Journal of Chromatography, 256 (1983) 378–380
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,409

Note

Thin-layer chromatographic separation of phosphonolipids from their phospholipid analogues

C. A. DEMOPOULOS*, M. C. MOSCHIDIS and L. G. KRITIKOU
Department of Food Chemistry, National University of Athens, Athens (Greece)
(Received September 3rd, 1982)

Several attempts have been made to separate phosphonolipids from their phospholipid analogues by thin-layer chromatography (TLC)[1–3], using solvent systems such as chloroform–methanol (4:4, 3:1 and 3:2), chloroform–glacial acetic acid (9:1), chloroform–92 % acetic acid (1:1) and chloroform–methanol–water (60:40:8) on silica gel H or G.

In this work, we used the solvent system methanol–water (2:1), previously employed for the separation of 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (AGEPC) from other lipids[4], for the complete separation of phosphonolipids.

EXPERIMENTAL

All reagents were of analytical-reagent grade from Merck or Mallinckrodt and were distilled before use.

a-Lecithin and lysolecithin were purchased from Merck, phosphatidylserine and cardiolipin from Serdary Research Labs. and phosphatidylethanolamine and sphingomyelin from Koch-Light.

AGEPC and the phosphono-analogues of sphingomyelin, phosphatidylcholine and phosphatidylethanolamine were synthesized in this laboratory[1,5–7]; the method for the synthesis of the phosphono-analogue of AGEPC will be published elsewhere[10].

Phosphatidylcholine and phosphatidylethanolamine in mixtures with their phosphonic acid analogues were also isolated from the lipid fraction of the protozoon *Tetrahymena pyriformis* after extraction of the total lipids using the Bligh–Dyer procedure[8], and subsequent purification by preparative TLC with chloroform–methanol–water (65:35:6) as the solvent system.

The phosphono-analogues of phosphatidylcholine and phosphatidylethanolamine were identified in the above mixtures by TLC using chloroform–glacial acetic acid–water (40:45:8)[3]. The pure phosphono-analogues were further identified by determining their phosphono-phosphorus[3].

The TLC plates were prepared using silica gel G (Merck) and the spots were revealed by exposure to iodine vapour and with ammonium molybdate spray[9].

0021-9673/83/0000-0000/$03.00 © 1983 Elsevier Scientific Publishing Company

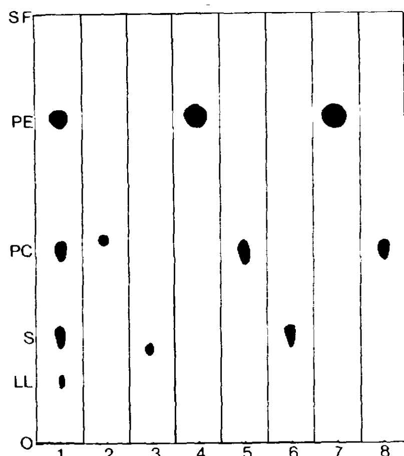

Fig. 1. TLC of various phospholipids in mixtures with their phosphono-analogues. SF = Solvent front; O = origin. The TLC plate was developed with chloroform–methanol–water (65:35:6). Lane 1: phospholipid standards, lysolecithin (LL, R_F = 0.15), sphingomyelin (S, R_F = 0.23), phosphatidylcholine (PC, R_F = 0.45) and phosphatidylethanolamine (PE, R_F = 0.76). Lane 2: phosphono-analogue of AGEPC (R_F = 0.47). Lane 3: AGEPC (R_F = 0.21). Lane 4: mixture of phosphono- and phosphatidylethanolamine from *Tetrahymena pyriformis*. Lane 5: mixture of phosphono- and phosphatidylcholine from *Tetrahymena pyriformis*. Lane 6: mixture of sphingomyelin and its synthetic phosphono-analogue. Lane 7: synthetic phosphono-analogue of phosphatidylethanolamine. Lane 8: synthetic phosphono-analogue of phosphatidylcholine. Spots were detected by exposure to iodine vapour.

