Isolation and identification of 1(3),2-diacylglyceryl-(3)-O-4′-(N,N,N-trimethyl)homoserine from the soil amoeba, *Acanthamoeba castellanii*

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Abstract A polar lipid accounting for 12.5% of the total lipid nitrogen has been isolated from the protozoan *Acanthamoeba castellanii.* On the basis of thin-layer chromatography and mass spectral analysis, the lipid has been identified as diacylglyceryltrimethylhomoserine (DGTS). Fast atom bombardment (FAB) mass spectra of DGTS are reported for the first time and are compared to the FAB mass spectra of phosphatidylcholines and the electron ionization (EI) and field desorption (FD) mass spectra of DGTS. Gas–liquid chromatographic–mass spectrometric (GLC–MS) analysis of the acyl chain composition of this lipid has shown that 87.5% consists of *cis*-9-octadecenoic acid. Plasma membrane isolated from this organism has shown that labeled DGTS appears in the plasma membrane but is not enriched in this fraction. DGTS has been isolated previously only from a limited number of green plants and one species of fungus. Identification of this lipid in *Acanthamoeba* indicates that this lipid is distributed among a diverse group of lower eucaryotes.—**Furlong, S. T., J. A. Leary, C. E. Costello, and E. A. Dawidowicz.** Isolation and identification of 1(3),2-diacylglyceryl-(3)-O-4′-(N,N,N-trimethyl)homoserine from the soil amoeba, *Acanthamoeba castellanii. J. Lipid Res.* 1986. **27:** 1182–1189.

Supplementary key words protozoan • fast atom bombardment • gas–liquid chromatography–mass spectrometry

There is a great degree of diversity in the lipid composition of eucaryotic microorganisms. While much of the lipid composition of protozoa, such as *Acanthamoeba castellanii,* appears typical of eucaryotes in general, other lipids are more restricted in their occurrence. For example, phosphonolipids, tetrahymenol and a taurine-containing lipid have been reported in *Tetrahymena,* but this combination of lipids appears to be unique to this organism (1–3). In bacteria as well, a number of unusual lipids have been reported and it has been suggested that lipid composition of these procaryotes may serve as a guide for classification (4). Recent examples of unusual bacterial lipids include macrocyclic glycerol diethers and ornithine-containing lipids (5–10). The distribution of both of these appears limited to specific groups of bacteria.

While the lipids of protozoa have not been studied as extensively as those of the bacteria, it is clear that in these organisms lipid composition is also indicative of phylogenetic relationships (1). During studies of membrane biogenesis in the soil amoeba, *Acanthamoeba castellanii,* a polar lipid was isolated which had not previously been described in this organism (11). In the present report we show that this lipid is diacylglyceryltrimethylhomoserine (DGTS), an unusual non-phosphorus-containing polar lipid that has been found in a limited number of green plants and one species of fungus (12–21). The presence of this lipid in *Acanthamoeba* suggests that DGTS may be more widespread in lower eucaryotes than previously thought, and, in particular, suggests a close phylogenetic relationship between this protozoan and cryptogamic green plants. A unique feature of this lipid in the present study is the near homogeneity of the acyl chain composition.

EXPERIMENTAL PROCEDURES

Cell growth and membrane isolation

Axenic cultures of *Acanthamoeba castellanii* were grown at 28°C as described by Korn (22). Labeling of cells, plasma membrane isolations, and characterization of

Abbreviations: DGTS, diacylglyceryltrimethylhomoserine; FAB, fast atom bombardment; EI, electron ionization; FD, field desorption; GLC–MS, gas–liquid chromatography–mass spectrometry; TLC, thin-layer chromatography; FAME, fatty acid methyl ester.

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fractions were carried out as previously described (23). For continuous label experiments of membrane fractions, cells were collected by centrifugation, washed in phosphate-buffered saline (PBS, pH 7.0), resuspended to a density of 7.5×10^7 cells/ml and incubated with 5 mCi of [^{3}H]acetic acid, sodium salt (sp act 2 Ci/mmol, ICN, Irvine, CA) at 23°C with occasional shaking. After the appropriate interval, 1.0 ml of cell suspension was added to 10.0 ml of ice-cold 10 mM Tris (pH 7.4) and cells were collected by centrifugation at 500 *g*. Following resuspension in 5.0 ml of 10 mM Tris (pH 7.4), labeled cells were homogenized with a Dounce homogenizer and an aliquot was removed for lipid analysis of whole cells. Plasma membranes were isolated from the remainder.

