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Distribution of Very-Long-Chain Fatty Acids between Molecular Species of Different Phospholipid Classes of Two Soft Corals

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Abstract

Fatty acids (FAs) of soft corals contain two very-long-chain tetracosapolyenoic acids (TPA, 24:5n-6 and 24:6n-3), which are chemotaxonomic markers of all species of the subclass Octocorallia. The distribution of TPA in molecular species of different phospholipid (PL) classes was investigated for the first time in the soft corals *Sinularia macropodia* and *Capnella sp*. From shallow waters of Vietnam. Phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI) were the major PL classes of *S. macropodia* and *Capnella sp*. More than thirty two molecular species of these four PL classes were determined by high resolution tandem mass spectrometry. 18:1e/20:4 PE, 18:0e/20:4 PC, 18:0e/24:5 PS, and 18:0/24:5 PI were the major molecular species of PL in both coral species. PE, PC, and PS mainly consisted of alkyl acyl and alkenyl acyl forms, but diacyl forms predominated in PI. TPA were the principal FAs in PS and PI, whereas 20:4n-6 was more abundant in PE and PC. Selective incorporation of TPA in the molecules of PS and PI are supposed to be a specific feature of the biosynthesis of PL in alcyonarians. To study the trophic and symbiotic relationships of soft corals, some molecular species of PS and PI with TPA may be applied as lipid molecular markers of coral polyps.

Keywords: Soft corals; Tetracosapolyenoic acids; Phospholipids; Molecular species; Lipid biosynthesis; High resolution tandem mass spectrometry

Introduction

Corals (Cnidaria: Anthozoa) are important components of both tropical and boreal benthic communities. Dry biomass of corals contain up to 30% of lipids [1]. Lipids take part in the majority of biochemical processes in corals [2]. Neutral lipids serve as long-term energy stores [3,4]. Polar lipids, first of all phospholipids (PLs), are the structural base of cell membranes. The content and composition of coral lipids depend on the annual cycle [5], light regimes [6], and the prevailing food [7]. In symbiotic coral species, lipids constitute a part of organic carbon transferred between coral host and their symbiotic dinoflagellates (zooxanthellae) [8-10].

Fatty acids (FAs) are a part of lipid molecules as acyl groups. FA profile is the main characteristic of coral total lipids. Several FAs are used as markers of trophic and symbiotic relationships of corals [11,12] and play an important role in the regulation of coral metabolism [13]. There are certain differences in FA profiles between coral subclasses; lipids of soft corals contain tetracosapolyenoic acids (TPA), namely, 24:5n-6 and 24:6n-3, which are absent in reefbuilding corals [1,14]. Svetashev and Vysotskii [15] suggested TPA as chemotaxonomic marker of soft corals and other taxonomic groups of the subclass Octocorallia. Comparison of FA compositions of zooxanthellate soft coral species and species without zooxanthellae showed that TPA are synthesized in coral polyp tissues and serve as the biochemical markers of the host [16,17]. Some C18-20 polyunsaturated FAs (PUFAs) can be evidently converted to TPA by the coral host [18]. Sprecher [19] summarized that arachidonic acid (20:4n-6) was elongated to 22:4n-6 and then to 24:4n-6, which in its turn was desaturated to yield 24:5n-6. Analogically, eicosapentaenoic acid (20:5n-3) was a substrate for synthesis of 24:6n-3. These pathways were found in several types of cells of terrestrial organisms and the presence of 24:4n-6 and 24:5n-3 as intermediate products was implied. The synthesis of TPA in cnidarians was not investigated, moreover, 24:4n-6 and 24:5n-3 were not found in soft corals [14]. A hypothesis about synthesis of 24:5n-6 from 22:5n-6 and 24:6n-3 from 22:6n-3 by Δ4 desaturase in soft corals has been suggested [20].

PUFAs are used as a substrate for phospholipid biosynthesis. The mechanism of this process is well described for medium- and long-chain PUFAs [21] but not for TPA. In soft corals, most TPA are known to concentrate in PLs [1,22] but the distribution of TPA between different PL classes is unclear and the data on molecular species composition of PLs containing TPA are very limited. Recently, the molecular species composition of phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI) has been determined in the soft coral Xenia sp. and a predomination of the molecular species with TPA in PS has been found [20]. To determine the features of the distribution of TPA between different PL classes, structures and compositions of PL molecular species in the soft corals *Sinularia macropodia* and *Capnella sp*. were investigated.

Materials and Methods

The colonies of the soft corals S. macropodia (Hickson & Hiles, 1900) (Anthozoa: Octocorallia: Alcyonacea: Alcyoniidae) and Capnella sp. (Anthozoa: Octocorallia: Alcyonacea: Nephtheidae) were collected in July 2014 at a depth of 1.5-2 m in Nha Trang Bay and Tonking Gulf, the South China Sea, Vietnam. To analyze lipids, three different colonies were taken. TL were extracted using a modified technique of [23]. Lipids were extracted by intensive homogenization in $CHCl_3$/MeOH (1:2, by vol) (30 mL per 10 g of coral wet wt). The homogenate obtained was filtered, and the residue was repeatedly extracted (6 h, 4 °C) in $CHCl_3$/MeOH (2:1, by vol) (2 × 30 mL). The extracts were then

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mixed and separated into layers by adding 35 mL of H_2O and 30 mL of $CHCl_3$. The lower layer was evaporated, and the TL obtained were dissolved in $CHCl_3$ and stored at -18°C.

