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Note

Rapid, large-scale purification of crude egg phospholipids using radially compressed silica gel columns

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Crude egg yolk phosphatidylcholine has been purified in the past by column chromatography on silicic acid or alumina. Hanahan *et al.*[1] used alumina as the adsorbent and eluted the egg yolk phosphatidylcholine in 95% ethanol. An obvious drawback to this procedure was the difficulty in separating lysophosphatidylcholine from phosphatidylcholine as well as a reported 1% degradation of phosphatidylcholine in 1 h at room temperature under these conditions[2]. In addition, phosphatidylethanolamine could not be purified using alumina as the adsorbent as it remained adsorbed on the column and extremely polar solvent systems were required in order to elute it. In 1955, Lea *et al.*[3] reported the purification of crude egg phosphatidylcholine using silicic acid as a chromatographic adsorbent and chloroform–methanol (8:2) as the elution solvent. With this method, pure phosphatidylcholine is often obtained in poor yield, and phosphatidylethanolamine is always contaminated with free fatty acids. Singleton *et al.*[4], in 1965, reported the use of alumina as an adsorbent and chloroform–methanol (9:1) to elute the phosphatidylcholine from the column. This modification of the Hanahan *et al.* procedure effectively eliminated the degradation of the phospholipid and allowed the use of increased flow-rates.

More recently, Geurts van Kessel *et al.*[5] reported the purification of phospholipids by high-performance liquid chromatography on silica gel columns. In another recent report, Kiuchi *et al.*[6] used an anion-exchange μBondapak-NH_2 column to purify phospholipids by high-performance liquid chromatography. Both of these methods are limited to approximately 1 g of lipid, and are therefore unsuitable for obtaining large amounts of pure phosphatidylcholine. MacDonald and Rempas[7] reported the use of silica gel dry-column chromatography for the purification of phospholipids. While this method is elegant, the need for special columns and the time required for development, location of the phospholipid bands and elution from the support preclude its use for large-scale purifications.

Following the introduction of the Waters Prep LC-500 liquid chromatography system, which uses radially compressed silica gel columns, we investigated its application to the rapid purification of large amounts of crude egg phospholipids in extremely short periods of time. We report here our results obtained using chloroform–methanol–water systems to elute the phospholipids.

MATERIALS

The crude egg phosphatidylcholine was purchased from Sigma (St. Louis, Mo., U.S.A.). Chloroform and methanol were Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.) "glass distilled" materials. All solvent mixtures were prepared by volume. Thin-layer chromatography (TLC) plates coated with silica gel were purchased from Brinkmann (Westbury, N.Y., U.S.A.). The Waters Assoc. Prep LC-500 liquid chromatograph, using Pre-Pack 500 cartridges containing silica gel, has been previously described[8]. Fatty acid compositions of the crude and purified egg phosphatidylcholine and phosphatidylethanolamine were obtained by gas–liquid chromatographic analyses of the *trans*-esterified fatty acid methyl esters. The elution of the phosphatidylcholine from the silica gel column was monitored by the differential refractometer on the instrument and by TLC in chloroform–methanol–water (60:30:4). Iodine and/or molybdenum spray[9] were used to detect the spots on the plates and the contents of each fraction were identified by comparison of the R_F values with those of authentic materials. Ninhydrin spray was used to identify phosphatidylethanolamine.

EXPERIMENTAL AND RESULTS

The silica gel column of the Prep LC-500 was pressurized to 700 p.s.i. with nitrogen and equilibrated with chloroform–methanol–water (60:30:4) at a flow-rate of 200 ml/min until a stable baseline was obtained. In one experiment, 5 g of crude egg yolk phosphatidylcholine dissolved in 10 ml of benzene were injected directly on to the column. The pump was started and the profile shown in Fig. 1A was obtained; TLC (Fig. 1B) of the collected fractions indicated that fractions 1–5 contained neutral lipids and phosphatidylethanolamine; fractions 6–8 contained an unidentified phospholipid and a small amount of phosphatidylcholine; fractions 9–12 contained phosphatidylcholine; fractions 13–15 contained phosphatidylcholine and sphingomyelins. Evaporation of the solvent from each pool and lyophilization of the residue from benzene gave the following amounts of lipid: fractions 1–5, 2.42 g; fractions 6–8, 0.09 g; fractions 9–12, 2.13 g; and fractions 13–15, 0.16 g. Fractions 9–12 (2.13 g) accounted for approximately 43% of the crude lipids loaded on to the column. The total recovery was 4.7 g, which represents 95% of the material loaded.

