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CHARACTERISTICS OF PHOSPHOLIPIDS IN MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

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Summary

Characteristics of lipids in the microvillar membranes of octopus photoreceptor cells were studied in order to obtain some information on the membrane environment with rhodopsin in the invertebrate.

(1) The membranes contain lipid and protein in almost equal proportion. The majority of lipids are phospholipids. Neutral lipids make up 16% of the total lipids, the major constituent of which is cholesterol.

(2) Phosphatidylethanolamine and phosphatidylcholine are the major phospholipids. Phosphatidylserine, ceramide 2-aminoethylphosphonate and sphingomyelin occur as minor components. An unidentified alkaline and acid stable phospholipid was found.

(3) The predominant fatty acids of phosphatidylethanolamine and phosphatidylcholine are highly unsaturated such as 22 : 6, 20 : 5 and 20 : 4. The 22 : 6 and 20 : 5 are exclusively linked at the 2-position, but the 20 : 4 is linked significantly at the 1-position of the phospholipids.

(4) Major molecular species are 16 : 0/22 : 6 (48.4%) and 16 : 0/20 : 4 (19.6%) in phosphatidylcholine, and 20 : 4/22 : 6 (50.7%) and 16 : 0/22 : 6 (25.6%) in phosphatidylethanolamine.

Introduction

Microvillar membranes in the outer segment of invertebrate photoreceptor cells are the sites of light reception and energy transduction to electrical activity in the visual system [1]. Rhodopsin is the major protein component of the microvillar membranes in the cephalopods and its chromopher, 11-*cis*-retinal, serves as the light reception [2]. Photolytic sequence of cephalopod rhodopsin

is clearly defined [3—6] and some intermediate processes may be associated with the visual excitation. Due to the strong hydrophobic nature of rhodopsin and apparent bilayer character of the membranes, the properties of the rhodopsin in the membrane should be partly dependent upon the physical and chemical nature of the lipid [7—12].

To elucidate the mechanism of photoreception in the microvillar membranes, it is of importance to characterize as fully as possible the lipid of microvillar membranes of photoreceptor cells. In this respect, lipid composition of photoreceptor membranes has well been studied for the vertebrates such as cattle [13—17] and frog [18]. Most of the fatty acids from photoreceptor membranes of vertebrates are polyunsaturated fatty acids, particularly 22 : 6 [14—17] which is believed to be critical in photoreceptor membranes [14]. However, only a few reports are available on the lipid analyses of invertebrate (squid and horseshoe crab) photoreceptor membranes [19—21], although there are morphological differences between vertebrate (discmembrane) and invertebrate (microvillar membrane).

Discrepant observations have been reported on the major fatty acids of phospholipids in the photoreceptor cells of squid. Mason et al. [19] report large percentages of short chain fatty acids and low levels of polyunsaturated fatty acids. But another report [20] describes that the levels of polyunsaturated fatty acids are highest among any other vertebrate or invertebrate retina examined so far. The present study was therefore made to elucidate the membrane environment with rhodopsin in micro of invertebrate photoreceptor cells, using octopus as the material; lipid analysis of which has not been carried out yet. In this paper we show the characteristics of phospholipids, and major lipids in the octopus microvillar membranes.

Materials and Methods

Chemicals

Ceramide 2-aminoethylphosphonate was a generous gift from Dr. Y. Hayashi, Department of Chemistry, Faculty of Science and Technology, Kinki University, Osaka, Japan. Ceramide aminoethylphosphate was also a gift from Dr. T. Hori, Department of Chemistry, Faculty of Education, Shiga University, Shiga, Japan. Reference standards of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin were isolated from the rat in our laboratory [22,31]. Authentic standards of fatty acid methylesters and phospholipase C from Clostridium Welchii and Bacillus cereus were purchased from Sigma Chem. Co. All solvents used here were flushed with nitrogen gas and contained 0.02% of 4-methyl-2,6-*tert*-butylphenol to avoid the oxidation of unsaturated lipids.