The TL were divided into neutral and polar lipid fractions by column chromatography on silica gel according to Rouser et al. [24]. Polar lipid compositions were analyzed by thin-layer chromatography (TLC) using the precoated silica gel plates (10 cm × 10 cm) Sorbfil PTLC-AFV (Sorbfil, Krasnodar, Russia) and the solvent system $CHCl_3$/MeOH/28% NH_4OH (65:35:5, by vol). Plates were sprayed with 10% H_2SO_4/MeOH and heated at 240°C for 10 min. The chromatograms were scanned using an image scanner (Epson Perfection 2400 PHOTO) in a grayscale mode. Using an image analysis program (Sorbfil TLC Videodensitometer, Krasnodar, Russia), the percentages of PL contents were determined by band intensity. Individual PL classes were isolated by preparative TLC as described above. The Bands of PLs were scraped, eluted with 10% H_2O/MeOH, dissolved in $CHCl_3$ and stored at -18°C. The HPLC separation of PLs was performed with a Shimadzu Prominence liquid chromatograph equipped with a Shim-Pack diol column (50 mm × 4.6 mm ID, 5 μm particle size) (Shimadzu, Kyoto, Japan) using the binary solvent gradient consisted of solvent mixture A: n-hexane/2-propanol/AcOH/Et_3N (82:17:1:0.08, by vol) and mixture B: 2- propanol/H2O/AcOH/Et_3N (85:14:1:0.08, by vol). The gradient started at 5% of mixture B and its percentage was increased to 80% over 25 min. This composition was maintained for 1 min, returned to 5% of mixture B over 10 min, and maintained at 5% for 4 min (the total run time was 40 min). The flow rate was 0.2 mL/min.

Lipids were detected by a high resolution tandem ion trap-time of flight mass spectrometry by a Shimadzu LCMS-IT-TOF instrument (Kyoto, Japan) operating both at positive and negative ion mode at electrospray ionization (ESI) conditions during each analysis. Ion source temperature was 200°C, the range of detection was m/z 100-1200, and potential in the ion source was -3.5 and 4.5 kV for negative and positive modes, respectively. The drying gas (N2) pressure was 200 kPa. The nebulizer gas (N2) flow was 1.5 L/min.

PL molecular species were identified as described earlier [20]. The quantification of individual molecular species within each PL class was carried out by calculating the peak areas for the individual extracted ion chromatograms [25].

Results and Discussion

PL profiles of two alcyonarian species investigated were quite similar. Phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI) were the major PL classes, which constituted in sum 75.5 and 82.3% of total PLs of *S. macropodia and Capnella sp*., respectively. The total percentages of these four PL classes in the species of the genera Sinularia, Lobophytum, and Sarcophyton collected previously in the same region and season were 72.8, 65.4, 71.1% of total PL, respectively [26]. The group of PLs (PE, PC, PS, and PI) seems to be a characteristic group of structural lipids of alcyonarians from shallow waters of Vietnam.

High performance liquid chromatography and high resolution mass spectrometry (HPLCHRMS) was applied for the analysis of chemical structures and amounts of molecular species of PE, PC, PS, and PI. The compositions of the major molecular species of these four PL classes obtained from *S. macropodia* and *Capnella sp*. are showed in Table 1.

PE, PC, and PS of both the coral species mainly consisted of alkyl acyl and alkenyl acyl forms, but diacyl forms predominated in PI. The same distribution of alkyl, alkenyl, and diacyl forms has been earlier found in PL molecular species of Xenia sp. (*Anthozoa: Octocorallia: Alcyonacea: Xeniidae*) [20] and PL classes of the genera Sinularia, Lobophytum, and Sarcophyton [26] collected in summer season. On the contrary, alkenyl forms of PL (plasmalogens) were not detected in coral colonies collected in winter season [26]. Delta-1'- desaturase is known to convert the alkyl form (1-alkyl-2-acyl-sn-glycerophospholipids) to the alkenyl form (1-alk-1'-enyl-2-acyl-sn-glycerophospholipids) [27]. We suppose that a seasonspecific low activity of delta-1'-desaturase leads to the low level of the PL alkenyl form in the Vietnamese alcyonarians in winter season.

