Journal of Chromatography, 577 (1992) 142–145
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6294

Short Communication

Separation of acidic and neutral lipids by aminopropyl-bonded silica gel column chromatography

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(First received December 12th, 1991; revised manuscript received January 29th, 1992)

ABSTRACT

The separation of acidic and neutral lipids by aminopropyl-bonded silica gel column chromatography is presented. Total lipid extracts from *Escherichia coli* and human spermatozoa were loaded onto pre-packed aminopropyl-bonded silica gel columns and the lipids separated into four fractions. Non-polar lipids including cholesterol esters, triglycerides, diglycerides, monoglycerides and cholesterol, were eluted with 4 ml of isopropanol–chloroform (1:2, v/v) (fraction 1); free fatty acids were eluted with 4 ml of 2% acetic acid in diethyl ether (fraction 2); neutral polar lipids, including phosphophatidylethanolamine, phosphatidylcholine, sphingomyelin and neutral glycolipids, were eluted with 4 ml of methanol (fraction 3); and, finally, polar acidic lipids, including phosphatidylglycerol, cardiolipin, phosphatidylinositol, phosphatidylserine, seminolipid lipid A and acidic glycosphingolipids, were eluted with 4 ml of chloroform–methanol–0.8 *M* sodium acetate (60:30:4.5, v/v/v) (fraction 4). The recoveries for the different lipids ranged between 89 and 98% and the intra-assay variation, expressed as the standard deviation, was $<5\%$.

INTRODUCTION

Current methodology for the separation of milligram amounts of lipids into acidic and neutral fractions involves DEAE-Sephadex (acetate form) A-25 column chromatography [1]. Using this methodology, various lipid classes, including glycolipids, phosphoglycerides and non-polar lipids, can be fractionated in that fashion. However, preparation of the gel in the acetate form is time-consuming, each individual column has to be packed separately, and the size and quality of the columns are difficult to reproduce from batch to batch. In this report, a new application for the aminopropyl-bonded silica gel column is introduced. In addition to its use as a means of fractionating different lipid classes, including triglycerides, cholesterol, diglycerides, monoglycerides and fatty acids, as reported by Kaluzny *et al.* [2] and Alvarez and Touchstone [3], this report describes the use of these columns as a means to fractionate polar lipids, including phosphoglycerides and glycolipids, into the neutral and acidic fractions. Since the aminopropyl columns are commercially available in the pre-packed form and they can be obtained in different sizes, they constitute a convenient and highly reproducible means of separation of neutral and acidic lipids from biological samples.

