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Membrane Lipids of a Marine Ciliate Protozoan *Uronema marinum*

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Lipid composition and fatty acid composition were characterized in the membrane of a marine ciliate protozoan (*Uronema marinum*). Phospholipids accounted for 70% of total lipid, and the remainder was neutral lipids. Total phospholipids were separated as phosphatidylcholine (24.26%), phosphatidylethanolamine (22.21%), phosphatidylinositol (6.14%), phosphatidylserine (5.11%), cardiolipin (3.07%) and unidentified phospholipids (28.72%) through high performance liquid chromatography (HPLC). Fatty acid composition of neutral lipids and phospholipids was determined by gas chromatography (GC), based solely on comparision of retention times. In neutral lipids, the most abundant fatty acid group was monounsaturated fatty acid (48.3% of total fatty acids) with oleic acid (18:1) and nervonic acid (24:1). Saturated fatty acids comprised 29.6% of total fatty acids, with palmitic acid (16:0), stearic acid (18:0) and myristic acid (14:0), and polyunsaturated fatty acid accounted for 33.0% with Di-homo-γ-linolenic acid (20:3) and linoleic acid (18:2). Wherease phospholipids predominantly contained the fatty acid group in the following order: polyunsaturated fatty acids (52.7% of total fatty acids) with linoleic acid (18:2) and γ-linolenic acid (18:3) > monounsaturated fatty acids (28.5% of total fatty acids) with oleic acid (18:1) and palmitoleic acid (16:1) > saturated fatty acids (25.5% of total fatty acids) with palmitic acid (16:0), stearic acid (18:0) and myristic acid (14:0).

Key words: Scuticociliate, *Uronema marinum*, Membrane phospholipid, Fatty acid

Introduction

Parasitic protozoa are surrounded by membrane structures that have a different lipid and protein composition relative to membranes of the host. The parasite membranes are essential structurally and also for parasite specific process, like host cell invasion, nutrient acquisition or protection against the host immune system. Furthermore, intracellular parasites can modulate membranes of their host, and trafficking of membrane components occurs between host membranes and those of the intracellular parasite. Phospholipids are major membrane components, and although many parasites scavenge these phospholipids from their host, most parasites also synthesise phospholipids de novo, or modify a large part of scavenged phospholipids (Bordmann et al., 1998). Previous studies on the lipid composition and metabolism of parasite suggest that lipids can be a drug or vaccine targets (Zidovetzki and Sherman, 1991; Florin-Christensen et al., 2000), and parasite's phosphatidylcholine biosynthesis may be a good target for a different chemotherapeutic approach to control the parasites (Bordmann et al., 1998). Therefore, it seems likely that parasite phospholipids may be important elements for parasite infection and parasite-host interaction.

Scuticociliate are facultative histophagus parasites capable of infecting a wide range of host cell. Three species are currently recognized: *Uronema*, *Miamiensis*, and *Philasterides*. The outbreak of scuticociliatosis by *U. marinum* have resulted in severe losses of cultured olive flounder (*Paralichthys olivaceus*) in Korea (Jee et al., 2001). These ciliates are characterized by their high potential for systemic infection and fish tissue destruction, leading to high mortalities in cultured fish. Despite their clinical

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importance, little information is available on the control or treatment of scuticociliate infection. Although there were some reports on the control or treatment of the scuticociliate infection by several chemotherapeutants (Yoshinaga and Nakazoe, 1993; Novotny et al., 1996; Cribb et al., 1999; Kwon et al., 2002). To date no effective chemotherapeutants exist for the control and treatment of scuticociliate infection. This may be due to no information concerning the membrane lipids of scuticociliate and their interaction with their eukaryotic host.

In this papers, we report the phospholipid and the fatty acid composition in membrane of scuticocilate, *U. marinum*.

