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Note

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Chromatographic fractionation of the lipids of *Tetrahymena pyriformis*

The free living ciliate *Tetrahymena pyriformis*, contains substantial amounts of glycerylphosphorylethanolamine (GPE) lipids and glycerol-(2-aminoethylphosphonate) [G-AEP] lipids[1] which for some time posed separation problems. Earlier attempts to achieve their separation by thin-layer chromatography (TLC) with a variety of solvent systems failed[2,3], until KAPOULAS[4] and THOMPSON[5] developed novel systems which contained high proportions of acetic acid and minimal amounts of methanol and/or water. However, this solvent system did not separate glycerylphosphorylcholine (GPC) lipids and minor lipids from one another and is somewhat unpleasant to handle because of its high acetic acid content. The present communication describes a fractionation procedure which not only avoids the use of acetic acid but also separates the lipids of *Tetrahymena* into ten polar components, affording the separation between GPE lipids and G-AEP lipids. It thus provides a better resolution than the systems mentioned above[4,5].

Experimental

Tetrahymena pyriformis E used in this work was originally obtained from Dr. G. A. THOMPSON, Jr. Materials and techniques for the maintenance and culture of the organism, lipid extraction and fractionation by TLC and ascending dry-column chromatography (ADCC), acid hydrolysis of lipids and identification of water-soluble products derived by acid hydrolysis of lipid have been described previously[6]. ^{32}P-labelled lipids were obtained by growing *Tetrahymena* for 19 h in 700 ml of low-phosphate medium containing 1 % proteose-peptone (difco), 0.1 % yeast extract, 1 % glucose, 0.1 m*M* Fe-EDTA complex and 40 μCi [^{32}P]orthophosphate. The cells were processed as described previously[6]. Kodak Royal Blue (RB-5G) medical X-ray film was used for autoradiography.

The new solvent system used for the fractionation of the lipids of *Tetrahymena pyriformis* either on Silica Gel F_{254} or by ADCC was chloroform–methanol–conc. ammonia (65:35:5), GPE lipids and G-AEP lipids for reference were obtained either by preparative TLC in THOMPSON's[5] system or by ADCC[6]. A mixture of GPE and G-AEP lipids was also obtained by preparative TLC using chloroform–methanol–water (70:30:5) as the developing solvent.

Results

The behaviour of the phospholipids of *Tetrahymena pyriformis* E on TLC in acidic and alkaline solvent systems are shown in Figs. 1 and 2, respectively. 50 to 100 μg of total lipids were spotted in increasing concentrations and, after development

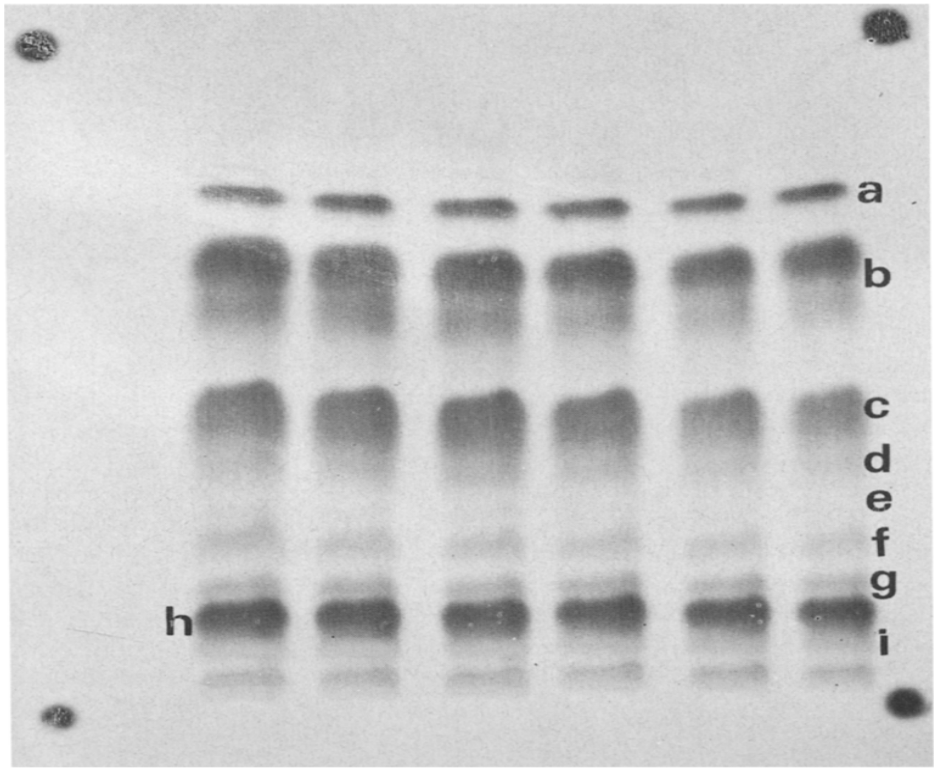


Fig. 1. TLC separation of ^{32}P-labelled total lipids of *Tetrahymena pyriformis* E. Adsorbent: Kieselgel F_{254}. Solvent: chloroform–acetic acid–methanol–water (75:25:5:1.5). Detection: autoradiography. Material spotted: 50 to 100 μg of total lipids with increments of 10 μg. The letters in the figure correspond to components listed in Table I.

