Journal of Chromatography, 232 (1982) 237—249
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1374

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METHYLATED PHOSPHOLIPIDS

S. SHI-HUA CHEN*,* and ANNE Y. KOU

Department of Pathology, Stanford University and Palo Alto Veterans Administration Medical Center, Stanford, CA 94305 (U.S.A.)

(Received March 24th, 1982)

SUMMARY

A rapid high-performance liquid chromatographic method for the separation of methylated phospholipids is described. The separation is accomplished on an amine column using acetonitrile—methanol—water as the eluting solvent and UV detection at 203 nm. The choice between gradient and isocratic elution for the separation depends upon the condition of column. The method is suitable for the isolation of phosphatidylcholine, sphingomyelin, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine and lysophosphatidylethanolamine from tissues. It is applicable to the study of reaction products in phosphatide methyltransferase assay mixtures. Choline and ethanolamine plasmalogens can be determined indirectly by converting them into lysophosphatidylcholine and lysophosphatidylethanolamine with exposure to hydrochloric acid fumes.

INTRODUCTION

Phosphatidylcholine (PC), a major constituent of mammalian cell membranes, can be synthesized by two pathways: by stepwise methylation of phosphatidylethanolamine (PE), and by the CDP-choline pathway [1]. The methylation pathway utilizes S-adenosyl-L-methionine (SAM) as the methyl donor, and is catalyzed by two methyltransferases with phosphatidyl-N-monomethylethanolamine (PMME) and phosphatidyl-N,N-dimethylethanolamine (PDME) as intermediates [2]. Recently Hirata and Axelrod [3] have demonstrated in many cell types an important role of phospholipid methylation in the transduction of receptor-mediated signals through the cell membranes. Phospholipid methyla-

*Address for correspondence: Laboratory Service (113), Veterans Administration Medical Center, 3801 Miranda Avenue, Palo Alto, CA 94304, U.S.A.

0378-4347/82/0000—0000/$02.75

tion is coupled to calcium influx and the release of arachidonic acid. A simple, rapid and quantitative analytical method for the isolation of PE, PMME, PDME and PC will be very useful for studying the function and regulation of phospholipid methylation. The isolation of the different methylated phospholipids by thin-layer chromatography (TLC) [4] is tedious and time consuming. Gas—liquid chromatographic analysis of the bases after acid hydrolysis of the lipids is also a laborious procedure [5]. Axelrod and co-workers [6, 7] recently reported a high-performance liquid chromatographic (HPLC) method for separation and characterization of the major phospholipids formed by methyltransferases in the rat liver. Using a silica gel column, a solvent system of chloroform—methanol—water—ethanolamine (77.8 : 20 : 2 : 0.2, v/v), a fraction collector and liquid scintillation counting, the method is suitable for the analysis of radiolabelled phospholipids. However, since the solvent system absorbs in the 200-nm range, direct monitoring of lipid separation with UV detection is not possible. In this report we describe a rapid and simple HPLC method which is suitable for the isolation of methylated phospholipids from tissues.

MATERIALS AND METHODS

Materials

Egg yolk phosphatidylcholine, sphingomyelin (SPH), lysophosphatidylcholine (LPC), phosphatidylethanolamine and lysophosphatidylethanolamine (LPE) were obtained from Sigma (St. Louis, MO, U.S.A.). Egg yolk PMME and PDME were purchased from Gibco (Grand Island, NY, U.S.A.). They were derivatives of egg yolk PC by the exchange of bases in the presence of phospholipase D. Dipalmitoyl PMME and PDME were synthetic products of Calbiochem-Behring (La Jolla, CA, U.S.A.). Acetonitrile and methanol were of HPLC grade from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). S-Adenosyl-L-[methyl-^{14}C]methionine (59 Ci/mol), and [dipalmitoyl-1-^{14}C]phosphatidylcholine (100 mCi/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.).

Tissue lipid extracts

Sprague-Dawley male rats weighing 150 g were used. They had access to the diet up to the time of sacrifice. Immediately after decapitation, heads and livers were placed in liquid nitrogen. A 1-g amount of rat tissue from cerebrum or right lobe of liver was homogenized in 30 ml of chloroform—methanol (2 : 1, v/v). After filtration the lipid extract was separated into two phases according to the procedure of Folch et al. [8]. An aliquot of the lower phase was injected into the chromatograph for analysis. Lipid phosphorus in tissue extracts was measured by the Bartlett procedure [9].

Hydrolysis of choline and ethanolamine plasmalogens

For the analysis of choline and ethanolamine plasmalogens present in tissues, an aliquot of the lipid extract was dried under nitrogen. The open vial was then inverted and held over an open bottle of concentrated hydrochloric acid for 10 min. After flushing the vial with nitrogen, chloroform was added in an amount identical with that of the original sample. An aliquot was injected into the chromatograph for analysis.