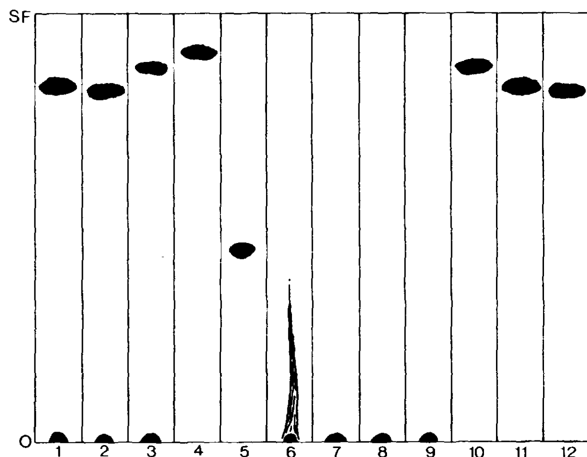

Fig. 2. TLC behaviour of various phospholipids and phosphonolipids. SF = Solvent front; O = origin. The TLC plates were developed with methanol–water (2:1). Lane 1: mixture of phosphono- and phosphatidylethanolamine from *Tetrahymena pyriformis* (R_F = 0.84 and 0.00 respectively). Lane 2: mixture of phosphono- and phosphatidylcholine from *Tetrahymena pyriformis* (R_F = 0.82 and 0.00 respectively). Lane 3: mixture of sphingomyelin and its synthetic phosphono-analogue (R_F = 0.00 and 0.87 respectively). Lane 4: phosphono-analogue of AGEPC (R_F = 0.91). Lane 5: AGEPC (R_F = 0.44). Lane 6: lysolecithin. Lane 7: phosphatidylserine. Lane 8: cardiolipin. Lane 9: neutral lipids. Lane 10: synthetic phosphonosphingomyelin (R_F = 0.87). Lane 11: synthetic phosphono-analogue of phosphatidylethanolamine (R_F = 0.84). Lane 12: synthetic phosphono-analogue of phosphatidylcholine (R_F = 0.82). Spots were detected by exposure to iodine vapour.

RESULTS AND DISCUSSION

Figs. 1 and 2 show that whereas the authentic corresponding phospholipids and phosphonolipids have the same R_F values using chloroform–methanol–water (65:35:6), they have widely differing R_F values using methanol–water (2:1). The phosphono-analogues migrate close to the solvent front whereas phospholipids and neutral lipids remain at the origin or show negligible migration or tailing. Only lysolecithin exhibits a similar mobility to that of AGEPC, but tends to tail.

Mixtures of the corresponding phospholipids and phosphonolipids isolated from *Tetrahymena pyriformis*, which appear as a single spot using chloroform–methanol–water (65:35:6) (Fig. 1), are completely separated using methanol–water (2:1) as two spots with widely differing R_F values (Fig. 2). The above mixtures of lipids from *Tetrahymena pyriformis* furnished two spots in the solvent system appropriate for the phospholipids[3] (see Experimental; data not shown).

The spot from the mixtures of *Tetrahymena pyriformis* lipids considered to be phosphonolipids (Fig. 2) were eluted from the silica gel with chloroform–methanol (1:1) and subjected to phosphonophosphorus determinations, which identified the compounds as being phosphonolipids. The corresponding spots at the origin (Fig. 2), when similarly tested, were free from phosphono-phosphorus.

In conclusion, it has been demonstrated that phosphonolipids migrate considerably faster than their phospholipid analogues and thus complete separation is effected.

REFERENCES

1 E. Baer and N. Z. Stanacev, *J. Biol. Chem.*, 240 (1965) 3754.
2 E. Baer and K. V. J. Rao, *Can. J. Biochem.*, 45 (1967) 317.
3 V. M. Kapoulas, *Biochim. Biophys. Acta*, 176 (1969) 324.
4 L. M. McManus, D. J. Hanahan, C. A. Demopoulos and R. N. Pinckard, *J. Immunol.*, 124 (1980) 2919.
5 C. A. Demopoulos, R. N. Pinckard and D. J. Hanahan, *J. Biol. Chem.*, 254 (1979) 9355.
6 V. M. Kapoulas and M. C. Moschidis, *Chem. Phys. Lipids*, 28 (1981) 357.
7 E. Baer and N. Z. Stanacev, *J. Biol. Chem.*, 239 (1964) 3209.
8 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
9 J. G. Dittmer and R. L. Lester, *J. Lipid Res.*, 5 (1964) 126.
10 M. C. Moschidis, C. A. Demopoulos and L. G. Kritikou, *Chem. Phys. Lipids*, in press.