Lipid analysis

Lipids were extracted by the procedure of Folch, Lees, and Sloane-Stanley (24) and for most analytical work, the polar lipids were separated by two-dimensional thin-layer chromatography (TLC) on silica gel H plates (Redicotes, Supelco) developed in chloroform–methanol–ammonia 65:25:5 in the first dimension and acetone–chloroform–methanol–acetic acid–water 40:30:10:10:5 in the second dimension (25). Individual polar lipids were identified by comparison with authentic standards (Avanti Polar Lipids, Inc., Birmingham, AL). In addition, for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, identification was confirmed by spraying with Draggendorff's and ninhydrin sprays (25). To quantitate radioactive polar lipids, the total labeled lipid was mixed with carrier lipid extracted from unlabeled cells. After chromatography, the lipids were visualized with iodine vapor (**Fig. 1**). Areas corresponding to the appropriate lipids were scraped from plates into scintillation vials and quantitated by liquid scintillation counting, after addition of 5.0 ml of scintillation cocktail (Ultrafluor, National Diagnostics, Somerville, NJ). Unlabeled phospholipids were separated as above and quantitated by the method of Ames (26). Nitrogen was determined by the total nitrogen microprocedure of Sloane-Stanley (27).

For isolation of larger amounts of the unidentified lipid, 0.5×10^{10} to 1.0×10^{10} cells were pelleted and resus-

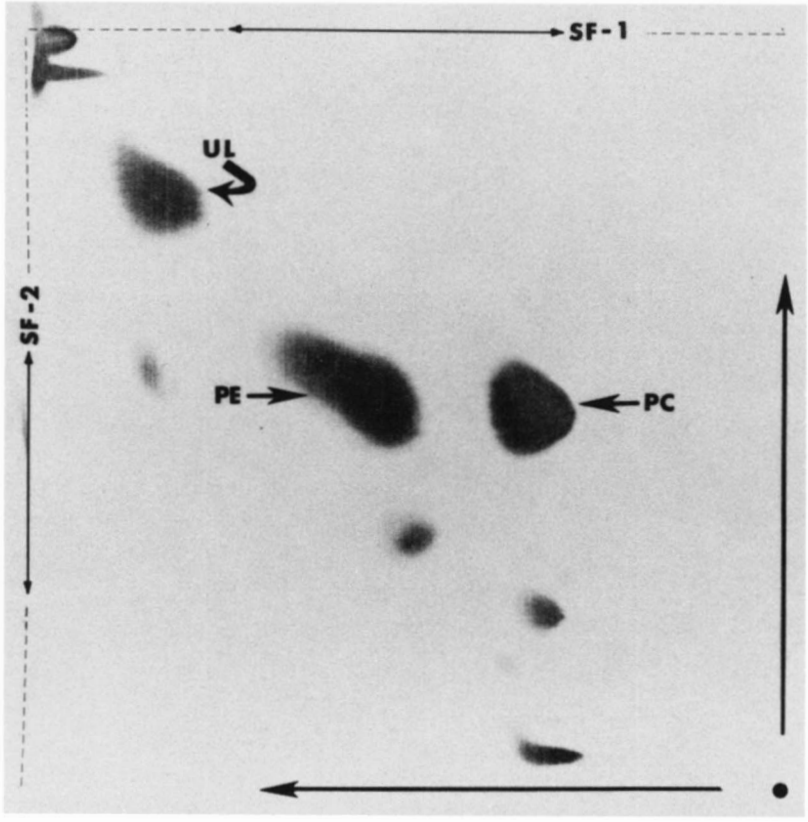


Fig. 1. Thin-layer chromatographic separation of whole cell lipid from *Acanthamoeba castellanii* spotted onto a 20 × 20 cm silica gel H plate. Lipids were separated with chloroform–methanol–ammonia 65:25:5 in the first dimension and acetone–chloroform–methanol–acetic acid–water 40:30:10:10:5 in the second dimension. Separated lipids were visualized with iodine vapor and recorded with a photocopy machine.

pended in PBS to make a 30% cell suspension. Cells were homogenized with a Dounce homogenizer and extracted for several hours with four volumes of chloroform–methanol 2:1. Following extraction, the organic phase was taken to dryness with a rotary evaporator and the dried lipid was resuspended in chloroform–methanol 90:10. The crude lipid suspension was added to a 55 × 2 cm column that had previously been packed with 50 g of Biosil 200–400 mesh and washed with chloroform. The column was eluted successively with 100 ml each of chloroform–methanol in the proportions 90:10, 80:20, 70:30, 60:40, and finally with 100% methanol. Fractions were monitored by TLC on silica gel H plates developed in chloroform–methanol–ammonia 65:25:5. The fractions containing the unidentified lipid were further purified by preparative TLC on silica gel H in chloroform–methanol–ammonia 65:25:5. While prefabricated plates were used for most of the work described, it was found for fast atom bombardment mass spectrometry (FABMS) that binder in the plates interfered with the signal. To overcome this, preparative thin-layer plates were coated with silica gel H (EM Reagents, Kieselgel 60H) that contained no binder.

Chemical analyses

Gas–liquid chromatography and gas-liquid chromatography–mass spectrometry (GLC–MS). Fatty acid methyl esters (FAMEs) were profiled using a Hewlett Packard gas chromatograph with a Silar 10-C column run isothermally at 175°C (oven temperature); the injector and flame ionization detector temperature was 195°C. For GLC–MS, solutions of samples to be analyzed were injected on-column into a Varian 3700 gas chromatograph equipped with a 15-m DB-5 fused silica capillary column with an internal diameter of 0.26 or 0.32 mm and a film thickness of 0.25 μm (J and W Scientific, Inc., Rancho Cordova, CA). The mass spectrometer used for all GLC–MS analyses was a Finnigan-MAT 212 double-focusing instrument of reversed Nier-Johnson geometry interfaced to a Finnigan-MAT SS200 data system. Instrument operating conditions were as follows: 70 eV electron energy, 4.0 A filament current, 3 kV accelerating voltage, and ion source temperature 180°C. The resolution was 1:1000 and the range from m/z 50 to 600 scanned in 2.2 sec with an interscan period of 0.8 sec.

