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Note

Thin-layer chromatography of phospholipids

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Thin-layer chromatography (TLC) is the most commonly used technique for the separation and identification of lipids. However, difficulties are encountered in the complete resolution of the complex lipid mixtures found in mammal tissues. One- and often two-dimensional TLC does not resolve such mixtures owing to either interference with phospholipid migration by galactolipids[1–7] or gangliosides or poor resolution of the different phospholipids[8–10]. In all instances a phase partition[11] of the whole lipid extract is necessary in order to prevent interferences by gangliosides and tailing due to the presence of salts or proteolipids.

We present here a new one-dimensional TLC system that allows a complete separation of the major galactolipids, neutral lipids and phospholipids found in brain tissue.

EXPERIMENTAL

Materials

Pre-coated silica gel plates (DC fertigplatten, 20 × 20 cm) and high-performance TLC) (HPTLC) plates (für nano-DC, 10 × 10 cm) were obtained from Merck (Darmstadt, G.F.R.). Standard phospholipids (DL-α-phosphatidylcholine dipalmitoyl, DL-α-phosphatidylethanolamine dipalmitoyl, L-α-phosphatidylinositol, L-α-phosphatidyl-L-serine, L-α-phosphatidic acid and sphingomyelin) were obtained from Sigma (St. Louis, Mo., U.S.A.). Cardiolipin (diphosphatidylglycerol) was obtained from Calbiochem (San Diego, Calif., U.S.A.).

Extraction of lipids from rat brain, cerebellum or myelin and partition of gangliosides were performed according to Suzuki[11]. Galactolipids from rat brain were prepared by the method of Vance and Sweeley[12] and purified by TLC[13].

The separation of the lipid mixtures was performed with the solvent methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:25:10:9) unless otherwise specified. Ascending chromatography was performed in conventional TLC tanks coated with filter-paper.

Samples were applied in streaks containing 0.5–3.0 μg/cm lipid phosphorus for HPTLC plates and 1.5–8 μg /cm of lipid phosphorus for conventional TLC plates. Chromatography was performed at room temperature until the solvent front reached

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the upper edge of the plates (50 min for HPTLC and 150 min for conventional plates).

Phospholipids, cerebrosides and sulphatides, and gangliosides were revealed with molybdate reagent[8], orcinol–sulphuric acid reagent[14] and orcinol–hydrochloric acid reagent[15], respectively. Simultaneous detection of all lipids was obtained by using iodine or rhodamine.

RESULTS AND DISCUSSION

Typical separations obtained with HPTLC or conventional pre-coated plates are shown in Fig. 1. Neutral lipids, cerebrosides, sulphatides, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine and sphingomyelin are well separated from each other. In addition, cerebrosides with non-hydroxylated fatty acid chains are separated from those with hydroxylated chains. The same is true for sulphatides. The minor brain phospholipids (phosphatic acid and diphosphatidylglycerol) migrate together but are well separated from phosphatidylethanolamine and phosphatidylinositol.

The results are the same whether a whole lipid extract or only the lower phase after lipid partition, is used. Chromatography of the upper phase or of whole lipid extracts (Fig. 2) show that gangliosides have a very low R_F value and are well separated

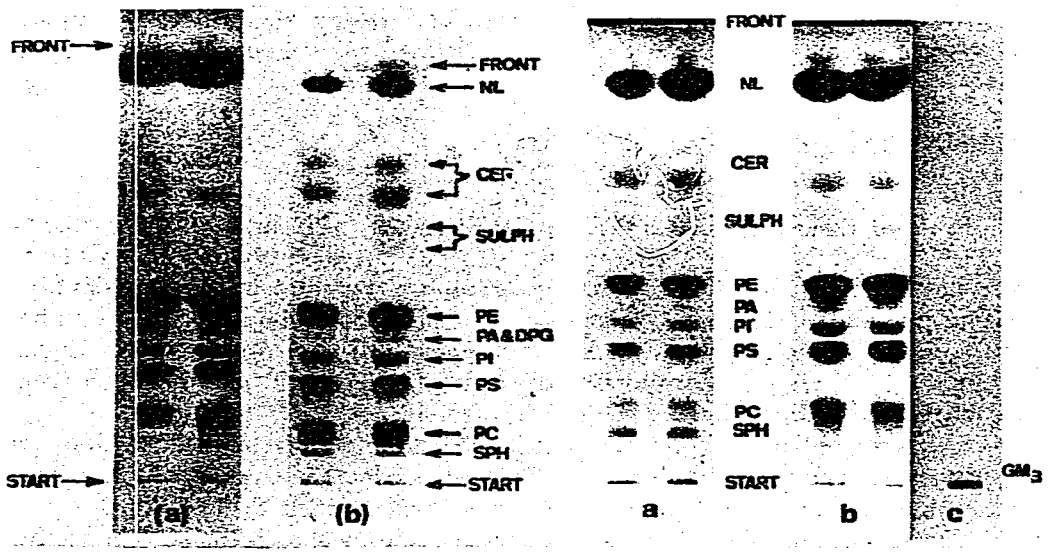

Fig. 1. One-dimensional separation of standard lipids in the solvent methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous KCl (25:25:25:10:9). (a) Separation on 10 × 10 cm HPTLC plates; (b) separation on classical pre-coated silica gel plates. Abbreviations: NL = neutral lipids; CER = cerebrosides; SULPH = sulphatides; PE = phosphatidylethanolamine; PA = phosphatidic acid; DPG = cardiolipin; PI = phosphatidylinositol; PS = phosphatidylserine; PC = phosphatidylcholine; SPH = sphingomyelin. Staining: molybdate reagent.