The column of the Prep LC-500 was flushed with chloroform–methanol–water (60:40:10) and re-equilibrated with 60:30:4 solvent using a flow-rate of 200 ml/min until a stable baseline was obtained. Twenty-six grams of crude egg phospholipids in 25 ml of benzene were injected. The phosphatidylcholine was eluted in fractions 4 and 5 of the elution profile in Fig. 2A, as shown by TLC (Fig. 2B); after concentration and lyophilization, the resulting solid amounted to 10 g. Fraction 3 contained phosphatidylcholine and an unidentified phospholipid. Fractions 1 and 2, containing the neutral lipids and phosphatidylethanolamine, were combined and evaporated.

The column was prepared for another run by flushing with chloroform–methanol–water (60:40:10) and re-equilibrating with 60:30:2 solvent at a flow-rate of 150 ml/min until a stable baseline was obtained. The lyophilized neutral lipids and phosphatidylethanolamine (fractions 1 and 2 in Fig. 2A) were dissolved in benzene and injected. The elution profile in Fig. 3A was obtained; TLC (Fig. 3B) indicated

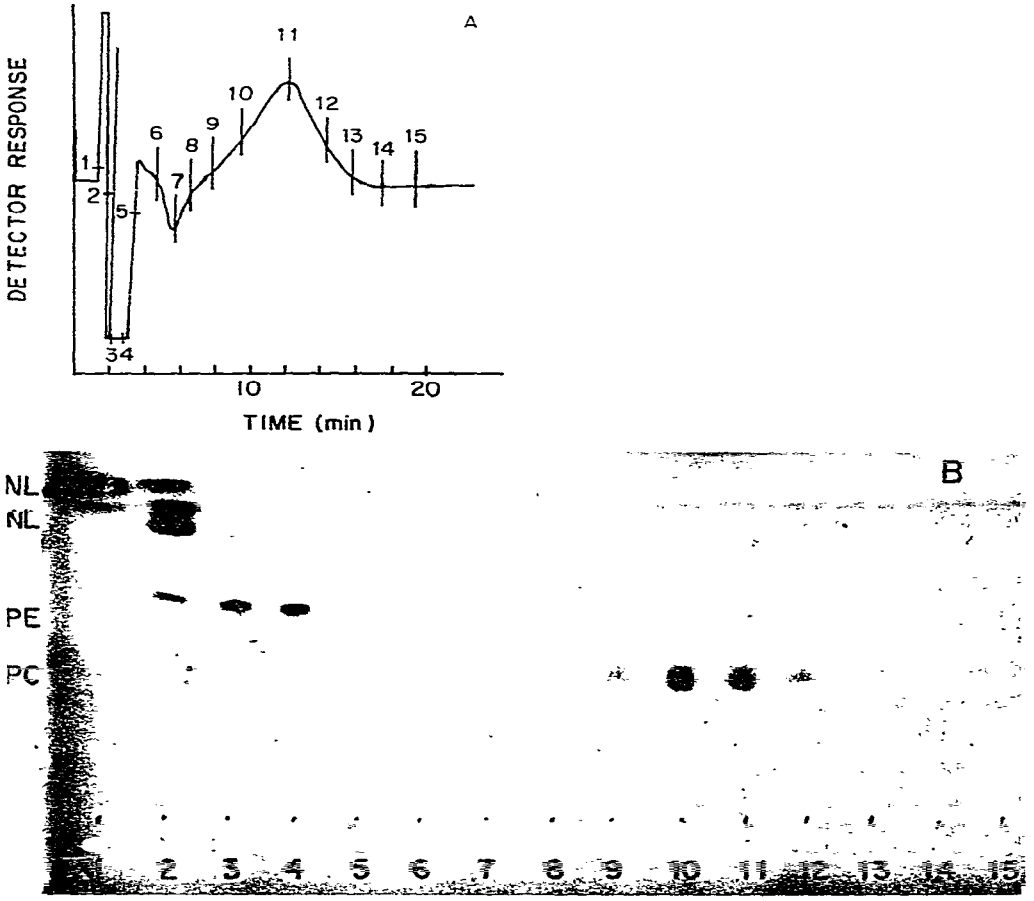

Fig. 1. (A) Separation of 5 g of crude egg yolk phosphatidylcholine. The radially compressed column was eluted with chloroform–methanol–water (60:30:4) at a flow-rate of 200 ml/min; the relative response of the refractometer was 5. (B) Thin-layer chromatography of fractions from the elution profile in A. The silica gel plate was developed in chloroform–methanol–water (60:30:4) and the spots were revealed with iodine. Neutral lipids (NL) were located at the solvent front. Phosphatidylethanolamine (PE) appeared in fractions 2, 3, 4 and 5 by ninhydrin and molybdenum sprays. Phosphatidylcholine (PC) appeared in fractions 9, 10, 11 and 12 by molybdenum spray.

that the phosphatidylethanolamine had eluted in fractions 6, 7 and 8. After evaporation and lyophilization, 3.84 g of pure phosphatidylethanolamine were obtained.

The fatty acid analyses of the crude egg phospholipids, purified phosphatidylethanolamine and phosphatidylcholine are shown in Table I. No residual silica was observed in any fractions, as often occurs when silicic acid columns are used to purify phosphatidylcholine.

DISCUSSION

We have demonstrated the use of preparative liquid chromatography on radially compressed columns of silica gel to purify large amounts of crude egg yolk

