COMPARATIVE INVESTIGATION OF THE FATTY ACID COMPOSITIONS OF THE PHOSPHOLIPIDS OF MARINE INVERTEBRATES

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The fatty acid (FA) compositions of the phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) of the muscle tissues of 13 species from seven types of marine invertebrates have been studied. In the PCs and PEs of all the animals the main FAs were the 20:5ω3, 20:4ω6, 16:0, 18:1, and 18:0 acids, although the representatives of each type had their own specific features in their FA composition. The main FAs in the PCs and PEs in the majority of animals investigated coincided, although there were exceptions. The most promising sources for the directed isolation of PCs or PEs enriched with certain FAs have been determined. The highest level of polyenic FAs and the highest unsaturation index were found in representatives of the *Coelenterata* and *Echinodermata* (stars) types. It was shown that the amount of polyenic acids as a fraction of the sum of the acyl and alkenyl radicals in the majority of animals investigated was greater in the PCs than in PEs.

We were induced to carry out the present investigation by the necessity for seeking sources of phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) with different fatty acid (FA) compositions for scientific-research and medicinal purposes. It is known that PCs and PEs are the main phospholipids (PLs) of marine invertebrates [1, 2] and have the smallest individual scatter in their quantitative amounts, particularly in the muscle tissue [3], and their FA composition is far less subject to variation under the action of external factors on the organism than the composition of the acids of the neutral lipids. The diversity of types of marine invertebrates and the levels of their development suggest a multiple nature of the adaptive mechanisms to the conditions of the surrounding medium and, as a consequence of this, different FA compositions of the main PLs in the structure of the membranes [2, 4]. The limited nature of literature information relating to the basic FA composition of the PLs of the muscle tissue of arthropods [5, 6], also stimulated the performance of the present investigation. We took muscle tissues of 13 animals of seven phyla of marine invertebrates (*Coelenterata, Annelida, Brachiopoda, Mollusca, Arthropoda, Echinodermata, Tunicata*) and studied the compositions of the FAs, PCs, and PEs isolated from them.

We took readily available and typical representatives of the phyla considered: three species from different classes of *Mollusca*, two species of *Echinodermata*, two species of simple and two species of colonial ascidians, and one species each of the other phyla.

The results of the investigation of the FA compositions of the PCs and PEs from lipid extracts of the marine invertebrates are given in Tables 1 and 2. In all the phyla of animals the main acids in the PCs and PEs were the 20:5ω3, 20:4ω6, 16:0, 18:1, and 18:0 acids. At the same time, in the representatives of each phylum there were specific features in the composition of the FAs of the PLs studied. In the PCs from *Metridium senile fimbriatum* we found the highest amounts of the 22:5ω3 acid (11.6%) and of the sum of the acids with chain lengths of more than 20 carbon atoms (27.1%), and in the PEs the 20:5ω3 acid (47.5%). The sum of the pentaenoic and polyenoic acids in the PCs and PEs of the sea anemone of (43.0 and 50.4%, and 73.6 and 82.6%, respectively) were also the highest. Results close to ours have been obtained by N. A. Romashina [7] in an investigation of the FA composition of the total lipid extract of *M. senile fimbriatum*.

We found the highest amounts of the 22:4ω6 acid (3.9 and 6.8%, respectively) in the PCs and PEs from *Chaetopterus variopedatus (Annelida)*. In the PEs of *Coptothyris grayi (Brachyopoda)* there was an unusually high amount of combined dienoic acids (20:2 and 22:2 — 37.9%). Similar acids but in somewhat smaller amount were found in the PEs of *Gasteropoda (Littorina*

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TABLE 1. Fatty Acid Compositions of the Phosphatidylcholines and Phosphatidylethanolamines of the Muscle Tissues of Marine Invertebrates

