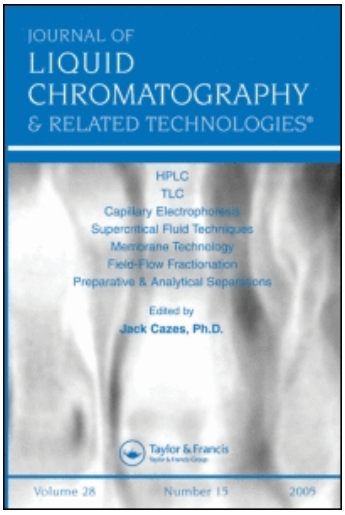

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Alkaline Hydrolysis of Phosphoglycerides on Thin Layer Plates In Situ

Juan G. Alvarez[a]; Isabel Lopez[a]; Bayard T. Storey[a]; Sidney S. Levin[a]; Joseph C. Touchstone[a]
[a] Department of Obstetrics and Gynecology and Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

To cite this Article Alvarez, Juan G. , Lopez, Isabel , Storey, Bayard T. , Levin, Sidney S. and Touchstone, Joseph C.(1986) 'Alkaline Hydrolysis of Phosphoglycerides on Thin Layer Plates In Situ', Journal of Liquid Chromatography & Related Technologies, 9: 16, 3495 — 3504
To link to this Article: DOI: 10.1080/01483918608077801
URL: http://dx.doi.org/10.1080/01483918608077801

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(16), 3495-3504 (1986)

ALKALINE HYDROLYSIS OF PHOSPHOGLYCERIDES ON THIN LAYER PLATES IN SITU

Juan G. Alvarez*, Isabel Lopez, Bayard T. Storey, Sidney S. Levin, and Joseph C. Touchstone
Department of Obstetrics and Gynecology and Physiology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

ABSTRACT

An alkaline hydrolysis method for selective cleavage of the acyl groups in phospholipids is described. The alkaline hydrolysis treatment was achieved with 0.8 N methanolic NaOH by incubation at 37°C in a chamber presaturated with aqueous methanol to saturate the ambient vapor phase and thus maintain the concentration of NaOH. Cleavage of acyl groups was quantitative while cleavage of ether groups was below the level of detection. There was no effect on the alkenyl ether linkages of plasmalogens.

INTRODUCTION

A procedure for selective hydrolysis of the acyl groups in phospholipids with no effect on either O -alkyl or O-alkenyl groups was required to give a

 0148-3919/86/0916-3495$3.50/0

more complete characterization of the plasmalogen types (1,2) found in sperm membrane phospholipids. A number of methods have been reported for alkaline hydrolysis of phospholipid acyl groups (3-7), but these have the disadvantages of requiring large samples, of being relatively harsh and unselective, or of requiring multiple manipulations including preliminary separation of the components on columns prior to final separation by TLC. In this paper, we report a selective and rapid technique for alkaline hydrolysis, which can be carried out directly on the TLC plate and so be used in conjunction with other hydrolytic methods such as the TCA/HCL and the phospholipase A_2 procedures. It showed that phosphatidyl choline of sperm is the 1-acyl rather than the 1-alkyl compound.

MATERIALS AND METHODS

Reagents: The synthetic standards, dipalmitoyl-sn-glycerol-3-phosphatidylethanolamine (DPPE), 1-palmitoyl-2-oleoyl- sn-glycerol-3-phosphocholine (POPC), bovine brain lysophosphatidyl ethanolamine (LPE) and purified palmitic acid (PA) were obtained from Avanti Biochemicals (Birmingham, Alabama). Phosphatidylethanolamine from bovine brain (PEB) was obtained from Supelco Co. (Bellefonte, Pennsylvania). Octadecenaldehyde, cholesteryl palmitate, tripalmitin, glycerylphosphoethanolamine (GPE), and glycerylphosphocholine (GPC) were obtained from Sigma Chemical Co. (St. Louis). The Schiff reagent was obtained from Accra-Lab Inc. (Bridgeport, New Jersey). Phospholipid standard solutions were prepared at concentrations between 0.5 and 2 mg/ml in chloroform-methanol (1:1, v/v). The purity of these standards was assessed by TLC in different mobile phases. Solvents were EM Science (Cherry Hill, New Jersey) chromatographic grade. Precoated silica LK5 plates (250 um thick) with preadsorbent zone of 500 um thickness were obtained from Whatman Inc. (Clifton, New Jersey). Inorganic salts were from J.T. Baker (Phillisburg, New Jersey) and of analytical grade.

