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Biosynthesis of 1,2-dieicosapentaenoyl-*sn*-glycero-3-phosphocholine in *Caenorhabditis elegans*

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Previously, we showed that lowering the growth temperature increased the level of eicosapentaenoic acid (EPA) in the phosphatidylcholine (PtdCho) of *Caenorhabditis elegans*. In this study, we investigated the molecular species composition of PtdCho of *C. elegans*, with an emphasis on EPA-containing species. *C. elegans* contained a substantial amount of 1,2-dipolyunsaturated fatty acid-containing PtdCho (1,2-diPUFA-PtdCho) species, such as arachidonic acid/EPA and EPA/EPA, which are unusual phospholipids in higher animals. The EPA/EPA-PtdCho content was significantly increased in *C. elegans* grown at a low temperature. To examine the possibility that the acyltransferase activity involved in the remodeling of phospholipids accounts for the production of 1,2-diPUFA-PtdCho, we investigated the substrate specificity of this enzyme in *C. elegans* and found that it did not exhibit a preference for saturated fatty acid for acylation to the *sn*-1 position of PtdCho. The efficacy of the esterification of EPA to the *sn*-1 position was almost equal to that of stearic acid. The lack of preference for a saturated fatty acid for acylation to the *sn*-1 position of PtdCho is thought to result in the existence of the unusual 1,2-diEPA-PtdCho in *C. elegans*.

Keywords: acyltransferase; *Caenorhabditis elegans*; eicosapentaenoic acid; phosphatidylcholine; nematode.

The free-living nematode *Caenorhabditis elegans* is the first multicellular animal to have its genome sequenced [1].

Previously, we investigated the fatty acid composition of *C. elegans* and found that the nematode contains abundant eicosapentaenoic acid (EPA) especially in phosphatidylcholine (PtdCho) fraction [2]. Our recent investigation [3] showed that *C. elegans* has the ability to synthesize polyunsaturated fatty acids (PUFAs) *de novo*, as do the other nematode species *Turbatrix aceti* [4] and *Steinernema carpocapsae* [5]. In this regard, three *C. elegans* genes encoding fatty acid desaturases have been cloned [6–8]. We also showed that growth temperature affected the fatty acid composition of *C. elegans*. A reduction in growth temperature from 25 °C to 15 °C caused the proportion of EPA to increase from 23.6% to 32.5% in the PtdCho fraction. Conversely, the levels of dihomo-γ-linolenic acid and arachidonic acid in the PtdCho fraction were reduced at the low growth temperature [3]. On the other hand, in the phosphatidylethanolamine (PtdEtn) fraction, the diacyl subclass of PtdEtn was markedly increased at low growth temperature [3].

In the present study, we investigated the effect of growth temperature on the molecular species composition of PtdCho of *C. elegans* with an emphasis on EPA-containing species. The results showed that the proportion of 1,2-dieicosapentaenoyl-*sn*-glycero-3-phosphocholine (1,2-diEPA-PtdCho), which is an unusual PtdCho species in higher animals, was significantly increased at a low growth temperature. We also investigated the substrate specificity of acylCoA : lysophospholipid acyltransferase activity of *C. elegans*. By comparison with acyltransferase of rat liver, we discuss the possibility that the lack of a preference for saturated fatty acid for acylation to the *sn*-1 of PtdCho underlies the existence of 1,2-diEPA-PtdCho in *C. elegans*.

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Abbreviations: AgTLC, argentation thin-layer chromatography; EPA, eicosapentaenoic acid; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid.

Enzymes: acylCoA, lysophospholipid acyltransferase; (EC 2.3.1.23 and EC 2.3.1.62); acylCoA synthetase, (EC 6.2.1.10); phospholipase A_2, (EC. 3.1.1.4); phospholipase C, (EC 3.1.4.3).

MATERIALS AND METHODS

Chemicals

Agar, peptone, tryptone and yeast extract were obtained from Difco Laboratories (Detroit, MI, USA). Glycerol and precoated thin-layer plates (Silica gel 60) were from Merck (Darmstadt, Germany). Phospholipase A_2 (EC 3.1.1.4, from *Crotalus adamanteus* venom), phospholipase C (EC 3.1.4.3, from *Bacillus cereus*) ATP, CoA and essentially fatty acid-free BSA were from Sigma Chemical Co. (St Louis, MO, USA). EPA was from Funakoshi Co. (Tokyo, Japan). Linoleic acid was from Doosan Serdary Research Laboratories (London, ON, Canada). Sep-Pak C18 cartridges were the product of Waters Associates (Milford, MA, USA). [1-^{14}C]EPA and [1-^{14}C]stearic acid were from NEN Life Science Products (Boston, MA, USA). [1-^{14}C]Linoleic acid was from Amersham Life Science (Arlington Heights, IL, USA). All other chemicals were analytical grade.