Fatty acid	Coelenterata		Annelides		Brachiopoda		Mollusca						Arthropoda	
	Metridium senile fimbriatum		Chaetopterus variopedatus		Coptothyris grayi		Crenomytilus grayanus		Acmea pallida		Littorina squalida		Hemigrapsus sanquineus	
	PC	PE	PC	PE	PC	PE	PC	PE	PC	PE	PC	PE	PC	PE
14:0	0,3	0,3	0,9	1,1	0,6	0,4	1,4	0,8	1,4	1,0	9,9	0,6	0,6	0,9
14:1	+	+	+	0,2	+	+	+	0,2	+	+	+	+	+	0,5
15:0	0,6	0,4	0,7	0,4	0,4	0,3	1,2	0,6	0,6	0,3	0,8	0,3	0,7	0,2
15:1	1,0	0,5	+	0,2	0,2	0,2	—	0,3	—	+	+	0,3	0,1	—
16:0	4,8	2,6	23,7	6,3	17,6	3,7	25,7	10,4	12,3	11,5	26,9	6,7	21,3	10,5
16:1	1,2	0,4	2,6	5,0	2,6	1,0	4,7	1,9	2,5	2,5	3 0	0,9	3,9	1,6
16:2	1,4	2,2	1,3	1,5	0,4	+	1,0	—	+	—	1,0	0,5	—	—
17:0	0,8	3,8	2,5	1,6	1,9	0,6	2,0	0,8	0,7	1,4	1,0	0,9	0,7	1,0
17:1	0,9	0,4	+	0,5	0,2	0,4	0,1	0,8	0,2	1,1	+	0,6	0,5	0,8
18:0	3,0	1,7	5,9	8,2	10,1	7,4	7,2	6,6	7,6	6,9	3,5	8,0	4,1	9,4
18:1	6,1	5,3	8,0	4,0	13,0	2,7	8,5	4,7	18,4	13,8	11,3	5,1	28,2	13,8
18:2 ω6	1,2	0,5	3,0	2,1	3,0	1,6	3,4	2,1	1,9	1,9	6,8	5,1	3,3	3,0
18:3 ω6	+	0,1	0,4	0,3	0,6	+	0,4	0,3	+	+	—	+	0,2	0,3
18:3 ω3	0,2	+	0,6	0,4	0,5	0,8	0,6	0,7	1,3	0,8	1,1	1,3	1,0	0,9
18:4 ω3	0,2	+	—	+	0,2	—	0,5	0,4	0,7	2,1	0,8	1,4	0,2	—
20:0	0,1	—	—	+	0,5	+	0,4	0,3	1,5	1,2	0,6	0,5	0,4	0,2
20:1	3,9	0,8	4,8	8,3	2,2	18,5	2,8	9,4	4,3	3,2	12,6	17,1	1,2	0,9
20:2	3,1	1,6	0,7	4,9	2,4	24,6	2,0	17,4	2,2	7,4	1,6	1,8	0,7	0,8
20:3	0,5	0,3	0,6	0,6	1,3	0,5	0,8	6,9	1,1	0,3	+	0,3	0,3	+
20:4 ω6	5,0	8,6	10,2	19,7	10,9	5,8	7,5	9,1	9,1	19,3	5,4	9,8	5,5	8,2
20:4 ω3	0,6	—	—	—	0,6	—	—	0,1	—	—	—	+	—	—
20:5 ω3	29,8	47,5	5,6	5,6	10,9	3,6	11,0	9,3	23,0	18,3	5,3	13,9	18,1	30,2
22:0	+	+	0,4	0,4	0,7	0,2	1,0	0,7	1,8	2,1	0,5	0,4	+	0,8
22:1	3,7	1,2	+	+	—	0,2	—	0,1	+	+	0,4	+	—	—
22:2	—	0,5	0,5	4,5	0,6	11,7	2 7	8,3	2,6	3,1	1,3	14 3	—	—
22:3	0,4	—	0,6	1,2	0,9	2,7	0,7	2,2	—	—	—	0,9	—	—
22:4 ω6	—	0,9	3,9	6,8	1,4	3,0	0,8	1,2	—	—	0,3	1,0	+	—
22:5 ω6	1,6	—	1,6	1,1	1,4	1,9	0,8	0,3	—	—	—	—	+	+
22:5 ω3	11,6	2,9	1,4	1,5	3,1	1,2	1,6	0,2	0,4	—	0,9	2,6	1,0	1,2
22:6 ω3	9,8	7,1	13,4	10,2	10,1	3,5	9,4	2,2	4,9	0,7	0,2	0,7	5,3	12,0
24:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unidentified	8,4	9,9	6,9	3,2	1,7	3,2	1,6	1,6	1,8	0,8	5,0	5,2	3,0	2,9
Saturated (SA)	9,6	8,8	34,1	18,0	21,8	12,6	38,9	20,2	25,9	24,4	43,2	17,4	27,8	23,0
Monoenoic (MA)	16,8	8,6	15,4	18,2	18,2	23,0	16,1	18,0	25,6	20,6	27,3	23,8	33,9	17,6
More than 20 carbon atoms	27,1	12,6	21,8	25,7	18,2	24,4	17,0	15,2	9,7	5,9	3,6	19,9	6 3	14,0
FA	6,4	44,3	5,8	44,0	1,4	45,9	2,2	41,7	5 7*	30,7*	2,3	40 2	4.8	16,4
PFA in % of the total FAs	73,6	82,6	50,5	63,8	50,0	64,4	45,0	61,8	48,6	55,0	29,5	58,8	38,3	59,4
PFA in % of the total radicals	68,9	46,0	47 6	35,7	49,3	34 8	44,0	36,0	45,6	38,1	28,9	35,2	36,5	49,7
UI	329	352	211	260	231	201	200	209	232	230	110	210	197	291