Chromatographic conditions

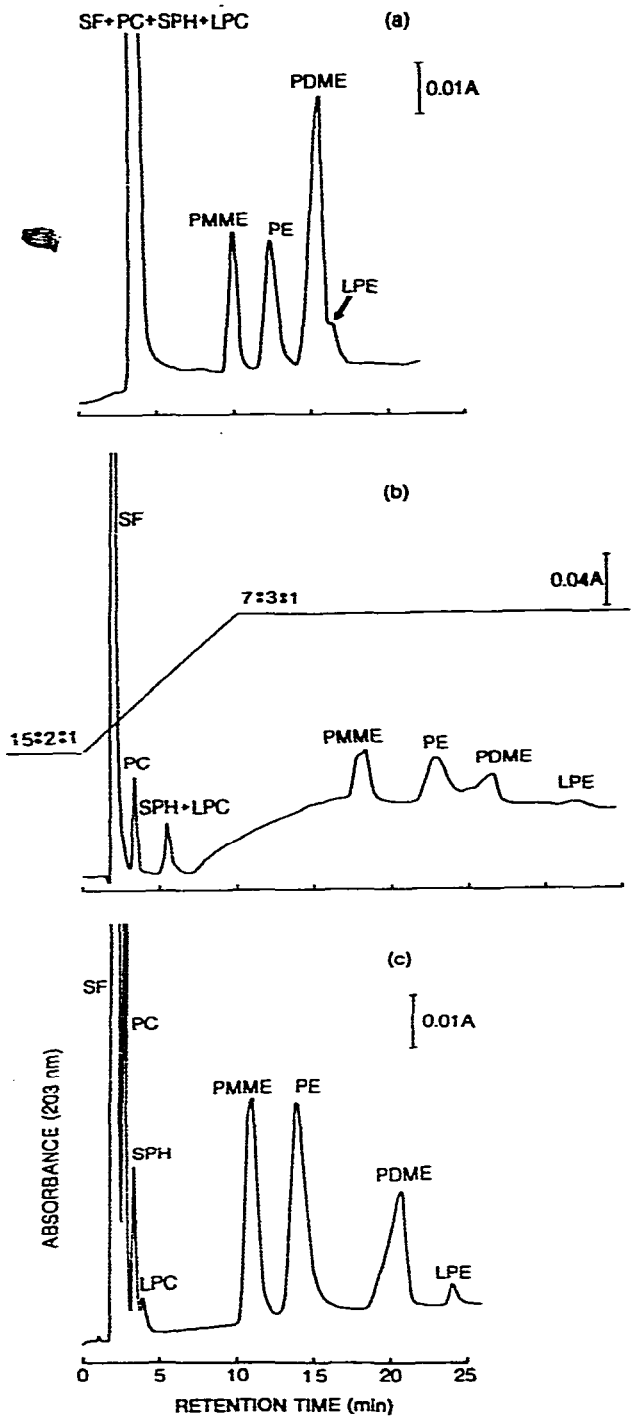
We used a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatographic system consisting of a Model 6000 solvent delivery system, a Model 660 solvent programmer, a Model U6K injector, a Model 450 variable-wavelength detector and a strip chart recorder. The column was a 30 cm × 3.9 mm I.D. prepacked stainless-steel column which contained μBondapak NH_2, particle size 10 μm (Cat. No. 84040). The packing material has an amino group chemically bonded to silica at 9% (w/w) (—Si—R—NH_2). Solvent composition, flow-rate, sample size and recorder response are indicated in the legends to the figures. The temperature was approximately 21°C. The detection was at 203 nm. The reference cell contained air. Each day after the analysis the column was washed successively with 30 ml each of methanol—water (1:1, v/v), methanol and dichloromethane before storing it overnight in *n*-hexane.

Analysis of reaction products in methyltransferase assay

The rat liver was minced and homogenized at 4°C in 3 volumes of 0.25 *M* sucrose with a Polytron (Brinkmann Instruments, Westbury, NY, U.S.A.) for 1 min. The homogenate was centrifuged for 20 min at 10,000 *g* to sediment cell debris, nuclei and mitochondria. Aliquots of the postmitochondria supernatant were used as enzyme suspensions in the phosphatide methyltransferase assays. The enzyme activity was assayed by measuring the incorporation of methyl group from [methyl-^{14}C]SAM into phospholipids as described by Tanaka et al. [10]. The assay mixture contained 5.9 μ*M* [methyl-^{14}C]SAM (0.1 μCi), 10 m*M* L-cysteine, 10% glycerol, 0.1 *M* Tris · HCl buffer (pH 8.8) and enzyme suspension (2.5 mg protein) in a total volume of 0.5 ml. The reaction was initiated by the addition of radioactive SAM. The mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 8 ml of chloroform—methanol (2:1, v/v). Lipids were extracted according to the Folch procedure [8]. After washing once with the pure solvent upper phase [8], the lower chloroform layer was dried down under nitrogen and redissolved in 30 μl of chloroform. An aliquot (20 μl) was injected into the chromatograph for the analysis of reaction products. We attempted to confirm that the reaction products were methylated phospholipids by comparing the retention times of ^{14}C-radioactivity peaks with those of phospholipids derived from various sources. The chromatogram of ^{14}C-labelled substances was obtained by collecting the effluent for 40 min into 80 scintillation vials, i.e. a 30-sec collection per fraction. The eluate was evaporated to dryness, dissolved in 0.5 ml of methanol and counted for β-emission in 15 ml of Liquifluor (New England Nuclear).

