CHROM. 5734

Thin-layer chromatography of bacterial lipids on sodium acetate-impregnated silica gel

The separation of polar lipids by thin-layer chromatography (TLC) is well documented[1,2]. Because of the diversity of lipid structures, however, the separation of the constituents of any particular lipid may well pose unique problems. We report here a TLC system involving silica gel impregnated with sodium acetate as adsorbent which we have found to have advantages for the chromatography of some bacterial lipids, which may contain glycolipids in addition to phospholipids.

Chromatographic systems

Plates were prepared from a slurry of Merck Silica Gel PF_{254} (40 g) in 100 ml of 0.2 % aqueous sodium acetate. The plates were allowed to dry overnight at room temperature; activation at $> 100°$ resulted in inferior separation.

The following solvents were employed for development of thin-layer chromatograms: (A) chloroform–methanol–water (65:25:4) (ref. 3) and (B) chloroform–acetic acid–methanol–water (80:18:12:5).

Universal spray reagents for the detection of lipids were 15 % aqueous ammonium sulphate[4] or a saturated solution of potassium dichromate in sulphuric acid[5] both followed by charring at 200°. Specific spray reagents employed were periodate-Schiff reagent for α-glycols[6–8], Zinzadze reagent for lipid phosphorus[9] and ninhydrin reagent for free amino groups.

Results

Fig. 1 illustrates both the separation in single dimensions for the two solvents employed and their two-dimensional combination as applied to the polar lipids of *Bacillus subtilis* W23 (grown as described previously for *B. cereus* T)[10].

In the single-dimensional system employing solvent A it will be noticed that diglycosyl diglyceride (DG) overlaps phosphatidylethanolamine (PE) but the other major lipids phosphatidylglycerol (PG) and cardiolipin (diphosphatidylglycerol, DPG) are separated. For the lipids of the majority of bacteria this system would be adequate since most Gram-positive bacteria usually contain DG but no PE and most Gram-negative bacteria have PE but no DG[10]. The main advantage of using sodium acetate impregnation with this very popular lipid TLC solvent is that the PG spot is of a compact shape compared with the elongated comet-shaped spot observed with unimpregnated plates.

Solvent B is designed to separate PE and DG but the latter now overlaps PG. The separation of PE and PG is better than in solvent A; system B would therefore be preferable for lipids lacking DG, which include those of most Gram-negative bacteria. In the lipids of certain bacteria, notably Bacilli, PE and DG are both encountered and single-dimensional solvent systems are inadequate.

The two-dimensional combination of solvents A and B resolves all the lipids under consideration. In addition bacterial lipids often contain small quantities of relatively more polar lipids notably amino acid esters of PG. In solvent A a spot assigned to a lysine ester of PG (lysyl-PG) gives an anomalous positive periodate-

Schiff reaction; in solvent B this spot is resolved into the lipoamino acid and an uncharacterized periodate-Schiff positive component (X), which is not a phospholipid. The two-dimensional system resolves these two lipids very efficiently, the area of the plate available for the separation of such polar lipids being greatly increased.