Phosphatidylcholine from rabbit epididymal spermatozoa (PCS) was obtained as described by Touchstone et al. (2).

In situ alkaline hydrolysis of phospholipids

The silica gel plates (20 x 20 cm; 250 um thick with 500 um thick preadsorbent zone) were washed by continuous development in chloroform/methanol 1:1, v/v). These were scored on a Schoeffel scoring device to give 1 cm lanes. Development was carried out in standard size tanks. Aliquots of 5 to 25 ul of the phospholipid solutions (0.5 to 2 mg/ml) were streaked on the preadsorbent zone. Then 25 ul aliquots of methanolic sodium hydroxide (NaOH) with final concentrations 0.2, 0.4, 0.6, 0.8 and 1.00 N were applied over the areas of sample. These solutions were made by dilution of 2.4 N aq. NaOH in methanol. When POPC was used, GPC, LPC, and palmitic acid were added to contiguous lanes for the identification of the products of hydrolysis. If PE or PEB were used, GPE, LPE, palmitic acid, and octadecenal were used as references.

Five ml of a methanol-water mixture in a ratio equivalent to that chosen for the NaOH reagent (from 1:1.2 to 1:11 aq. NaOH:methanol v/v) were placed in standard size tanks and then incubated in an oven at 37°C to saturate the air with methanol-water vapor. Immediately after addition of the 25 ul of the NaOH/methanol to the phospholipid sample, plates were placed in the presaturated tanks and incubated at 37°C for 20 min. This step avoids preferential evaporation of methanol and maintains constant the NaOH concentration of the reagent throughout the incubation. After incubation, plates were predeveloped once in chloroform-methanol 1:1 to the zone juncture, then air dried for a time sufficient to complete evaporation of the solvent. The chromatograms were then developed in a mobile phase of chloroform/ethanol/triethylamine/water (30:34:30:8 v/v/v/v) (8). Development

proceeded until the mobile phase reached 2 cm from the top of the plate. After development, the plates were air dried, followed by heating in an oven at 170°C for 2 min to remove residual solvent.

Lipids were detected by spraying with a solution of 10% $CuSO_4$ in 8% H_3PO_4, drying for 8 min at room temperature, then heating in an oven at 120°C for 5 min, followed by 170°C for 10 min (9). Separate plates to which PEB was applied were sprayed with a 0.2% solution of ninhydrin in acetone for identification of LPEB, GPE, and unreacted LPEB. Separate plates to which phospholipids containing vinyl-ether moieties were applied (PEB and PES) were sprayed with the Schiff reagent for free aldehyde identification (2) after the TCA/HCl reaction for vinyl ethers.

The developed chromatograms were scanned in a Kontes Fiber Optic Scanner (Model 800) using a 440 nm peak bandpass filter. A Hewlett-Packard 3390 A integrator provided integration. The scanning was carried out in the transmission mode using double beam operation. Details of this procedure are given in ref. (2).

RESULTS AND DISCUSSION

Treatment of POPC, PEB and PCS with methanolic NaOH at concentrations between 0.2 and 1.00 N NaOH resulted in different degrees of hydrolysis of the acyl groups, as shown in Fig. 1. Complete hydrolysis was observed between 0.8 and 1.0 N. At 1.0 N NaOH, attack on the vinyl ether was above the limit of detection, but at 0.8 N, a reaction could not be detected. Methanolic NaOH obtained by diluting 2.4 N aqueous NaOH with methanol to 0.8 N (1:2 v/v) provided the desired selective hydrolysis of the acyl groups. When POPC was treated with 0.8 N methanolic NaOH reagent, free fatty acid (FA) (Fig.2) and glyceryl phosphatidyl choline (GPC), as identified separately by $CuSO_4$ and