0378-4347/92/$05.00

EXPERIMENTAL

Escherichia coli and human spermatozoa cell suspensions were centrifuged at 800 *g* for 8 min, the supernatants removed, and the resulting pellets resuspended in isotonic phosphate buffer and centrifuged at 800 *g* for 8 min. The final pellets (*ca.* 1 ml of packed cells) were extracted with twenty volumes of a mixture of chloroform–methanol–water (C–M–W) (10:10:1, v/v/v). This procedure was repeated twice. The combined extracts were evaporated to dryness, the residue was redissolved in 200 μl of chloroform containing 0.1 μCi each of [^{3}H]cholesteryl oleate, [^{3}H]cholesterol, [^{3}H]triolein, [^{14}C]phosphatidylinositol, [^{14}C]phosphatidylethanolamine and [^{3}H]oleic acid (DuPont NEN, Boston, MA, USA) and loaded onto an aminopropyl column (7.5 cm × 1.0 cm, 500 mg per 1.8 cm pre-packed column from United Chemical Technologies, Horsham, PA, USA) conditioned with 4 ml of hexane. The lipid standards, including galactosylceramide, lactosylceramide, GM1, GD1b, cholesteryl oleate, triolein, cholesterol, lipid A from *E. coli* (Sigma, St. Louis, MO, USA) and seminolipid from bovine spermatozoa, were loaded onto another aminopropyl column at a concentration of 1 mg/ml (total volume of 200 μl, corresponding to 1.8 mg). Non-polar neutral lipids, fatty acids, neutral phospholipids and glycolipids, and acidic phospholipids and glycolipids, were eluted with 4-ml fractions of isopropanol–chloroform (1:2, v/v) (fraction 1), 2% acetic acid in diethyl ether (fraction 2), methanol (fraction 3), and C–M–0.8 *M* sodium acetate (60:30:4.5, v/v/v) (fraction 4), respectively. The lipid fractions were evaporated to dryness, redissolved in 100 μl of C–M (1:1, v/v) and 4-μl aliquots streaked onto Whatman (Clifton, NJ, USA) silica gel HP-K plates (10 cm × 10 cm, 200 μm thickness). Aliquots of 4 μl of the lipid standards (1 mg/ml) (Sigma) were streaked in separate lanes. The plates were predeveloped in C–M (1:1, v/v) followed by development with chloroform–ethanol–triethylamine–water (30:34:30:8, v/v/v/v) (solvent 1) for 4 cm, thoroughly dried and developed in hexane–diethyl ether (50:5, v/v) (solvent 2) for separation of polar and non-polar lipids, respectively. The developed chromatograms were sprayed with the $CuSO_4$ reagent [4], heated in an oven with initial and final temperatures of 24 and 125°C, respectively, and the stained chromatograms scanned with a Shimadzu CS-9000 spectrodensitometer at 400 nm in the reflectance mode [5]. Aliquots of 4 μl of the phosphoglycerides and glycolipids from human spermatozoa, eluted in fractions 3 and 4 of the aminopropyl column, respectively, were also applied to Whatman plates, predeveloped in C–M (1:1, v/v), developed in solvent 1 and stained with the $CuSO_4$ reagent, as indicated above. The developed chromatograms, containing the radiolabeled lipids, were scanned with a Bioscan 200 imaging scanner. When indicated, the developed chromatograms were sprayed with either the fluorescamine (Whatman), molybdenum blue or orcinol reagents (Sigma) and the lipids visualized at room temperature or following heating in an oven, respectively. The resulting bands were then scanned at 254 nm in the fluorescence mode [5], at 600 nm in the reflectance mode [6] and at 500 nm [7] in the transmittance mode, respectively.

RESULTS AND DISCUSSION

The $CuSO_4$-stained chromatogram corresponding to the separation of the different lipid classes from *E. coli* by aminopropyl-bonded silica gel chromatography is shown in Fig. 1. The $CuSO_4$-stained chromatogram corresponding to the separation of phosphoglycerides and glycolipids from human spermatozoa into neutral and acidic is shown in Fig. 2. The identification of these lipids was based on: (1) comigration with the authentic standards; (2) high-performance thin-layer chromatography (HPTLC)–fluorescence spectrodensitometry following spraying of the developed chromatograms with the fluorescamine reagent (phophatidylethanolamine and phosphatidylserine) [5]; (3) HPTLC–reflectance spectrodensitometry after spraying with the molybdenum blue reagent (phosphoglycerides and lipid A) [6]; (4) HPTLC–transmittance spectrodensitometry following spraying with the orcinol

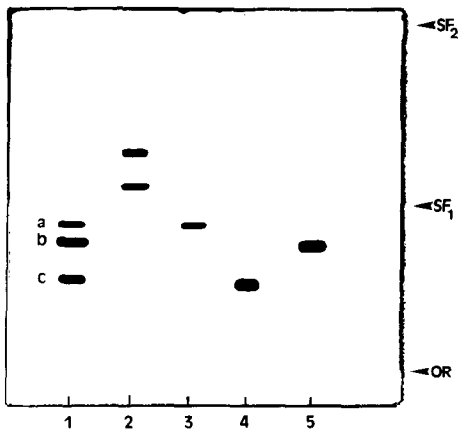

Fig. 1. $CuSO_4$-stained HPTLC chromatogram of lipids from *E. coli* separated by aminopropyl column chromatography. (1) Standard mixture, including oleic acid (a), phosphatidylglycerol (b) and phosphatodylethanolamine (c); (2) fraction 1 (non-polar lipids) from *E. coli* separated by aminopropyl column chromatography; (3) fraction 2 (free fatty acid); (4) fraction 3 (phosphatidylethanolamine); and (5) fraction 4 (phosphatidylglycerol). OR = origin; SF_1 = solvent front for the first development (solvent 1); SF_2 = solvent front for the second development (solvent 2).