Growth and isolation of *C. elegans*

The standard wild-type strain N2 of *C. elegans* was grown at 15 °C, 20 °C or 25 °C on 90-mm nematode-growing-medium agar plates seeded with *Escherichia coli* (OP50 strain) [2]. When microscopic inspection showed the plates were rich with adult worms, they were washed off with M9 buffer (22 mM KH_2PO_4,

were then subjected to lipid extraction or french press for enzyme assay.

Molecular species analysis of PtdCho

Purified live *C. elegans* were homogenized by grinding in a mortar with a pestle in a small amount of chloroform/methanol mixture (1 : 2, v/v). Total lipids were extracted from the homogenate according to the method of Bligh and Dyer [9]. The PtdCho and PtdEtn fractions of *C. elegans* were prepared by thin-layer chromatography (TLC) using the solvent chloroform/methanol/H_2O (65 : 35 : 6, v/v) (solvent system A) [2]. Detection was done with 0.01% primuline (in acetone/water, 4 : 1, v/v) under ultraviolet light. The PtdCho fraction (1 μmol) from *C. elegans* was subjected to phospholipase C treatment. As a large portion of PtdCho of *C. elegans* is accounted for by the diacyl subclass (96.4%) [2], almost all of the resulting glyceride is diacylglycerol. The diacylglycerol was converted to the monoacetyldiacylglyceride derivative or dinitrobenzoyl derivative [10]. For acetylation, diacylglycerol dissolved in dehydrated pyridine (0.5 mL) was mixed with 1 mL of acetic anhydride at 30 °C. After 15 h, 2.5 mL of chloroform/methanol (1 : 2, v/v) and 1.5 mL of water was added to the solution. The monoacetyldiglyceride was recovered from the chloroform phase and subjected to argentation TLC (AgTLC) on chromatoplates prepared as described previously [11]. The developing system of AgTLC was benzene/chloroform/methanol (80 : 20 : 10, v/v), and detection was performed with 0.2% 2,7-dichlorofluorescein (in ethanol) under ultraviolet light. Monoacetyldiglyceride was recovered from each of the zones of silica gel by the method of Bligh and Dyer [9] after spotting the authentic monoacetyldiglyceride (20 : 0/20 : 0) (10 μg as fatty acid) in each zone of silica gel. The recovered monoacetyldiglyceride was then dissolved in 5% methanolic HCl solution for methanolysis [3]. The fatty acid methyl esters derived from monoacetyldiglyceride were determined by gas chromatography (GC) using 20 : 0 as the internal standard as described below.

Positional distribution of fatty acid in the PtdCho and PtdEtn fraction

The PtdCho or PtdEtn of *C. elegans* was hydrolyzed with phospholipase A_2, and the resulting free fatty acid and lysophospholipid fractions were isolated by TLC [3]. After methanolysis of each fraction, the fatty acid composition was determined by GC.

GC and HPLC

Fatty acid methyl esters were analyzed by GC (Shimadzu GC-14 A, Kyoto, Japan) equipped with a capillary column coated with a 0.25-μm film of polar CBP 20 (0.22 × 50 m; Shimadzu). The temperature of both the injector and the flame ionization detector was 250 °C. The initial column temperature was set at 170 °C and then raised to 225 °C at 5 °C·min^{-1}.

The dinitrobenzoyl derivative of diacylglycerol was analyzed by high-performance liquid chromatography (HPLC) (Tosoh CCPD, Tokyo, Japan) equipped with a 0.45 × 15-cm TSK-gel ODS-80 TM column (Tosoh) using acetonitrile-isopropanol (90 : 10, v/v) as eluent. The flow rate was 0.7 mL·min^{-1}. Several EPA-containing PtdCho species were chemically synthesized as standard PtdCho for HPLC analysis. They were prepared by condensation of 1-acyl-2-lyso-PtdCho with [illegible] eicosapentaenoyl chloride was prepared using EPA and oxalyl chloride [12].