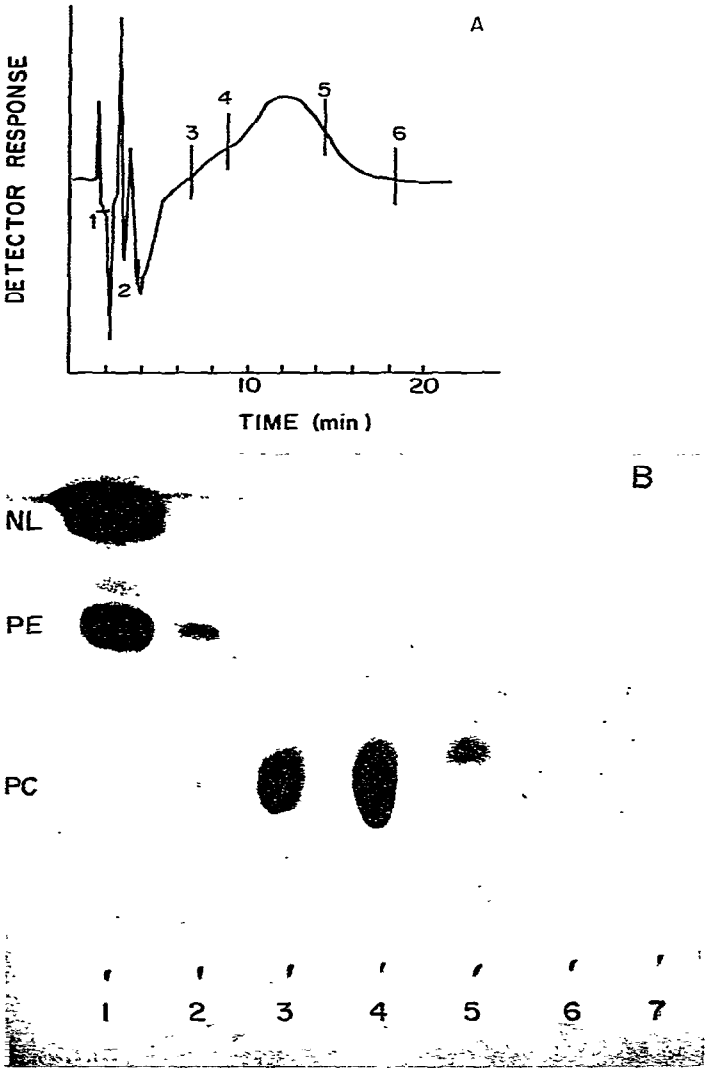

Fig. 2. (A) Elution profile of 26 g of crude egg yolk phosphatidylcholine. The column was eluted as above; the relative response was 1. (B) Thin-layer chromatography of fractions collected in A. The plate was developed and sprayed as in Fig. 1B. Fractions 1 and 2 contained phosphatidylethanolamine. Fraction 3 contained phosphatidylcholine and was slightly contaminated with an unknown phospholipid. Fractions 4 and 5 contained pure phosphatidylcholine.

phosphatidylcholine in extremely short periods of time. We have also used this procedure to purify synthetic phosphatidylcholines prepared by the acylation of lysophosphatidylcholine or glycerophosphorylcholine. The same column has been used for more than 40 purifications without any loss in resolution. It is assumed that by using both columns of the Prep LC-500 system and/or additional column modules even larger amounts of crude phosphatidylcholine could be purified on a commercial scale.

