyl groups (tetra-acylated glycerophosphorylinositol dimannoside), but with similar chromatographic properties to the triacylated dimannophosphoinositide obtained from *Mycobacterium phlei* (Brennan & Ballou, 1967). The second is a phosphoinositide with three acyl groups (triacylated glycerophosphorylinositol), but similar in chromatographic properties to phosphatidylinositol from yeast.

C. xerosis (from the Department of Bacteriology, Trinity College, Dublin) was grown in a New Brunswick Fermentor in a medium modified slightly from that of Rose & Evison (1965). The organisms were harvested by centrifugation, washed with deionized water and stored at −20°. Lipids were extracted from acetone-dried powders with chloroform–methanol–water (16:6:1, by vol.) (Brennan & Ballou, 1968*a*). The extracts were evaporated to dryness, and the residues were triturated repeatedly with acetone, washed by the method of Folch, Lees & Sloane-Stanley (1957) and converted into the sodium form (Pangborn & McKinney, 1966). Finally the lipid was emulsified with water and the mixture was dialysed against water for several days and freeze-dried. This phospholipid fraction constituted about 7% of the dry weight of the cell, and acetone-soluble lipids constituted about 5%.

Thin-layer chromatography was carried out on plates (40cm. × 20cm.) of silica gel H as a layer 0·5mm. thick. The following solvents were used: *A*, chloroform – methanol – acetic acid – water (30:15:4:2, by vol.); *B*, chloroform–methanol–water (65:25:4, by vol.); *C*, chloroform–methanol–7N-NH_3 (12:7:1, by vol.). For preparative thin-layer chromatography lipids were located by spraying plates with water (Tate & Bishop, 1962) or by exposure to iodine vapour. Lipids were eluted from silica gel with chloroform–methanol (1:2, v/v), filtered through glass-fibre paper and washed by the method of Folch *et al.* (1957). The molybdenum blue reagent of Dittmer & Lester (1964) was used to detect phosphorus-containing lipids, and a 0·1% solution of ninhydrin in acetone was used to detect nitrogen-containing compounds.

Paper chromatography was carried out on Whatman no. 1 or no. 3MM paper in the following solvents: *D*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4, by vol.); *E*, butan-1-ol–pyridine–water (3:1:1, by vol.); *F*, water-saturated butan-1-ol; *G*, propan-2-ol–aq. NH_3 (sp.gr. 0·88) (2:1, v/v); *H*, ethyl acetate–pyridine–water (5:3:2, by vol.); *I*, propan-2-ol–formic acid–water (50:13:7, by vol.). Spots on paper chromatograms were revealed with the $AgNO_3$–NaOH dip reagent (Anet & Reynolds, 1954).

Fatty acid esters were determined by the method of Stern & Shapiro (1953) with methyl myristate as the standard. Phosphorus was determined by the method of Bartlett (1959), hexoses were determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956) and glycerol was determined by the method of Renkonen (1962). For the determination of inositol, hydrolysed lipid samples were subjected to preparative paper chromatography on Whatman no. 50 paper in 90% (v/v) acetone; inositol was eluted and determined by the periodate oxidation method of Gaitonde & Griffiths (1966).

The preparation of the dimannophosphoinositides of *M. phlei* and of their deacylation product was as described by Brennan & Ballou (1967). Yeast phosphatidylinositol was prepared by the method of Trevelyan (1966) and finally purified by preparative thin-layer chromatography as described above.

Acid hydrolysis (Brennan & Ballou, 1967) of the phospholipid fraction of *C. xerosis* and chromatography of the products in solvent *D* revealed the presence of inositol, mannose, glucose, arabinose and glycerol. This was confirmed by chromatography in solvent *E*, except that this did not clearly resolve

Table 1. *Analyses of phosphoinositides from* C. xerosis

The results for acyl groups are given as means ± S.E.M. with the numbers of determinations in parentheses; for phospholipid I results of analyses on two separate preparations are given.

Lipid	Composition (molar proportions)				
	Phosphate	Mannose	Inositol	Glycerol	Acyl groups
Dimannophosphoinositide (phospholipid I)	1	2·02	0·98	0·92	4·01 ± 0·24 (4), 3·28 ± 0·24 (4)
Phosphoinositide (phospholipid II)	1	0·00	1·02	1·05	2·83 ± 0·20 (7)
Yeast phosphatidylinositol	1	—	—	—	2·03 ± 0·23 (7)

mannose and arabinose. Preparative thin-layer chromatography of the phospholipid fraction in solvent *A* and location of the lipid with either water or iodine showed the presence of two closely associated bands with R_F 0·46 (phospholipid I) and R_F 0·54 (phospholipid II). Both of these lipids were shown to contain phosphorus. All other lipids either remained at the origin or travelled to the front in this solvent system. No ninhydrin-positive lipid was detected on these chromatograms, nor could ethanolamine or serine be detected on hydrolysis of the phospholipid and chromatography in solvent *G*. Phospholipids I and II were eluted from the silica gel and rechromatographed in solvent *A*. A portion of each of the lipids obtained was hydrolysed and chromatographed in solvents *D*, *E* and *F*. Phospholipid I yielded inositol, mannose and glycerol, and phospholipid II inositol and glycerol only. Chromatography of the deacylated products in solvents *G*, *H* and *I* showed that phospholipid I yielded only glycerophosphorylinositol dimannoside, identical with the product from *M. phlei*, and phospholipid II yielded only glycerophosphorylinositol, identical with the product from yeast. The pure lipids were analysed for carbohydrate (as mannose), phosphorus, glycerol, inositol and acyl groups (Table 1). The results confirm the chromatographic evidence for the presence of a phosphoinositide and a dimannophosphoinositide in *C. xerosis*.

The finding of three acyl groups in the phosphoinositide (Table 1) is surprising on two grounds. First, as far as we know a triacylated phosphoinositide without sugar residues has not previously been reported. Secondly, its chromatographic properties did not appear to conform to the degree of acylation found: the phosphoinositide from *C. xerosis* had chromatographic properties in solvents *A*, *B* and *C* identical with those of yeast phosphatidylinositol, which has only two acyl groups.

It was difficult to establish clearly the number of acyl groups attached to the dimannophosphoinositide. In a first experiment results indicating four acyl groups were obtained (Table 1). However, further purification and analysis of more of the dimannophosphoinositide resulted in values intermediate between three and four. This suggests that under certain conditions some loss of the fourth ester group can take place. A tetra-acylated dimannophosphoinositide has been found in mycobacteria (Pangborn & McKinney, 1966; Brennan & Ballou, 1967), but the dimannophosphoinositide from *C. xerosis* has a chromatographic mobility identical with that of the triacylated dimannophosphoinositide from *M. phlei* in solvents *A*, *B* and *C* and moved appreciably slower than the tetra-acylated compound.

The apparent discrepancy between the number of acyl groups in the phospholipids of *C. xerosis* and their chromatographic properties may be accounted for by some unusual structural feature in one or more of the acids, such as very short chain length, as in the acetylated sugar derivatives described by Welsh, Shaw & Baddiley (1968).

This work establishes that *C. xerosis*, as well as *M. phlei*, *M. tuberculosis* and *Propionibacterium shermanii* (Brennan & Ballou, 1968*b*), contains mannophosphoinositides, thus providing further evidence for a close relationship between the mycobacteria, propionibacteria and corynebacteria.

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