Materials and Methods

Isolation and culture of *Uronema marinum*

U. marinum was isolated from the brain of an infected flounder (*P. olivaceus*) and were washed with hanks balanced salt solution (HBSS, Sigma) three times by centrifugation (1,000×g, 5 min). The parasite from the brain tissues of the infected fish was aseptically inoculated to CHSE-214 cells cultured in minimum essential medium (MEM, Sigma) containing 10% fetal bovine serum and penicillin (100 units/mL, Sigma) and streptomycin (100 units/mL, Sigma) at 18℃.

Parasites harvested from cultures were washed three times in HBSS by centrifugation (1000×g, 4℃, 5 min) and washed parasites were either used fresh or stored at -80℃ (less than 6 months) prior to analysis.

Extraction of phospholipids and neutral lipids

We used a modification of the method of Horwitz and Perlman (1987) and Soderberg et al. (1992). Parasites (10^9/mL) were homogenized in 0.15 M KCl, and lipids were extracted in chloroform/methanol/water (CMW, 1/1/0.3, v/v/v) at 40℃ for 60 min with magnetic stirring. Phase separation was achieved by the addition of chloroform, followed by centrifugation at 1000×g, 10 min). The upper phase was discarded and the lower phase was washed once with CMW (3/48/47, v/v/v) before evaporation under nitrogen. The lipid residue was dissolved in 2 mL of chloroform and applied to a silica SepPak cartridge (Zorbax, Co, U.S.A) preequilibrated in chloroform. Neutral lipids were eluted through with 12 mL of chloroform and phospholipids were eluted with 20 mL of methanol. Each fractions (neutral lipids and phospholipids) was evaporated under nitrogen, redissolved and then analyzed through high performance liquid chromatography (HPLC) and gas chromatography (GC) system. All organic solvents were supplemented with 5 μM butylated hydroxytoluene (Sigma) in order to prevent lipid peroxidation.

Analysis of phospholipid class

Phospholipid analysis were prepared as described by Guan et al. (2001). The separation was performed on a Zorbax Rx-SIL (25 cm×4.6 mm i.d., 5 μm particle size) column (Zorbax, Co, U.S.A) with a Zorbax Rx-SIL guard column (12.5×4.6 mm i.d., 5 μm particle size). The column was maintained at 28℃ with a column oven and the absorption of the eluate was monitored at 205 nm. The HPLC system consisted of HP1100 series and Chemstation software (Hewlett Packard Co.). Solvent A was hexane/isopropanol (3/2, v/v). Solvent B was prepared by weighting out 55.4 g of a 5 mM ammonium sulfate solution in water (pH 6.0) and mixing it with solvent A to give a total volume of 1 L. The solvents were degassed by sonication. The flow rate was 1.5 mL/min and an sample (20 μL, 0.5 μmol of lipid phosphorus) was injected on the column in order to achieve good separation. The elution started with a linear gradient from 50 to 78% B over a period of 23 min. The latter percentage of solvent B was the maintained for 4 min after which solvent B was increased to 100% over the following 9 min and maintained at this level for an additional 3 min. Finally, the washing solvent was changed linearly to 50% B over a period of 11 min. The total analysis time was 50 min. Standard peaks were collected by HPLC phospholipid standard mixture (Sigma).

Analysis of fatty acids

The fatty acids were converted to fatty acid methyl esters (FAME) with 1 mL of 14% boron trifluoride (Sigma) in methanol at 90℃ for 30 min. After the reaction, the tubes were cooled on ice to 0℃ and 4 mL of ice-cold pentane and 1 mL of 5 M NaOH were added. The mixture was shaken by vortex and the pentane phase was collected. The sample were analyzed by a ATD400 GC (Perkin Elmer, U.S.A) with a flame ionization detector. Nitrogen was used as carrier gas and the column was of the fused silica capillary type, with DB-5 as the stationary phase (30 m×0.25 mm i.d., df = 0.18 μm). The temperature was programmed from 130 to 190℃ and the total

time per run was 45 min. Quantitation was achieved by integrating the peaks on a Perkin Elmer Software. Pure standards of fatty acids (Sigma) were used for identification, based on comparison of retention times.