Preparation of lyso-phospholipid

First, 1,2-diEPA-PtdCho was prepared by condensation of l-α-glycerophosphorylcholine with EPA anhydride [13] and purified by silicic acid chromatography and TLC with solvent system A. 1-EPA-2-lyso-PtdCho was prepared by hydrolysis of 1,2-diEPA-PtdCho with phospholipase A_2 [3], and purified by TLC with solvent system A. 1-Alkenyl(alkyl)-2-EPA-PtdCho was prepared by the condensation of 1-alkenyl(alkyl)-2-lyso-PtdCho with EPA anhydride [14]. 1-Alkenyl(alkyl)-2-EPA-PtdCho was subjected to mild acid hydrolysis and resulting 1-lyso-2-EPA-PtdCho was purified by TLC with solvent system A. 1-Lyso-2-acyl-PtdEtn was obtained by acid hydrolysis of 1-alkenyl-2-acyl-PtdEtn of bovine heart, and 1-acyl-2-lyso-PtdEtn was prepared from egg yolk PtdEtn with phospholipase A_2.

Determination of the acyl migration of 1-lyso-2-EPA-PtdCho

It has been pointed out that the acyl group of the *sn*-2 position of lysophospholipid is liable to migrate to the *sn*-1 position to form 1-acyl-2-lyso-PtdCho [15]. Therefore, we checked the extent of the migration of EPA residue in 1-lyso-2-EPA-PtdCho. The lyso-PtdCho was acetylated, treated with phospholipase C and converted to *tert*-butyldimethylsilil derivative for determination of isomer ratio by GC [14]. During 24 h storage at −20 °C after preparation of 1-lyso-2-EPA-PtdCho, the formation of 1-EPA-2-lyso-PtdCho was about 10%. Lysophospholipids were used within 24 h. We also checked the extent of migration under our acyltransferase assay conditions. The migration of EPA residue from *sn*-2 to *sn*-1 during the 10 min incubation was estimated to be 20% at pH 7 at 20 °C. At higher pHs, the migration rate was pronounced (50% at pH 8). On the other hand, it was reduced at acidic pH (10% at pH 6), so the assay was conducted at a slightly acidic pH (pH 6.8) at 20 °C for 10 min

Preparation of membrane fraction of *C. elegans*

The purified *C. elegans* (2 g, wet weight) was suspended in 8 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1.5 mM glutathione, 0.15 M KCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride and 0.25 M sucrose (homogenizing buffer), and subjected to the french press. Because the enzyme activity was impaired above 200 kg·cm^{-2} in preliminary experiments, the french press was conducted at a pressure lower than 200 kg·cm^{-2}. The *C. elegans* homogenate was centrifuged at 11 000 ***g*** for 30 min. The resulting supernatant was further centrifuged at 105 000 ***g*** for 60 min. The pellet was suspended in the homogenizing buffer (omitting EDTA, dithiothreitol and phenylmethanesulfonyl fluoride) and immediately used for the enzyme assay described below. The protein content was estimated by the method of Lowry *et al*. [16] using BSA as a standard.

Preparation of microsome fraction of rat liver

Male Sprague–Dawley rats (250–300 g) were killed, and their livers were perfused with 0.9% NaCl to remove contaminating hemoglobin. The tissue was homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1.5 mM glutathione, [illegible]

was prepared by sequential centrifugation [17]. The final microsomal pellet was suspended in the homogenizing buffer (omitting EDTA) and immediately used for the enzyme assay.

Acyltransferase assay

Each incubation consisted of 32 nmol of lysoPtdCho or lysoPtdEtn, 0.5 mM nicotinamide, 1.5 mM glutathione, 0.15 M KCl, 5 mM $MgCl_2$, 0.25 M sucrose, 7.5 mM ATP, 0.4 mM CoA, 50 mM potassium phosphate buffer (pH 6.8), 0.8 mg protein of the membrane fraction of *C. elegans*, and the desired fatty acid in a total volume of 2.0 mL. The fatty acids were added as a fatty acid/albumin complex [18], and the specific activity was 0.05 μCi/50 nmol. The incubation was conducted at 20 °C for 10 min, and the reaction was stopped by mixing with 7.5 mL of chloroform/methanol (1 : 2, v/v). The lipids were extracted by the method of Bligh and Dyer [9], and PtdCho or PtdEtn was isolated by TLC with solvent system A. The isolated phospholipids were hydrolyzed with phospholipase A_2, and the resulting lysophospholipids and free fatty acid were separated by TLC [14]. Each zone of silica gel was scraped off the plate and radioactivity was determined. The assay for acyltransferase activity of the rat liver microsomes was conducted similarly, except for the temperature of the incubation (37 °C), microsomal protein (0.1 mg), and the total volume of the incubation (1 mL).