Fast atom bombardment mass spectrometry. For FABMS, methanolic solutions of the samples to be analyzed were mixed 1:1 with the glycerol matrix and then 0.5 μl of mixture was applied to a 3-mm diameter stainless-steel probe tip. Measurements were carried out using a Varian MAT 731 mass spectrometer of Mattauch-Herzog geometry operated at 8 kV accelerating voltage. The xenon atom beam was produced by an Ion-Tech B12N atom gun with 7 kV acceleration energy. Low resolution mass spectra were recorded at 1:1000 resolution during magnetic scans. Accurate mass measurements were made by peak-matching against standards or against glycerol cluster ions at 1:10,000 resolution.

Determination of acyl chain composition of unidentified lipid

To determine acyl chain composition, lipid was subjected to mild alkaline hydrolysis. Methyl esters were generated by subsequent treatment of the free fatty acids with methanolic HCl and were extracted into hexane (28). The FAMEs were analyzed by GLC and GLC–MS. For the GLC–MS analysis, the column was held isothermal at 45°C for 45 sec and then programmed at 4°C/min to 280°C and held for 2 min. In order to determine the double bond position, the method of Kidwell and Biemann (29) was used, with slightly modified reaction conditions. The FAMEs were dissolved in hexane and the solution was placed in a 5 × 150 mm Pyrex tube (without sprinkling the wall). To this solution was added 5,5-dimethoxy-1,2,3,4-tetrachlorocyclocentadiene (630 μg) (Aldrich) in hexane. The tube was sealed and heated for 19 hr at 200°C. After it cooled, the tube was opened and 1 μl of the solution was used for GLC–MS analysis. The gas chromatograph oven was programmed from 70–300°C at 10°C/min and held at 300°C for 5 min. A sample of methyl *cis*-9-octadecenoate (Fluka) was analyzed for comparison with the isolated material.

RESULTS

Polar lipid composition of *Acanthamoeba*

The determination of polar lipid composition of *A. castellanii* in this work gave a result similar to that described previously (30). Phosphatidylethanolamine and phosphatidylcholine are the predominant phospholipids in whole cell extracts, making up 38.6% and 30.0%, respectively, of the total lipid. In addition to the phospholipids previously described, we have detected another polar lipid that appears in significant quantity in whole cell extracts. This lipid elutes from silicic acid columns in the 70:30 chloroform–methanol fraction and, in two-dimensional TLC (Fig. 1), has an R_f of 0.79 in the first solvent and an R_f of 0.82 in the second dimension. It was determined that this lipid did not stain with ninhydrin reagent for primary amines or anthrone reagent for glycolipids (31). It did, however, stain with Draggendorff's reagent for detecting quaternary amines. Lipid phosphorus determination showed that this lipid has no phosphorus, while lipid nitrogen determination showed that this lipid makes up 12.5% of the total cellular lipid nitrogen. Phosphatidyl-

ethanolamine and phosphatidylcholine were shown to contain 21.8% and 17.1%, respectively, of lipid nitrogen.

Acyl chain composition

Gas–liquid chromatography of the saponified unknown lipid showed a single major peak with a retention time identical to that of a C18:1 lipid standard. GLC–MS confirmed that the acyl chain composition was 87.5% C18:1. Two other minor peaks were also detected and identified as C16:0 (7.0%) and C18:0 (5.5%). A sample of egg phosphatidylcholine treated identically showed the major characteristic peaks of this lipid corresponding to C16:0, C18:0, and C18:1.

The 2,3,4,5-cyclopentadienyl Diels-Alder adducts of the fatty acid methyl esters from the lipid were prepared. The GLC retention time and mass spectrum of the major component were identical to those of the derivative obtained from methyl *cis*-9-octadecenoate. The mass spectrum of this derivative has been shown to be characteristic for both the position and the orientation of the double bond (29).

FAB mass spectral analysis of intact lipid and hydrolysis products

The FAB mass spectrum of the intact unidentified lipid showed a parent ion (MH^+) at m/z 764 (**Fig. 2**). High resolution FABMS peak-matching of the m/z 764 ion against the MH^+ of egg phosphatidylcholine (m/z 760) determined the value m/z 764.6404 corresponding to the elemental composition $C_{46}H_{86}NO_7$ (diff. = 0.0 mmu). Assignments of the major fragment ions of the unidentified lipid are indicated in Scheme 2 (see Discussion).