Here and in Table 2: (*) Dembitskii's figures [29], obtained from extracts of whole organisms were used for the calculation; (+) FA found in traces; (—) FA not found.

previously that the presence of the 20:2 and 22:2 dienoic acids is characteristic for the PLs of molluscs [8-11]. The PEs of *C. grayanus* contained a large amount of the 20:3 acid [6, 9]; in the other animals investigated its amount did not exceed 2%. The PCs of the gastropod mollusc *L. squalida* had the highest amount of the 14:0 and 16:0 saturated acids (9.9% and 26.9%, respectively). For the PCs of the shore crab *Hemigrapsus sanguineus* there was the highest amount of the 18:1 acid (28.2%) and of combined monoenoic acids (33.9%), and in the PEs the highest amount of the 22.6ω3 acid (12%). The similar distributions of the FAs in the PCs and PEs of other marine *Crustacea* (crabs, lobsters, shrimps, and other decapods) [5, 6, 12-14] show the regularity of such a composition in the *Arthropoda*. The PCs of the star *Distolasterias nipon* have the highest level of the 20:5ω3 acid (41.7%) and its PEs the highest level of the 20:4ω6 acid (37.3%), with the highest level of total polyenoic acids (78.6 and 85.3%), and the smallest amount of acids with chains longer than 20 carbon atoms. In the PEs of the star *D. nipon* and the urchin *Strongylocentrotus intermedius* we found the highest amounts of the 20:2 acid (17.4 and 18.2%, respectively). In the PCs and PEs of *Tunicata* there was an unusually large amount of the 18:2 acid (18.4 and 14.4% on an average for the

