A modified procedure for thin-layer chromatography of phospholipids

David Allan and Shamshad Cockcroft

Department of Experimental Pathology, University College London, Faculty of Clinical Sciences, School of Medicine, University Street, London WC1E 6JJ

Summary We have found that when lipid samples from a variety of tissues are run on EDTA-impregnated plates in a solvent system which contains a reduced amount of acetic acid and water compared with that used by Skipski et al. (1964. *Biochem. J.* **90:** 374–378), there is a clear separation among sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, and phosphatidate. This modified procedure appears to offer significant advantages over the original method described by Skipski et al. particularly for studies of the metabolism of anionic phospholipids.—**Allan, D., and S. Cockcroft.** A modified procedure for thin-layer chromatography of phospholipids. *J. Lipid Res.* 1982. **23:** 1373–1374.

Supplementary key words anionic phospholipids

A large number of procedures have been described for the separation of phospholipids by thin-layer chromatography but, in general, only separation in two dimensions are capable of resolving all of the different phospholipid classes (1, 2). Of the separations in one dimension, the most widely used procedure has been that of Skipski, Peterson, and Barclay (3) which produces a good separation of the main phospholipid classes present in animal tissues. However, it is commonly found that some of the minor lipids, especially the anionic ones such as phosphatidylinositol, phosphatidylserine, and phosphatidic acid are not completely and reproducibly resolved by the procedure of Skipski et al. (3). Our particular interest in these lipids led us to investigate modifications of this procedure that would enable us to resolve the anionic lipids while still retaining the separation of the major phospholipids originally described (3).

Abbreviations: TLC, thin-layer chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphatidylinositol.

METHODS

Glass plates (20 cm^2) were coated with a 250-μm layer of silica gel HR (BDH Ltd. Poole, Dorset, U.K.) using a Unoplan spreader (Shandon Southern Instruments Ltd.). The slurry was made up by adding 30 g of dry silica gel to 70 ml of 1 mM EDTA. (BDH Analar reagent). Plates were air-dried overnight and activated prior to use by heating for at least 1 hr at 120°C. Standard phospholipids were obtained either from Sigma (London) Chemical Co., Poole, Dorset, U.K. (PC, PE, PG, cardiolipin, ceramide monohexoside, and ganglioside) or from Lipid Products, South Nutfield, Surrey, U.K. (PA, PS, PI, lysoPC, lysoPE, lysoPS).

Samples of mixed lipids were obtained by extraction of human and chicken erythrocytes, rat liver, and human and rabbit polymorphonuclear leukocytes according to the method of Bligh and Dyer (4) but with the substitution of 2 M KCl for water in the phase separation. A bovine brain lipid subfraction whose main components were phosphatidylserine, diphosphoinositide, and triphosphoinositide was obtained from Dr. R. H. Michell, University of Birmingham, UK.

All lipids were dissolved in chloroform and 5- or 10-μl samples were applied as elliptical spots to the origin of the TLC plate. Spots were dried under a flow of N_2 gas and plates were developed at 20°C by ascending chromatography in a solvent (prepared daily) consisting of chloroform–methanol–acetic acid–water 75:45:3:1 (by volume). In a tank lined with filter paper running time was 70–90 min. Plates were air-dried and either stained with iodine vapor or sprayed with 20% ammonium sulfate prior to charring of spots by heating for 30 min at 190°C. With radioactive samples, plates were radioautographed prior to staining. Glycolipids were revealed by spraying with orcinol/sulfuric acid and heating at 100°C for 10 min (2).

RESULTS AND DISCUSSION

The separations obtained using the modified procedure are shown in **Fig. 1**. Although the pattern is

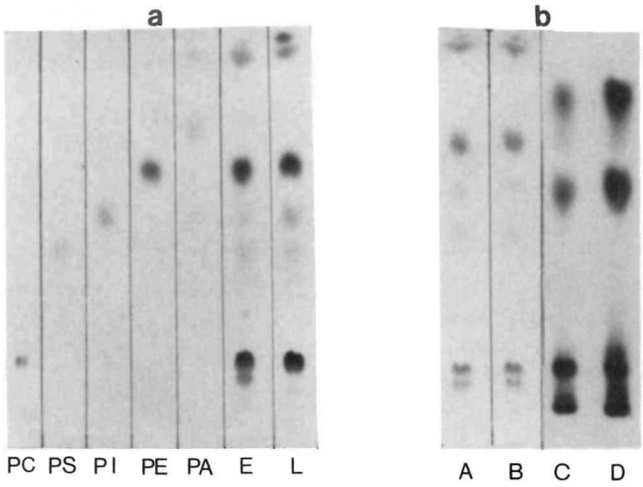

Fig. 1. a), Chromatogram of standard lipids and lipid mixtures on EDTA-impregnated plates. E, chicken erythrocyte lipids; L, rat liver lipids. b), Chromatogram of lipid extracts from rabbit polymorphonuclear leukocytes labeled with ^{32}P. A, control untreated; B, treated with F-met-leu-phe (see ref. 9). C and D are radioautograms of A and B, respectively. About 200 nmol of mixed phospholipids or 50 nmol of individual phospholipid was applied to the chromatograms.

broadly similar to that obtained by Skipski et al (3), there are two important differences. First, phosphatidate is well separated from phosphatidylethanolamine and from neutral lipids; and second, phosphatidylserine migrates considerably more slowly in the modified system and separates clearly and reproducibly from phosphatidylinositol. These separations are of particular significance for studies of phosphatidylinositol and phosphatidate metabolism, which have been the subject of much attention in recent years (5, 6). However, phosphatidylglycerol and cardiolipin, lipids that are characteristically present in mitochondria, were not separated from phosphatidylethanolamine by this procedure. Lysolecithin (migrating between the origin and sphingomyelin) and lysophosphatidylethanolamine (migrating between phosphatidylserine and phosphatidylcholine) were generally well separated from other phospholipids, but lysophosphatidylserine did not separate from phosphatidylcholine. Polyphosphoinositides remained at the origin and probably accounted for the nonmigrating radioactive spot in Fig. 1b that was observed even in samples which had been repeatedly washed with aqueous phase. The identity of the radioactive spot migrating in front of phosphatidylcholine in Fig. 1b is not known, but it could represent CDP-diglyceride.

The presence of EDTA in the silica gel used for the TLC plates did not, so far as we could judge, have any effect whatsoever on the extractability of spots with organic solvents or on phosphate analysis following digestion with perchloric acid.

Of the glycolipids tested, ceramide monohexoside ran at the front, and lactosyl ceramide, the major glycolipid of human leukocytes (7), was not separated from phosphatidate. The major glycolipid of human erythrocytes, a globoside (8), migrated with phosphatidylserine, and gangliosides remained at or close to the origin.

The TLC procedure described here represents only a small modification to a previously published method (3) but nonetheless seems to offer significant advantages, particularly for the separation of the metabolically active anionic phospholipids. It should therefore be recommended for metabolic studies of phospholipids where large numbers of samples make two-dimensional separations onerous or expensive.

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