マダラのホスファチジルコリン及びホスファチジルエタノールアミンの脂肪酸の組合せ

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Estimation of Possible Fatty Acid Combinations in Phosphatidylcholine and Phosphatidylethanolamine of Cod

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Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of cod *Gadus morhua* were separated by HPLC and analyzed for the fatty acid compositions and the compositions based on total carbon in fatty acyl chains by GLC. From the results obtained, the possible fatty acid combinations of PC and PE were estimated.

Without regard to the number of double bonds in the fatty acid, eight possible fatty acid combinations: (C_{16}, C_{22}), (C_{16}, C_{20}), (C_{18}, C_{18}), (C_{20}, C_{20}), (C_{20}, C_{22}), (C_{18}, C_{22}), (C_{22}, C_{22}), and (C_{14}, C_{20}), were estimated for the PC. The combination of (C_{16}, C_{22}) was most prominent, accounting for 44.2% in the PC. Similarly, nine possible fatty acid combinations: (C_{18}, C_{22}), (C_{16}, C_{22}), (C_{20}, C_{20}), (C_{20}, C_{22}), (C_{18}, C_{20}), (C_{22}, C_{22}), (C_{14}, C_{22}), (C_{16}, C_{20}), and (C_{18}, C_{18}), were estimated for PE. The combination of (C_{18}, C_{22}) was most prominent, accounting for 23.5% in the PE.

From the fatty acid compositions, the major fatty acid combinations of PC and PE were tentatively estimated to be as follows: ($C_{16:0}$, $C_{22:6}$), ($C_{16:0}$, $C_{20:5}$), ($C_{18:1}$, $C_{18:1}$), ($C_{20:5}$, $C_{20:5}$), ($C_{20:5}$, $C_{22:6}$), ($C_{18:1}$, $C_{22:6}$), ($C_{22:6}$, $C_{22:6}$), and ($C_{14:0}$, $C_{20:5}$) for PC and ($C_{18:1}$, $C_{22:6}$), ($C_{16:0}$, $C_{22:6}$), ($C_{20:5}$, $C_{20:5}$), ($C_{20:5}$, $C_{22:6}$), ($C_{18:1}$, $C_{20:5}$), ($C_{22:6}$, $C_{22:6}$), ($C_{14:0}$, $C_{22:6}$), ($C_{16:0}$, $C_{20:5}$), and ($C_{18:1}$, $C_{18:1}$) for PE.

Thin layer chromatography (TLC), gas liquid chromatography (GLC), and liquid liquid chromatography (LLC) are current methods for phospholipid analysis. TLC impregnated with silver nitrate was used successfully for separation of phospholipids of bovine liver[1] and *Escherichia coli*[2] according to their degree of unsaturation. Egg yolk and rat liver phospholipids were also separated into four fractions of different fatty acid composition by LLC on hydroxypropyl Sephadex with hydroxyalkyl (C_{11}-C_{14}) group.[3]

In recent years, high-performance liquid chromatography (HPLC) is widely used for the separation of many naturally occurring compounds. PORTER *et al.*[4] and CRAWFORD *et al.*[5] separated egg yolk and soy bean phosphatidylcholine (PC) using HPLC on reversed phase column and estimated the molecular species of these phospholipids. More recently, SMITH and JUNGALWALA[6] reported that PC species from egg, bovine liver, and porcine liver were resolved into 11 to 13 separated peaks by HPLC. PATTON *et al.*[7] isolated rat liver phospholipids into individual molecular species of 30 to 35 without any derivatization of phospholipid using HPLC on reversed phase column. However, application of the HPLC proposed by PATTON *et al.* to fish phospholipid analysis was considered difficult, because the fish phospholipids are composed of a great variety of fatty acids in regard to chain length and number of double bond.

The present study was carried out in order to search the procedure which would provide detailed information concerning the molecular species of fish phospholipids. Phosphatidylcholine and phosphatidylethanolamine (PE), major phospholipids of cod *Gadus morhua*, were isolated by HPLC and analyzed by GLC. From the results obtained, possible fatty acid combinations of PC and PE were tentatively estimated. In the estimation, all unsaturated fatty acids were treated as corresponding saturated fatty acids.