TABLE I

THIN-LAYER CHARACTERIZATION OF THE PHOSPHOLIPIDS OF *Tetrahymena pyriformis* E

Component	*Fig. 1*			*Fig. 2*		
	Reagent		*Characterization*	*Reagent*		*Characterization*
	P	NH_2		*P*	NH_2	
a	+	−	Diphosphatidyl glycerol	+	−	Diphosphatidyl glycerol
b	+	+	G-AEP lipid	+	+	GPE lipid
c	+	+	GPE lipid	+	+	G-AEP lipid
d	+	+	Under investigation	+	−	GPC lipid
e	+	−	Under investigation	+	−	Under investigation
f	+	+	Ceramide-MMAEP	+	−	Under investigation
g	+	+	Ceramide-AEP	+	+	Ceramide-MMAEP[a]
h	+	−	GPC lipid	+	+	Ceramide-AEP[b] plus phospholipid 'd' of Fig. 1
i	+	−	Under investigation	+	−	Under investigation
j				+	−	Under investigation

[a] MMAEP = monomethylaminoethylphosphonate.
[b] AEP = aminoethylphosphonate.

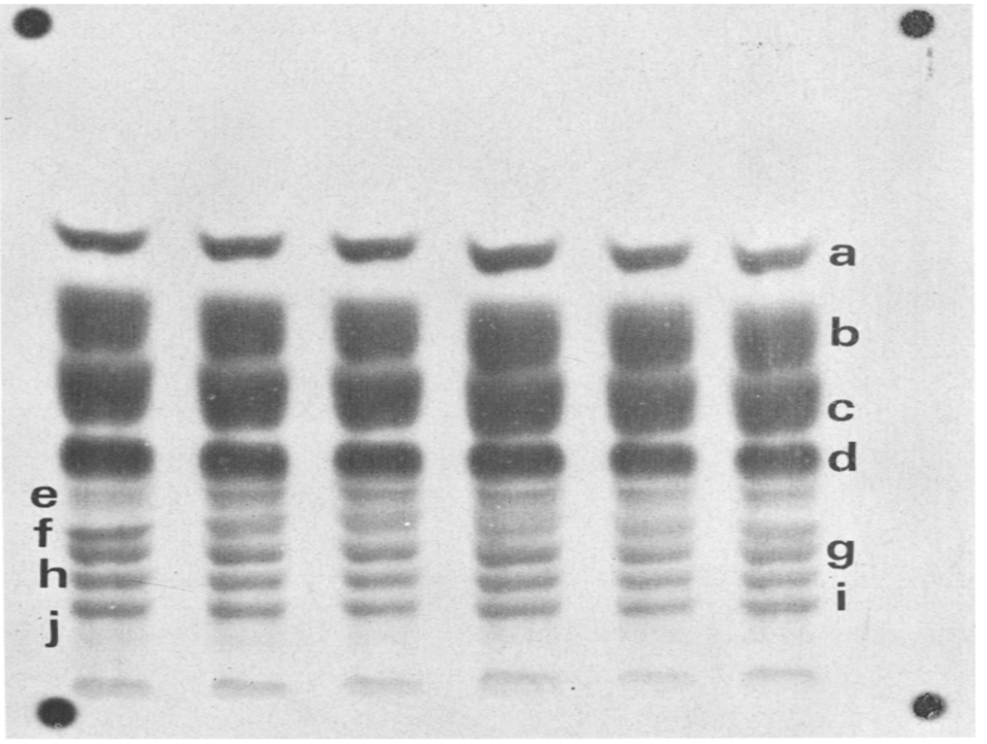
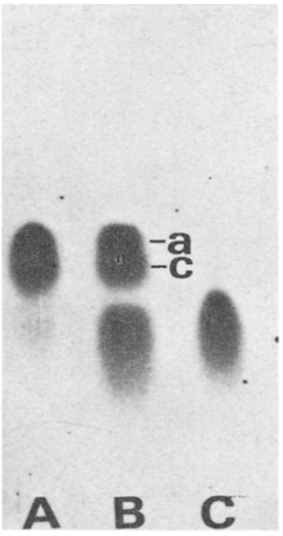