Quantitative analysis of phospholipids in the rat liver and brain extract

Lipid extracts were injected into the chromatograph for analysis. Phospholipid classes were quantitated by collecting the effluent under the peaks and measuring the lipid phosphorus with malachite green [11]. The effluent of PMME peak was also quantified by a Dns derivatization procedure which we previously described [12]. Briefly, the lipid reacted with 1-dimethylaminonaphthalene-5-sulfonyl chloride (Dns-Cl) to form a fluorescent derivative. The derivatization mixture was analyzed by a second high-performance liquid chromatograph using a silica gel column and gradient elution. Dns-PMME was measured by fluorescence detection.


SF+PC+SPH+LPC
(a)
PDME
0.01A
PMME
PE
LPE
(b)
SF
0.04A
7:3:1
15:2:1
PC
SPH+LPC
PMME
PE
PDME
LPE
(c)
SF
PC
0.01A
PMME
PE
SPH
PDME
LPC
LPE
ABSORBANCE (203 nm)
0
5
10
15
20
25
RETENTION TIME (min)

RESULTS

HPLC of phospholipid standards

Aliquots of phospholipid solution, containing egg yolk PC, SPH, LPC, PE, PMME, PDME and LPE were injected onto an amine column for analysis, and acetonitrile—methanol—water mixtures were used as eluting solvents. The selection of solvent composition and the choice between gradient and isocratic elution for the separation depended upon the condition of the column. When the column was new, PE, PMME, PDME and LPE were strongly retained. Only eluting solvents with relatively high methanol and water contents could elute these lipids within a short time. On the other hand, the separation of PC, SPH and LPC required a solvent system of very low polarity. Fig. 1a shows that with an acetonitrile—methanol—water (3:2:1, v/v/v) solvent mixture, PE, PMME and PDME were readily separated. PC, SPH and LPC were eluted with the solvent front (SF), while LPE co-eluted with PDME. To separate all of the methylated phospholipids in a single run, it is necessary to use gradient elution. As shown in Fig. 1b, choline-containing phospholipids were eluted first, while the others were not eluted until the polarity of the solvent was raised. However, after the column was used repeatedly for the analysis of tissue extracts, the ability of stationary phase to retain ethanolamine-containing phospholipids decreased. The polarity of eluting solvent that was required for a rapid separation of these lipids became lower and lower with increasing use of the column. In contrast, the retention times of choline-containing phospholipids tended to increase. In time the use of a solvent system of relatively low polarity (acetonitrile—methanol—water, 13:7:1, v/v/v) in isocratic elution could separate all of the methylated phospholipids rapidly (Fig. 1c). The number of theoretical plates of the column was approximately 1500 in the beginning and decreased to 900 at the time the isocratic elution was employed. Despite the decrease in column efficiency, good resolution was achieved by adjusting the proportion of acetonitrile, methanol and water in the eluting solvent. These results were reproducible in all the three columns that we utilized in this present study. Recoveries of phospholipids applied to the column were determined by measuring the amount of phosphorus in the eluted phosphorus peaks. They were consistently greater than 95%, except for the recovery of PE which was 85%. The quantitative recovery of PC was also confirmed by counting the radioactivity in the eluted PC peak after injecting [dipalmitoyl-1-^{14}C] PC into the column.

Fig. 1. Chromatograms of egg yolk phospholipids. Chromatographic conditions: (a) new column, isocratic elution with acetonitrile—methanol—water (3:2:1, v/v/v), flow-rate, 1.0 ml/min, recorder response, 0.1 a.u.f.s.; (b) new column, gradient elution with acetonitrile—methanol—water mixtures from 15:2:1 to 7:3:1 (v/v/v) linearly in 10 min, flow-rate, 1.5 ml/min, recorder response, 0.4 a.u.f.s.; (c) a column in daily use for 1 month; isocratic elution with acetonitrile—methanol—water (13:7:1, v/v/v), flow-rate, 1.6 ml/min, recorder response 0.1 a.u.f.s. The amount injected was approximately 1 μg of each lipid in (a), 6 μg of each lipid in (b), and 2 μg of each lipid in (c). Peaks: SF = solvent front: PC = phosphatidylcholine; SPH = sphingomyelin; LPC = lysophosphatidylcholine; PE = phosphatidylethanolamine; PMME = phosphatidylmonomethylethanolamine; PDME = phosphatidyldimethylethanolamine; LPE = lysophosphatidylethanolamine.