Materials and Methods

Sample

Cod *Gadus morhua* was obtained from Tokyo whole-sale market and kept at −70°C until use.

Extraction and Fractionation of Lipid

Total lipid (TL) was extracted from the minced flesh of cod with chloroform- MeOH according to the method of BLIGH and DYER.[8] Polar lipid fraction (PL) was separated from the TL by

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column chromatography on Biobeads S-X2[9] (65 cm×2.6 cm i.d.) using benzene as a solvent at a flow rate of 70 ml/h.

HPLC for Separation of Phospholipids

To separate PL into phospholipid classes, the PL dissolved in chloroform was subjected to HPLC (Shimadzu LC-3A) on Partisil 10 SCX column (25 cm×4.6 mm i.d., Whatman Inc.), using a mixture of CH_3CN/MeOH/water (60/30/7, v/v) as a mobile phase at a flow rate of 1.5 ml/min. The column and a reflactive index detector were held at 40°C by means of circulated water. PC and PE fractions were collected separately by repeated HPLC.

Hydrolysis of PC and PE by Phospholipase C

PC and PE fractions collected were hydrolyzed by phospholipase C (EC 3.1.4.3.) and 1,2-diglycerides (1,2-DG) formed were isolated from the hydrolyzate according to the method of HANAHAN and VERCAMER.[10] The PC or PE fraction was dissolved in 1 ml of a mixture of EtOH/ether (2/98, v/v). To the solution 1.5 ml of 0.1 M Tris-HCl buffer (pH 7.2) and 0.5 ml of 20 mM $CaCl_2$ were added. The mixture was homogenized for 30 sec at 35°C. Phospholipase C from *Bacillus cereus* (Boehringer Mannheim GmbH, G.F.R.) which was 1,000 units/mg in specific activity was suspended in 10 volume of Tris-HCl buffer. To the sample mixture 0.3 ml of this enzyme suspension was added. After blending vigorously for 30 sec, the mixture was kept at 35°C for 90 min. The 1,2-DG formed was extracted from the reaction mixture with a mixture of chloroform/MeOH/water (1/1/0.8, v/v) and the separated chloroform layer was evaporated to a small volume. The concentrated extract was spotted on a silica gel H plate and developed in a mixture of n-hexane/Et_2O/AcOH (80/30/1, v/v). The 1,2-DG was visualized by spraying 0.2% 2′,7′-dichlorofluorescein in EtOH under UV light, scraped off from the developed plate, and extracted with a mixture of MeOH/chloroform (95/5, v/v).

GLC for Analysis of 1,2-DG Compositions

Prior to subject to GLC, the 1,2-DG formed by the action of phospholipase C was hydrogenated in the presence of platinum black catalyst and then converted into trimethyl silyl (TMS)-derivatives using both hexamethyldisilazane and trimethylchlorosilane in dry pyridine. The TMS-derivatives of 1,2-DG was subjected to GLC to analyze total carbons of their fatty acyl chains. Shimadzu GC-4BM gas chromatograph equipped with a flame ionization detector (FID) and a glass column (50 cm×3 mm i.e.) packed with 1.5% OV-1 on Shimalite W (AW, DMCS, 80–100 mesh) was used. Column temperature was programmed from 230° to 300°C at a rate of 2°C/min.

GLC for Analysis of Fatty Acid Composition

Fatty acid composition of lipid was analyzed by GLC. Fatty acid methyl esters of lipids obtained by interesterification in 14% BF_3-MeOH were subjected to GLC (Shimadzu GC-6A) equipped with a FID and a glass column (2 m×3 mm i.d.) packed with 20% DEGS on Chromosorb P (AW, DMCS, 60–80 mesh). Column temperature was held at 195°C.