TABLE 2

Fatty acid	Echinodermata				Tunicata							
	Distolasterias nipon		Strongylocentrotus intermedius		Halocynthia aurantium		Stiela clava		Botryllus tuberatus		Botrylloides elligulatum	
	PC	PE	PC	PE	PC	PE	PC	PE	PC	PE	PC	PE
14:0	0,1	0,1	2,4	0,9	5,3	2,4	1,8	2,4	2,9	1,9	1,5	1,4
14:1	+	—	0,1	+	0,3	0,4	0,3	0,3	0,3	0,2	0,2	0,1
15:0	+	+	0,8	0,5	1,8	1,5	0,8	1,1	1,5	1,2	1,0	0,6
15:1	+	—	0,1	+	0,3	0,5	—	0,2	0,3	0,2	0,4	0,1
16:0	2,2	1,8	15,6	8,1	19,3	11,3	12,8	11,1	13,7	11,4	13,9	12,0
16:1	0,4	0,3	3,3	1,4	5,7	3,9	5,2	5,0	4,5	3,4	3,4	7,6
16:2	—	+	—	—	0,4	—	—	—	—	0,4	0,4	0,9
17:0	+	1,4	0,8	0,5	1,2	2,0	1,4	1,8	1,4	1,2	1,5	1,7
17:1	—	—	0,4	0,3	0,5	3,8	0,4	0,6	0,4	0,5	0,3	0,9
18:0	5,9	3,4	4,8	5,0	4,4	9,0	6,3	6,3	6,7	7,2	3,4	6,5
18:1	2,4	2,8	9,4	6,9	16,3	17,5	10,9	13,4	14,5	16,5	19,2	16,1
18:2 ω 6	0,9	1,9	1,8	0,9	9,5	5,7	18,4	6,8	17,1	23,1	28,5	21,9
18:3 ω 6	+	0,1	0,2	0,1	0,6	0,5	1,0	0,5	1,5	0,8	0,8	0,5
18:3 ω 3	0,7	0,6	0,5	0,3	1,2	0,6	1,2	0,8	2,2	0,7	1,4	0,5
18:4 ω 3	1,1	0,7	0,2	0,1	1,2	0,6	1,4	1,2	2,7	0,6	0,7	0,2
20:0	+	0,2	+	+	0,6	0,8	0,9	0,5	1,2	0,8	0,8	0,8
20:1	7,4	2,8	8,5	7,0	1,2	0,8	1,3	1,9	0,7	0,7	0,4	0,6
20:2	5,0	17,4	5,0	18,2	0,5	—	0,9	0,4	—	0,2	0,4	—
20:3	0,8	0,5	1,9	0,8	0,3	—	+	0,1	1,1	—	0,5	—
20:4 ω 6	19,6	37,3	18,9	28,4	1,8	6,4	5,2	9,3	3,1	3,8	2,1	5,9
20:4 ω 3	—	—	0,4	0,5	0,4	+	+	0,4	—	—	—	—
20:5 ω 3	41,7	24,0	18,7	15,5	15,5	24,2	20,0	24,6	13,0	13,3	10,4	16,1
22:0	0,8	0,9	1,2	0,6	0,9	—	+	1,0	1,4	—	1,1	0,8
22:1	1,1	0,5	1,8	1,2	+		0,7	0,5	0,8	0,1	0,4	—
22:4	—	—	—	—	0,4	+	0,4	0,2	—	—	0,4	—
22:5 ω 6	—	—	—	0,2	—	—	0,8	1,0	—	+	—	—
22:5 ω 3	0,8	—	0,2	—	—	0,4	0,4	0,5	—	+	—	—
22:6 ω 3	4,4	+	1,4	1,3	8,0	4,7	6,7	7,1	7,3	11,6	4,9	3,4
24:1	1,1	—	0,4	0,5	—	0,2	+	—	—	+	—	—
Unidentified	3,0	3,2	0,9	0,8	2,3	2,7	1,3	0,3	1,4	0,1	1,9	2,1
Saturated (SA)	9,0	7,8	25,6	15,6	33,5	27,0	24,0	24,2	28,8	23,7	23,2	23,8
Monoenoic (MA)	12,4	5,9	24,0	17,3	24,3	27,1	18,8	21,9	21,5	21,6	24,3	25,4
More than 20 carbon atoms FA	8,2	1,4	5,0	3,8	9,3	5,3	9,0	10,3	9,5	11,7	6,8	4,2
PFA in % of the total FAs	4,8	46,9	3,2	45,5	7,7	37,0	6,0*	27,8*	5,6	30,9	5,3	28,0
PFA in % of the total radicals	78,6	86,3	50,4	67,1	42,2	45,9	57,2	53,9	49,7	54,7	52,5	50,8
UI	74,8 350	45,8 320	48,8 226	36,6 261	39,0 192	28,9 221	53,8 238	38,9 256	46,9 202	37,8 226	49,8 185	36,6 198

