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A Novel Phosphatidylcholine Which Contains Pentadecanoic Acid at *sn-1* and Docosahexaenoic Acid at *sn-2* in *Schizochytrium* sp. F26-b

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Docosahexaenoic acid (DHA, 22:6*n*-3)–containing phospholipids are a ubiquitous component of the central nervous system and retina, however their physiological and pharmacological functions have not been fully elucidated. Here, we report a novel DHA-containing phosphatidylcholine (PC) in a marine single cell eukaryote, *Schizochytrium* sp. F26-b. Interestingly, 31.8% of all the fatty acid in F26-b is DHA, which is incorporated into triacylglycerols and various phospholipids. In phospholipids, DHA was found to make up about 50% of total fatty acid. To identify phospholipid species containing DHA, the fraction of phospholipids from strain F26-b was subjected to normal phase high-performance liquid chromatography (HPLC). It was found that DHA was incorporated into PC, lyso-PC, phosphatidylethanolamine, and phosphatidylinositol. The major DHA-containing phospholipid was PC in which 32.5% of the fatty acid was DHA. The structure of PC was analyzed further by phospholipase A_2 treatment, fast atom bombardment mass spectrometry, and ^{1}H- and ^{13}C-NMR after purification of the PC with reverse phase HPLC. Collectively, it was clarified that the major PC contains pentadecanoic acid (C15:0) at *sn*-1 and DHA at *sn*-2; the systematic name of this novel PC is therefore "1-pentadecanoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine."

Key words: DHA, odd-chain fatty acid, PC-DHA, phosphatidylcholine, phospholipid, Thraustochytrid.

Abbreviations: C15:0, pentadecanoic acid; DHA, docosahexaenoic acid; GC, gas chromatography; GC-MS, gas chromatography mass spectrometry; FAB-MS, fast-atom bombardment mass spectrometry; FAME, fatty acid methyl ester; HPLC, high performance liquid chromatography; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TEA, triethylamine; TLC, thin-layer chromatography.

Docosahexaenoic acid (DHA) is the most highly unsaturated and one of the most abundant polyunsaturated fatty acids (PUFAs) in membrane phospholipids of the central nervous system and retina (*1*, *2*). DHA seems to be essential for brain maturation and function, because a deficiency of DHA in both rodents and humans impairs learning, memory, spatial learning *etc.* (*3*). Recently, DHA was identified as a ligand for the retinoid X receptor in mouse brain (*4*) and as a positive modulator of Akt signaling in neural survival (*5*). These results suggest that DHA may influence neural function through modulation of a retinoid X receptor and/or an Akt signaling pathway. Newly synthesized DHA phospholipids were reported to be co-transported with rhodopsin-bearing post-Golgi vesicles to rod outer segments (*6*). The disruption of G-protein-coupled signaling in rod outer segments was caused by a deficiency of DHA, which may lead to the reduction in visual activity (*7*). Several lines of evidence also suggest pharmacological benefits from DHA and DHA-containing phospholipids in the treatment of hypertension, arthritis, atherosclerosis, depression, thrombosis, and cancer (*8*–*12*), although the mechanism underlying these effects has not been elucidated.

It should be noted that in humans, little DHA is produced *de novo* and most is supplied by dietary sources. At present, the main commercial source of DHA and DHA-containing phospholipids is marine fish oils, however, the supply of fish oils is hindered by seasonal variation, marine pollution, and high processing costs. Alternatively, the production of DHA by microorganisms seems to be promising, and in fact some microbes have been industrialized as a new source of DHA (*13*). Among them, single cell oils from marine thraustochytrids, which are widely distributed in the oceans and taxonomically aligned with the heterokont algae (*14*), could be a satisfactory alternative to fish oils as a source of DHA (*15*). Although thraustochytrids produce DHA and DHA-containing phospholipids, a detailed structural analysis of their phospholipids has yet to be performed.

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Here, we report that the major phospholipid in *Schizochytrium* sp. strain F26-b is a novel PC which contains pentadecanoic acid (C15:0) at *sn*-1 and DHA at *sn*-2, *i.e.* the systematic name of the phospholipid is "1-pentadecanoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine."

MATERIALS AND METHODS

Materials—Egg yolk phosphatidylcholine (PC) was purchased from Avanti Polar Lipids, Inc (Birmingham, Alabama, USA). Bovine brain phosphatidylethanolamine (PE), and tetracosanoic acid (C24:0) were obtained from SIGMA-ALDRICH, Inc. (St Louis, MO, USA). Porcine liver phosphatidylinositol (PI) and bovine brain phosphatidylserine (PS) were obtained from Doosan Serdary Research Laboratories (London, ON, Canada). Lysophosphatidylcholine (LPC) (1-palmitoyl-*sn*-glycero-3-phosphocholine) was obtained from Matreya, Inc (USA). Precoated Silica Gel 60 TLC plates were obtained from Merck (Darmstadt, Germany). Sep-Pak Plus Silica was obtained from Waters (USA). All other reagents were of the highest quality available.