For comparison, the FAB spectrum of a sample of purified egg phosphatidylcholine was measured under the same conditions used for the unknown. The molecular protonated ion of the major component, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine 2 (Scheme 1, see Discussion) was observed at m/z 760. This represented 58% of the total molecular ion abundances. The other MH^+ and their contributions to the total were: m/z 758 (19%), m/z 786 (9%), and m/z 788 (14%). Fragment ions and their relative abundances were as follows: m/z 496 (15%), m/z 480 (19%), m/z 224 (7%), m/z 184 (100%), m/z 166 (11%), m/z 104 (16%), m/z 86 (86%), and m/z 72 (60%). Assignments of the fragment ions are indicated in Scheme 1 (see Discussion).

The FAB mass spectrum of the hydrolysis products of the unknown had the base peak m/z 236. High resolution FAB measurement determined the value m/z 236.1499 corresponding to the elemental composition $C_{10}H_{22}NO_5$ (diff. = 0.1 mmu). The structure 3 (see Discussion) was assigned to this compound.

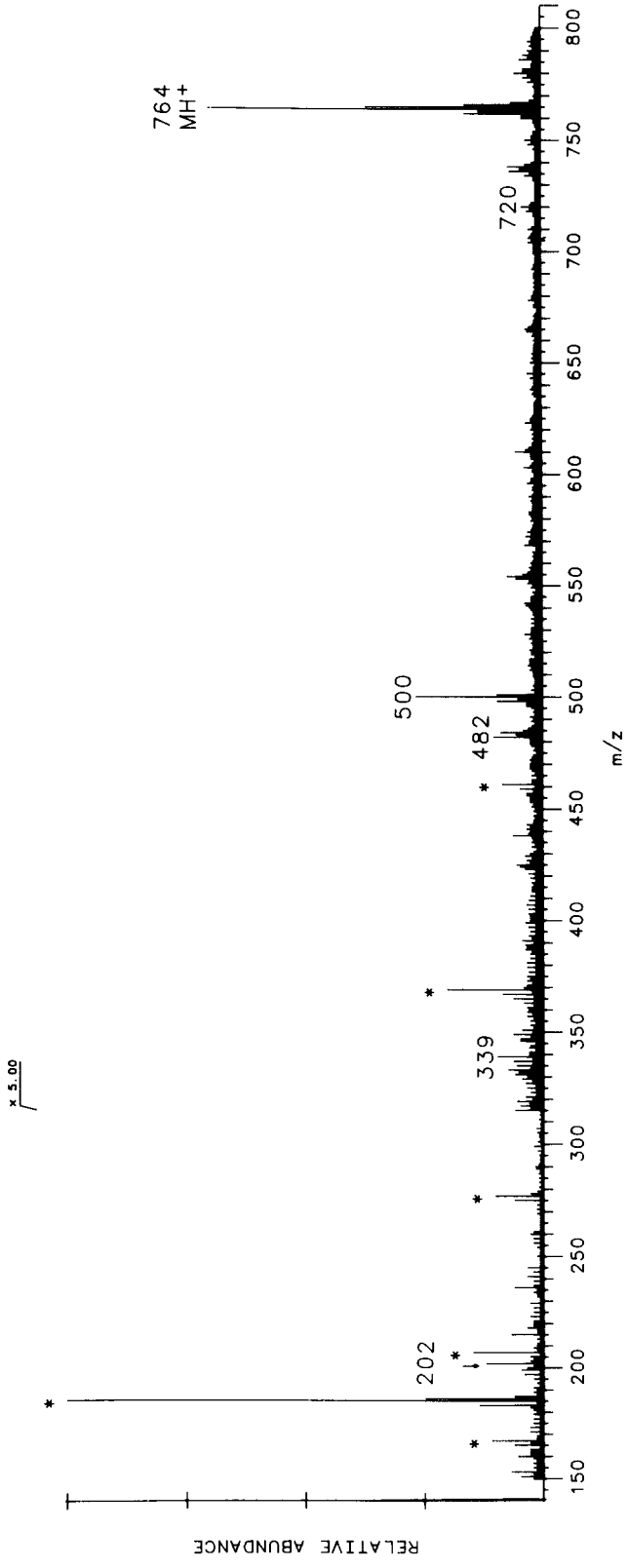


Fig. 2. Positive ion fast atom bombardment mass spectrum of *Acanthamoeba* lipid dissolved in methanol–glycerol 1:1. See Scheme 2 for peak assignments.

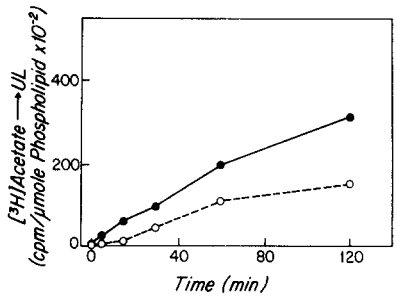


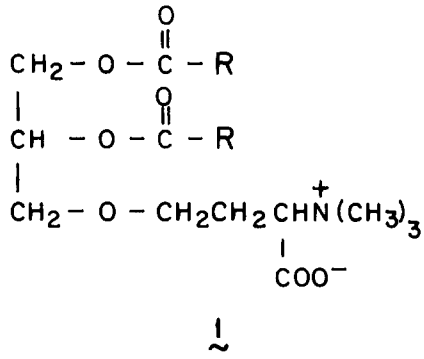
Fig. 3. Labeling of unidentified lipid. Cells were labeled with [^{3}H]acetate and plasma membranes were isolated as described in Experimental Procedures. Labeled lipid plus carrier lipid were separated by two-dimensional thin-layer chromatography and spots corresponding to the unidentified lipid were scraped and counted. Closed circles indicate whole cell; open circles indicate plasma membrane; UL, unidentified lipid.