AcylCoA synthetase assay

The incubation was conducted in the same manner as the assay for acyltransferase except for the omission of lysophospholipid from the reaction mixture. After incubation, 10 mL of isopropanol/*n*-heptane/1 M sulfuric acid (40 : 10 : 1, v/v) was added. Then, 2 mL of water and 10 mL of *n*-heptane were added for phase separation. The lower phase was washed twice with 10 mL of *n*-heptane, and radioactivity of the lower phase was measured to determine acylCoA formation.

RESULTS

Effect of growth temperature on the EPA-containing molecular species composition of PtdCho

The monoacetyldiglyceride derivative obtained from the PtdCho of *C. elegans* was separated on AgTLC by degree of unsaturation (total unsaturation 10–0). Typical results of fatty acid analyses of fractions 1 (total unsaturation 10) through 6 (total unsaturation 5) are shown in Table 1. Because of the tailing of monoacetyldiglycerides on AgTLC plate, the streaked monoacetyldiglycerides contaminated to the next fraction. Therefore, the number of nmol of EPA detected as fatty acid was slightly higher than the sum of the number of nmol of counterpart fatty acids in each fraction except for fraction 1. The PtdCho of *C. elegans* contained substantial amounts of 1,2-diPUFA-PtdChos, such as 20 : 4(n-6)/20 : 5 and 20 : 5/20 : 5 which are unusual phospholipids in higher animals. Among them, 20 : 5/20 : 5-PtdCho was significantly increased in *C. elegans* grown at 15 °C (Fig. 1).

We also investigated the molecular species composition of PtdCho as dinitrobenzoyl derivative by HPLC. Many peaks were detected, and peaks corresponding to dinitrobenzoyl derivative of 18 : 0/20 : 5, 18 : 1/20 : 5, 20 : 4/20 : 5 and 20 : 5/20 : 5 were identified by comparison with synthetic

Table 1. Typical results of molecular species analysis of PtdCho of *C. elegans* grown at 15 °C by AgTLC coupled with GC. PtdCho from *C. elegans* was converted to monoacetyldiacylglyceride derivative and fractionated by AgTLC. Fatty acids in each fractions were determined by GC using 20 : 0 as internal standard.

Fraction No.	Degree of unsaturation	Fatty acid detected	(nmol)	Possible molecular species	(nmol)
1	10	20 : 5(n-3)	163	20 : 5(n-3)/20 : 5(n-3)	82
2	9	20 : 5(n-3)	54	20 : 4(n-3)/20 : 5(n-3)	30
		20 : 4(n-3)	30	20 : 4(n-6)/20 : 5(n-3)	23
		20 : 4(n-6)	23		
3	8	20 : 5(n-3)	30	20 : 3(n-6)/20 : 5(n-3)	22
		20 : 4(n-3)	10	20 : 4/20 : 4	
		20 : 4(n-6)	8		
		20 : 3(n-6)	22		
4	7	20 : 5(n-3)	55	18 : 2(n-6)/20 : 5(n-3)	32
		20 : 4(n-3)	6	20 : 3(n-6)/20 : 4(n-3)	
		20 : 3(n-6)	3		
		18 : 2(n-6)	32		
5	6	20 : 5(n-3)	104	18 : 1(n-7)/20 : 5(n-3)	71
		20 : 4(n-3)	9	18 : 1(n-9)/20 : 5(n-3)	13
		18 : 2(n-6)	8	18 : 2(n-6)/20 : 4(n-3)	
		18 : 1(n-7)	71		
		18 : 1(n-9)	13		
6	5	20 : 5(n-3)	167	16 : 0/20 : 5(n-3)	11
		20 : 4(n-3)	15	18 : 0/20 : 5(n-3)	76
		20 : 4(n-6)	5	19 : Δ/20 : 5(n-3)	12
		20 : 3(n-6)	8	17 : 0iso/20 : 5(n-3)	13
		18 : 2(n-6)	7	16 : 0iso/20 : 5(n-3)	7
		18 : 1(n-7)	12	17 : 0/20 : 5(n-3)	5
		18 : 1(n-9)	5	17 : Δ/20 : 5(n-3)	7
		19 : Δ[a]	12	18 : 2(n-6)/20 : 3(n-6)	
		18 : 0	76	18 : 1/20 : 4	
		17 : Δ[b]	7		
		17 : 0	5		
		17 : 0iso	13		
		16 : 0	11		
		16 : 0iso	7		

