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DO LYSOPHOSPHOLIPIDS REALLY EXIST IN *TETRAHYMENA PYRIFORMIS?*

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SUMMARY

Lyso-compounds derived from the major phosphorus-containing lipids of *Tetrahymena pyriformis* have been characterized by their chromatographic mobilities, chemical analysis and infrared and nuclear magnetic resonance spectroscopy.

INTRODUCTION

Thompson[1] reported potent systems of a mixture of phospholipases, including phospholipase A (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5) and phospholipase C (EC 3.1.4.3) in *Tetrahymena pyriformis*, which hydrolyzed its endogenous phospholipids unless proper care was exercised in preparing its organelles for *in vitro* studies. Reports on the occurrence of lysophospholipids in this organism have been frequent[2–4], but these have not been sufficiently well characterized chemically. For instance, criteria advanced for the identification of lyso-(2-aminoethylphosphonoglyceride)[3] could equally well apply to ceramide-2-aminoethylphosphonate[5].

Recent investigations[6,7] in this laboratory have failed to detect the presence of any lysophospholipids in this organism, although a number of minor lipids have still eluded their complete characterization.

This paper describes the preparation, chromatographic mobilities, infrared (IR) and nuclear magnetic resonance (NMR) properties of some of the lyso-compounds derived from the major phospholipids of *Tetrahymena pyriformis*. These criteria of characterization may be useful in future investigations on the *Tetrahymena* lipids.

EXPERIMENTAL

Materials

Plates pre-coated with silica gel F_{254} and cellulose (E. Merck, Darmstadt, G.F.R.) were used for thin-layer chromatographic (TLC) studies. All chemicals used, including solvents, were of analytical-reagent grade and not further purified.

Methods

A mixture of 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine (alkylacyl PC) and 1,2-diacyl-*sn*-glycero-3-phosphorylcholine (diacyl PC) and a mixture of 1-alkyl-2-

acyl-*sn*-glycero-3-(2-aminoethylphosphonate) (alkylacyl PnE) and 1,2-diacyl-*sn*-glycero-3-(2-aminoethylphosphonate) (diacyl PnE) were obtained by the procedure described previously[6]. Pure ceramide-2-aminoethylphosphonate (Cer-AEP) and ceramide-monomethylaminoethylphosphonate (Cer-MMAEP) were isolated as described previously[8]. Lysophosphatidyl ethanolamine (L-PE), which was an artifact produced during the isolation of lipids from cells of *Escherichia coli*, was isolated by the ascending dry-column chromatographic technique[6]. The lyso-compounds L-alkyl PC and L-alkyl PnE were prepared from mixtures of alkylacyl PC and diacyl PC, and alkylacyl PnE and diacyl PnE by the procedure described by Berger *et al.*[9].

The chromatographic behaviour of the lyso-compounds was studied by adsorption TLC in the alkaline solvent system described earlier[7].

IR spectra were determined on a liquid film with sodium chloride optics on a Unicam SP 200 instrument.

NMR spectra were recorded on 10% solution in C^3HCl_3 using a Varian HA-100 instrument (100 MHz). The ambient temperature was 34°. Chemical shifts were measured in parts per million downfield from the internal standard, tetramethylsilane ($\tau = 10$). All assignments were supported by integration of the areas under the spectral curve, giving the number of protons responsible for each peak.

Hydrolysis of the various lipid fractions was carried out with aqueous 2 or 6 *N* HCl at 110° for 48 h in 100 × 13 mm tubes with PTFE-lined screw-caps.

TLC on cellulose plates with butanol–acetic acid–water (60:15:25, by volume) was used to identify either ethanolamine, ethanolamine phosphate, choline or 2-aminoethylphosphonate[5].

Specific group reagents used for the detection of lipids on TLC plates were: 0.25% ninhydrin in acetone for aminolipids; 0.25% *cis*-aconitic anhydride in acetic anhydride[10] for choline lipids; and molybdenum blue reagent for phospholipids[11].

The ester contents of various lipid-fractions were determined by the procedure of Snyder and Stephens[12].

Lipid phosphorus was determined by the procedure of Ernster *et al.*[13] after digesting the lipids with 70% perchloric acid for 1 h.

Glycerol was determined by the procedure of Renkonen[14].