Appearance of labeled lipids in whole cell and plasma membrane

In order to determine whether the unidentified lipid represents a membrane lipid in this organism and to estimate the relative proportion at the cell surface, cells were labeled with [^{3}H]acetate and plasma membrane fractions were isolated and lipid was extracted. After separation of the total polar lipids by two-dimensional TLC, a significant amount of tritium was incorporated into the unidentified lipid (**Fig. 3**). Labeled acetate was incorporated into this lipid in whole cell and plasma membrane at rates of 9.0×10^{-12} and 4.4×10^{-12} mol/μmol lipid phosphorus per hr, respectively (Fig. 3).

DISCUSSION

The thin-layer chromatography, staining behavior with spray reagents, lipid phosphorus and lipid nitrogen determinations, and mass spectral data have shown that the unknown lipid from *Acanthamoeba* is diacylglyceryltrimethylhomoserine 1. As would be expected from the structure of this lipid and from previous work (13, 16, 20), the DGTS from *Acanthamoeba* stains positively with Dragendorff's reagent, but did not stain with ninhydrin. There was no detectable lipid phosphorus from isolated DGTS but *Acanthamoeba* DGTS was found to account for 12.5% of the total cellular lipid nitrogen. There is only one previous report in which the membrane distribution of this lipid had been studied. In *Chlamydomonas*, 40% of the total DGTS was found in the photosynthetic lamellae of the thylakoid membrane. We have shown that after labeling cells with [^{3}H]acetate for 2 hr, the distribution of labeled phosphatidylethanolamine and phosphatidylcholine between plasma membranes and whole cells reflects the distribution of the unlabeled molecules (11). After a similar incubation with precursor, the distribution of labeled unidentified lipid is intermediate between that of phosphatidylethanolamine and phosphatidylcholine. On the basis of this labeling it may be estimated that DGTS makes up approximately 5–6% of the polar lipid fraction in the plasma membrane.



GLC–MS analysis of the FAMEs obtained by saponification of DGTS from *Acanthamoeba* and esterification of the released fatty acids showed the composition to be C16:0 (7.0%), C18:1 (87.5%), C18:0 (5.5%). Only trace amounts of other FAMEs were found. The GLC retention time and mass spectrum of the 2,3,4,5-tetrachloropentadienyl Diels-Alder adduct of the methyl ester of C18:1 were identical to those of the derivative of methyl *cis*-9-octadecenoate. The acyl chain composition of DGTS isolated from *Acanthamoeba* is apparently more homogeneous than DGTS from any other reported source. DGTS isolated from all other sources thus far examined exhibits considerable molecular heterogeneity (12, 13, 15, 16, 17, 20).

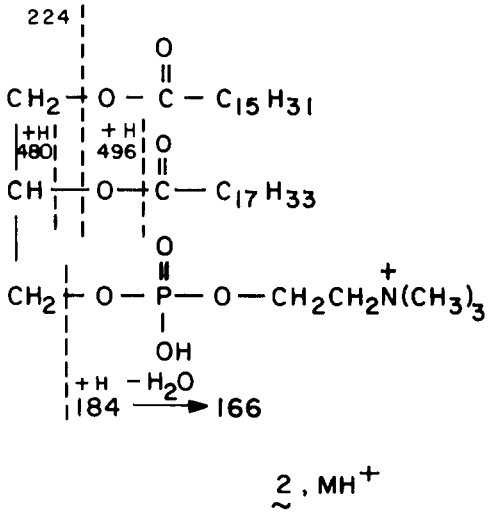
Because FAB mass spectrometry shows great sensitivity for the analysis of polar compounds, and often provides both molecular weight related ions and fragment ions indicative of structural detail, it was the method of choice for the analysis of the intact unknown lipid. The FAB mass spectrum shown in Fig. 2 and the elemental composition assigned on the basis of the exact mass measurement of the molecular protonated ion led us to consider the DGTS structure for the unknown lipid. Since FAB mass spectra of DGTS lipids have not previously been reported, the FAB mass spectra of related compounds and DGTS mass spectra determined using other ionization methods were compared with that of the unknown in order to support the assignments. For this purpose, the FAB mass spectrum of egg phosphatidylcholine (egg PC) was determined under conditions identical to those used for the *Acanthamoeba* lipid. The most abundant component of egg PC is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-

choline 2. Assignments of fragments observed above m/z 150 in this spectrum are indicated in **Scheme 1.** Abundances of these and of lower mass fragments are listed in the Results Section.