[a] 19:Δ, *cis*-11, 12-methyleneoctadecanoic acid. [b] 17:Δ, *cis*-9, 10-methylenehexadecanoic acid.

molecular species of *C. elegans* grown at 25 °C and 15 °C were 6.3 ± 1.2% and 10.0 ± 1.9%, respectively.

Positional distribution of fatty acid in PtdCho and PtdEtn of *C. elegans*

In *C. elegans*, PtdEtn had a preponderance of PUFAs at the *sn*-2 position (Table 2). In contrast, substantial amounts of PUFAs were found at the *sn*-1 position of PtdCho (Table 2). This indicates that the positional distribution of fatty acid in PtdCho molecule of *C. elegans* is not so strictly regulated as the phospholipids of higher animals, which show a strict PUFA-distribution toward the *sn*-2 position. Therefore, the mechanism that regulates the positional distribution of PUFA of PtdCho may be different from that of PtdEtn in *C. elegans*.

Substrate specificity of acylCoA: lysophospholipid acyltransferase activity

To examine the possibility that the acylCoA:lysophospholipid acyltransferase involved in the deacylation/reacylation of phos-

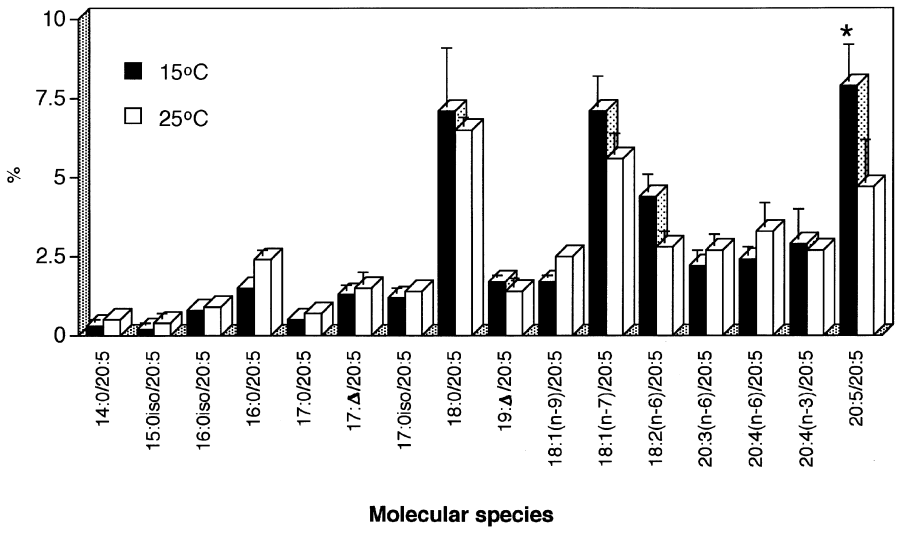

Fig. 1. Effect of growth temperature on the proportion of EPA-containing PtdCho from *C. elegans* analyzed by AgTLC coupled with GC. The PtdCho from *C. elegans* grown at 25 °C and 15 °C was converted to monoacetyldiacylglyceride derivative and fractionated by AgTLC. The fatty acid analysis of each fraction was conducted by GC. Values are percentage of total molecular species of PtdCho and means ± SD (three harvests of nematodes grown at 15 °C and 25 °C, respectively). * Indicates significantly different from corresponding molecular species grown at 25 °C using Student's *t*-test ($P < 0.05$).

