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A method of column chromatographic isolation of major phospholipid components on Escherichia coli*

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Abstract

For the column chromatographic isolation of individual phospholipids from the total phospholipid mixture, silicic acid, DEAE cellulose, alumina and others, have been used as adsorbent. However, it must be emphasized that silicic acid (1, 2, 3, 4) is the most useful adsorbent for the separation of the total phospholipid mixture from each other in reasonable purity. VAN DEENEN reported that pure phosphatidyl glycerol was obtained from the lipid fraction of spinach leaves after repeated chromatography on silicic acid column (5). The phospholipid extracted from Escherichia coli B consists of abundant phosphatidyl ethanolamine (70-80 %), cardiolipin, phosphatidyl glycerol and other minor components as described in the previous paper (6). The high percentage content of phosphatidyl ethanolamine renders it difficult to separate the phospholipids by the column chromatography. Therefore, repeated chromatographies on the silicic acid column treated with sodium bicarbonate (7) and normal silicic acid column were employed for the isolation of the major components from the total phospholipid of E. coli B. Stepwise elution (4) was carried out with chloroform containing increasing proportions of methanol, and the eluent was divided into several fractions according to experience with thin-layer chromatography.

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A METHOD OF COLUMN CHROMATOGRAPHIC ISOLATION OF MAJOR PHOSPHOLIPID COMPONENTS OF ESCHERICHIA COLI

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For the column chromatographic isolation of individual phospholipids from the total phospholipid mixture, silicic acid, DEAE cellulose, alumina and others, have been used as adsorbent. However, it must be emphasized that silicic acid (1, 2, 3, 4) is the most useful adsorbent for the separation of the total phospholipid mixture from each other in reasonable purity. VAN DEENEN reported that pure phosphatidyl glycerol was obtained from the lipid fraction of spinach leaves after repeated chromatography on silicic acid column (5).

The phospholipid extracted from *Escherichia coli* B consists of abundant phosphatidyl ethanolamine (70—80 %), cardiolipin, phosphatidyl glycerol and other minor components as described in the previous paper (6). The high percentage content of phosphatidyl ethanolamine renders it difficult to separate the phospholipids by the column chromatography. Therefore, repeated chromatographies on the silicic acid column treated with sodium bicarbonate (7) and normal silicic acid column were employed for the isolation of the major components from the total phospholipid of *E. coli* B. Stepwise elution (4) was carried out with chloroform containing increasing proportions of methanol, and the eluent was divided into several fractions according to experience with thin-layer chromatography.

First chromatographic isolation on the $NaHCO_3$-treated silicic acid column

Silicic acid (MALLINKRODT, analytical grade) containing half portion of cleaned Hyflo supercel was treated with IN $NaHCO_3$ by the method of RATHBORN (7) and activated by heating at 120°C overnight. Column was made from a thin slurry of the above-mentioned silicic acid and washed with chloroform. A sample of 1.37 g of the phospholipids dissolved in 270 ml of $CHCl_3$ was introduced onto a column containing 120 g of the above-mentioned silicic acid (column 45 × 4cm). By the assay on thin-layer plates,

two crude fractions were collected (Table 1). One of them containing cardiolipin as main substance was isolated at the elution with chloroform-methanol (93: 7 and 92: 8, v/v) and was named as "CL fraction". In the same way, "PE+PG fraction" containing phosphatidyl ethanolamine and phosphatidyl glycerol was obtained by the elution with chloroform-methanol (91: 9~80: 20, v/v).

Table I COLUMN CHROMATOGRAPHY ON SILICIC ACID TREATED WITH SODIUM BICARBONATE

$CHCl_3$-MeOH (v/v)	Eluted volume (ml)	Thin-layer chromatographic analysis					Collected fraction
		Neutral lipid	Cardio-lipin	Unknown spot*	Phospha-tidyl glycerol	Phospha-tidyl ethanol-amine	
100 : 0	300						
99 : 1	100						
98 : 2	100						
97 : 3	100	trace					
96 : 4	200	+					
95 : 5	200	+					
94 : 6	200	trace	trace				
93 : 7	300		++	+			"CL fraction"
92 : 8	300		+++	trace	trace		"CL fraction"
91 : 9	200		trace		+		"CL fraction"
91 : 9	200				+++	trace	"PE+PG fraction"
90 : 10	200				++	++	"PE+PG fraction"
95 : 15	200				+	++++	"PE+PG fraction"
82 : 18	200				trace	+++	"PE+PG fraction"
80 : 20	200					++	"PE+PG fraction"
75 : 25	200					+	"PE+PG fraction"
70 : 30**	200					trace	"PE+PG fraction"

* This spot was positive for the molybdate reagent and negative for the ninhydrin reagent.
** When the elution is continued with the solvent containing higher ratio of methanol, there appear minor components. However, these data are omitted from the table.