HPLC of tissue extracts

Aliquots of lipid extracts from rat brain and liver were injected into the chromatograph, and the isocratic technique was used to elute the lipids. Despite the use of crude Folch extracts [8], chromatograms in Fig. 2a and Fig. 3a reveal peaks of PC, SPH and PE free of interferences by other materials. Neutral lipids were eluted in the solvent front. Phosphatidylinositol (PI) and phosphatidylserine (PS) could not be eluted. These chromatographic patterns were consistent with the published results on the relative abundance of phospholipids in various organs of rats [13]. Peaks of PMME, PDME and LPE were detectable only when a larger aliquot was injected and when the sensitivity of the detector was increased (Fig. 3b).

Analysis of plasmalogens

The chromatographic condition described here did not separate choline plasmalogen from PC nor ethanolamine plasmalogen from PE. Plasmalogens

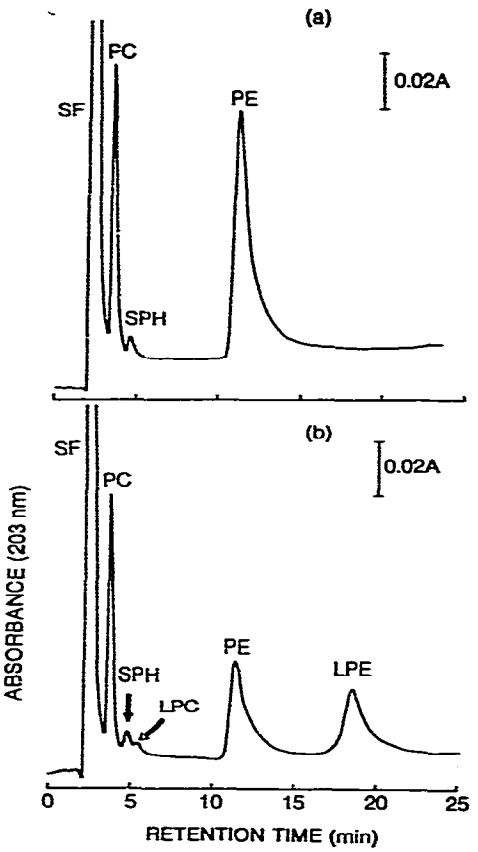


Fig. 2. HPLC analysis of the lipid extract of rat brain before (a) and after (b) exposure to hydrogen chloride fumes. An aliquot of the Folch lipid extract [8], containing 15 μg of total phospholipids, was injected directly into the chromatograph. Another aliquot, also containing 15 μg, was dried, exposed to hydrogen chloride fumes, redissolved in chloroform and then injected. Chromatographic conditions: isocratic elution with acetonitrile—methanol—water (13 : 7 : 1, v/v/v); flow-rate, 1.6 ml/min; and recorder response, 0.2 a.u.f.s.

