Resolution of intact phosphatidylinositols by argentation thin-layer chromatography

B. J. Holub and A. Kuksis

Department of Biochemistry, and Banting and Best Department of Medical Research, University of Toronto, Toronto 101, Canada

SUMMARY An argentation thin-layer chromatographic method is described for the separation of intact phosphatidylinositols on the basis of the degree of unsaturation of their component fatty acids. The system is applicable for metabolic studies using radioactive precursors of phosphatidylinositol.

SUPPLEMENTARY KEY WORDS molecular species · gas–liquid chromatography of fatty acids · acetolysis

Recently, we reported (1) the complete determination of the molecular species of the phosphoinositides from bovine brain. The method was based upon the identification of the diglycerides released from the mono-, di-, and triphosphoinositides by a brain phosphodiesterase. Like the analyses of other glycerophosphatides based on the hydrophobic moieties of the molecules, this method does not allow metabolic studies using radioactive markers in the polar residues of the phosphatide. The present report describes the separation of intact natural phosphatidylinositols into different classes of unsaturation by means of argentation thin-layer chromatography. The chromatographic system does not effectively resolve other acidic glycerophosphatides, or phosphatidylcholine and phosphatidylethanolamine, which require different chromatographic conditions (2, 3).

Isolation of Phosphatidylinositol. Rat livers were homogenized with 20 vol of chloroform–methanol 2:1 (v/v), and total lipid extracts were prepared according to Folch, Lees, and Sloane Stanley (4). The lipids were applied to a column of alumina (1 mg of phosphorus/g of alumina) in chloroform–methanol 1:1 (v/v) and were eluted according to Dawson (5). The fraction rich in phos-

phatidylinositol was recovered with ethanol–chloroform–water 5:2:2 (v/v/v). The eluate was taken to near dryness under nitrogen and redissolved in chloroform–methanol 4:1 (v/v). Pure phosphoinositide was isolated by thin-layer chromatography in chloroform–methanol–acetic acid–water 25:15:4:2 (v/v/v/v) using 20 × 20 cm plates of silica gel H with a 0.50 mm thick adsorbent layer (6). The lipids were detected by spraying the plates with a solution of 2′,7′-dichlorofluorescein in ethanol and examining them under ultraviolet light. The phosphatidylinositol was eluted from the gel with chloroform–methanol–acetic acid–water 50:39:1:10 (v/v/v/v) as described elsewhere (3), and its identity was confirmed (1).

Subfractionation of Phosphatidylinositol. Thin-layer plates (20 × 20 cm, 0.5 mm thick layer) were spread from a slurry of 40 g of silica gel H (Merck and Co.) and 4 g of silver nitrate in 120 ml of water. After drying overnight at room temperature and atmosphere, they were activated at 120°C for 20 hr prior to use. The purified phospholipid (1–2 mg) was applied as a narrow band across the plate about 2.5 cm from the lower edge. The chromatoplate was developed in chloroform–methanol–water 65:35:5 (v/v/v). The separated bands were located by viewing the plates under ultraviolet light after spraying with 2′,7′-dichlorofluorescein. Fig. 1 shows a typical resolution obtained for rat liver phosphatidylinositol. The composition of the solvent system and the amount of silver nitrate appeared critical, since a limited number of simple variations failed to give comparable resolution. Table 1 gives the quantitative composition of the fatty acid patterns of the subfractions as revealed by transmethylation and gas–liquid chromatography. The proportions of the fractions were ascertained by adding methyl heptadecanoate as an internal standard.

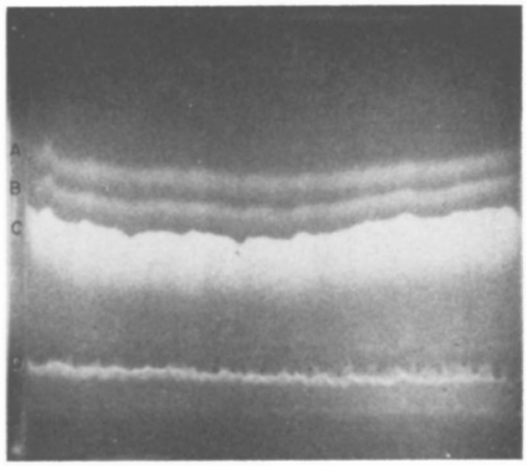

FIG. 1. $AgNO_3$–TLC separation of intact rat liver phosphatidylinositols. Plates: 200 × 200 × 0.5 mm silica gel layers prepared from a slurry of 40 g of silica gel H and 4 g of silver nitrate in 120 ml of water. Solvent: chloroform–methanol–water 65 : 35 : 5 (v/v/v). Spray: 0.05% 2,7-dichlorofluorescein in 50% methanol. Bands identified as follows: *A*, monoenes + dienes; *B*, trienes; *C*, tetraenes; *D*, polyenes.