Isolation of the microvillar membranes of the octopus photoreceptor cells

Paroctopus defleini, the common octopus in Hokkaido, Japan was used as material. Live octopi were decapitated at once in dim light and their eyeballs, kept in the dark at —80°C, were brought to the laboratory. Within a day we started to isolate the microvillar membrane by a method described previously [6]. The bisected eyes were shaken in 10 mM imidazole buffer (pH 7.2) con-

taining 1 mM $MgCl_2$ and 500 mM NaCl. The microvillar membranes were isolated by repeated discontinuous sucrose gradient centrifugation. The outer segments containing sucrose (density of 1.20 g/ml) were layered at the bottom of a discontinuous gradient of sucrose in 10 mM phosphate (pH 6.8) containing 1 mM $MgCl_2$ and 10 mM NaCl. The gradient was formed by layering 2 ml of sucrose solutions of different densities (1.15, 1.13 and 1.11 g/ml). After centrifugation at 21 000 × *g* for 2 h at 5°C, a dense orange band appeared at the 1.13/1.15 g/ml interface, which was collected with a syringe with a long needle (No. 18). This discontinuous gradient centrifugation was repeated twice. The isolated outer segments were then washed repeatedly with distilled water, 10 mM KH_2PO_4, 100 mM Na_2HPO_4, and finally, with 67 mM phosphate buffer (pH 6.8) by centrifugation.

Lipid extraction and analysis of lipids by two dimensional thin-layer chromatography

Microvillar membranes of octopus photoreceptor cells were homogenized with 0.1 M Tris-HCl buffer (pH 7.4) and an aliquot was taken for determination of protein. The lipids were extracted from the homogenates by the method of Bligh and Dyer [23]. For the analysis of phospholipid composition, individual phospholipid was separated by two-dimensional thin-layer chromatography as described by Rouser et al. [24]. Identification of the phospholipid on the plates was made on the basis of ninhydrin [25], and Dittmer's reagent [26] spray, mild alkaline and acid hydrolysis [27], and comparison with migration of reference standards. After chromatography, the spots on the plates were detected by iodine vapor and individual spots were scraped off and transfered into test tubes. Lipid phosphorus of the spots was then analyzed by the method of Aalbers and Bieber [28]. The spot with the gel was digested with 0.4 ml of 70% $HClO_4$ at 175°C for 15 h. After cooling, 0.3 ml of 10 N H_2SO_4 and 2 drops of 30% H_2O_2 were added and digested again at 175°C for 2 h and then phosphorus was determined according to the method of Bartlett [29].

Isolation of pure phospholipids

For isolation of pure phospholipids, the combined method of column chromatography on DEAE-cellulose and preparative thin-layer chromatography was employed as reported previously [30,31]. After elution of neutral lipids with chloroform, the nonacidic phospholipids were eluted with methanol by chromatography on DEAE-cellulose. Phosphatidylcholine and phosphatidylethanolamine were then isolated from the neutral phospholipid fraction by means of thin-layer chromatography on Silica gel G plates with a solvent system of chloroform/methanol/water (70 : 30 : 5 v/v). Each band on the plate of thin-layer chromatography was scraped and eluted with chloroform/methanol/acetic acid/water (50 : 39 : 1 : 10, v/v) as described by Arvidson [32] and the extract was washed with 4 N ammonia and 50% methanol. The recovery of phosphatidylcholine and phosphatidylethanolamine was 94.2 ± 2.3% and 92.7 ± 3.5%, respectively when examined by the separate experiment using radioactive phospholipid samples.

Resolution of phospholipids into molecular species

Phosphatidylcholine and phosphatidylethanolamine were converted into 1,2-diacylglycerol by phospholipase C from *Clostrium Welchii* and *Bacillus cereus*, respectively [33]. The 1,2-diacylglycerols formed from the phospholipids were immediately acetylated with acetic anhydride and anhydrous pyridine as described by Soodsma et al. [34]. The diacylglycerol acetates were purified by thin-layer chromatography with a solvent system of hexane/ether/formic acid (60 : 20 : 1.5, v/v) and recovered as described by Kuksis et al. [35]. No significant loss of polyunsaturated fatty acids occurred in this acetylation procedure.

The diacylglycerol acetates were resolved into molecular classes according to the degree of unsaturation by means of argentation thin-layer chromatography. The silica gel H plates containing 10% $AgNO_3$ were first developed to a height of 6 cm with chloroform/methanol/water (55 : 33 : 7, v/v) and after drying with N_2 stream were further developed to a height of 12 cm with chloroform/methanol (90 : 10, v/v). Finally the plates were developed to a height of 18 cm with hexane/ether (4 : 1, v/v). By this three step development, the diacylglycerol acetates from the phospholipids were separated into six subfractions designated as saturates, monoenes, dienes, tetraenes, polyene I and polyene II. The diacylglycerol acetates were recovered from the gel by the method of Arvidson [32]. The relative proportion of the diacylglycerol acetates separated on argentation thin-layer chromatography was estimated by glycerol determination of each subfraction.