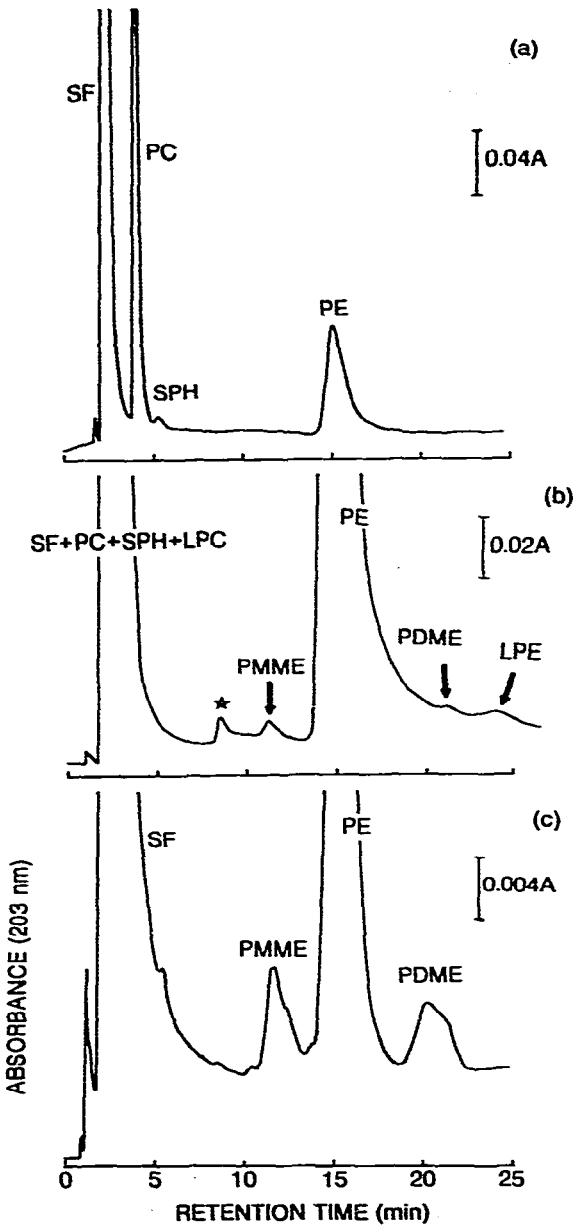


Fig. 3. HPLC analysis of the lipid extract of rat liver. Chromatographic conditions: isocratic elution with acetonitrile—methanol—water (13:7:1, v/v/v); and flow-rate, 1.6 ml/min. (a) An aliquot of the Folch lipid extract [8] containing 15 μg of total phospholipids; recorder response, 0.4 a.u.f.s.; (b) an aliquot containing 360 μg of total phospholipids in 25 μl of chloroform, recorder response, 0.2 a.u.f.s.; (c) isolation of PMME and PDME from rat liver. PMME and PDME peaks were collected and reinjected into the chromatograph for the removal of PE contaminant, see text for explanation. ★ = An unidentified peak.

could be analyzed by comparing the chromatogram of the original lipid extract (Fig. 2a) with that of the lipid extract that had been exposed to hydrogen chloride fumes before the HPLC analysis (Fig. 2b). This is based on previous observations that hydrogen chloride fumes quantitatively hydrolyze the alk-1-enyl group from phospholipids and neutral lipids [14]. The chromatogram of the original lipid extract reveals no detectable amounts of lyso-PC and lyso-PE in rat brain. The exposure to hydrogen chloride fumes converted choline plasmalogen into lyso-PC and ethanolamine plasmalogen into lyso-PE, since Fig. 2b shows that the peaks corresponding to PC and PE decreased while the peaks corresponding to lyso-PC and lyso-PE appeared. Table I shows the results of analysis of ethanolamine plasmalogens in the rat brain extract. They are compared with the results obtained by other HPLC and TLC methods. Good agreement between these results is evident.

TABLE I

ANALYSIS OF ETHANOLAMINE PLASMALOGENS IN RAT BRAIN

The values in the present analysis, percentages of the total lipid phosphorus, are mean ± S.D. obtained from three rats. Aliquots of lipid extracts containing 32 μg of total phospholipids were injected into the chromatograph as described in Fig. 2. PE and lyso-PE peaks were collected from the effluent and quantitated by measuring the lipid phosphorus. Ethanolamine plasmalogens were determined by the decrease in PE fraction and the increase in lyso-PE after exposure of the lipid extract to hydrogen chloride fumes.

	Present analysis	Dns derivatization and HPLC by Chen et al. [12]	HPLC by Jungalwala et al. [15]	TLC by Clarke and Dawson [16]
Before exposure to hydrogen chloride				
PE	40.0 ± 2.8	43.6 ± 1.2	41.6 ± 2.6	38.2
Lyso-PE	None detected			
After exposure to hydrogen chloride				
PE	19.2 ± 2.0			15.4
Lyso-PE (derived from plasmalogens)	19.9 ± 0.9	18.9 ± 0.6	22.9 ± 1.3	21.3

Isolation of PMME and PDME from rat liver

The isolation of PMME and PDME from tissues was complicated by the fact that the content of these lipids in the crude lipid extract is extremely small [5, 17]. For PMME and PDME peaks to be detectable with UV detection, a relatively large amount of lipid extract had to be injected into the chromatograph. Because the content of PE in the rat liver is about 1000-fold more than those of PMME and PDME [17], the resolution of ethanolamine-containing phospholipids is sacrificed when the sample size is increased. We used the following procedure to isolate PMME and PDME from the rat liver. In order to avoid excessive contamination by PE, in each injection the sample size of the lipid extract was limited to 360 μg of total phospholipids. PMME and PDME peaks were collected from the effluent. Collections from several injections were combined, evaporated to dryness under nitrogen, and redissolved in 30 μl of chloroform. It was injected for the second time into the chromatograph for the

TABLE II

ANALYSIS OF PMME AND PDME IN THE RAT LIVER AND BRAIN EXTRACTS

The values are the amount of each lipid per mg of total phospholipids in lipid extracts. They are mean ± S.D. obtained from three rats. The method of analysis was described in the text and Fig. 3. PE, PMME and PDME peaks were collected from the effluent and quantitated by measuring the lipid phosphorus with malachite green [11].