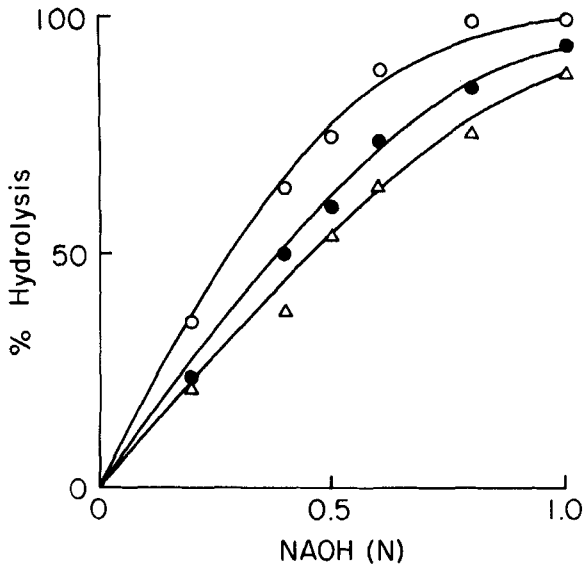

FIG. 1. Hydrolysis of the three phospholipids POPC (o), PEB (●), and PCS (Δ) as a function of NaOH concentration. Hydrolysis conditions were 37°C for 20 min in a chamber saturated with aqueous methanol vapor to maintain constant the NaOH concentration (see Materials and Methods).

ninhydrin staining (2), resulted as the major reaction products. In addition, a third component was found which comigrated with fatty acyl methyl ester (FME) in hexane:ether (96:4) (data not shown); this is the other solvolysis product expected in this system. Its migration with the solvent front in the standard solvent system (Fig. 2) conveniently removes it from the chromatogram.

The test substance used in the previous study to verify the efficacy of the TCA/HCl and phospholipase A_2 methods for plasmalogen characterization was the phosphatidylethanolamine plasmalogen from bovine brain (PEB) (2). This substance consists of 15% 1,2-diacyl PE, 20% 1,2 -di(O-1′-alkenyl)PE, and 65%

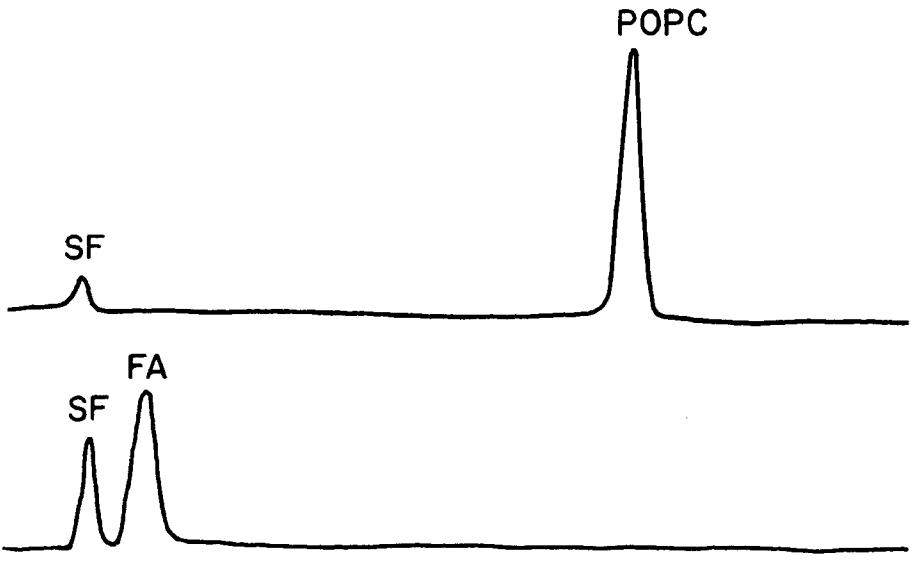

FIG. 2. Densitometric traces of TLC with 1-palmitoyl-2-oleoyl-sn-3-phosphatidylcholine (POPC) separated and treated with $CuSO_4$. The untreated sample gave the upper trace with the single component indicated by PC and the solvent front by SF. The lower trace was obtained by treatment with 0.8 N methanolic NaOH as described in Materials in Methods, followed by separation and $CuSO_4$ reaction. The original phospholipid has been quantitatively hydrolyzed; the free fatty acid (FA) was the only component detected on the plate.