Determination of total lipid phosphous

Lipid phosphous were prepared as described by Valtersson and Dallner (1982). The sample were evaporated under nitrogen, dissolved in 0.3 mL of 70% perchloric acid and heated at 180℃ for 60 min. After cooling, each sample was supplemented with 400 μL of 5% ascorbic acid, and water to give a final volume of 1.8 mL. This mixture was heated in boiling water for 5 min and the absorbance at 797 nm was measured after 30 min.

Results and Discussion

In this study, we present a characterization of the lipid composition and fatty acid composition of *U. marinum*. The purified extractable lipids obtained from *U. marinum* were fractionated into phospholipid fraction and neutral lipid fraction. Phospholipids accounted for 70% of total lipid and the remainder was neutral lipids. The total phospholipids were separated as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipin (CL) and unidentified phospholipids by HPLC (Fig. 1). The relative amounts of the individual phospholipids from *U. marinum* was a 24.26% of PC, 22.21% of PE, 6.14% of PI, 5.11% of PS, 3.07% of CL and 28.72% of unidentified phospholipids (Table 1). PC, PE and unidentified phospholipids were the major phospholipids while PI, PS and CL were the minor phospholipids. The composition and relative amounts of the individual phospholipids obtained from *U. marinum* were with the exception of the presence of CL and the relative high level of PC almost similar to those of the other groups of ciliated protozoa, *Parauronema acutum* which is a marine ciliated protozoa, and several species of the genus *Paramecium* and *Tetrahymena*. PC and PE were the major phospholipids, while PS, PI and CL were the minor phospholipids in *P. acutum* (Sul and Erwin, 1997) and the species of both *Paramecium* and *Tetrahymena* (Kates and Volcani, 1966; Jonaha and Erwin, 1971; Berger et al., 1972; Rhoads and Kaneshiro, 1979). Therefore, our results indicate that the composition of phospholipids in scuticociliate is typical for many ciliated protozoa.

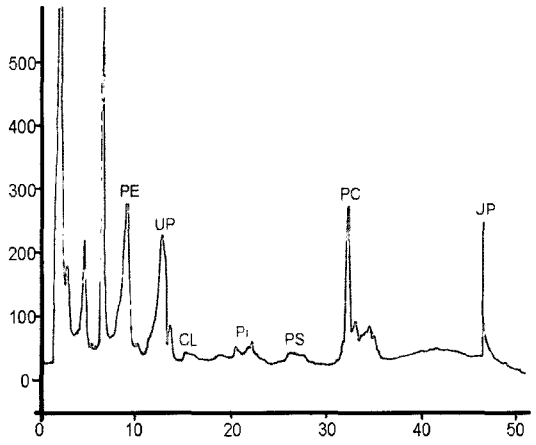

Fig. 1. HPLC chromatogram of phospholipids extracted from scuticociliate. The samples (0.4 μmol of lipid phosphorus) were injected into a Zorbax Rx-SIL column. The standard phospholipid mixture was used the mixture of cardiolipin, phosphatidylserine and soybean (L-α phosphatidylcholine, L-α phosphatidylinositol, L-α phosphatidylethanolamine). CL: cardiolipin, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phospatidylserine, UP: unknown phospholipid.

Table 1. Phospholipid composition of *Uronema marinum*. Data are mean values ± S.D. from five independent samples.