The secondary ion mass spectrum (SIMS) and FAB mass spectrum of dipalmitoylphosphatidylcholine have been reported by Aberth, Straub, and Burlingame (32). Ayanoglu et al. (33) have studied the ammonia desorption chemical ionization and FAB mass spectra of a series of phospholipids. Scheme 1 is consistent with the results of both these groups.

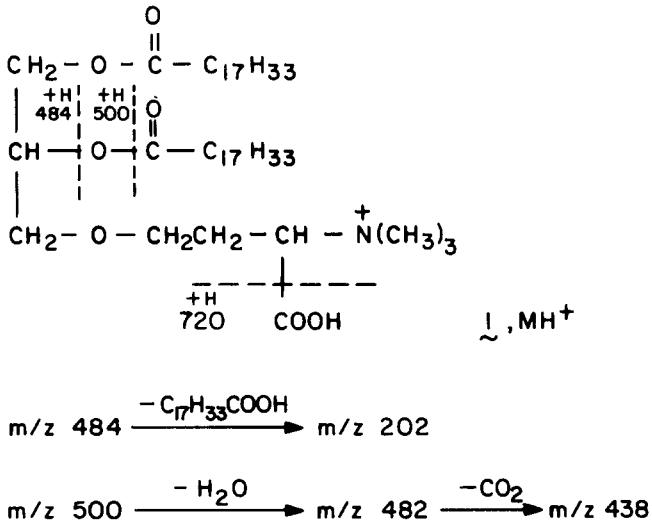
FABMS established the molecular weight of the unknown lipid as 763 and indicated the elemental composition $C_{46}H_{86}NO_7$ for the molecular protonated ion. The low resolution positive-ion FAB mass spectrum of the unknown is shown in Fig. 2. Ions that are characteristic for compounds containing the choline moiety (32, 33) were not observed in this spectrum. However, the spectrum could be interpreted by assignments of the major fragments as shown in **Scheme 2,** a result that provided evidence that the structure was 1 (acyl = *cis*-9-octadecenoyl, oleoyl).

Previous investigators have reported electron ionization (EI) (14) and field desorption (FD) mass spectral data for members of the DGTS series of compounds. Eichenberger (14) has reported the EI mass spectra of several hydrogenated DGTS. The spectra include molecular ions of low abundance and show extensive fragmentation. In each of these EI mass spectra, the dominant high mass ion corresponds to $[M - N(CH_3)_3]^+$. The molecular protonated ion was the base peak of our FAB mass spectrum (Fig. 2), and loss of trimethylamine was not observed. Evans, Kates,



2, MH^+

Scheme 1. FABMS fragmentation of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine 2, MH^+ = m/z 760.



1, MH^+

m/z 484 $\xrightarrow{-C_{17}H_{33}COOH}$ m/z 202

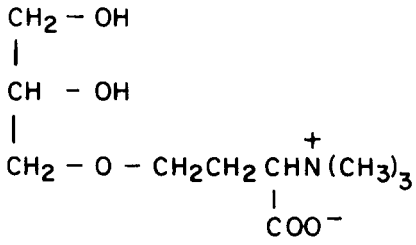
m/z 500 $\xrightarrow{-H_2O}$ m/z 482 $\xrightarrow{-CO_2}$ m/z 438

Scheme 2. Assignments of fragment ions observed in the FAB mass spectrum of the *Acanthamoeba* lipid 1 (acyl = oleoyl), MH^+ = m/z 764 (Fig. 2).

and Wood (16) have discussed FD mass spectra of various DGTS analogs. In FABMS as in FDMS, the distribution of the different acylated species can be determined from the relative abundances of the molecular protonated ion and other cluster ions. Like the FD mass spectrum reported for dipalmitoyl-DGTS (16), the FAB mass spectrum of the *Acanthamoeba* lipid shows as its most abundant fragment the ion that corresponds to loss of one acyl group, with transfer of a hydrogen to the glycerol oxygen. (In the FD mass spectrum of a complex DGTS mixture (16), both the protonated (7.5%) and sodium cationized (32.5%) fragment were observed.) Cleavage on the other side of the oxygen was not observed in the FD spectra of DGTS, although it was observed in the FAB spectra of the unknown lipid and of egg phosphatidylcholine, and has been reported by others (32, 33) in the FAB mass spectra of phosphatidylcholines. The FD mass spectra of a series of DGTS (16) had abundant (20–50%) fragment ions due to loss of CO_2 from MH^+ and less abundant (2–16%) fragments due to the combined loss of CO_2 and $N(CH_3)_3$. Loss of CO_2 was observed as a peak of low abundance at m/z 720 in the FAB mass spectrum of the unknown. No peak corresponding to the loss of $N(CH_3)_3$ or the combined loss of this group and CO_2 was observed by FAB. The FD mass spectra of DGTS included an extensive set of low abundance fragments that could be assigned to the homoserine moiety (16), but no corresponding set of fragments was observed in the FAB mass spectrum of the lipid, even though there was little interference from matrix ions at the masses where these fragment ions would occur. Evans et al. (16) reported abundant adduct ions in the DGTS FD mass spectra, and assigned these to

$[M + CH_3 - H]^+$ and $(M + Na)^+$. These ions were not observed in the FAB mass spectrum.