In order to determine the detailed molecular species, the carbon number distribution of the diacylglycerol acetates separated on the basis of the degree of unsaturation, was analyzed by gas chromatography [35]. The diacylglycerol acetates were analyzed on a 50 cm × 4 mm (outer diameter) pyrex column packed with 1% silicone OV-1 on Gaschrom Q at 290°C. Peak identity of the diacylglycerol acetates was made by comparison with calibration standards composed of dimyristoyl, dipalmitoyl, distearoyl and diarachidoyl acetates. The carbon number on each diacylglycerol acetate has been defined as the sum of the carbon atoms in the fatty acid moieties.

Analytical methods

The fatty acid methylesters of phospholipids were prepared with BF_3/methanol [36]. The fatty acid methylesters were analyzed on a 2 m × 4 mm outer diameter pyrex column packed with 10% diethyleneglycol succinate on Chromosorb W at 185°C. Identification of fatty acid methylesters was made by comparison with retention time of reference standards. Hydrogenation and argentation thin-layer chromatography were also employed to identify fatty acid methylesters. Hydrogenation of fatty acid methylesters was carried out with panadium charcoal as reported previously [22]. Argentation thin-layer chromatography on plates of 10% $AgNO_3$ in silica gel G was employed to identify fatty acid methylesters with a solvent of hexane/ether (4 : 1, v/v) [37]. The positional distribution of fatty acids in the original phospholipids was determined by hydrolysis with phospholipase A_2 according to the method of Long and Penny [38].

Lipid phosphorus was determined by the method of Aalbers and Bieber [28], and Bartlett [29]. Glycerol was determined by the method of Van

Handel and Zilversmit [39], total cholesterol by the method of Zak et al. [40] and free fatty acid by the method of Itaya and Ui [41]. Protein was determined by the method of Lowry et al. [42]. All gas chromatographies were done with a Shimadzu GC-5A gas chromatograph, equipped with a flame ionization detector, in conjunction with Shimadzu chromatopac-E1A.

Results

The lipid content of microvillar membranes of octopus photoreceptor cells is given in Table I. The ratio of total lipid to protein was about 1, almost the same as the values obtained from squid rhabdome [19] and bovine rod outer segments [13]. Most of the lipid was phospholipid (84%). Neutral lipids made up 16%, the major one of which was free cholesterol (10%).

Two-dimensional thin-layer chromatography resolved the octopus outer segment lipids into ten or eleven spots (Fig. 1). However, seven of the spots were Dittmer's reagent-positive, that is phospholipids (Table II). Phosphatidylethanolamine and phosphatidylcholine were the major phospholipids and together they accounted for about 84% of the phospholipids. The content of phosphatidylserine was relatively low (3.7%) as compared with that of bovine outer segment (13—15%) [13,17]. In contrast, three spots (spots 4, 5 and 6 in Fig. 1) of alkaline and acid stable phospholipids were detectable. One of the phospholipids, spot 4, was identified as ceramide 2-aminoethylphosphonate, since it gave ninhydrin positive and co-chromatographed with the reference standard. In the same manner, ceramide aminoethylphosphate was not found to occur in this sample, the authentic standard of which migrated between spots 3 and 4 in this solvent system. These findings are consistent with those reported by Hori et al. [43,44], who described that cephalopods contain ceramide 2-aminoethylphosphonate and sphingomyelin, but not ceramide aminoethylphosphate. Spot 6 was co-chromatographed with reference phosphatidylinositol, but it gave ninhydrin positive, and was alkaline and acid stable. Hence this phospholipid was not identified. The Dittmer's reaction was weak but certainly positive in spot 1. It was also unidentified. No glycolipids were observed by specific glycolipid strain on the plates of thin-layer chromatography. Phosphatidylcholine was of the diacyl type alone and only

TABLE I

LIPID CONTENT OF MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

	Lipid content	
	mg/mg protein	%
Phospholipid **	0.95 ± 0.11 *	84.1
Cholesterol ***	0.11 ± 0.005	9.7
Triglyceride	0.02 ± 0.01	1.8
Free fatty acid	0.05 ± 0.02	4.4

* Values are means ± S.D., from four different samples.