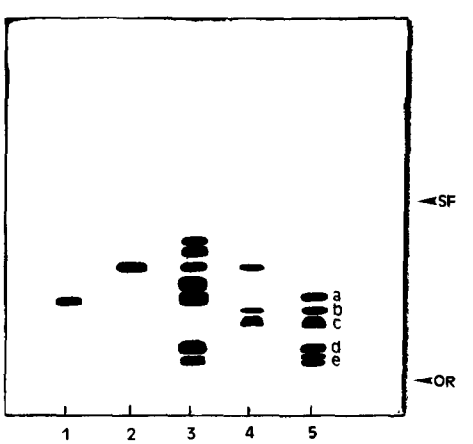

Fig. 2. $CuSO_4$-stained HPTLC chromatogram of the polar lipids from human spermatozoa separated by aminopropyl column chromatography. (1) and (2) correspond to phosphatidylethanolamine and phosphatidylglycerol standards, respectively; (3) fraction 3 from aminopropyl column; (4) fraction 4; (5) standard mixture of phospholipids, including phosphatidylethanolamine (a), phosphatidylinositol (b), phosphatidylserine (c), phosphatidylcholine (d) and sphingomyelin (e). OR = origin; SF = solvent front.

reagent (glycolipids) [7]; and (5) HPTLC–reflectance spectrodensitometry following spraying with the $CuSO_4$ reagent (all lipids) [4]. The recoveries for the lipids eluted from the aminopropyl column were calculated from the values obtained following quantification by HPTLC–fluorescence/reflectance/transmittance spectrodensitometry and HPTLC–radioscanning. These values, expressed as the percentage recovery, are shown in Table I. The intra-assay variation, expressed as the standard deviation, was $<5\%$.

These results indicate that the aminopropyl-bonded silica gel column behaves as an anion-exchange stationary phase and that it is suitable for the isolation of neutral and acidic lipids from biological samples and, in particular, phosphoglycerides and glycolipids.

TABLE I

RECOVERIES FOR THE DIFFERENT LIPIDS SEPARATED BY AMINOPROPYL-BONDED SILICA GEL COLUMN CHROMATOPGRAPHY

Lipid	Recovery[a] (%)		Fraction No.
	$CuSO_4$	Radiolabel	
Phosphatidylglycerol	97	95	4
Phosphatidylinositol	89	90	4
Oleic acid	98	98	2
Phosphatidylethanolamine	98	N.D.[b]	3
Phosphatidylserine	90	N.D.	4
Phosphatidylcholine	92	N.D.	3
Sphingomyelin	89	N.D.	3
Cholesteryl oleate	99	98	1
Triolein	98	97	1
Cholesterol	99	97	1
Galactosylceramide	96	N.D.	3
Lactosylceramide	92	N.D.	3
Seminolipid	91	N.D.	4
GM1[c]	95	N.D.	4
GD1b[c]	98	N.D.	4
Lipid A	97	N.D.	4

[a] Values represent the mean of five experiments performed with five different aminopropyl columns. Standard deviations ranged between 1 and 3%.

[b] Not determined.

[c] GM1 = $II^3\alpha NeuAcGgOse_4Cer$; GD1b = $II^3\alpha(NeuAc)_2$-$GgOse_4Cer$.

ACKNOWLEDGEMENT

This work was supported by NIH Grant HD-15842.

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