phylum), the level of which in the PCs of some ascidians reaches 28%. The amounts of arachidonic acid (3.0 and 6.4% as averages for the phylum) in the PCs and PEs, respectively, were lowest in the *Tunicata*. The 18:2ω6 acid that is characteristic for plant organisms is usually found in marine invertebrates in amounts not exceeding 3% [15]. Only in representatives of the arctic zooplankton has from 7 to 13% of the 18:2 acid been found in the PL fraction [16], which, in the opinion of the author concerned, is connected with its feeding on phytoplankton rich in this acid. It is not excluded that in ascidians, as well, the high level of the 18:2 acid is connected with their feeding on phytoplankton and *Protozoa*, which are rich in this acid [15, 17, 18]. With respect to the relative amounts of the 16:0, 18:2, and 20:5ω3 acids the lipids of ascidians are close to the typical lipids of phytoplankton [17]. The amount of the 22:6ω3 acid, which is characteristic for zooplankton, is low.

The main fatty acids in the PCs and PEs of the majority of the animals investigated (*Coelenterata, Annelida, Mollusca — A. pallida, Arthropoda, Echinodermata,* and *Tunicata*) agree, although there are also exceptions (*Brachiopoda, Mollusca — C. grayanus, L. squalida*). In our opinion, the analysis that has been performed of the fatty acid compositions of the PCs and PEs of marine invertebrates would not be complete without taking into account the alkenyl radicals that, according to the literature, are present in considerable amounts in the PLs of marine invertebrates [19]. In view of the fact that in the plasmalogenic form of the PLs half the radicals are represented by FAs and half by fatty aldehydes (FAlds), the amount of FAlds has been given in the Table from the formula

$$\text{FAld}\,\% = \frac{1}{2}\,\text{PlL}\,\%,$$

where PlL is the amount of the plasmalogenic form of the PLs as a percentage of the total of the forms of the class.

The amount of PlLs was determined by reaction micro-TLC [20]. The FAld values reflect molar percentages and not weight percentages and their magnitude may therefore differ somewhat from the FAld content determined by GLC. In order to take into account the contribution of the FAlds in the characterization of the PC and PE radicals, a number of additional indices have been given in the tables. The sum of the polyenic FAs (PFAs) as a percentage of the sum of the radicals was calculated by means of the formula

$$\sum_{i=1}^{n} \text{PFA} \quad \% = \frac{100 - \text{FA}}{100} \times \sum_{i=1}^{n} N_i \%,$$

where N_i represents the FAs with not more than two double bonds.

The unsaturation index (UI) of the FAs was calculated from the formula [21] UI = $\sum_{i=1}^{n}$ [(number of double bonds in each unsaturated acids) × (percentage of this acid)].