Microorganism Culture—Schizochytrium sp. strain F26-b, isolated from Ishigaki island in Japan, was inoculated into a GY medium (3% glucose, 1% yeast extract in 50% artificial sea water, and 50% distilled water, pH 6.0). The culture was shaken on a rotary shaker at 150 rpm in a 500-ml flask containing 200 ml of the GY medium at 25°C for 5 days. The cells were harvested by centrifugation at 3,000 × *g*, washed twice with 0.9% NaCl, and once with distilled water, and then freeze-dried.

Lipid Extraction—Lipids were extracted from lyophilized cells with 25 ml of chloroform/methanol (2:1, v/v) with 2 min of sonication. Cell debris was removed by centrifugation at 3,000 rpm for 15 min. The extraction process was repeated 4 times and the extracts were pooled (total lipid fraction). The total lipid extract was dried by evaporation.

Separation of Extracted Lipids—The separation of total lipids into neutral lipid, glycolipid, and phospholipid fractions was performed using a method described previously (*16*) with minor modifications. Briefly, 20 mg of the extracted lipids was dissolved in 2 ml of chloroform, and applied to a Sep-Pak Plus Silica cartridge (2 ml). The cartridge was eluted with chloroform (30 ml), then acetone (50 ml), and finally methanol (20 ml). This order of elution produced the fractions containing neutral lipids, glycolipids, and phospholipids, respectively.

TLC (Thin-Layer Chromatography)—The lipid fractions eluted from Sep-Pak Plus Silica were analyzed by TLC using a Silica Gel 60 plate. The developing solvent and staining reagent for neutral lipids were *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) and H_2SO_4/distilled water (1:1, v/v) solution, respectively. For glycolipids and phospholipids, chloroform/methanol/distilled water (65:25:4, v/v/v) (solvent I) and orcinol-H_2SO_4 reagent, and solvent I and Dittmer reagent were used as the developing solvent and staining reagent, respectively.

Fatty Acid Analysis—Fatty acids were analyzed by gas-liquid chromatography (GC) as fatty acid methyl esters (FAME), which were prepared by treatment with 3 N methanolic HCl with addition of tetracosanoic acid (C24:0) as an internal standard. The samples were subjected to chromatography with a Shimadzu GC-14 gas-liquid chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector and a capillary column (HR-SS-10, 30 m × 0.25 mm; Shinwa Chemical Ind. Ltd., Japan). The column temperature was programmed to increase at 2°C/min from 150°C to 220°C, then maintained for 10 min. The injection and detector port temperatures were 250°C and 300°C, respectively. The methyl esters on GC were identified by conventional methods using the retention time of a PUFA-3 standard mixture. To confirm the identification of PUFA, some FAMEs were analyzed by GC-mass spectrometry (GC-MS) using a Shimazu GCMS-QP 5000 spectrometer (Shimazu, Kyoto, Japan) according to the method described in Ref. *17*.

Phospholipase A_2 Treatment of Phospholipids—Ten milligrams of phospholipids were treated with 5 units of phospholipase A_2 from snake venom (Sigma) in 20 mM Tris-HCl, pH 8.0, containing 1 mM $CaCl_2$ at 37°C for 16 hours. After treatment, fatty acids released were separated from lyso-phospholipids by Sep-Pak Silica cartridge and subjected to fatty acid analysis by GC.

Separation of Phospholipid Classes by HPLC—The phospholipid classes were separated by high performance liquid chromatography (HPLC) with UV detection (*18*). The dried phospholipids, dissolved in 20 μl of *n*-hexane/2-proparnol (3:1, v/v), were injected into a Wakosil 5 NH_2 column (250 × 4.6 mm; Wako pure Chem., Japan) which was equipped with a Hitachi HPLC system (Hitachi Co., Tokyo, Japan). The mobile phase was acetonitrile/methanol/0.2% triethylamine (TEA) (67:22:11, v/v/v, pH 4.0). The flow rate was 1 ml/min and the column temperature was maintained at 40°C. Phospholipids were detected by measuring UV at 210 nm.

Reverse Phase HPLC of PC—The PC fraction was further fractionated by reverse phase HPLC. Ten milligrams of the PC was dissolved in 1 ml of chloroform and an aliquot of the sample (20 μl) was injected into an Inertsil ODS-3 column (250 × 4.0 mm; GL Science Inc., Tokyo, Japan), which was eluted with a mixture of methanol and 1 mM potassium phosphate buffer, pH 7.4 (9.5:0.5, v/v) at a flow rate of 1 ml/min (*19*).

Fast Atom Bombardment Mass Spectrometry (FAB-MS)—The purified PC was mixed with triethanolamine and *m*-nitrobenzylalchol as a matrix and subjected to fast atom bombardment mass spectrometry (FAB-MS) using a Jeol SX102A mass spectrometer in both positive and negative ion modes (Joel, Tokyo, Japan).

