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Note

Separation of glycerylphosphorylinositol mannosides by thin-layer chromatography

G. K. KHULLER and B. BANERJEE

Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh (India)

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Mannophosphoinositides appear to be widespread among the Actinomycetales. In addition to the well known mycobacterial source, they have been identified in streptomyces, microbispora, nocardias and corynebacteria[1]. These phospholipids differ in two respects, *i.e.*, they contain from one to six mannose units and there can be considerable variation in the number of attached acyl groups[2,3]. The best approach for characterizing the mannophosphoinositides involves deacylation and paper chromatography of the aqueous products for 3 days[4,5]. In view of the biological importance of mannosides[6], a rapid method for their separation as glycerylphosphoryl derivatives would be of great value in characterizing these phospholipids. This paper describes a simple one-dimensional thin-layer chromatographic (TLC) method for the separation of different classes of glycerylphosphorylinositol mannosides from the deacylated total lipids of *Streptomyces griseus*.

EXPERIMENTAL

The total lipids were extracted from large amounts of *S. griseus* obtained from Dr. Y. Okami (Institute of Microbial Chemistry, Tokyo, Japan) grown on liquid media containing glucose, beef extract and peptone for 8 days[7]. Lipids were purified as described elsewhere[8]. Lipids were subjected to mild alkaline hydrolysis according to the method of Tarlov and Kennedy[9]. The TLC of the deacylated total lipids was carried out on silica gel H plates. A deacylated lipid sample containing 20–30 μg of lipid-P was spotted and developed with isopropanol–ammonia solution (sp. gr. 0.91) (1:1). Phosphorus-containing lipids were located by using Hanes–Isherwood spray[10]. Carbohydrate-containing lipids were detected by spraying with α-naphthol–sulphuric acid reagent[11] and heating the plates at 100° for 3–6 min.

For quantitative isolation of mannosides, preparative TLC was used. In preparative TLC, marker plates were run simultaneously to locate the position of the phospholipids. Deacylated lipids were eluted from the silica gel with water. The extracts were evaporated to dryness at 50–60° under reduced pressure and the residue was dissolved in an aliquot of water. The isolated lipids were analysed qualitatively for glycerol, mannose and inositol by acid hydrolysis followed by paper chromato-

graphy[8]. Methods for the detection of polyols on paper chromatograms have been described previously[8].

RESULTS

A typical separation of the deacylated total lipids of *S. griseus* on a silica gel H plate is shown in Fig. 1. It can be seen that deacylated lipids were separated clearly on these plates. The five components separated from the total lipid extract were carbohydrate positive. The five components were eluted separately and on quantitative analysis components I–IV were found to contain glycerol, mannose and inositol. Component V was found to contain glycerol and glucose, which was due to the presence of acyl glucoses in *S. griseus*.

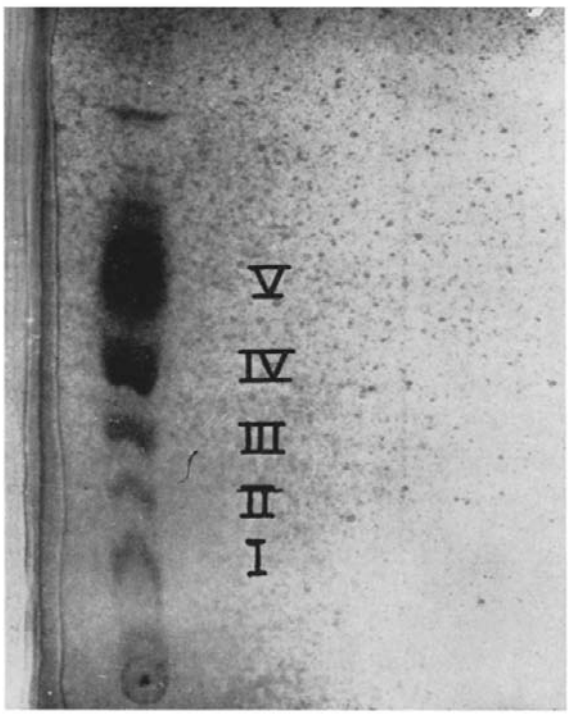

Fig. 1. Separation of glycerylphosphorylinositol mannosides from deacylated total lipids on a silica gel H plate. Spots developed with α-naphthol–sulphuric acid spray.

Further characterization of these components is in progress and will be reported elsewhere.

REFERENCES

1 R. T. Ambron and R. A. Pieringer, in G. M. Ansell, J. N. Hawthorne and R. M. C. Dawson (Editors), *Form and Function of Phospholipids*, Elsevier, New York, 1973, p. 289.
2 M. C. Pangborn and J. A. Mckinney, *J. Lipid Res.*, 7 (1966) 627.
3 P. Brennan and C. E. Ballou, *J. Biol. Chem.*, 242 (1967) 3046.

4 Y. C. Lee and C. E. Ballou, *Biochemistry*, 4 (1965) 1395.
5 G. K. Khuller and P. J. Brennan, *Amer. Rev. Respir. Dis.*, 106 (1972) 892.
6 G. K. Khuller and D. Subrahmanyam, *Immunochemistry*, 8 (1971) 251.
7 T. Kataoka and S. Nojima, *Biochim. Biophys. Acta*, 144 (1967) 681.
8 G. K. Khuller and P. J. Brennan, *Biochem. J.*, 127 (1972) 369.
9 A. R. Tarlov and E. P. Kennedy, *J. Biol. Chem.*, 240 (1965) 49.
10 C. S. Hanes and F. A. Isherwood, *Nature (London)*, 164 (1949) 1107.
11 H. Jacin and A. R. Mishkin, *J. Chromatogr.*, 18 (1965) 170.