The FAB mass spectrum of the hydrolysis products was dominated by an ion at m/z 236, whose elemental composition was determined to be $C_{10}H_{21}NO_5$, appropriate for the deacylated species 3.



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The combination of measurements made by GLC–MS and by FAB mass spectrometry thus show that lipid isolated from *Acanthamoeba* is quite homogeneous and that oleoyl is the dominant acyl substituent. The FAB mass spectrum differs markedly from the FAB mass spectra of phosphatidylcholines, both as determined in this work, and as reported by others. There is some similarity in fragmentation between the FAB mass spectrum of the *Acanthamoeba* lipid and the FD mass spectra reported for DGTS. The molecular ion and fragment ions observed in the FAB mass spectrum of the intact lipid provide evidence for the structure 1 (acyl = oleoyl). Hydrolysis of the acyl groups leads to a species whose composition is that expected for glyceryl-trimethylhomoserine. On the basis of this data, we therefore have assigned the DGTS structure 1 (acyl = oleoyl) to the *Acanthamoeba* lipid.

No clear pattern has yet emerged with respect to functional distribution of DGTS within cells containing this lipid nor does the occurrence of this lipid appear to be strictly correlated with phylogeny (14). The relatively high concentration in which DGTS is found in *Acanthamoeba* and other species (12–15, 20), however, suggests an important role in basic cellular function. It has been suggested that an important function of DGTS is to contribute charge to the photosynthetic membrane in organisms such as *Chlamydomonas reinhardtii* (20). The relative amount of DGTS in the thylakoid membrane, however, is only slightly higher than in the cell as a whole (20), so it is unlikely that this is the sole function. Also, membranes with photosynthetic activity would not be expected in organisms such as *Acanthamoeba* or *Epidermophyton* (13). As shown in Fig. 3, DGTS is found in the plasma membrane of *Acanthamoeba* but it is not enriched in this fraction.

It now seems clear that DGTS is widely distributed among cryptogamic green plants (15). It is less clear what the distribution of this lipid is among the lower eucaryotes. Among the fungi, DGTS has been reported in the dermatophyte *Epidermophyton floccosum* but was not found in the dermatophytes *Microsporum cookei* or *Trichophyton rubrum* (13). DGTS has been reported in Phytomastigophoreans such as *Chlamydomonas, Ochromonas,* and *Volvox,* but there have been no reports of this lipid in Zoomastigophoreans. We have examined the lipid composition of one such protozoan, the human pathogen *Leishmania mexicanum* but found no evidence for the existence of DGTS in this organism. As it has been suggested that the Mastigophora are ancestral to the Sarcodina such as *Acanthamoeba* (34), it is tempting to speculate that the presence of DGTS is a reflection of this relationship. As there is a great degree of diversity among the lipids of protozoa (1), the presence of DGTS may serve as another criterion through which to clarify the taxonomy of these organisms.

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REFERENCES

1. Thompson, G. A., and Y. Nozawa. 1972. Lipids of protozoa: phospholipids and neutral lipids. *Annu. Rev. Microbiol.* **26:** 249–278.
2. Rosenberg, H. 1973. Phosphonolipids. *BBA Libr.* **3:** 333–334.
3. Kaya, K., K. Uchida, and T. Kusumi. 1985. Identification of taurine-containing lipid in *Tetrahymena pyriformis NT-1. Biochim. Biophys. Acta.* **835:** 77–82.
4. Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. *Adv. Appl. Microbiol.* **17:** 63–108.
5. Comita, P. B., and R. B. Gagosian. 1983. Membrane lipid from deep-sea hydrothermal vent methanogen: a new macrocyclic glycerol diether. *Science.* **122:** 1329–1331.
6. Comita, P. B., R. B. Gagosian, H. Pang, and C. E. Costello. 1984. Structural elucidation of a unique macrocyclic membrane lipid from a new, extremely thermophilic, deep-sea hydrothermal vent archebacterium, *Methanococcus jannaschii. J. Biol. Chem.* **259:** 15234–15241.
7. Thiele, O. W., J. Oulevey, and D. H. Hunneman. 1984. Ornithine-containing lipids in *Thiobacillus A2* and *Achromobacter sp. Eur. J. Biochem.* **139:** 131–135.
8. Kawai, Y., and I. Yano. 1983. Ornithine-containing lipid of *Bordetella pertussis,* a new type of hemagglutinin. *Eur. J. Biochem.* **136:** 531–538.