Phospholipid class	Relative amount (%)
Phosphatidylcholine	24.26 ± 2.09
Phosphatidylethanolamine	22.21 ± 0.31
Phosphatidylinositol	6.14 ± 8.45
Phosphatidylserine	5.11 ± 3.71
Cardiolipin	3.07 ± 2.34
Unidentified phospholipid	28.72 ± 0.23

The fatty acid profile of each lipid is presented in Table 2 and Table 3. Some differences were observed in the fatty acid composition. In neutral lipids, the most abundant fatty acid group was monounsaturated fatty acid (48.3% of total fatty acids) with oleic acid (18:1) and nervonic acid (24:1). Saturated fatty acids comprised 29.6% of total fatty acids, with palmitic acid (16:0), stearic acid (18:0), and myristic acid (14:0). and polyunsaturated fatty acid accounted for 33.0% with Di-homo-γ-linolenic acid (20:3) and linoleic acid (18:2). Whereas phospholipids predominantly contained the fatty acid group in the following order: polyunsaturated fatty acids (52.7% of total fatty

Table 2. Fatty acid composition of neutral lipid from scuticociliate. Identity of fatty acids was based on retention times of the used standard fatty acids. Data are mean values ± S.D. from five independent samples.

Fatty acid	Relative amount wt. (%)
Oleic acid (18:1)	31.7
Linoleic acid (18:2)	30.8
Palmitic acid (16:0)	19.0
Nervonic acid (24:1)	16.6
Stearic acid (18:0)	10.2
Di-homo-γ-linolenic acid (20:3)	3.0
Myristic acid(14:0)	0.4

Table 3. Fatty acid composition of phospholipid from scuticociliate. Identity of fatty acids were based on retention times of the used standard fatty acids. Data are mean values ± S.D. from five independent samples.

Fatty acid	Relative amount wt. (%)
Linoleic acid (18:2)	45.0
Oleic acid (18:1)	22.5
Palmitic acid (16:0)	16.8
γ-Linolenic acid (18:3)	7.7
Stearic acid (18:0)	7.0
Palmitoleic acid (16:1)	6.0
Myristic acid (14:0)	1.7

acids) with linoleic acid (18:2) and γ-linolenic acid (18:3) > monounsaturated fatty acids (28.5% of total fatty acids) with oleic acid (18:1) and palmitoleic acid (16:1) > saturated fatty acids (25.5% of total fatty acids) with palmitic acid (16:0), stearic acid (18:0) and myristic acid (14:0). With exception of these difference, neutral lipids and phospholipids have almost the similar fatty acid composition pattern. They contained palmitic acid (16:0) as major saturated fatty acid and linoleic acid (18:2) as major polyunsaturated fatty acid. However, Both of them did not contained polyunsaturated fatty acids of omega-3 family including 18:4, 20:5 and 22:6. This result is inconsistent with the results from the other marine ciliated protozoan (*P. acutum*) which showed the presence of omega-3 polyunsaturated fatty acids in the total lipids of membrane (Sul and Erwin, 1997). It has been reported that the mem- brane lipids of marine organisms characteristically contained poly-unsaturated fatty acids of omega-3 family and the presence of these fatty acids might represent one of the adaptation mechanisms in marine organisms subjected to changes in marine environmental factors such as salinity and temperature (Kates and Volcani, 1966; Parrish et al., 1991; Sul and Erwin, 1997). In the view of these consideration, it would be assumed that the adaptation mechanism of scuti-cociliate (*U. marinum*) to changes in the environ-mental factors might be distinct from that of other marine ciliated protozoan (*P. acutum*).

In summary, our results show that the scuticociliate (*U. marinum*) unlike other marine ciliated protozoa, has a unique fatty acid profile without omega-3 family polyunsaturated fatty acid, although its lipid com-position is similar to that of other marine ciliated protozoa.

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References

Berger, H., P. Johns and D.J. Hanahan. 1972. Structural studies on lipids of *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 260, 617-629.

Bordmann G, W. Rudin and N. Favre. 1998. Immnuization of mice with phosphatidylcholine drastically reduced the parasitaema of subsequent *Plamodium cabaudi chabaudi* blood-stage infections. Immunol., 94, 35-40.