TLC-Densitometry of Phospholipid

The PL separated with column chromatography was analyzed by TLC. The silica gel G plate was developed in a solvent system of chloroform/MeOH/water/28% ammonia (130/70/8/0.5, v/v), charred at 130°C for 20 min after spraying 50% sulfuric acid, and subjected to densitometry using Shimadzu TLC Chromatoscanner CS-920. Under the TLC conditions used, lysophosphatidylcholine (LPC), sphingomyelin (SPM), and PE were well separated but PC and phosphatidylserine (PS) could not be resolved. Therefore, the PC and PS were scraped off from the plate and rechromatographed on silica gel G plate using a mixture of chloroform/Et_2O/MeOH/AcOH/water (100/40/20/20/10, v/v) as a solvent system.

Standard

Phosphatidylcholine, PE, LPC, SPM, dipalmitin, distealin, and cholesteryl acetate were purchased from Sigma Chemical Company. Phosphatidylserine was from Serdary Research Lab. Diarachidin and dibehenin were from Nu Chek Prep, Inc. (Copenhagen, Denmark).

Results and Discussion

Lipid Content and Fatty Acid Composition

The TL content of cod was determined to be 850 mg/100 g of the flesh. The PL content was 570 mg/100 g of the flesh accounding for 67% of TL.

The fatty acid compostions of TL, NL, and PL were shown in Table 1. Polyunsaturated fatty

Table 1. Fatty acid compositions of total lipid, polar lipid, and non-polar lipid of cod

(wt%)

Fatty acid	TL*1	PL*2	NL*3
14:0	1.5	1.3	3.2
16:0	24.9	24.8	26.2
:1	3.9	3.3	6.8
:2	0.2	0.3	0.3
17:0	—	0.5	—
18:0	4.2	4.3	6.5
:1	13.5	12.9	15.2
:2	0.7	0.8	1.4
:3	1.7	1.4	2.6
20:0	0.2	0.1	tr
:2	0.4	0.3	2.7
:4	2.7	2.2	1.0
:5	20.5	19.9	17.9
22:4	0.2	0.8	tr
:5	1.2	1.8	tr
:6	24.2	25.3	16.2
Total	100.0	100.0	100.0

*1 TL: total lipid.
*2 PL: polar lipid.
*3 NL: non-polar lipid.

acids such as $C_{20:5}$ and $C_{22:6}$ and $C_{18:1}$ fatty acid were contained at higher level in all lipid classes. Both $C_{16:0}$ and $C_{18:1}$ fatty acids were also prominent. The fatty acid composition of TL coincided essentially with those reported by ACKMAN and BURGHER[11] and GRUGER.[12]

Phospholipid Composition

In the TLC using chloroform/MeOH/water/28% ammonia (130/70/8/0.5, v/v) as a solvent system, PC and PS could not be separated, as mentioned above. Therefore, a mixture of chloroform/Et_2O/MeOH/AcOH/water (100/40/20/20/10, v/v) was used as a solvent system to separate PC and PS. The results obtained are shown in Fig. 1 and Table 2. The prominent phospholipid classes were PE and PC, though, their contents were slightly lower than those reported by ROUBAL[13] on the lipid of Pacific cod *Gadus macrocephalus*.

Separation of PC and PE Fraction with HPLC

As shown in Fig. 2, the cod phospholipids were separated into two fractions by HPLC on Partisil 10 SCX column. From the comparison of their retention times and Rf values in the TLC with those of pure references compounds, the peaks at about 5 min and 16 min were identified as those of PE and PC, respectively. PS and LPC were not detected under the experimental conditions used. This seems to be due to small amounts of these phospholipids in PL as shown in Table 2. GROSS and SOBEL[14] reported that PE and PS of rabbit myocardial lipid were not separated completely in the HPLC on the same column. In our