Rechromatography of "CL fraction"

"CL fraction" (about 145 mg in 50 ml of chloroform) was loaded on a column of 10 g of silicic acid and 5 g of Hyflo supercel (column 15×1.5 cm). After the stepwise elution with solvent mixture increasing proportions of methanol in chloroform, two phospholipid fractions were obtained (Table II). The elution with chloroform-methanol (96: 4, v/v) yielded a main fraction (ca. 75 mg), identified as cardiolipin in the previous report

(6). Another substance (ca. 40 mg) eluted with chloroform-methanol (95: 5 and 93: 7, v/v) was not identified but preliminary tests showed that it was a phosphatidyl derivative.

Table II COLUMN CHROMATOGRAPHY OF "CL FRACTION" ON A SILICIC ACID COLUMN

$CHCl_3$-MeOH (v/v)	Eluted volume (ml)	Thin-layer chromatographic analysis					Collected fraction
		Neutral lipid	Cardiolipin	Unknown spot	Phoshhatidyl glycerol	Phosphatidyl ethanolamine	
100 : 0	150	trace					
98 : 2	150						
97 : 3	100		trace				
96 : 4	100		++				Cardiolipin
96 : 4	100		+				Cardiolipin
95 : 5	100		trace	trace			
95 : 5	100			++			Unknown fraction
93 : 7	100			trace			Unknown fraction
93 : 7	100						
90 : 10	150				trace	trace	
85 : 15	200				+	+	

Table III COLUMN CHROMATOGRAPHY OF "PE+PG FRACTION" ON A SILICIC ACID COLUMN

$CHCl_3$-MeOH (v/v)	Eluted volume (ml)	Thin-layer chromatographic analysis					Collected fraction
		Neutral lipid	Cardiolipin	Unknown spot	Phosphatidyl glycerol	Phosphatidyl ethanolamine	
100 : 0	200						
98 : 2	100						
95 : 5	300	trace					
93 : 7	300		trace				
91 : 9	200					trace	
91 : 9	200					+	
91 : 9	300					+++	Phosphatidyl ethanolamine
91 : 9	300					++++	Phosphatidyl ethanolamine
90 : 10	300					++	Phosphatidyl ethanolamine
85 : 15	400				trace	+	
85 : 15	400				+	+	
80 : 20	350				+	trace	
75 : 25	400				++		Phosphatidyl glycerol
70 : 30	250				++		Phosphatidyl glycerol
60 : 40	300				+		
50 : 50	200				trace		

Rechromatography of "PE+PG fraction"

"PE + PG fraction" (about 1000 mg in 300 ml of chloroform) was separated on a column of 80 g of silicic acid and 25 g of Hyflo supercel in the stepwise manner into two components, phosphatidyl ethanolamine (ca. 620 mg) and phosphatidyl glycerol (ca. 140 mg), each of which was eluted with chloroform-methanol (91 : 9~90 : 10, v/v) and chloroform-methanol (75 : 25~70 : 30, v/v), respectively (Table III).

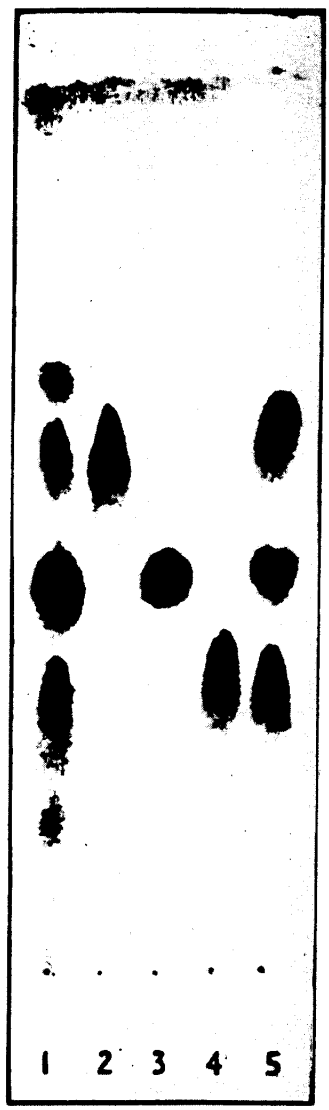

Fig. 1 Comparison between the total phospholipid fraction from *E. coli* B and the isolated individual components on a thin-layer chromatoplate