	Liver	Brain
PE	0.28 ± 0.01 mg	0.40 ± 0.03 mg
PMME	0.60 ± 0.04 μg	
	0.76 ± 0.17 μg*	0.50 ± 0.08 μg*
PDME	1.00 ± 0.16 μg	1.94 ± 0.20 μg

*Lipid quantitated by Dns derivatization [12].

removal of PE contamination (Fig. 3c). Identities of the isolated materials, PMME and PDME, were confirmed by comparing their retention times with those of the ^{14}C-labelled phospholipids of the reaction products in the phosphatide methyltransferase assay as described in the next experiment. Table II shows the results of analysis of PE, PMME and PDME in the rat liver and brain extracts. The liver results are in general agreement with those obtained by TLC method as reported by Katyal and Lombardi [17]. We have previously described an HPLC procedure for the analysis of amino group-containing phospholipids in tissues [12]. It involves Dns derivatization of phospholipids followed by HPLC separation of fluorescent derivatives. The usefulness of this procedure for the analysis of methylated phospholipids is limited, because the gradient elution does not separate Dns-PMME from Dns-PE, and because PDME cannot be derivatized with Dns. However, after PMME has been isolated from the tissue extract and PE contamination removed, dansylation can be used for the quantitation of PMME. The major advantage is that because of its sensitivity the effluent collected from only one single injection of the lipid extract is sufficient for quantitation. Table II shows that PMME values obtained by measuring lipid phosphorus with malachite green and by the derivatization procedure were in agreement.

Analysis of reaction products in methyltransferase assay

Experiments were performed in order to determine the usefulness of HPLC in the assay of phosphatide methyltransferases. Intermediates and the product of the methylation pathway in the biosynthesis of PC from PE were labelled by [methyl-^{14}C]SAM using liver homogenates. Previous studies [4, 6, 7] using TLC and HPLC revealed that the labelled reaction products in the assay mixture were PMME, PDME and PC. PE was not significantly labelled. With the method described here the chromatogram of lipid extract (Fig. 4d) shows four peaks of radioactivity. Under the same chromatographic condition, we also injected into the chromatograph separately mixtures of PE, PMME and PDME derived from various sources (Fig. 4a, b and c). The comparison of these chromatograms shows that radioactivity peaks A, B, C and D corresponded to SF, PC, PMME and PDME, respectively. Phospholipids from different sources show-