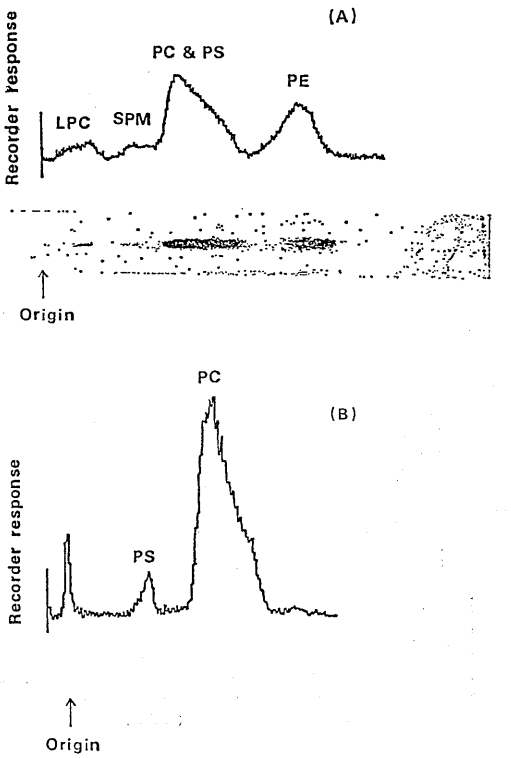

Fig. 1. Thin layer chromatograms of cod phospholipid recorded with a densitometer.
A: sample, PL of cod; solvent system, chloroform/MeOH/water/28% ammonia (130/70/8/0.5, v/v).
B: sample, fraction of PC and PS shown in chromatogram (A); solvent system, chloroform/Et_2O/MeOH/AcOH/water (100/40/20/20/10, v/v).

Table 2. Phospholipid composition of cod determined with densitometry

Phospholipid*	Content (%)
LPC	2.7
SPM	2.3
PC	54.2
PS	6.4
PE	34.5
Total	100.1

* LPC: lysophosphatidylcholine, SPM: sphingomyelin, PC: phosphatidylcholine, PS: phosphatidylserine, PE: phosphatidylethanolamine.

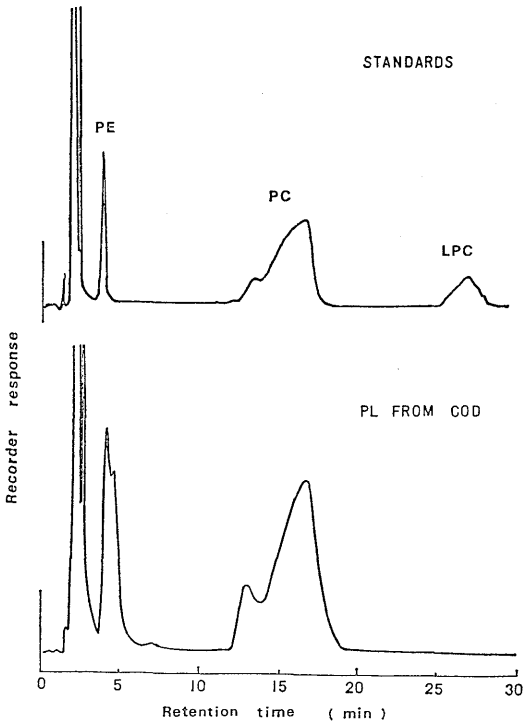

Fig. 2. Elution patterns of phospholipids of cod and standard mixture in HPLC.

solvent system, however, PS was eluted at a solvent front. Each PC and PE collected separately by means of repeated HPLC showed only one spot by two-dimensional development on silica gel G plate: 1st dimension with a solvent system of chloroform/MeOH/water/28% ammonia (130/70/8/0.5, v/v) and second one with that of chloroform/Et_2O/MeOH/AcOH/water (100/40/20/20/10, v/v).

In the past, column chromatography on silicic acid[15] or ion exchange column chromatography on DEAE-cellulose[16] has been widely used for the separation of phospholipid classes. These chromatographic techniques are, however, time-consuming and troublesome and are not able to separate PE from PS completely. From this point of view, HPLC on Partisil 10 SCX column seems to be convenient method for the analysis of PE and PC.

PC and PE Compositions on the Basis of Total Carbon in Fatty Acyl Chains

In the GLC analysis of TMS-DG, it was found that the peak ascribed to the TMS-DG containing double bonds in fatty acyl chains such as TMS-diolein appeared faster than that containing no double bond such as TMS-distearin on the chromatogram under the same analytical conditions. However, the influence of degree of unsaturation on the retention time of TMS-DG in GLC analysis was not clear. In the present study, therefore, all DG derived from PC and PE with the action of phospholipase C were catalytically hydrogenated prior to GLC analysis.