9. Thiele, O. W., C. J. Biswas, and D. H. Hunneman. 1980. Isolation and characterization of an ornithine-containing lipid from *Paracoccus denitrificans*. *Eur. J. Biochem.* **105:** 267–274.
10. Batrakov, S. G. 1982. Ornithine lipids: a new class of microbial lipids. *Izv. Akad. Nauk. SSSR (Biol.)* **(4):** 591–604.
11. Furlong, S. T., J. T. Mills, and E. A. Dawidowicz. 1984. Intracellular translocation and sorting of newly synthesized polar lipid. *J. Cell Biol.* **99:** 97 (Abstract).
12. Brown, A. E., and J. Elovson. 1974. Isolation and identification of a novel lipid, 1(3),2-diacylglyceryl-(3)-O-4′(N,N,N-trimethyl)homoserine, from *Ochromonas danica*. *Biochemistry.* **13:** 3476–3482.
13. Yamada, T., and Y. Nozawa. 1979. An unusual lipid in the human pathogenic fungus *Epidermophyton floccosum*. *Biochim. Biophys. Acta.* **574:** 433–439.
14. Eichenberger, W. 1982. Distribution of diacylglyceryl-O-4′-N,N,N-trimethylhomoserine in different algae. *Plant Sci. Lett.* **24:** 91–95.
15. Sato, N., and M. Furuya. 1985. Distribution of diacylglyceryltrimethylhomoserine and phosphatidylcholine in non-vascular green plants. *Plant Sci.* **38:** 81–85.
16. Evans, R. W., M. Kates, and G. W. Wood. 1982. Identification of diacylglycerol-O-(N,N,N-trimethyl)-homoserine in the halotolerant alga, *Dunaliella parva*. *Chem. Phys. Lipids.* **31:** 331–338.
17. Eichenberger, W., and A. Boschetti. 1978. Occurrence of 1(3),2-diacylglyceryl-(3)-O-4′-(N,N,N-trimethyl)-homoserine in *Chlamydomonas reinhardtii*. *FEBS Lett.* **88:** 201–204.
18. Sato, N., and M. Furuya. 1984. Distribution of diacylglyceryltrimethylhomoserine in selected species of vascular plants. *Phytochemistry.* **23:** 1625–1627.
19. Sato, N., and M. Furuya. 1983. Isolation and identification of diacylglycerol-O-4′-(N,N,N-trimethyl)-homoserine from the fern *Adiantum capillus-verneris* L. *Plant Cell Physiol.* **24:** 1113.
20. Janero, D. R., and R. Barrnett. 1982. Isolation and characterization of an ether-linked homoserine lipid from the thylakoid membrane of *Chlamydomonas reinhardtii* 137^{+}. *J. Lipid Res.* **23:** 307–316.
21. Moseley, K. R., and J. A. Thompson. 1980. Lipid composition and metabolism of *Volvox carteri f. nageriensis*. *Plant Physiol.* **65:** 260–265.
22. Korn, E. D. 1974. The isolation of the amoeba plasma membrane and the use of latex beads for the isolation of phagocytic vacuole (phagosome) membranes from amoebae including the culture techniques for amoebae. *Methods Enzymol.* **31:** 686–698.
23. Mills, J. T., S. T. Furlong, and E. A. Dawidowicz. 1984. Plasma membrane biogenesis in eucaryotic cells: translocation of newly synthesized lipid. *Proc. Natl. Acad. Sci. USA.* **81:** 1385–1388.
24. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissue. *J. Biol. Chem.* **226:** 497–509.
25. Kates, M. 1972. Techniques of lipidology. *In* Laboratory Techniques in Biochemistry and Molecular Biology. T. S. Work and E. Work, editors. North Holland Publishing Co., Amsterdam. 269–610.
26. Ames, B. N. 1966. Assay of inorganic phosphate total phosphate and phosphatases. *Methods Enzymol.* **8:** 115–118.
27. Sloane-Stanley, G. H. 1967. A simple procedure for the estimation of very small amounts of nitrogen in lipids. *Biochem. J.* **104:** 293–295.
28. Hennessey, T. M., D. Andrews, and D. L. Nelson. 1983. Biochemical studies of the excitable membrane of *Paramecium tetraurelia*. VII. Sterols and other neutral lipids of cells and cilia. *J. Lipid Res.* **24:** 575–587.
29. Kidwell, D. A., and K. Biemann. 1982. Determination of double bond position and geometry of olefins by mass spectrometry of their Diels-Alder adducts. *Anal. Chem.* **54:** 2462–2465.
30. Ulsamer, A. G., P. L. Wright, M. G. Wetzel, and E. D. Korn. 1971. Plasma and phagosome membranes of *Acanthamoeba castellanii*. *J. Cell Biol.* **51:** 193–215.
31. Heinz, E. 1968. Über die enzymatische Bildung von Acylgalaktosyldiglycerid. *Biochim. Biophys. Acta.* **144:** 333–343.
32. Aberth, W., K. M. Straub, and A. L. Burlingame. 1982. Secondary ion mass spectrometry with cesium ion primary beam and liquid target matrix for analysis of bioorganic compounds. *Anal. Chem.* **54:** 2029–2034.
33. Ayanoglu, E., A. Wegmann, O. Pilet, G. D. Marbury, J. R. Hass, and C. Djerassi. 1984. Mass spectrometry of phospholipids. Some applications of desorption chemical ionization and fast atom bombardment. *J. Am. Chem. Soc.* **106:** 5246–5251.
34. R. D. Barnes. 1974. Invertebrate Zoology. W. B. Saunders Co., Philadelphia. 24.