Fig. 2. One-dimensional TLC on HPTLC plates of whole lipid extracts.(a) Whole lipid extract of crude myelin; (b) whole lipid extract of rat cerebellum (20 days old); (c) TLC of standard gangliosides. Abbreviations as in Fig. 1.

from other lipids. The presence of proteolipids has no effect on the separation, as demonstrated in Fig. 2, when a whole lipid extract of crude myelin is chromatographed.

Vapour saturation of the chromatographic tank and temperature do not have a significant effect on the reproducibility of the separation ,which is always excellent provided that appropriate amount of lipids are applied to the plates (see above).

Our solvent system in conjunction with home-made silica gel H plates can be used for preparative separation, in a single chromatographic run and starting from a whole lipid extract, of cerebrosides, sulphatides, phosphatidyl-choline, -ethanolamine, -inositol and -serine, sphingomyelin and neutral lipids. This separation is complete and tailing or degradation due to acidic or basic solvents are absent, as shown by re-chromatography of each phospholipid and galactolipid spot (Fig. 3).

Chromatography in the present solvent followed by hydrolysis on the plate[6] and chromatography in the second dimension with the solvent chloroform–methanol–acetone–acetic acid–water (75:15:30:15:7.5)[16] demonstrate that, in our solvent system, plasmalogens are not separated from the corresponding phospholipids, but they can be easily separated by this technique (Fig. 4). Otherwise, phosphatidic acid can easily be separated from cardiolipin by chromatography in the second dimension in a classical solvent[2].

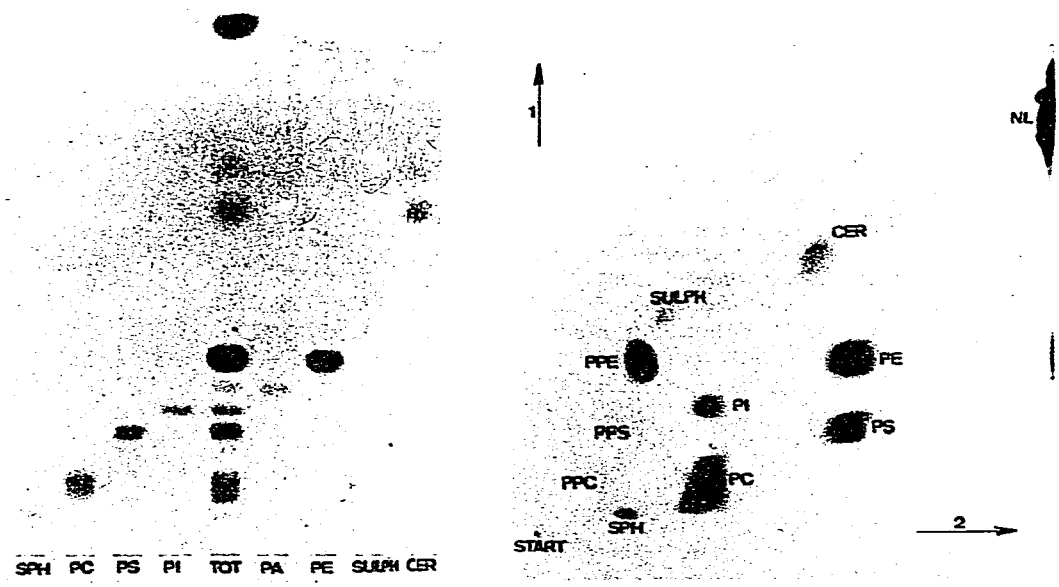

Fig. 3. Re-chromatography on HPTLC plates of compound separated by one-dimensional preparative chromatography in our solvent. Abbreviations as in Fig. 1.

Fig. 4. Two-dimensional separation of plasmalogen on conventional TLC plates. First chromatography (1) in our solvent followed by hydrolysis[6] and second chromatography (2) in another solvent[16]. PPE, PPS, PPC correspond to plasmalogen of PE, PS and PC, respectively. In order to reveal the trace amount of PPS and PPC present in lipid of rat cerebellum, the plate was overloaded so that some tailing is seen.Abbreviations as in Fig. 1.

It is necessary to emphasize that some spots (in particular phosphatidylethanolamine and phosphatidylcholine) appeared to be asymmetrical. This is probably due to differences in the fatty acid composition of the lipids. In fact, commercially available dipalmityol derivatives show slightly lower R_F values than the bulk of corresponding brain phospholipids, which are known to be richer in unsaturated fatty acid chains[2]. The phenomenon is particularly evident with HPTLC (which appeared to have higher resolution).

In conclusion, we believe that our solvent system has the following considerable advantages over classical methods: (*i*) improved resolution; (*ii*) possibility of analysing directly the whole lipid extract; (*iii*) substantial time saving; and (*iv*) absence of interference by gangliosides.

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