RESULTS

The relative chromatographic mobilities of the three lyso-compounds are shown in Fig. 1. The compound with the lowest mobility was L-alkyl PC, with intermediate mobility L-alkyl PnE, and the compound with the highest chromatographic mobility was characterized as 1-acyl-*sn*-glycero-3-phosphorylethanolamine (L-acyl PE) by hydrolytic techniques and by NMR spectroscopy. In this alkaline solvent system, chromatographic mobilities of L-acyl PE, L-alkyl PnE and L-alkyl PC corresponded exactly to those of Cer-MMAEP, Cer-AEP and an unknown phospholipid, respectively, from *Tetrahymena pyriformis*.

The lyso-compounds were also characterized by NMR spectroscopy[15,16]. For comparison, the corresponding diradyl phospholipids were also studied. The results are given in Table I.

The results of NMR spectroscopy can be briefly interpreted as follows.

For the protons of fatty chains of phospholipids, the absence of absorption

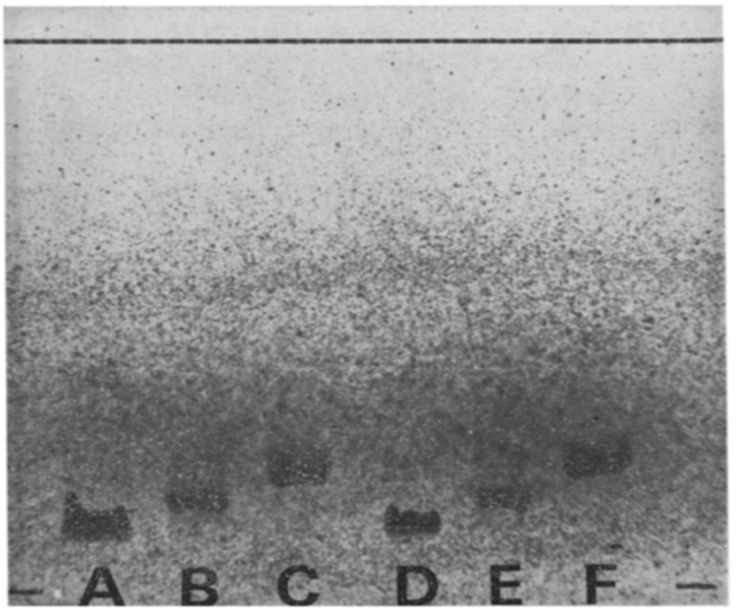

Fig. 1. TLC separation of minor lipids and lyso-compounds derived from the major phosphorus-containing lipids of *Tetrahymena pyriformis*. Adsorbent: Kieselgel F_{254}. Sovent: chloroform–methanol–concentrated ammonia (65:35:5 by volume). Spray reagent: molybdenum blue reagent of Dittmer and Lester[11]. Materials spotted: A, Lysophosphatidylcholine (L-alkyl PC); B, lyso-(2-aminoethylphosphonoglyceride) (L-alkyl PnE); C, lysophosphatidylethanolamine (L-acyl PE); D, unknown phospholipid; E, ceramide-AEP; F, ceramide-MMAEP.

in the regions of 4.6, 7.2 and 8.0 ppm indicates that the alkyl chains of L-alkyl PC and L-alkyl PnE are saturated. This result supports the finding of Thompson[2] that chimyl alcohol accounts for more than 98 mole% of glyceryl ethers derived from the lipids of *Tetrahymena*. The absence of absorption in the region of 7.7 ppm in both L-alkyl PC and L-alkyl PnE indicates that these lyso-compounds are only ether analogues, compared with the L-acyl PE, which is an ester analogue.

For the protons of the glyceryl residue of phospholipids, the absence of absorption in the 6.0–6.2 ppm region in both L-alkyl PC and L-alkyl PnE indicates the absence of their respective 1-acyl analogues. The presence of absorption in the region of 6.3 ppm and the absence of absorption in the region of 4.8 ppm in all the three lyso compounds indicates that all of the lyso-compounds are 1-isomers.

For the protons of polar head-groups of phospholipids, the choline lipids are characterized by a typical singlet absorption in the 6.6 ppm region due to the $N^+(CH_3)_3$-group protons. The aminolipids (PE and PnE), on the other hand, are characterized by a typical absorption in the 6.5–6.9-ppm region due to the protons of the CH_2–N group and in the 1.6–1.8-ppm region due to protons of the N^+H_3 group.

It is noteworthy that the absorption due to the CH_2 group of the glyceryl residue and the fatty chain adjacent to the ether oxygen in these lipids is masked by the absorption peak between 6.5 and 7.0 ppm characteristic of aminolipids (PE and PnE) and choline lipid (PC).