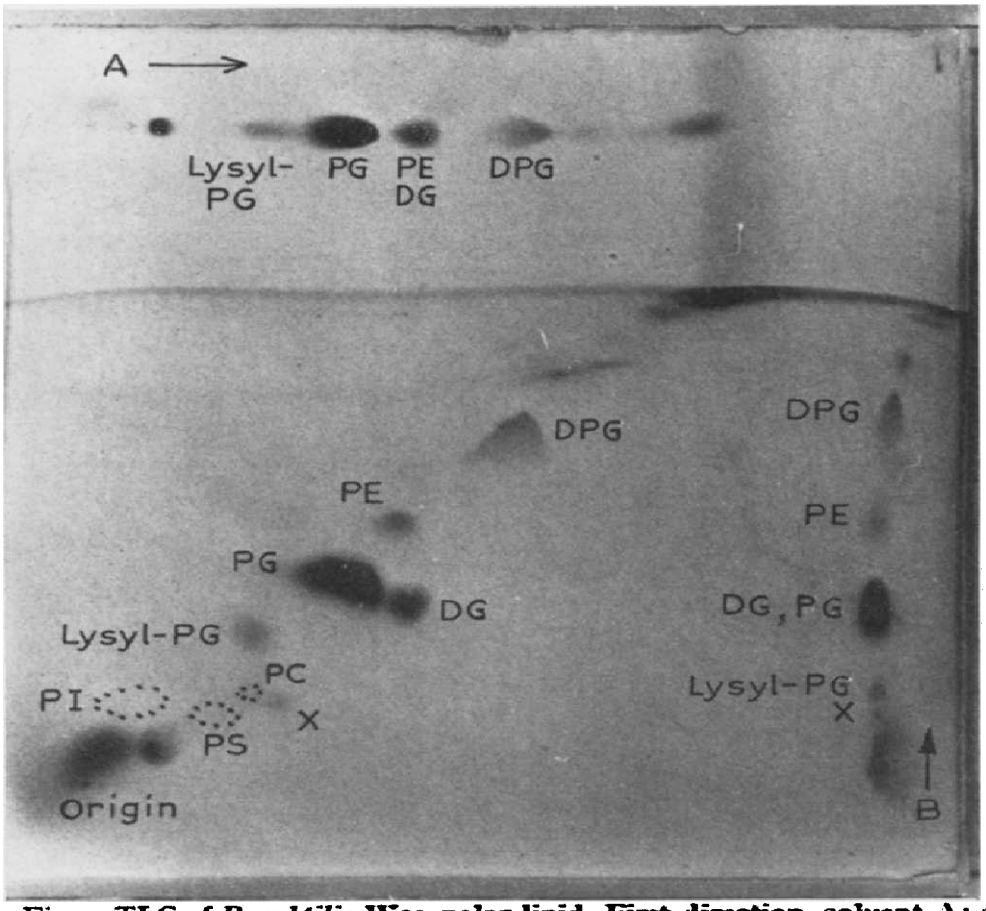

Fig. 1. TLC of *B. subtilis* W23 polar lipid. First direction, solvent A; second direction, solvent B. The spots are identified in the text.

Phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI) are rarely encountered in bacterial lipids but are widespread in the lipids of many other organisms. Samples of these lipids (Sigma Chemical Co. Ltd.) were examined in the two-dimensional system and their approximate positions are indicated in Fig. 1. The common bacterial lipids DPG, PE, PG and DG are therefore conveniently resolved in an area of the plate distinct from that in which the less common bacterial lipids occur.

These procedures outlined above are a further example of the advantages of modifying TLC conditions so that particular separations can be achieved efficiently. Recently silica gel plates impregnated with borax have been used to resolve complex mixtures of glycosyl diglycerides from Mycoplasma[11] and boric acid impregnation enabled efficient separations of DPG, PE and PG from *Escherichia coli* to be obtained[12]; this latter separation may also be easily carried out on the plates described here especially using solvent B. Brain phospholipids have been chromatographed on silica gel plates impregnated with a variety of materials including sodium acetate but the degree of impregnation (3 g of sodium acetate in 60 ml water slurried with 27 g silica

gel) was much greater[13]. Such systems were found to be unsuitable for our purpose as were the procedures devised for plant polar lipids[7] which have similarities in composition to bacterial lipids.

Regarding detection reagents, both periodate-Schiff and Zinzadze reagents tend not to give a very permanent stain to the chromatogram. We have found, however, that following periodate-Schiff application, which locates DG and PG, the Zinzadze reagent may then be used to reveal the remaining phospholipids, for example, PE and DPG. The glycolipids remain coloured and thus all polar lipids are conveniently detected by this procedure; the resulting spots are stable for several weeks.

We thank Professor J. BADDILEY, F.R.S., for his encouragement.

Microbiological Chemistry Research Laboratory,
School of Chemistry, The University,
Newcastle upon Tyne NE1 7RU (Great Britain)

D. E. MINNIKIN
H. ABDOLRAHIMZADEH

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Received August 12th, 1971