1-(O-1′-alkenyl)-2-acyl PE. The percentages are based on moles of long chain acyl and alkyl groups as determined by standards (2). Treatment of PEB with 0.8 N methanolic NaOH, followed by chromatography (Fig.3), gave the following products as mole percent long chain acyl and alkyl groups: PEB_1, corresponding to previously identified 1,2-di(O-1′-akenyl)PE, 19%; FME, 29%; free FA, 20%; lyso PE corresponding to $LPEB_2$ previously identified as 1-(O-1′-alkenyl)-lyso PE, 32%. The 19% recovery of the dialkenyl plasmalogen as compared to 20%

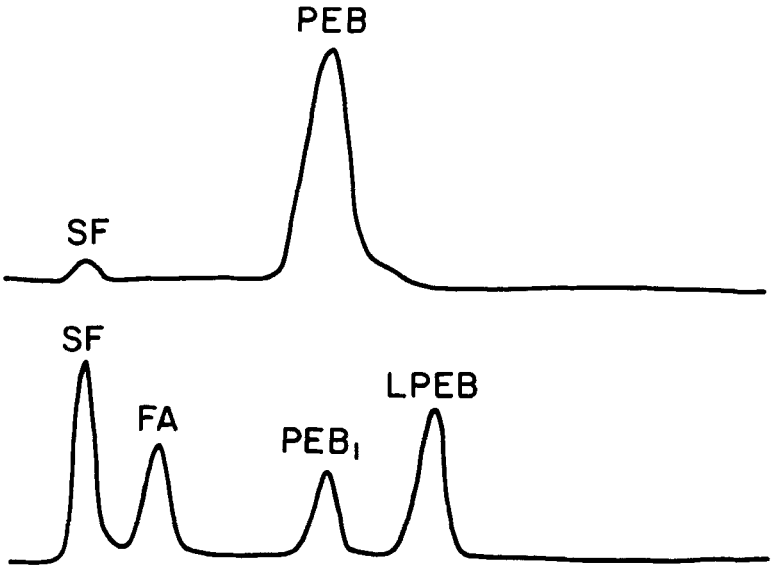

FIG. 3. Densitometric traces of TLC with phosphatidylethanolamine from bovine brain (PEB). The upper trace shows only the untreated phosphoslipid as a single, somewhat broad peak and the solvent front (SF). The lower trace shows the effect of treatment with 0.8 N methanolic NaOH. Hydrolysis has occurred to give three peaks: unreacted diplasmalogen PE (PEB_1), lysophosphitidylethanolamine (LPEB), and free fatty acid (FA).

previously found (2) shows that this method cleaves less than 5% of diplasmalogens. From the percentages of monacyl and diacyl PE in PEB, one calculates that the sum of FME and free FA should amount to 48%, compared to 49% observed; and that $LPEB_2$ should account for 33%, compared to 32% observed. These results gave evidence that 0.8 N methanolic NaOH cleaved the acyl chains of phospholipid plasmalogens in yields sufficient for quantitation, while leaving the ether links intact. A further test of the specificity of the methanolic NaOH reagent was carried out with the neutral lipids, tripalmitin