Cribb, A.E., R.J. Despres and T. Cawthorn. 1999. Tetrazolium-based cytotoxicityassay to determine anti-protozoal activity against the scuticociliate *Anohryoides haemopholia*. Dis. Aquat. Org., 35, 213-219.

Florin-Christensen, J., C.E. Suarez, M. Florin-Christensen, S.A. Hines, T.F. McElwain and G.H. Palmer. 2000. Phosphatidylcholine formation is the predominant lipid biosynthetic event in the hemoparasite *Babesia bovis*. Mol. Biochem. Parasitol., 106, 147-156.

Guan, Z., J. Grunler, S. Piao and P.J. Sindelar. 2001. Separation and quantitation of phospholipids and their ether analogues by high-performance liquid chroma-tography. Anal. Biochem., 297, 137-143.

Harrington, G.W., D.H. Beach, J.E. Dunham and G.G. Holz, Jr. 1970. The polyunsaturated fatty acids of marine dinoflagellates. J. Protozool., 17, 213-219.

Horwitz, J. and R.L. Perlman. 1987. Phospholipid meta-bolism in PC12 pheochromocytoma cells. Meth. Enzymol., 141, 169-175.

Jee, B.Y., Y.C. Kim and M.S. Park. 2001. Morphology and biology of parasite responsible for scuticociliatosis of cultured olive flounder *Paralichtys olivaceus*. Dis.

Aquat. Org., 47, 49-55.
Jonaha, M. and J.A. Erwin. 1971. The lipids of membraneous cell organelles isolated from the ciliate, *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 231, 80-92.
Kates M. and B.E. Volcani. 1966. Lipid components of diatoms. Biochim. Biophys. Acta. 4, 116, 264-278.
Kwon, S.R., J.K. Chung, H.H. Lee and K.H. Kim. 2002. Effects of oxytetracycline treatments on the infection potential of scuticociliates in cultured olive flounder, *Paralichthys olivaceus*. J. Fish. Sci. Tech., 5, 1-4.
Novotny, M.J., R.J. Cawthorn and B. Despres. 1996. *In vitro* effects of chemotherapeutants on the lobster parasite *Anophryoides haemophila*. Dis. Aquat. Org., 24, 233-237.
Parrish, C.C., A.S.W. DeFreitas, G. Bodennec, E.J. Macpherson and R.G. Ackman. 1991. Lipid composition of the toxic marine diatom, *Nitzschia pungens*. Phytochemistry 30, 113-116.
Rhoads, D.E. and E.S. Kaneshiro. 1979. Characterizations of phospholipids from *Paramecium tetraurelia* cells and cilia. J. Protozool., 26, 329-338.
Soderberg, M., C. Edlund, I. Alafuzoff, K. Kristensson and G. Dallner. 1992. Lipid composition in different regions of the brain in Alzheimer's disease/senile dementia of Alzheimer's type. J. Neurochem., 59. 1646-1653.
Sul, D. and J.A. Erwin. 1997. The membrane lipids of the marine ciliated protozoan *Parauronema acutum*. Biochim. Biophys. Acta, 1345, 162-171.
Valtersson, C. and G. Dallner. 1982. Compartmentalization of phosphatidylethanolamine in microsomal membranes from rat liver. J. Lipid Res., 23, 868-875.
Yoshinaga, T. and J. Nakazoe. 1993. Isolation and *in vitro* cultivation of an unidentified ciliate causing scuticociliatosis in Japanese flounder, *Paralichthys olivaceus*. Gyobyo Kenkyu, 28, 131-134.
Zidovetzki R. and I.W. Sherman. 1991. Lipid composition of the membranes of malaria-infected erythrocytes and the role of drug-lipid interactions in the mechanism of action of chloroquine and other antimalarials. In: Coombs G, North M, eds. Biochemical Protozoo- logy. Taylor and Francis, London, pp. 8-99.

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