NMR—^{1}H and ^{13}C NMR spectra of the purified PC were obtained using a Varian Unity-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR).

RESULTS

Production of Lipids in Schizochytrium sp. Strain F26-b—The strain was cultured at 25°C for 5 days in a GY medium, and the cells were harvested by centrifugation (3,000 × *g* for 15 min). In a typical experiment, 3.5 g of cells (dry weight) was obtained from 1 liter of culture, from which 915 mg of lipids was extracted (total lipid fraction). Overall, 601 mg of neutral lipid fraction (chloroform-eluted fraction), 67.5 mg of glycolipid fraction (acetone-eluted fraction), and 204 mg of phospholipid fraction (methanol-eluted fraction) were obtained from

Table 1. **Composition of total fatty acids of strain F26-b.**

Fatty acid molecules	Percentage of GC area (%)
C13:0	1.0
C14:0	0.6
C15:0	35.3
C16:0	6.9
C16:2*n*4	8.7
C18:0	0.7
C18:1*n*7	1.7
C20:4*n*3	0.6
C20:5*n*3	1.2
C22:4*	5.7
C22:5*n*3	0.5
C22:6*n*3	31.8
Others	5.3
Total	100

*Determined by GC-MS.

Table 2. **Composition of fatty acids in each lipid fraction from strain F26-b.**

Fatty acid molecules	Percentage of GC area (%)		
	Neutral lipid fraction	Glycolipid fraction	Phospholipid fraction
C13:0	1.4	8.6	0.2
C14:0	0.8	–*	0.3
C15:0	40.4	21.7	21.5
C16:0	7.0	7.8	6.7
C16:2*n*4	10.1	4.9	4.9
C18:0	0.6	–	0.7
C18:1*n*7	1.7	4.7	1.3
C20:4*n*3	0.6	–	0.5
C20:5*n*3	0.9	–	0.2
C22:4**	4.1	4.7	9.3
C22:5*n*3	0.4	10.0	0.6
C22:6*n*3	23.4	36.8	50.3
Others	8.6	0.8	3.5
Total	100.0	100.0	100.0

*Below the limit of detection. **Determined by GC-MS.

the total lipids by using Sep-Pak Plus Silica cartridge, although an aliquot of total lipids (20 mg/one experiment) was applied to the cartridge as described in the "MATERIALS AND METHODS." Triacylglycerol was identified as the predominant neutral lipid while alkaline stable, glucose-containing glycolipids were found in the glycolipid fraction. The molecular species of the phospholipid fraction will be described below.

Fatty Acid Composition of Strain F26-b—As shown in Table 1, the major fatty acids of the total lipid fraction are C15:0 (pentadecanoic acid) and C22:6*n*-3 (DHA), together making up 67.1% of all the fatty acids. Fatty acid components of the neutral lipid, glycolipid, and phospholipid fractions are summarized in Table 2. Although C15:0 and DHA were predominant in all fractions, the ratio of these fatty acids to the others differed in each lipid fraction. The highest proportion of DHA was found in the phospholipid fraction, in which about 50% of the fatty acid was DHA, whereas the highest percentage of C15:0 was found in the neutral lipid fraction.

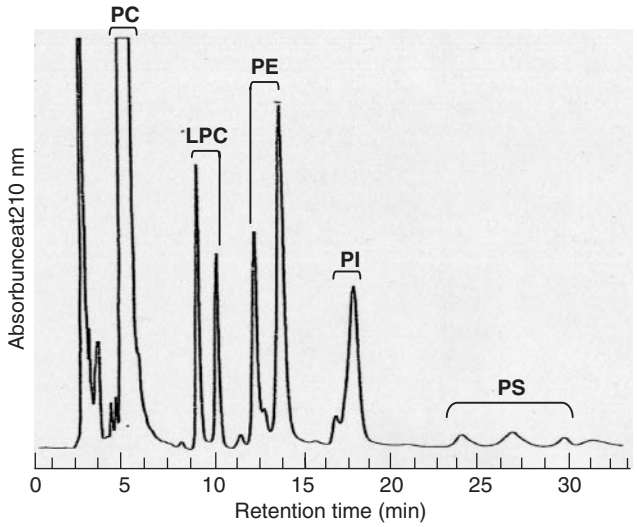

Fig. 1. **Separation of phospholipid classes from strain F26-b by normal phase HPLC.** Phospholipids (200 μg) of strain F26-b were dissolved in 20 μl of *n*-hexane/2-propanol (3:1, v/v), and then injected into a Wakosil 5 NH_2 column (250 × 4.6 mm) which was eluted with acetonitrile/methanol/0.2% TEA, pH 4.0 (67:22:11, v/v) at a flow rate of 1 ml/min. The phospholipids were monitored using UV at 210 nm. PC was eluted at 5–6 min, followed by LPC, PE, PI, and PS in this order. Molecular species of phospholipids were determined by TLC using solvent I as a developing solvent and appropriate standards as described in "MATERIALS AND METHODS."

