Selective Extraction of Phospholipids from Egg Yolk

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ABSTRACT

Solubility differences of phospholipids and triglycerides in methanol have been used with advantage for the selective quantitative extraction of phospholipids, almost free of triglycerides, from egg yolk. Cholesterol, a comparatively minor component of egg yolk lipids, is easily removed from the methanolic solution of phospholipids by low temperature crystallization (5 C), if required.

INTRODUCTION

Egg yolk is one of the rare biological materials rich in phospholipids, and hence has been used in the large scale preparation of phospholipids (1,2). The major lipid components of hen egg yolk are phospholipids (12% by weight of wet tissue) and triglycerides (24% by weight of wet tissue). Cholesterol is only a minor component (1.5% by weight of wet tissue). Isolation of egg yolk phospholipids conventionally involves extraction of total egg yolk lipids by the Folch procedure (3) followed by acetone precipitation (2). Alternatively, with the use of chromatography, the same type of fractionation has been achieved from silicic acid columns by either sequential elution with chloroform and methanol (4) or with petroleum ether and ethyl ether (5).

Recently, this laboratory reported a simple procedure for the preparation of egg yolk phospholipids free of accompanying triglycerides (6). In that method, egg yolk was adsorbed on activated thin layer grade silicic acid and was sequentially extracted with solvents of increasing polarity to fractionate total egg yolk lipids into nonpolar and polar components. The procedure could thus achieve simultaneous extraction and preparative fractionation of egg yolk lipids into its major components (triglycerides and phospholipids).

Ethanol (7) and methanol (3) have been used as components of lipid-extracting solvent systems mainly with the idea of denaturing the lipoprotein complexes occurring in nature. The bound lipids thus released are subsequently solubilized by the other component of the extraction solvent systems – ethyl ether (7) and chloroform (3), respectively. In this communication we report a simpler approach in which the solubility differences of phospholipids and triglycerides in methanol have been used for the selective quantitative extraction of phospholipids almost free of triglycerides from egg yolk.

EXPERIMENTAL PROCEDURES

Hen eggs were purchased locally. Methanol, commercial grade, was distilled before use. Thin layer chromatography (TLC) grade silicic acid was prepared in the laboratory (6). Reagent grade solvents were used for chromatography.

Yolk, 65.6 g, from three hen eggs, was separated from egg white and a homogeneous sample prepared. Two-thirds of this sample was then suspended in 150 ml of methanol. The suspension was swirled intermittently, and after two hours of standing, filtered by decantation through a cotton plugged funnel. Four more extractions were needed for the complete extraction of phospholipids. The pooled methanol extracts were concentrated under reduced pressure to 50 ml, when two layers were observed. The top layer was of methanol diluted with water from egg yolk, while the bottom one was of phospholipids in methanol. To this concentrate was added 5 ml of 5% salt solution, 50 ml of water and 50 ml of chloroform, and the solution was thoroughly mixed. This yielded a lower layer of chloroform containing phospholipids and an upper layer of aqueous methanol containing nonlipids extracted from egg yolk. The chloroform layer was completely freed of nonlipids by two more washes with 50 ml of 0.5% aqueous salt solution, dried over anhydrous sodium sulfate, and then the chloroform recovered under reduced pressure by distillation to yield phospholipid residue, which was weighed. The phospholipid-free egg yolk residue was then further subjected to 5 more extractions, each time with a 150 ml mixture of chloroform and methanol (1:1 by vol.), extracts concentrated to 50 ml and then treated as above to recover the triglycerides.

The remaining one-third of the sample was subjected to the Folch extraction procedure (3). The phospholipid content was determined by the standard procedure (8)

The lipid components of all the extracts were separated and visualized by TLC on glass plates (20 x 5 cm) coated with silicic acid without binder by the dip method (9). The separation between all the egg yolk lipid components was achieved (Fig. 1) by unidirectional sequential development in two different solvent systems. The TLC plate was developed to its full length in the first solvent system consisting of only chloroform. After removing the chloroform from the TLC plate by its exposure to an inert atmosphere of carbon dioxide for 5 minutes, the plate was redeveloped, but only to three-fourths its length in a second solvent system comprised of chloroform, methanol and water (400: 90:5, v/v/v). The second solvent system alone was used

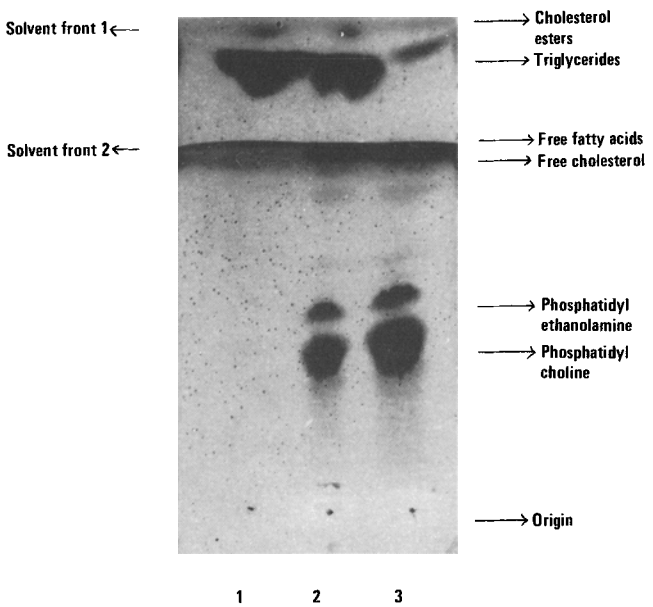

FIG. 1. TLC of lipids obtained from egg yolk by extraction with different solvents. Spotting of lipids obtained from egg yolk at: 1) by the last step (B) of sequential extraction procedure; 2) by the Folch extraction procedure; and 3) by the first step (A) of sequential extraction procedure.