Relationship between the amount of TMS-DG injected to GLC and the area of peak appeared on the chromatogram was examined using various standard DG prior to GLC analysis of the DG prepared from cod phospholipids. Standard dipalmitin, distearin, diarachidin and dibehenin were dissolved in chloroform in such a way that all diglycerides give the same concentration. Aliquot samples of this solution were subjected to trimethylsilylation. After the derivatization, the sample was injected to GLC under various conditions and the ratios of peak area of distearin, diarachidin, and dibehenin to that of dipalmitin which was regarded as an internal standard were calculated. The results obtained are shown in Table 3. In general, the ratios of peak areas of TMS-dibehenin to that of TMS-dipalmitin were smaller than those of the other TMS-DG. This tendency seemed to be marked when a freshly prepared GLC column was used. Therefore, in the GLC analysis of cod phospholipids, the diglyceride compositions were calculated from the peak areas using correction factors which were obtained using various standard DG.

PC and PE compositions on the basis of the total carbons thus obtained are shown in Fig. 3 and Table 4. The total carbons in fatty acyl chains of PC and PE were from 34 to 44 and from 36 to 44, respectively. The most prominent components of PC and PE were those having total carbons of 38 and 40, respectively.

Fatty Acid Compositions of PC and PE

The fatty acid compositions of cod PC and PE were determined before and after catalytic hydrogenation and are shown in Table 5. In the fatty acid composition of PC, $C_{16:0}$, $C_{18:1}$ $C_{20:5}$, and $C_{22:6}$ fatty acids were major ones. Similar fatty acid composition was obtained on the PE, although the PE was different from the PC in the fatty acid which showed a highest percentage: $C_{16:0}$ in PC and $C_{22:6}$ in PE.

In both PC and PE, the fatty acid compositions determined before hydrogenation did not completely agree with those after hydrogenation. For example, percentage of C_{16}-fatty acids, $C_{16:0}$,

Table 3. Ratios of peak areas of trimethyl silylated-distearin, -diarachidin, and -dibehenin to that of trimethyl silylated-dipalmitin in the GLC analysis

Column temp.	Number	Standard 1*2				Standard 2*2			
		32	36	40	44	32	36	40	44
220–300°C	1	1	1.03	1.05	0.96	1	1.03	1.05	1.15
Column A*1	2	1	1.02	1.05	0.98	1	1.02	1.05	0.99
	3	1	1.01	1.05	0.95	1	1.03	1.07	1.00
	4	1	1.07	1.02	0.99	1	1.10	1.05	1.05
	5	1	1.09	1.02	0.99	—	—	—	—
	6	1	1.07	1.03	1.03	1	1.06	1.03	1.02
	7	1	1.07	1.02	1.01	1	1.07	1.02	0.99
230–300°C	1	1	1.00	1.01	0.92	1	1.01	1.03	0.95
Column A*1	2	1	0.99	1.02	0.89	1	1.02	1.02	0.95
	3	1	1.02	1.02	0.93	—	—	—	—
	4	1	1.02	0.99	0.95	1	1.01	1.02	0.95
	5	1	1.05	1.01	0.98	1	1.06	1.00	0.99
	6	1	1.08	1.03	1.02	1	1.07	0.99	1.01
	7	1	1.07	1.02	1.00	1	1.07	1.02	1.01
	8	1	1.06	1.03	0.99	1	1.06	1.02	1.00
220–300°C	1	1	0.98	0.99	0.89	1	0.98	0.94	0.81
Column B*1	2	1	0.98	1.01	0.92	1	0.98	0.97	0.88
	3	1	1.06	1.03	1.00	1	1.12	1.05	0.95
230–300°C	1	1	1.07	1.07	0.83	1	0.99	1.01	0.88
Column B*1	2	1	1.01	1.02	0.85	1	1.00	1.00	0.87
	3	1	0.89	1.02	0.93	1	1.01	1.02	0.89
240–300°C	1	1	1.03	1.05	0.89	1	1.02	1.01	0.85
Column B*1	2	1	1.01	1.04	0.92	1	1.04	1.04	0.88

*1 Column A and column B is used after conditioning for 36 hr and 18 hr at 340°C, respectively.
*2 Standard 1 and standard 2 have the same composition of diglycerides, though they are TMS-derivatized separately.