Analysis of Phospholipids in Strain F26-b—As shown in Fig. 1, the phospholipid fraction was further separated into PC, LPC, PE, PI, and PS fractions using normal phase HPLC by the method described in "MATERIALS AND METHODS." Phospholipid classes were determined by TLC using appropriate standards according to the method described in "MATERIALS AND METHODS." The major phospholipid of strain F26-b was PC accounting for about 70% of all phospholipids when calculated from the peak area on the chromatograph (Fig. 1). All phospholipids except PS contained DHA as the predominant fatty acid while saturated fatty acids such as C16:0 and C18:0 were predominant in the PS fraction (Table 3). Collectively, the major DHA-containing phospholipid in strain F26-b was PC in which 23% of all the DHA in the phospholipid fraction was incorporated.

Separation of PC Molecules by Reverse Phase HPLC—The PC fraction was further separated into six fractions by reverse phase HPLC possibly due to the variation in fatty acid components (Fig. 2A). Each peak was apparently one band on TLC and their Rf correspond to that of an authentic PC (Fig. 2B). The major fractions, peaks 2 and 3, were pooled separately and subjected to a GC-based analysis. As shown in Fig. 2C and D, the major fatty acids of fraction 2 and fraction 3 were C16:0, C18:0, and DHA (molar ratio, 0.31:0.23:1.0), and C15:0 and DHA (molar ratio, 1:1), respectively. This result suggests that fraction 3 is more homogenous than fraction 2. Thus, further analysis of the PC was performed using fraction 3.

Phospholipase A_2 Treatment of the PC—To determine which carbon in the glycerol frame of the PC is occupied by DHA, fraction 3 was treated with phospholipase A_2 which can cleave the fatty acid residues at *sn*-2, but not

Table 3. **Fatty acid profile of phospholipids extracted from strain F26-b.**

Phospholipids Position	Percentage of GC area (%)								
	PC		LPC	PE		PI		PS	
	sn-1[#]	*sn*-2[#]	–	*sn*-1[#]	*sn*-2[#]	*sn*-1[#]	*sn*-2[#]	*sn*-1[#]	*sn*-2[#]
Fatty acids									
C13:0	–*	0.3	–	–	0.8	–	–	–	–
C14:0	–	0.3	–	1.1	–	1.5	–	5.7	–
C15:0	54.1	17.9	13.8	17.8	8.2	11.2	9.3	5.7	12.2
C16:0	17.6	4.3	10.7	25.9	15.1	8.4	11.4	14.0	29.4
C16:2*n*4	14.6	4.0	3.3	5.1	3.3	3.4	–	–	–
C18:0	1.6	0.9	–	7.6	9.6	1.5	7.8	4.6	23.2
C18:1*n*7	1.8	1.4	–	–	1.1	–	–	–	–
C20:4*n*3	–	0.7	–	–	–	–	–	0.0	–
C20:5*n*3	2.0	2.0	–	–	2.1	–	–	0.0	–
C22:4**	–	9.9	9.5	1.5	6.1	3.6	15.4	–	–
C22:6*n*3	8.2	56.8	49.1	15.9	43.3	11.3	46.5	–	–
Others	–	1.5	13.6	24.9	11.4	58.9	9.5	70.1	35.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine. *Below the limit of detection. **Identified by GC-MS. [#]The fatty acids at *sn*-2 were analyzed by GC after removal from phospholipids with phospholipase A_2 and those at *sn*-1 were calculated from the fatty acids of lyso-phospholipids generated. Details are described in "MATERIALS AND METHODS."