18:1e/20:4 PE, 18:0e/20:4 PC, 18:0e/24:5 PS, and 18:0/24:5 PI were the major molecular species of PL in both coral species studied (Table 1). In *Xenia sp*. described recently, 18:1e/20:4 PE, 18:0e/20:4 PC, and 18:0e/24:5 PS constituted 86.4, 51.9, and 68.4% of the respective PL classes [20]. Thus, three soft corals (*S. macropodia, Capnella sp.,* and *Xenia sp*.) had similar compositions of the molecular species of PE, PC, and PS. Most species-specific variations were found in the composition of PI molecular species. Nevertheless, 18:0/22:4, 18:0/24:5, and 18:0/24:6 prevailed in PI molecular species of all three soft corals mentioned above. We suppose that the composition of diacyl PC is under the influence of PC of zooxanthellae [20], whereas the composition of diacyl PI mostly depends on lipids and FAs obtained from food.

An evident imbalance in the distribution of TPA and 20:4n-6 between PL classes was found in *S. macropodia* and *Capnella sp.* (Table 1). All identified PS molecular species of two species, 79.9% of PI

PE			PC			PS			PI		
Molecular species	**SM**	**CS**	**Molecular species**	**SM**	**CS**	**Molecular species**	**SM**	**CS**	**Molecular species**	**SM**	**CS**
16:0e/20:4*	2.4***	-	16:0e/16:2	3.6	-	16:0e/24:5	0.8	-	18:0e/20:4	-	8.3
18:0e/20:4	16.7	23.3	16:0e/18:2	5.1	4.4	18:0e/24:5	83.9	77.9	18:0e/22:4	-	3.9
18:0e/24:5	-	2.1	16:0e/18:3	6.8	2.1	18:0e/24:6	15.3	22.1	18:0e/24:5	-	4.2
18:1e/20:4**	56.0	41.1	16:0e/18:4	8.0	2.1				18:0e/24:6	-	0.1
18:1/20:4	-	2.8	16:0e/20:4	23.9	10.8				16:0/18:1	-	2.6
18:2/20:4	17.1	30.5	18:0e/16:2	2.7	-				16:0/18:2	-	1.8
Other	7.8	0.2	18:0e/18:1	1.5	-				16:0/22:6	-	11.5
			18:0e/18:2	4.9	4.1				16:0/24:5	6.0	-
			18:0e/18:3	9.7	4.3				18:0/20:3	-	2.6
			18:0e/20:4	29.6	63.1				18:0/22:4	20.1	26.5
			19:1/20:4	-	1.4				18:0/24:5	63.0	38.5
			Other	4.2	7.7				18:0/24:6	10.9	-

* Alkyl acyl form; ** Alkenyl acyl form (plasmalogen); ***Values are presented as mean of triplicate analysis, SD do not exceed 10% of the means.

Table 1: Composition of the Major Molecular Species of Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), Phosphatidylserine (PS), and Phosphatidylinositol (PI) (mol % of Each Lipid Class) of the Soft Corals *Sinularia macropodia* (SM) and *Capnella* sp. (CS)

molecular species of *S. macropodia*, and 42.8% of PI molecular species of *Capnella sp.* contained TPA. Acid 20:4n-6 was abundant in PE and PC, but molecular species with TPA consisted less than 3% of PE and PC. In Xenia sp. investigated recently, PS contained 76.6% of molecular species with TPA; PI contained 30.2% of three diacyl molecular species (16:0/24:5 PE, 18:0/24:6 PE, 18:0/24:5 PE) and 1.7% of 18:0e/24:5 PE [20].

We compared three soft coral species belonging to three different families (Alcyoniidae, Nephtheidae, and Xeniidae) of the order Alcyonacea. The high concentration of TPA in PS and PI may be considered as a taxonomic indicator of zooxanthellate alcyonarians. Recently, several highly unsaturated molecular species of galactolipids have been proposed as lipid molecular markers of *zooxanthellae* in a symbiont-host association of corals [28]. Contrary to *zooxanthellae*, soft coral polyps have no galactolipids but can synthesize TPA. To study trophic and symbiotic relationships of soft corals, some molecular species of PS and PI with TPA may be applied as lipid molecular markers of soft coral polyp tissues.

Ether lipids, which contain alkyl or alkenyl group in sn-1 position and 20:4n-6 in sn-2 position, were the major PC and PE molecular species in *S. macropodia, Capnella sp*., and *Xenia sp*. The profiles of the major PC and PE were similar in all three species. This similarity is easily explained by biosynthesis of ether lipids, which assumes that both 1-alkyl-2-acyl-sn-glycero-3-phosphoethanolamine and 1-alkyl-2-acyl-sn-glycero-3-phosphocholine are synthesized from the same 1-alkyl-2-acyl-sn-glycerols (AAG) [29]. Alkyl lipids can be then converted to the alkenyl lipids.

The very high level of TPA in PS molecular species allowed us to postulate that PS and PE (PC) synthesized from two different groups of AAG distinguished by their FA compositions. AAG with TPA are used for the synthesis of PS, whereas PE (PC) synthesized from AAG contained 20:4n-6. Theoretically, soft corals can use full set of FAs for the synthesis of diacyl PI, although soft coral PI are rich in TPA. Selective incorporation of TPA in the molecules of PS and PI are supposed to be a specific feature of the biosyntheses of PL in alcyonarians.

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