investigated the substrate specificity of this enzyme of *C. elegans.* We also compared the enzyme activity to that in rat liver. Because EPA is an abundant fatty acid at both *sn*-1 and *sn*-2 of PtdCho of *C. elegans* (Table 2), the acyl acceptor used in this study was 1-lyso-2-EPA-PtdCho or 1-EPA-2-lyso-PtdCho. When 1-lyso-2-EPA-PtdCho was used as the acyl acceptor, stearic acid and EPA were incorporated into the *sn*-1 position of PtdCho in a dose-dependent manner. The saturated level was around 2 nmol per 10 min·mg^{-1} protein in both fatty acids (Fig. 2A, Table 3). In contrast, the level of acylation of 1-EPA-2-lyso-PtdCho with EPA was over 10 times that with stearic acid at any concentration of the fatty acid (Fig. 2B). The substrate specificity of acyltransferase activity of the *C. elegans* membrane fraction was compared with that of rat liver microsomes using fixed concentrations of stearic acid, linoleic acid and EPA. When the 1-EPA-2-lyso-PtdCho was used as acyl acceptor, the acyltransferase activity of rat liver microsomes acylated linoleic acid and EPA to the *sn*-2 position of PtdCho more effectively than stearic acid (Table 4). Likewise, the acyltransferase activity of the *C. elegans* membrane fraction preferred PUFA to stearic acid for acylation

Table 2. Positional distribution of fatty acid in PtdCho and PtdEtn of *C. elegans*. PtdCho or PtdEtn of *C. elegans* grown at 20 °C was hydrolyzed with phospholipase A_2, and fatty acid composition of resulting free fatty acid and lysophospholipid fractions was analyzed by GC. ND, not detected.

	PC (%)		PE (%)	
Fatty acid	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14 : 0	1.1 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	1.0 ± 0.1
15 : 0iso	1.5 ± 0.2	Trace	0.9 ± 0.3	Trace
15 : 0ante	0.6 ± 0.1	Trace	Trace	Trace
16 : 0iso	2.3 ± 0.3	0.5 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
16 : 0	5.1 ± 1.1	2.0 ± 0.5	9.1 ± 1.7	3.9 ± 1.8
16 : 1(*n*-7)	1.0 ± 0.4	1.0 ± 0.4	1.9 ± 0.9	1.2 ± 0.9
17 : 0iso	2.9 ± 0.3	0.7 ± 0.1	6.3 ± 1.4	1.1 ± 0.3
17 : 0	1.2 ± 0.1	Trace	3.3 ± 0.1	Trace
17 : Δ[a]	6.2 ± 2.4	1.2 ± 0.2	4.7 ± 1.0	2.3 ± 0.6
18 : 0DMA[b]	Trace	ND	14.6 ± 3.1	ND
18 : 0	5.4 ± 0.3	1.4 ± 0.2	20.4 ± 1.7	1.6 ± 0.6
18 : 1(*n*-9)	3.9 ± 0.8	3.8 ± 0.5	2.4 ± 0.8	3.8 ± 0.8
18 : 1(*n*-7)	16.4 ± 0.4	5.0 ± 0.8	11.7 ± 0.6	24.4 ± 4.6
18 : 2(*n*-6)	7.8 ± 0.6	10.3 ± 0.9	1.9 ± 0.8	14.1 ± 1.5
19 : Δ[c]	5.3 ± 1.1	1.3 ± 0.2	1.6 ± 0.5	1.6 ± 0.1
20 : 3(*n*-6)	1.5 ± 0.5	6.7 ± 0.5	1.1 ± 0.1	3.8 ± 0.6
20 : 4(*n*-6)	3.7 ± 1.3	7.6 ± 0.5	0.2 ± 0.4	2.0 ± 1.7
20 : 4(*n*-3)	4.0 ± 0.9	7.8 ± 0.9	0.4 ± 0.5	3.9 ± 1.0
20 : 5(*n*-3)	13.8 ± 5.2	42.9 ± 4.0	0.6 ± 0.2	17.5 ± 2.5

Table 3. Incorporation of various fatty acids into exogenously added lysoPtdCho and lysoPtdEtn in *C. elegans* membrane fraction. The incubation was conducted at 20 °C for 10 min with 0.8 mg protein of *C. elegans* membrane fraction and 50 nmol of fatty acid. The acyl acceptors used were 32 nmol (a)1-lyso-2-EPA-PtdCho, (b)1-EPA-2-lyso-PtdCho, (c) 1-lyso-2-acyl-PtdEtn, and (d)1-acyl-2-lyso-PtdEtn. Values are means ± SD (three harvests of nematodes grown at 20 °C).