** The amount of phospholipid was calculated as lipid phosphorus × 25.

*** The value of cholesterol contains free and esterified types, but the latter spot was not detected in thin-layer chromatography.

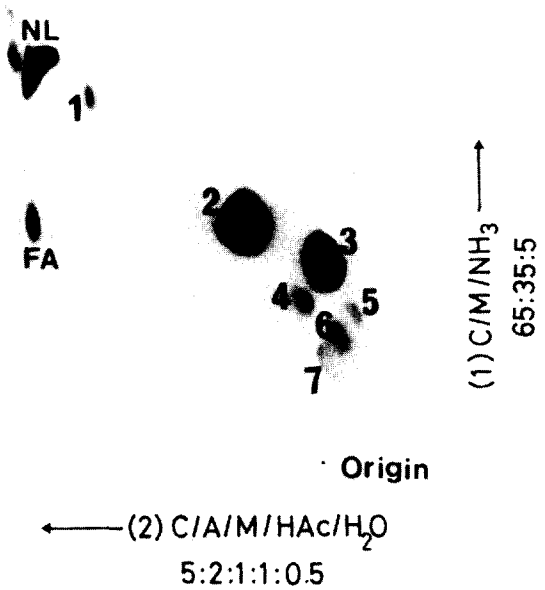

Fig. 1. Two-dimensional thin-layer chromatograph of the lipids of microvillar membranes of octopus photoreceptor cells. The chromatogram was developed with chloroform/methanol/28% aqueous ammonia (65 : 35 : 5, v/v) followed by chloroform/acetone/methanol/acetic acid/water (5 : 2 : 1 : 1 : 0.5, v/v). Spots were visualized with the formaldehyde/sulfuric acid (3 : 97) char procedure. NL, neutral lipids; FA, free fatty acid.

trace amounts of alkenyl type occur in phosphatidylethanolamine, which were noticed by the detection of dimethyl acetals on gas chromatograms.

The results of the fatty acid analysis of the major phospholipids, phosphatidylcholine and phosphatidylethanolamine, in the microvillar membranes are presented in Table III. The fatty acids of the two phospholipids were found

TABLE II

PHOSPHOLIPID COMPOSITION IN MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

Spot No. **	Phospholipid	Mol% of phosphate (n = 6)
1	Unidentified	0.4 ± 0.2 *
2	Phosphatidylethanolamine	45.5 ± 2.9
3	Phosphatidylcholine	38.8 ± 1.2
4	Ceramide 2-aminoethylphosphonate	5.1 ± 1.1
5	Sphingomyelin	2.5 ± 0.8
6	Unidentified	4.0 ± 1.9
7	Phosphatidylserine	3.7 ± 1.6

* Values are means ± S.D., from six different samples.

** Spot numbers represent numbers shown in Fig. 1.

TABLE III

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

Fatty acid **	Fatty acid composition (mol%) *							
	Phosphatidylcholine				Phosphatidylethanolamine			
	Total	1-Position	2-Position	Hydrogenated total	Total	1-Position	2-Position	Hydrogenated total
14 : 0	0.4	1.1	0.3	1.3	0.3	0.2	0.1	1.5
16 : 0	36.0	52.4	16.3	37.1	6.1	13.8	3.1	7.1
17 : 0	0.5	1.9	1.5	0.4	0.4	2.7	0.5	0.5
18 : 0 aldehyde	—	—	—	—	0.5	0.9	—	0.5
18 : 0	1.8	2.1	0.6	9.2	1.8	4.4	0.5	6.7
18 : 1	5.1	4.8	6.0	—	4.5	8.4	1.1	—
18 : 2	1.4	1.8	0.7	—	0.3	1.3	0.1	—
20 : 0	—	—	—	23.3	—	—	—	43.4
20 : 1	1.1	3.0	0.8	—	3.3	7.9	1.0	—
20 : 4	14.9	25.1	3.5	—	27.7	45.7	8.2	—
20 : 5	7.6	2.4	12.6	—	13.4	6.2	18.8	—
22 : 0	—	—	—	28.7	—	—	—	40.3
22 : 5	—	—	0.5	—	—	—	—	—
22 : 6	30.1	1.5	57.2	—	41.7	5.8	66.6	—

* Values represent the mean of triplicate analyses from each preparation of the microvillar membranes of photoreceptor cells from about 100 octopi.