TABLE I

NMR SIGNALS OF PROTONS OF FATTY CHAINS, GLYCERYL RESIDUES AND POLAR HEAD-GROUPS OF PHOSPHOLIPIDS

Compounds	*Protons of fatty chains*						*Protons of glyceryl residues and polar head-groups*						
	$-C\underline{H}_3$	$-C\underline{H}_2$	$-C\underline{H}_2-CH=$	$-C\underline{H}_2-COO$	$CH=C\underline{H}_2-CH=$	$-C\underline{H}-C\underline{H}$	$-C\underline{H}_2-N$	$'N'^+-\underline{Me}_3$	$C\underline{H}_2-O-CO$	$C\underline{H}-OH$	$C\underline{H}_2-O-PO$	$C\underline{H}-O-CO$	$\overset{+}{N}\underline{H}_3$
Phosphatidylcholine (PC)	9.1	8.7	8.0	7.65	7.2	4.65	–	6.65	6.15	–	5.7	4.85	–
Lysophosphatidylcholine (L-alkyl PC)	9.1	8.7	–	–	–	–	–	6.7	–	6.3	6.2	–	–
2-Aminoethylphosphono-glyceride (PnE)	9.1	8.7	7.95	7.7	7.2	4.65	6.6	–	6.0	–	5.8	4.8	1.8
Lyso-(2-aminoethylphosphono)-glyceride (L-alkyl PnE)	9.1	8.7	–	–	–	–	6.55	–	–	6.3	6.0	–	1.8
Phosphatidylethanolamine (PE)	9.1	8.7	7.95	7.7	–	4.65	6.85	–	6.0	–	5.7	4.8	1.65
Lysophosphatidylethanolamine (L-acyl PE)	9.1	8.7	8.0	7.7	–	4.65	6.75	–	6.1	6.3	5.9	–	1.70

TABLE II

INFRARED CHARACTERISTICS OF VARIOUS PHOSPHOLIPIDS

Compound	*Ester region* (cm^{-1})	*'NH' group region* (cm^{-1})	*Phosphate phosphonate region* (cm^{-1})	*Choline region* (cm^{-1})
Phosphatidylcholine (PC)	1730	–	1240, 1100, 1065	980
Lysophosphatidylcholine (L-alkyl PC)	–	–	1240, 1100, 1065	980
2-Aminoethylphosphono-glyceride (PnE)	1735	1550	1200, 1050	–
Lyso-(2-aminoethylphosphono)-glyceride (L-alkyl PnE)	–	1550	1190, 1075	–
Phosphatidylethanolamine (PE)	1735	1550	1230, 1080, 1030	–
Lysophosphatidylethanolamine (L-acyl PE)	1735	1550	1230, 1080, 1030	–

The IR spectra[17] of diradyl phospholipids and their corresponding lyso-derivatives (Table II) substantiate earlier NMR observations regarding the absence of ester analogues in L-alkyl PC and L-alkyl PnE. In addition, the IR spectra show a clear distinction between the phospholipids and phosphonolipids[8] in the region 1250–1000 cm^{-1}.

The analytical results show that L-acyl PE contained glycerol, fatty acids and phosphorus in the ratio 1.1 : 1 :1 and that it liberated ethanolamine on acid hydrolysis. Reduction of L-alkyl PnE with lithium aluminium hydride yielded the glyceryl ether while acid hydrolysis at 110° for 48 h with 6 *N* HCl yielded 2-aminoethylphosphonate. L-alkyl PC was characterized by its typical colour reaction with *cis*-aconitic acid anhydride[10] and by the liberation of choline, identified by cellulose TLC[18], on acid hydrolysis. This compound also yielded the glyceryl ether when subjected to reduction with lithium aluminium hydride.

DISCUSSION

The lyso-derivatives of phosphatidylcholine, phosphatidylethanolamine and 2-aminoethylphosphonoglyceride have been characterized by chromatographic mobilities, chemical analysis and IR and NMR spectroscopy. The similarity of the chromatographic mobilities of lysophospholipids and sphingolipids indicates the necessity for the exhaustive characterization of lipids with the aid of other techniques such as IR and NMR spectroscopy, chemical reactions and the identification of individual components. The usefulness of NMR spectroscopy in establishing that all of the lysophospholipids are 1-isomers is re-emphasized[15].

It was pointed out in a previous paper[7] that the relative mobilities of phosphatidylethanolamine and 2-aminoethylphosphonoglycerides in an acidic solvent system are reversed when these compounds are separated in an alkaline solvent system. The same behaviour, as expected, was noted for the corresponding lyso-compounds.

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