Table 4. Phosphatidylcholine and phosphatidylethanolamine compositions on the basis of total carbon in fatty acyl chains

(mol %)

Total carbon in fatty acyl chains	PC*1	PE*2
34	1.7	tr
36	33.9	10.1
38	44.2	31.7
40	12.2	35.9
42	5.6	12.4
44	2.3	9.8
Total	99.9	99.9

*1 PC: phosphatidylcholine.
*2 PE: phosphatidylethanolamine.

$C_{16:1}$, and $C_{16:2}$ in the fatty acid composition before hydrogenation amounted to 37.1%, differing from 35.0% of $C_{16:0}$ fatty acid determined after hydrogenation. The fatty acid compositions determined after hydrogenation seem to be more reliable than those determined before hydrogenation, because the errors introduced from the integration of peak areas are considered to be smaller in the chromatogram of the hydrogenated samples which revealed a few peaks with shorter retention times. Therefore, the fatty acid compositions obtained after hydrogenation were used to estimate possible fatty acid combinations in cod PC and PE.

Estimation of Fatty Acid Combinations in PC and PE

Possible fatty acid combinations of cod PC and PE were estimated from the fatty acid compositions obtained on the hydrogenated samples (Table 5) and phospholipid compositions based on the total carbons in fatty acyl chains (Table 4) and shown in Table 6. The estimation method, when the PC is used as an example, is as follows. The PC was composed of six components with total carbons of 34 (1.7%), 36 (33.9%), 38 (44.2%), 40 (12.2%), 42 (5.6%), and 44 (2.3%) in their fatty acyl chains and the fatty acid compositions of this PC in regard to carbon numbers were as follows; C_{14} (3.1%), C_{16} (35.0%), C_{18} (11.3%), C_{20}

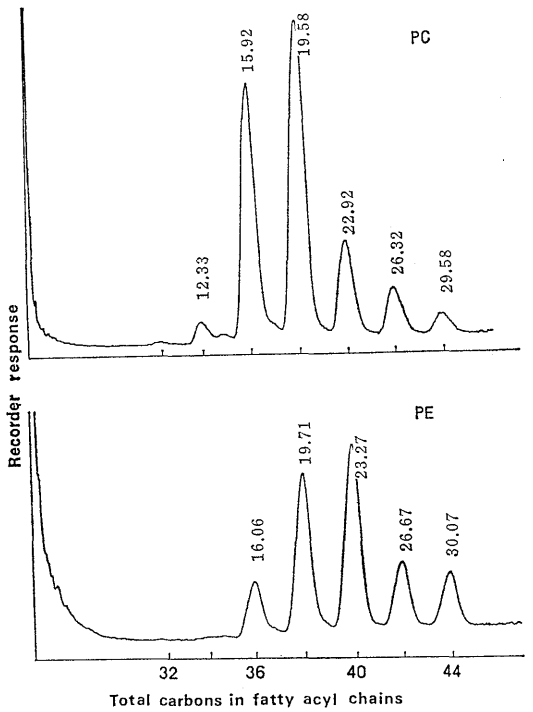

Fig. 3. Gas liquid chromatograms of trimethyl silylated diglycerides derived from cod phosphatidylcholine and phosphatidylethanolamine after hydrogenation.