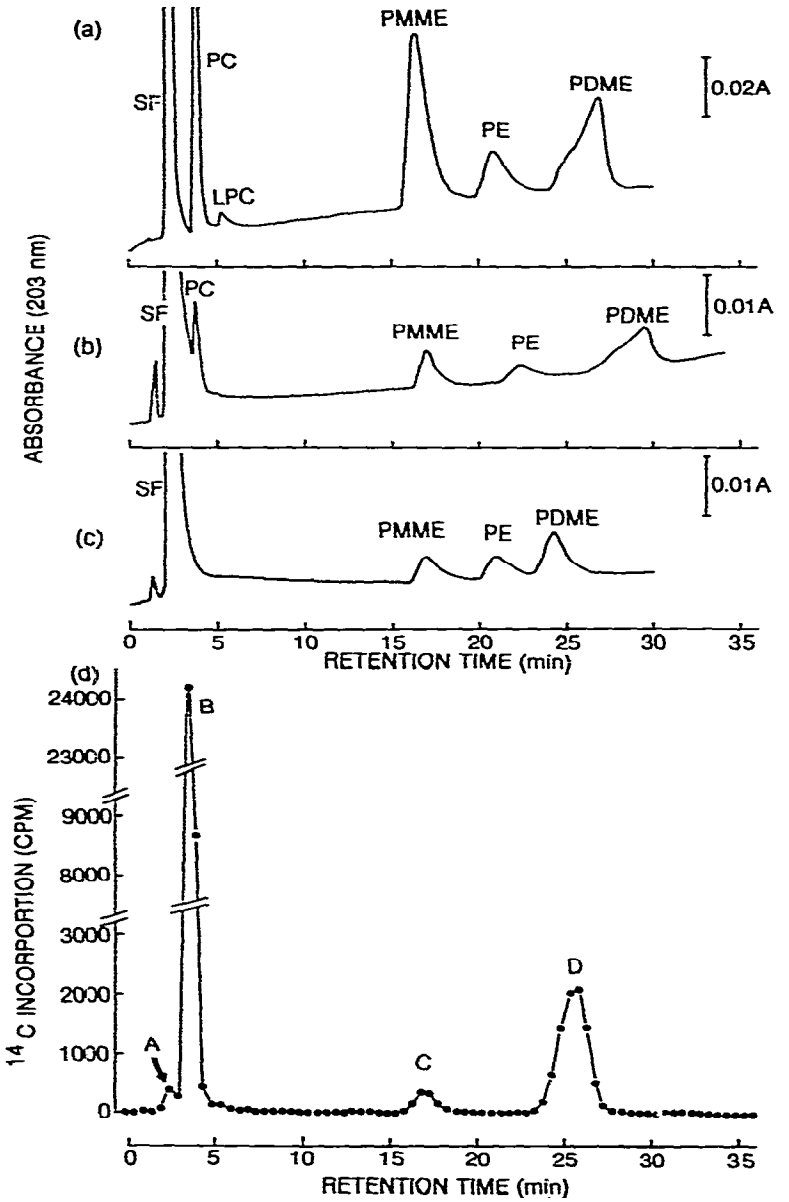


Fig. 4. HPLC analysis of the ^{14}C-labelled lipids of reaction products in the methyltransferase assay mixture, and its comparison with the chromatographic patterns of methylated phospholipids from various sources. Chromatographic conditions: isocratic elution with acetonitrile—methanol—water (110:65:3, v/v/v); flow-rate, 1.5 ml/min. (a) Egg yolk phospholipid mixture, containing approximately 3 μg of PE and 8 μg each of the others; (b) synthetic phospholipid mixture, containing dipalmitoyl PC (15 μg), dipalmitoyl PE (15 μg), dipalmitoyl PMME (30 μg) and dipalmitoyl PDME (30 μg); (c) rat liver PE, PMME and PDME, approximately 1 μg each; (d) reaction products in the stepwise methylation of PE to PC in the rat liver were labelled by [methyl-^{14}C]SAM in vitro as described under Materials and methods. The lipid extract of the assay mixture was injected into the chromatograph. The radioactivity of the effluent was measured. Each dot represents cpm in a 30-sec collection of effluent.

ed variations in the retention time and the peak shape. This is evident particularly in the elution of PDME. This most likely reflected the influence of fatty acid composition on the retention time.

DISCUSSION

The packing material, μBondapak NH_2, contains amino groups chemically bonded to silicas (—Si—R—NH_2). It is a weak anion exchanger (pK_b 6.5) when the pH of the mobile phase is 5.0 or below, but in this present study it was used in a partition mode to separate phospholipid components. The choice of this stationary phase was based on the rule, like-attracts-like. Indeed, ethanolamine-containing phospholipids were eluted from the column much later than choline-containing phospholipids. When the column was new, the differing retention by the stationary phase between choline-containing and ethanolamine-containing phospholipids was so great that gradient elution was required for a rapid separation of all of the methylated phospholipids in a single run. However, with increasing use of the column, the retention of ethanolamine-containing phospholipids decreased gradually. In contrast, the retention of choline-containing phospholipids was increased. It became feasible to use a low-polarity solvent to elute all of the methylated phospholipids isocratically. This most likely was due to a decrease in the number of amino group functionality on the stationary phase resulted from either deactivation of the amino function by peroxides, ketones and aldehydes or the slow accumulation of organic compound contaminants.

The reduction in amino group functionality on the stationary phase enables lipid components to be analyzed by chromatography using isocratic elution without needlessly long separation times. Compared with the analysis using gradient elution, isocratic technique not only is faster, but also avoids a steeply sloped baseline. UV detection with a straight baseline is a prerequisite for operating the chromatograph at maximum sensitivity. A brand new μBondapak NH_2 column was unsuitable for the simultaneous analysis of choline- and ethanolamine-containing phospholipids in tissue extracts, because gradient elution precluded the use of high-sensitivity detection (Fig. 1b). In this present study it was necessary to operate the chromatograph at high sensitivity, because contents of PMME and PDME were very low. For this reason, we did not consider the deactivation and the reduction of amino group functionality in the early stage of the column life to be undesirable. However, after a column has been deactivated to the extent that isocratic elution technique becomes applicable, precautions must be taken to avoid contamination and prevent further reduction in amino group functionality. To minimize the change with time in separation patterns, sample purification prior to HPLC analysis with Sep-Pak silica gel cartridges (Waters Assoc.) and cleaning of the column after use by washing with organic solvents (see Materials and methods) are effective and good practices. The use of a guard column which is changed regularly prolongs the column life, provided that it is packed with suitable material. We have observed that the packing material for guard column recommended by the vendor (Waters Assoc.), Bondapak AX/Corasil, broadens the PE peak. This may be related to the fact that Bondapak AX/Corasil, a strong anion exchanger,

ionizes in a pH range from 1 to 13. This is incompatible with an analytic column which operates in a partition mode.

Because of the different fatty acid composition, phospholipid component derived from various sources may show variations in retention time. This is evident particularly in the elution of PDME and lyso-PE. The tentative identification of chromatographic peaks based on retention times, the elution order and relative abundance of phospholipids in tissues should be confirmed by other independent methods. In this present study the identity of peaks was confirmed by the following methods: (a) the exposure of lipid extracts to hydrogen chloride fumes caused the peaks corresponding to PC and PE to decrease, while peaks corresponding to lyso-PC and lyso-PE appeared (Fig. 2); (b) peaks corresponding to PE, PMME and lyso-PE were collected and confirmed by Dns derivatization and a second HPLC with fluorescence detection [12]; and (c) putative PC, PMME and PDME peaks in the chromatogram of rat liver were confirmed by comparing their retention times with those of the radiolabelled phospholipids of the reaction products in the phosphatide methyltransferase assay (Fig. 4). Previous TLC and HPLC studies [4, 6, 7] have shown that only these three phospholipids became radiolabelled in the assay.