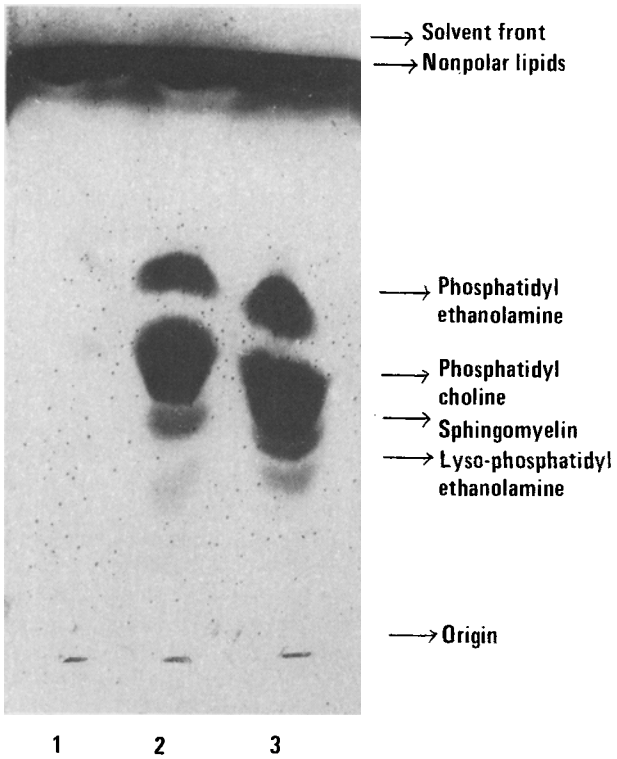

FIG. 2. TLC of lipids obtained from egg yolk by extraction with different solvents. Spotting of lipids obtained from egg yolk at: a) by the last step (B) of sequential extraction procedure; 2) by the Folch extraction procedure; and 3) by the first step (A) of sequential extraction procedure.

when only the phospholipid components of egg yolk were to be separated and visualized (Fig. 2). The detection of various lipids was achieved by spraying the TLC plates with either the phosphorus reagent (10) or aqueous sulphuric acid (50% by Vol.). The former reagent reacted specifically with phospholipids and produced blue spots at room temperature immediately. When heated, the TLC plates sprayed with either of the reagents produced black spots as a consequence of charring of the different lipid components (Fig. 1,2).

RESULTS AND DISCUSSION

Recoveries of total lipids by the sequential extraction and Folch extraction procedures are given in Table I. The phospholipid content determined in the Folch extract was recovered quantitatively in the methanol extract of the sequential procedure. Thus, only trace amounts of phospholipids (0.4% of total lipids) could be detected in the chloroform-methanol extract of the sequential procedure. The nonphosphorus lipids (19.05% of total methanol soluble lipids) in the methanol extract of the sequential procedure was comprised of triglycerides, free fatty acids and free cholesterol, while those (99.6% of total chloroform-methanol soluble lipids) in the chloroform-methanol extract of the sequential procedure were made up of mainly triglycerides with trace amounts of free fatty acids and cholesterol esters (Fig. 1). Since all the free cholesterol was extracted in methanol (Fig. 1), around 12% of the nonphorus lipids in methanol is cholesterol (6). Thus, the remaining 7% of the nonphosphorus lipids in methanol extract was comprised of triglycerides and free fatty acids. When necessary, cholesterol up to 90% could be removed by low temperature crystallization (5 C) from the concentrated methanolic solution of phospholipids (unpublished observations). Figure 2 shows the separation between extracted phospholipids. The minor phospholipid components of egg yolk, like lysophospholipids and sphingomyelin, are seen in both the Folch extract and methanol extract of the sequential procedure, when the plates were overloaded and developed with the usual phospholipid solvent. Phosphatidyl choline was the major component.

The present method thus yields egg yolk phospholipids almost free of triglycerides in one step and does not require the pre-extraction of total lipids as is conventional (2,4,5). This method has been successfully used in our laboratory in the selective isolation of phospholipids from animal (liver, heart, and brain) and plant tissues (oil-bearing seeds, leaves). The details of these investigations will be the subject of another communication.

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TABLE I

Solvent Fractionation of Egg Yolk Lipids

Method of extraction	Total lipids (g)[a] in 44.1 g of egg yolk	Amounts of various lipid[b] components in 44.1 g of egg yolk (g)	
		Triglycerides[c]	Phospholipids
Sequential extraction			
A) Methanol extract	6.677 (15.14)	1.273 (19.05)	5.404 (80.95)
B) Chloroform/methanol (1:1 by vol.)	9.630 (21.83)	9.592 (99.60)	0.038 (0.4)
C) A + B	16.307 (36.97)	10.865 (66.6)	5.442 (33.4)
Direct extration[d] (Folch procedure)	15.7 (35.65)	10.497 (68.1)	5.203 (31.9)

[a] Figures in parentheses in this column represent per cent lipids in wet tissue.
[b] Figures in parentheses in the two subcolumns represent per cent lipid components in total lipids obtained by various extraction solvents.
[c] The figures for triglycerides also include cholesterol and free fatty acids as minor components. These figures are calculated as difference between total lipids by weight minus phospholipids by estimation.
[d] Although 21.5 g of egg yolk was treated by the Folch procedure, the figures obtained were converted to represent lipid composition of 44.1 g of egg yolk.

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