Table 5. Fatty acid compositions of phosphatidylcholine and phosphatidylethanolamine determined before and after hydrogenation

(mol%)

Fatty acid	PC*1	PC(H)*2	PE*3	PE(H)*4
14:0	2.8	3.1	0.3	5.1
sum	2.8	3.1	0.3	5.1
16:0	32.3	35.0	11.8	15.6
:1	4.5	—	1.1	—
:2	0.3	—	0.1	—
sum	37.1	35.0	13.0	15.6
17:0	—	—	0.2	tr
sum	—	—	0.2	tr
18:0	0.7	11.3	6.4	21.9
:1	8.8	—	17.8	—
:2	0.8	—	0.8	—
:3	0.2	—	2.4	—
sum	10.5	11.3	27.4	21.9
20:0	—	24.2	tr	20.3
:2	1.6	—	2.2	—
:4	1.8	—	1.5	—
:5	22.0	—	17.7	—
sum	25.4	24.2	21.4	20.3
22:0	—	26.5	—	37.0
:4	0.5	—	—	—
:5	1.5	—	—	—
:6	22.2	—	37.8	—
sum	24.2	26.5	37.8	37.0
Total	100.0	100.1	100.1	99.9

*1 PC: phosphatidylcholine.
*2 PC(H): hydrogenated phosphatidylcholine.
*3 PE: phosphatidyletanolamine.
*4 PE(H): hydrogenated phosphatidylethanolamine.

Table 6. Explanation for estimation method of possible fatty acid combinations in phosphatidylcholine of cod

Fatty Acid Composition	Total Carbon in Fatty Acyl Chains						(mol%)	
	C_{34}	C_{36}	C_{38}	C_{40}	C_{42}	C_{44}	Calculated	Analyzed
C_{14}	0.8 a	0 c					0.8	3.1**
C_{16}	0 b	12.9 d	22.1 f				35.0	35.0
C_{18}	0 b	4.5 e 4.5	0 g	2.2 h			11.2	11.3
C_{20}	0.8 a	12.9 d	0 g	3.8 i 3.8	2.8		24.1	24.2
C_{22}		0 c	22.1 f	2.2 h	2.8	1.2 1.2	29.5	26.5
Calculated	1.6	34.8	44.2	12.0	5.6	2.4		
Analyzed	1.7*	33.9	44.2	12.2	5.6	2.3		

*,** See text.

(24.2%) and C_{22} (26.5%). The possible fatty acid combinations of PC with total carbons of 44 and 42 are considered to be those of (C_{22}, C_{22}) and (C_{20}, C_{22}), respectively, because no fatty acid having carbon numbers of more than 24 was contained in this PC. This implies that the sample contains the PC having fatty acid combinations of (C_{22}, C_{22}) and (C_{20}, C_{22}) at 2.4% (1.2%×2) and 5.6% (2.8%×2), respectively.

The fatty acid combinations of PC with total carbons of 36 are restricted to those of (C_{14}, C_{22}), (C_{16}, C_{20}), and (C_{18}, C_{18}) from the fatty acid composition. Similarly, the fatty acid combinations of PC with total carbons of 38 and 40 are restricted to the followings; (C_{16}, C_{22}) and (C_{18}, C_{20}) for 38 and (C_{18}, C_{22}) and (C_{20}, C_{20}) for 40. However, calculation of percentage of individual PC with these fatty acid combinations was more complex. So the following equations were used for the calculation.

$$2a+2b=1.7$$
$$2c+2d+2e=33.9$$
$$2f+2g=44.2$$
$$2h+2i=12.2$$
$$a+c=3.1$$
$$b+d+f=35.0$$
$$b+e+g+h=11.3$$
$$a+d+g+i+2.8=24.2$$
$$c+f+h+2.8+2.4=26.5$$

where a−i are molar percentages of fatty acids having carbon numbers of C_{14}–C_{22} fatty acids in the PC, as shown in Table 6. For example, a is percentages of C_{14}- and C_{20}-fatty acids contained in the PC having total carbons of 34. Similarly, b is percentages of C_{16}- and C_{18}-fatty acids in the PC having the same total carbons. As a result, the above-mentioned equation $2a+2b=1.7$* is derived. Further, since c is percentages of C_{14}- and C_{22}-fatty acids in the PC having total carbons of 36 and C_{14} fatty acid is contained only in the PC having total carbons of 34 and 36, the equation $a+c=3.1$** results. The figures in Table 6 are the results derived from the calculations of all above-mentioned equations using FACOM M-140F computer. In these calculations, the results showing minus was treated as 0.