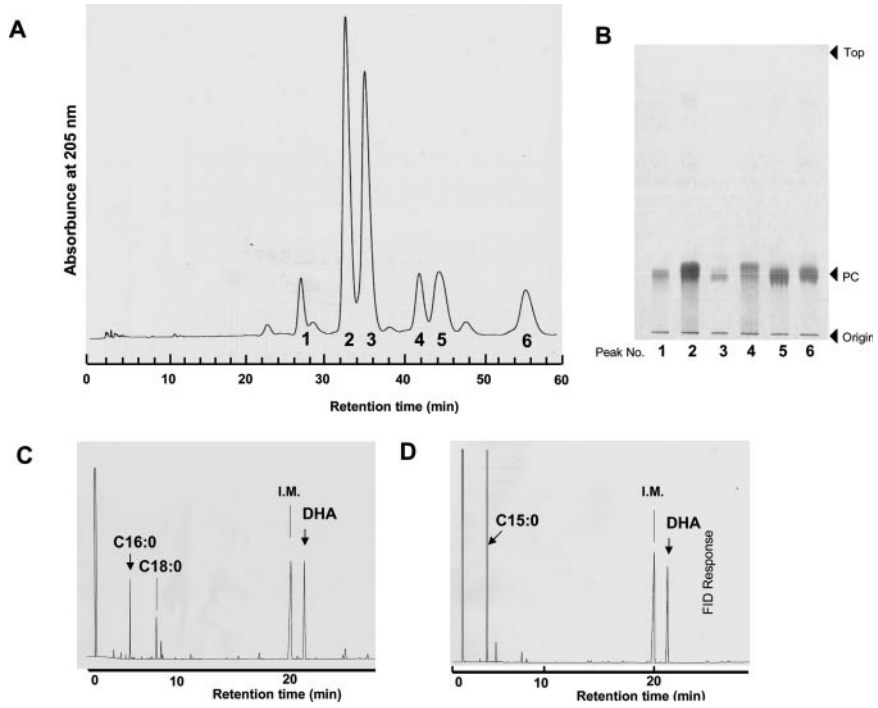

Fig. 2. **Separation of the PC fraction by reverse phase HPLC.** (A) The PC fraction obtained by normal phase HPLC was applied to an Inertsil ODS-3 column which was eluted with methanol/1 mM phosphate buffer (9.5:0.5, v/v, pH 7.4) at a flow rate of 1 ml/min. (B) TLC of each peak from the normal phase HPLC. TLC was developed with solvent I and phospholipids were visualized by Dittmer reagent. (C, D) GLC-based analysis of fatty acid methyl esters of fractions No. 2 and No. 3 of normal phase HPLC (A), respectively. Details for HPLC, TLC and GLC are described under "MATERIALS AND METHODS."

sn-1, of PC (*20*). It was found that the ratio of DHA to C15 was about 5:1 when the released fatty acids were analyzed by GC, indicating DHA is mainly bound to the carbon at *sn*-2 of the PC. This result also suggests that the carbon at *sn*-1 is mainly occupied with C15:0 fatty acid.

FAB-MS and NMR of the PC—Fraction 3 was further analyzed by FAB-MS using both positive and negative ion modes. In the positive ion mode (Fig. 3A), a parent ion $[M+H]^+$ and an ion corresponding to phosphocholine (head group of PC) were observed at *m*/*z* 792 and 184, respectively. In the negative ion mode (Fig. 3B), three fragment ions of PC were observed at *m*/*z* 776, 731, and 705 corresponding to $[M-15]^-$, $[M-60]^-$, and $[M-86]^-$, respectively, which are known to be typical fragment ions of phosphocholine-containing lipids in FAB-MS (*21*). Furthermore, two ion peaks corresponding to DHA and C15:0 were detected in the negative ion mode at *m*/*z* 327 and 241, respectively. The analysis of fraction 3 with ^{1}H-NMR spectroscopy revealed typical signals for PC (Fig. 4), *i.e.* ω-CH_3 at 0.95 ppm, $(CH_2)_n$ at 1.23 ppm, β-CH_2 at 1.65 ppm, α-CH_2 at 2.52 ppm, $N(CH_3)_3$ at 3.47 ppm, PO_3CH_2 at 3.98 ppm, CH_2O at 4.42 ppm and HC=CH at 5.05 ppm. Furthermore, ω-terminal methyl groups of DHA (DHA-CH_3) and C15 (C15-CH_3) were