	Acylation (nmol·10 min^{-1}·mg^{-1} protein)			
	To lysoPtdCho		To lysoPtdEtn	
Fatty acid	*sn*-1[a]	*sn*-2[b]	*sn*-1[c]	*sn*-2[d]
Stearic acid	1.8 ± 0.2	0.9 ± 0.4	2.3 ± 0.7	0.1 ± 0.1
Linoleic acid	3.4 ± 0.5	11.6 ± 1.5	2.2 ± 0.4	0.5 ± 0.2
EPA	2.1 ± 0.5	18.3 ± 3.8	0.9 ± 0.4	0.4 ± 0.2

Table 4. Incorporation of various fatty acids into exogenously added lysoPtdCho in rat liver microsomal fraction. The incubation was conducted at 37 °C for 10 min with 0.1 mg protein of rat liver microsome and 50 nmol fatty acid. The acyl acceptors used were 32 nmol: (a) 1-lyso-2-EPA-PtdCho, (b) 1-EPA-2-lyso-PtdCho, (c) 1-lyso-2-acyl-PtdEtn, and (d) 1-acyl-2-lyso-PtdEtn. Values are means ± SD (three microsome preparations from different rats).

	Acylation (nmol·10 min^{-1}·mg^{-1} protein)			
	To lysoPtdCho		To lysoPtdEtn	
Fatty acid	*sn*-1[a]	*sn*-2[b]	*sn*-1[c]	*sn*-2[d]
Stearic acid	182.3 ± 17.7	8.6 ± 2.1	111.5 ± 1.8	1.6 ± 0.6
Linoleic acid	19.1 ± 1.4	135.7 ± 7.8	10.3 ± 3.6	7.9 ± 0.7

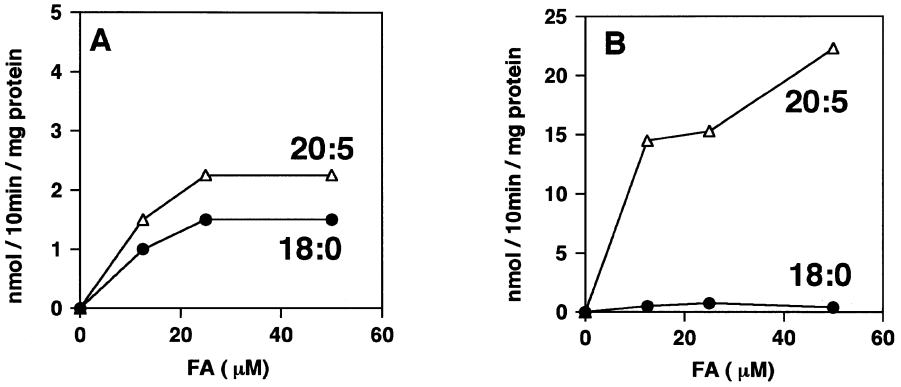

Fig. 2. Substrate concentration-dependent incorporation of stearic acid and EPA into 1-lyso-2-EPA-PtdCho (A) and 1-EPA-2-lyso-PtdCho (B). The incubation was conducted at 20 °C for 10 min with 0.8 mg protein of *C. elegans* membrane fraction, 32 nmol of lysoPtdCho and increasing amounts of stearic acid or EPA. The PtdCho extracted from reaction mixture was hydrolyzed with phospholipase A_2 and radioactivity of lysoPtdCho (1-lyso-2-EPA-PtdCho as acyl acceptor) or free fatty acid (1-EPA-2-lyso-PtdCho as acyl acceptor) was determined.

to the *sn*-2 position of PtdCho (Table 3). When 1-lyso-2-EPA-PtdCho was used as the acyl acceptor, a quite distinct preference for stearic acid over linoleic acid and EPA for acylation to *sn*-1 of PtdCho was observed with rat liver microsomes (Table 4). In contrast, the acyltransferase activity of *C. elegans* did not exhibit such a strict preference to stearic acid for acylation to the *sn*-1 position of PtdCho. The efficacy of the acylation was highest with linoleic acid, and that of stearic acid and EPA was almost equal (Table 3).

When 1-lyso-2-acyl-PtdEtn was used as acyl acceptor, the level of incorporation of fatty acid into the *sn*-1 position of PtdEtn was higher with stearic acid and linoleic acid, and EPA was a poor acyl donor in *C. elegans* preparation (Table 3). These results with 1-lyso-2-acyl-PtdEtn differed from those obtained with 1-lyso-2-EPA-PtdCho as the acyl acceptor in *C. elegans*. Both in *C. elegans* and rat liver preparations, acylation of the *sn*-2 of PtdEtn did not occur to a significant extent compared to that of the *sn*-2 of PtdCho. These results with rat liver microsomes were consistent with the results using rat liver hepatocytes [19,20].