Kiuchi et al. [18] previously used flame ionization detection, a μBondapak NH_2 column and an isocratic solvent system of chloroform—methanol—water to separate lipid mixtures. Using a solvent proportion of 75 : 25 : 4 (v/v/v), the order of lipids eluted were triglycerides, phosphatidic acid, PC, phosphatidylglycerol, PE, PS and PI. Hanson et al. [19] previously performed HPLC of egg yolk lipids with a similar amine column, Ultrasil-NH_2 (Altex, Berkeley, CA, U.S.A.). Using gradient elution of hexane—isopropanol—water mixtures they separated the lipid extract into neutral lipids, PC, SPH, lyso-PC and PE fractions. PS and PI were not eluted. Comparisons of these previous methods which also employed amine columns with the method described here reveal similarity in the order of phospholipid elution.

In the biosynthesis of PC from PE, PMME is the product of methyltransferase I, while PDME and PC are the products of methyltransferase II. HPLC offers an easier and more accurate method for analyzing the reaction products in phosphatide methyltransferase assays than TLC. With the method described here the dynamics of phospholipid methylation in cellular activation can be more readily studied. The method is also well suited for the quantitative analysis of methylated phospholipids and plasmalogens in tissue extracts. For the quantitation of phospholipid components previous investigators [20] suggested that specific peaks are collected and quantified by the analysis of phosphorus. Being sensitive and non-destructive, UV detection is ideal for monitoring the separation of lipids by HPLC. Approximately 1 μg of phospholipid can be detected. However, it is complicated to use UV response directly for lipid quantitation. UV absorption by lipids at the 200-nm region is due largely to the presence of double bonds [20]. The absorption by other functional groups, such as ester carbonyl and amino, also occurs, but it is small in extent. PS and PI possibly were retained by the stationary phase and could not be eluted with an amine column and the solvent systems described in this report. With a differ-

ent solvent, chloroform—methanol—water (75:25:4, v/v/v), PS and PI were eluted behind PE as reported by Kiuchi et al. [18]. They also can be analyzed by HPLC using the methods that we reported previously [12, 21].

ACKNOWLEDGEMENT

This investigation was supported by the Veterans Administration.

REFERENCES

1 H. van den Bosch, Ann. Rev. Biochem., 43 (1974) 243.
2 J. Bremer and D.M. Greenberg, Biochim. Biophys. Acta, 46 (1961) 205.
3 F. Hirata and J. Axelrod, Science, 209 (1980) 1082.
4 F. Hirata, W.J. Strittmatter and J. Axelrod, Proc. Nat. Acad. Sci. U.S., 76 (1979) 368.
5 R.L. Lester and D.C. White, J. Lipid Res., 8 (1967) 565.
6 B.V.R. Sastry, C.N. Statham, J. Axelrod and F. Hirata, Arch. Biochem. Biophys., 211 (1981) 762.
7 B.V.R. Sastry, C.N. Statham, R.G. Meeks and J. Axelrod, Pharmacology, 23 (1981) 211.
8 J. Folch, M. Lees and G.H.S. Stanley, J. Biol. Chem., 226 (1957) 497.
9 G.R. Bartlett, J. Biol. Chem., 234 (1959) 466.
10 Y. Tanaka, O. Doi and Y. Akamatsu, Biochem. Biophys. Res. Commun., 87 (1979) 1109.
11 A. Chalvardjian and E. Rudnicki, Anal. Biochem., 36 (1970) 225.
12 S.S.-H. Chen, A.Y. Kou and H.-H.Y. Chen, J. Chromatogr., 208 (1981) 339.
13 G. Rouser, G. Simon and G. Kritchevsky, Lipids, 4 (1969) 599.
14 L.A. Horrocks, J. Lipid Res., 9 (1968) 469.
15 F.B. Jungalwala, R.J. Turel, J.E. Evans and R.H. McCluer, Biochem. J., 145 (1975) 517.
16 N.G. Clarke and R.M.C. Dawson, Biochem. J., 195 (1981) 301.
17 S.L. Katyal and B. Lombardi, Lipids, 11 (1976) 513.
18 K. Kiuchi, T. Ohta and H. Ebine, J. Chromatogr., 133 (1977) 226.
19 V.L. Hanson, J.Y. Park, T.W. Osborn and R.M. Kiral, J. Chromatogr., 205 (1981) 393.
20 F.B. Jungalwala, J.E. Evans and R.H. McCluer, Biochem. J., 155 (1976) 55.
21 S.S.-H. Chen and A.Y. Kou, J. Chromatogr., 227 (1982) 25.