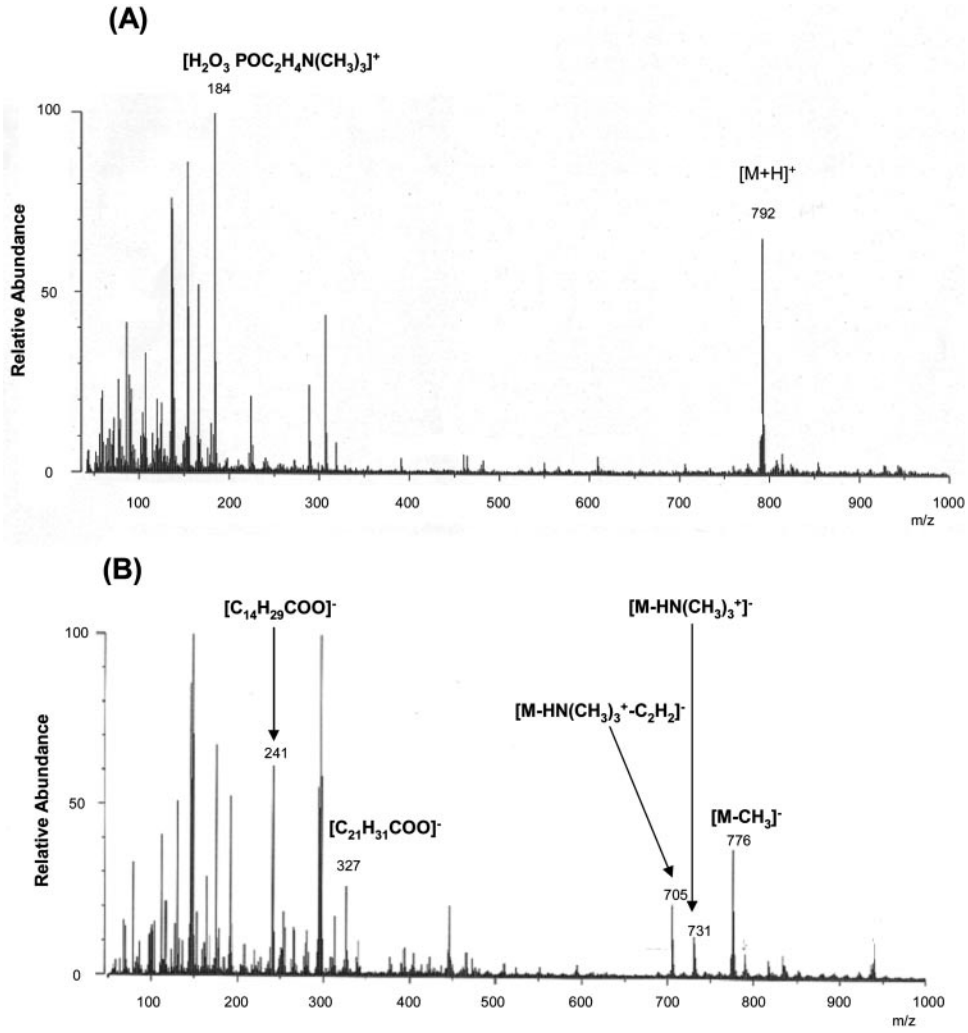

Fig. 3. **FAB-MS spectra of the PC from strain F26-b in positive (A) and negative (B) ion modes.** FAB-MS of the PC was conducted in the positive ion mode using *m*-nitrobenzylalchol and negative ion mode using triethanolamine as a matrix. Details are described in "MATERIALS AND METHODS."

observed at 0.95 ppm and 0.88 ppm, respectively. However, branching of fatty acids was not likely to be present in the PC, because in the Heteronuclear Single Quantum Correlation spectrum (HSQC spectrum), the correlations of terminal methyl groups of the acyl chains were observed at between δ_H0.88–0.96 ppm and δ_C14.0 ppm (*22*). Taken together, it was concluded that the major phospholipid of strain F26-b is a novel PC which contains an odd fatty acid, C15:0, at *sn*-1 and a DHA at *sn*-2; the systematic name of this PC is therefore "1-pentadecanoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine" (Fig. 5).

DISCUSSION

The production of DHA and DHA-containing phospholipids has recently drawn tremendous attention because of its beneficial effect on human health, and fish oils are usually employed for their production. However, there are severe problems with the use of fish oils as a source of DHA and DHA-containing phospholipids, since environmental man-made pollutants, such as dioxins, PCBs, and heavy metals including mercury compounds, could be concentrated in fish oils (*23*). Indeed some countries have an outright ban on fish oils being given to infants and children for these reasons. Therefore, the microbial production of DHA and DHA-containing phospholipids is expected to supply safe and convenient alternatives to fish oils. One of the candidate sources for this purpose is marine thraustochytrids; *i.e. Sphizochytrium* sp. F26-b was found to be easy to cultivate and produce an abundance of DHA and DHA-containing phospholipids as shown in the present study.

It was revealed in this study that about 32% of all the fatty acid in *Schizochytrium* sp. strain F26-b was DHA, which was mainly incorporated into triacylglycerols and various phospholipids. To our knowledge, there have been very few reports of molecular species of phospholipids that contain DHA in microorganisms including thraustochytrids, and thus we performed a structural analysis of DHA-containing phospholipids from strain F26-b. As a result, it was disclosed that the major phospholipid of strain F26-b is a novel PC which contains an odd-numbered fatty acid, C15:0, at *sn*-1 and DHA at *sn*-2 (Fig. 5). It should be emphasized that this is the first report of a PC which contains both an odd-numbered fatty acid and a DHA.

Odd-numbered fatty acids are not usually found in mammals, but they have been detected in the nervous system of a patient with an impaired vitamin B12 metabolism (*24*, *25*). Interestingly, odd-numbered fatty