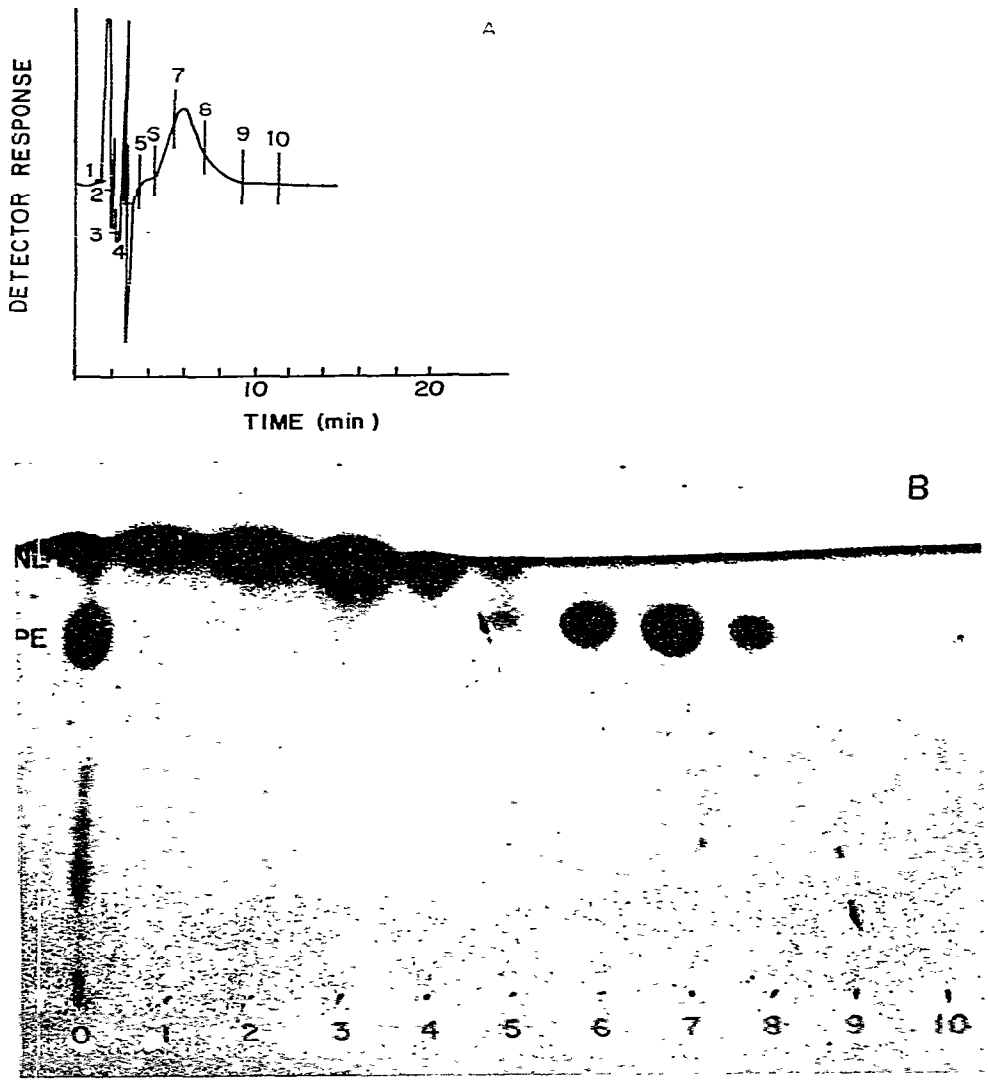

Fig. 3. (A) Elution profile of the pooled neutral lipid and phosphatidylethanolamine fractions from Fig. 2A. The silica gel column was re-equilibrated with chloroform–methanol–water (60:30:2) at a flow-rate of 150 ml/min; the relative response was 5. (B) Thin-layer chromatography of fractions in A. The plate was developed and sprayed as in Fig. 1B. Fraction 0 was the crude lipids from fractions 1 and 2 in Fig. 2A: fractions 1–4 contained neutral lipids; fractions 6, 7 and 8 contained pure phosphatidylethanolamine.

The purification procedure can be carried out stepwise by equilibrating the column first with chloroform–methanol–water (60:30:2) and injecting the crude egg phosphatidylcholine. After the neutral lipids and phosphatidylethanolamine are eluted, switching to 60:30:4 solvent elutes the phosphatidylcholine. A drawback of this modification is the loss of the refractive index detection owing to the large difference in the refractive indices of the two solvent systems.

TABLE I

% FATTY ACID COMPOSITION OF CRUDE AND PURE EGG PHOSPHOLIPIDS

Fatty acid	*Crude egg yolk phosphatidylcholine (%)*	*Pure phosphatidylethanolamine (%)*	*Pure phosphatidylcholine (%)*
14:0	0.07	0.06	0.08
16:0	20.70	13.20	29.80
16:1	1.10	0.63	1.80
18:0	13.10	26.40	15.10
18:1	28.50	19.10	31.30
18:2	10.20	11.60	13.70
20:4	7.10	13.10	1.86