The indices given in the tables permitted a new evaluation of the degree of unsaturation of the PC and PE radicals. On the basis of literature information for the composition of the FAs of the PLs of marine invertebrates, in the majority of cases the most unsaturated of the PLs are the PEs [5, 8, 13, 14, 22, 23], although they may sometimes include other PLs: PSs [24, 25], LPEs [22]. At the same time, the contribution made by the alkenyl residues of the plasmalogenic forms of the PLs is not usually taken into account. However, this contribution is fairly large and, as will be shown below, it may change the idea that has become traditionally established concerning the saturated nature of the PEs and PCs of marine organisms. The index of the total PFAs as a percentage of the sum of the FAs given in the Tables basically confirms the traditional idea of the PEs as the most saturated PLs. However, on passing to a consideration of the index of the total PFAs as a percentage of the sum of the radicals (FAs and FAlds), this idea changes fundamentally. The combination of the FAlds with the sum of the UAs and MAs is justified, in our opinion, since, judging from the available literature information, alkenyl radicals are usually represented by saturated and monoenic FAlds. Thus, the main FAlds present in the PC- and PE-plasmalogens of the nerve cells of the legs and sarcoplasmic reticulum of the lobster are the 18:0 and 16:0 compounds, their sum amounting to 80-99% of all the FAlds [12, 23]; in the PEs of sponges, the FAlds from 14:0 to 20:0 amount to 57.4% of all the alkenyl radicals [26]. In 11 out of 13 animals the index of the total PFAs as a percentage of the total FA and FAld radicals for the PCs was higher (sometimes substantially) than for the PEs. In only two cases (*L. squalida* and *H. sanguineus*) was the saturation of the residues in the PCs statistically higher than in the PEs. It must be mentioned that the last two animals, unlike the others, spend a considerable part of their time (during ebb tides) on dry land.

Passing to a discussion of the UI, it must be mentioned that in 3 out of the 13 animals investigated the UI was higher in the PCs than in the PEs. The absence of concrete information on the ratio of saturated and monoenic FAlds in the animals that we investigated does not permit us to calculate the UIs of the sums of the radicals (FAs and FAlds), but, judging from the amount of PFAs as a percentage of the total radicals and as a percentage of the total FAs, it may be assumed that the UI of the sum of the radicals (FAs and FAlds) in the majority of animals will be greater in the PCs than in the PEs.

The investigation performed has shown the promising nature of the use of marine invertebrates for obtaining PCs and PEs with definite FA compositions. In practically each phylum studied there were animals having in their PCs or PEs a relatively high amount of specific (for the phylum) FAs or possessing a unique FA composition with the maximum amount of saturated, monoenoic, or polyenoic acids or acids with chain lengths greater than 20 carbon atoms, and different UIs.

EXPERIMENTAL

The animals were collected in August to beginning of September in Posyet Bay, Sea of Japan. We used muscle tissues to prepare the lipid extracts. The extraction, the isolation of the PCs and PEs, and the analysis of the FAs with the aid of GLC were carried out as described previously [2, 27]. The quantitative analysis of the plasmalogens was carried out by reaction MTLC [20].

SUMMARY

1. The FA compositions of the PCs and PEs of the muscle tissue of 13 species of marine invertebrate animals from seven phyla have been studied. In the PCs and PEs of these animals the main FAs are the 20:5ω3, 20:4ω6, 16:0, 18:1, and 18:0 acids, although in the representatives of each phylum there are specific features in the composition of the FAs of the phospholipids studied.

2. The main FAs in the PCs and PEs of the majority of animals investigated coincide, although there are also exceptions.

3. The most promising sources for the directed isolation of PCs or PEs enriched with the following FAs have been determined: 14:0 (PCs), 16:0 (PCs), 18:1 (PCs), 18:2ω6 (PCs, PEs), 20:2 (PEs), 22:2 (PEs), 20:3 (PEs), 20:4ω6 (PCs), 20:5ω3 (PCs, PEs), 22:4ω6 (PEs), 22:5ω3 (PCs), 22:6ω3 (PEs).

4. Animals have been found with maximum or minimum amounts of saturated, monoenoic, and polyenoic acids and acids with chain lengths greater than 20 carbon atoms and with different unsaturation indices. The highest levels of polyenoic FAs and of the unsaturation index are found in representatives of the phyla *Coelenterata* and *Echinodermata* (stars).

5. The sum of polyenoic fatty acids in the PCs and PEs as a proportion of the sum of the acyl and alkenyl radicals has been determined and it has been shown that the unsaturation of the PC radicals is greater than that of the PE radicals in the majority of animals studied.

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