** Number of carbons: number of double bonds.

to be highly unsaturated. About 90% in phosphatidylethanolamine and about 60% in phosphatidylcholine were unsaturated fatty acids, the majority of which were 20 : 4, 20 : 5 and 22 : 6, particularly about a half of the unsaturated fatty acids were 22 : 6. The positional specificity of the unsaturated fatty acids should also be noted. The 22 : 6 and 20 : 5 were almost exclusively linked at the 2-position of glycerol, but the 20 : 4 was significantly linked at the 1-position. The identification of the major unsaturated fatty acid methylesters was made by the comparison of retention time with reference standards, hydrogenation, and combination of argentation thin-layer chromatography and gas chromatography. After hydrogenation, the peaks of unsaturated fatty acid methylesters disappeared and the corresponding increases in 18 : 0, 20 : 0 and 22 : 0 were found on gas chromatograms (Table III). The tetraenoic, pentaenoic and hexaenoic fatty acid methylesters isolated by argentation thin-layer chromatography were 20 : 4, 20 : 5 and 22 : 6 respectively (data not shown). The saturated fatty acids of short carbon chains were not found. These fatty acid patterns of the phospholipids in microvillar membranes of octopus photoreceptor cells were very similar with data of squid retina reported by Anderson et al. [20], but different from those of squid rhabdomes reported by Mason et al. [19] in high concentrations of polyunsaturated fatty acids, and from those of bovine rod outer segments [13] in their positional specificity.

Table IV gives the proportion of molecular classes, characterized by their degree of unsaturation, of phosphatidylcholine and phosphatidylethanolamine

TABLE IV

COMPOSITION OF MOLECULAR CLASSES OF PHOSPHOLIPIDS IN MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

Values represent mean ± S.D. from each phospholipid isolated from three preparations of the membranes of octopus photoreceptor cells.

Content (μmol/mg protein)	Phosphatidylcholine 0.43 ± 0.01	Phosphatidylethanolamine 0.53 ± 0.03
Saturates	3.0 ± 0.6 (%)	0.9 ± 0.2 (%)
Monoenes	16.0 ± 1.8	2.9 ± 0.4
Dienes	5.4 ± 1.1	3.2 ± 1.2
Tetraenes	20.0 ± 2.7	8.1 ± 1.9
Polyene I	52.0 ± 4.3	32.1 ± 2.3
Polyene II	3.6 ± 1.4	51.7 ± 3.4

from the microvillar membranes of octopus photoreceptor cells. The most striking characteristics of the molecular classes in the two phospholipids were the high concentration of polyunsaturated ones, that is, polyenes I and II. However, some differences in molecular classes were found between phosphatidylcholine and phosphatidylethanolamine. The major molecular classes in phosphatidylcholine were monoenes (16.0%), tetraenes (20%) and polyenes I (52.0%) and only small proportions (3.6%) of polyenes II were seen, while

TABLE V

COMPOSITION OF INDIVIDUAL MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

Molecular class	Carbon number	Phosphatidyl-choline (%)	Phosphatidyl-ethanolamine (%)	Corresponding molecular species *
Saturates	30	0.2	0.1	14 : 0/16 : 0
	32	2.8	0.8	16 : 0/16 : 0
	34	0.1	—	16 : 0/18 : 0
Monoenes	32	4.2	0.3	16 : 0/16 : 1
	34	10.5	2.1	16 : 0/18 : 1
	36	1.3	0.5	18 : 0/18 : 1
Dienes	34	2.9	2.0	16 : 0/18 : 2
	36	1.9	1.2	18 : 0/18 : 2, 18 : 1/18 : 1
	38	0.2	—	18 : 1/20 : 1
Tetraenes	34	0.2	0.1	14 : 0/20 : 4
	36	19.6	7.9	16 : 0/20 : 4
	38	0.6	0.2	18 : 0/20 : 4
Polyenes I	36	3.6	1.1	16 : 0/20 : 5
	38	48.4	25.6	16 : 0/22 : 6
	40	—	5.4	18 : 0/22 : 6
Polyenes II	36	0.2	—	
	38	0.7	—	
	40	0.6	1.0	18 : 2/22 : 6
	42	2.1	50.7	20 : 4/22 : 6

* No positional specificities are implied in the symbolism of molecular species. The main molecular species are listed on the basis of fatty acid analysis of each molecular class.

phosphatidylethanolamine contained polyenes I (32.1%) and II (51.0%) as the predominant molecular classes.