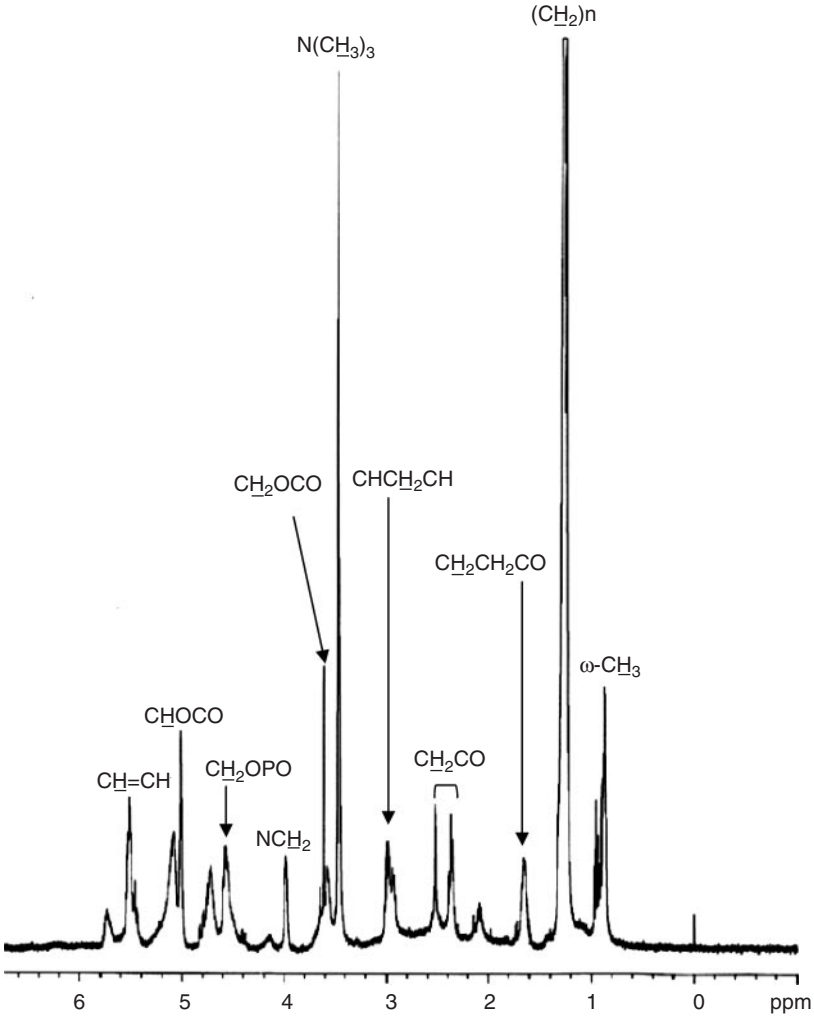

Fig. 4. **^{1}H-NMR spectra of the PC from strain F26-b.** Three milligrams of the sample was analyzed with a Varian Unity-500 spectrometer (500 MHz).

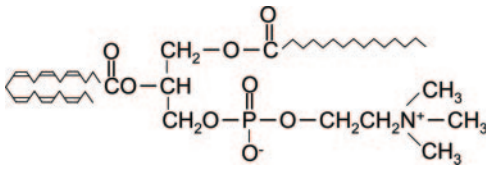

Fig. 5. **The proposed structure of the PC from strain F26-b.**

acids have been shown to improve the symptoms of impaired long-chain fatty acid oxidation (*26*, *27*). Since C15:0 (pentadecanoic acid) was found to make up about 35% of all the fatty acid in F26-b, this strain is useful as a source of not only DHA but also odd-numbered fatty acids, although the physiological function and benefit of pentadecanoic acid have yet to be clarified.

Only a few reports have described the pharmacological benefits of DHA-containing phospholipids, *e.g*. DHA-containing PC vesicles brought about a dose-dependent decrease in tumor cell viability (*28*) and DHA- and EPA-enriched liposomes enhanced the permeability, transport, and uptake of phospholipids in Caco-2 cells (*29*). The pharmacological effects of the novel PC containing DHA described in this article are of interest and should be clarified.

REFERENCES

1. Crawford, M.A., Costeloe, K., Phylactos, A., Skirvin, L., and Stacey, F. (1997) Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *Am. J. Clin. Nutr.* **66**, 1032S–1041S
2. Giusto, N.M., Pasquare, S.J., Salvador, P.I., Roque, M.E., and Ilincheta de Boschero M.G. (2000) Lipid metabolism in vertebrate retinal rod outer segments. *Prog. Lipid Res.*, **39**, 315–391
3. Uauy, R., Hoffman, D.R., Peirano, P., Birch, D.G. and Birch, E.E. (2001) Essential fatty acids in visual and brain development. *Lipids* **36**, 885–895
4. de Urquiza, A.M., Liu, S., Sjoberg, M., Zetterstrom, R.H., Griffiths, W., Sjovall, J., and Perlmann, T. (2000) Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* **290**, 2140–2144
5. Akbar, M., Calderon, F., Wen, Z., and Kim, H.Y. (2005) Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc. Natl. Acad. Sci. USA*, **102**, 10858–63
6. Rodriguez de Turco, E.B., Deretic, D., Bazan, N.G., and Papermaster, D.S. (1997) Post-Golgi vesicles cotansport docosahexaenoyl-phospholipids and rhodopsin during frog photoreceptor membrane biogenesis. *J. Biol. Chem.* **272**, 10491–10497
7. Niu, S.-L., Mitchell, D.C., Lim, S.-Y., Wen, Z.-M., Kim, H.-Y., Salem Jr., N., and Litman, B.J. (2004) Reduced G protein-coupled signaling efficiency in retinal rod outer segments in