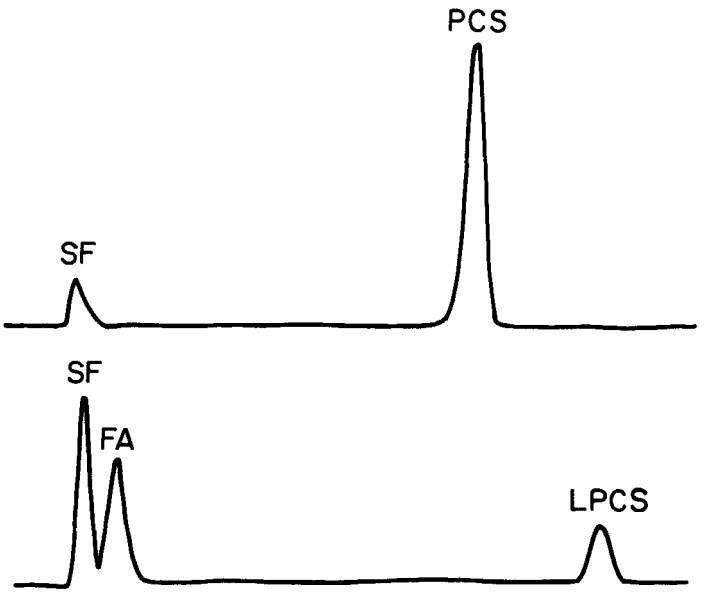

FIG. 4. Densitometric traces of TLC with rabbit epididymal sperm phosphatidylcholine (PCS). The upper trace shows the untreated phospholipid as a single peak (PCS) and the solvent front (SF). The lower trace shows the effect of treatment with 0.8 N methanolic NaOH. Hydrolysis has occurred to give a form of lypophosphatidylcholine (LPCS) and free fatty acid (FA); no detectable parent peak of PCS was detectable.

and cholesteryl palmitate. Triglycerides, cholesteryl esters or sphingomyelin were not cleaved under the conditions which gave quantitative cleavage of the acyl groups from the phospholipids.

Treatment of PCS with 0.8 N methanolic NaOH, followed by chromatography and staining with $CuSO_4$ (Fig.4), gave the following products: FME, 30%; free FA, 45%, and lyso PC (LPCS), 25%. These are products to be expected for hydrolysis of the acyl groups while leaving the 2-(O-1′-alkenyl) group intact

on LPCS. Were 1-(O-1'-alkyl) groups to be present in a significant proportion of the plasmalogen molecules, a lower yield of FME plus free FA and a higher yield of LPCS, comprising two different compounds, would have been observed. This characterization of PCS shows that the three hydrolysis methods: TCA/HCL, phospholipase A_2, and 0.8 N methanolic NaOH, can be used in parallel to give a characterization of phospholipids. The convenience, rapidity and applicability in situ on the thin layer make the approach a particularly useful one.

ACKNOWLEDGMENTS

This study was supported in part by NIH grants HD-15842, HL-19737, and HL-07027. Dorothy Rivers provided excellent technical help, while Elvira Walker and Neisha Son gave skilled secretarial assistance.

REfERENCES

1. Mann, T., and Lutwak - Mann, C. Male Reproductive Function and Semen, pp.207-210, Springer Verlag, Berlin-Heidelberg-New York. 1981, p. 207-210.

2. Touchstone, J.C., Alvarez, J.G., Levin, S.S. and Storey, B.T. Lipids 20, 869-876 (1985).

3. Viswanathan, C.V. Basilio, M. Hoenet, S.P., and Lundberg, W.B., J. Chromatogr. 34, 241 (1968).

4. Kates, M. Laboratory Techniques in Biochemistry and Molecular Biology, North Holland American Elsevier, New York, 1972, p. 416, 420.

5. Tsai, S.C., Avigan, J., and Steinberg, D. J. Biol. Chem. 244, 2682-2692, (1969).

6. Sweeley, C.G. J. Lipid Res. 4, 402-406, (1963).

7. Do, U.H. and Ramachandran, S. J. Lipid Res. 21, 888-894, (1980).

8. Touchstone, J.C., Chen, J.C., and Beaver, K.M., Lipids 15, 61-62, (1979).

9. Touchstone, J.C., Levin, S.S. Dobbins, M.P., and Beers, P.C. J. Liquid Chromatogr. 6, 179-192, (1983).