From the analyses of the fatty acids, it is obvious that the separations depend upon the degree of unsaturation of the component acids, as noted by Arvidson for intact lecithins (2) and cephalins (3). Like the systems of Arvidson (2, 3), the present method also failed to give

TABLE 1 FATTY ACID COMPOSITION OF VARIOUS CHEMICAL CLASSES OF RAT LIVER PHOSPHATIDYLINOSITOLS*

Fatty Acids	Total		Chemical Classes			
	Original	Reconstituted†	Monoenes + Dienes	Trienes	Tetraenes	Polyenes
			mole %			
14:0	tr‡	tr				
16:0	6.7	6.0	22.5	9.7	3.6	9.9
18:0	39.6	43.9	32.3	41.4	46.6	33.2
18:1 (n − 9)	2.9	3.0	7.3	6.9	2.0	5.3
18:2 (n − 6)	3.3	2.7	35.3	4.4		
20:2 (n − 6)	tr	0.2	2.6			
20:3 (n − 9) 20:3 (n − 6)	3.0	2.1		37.6		
20:4 (n − 6)	40.2	37.1			46.9	9.5
20:5 (n − 3)	0.4	0.2				1.9
22:3 (n − 9)	0.5	0.7			0.9	
22:5 (n − 3)	1.0	1.1				11.0
22:6 (n − 3)	2.4	3.0				29.2

* Original isolation from a pooled sample of two livers; subfractions estimated as averages of at least two thin-layer and gas–liquid chromatographic separations.

† Reconstituted composition derived from the fatty acid values of individual chemical classes and their contribution to the total unfractionated phospholipid. The monoene + diene, triene, tetraene, and polyene fractions constituted an average of 7.1, 5.5, 77.0, and 10.4%, respectively, of the total mixture.

‡ Trace.

reproducible resolution of the monoenoic and dienoic phosphatides. There was a surprisingly good resolution, however, of the trienoic species, which has not been realized in previous work. The absence of any saturated species was confirmed by argentation chromatography of the diglyceride acetates derived from the phosphatides by acetolysis (7, 8). The proportions of the various unsaturation classes estimated by direct chromatography gave higher recoveries of the polyenoic species than reported following acetolysis (8). The good agreement between the fatty acid compositions of the original and reconstituted phosphatidylinositols suggested that argentation chromatography of intact phosphoinositides was not accompanied by significant losses of any molecular species. The identification and quantitative estimation of the individual molecular species of the phosphatidylinositols has been determined[1] along with that of the other rat liver glycerophosphatides. Further studies with labeled phosphate, *myo*-inositol, glycerol, and fatty acids using the above method are now feasible, as are determinations of the relationship of the in vivo biosynthesis of the glycerophosphatides to their diglyceride and phosphatidic acid precursors.

[1] Holub, B. J., and A. Kuksis. Unpublished data.

This work was supported by grants from the Ontario Heart Foundation, Toronto, Ontario, and the Medical Research Council of Canada. The Medical Research Council provided a studentship to B. J. Holub.

Manuscript received 30 November 1970; accepted 5 March 1971.

References

1. Holub, B. J., A. Kuksis, and W. Thompson. 1970. Molecular species of mono-, di-, and triphosphoinositides of bovine brain. *J. Lipid Res.* **11:** 558–564.
2. Arvidson, G. A. E. 1965. Fractionation of naturally occurring lecithins according to degree of unsaturation by thin-layer chromatography. *J. Lipid Res.* **6:** 574–577.
3. Arvidson, G. A. E. 1968. Structural and metabolic heterogeneity of rat liver glycerophosphatides. *Eur. J. Biochem.* **4:** 478–486.
4. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
5. Dawson, R. M. C. 1958. The identification of two lipid components in liver which enable *Penicillium notatum* extracts to hydrolyze lecithin. *Biochem. J.* **68:** 352–357.
6. Skipski, V. P., R. F. Peterson, and M. Barclay. 1964. Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J.* **90:** 374–378.
7. Breckenridge, W. C., and A. Kuksis. 1968. Specific distribution of short-chain fatty acids in molecular distillates of bovine milk fat. *J. Lipid Res.* **9:** 388–393.
8. Akino, T., and T. Shimajo. 1970. On the metabolic heterogeneity of rat liver phosphatidylinositol. *Biochim. Biophys. Acta.* **210:** 343–346.