1. total phospholipid fraction
2. isolated cardiolipin
3. isolated phosphatidyl ethanolamine
4. isolated phosphatidyl glycerol
5. reconstruction of 2, 3 and 4

Solvent system: chloroform-methanol-water (70 : 25 : 4, v/v/v)

Spots were revealed by charring after spraying with 10 % H_2SO_4.

Checking on the isolated major phospholipids

The checking of purity and the determination of isolated phospholipids have been carried out by different chromatographic methods. The

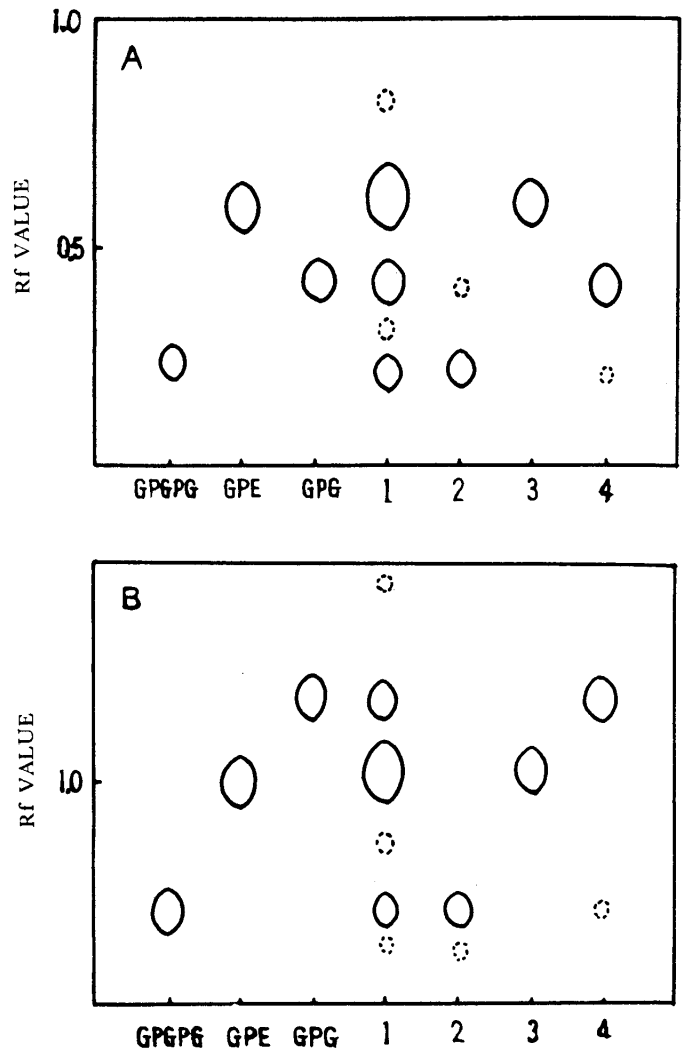

Fig. 2 Tracing of paper chromatograms of water soluble products of the individual phospholipids of *E. coli* B after Dawson's hydrolysis

A; Phenol-water-acetic acid-ethanol (80:20:10:12) ascending

B: Isopropanol-ammonia (3:1) descending

Abbreviations: GPGPG; bis (glyceryl-phosphoryl) glycerol, GPE; glyceryl-phosphoryl-ethanolamine, GPG: glyceryl-phosphoryl-glycerol, 1; total phospholipid, 1; isolated cardiolipin, 3; isolated phosphatidyl ethanolamine, 4; phosphatidyl glycerol

separated phospholipids, cardiolipin, phophatidyl ethanolamine and phosphatidyl glycerol, revealed respective individual spots on the thin-layer chromatoplate corresponding exactly to major components of the original total lipid mixture (Fig. 1). Furthermore, the water-soluble hydrolysis products of the isolated phospholipids by Dawson's method (8) were chromatographed on Whatman No. 1 paper to compare their Rf values with those of the reference compounds (Fig. 2). These results further made it possible to confirm the purity and identify individual components.

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