Our procedure provides a rapid and simplified method for obtaining chromatographically pure phospholipids for further studies or for conversion into intermediates for synthesis. This system offers a significant saving of time over the previous procedures using silicic acid or alumina columns and spotting fractions from the column on to thin-layer plates to locate the phosphatidylcholine. Furthermore, degradation of the phosphatidylcholine on the column is essentially eliminated by the short separation times. We feel that this instrumental procedure will advance the work of the lipid chemist by significantly decreasing the time spent on routine purifications.

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REFERENCES

1 D. J. Hanahan, M. B. Turner and M. E. Jayko, *J. Biol. Chem.*, 192 (1951) 623.
2 O. J. Renkonen, *J. Lipid Res.*, 3 (1962) 181.
3 C. H. Lea, D. N. Rhodes and R. D. Stoll, *Biochem. J.*, 60 (1955) 353.
4 W. S. Singleton, M. S. Gray, M. L. Brown and J. L. White, *J. Amer. Oil Chem. Soc.*, 42 (1965) 53.
5 W. S. M. Geurts van Kessel, W. M. A. Hax, R. A. Demel and J. De Gier, *Biochim. Biophys. Acta*, 486 (1977) 524.
6 K. Kiuchi, T. Ohta and H. Ebine, *J. Chromatogr.*, 133 (1977) 226.
7 R. C. MacDonald and S. P. Rempas, *J. Chromatogr.*, 131 (1977) 157.
8 J. N. Little, R. L. Cotter, J. A. Prendergast and P. D. McDonald, *J. Chromato gr.*, 126 (1976) 439.
9 J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, 5 (1964) 126.