Fig. 2. TLC separation of ^{32}P-labelled total lipids of *Tetrahymena pyriformis* E. Adsorbent: Kieselgel F_{254}. Solvent: chloroform–methanol–conc. ammonia (65:35:5). Detection: autoradiography. Material spotted: 50 to 100 μg of total lipids with increments of 10 μg. The letters in the figure correspond to components listed in Table I.

in respective solvents, the plates were autoradiographed. The reaction of the various separated phopholipids on the two TLC plates, with the 'phosphorus' reagent of DITTMER AND LESTER[7] and ninhydrin reagent, as well as their chemical characterization, wherever possible, are briefly summarized in Table I.

It is interesting to observe, not only the clear resolution of the phospholipid components of *Tetrahymena* in the alkaline solvent system (Fig. 2), but also the reversed relative mobilities of G-AEP lipids and GPE lipids in the two solvent systems (Figs. 1 and 2). This is further shown in Figs. 3 and 4. In Fig. 3 the G-AEP spotted at A has a higher mobility than the GPE lipid spotted at C. A clear separation between a mixture of G-AEP lipid and GPE lipid spotted at B is seen. This system also achieves a partial separation between alkyl acyl G-AEP (a) and diacyl G-AEP (b). In Fig. 4, on the other hand, one observes a lower mobility of G-AEP lipid at A as against higher mobility of GPE lipid at C while a clear separation of a mixture of G-AEP lipid and GPE lipid is seen at B. Although a better resolution between the analogs of G-AEP lipids is achieved in the alkaline solvent system (Fig. 4) than in the acidic solvent system of THOMPSON[5] (Fig. 3), in both solvent systems the alkyl acyl G-AEP (a) had a higher mobility than diacyl G-AEP (b).



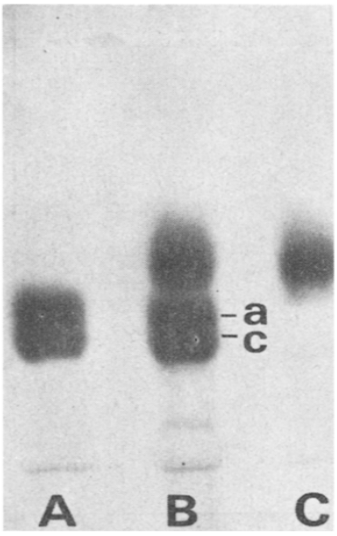


Fig. 3. TLC separation of G-AEP and GPE lipids. Adsorbent: Kieselgel F_{254}. Solvent: chloroform–acetic acid–methanol–water (75:25:5:1·5). Spray reagent: ninhydrin. Material spotted at: (A) G-AEP lipids, (C) GPE lipids, and (B) A + C.

Fig. 4. TLC separation of G-AEP and GPE lipids. Adsorbent: Kieselgel F_{254}. Solvent: chloroform–methanol–conc. ammonia (65:35:5). Spray reagent: ninhydrin. Material spotted at: (A) G-AEP lipids, (C) GPE lipids, and (B) A + C.

Discussion

Although BAER AND STANACEV[2] observed higher TLC mobilities for phosphonic acid analogs of GPC lipids compared with the corresponding GPC lipids (1.09 relative mobility factor) in the solvent system chloroform–methanol–water (65:25:4), the two components could not be separated chromatographically. However, modification of this solvent system by increasing the methanol content and replacement of water with concentrated ammonia (28 % w/v) produced a good chromatographic separation between GPE lipids and G-AEP lipids (Fig. 4). The comment of KAPOULAS[4] that these components can only be separated when the solvent system contains methanol as a minor component, does not apply to the alkaline solvent described here.

The higher chromatographic mobility of GPC lipid in the alkaline solvent system revealed six minor phosphorus-containing lipids some of which otherwise remained obscured by GPC lipid in the acidic solvent system. This resulted in our recent characterization of two minor lipids 'g' and 'h' (Fig. 2) as ceramide-monomethlayminoethylphosphonate and ceramide-aminoethylphosphonate[8].

A still better TLC resolution of the alkyl acyl and diacyl analogs of G-AEP lipids is achieved when these are applied in a mixture, with or without GPE lipids (Figs. 3 and 4), but free of the other lipids (Figs. 1 and 2). A complete separation of substantial amounts of total lipids is possible by the ADCC technique[6], but even this procedure is greatly improved by the substitution of the new solvent as it avoids the use of acetic acid and yields pure GPC lipid as well as six minor lipids. The characterization of the remaining four minor lipids is in progress.

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