response to *n-3* fatty acid deficiency. *J. Biol. Chem.*, **279**, 31098–31104
8. Armitage, J.A., Pearce, A.D., Sinclair, A.J., Vingrys, A.J., Weisinger, R.S., and Weisinger, H.S. (2003) Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. *Lipids* **38**, 459–464
9. Simopoulos, A.P. (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* **21**, 495–505
10. Das, U.N. (2005) Long-chain polyunsaturated fatty acids, endothelial lipase and atherosclerosis. *Prostaglandins Leukot. Essent. Fatty Acids.* **72**, 173–179
11. Denys, A., Hichami, A., and Khan, N.A. (2005) n-3 PUFAs modulate T-cell activation via protein kinase C-alpha and -epsilon and the NF-kappa B signaling pathway. *J. Lipid Res.* **46**, 752–758
12. Nordoy, A., Marchioli, R., Arnesen, H., and Videbaek, J. (2001) n-3 polyunsaturated fatty acids and cardiovascular diseases. *Lipids* **36**, S127–129
13. Li, Z.Y. and Ward, O.P. (1994) Production of docosahexaenoic acid by *Thraustochytrium roseum*. *J. Ind. Microbiol.* **13**, 238–241
14. Weete, J.D., Kim, H., Gandhi, S.R., Wang, Y., and Dute, R. (1997) Lipids and ultrastructure of *Thraustochytrium* sp. ATCC 26185. *Lipids* **32**, 839–845
15. Lewis, T.E., Nichols, P.D., and McMeekin, T.A. (1999) The biotechnological potential of Thraustochytrids. *Mar. Biotechnol.* **1**, 580–587
16. Kendrick, A.and Ratledge, C. (1992) Lipids of selected molds grown for production of n-3 and n-6 polyunsaturated fatty acids. *Lipids* **27**, 15–20
17. Chisada, S., Horibata, Y., Hama, Y., Inagaki, M., Furuya, N., Okino, N., and Ito, M. (2005) The glycosphingolipid receptor for *Vibrio trachuri* in the red sea bream intestine is a GM4 ganglioside which contains 2-hydroxy fatty acids. *Biochem. Biophys. Res. Commun.* **333**, 367–373
18. Mawatari, S. and Murakami, K. (1998) Analysis of membrane phospholipid peroxidation by isocratic high-performance liquid chromatography with ultraviolet detection. *Anal. Biochem.* **264**, 118–23
19. Smith, M. and Jungalwala, F.B. (1981) Reversed-phase high performance liquid chromatography of phosphatidylcholine: a simple method for determining relative hydrophobic interaction of various molecular species. *J. Lipid Res.* **22**, 697–704
20. Huang, P. and Mackessy, S.P. (2004) Biochemical characterization of phospholipisa A_2 (trimorphin) from the venom of the Sonoran Ryre Snake *Trimorphodon biscutatus lambda* (family *Colubridae*). *Toxicon*, **44**, 27–36
21. Jensen, N.J., Tomer, K.B., and Gross, M.L. (1986) Fast atom bombardment and tandem mass spectrometry of phosphatidylserine and phosphatidylcholine. *Lipids*, **21**, 580–588
22. Inagaki, M., Nakata, T., and Higuchi, R. (2006) Isolation and structure of a galactocerebroside molecular species from the starfish *Culcita novaeguneae*. *Chem. Pharm. Bull.* **54**, 260–261
23. Kathryn, K.R. and Mahaffey, R. (2004) Fish and shellfish as dietary sources of methylmercury and the ω-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. *Environ. Res.* **95**, 414–428
24. Kishimoto, Y., Williams, M., Moser, H.W., Hignite, C., and Biermann, K. (1973) Branched-chain and odd-numbered fatty acids and aldehydes in the nervous system of a patient with deranged vitamin B 12 metabolism. *J. Lipid Res.* **14**, 69–77
25. Ramsey, R.B., Scott, T., and Banik, N.L. (1977) Fatty acid composition of myelin isolated from the brain of a patient with cellular deficiency of co-enzyme forms of vitamin B_{12}. *J. Neurol. Sci.* **34**, 221–232
26. Roe, C.R., Sweetman, L., Roe, D.S., David, F., and Brunengraber, H. (2002) Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. *J. Clin. Invest.* **110**, 259–269
27. Jones, P.M., Butt, Y.M., and Bennett, M.J. (2004) Effects of odd-numbered medium-chain fatty acids on the accumulation of long-chain 3-hydroxy-fatty acids in long-chain L-3-hydroxyacyl CoA dehydrogenase and mitochondrial trifunctional protein deficient skin fibroblasts. *Mol. Genet. Metab.* **81**, 96–99
28. Zerouga, M., Stillwell, W., Stone, J., Powner, A., and Jenski, L.J.P. (1996) hospholipid class as a determinant in docosahexaenoic acid's effect on tumor cell viability. *Anticancer Res.* **16**, 2863–2868
29. Hossain, Z., Kurihara, H., Hosokawa, M., and Takahashi, K. (2006) Docosahexaenoic acid and eicosapentaenoic acid-enriched phosphatidylcholine liposomes enhance the permeability, transportation and uptake of phospholipids in Caco-2 cells. *Mol. Cell. Biochem.* **14**, 1–9