The possible fatty acid combinations of PC and PE obtained are shown in Table 7. The most prominent combination in the PC was that of (C_{16}, C_{22}) accounting for 44.2%. In the fatty acid composition of PC shown in Table 5, major fatty acid in the C_{16}-fatty acids was $C_{16:0}$ accounting for 87.1%. Similarly, major fatty acid in C_{22}-fatty acids was $C_{22:6}$ accounting for 91.7%. Therefore, most of PC having fatty acid combination of (C_{16}, C_{22}) was considered to be that of ($C_{16:0}$, $C_{22:6}$). From the same reason, major fatty acid combinations of (C_{16}, C_{20}), (C_{18}, C_{18}), (C_{20}, C_{20}), (C_{20}, C_{22}), (C_{18}, C_{22}), (C_{22}, C_{22}), and (C_{14}, C_{20}) in PC could be estimated to be those of ($C_{16:0}$, $C_{20:5}$), ($C_{18:1}$, $C_{18:1}$), ($C_{20:5}$, $C_{20:5}$), ($C_{20:5}$, $C_{22:6}$), ($C_{18:1}$, $C_{22:6}$), ($C_{22:6}$, $C_{22:6}$), and ($C_{14:0}$, $C_{20:5}$), respectively.

Table 7. Possible fatty acid combinations in phosphatidylcholine and phosphatidylethanolamine of cod

Total carbon in fatty acyl chains	Possible combination of fatty acid	Content (mol%)	
		PC*1	PE*2
34	C_{14}, C_{20}	1.6	—
36	C_{14}, C_{22}	—	6.1
	C_{16}, C_{20}	25.8	2.4
	C_{18}, C_{18}	9.0	1.6
38	C_{16}, C_{22}	44.2	21.8
	C_{18}, C_{20}	—	9.9
40	C_{18}, C_{22}	4.4	23.5
	C_{20}, C_{20}	7.6	12.4
42	C_{20}, C_{22}	5.6	12.4
44	C_{22}, C_{22}	2.4	6.3
Total	—	100.6	96.4

*1 PC: phosphatidylcholine.
*2 PE: phosphatidylethanolamine.

Nine possible fatty acid combinations were estimated for PE, as shown in Table 7. The most prominent combination was that of (C_{18}, C_{22}). This implies that the PE having fatty acid combination of ($C_{18:1}$, $C_{22:6}$) is major one, because the fatty acids $C_{18:1}$ and $C_{22:6}$ accounted for 65.0% of the C_{18}-fatty acids and 100% of C_{22}-fatty acids, respectively. Similarly, ($C_{16:0}$, $C_{22:6}$), ($C_{20:5}$, $C_{20:5}$), ($C_{20:5}$, $C_{22:6}$), ($C_{18:1}$, $C_{20:5}$), ($C_{22:6}$, $C_{22:6}$), ($C_{14:0}$, $C_{22:6}$), ($C_{16:0}$, $C_{20:5}$), and ($C_{18:1}$, $C_{18:1}$) might be major fatty acid combinations in cod PE.

In general, fish phospholipids are mainly composed of C_{16}-, C_{18}-, C_{20}-, and C_{22}-fatty acids, in which $C_{16:0}$, $C_{18:1}$, $C_{20:5}$, and $C_{22:6}$ fatty acids are major ones, respectively, though there is a few exception. For example, a prominent fatty acid $C_{18:1}$ accounted for 89.0% of the total of C_{18}-fatty

*,** As shown in Table 6, figures of 1.7 and 3.1 are percentages of DG having total carbons of 34 and of fatty acid C_{14}, respectively.

acids in the PC of northern blenny.[17] Similarly, other prominent fatty acids $C_{16:0}$, $C_{20:5}$, and $C_{22:6}$ also accounted for 93.1%, 71.7%, and 96.1%, respectively. Similar fatty acid compositions were found in the PC and PE of atka mackerel,[17] cod,[17] and trout,[18] and PC of salmon,[19] menhaden,[19] tuna,[19] and cod.[20] From this point of view, the method described in the present study is applicable to estimation of the possible fatty acid combinations of PC and PE of various fishes. Further study is necessary for distinguishing the position of fatty acyl chains on glycerol moiety.

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