The mass distribution of individual molecular species of the two phospholipids is presented in Table V. The predominant carbon number of diacylglycerol acetates from polyenes I was 38, which were mainly composed of 16 : 0 and 22 : 6. Polyenes II in phosphatidylethanolamine contained predominantly carbon number 42, the fatty acids of which were significantly 20 : 4 and 22 : 6. The polyenes I and II are, therefore, considered to be hexaenes and decaenes, respectively. The major molecular species were 16 : 0/22 : 6, 16 : 0/20 : 4 and 16 : 0/18 : 1 in phosphatidylcholine, and 20 : 4/22 : 6 and 16 : 0/22 : 6 in phosphatidylethanolamine. It was also noted that monoenes, dienes or tetraenes occurred predominantly in combination with 16 : 0 as saturated acids. Tetraenoic phospholipids in mammalian tissues contain generally significant amounts of stearic acid as saturated acids [46], and preferential combinations between stearic and arachidonic acids have been reported in certain phospholipids from mammalian tissues [45]. However, apparently only small amounts of 18 : 0/20 : 4 species occurred in the phospholipids of this preparation from the octopus.

Discussion

The phospholipids occurring in natural sources are composed of complicated mixtures of molecular species which contain a pairing of various fatty acids. Recent observations revealed that different molecular species of phospholipids are synthesized differently via their characteristic routes [46—48] and phospholipids function as a unit of molecular species in the membrane [49]. Therefore, more detailed information on the characteristics of lipids in the membrane will be obtained by the analysis of molecular species of microvillar membrane phospholipid classes than their fatty acid analysis alone. In this respect, the positional distribution of fatty acids and the molecular species of phosphatidylcholine and phosphatidylethanolamine in microvillar membranes of octopus photoreceptor cells were analyzed in the present work, which have not been performed previously except for the positional analysis of fatty acids in the phospholipids of vertebrate photoreceptors [50,51]. Very interesting is the fact that 22 : 6 and 20 : 5 are exclusively linked at the 2-position, but 20 : 4 significantly linked at the 1-position of the phospholipids, and 16 : 0 are distributed in both positions, especially in phosphatidylcholine. Major molecular species are 16 : 0/22 : 6 and 16 : 0/20 : 4 in phosphatidylcholine, and 20 : 4/22 : 6 and 16 : 0/22 : 6 in phosphatidylethanolamine. Although no positional specificities were determined in each molecular species, the predominant tetraenoic species, 16 : 0/20 : 4, seems to be mainly composed of 20 : 4 at the 1-position and 16 : 0 at the 2-position.

As mentioned above, the most striking feature of lipids of the octopus microvillar membranes is that they contain highly unsaturated molecular species of phosphatidylcholine and phosphatidylethanolamine as the major lipid classes. These facts suggest that the microvillar membranes of octopus photoreceptor cells are liquid-like in nature and rich in fluidity, which seems to be similar with the nature of vertebrate photoreceptor cells [13—17]. However,

discrepant observations have been reported on characteristics of lipids in photoreceptor cells of invertebrate (squid). Mason et al. [19] described that squid rhabdom is different from the vertebrate photoreceptor in neutral lipid content and predominant fatty acids; small quantities of 22 : 6, large quantities of the short-chain fatty acids in all lipid classes, and relatively high content of cholesterol are found in squid photoreceptor. They suggest that the squid photoreceptor membrane is more rigid than the vertebrate photoreceptor membrane and consequently that squid rhodopsin has a potentially lower mobility in the visual pigment membranes. In sharp contrast, Anderson et al. [20] reported that unusually large amounts of polyunsaturated fatty acids (20 : 4, 20 : 5 and 22 : 6) occur in each of the lipid classes of the squid photoreceptor membranes; phosphatidylethanolamine contain almost 90% polyunsaturated fatty acids, the most abundant of which is 22 : 6, while phosphatidylcholine contain 58% long chain polyunsaturates. The present results with microvillar membranes of octopus, belonging to the same cephalopods as squid, are very similar with the findings of Anderson et al. [20] in the types and percentages of fatty acids of phosphatidylcholine and phosphatidylethanolamine. However, it should also be noted that the octopus membranes contain relatively large parts (about 16% of total lipid) of cholesterol and sphingolipids (ceramide 2-aminoethylphosphonate and sphingomyelin), which are considered to have a much more rigid and compact nature than the unsaturated molecular species of phospholipids in the membrane organization [52].

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