Substrate specificity of acylCoA synthetase of *C. elegans*

In the acyltransferase assay used in this study, the added fatty acid was first converted to acylCoA by acylCoA synthetase, and then was transferred to lysophospholipid. Therefore, the substrate specificity of acylCoA synthetase affects the acyltransferase activity. The conversion rates of EPA and stearic acid to its CoA form were 11.1 ± 2.9 and 8.0 ± 3.3 nmol·10 min^{-1} mg^{-1} protein, respectively. The difference was statistically insignificant.

DISCUSSION

Previously, we showed that the nematode *C. elegans* contained abundant EPA [2]. Our recent investigation revealed that palmitic, oleic, dihomo-γ-linolenic and arachidonic acid were decreased and linoleic acid and EPA were increased in the PtdCho fraction when *C. elegans* was grown at a low temperature [3]. In this study, we investigated the effect of growth temperature on the molecular species composition of PtdCho of *C. elegans*. The AgTLC system and subsequent GC analysis showed the existence of as many as 16 molecular species of EPA-containing PtdCho in *C. elegans*. Consistent with our previous report, proportions of EPA-containing PtdCho molecules with palmitic, oleic, dihomo-γ-linolenic and arachidonic acid all tended to decrease, and 1,2-diEPA-PtdCho increased significantly at low growth temperature. The 1,2-diPUFA-PtdCho such as 1,2-diEPA-

In the fish species carp, it has been reported that the proportion of oleic acid at position *sn*-1 of PtdEtn was increased at low temperature and 18 : 1(n-9)/22 : 6(n-3)-PtdEtn exerted a membrane disordering effect [21]. In *C. elegans*, the extent of the change in the fatty acid composition due to growth temperature was greater in PtdCho than that in PtdEtn [3], and 1,2-diEPA molecular species were only detected in the PtdCho fraction. Therefore, 1,2-diEPA-PtdCho might be one of the molecules that plays a role in the regulation of physiological properties of *C. elegans* membranes.

The asymmetrical distribution of fatty acids in mammalian phospholipid is due to the substrate specificity of acyltransferases involved in the phospholipid biosynthesis, by either the *de-novo* or the remodeling route. For example, it has been shown that linoleic acid and docosahexaenoic acid are considered to be utilized primarily for *de novo* synthesis and arachidonic acid is reported to enter through the remodeling pathway for acylation at *sn*-2 [22–25]. In *C. elegans*, PUFAs in PtdCho (but not PtdEtn) do not show a strict asymmetrical distribution. In the other free-living nematode species *Tubatrix aceti*, PUFAs such as linoleic acid and dihomo-γ-linolenic acid are found in *sn*-1 position of PtdCho, whereas fatty acid of PtdEtn shows strict asymmetrical distribution like mammalian phospholipid [26]. The difference in the pattern of PUFA distribution between *C. elegans* PtdCho and mammalian PtdCho or *C. elegans* PtdEtn could be partially explained by the nature of acyltransferase involved in the remodeling of the respective phospholipid at *sn*-1. The most notable difference in the enzyme activity between the *C. elegans* and rat liver was that the acyltransferase of *C. elegans* did not show the strict preference for stearic acid for acylation to the *sn*-1 position of PtdCho. In *C. elegans,* the efficacy of utilization of EPA for acylation to *sn*-1 of PtdCho was almost equal to that of stearic acid. In contrast, the acylation of *sn*-1 of PtdEtn tended to occur with less unsaturated fatty acid in *C. elegans* preparations. These results are consistent with the fact that EPA was found abundantly in *sn*-1 of PtdCho and stearic acid was the predominant fatty acid in *sn*-1 of PtdEtn (Table 2).

In experiments using rat hepatocytes, it has been reported that phospholipids with PUFAs both in the *sn*-1 and *sn*-2 position were formed by the *de novo* pathway when the cells were incubated in relatively higher concentrations of PUFA [27–29]. However, the PUFA residue in *sn*-1 of the 1,2-diPUFA-phospholipid was then replaced mainly with stearic acid by the remodeling pathway during the subsequent incubation without exogenous PUFA [30–32]. This indicates that even in higher animals the *de-novo*

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